Final Report

Investigating the importance of latent infection in causing tuber breakdown during storage and transit

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1. SUMMARY

1.1. Aim

This project aims to determine the importance of latent infection in seed potato consignments destined for export markets and to establish whether stocks which are at high risk of quality decline can be identified prior to dispatch. The effect of tuber storage and transit on the quality of seed tubers and, more importantly, on the maintenance of quality during export transit was studied.

1.2. Methodology

Seed potato crops intended for export were submitted by exporters and three two hundred tuber samples were collected from each lot by Scottish Government Plant Health inspectors. One 200 tuber sample was processed immediately (harvest), another was stored in a potato store at SASA to be processed when the exported tubers arrived back in Scotland (stored), and the final 200 tuber sample was exported (exported). A basic data logger that records temperature, relative humidity and dew point measurements was placed in a netted bag with a sample of the seed lot and these were included with the consignment for export. The harvest tuber samples were visually assessed for blemish diseases and internal rots immediately after collection from the exporter and peel and heel-end core samples collected for molecular diagnostics. Latent infection was determined using molecular diagnostics following DNA extraction from samples collected from bulks of ten tubers. Exported tuber samples were returned to the UK from the customer in the importing country via courier (air freight) and processed in parallel with the stored tuber samples as described for the harvest samples. Transit condition data was downloaded from loggers for analysis and used to design an experiment under controlled conditions to mimic export and evaluate the effects of the transit environment on tuber quality of high and low tuber health stocks.

1.3. Key findings

Seed tuber quality showed little deterioration in the crops assessed over three years of the project. In all cases the storage conditions during transit appear to have been maintained at the prescribed levels indicating no problems with the haulage providers' facilities during export. Occasional increases in surface blemish diseases known to increase during storage were observed on those tubers that were stored in the UK or exported. However, no consistent increases in DNA levels of the pathogens responsible for these diseases was associated with increased disease incidence. The most consistent changes in pathogen DNA levels were observed for *Pectobacterium atrosepticum* DNA in tuber heel end tissue. Compared to tubers assessed after harvest, DNA levels of *P. atrosepticum* generally increased in samples that had been stored in the UK or had been exported with levels typically highest in the exported samples. Stocks of a higher seed health pose a reduced risk of increasing *P. atrosepticum* DNA levels during the export process.

1.4. Practical recommendations

Data suggests that if tubers are stored under recommended conditions during transit then the risks of latent pathogen infections resulting in disease expression are minimal. Storage conditions on arrival in the importing country appear much more variable. Exporters may be advised to request that the official inspection of consignments is performed once the tubers arrive in the importing country to prevent losses after the transit process. Maintaining the highest levels of seed health prior to export remains a key requirement to limit the risk of tuber quality deterioration during export.

2. INTRODUCTION

The Scottish seed potato industry underpins UK production, with more than 75% of UK crops derived from Scottish seed and contributing in excess of £100 million per annum to the Scottish economy (Potato Council, 2014). Over 70,000 tonnes of seed potatoes are marketed to non-European countries each year (Fig. 1A), many of which have more strict disease tolerances than the EU market, highlighting the importance of producing disease-free crops. Crop health is paramount, and Scotland is one of seven regions recognised within the EU as a Community Grade region for seed potato production, applying stricter plant health standards than in other regions. Despite the strict plant health regulations for non-EU markets, few consignments of seed potatoes exported from Scotland are rejected by the importing country for plant health reasons (Fig. 1B).

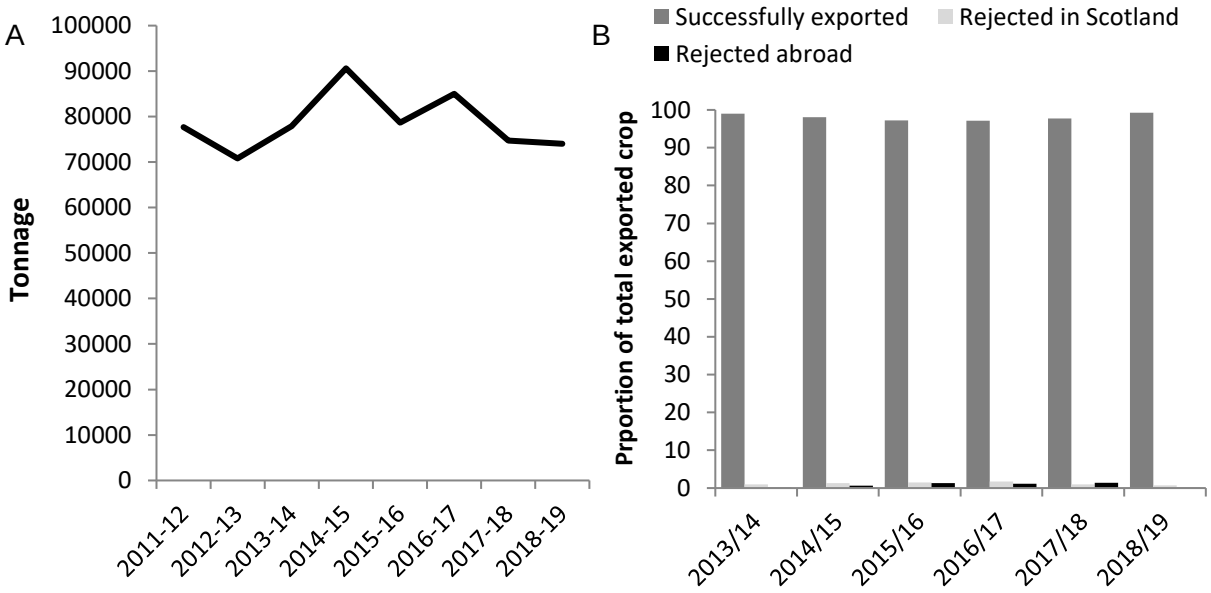

Fig. 1 Export statistics of Scottish seed potatoes to non-EU destinations over the five-season period 2013-2019. A. Volume of seed potato exports (tonnes) from Scotland to non-EU countries. B. Proportion of seed potato consignments successfully exported, rejected for export in Scotland (at inspection) for failing to meet the destination country's import requirements, or rejected at final export destination.

Despite low numbers of official rejections by importing countries, rejected consignments still pose an important threat to the UK potato industry, through both economic loss and loss of confidence in the quality of exports. Since 2013, the presence of common scab or powdery scab was the main reason for rejection by officials in importing countries which may be explained by the strict tolerances for both scab diseases in Egypt, the major export destination for Scottish seed crops. The exporter is responsible for re-exporting consignments back to the UK if the consignment cannot be destroyed in the importing country. This places a huge financial burden on the exporter. For example, returning a 25-tonne container of rejected seed potatoes from the Canary Islands costs approximately £15,000. Costs to return consignments from the major importers of Scottish potatoes in North Africa are likely to be higher.

Determining what factors influence tuber quality, and whether this is associated with increased disease development resulting from latent infections which manifest during transit, is essential to protect the reputation of the Scottish seed potato industry. Optimum storage conditions for seed potatoes are well documented throughout the

industry and advice on minimising the risk of storage diseases is available (Cunnington and Pringle, 2012). Unfortunately, no single specific store condition will prevent the development of all the possible diseases that can affect post-harvest potato quality (Fiers *et al*., 2012) but conditions are set to minimise the risk of disease development as much as is practical.

Although the effects of storage conditions on disease development are relatively well understood, there is little information on the effect of transport conditions and latent infection on disease development in seed potatoes during transit. Temperature, humidity and ventilation are critical in delivering quality seed potatoes to their final destination. Contractors employed to transport seed potato consignments to export destinations are responsible for storage conditions during transit. Exporters frequently monitor temperature in containers during transit but do not necessarily monitor any other factors that may influence disease development such as relative humidity, movement in transit or changes in condensation levels on tubers. It is not yet known at what stage in the supply chain symptom development occurs; however, in most cases of official rejections, pre-shipment inspection reports from the UK show marked differences with inspection reports on arrival in the importing country suggesting that the quality of the seed has significantly deteriorated in transit. Transit duration and conditions can be prolonged and uncontrolled depending on the destination of the consignment. Temperature and humidity are known to affect disease development (Bartz and Kelman, 1984; Du Raan *et al*., 2016; Kushalappa and Zulfiqar, 2001; Smadja *et al*., 2004; Moh *et al*., 2012; Pringle, 1996; Pringle *et al*., 1991) and there is some evidence of the effect of atmosphere, specifically changes in oxygen and carbon dioxide concentrations in the local environment holding the tubers on soft rot bacteria (De Boer and Kelman, 1978; Lund and Wyatt, 1972; Lund and Nicholl, 1970). Previous studies have shown that increased respiration may also accelerate the development of soft rot in tubers during transit (Nielsen, 1968). It is not always clear however, if disease development in store and transit can be directly attributable back to the health of the crop in the field and conditions at harvest, or whether sub-optimal conditions favour the amplification and of previously inactive members of the microbial community resident on the tuber surface. It may also be the case that the manner in which the seed has been handled prior to export affects how the seed will react during transport and transit conditions cannot reverse damage that has already been done prior to loading.

Determining whether fluctuations in storage conditions during the export process can influence the accumulation of pathogen biomass, and subsequent development of disease symptoms in transit, will provide novel insights into the causes of consignment rejections on arrival in the importing country. This project sought to assess the role of latent infections of seed potatoes in affecting tuber quality during transit. By monitoring changes in pathogen levels during transit and recording storage conditions during the export process, the project aimed to develop a model to aid in the identification of those consignments that are of high risk of tuber quality degradation during export.

Objectives

Objective 1: Determine the relative importance of latent infection in seed potato consignments destined for export.
Objective 2: Identify and monitor pathogens that are responsible for causing quality deterioration of seed potato consignments during transit.
Objective 3: Determine the effect of storage and transit on the quality of seed tubers by monitoring fluctuations in CO_2, temperature, RH and movement.
Objective 4: Develop a risk model to identify high risk consignments prior to export and an action plan to mitigate risk.

3. MATERIALS AND METHODS

3.1. Export crops

3.1.1 Year 1 export crops

Seven export consignments derived from five Scottish crops were selected. Four of the consignments were derived from two crops of cv. Atlantic, with seed lots from both of these crops shipped to different countries in southeast Asia. One additional seed lot of cv. Atlantic due for export to southeast Asia was also included, as were consignments of crops of cv. Desiree and cv. Winston destined for North Africa and The Middle East, respectively (Table 1).

Table 1 Details of 2017-18 season seed potato consignments used in Year 1 of this study

Code	Variety	Export destination
SEA1_2017A	Atlantic	Southeast Asia (SEA1)
SEA1_2017B	Atlantic	Southeast Asia (SEA1)
ME_2017	Winston	Middle East (ME)
SEA2_2017A	Atlantic	Southeast Asia (SEA2)
SEA2_2017B	Atlantic	Southeast Asia (SEA2)
SEA2_2017C	Atlantic	Southeast Asia (SEA2)
NA1_2017	Desiree	North Africa (NA1)

Cultivar Atlantic is a popular processing variety. It is particularly susceptible to foliar and tuber late blight, gangrene, PVY and spraing whereas cv. Winston, a very early variety, is susceptible to powdery scab but moderately resistant to tuber and foliar blight. Both varieties exhibit intermediate resistance to blackleg. Cultivar Desiree is a main crop variety that shows good resistance to powdery scab but is susceptible to foliar blight, blackleg and common scab (Table 2).

Table 2 Disease resistance ratings from the AHDB Potato variety database for the potato varieties used in year 1 of this study

Disease (Pathogen)	**Variety**		
	Atlantic	**Winston**	**Desiree**
PVY O	3 [a]	7	7
PLRV	6	5	4
Common scab (*Streptomyces* scabies)	6	5	4
Blackleg (*Pectobacterium* spp.)	5	6	4
Powdery scab (*Spongospora subterranea)*		4	7
Tuber blight (*Phytophthora infestans*)	3	5	5
Foliar blight (*Phytophthora infestans*)	3	5	4

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

Tuber samples from each seed lot were collected by SG Plant Health inspectors and sent to SASA prior to dispatch of the consignment. Year 1 samples were stored in a cold store at SASA at 4°C until the exported samples from the consignment were returned to Scotland. Stored tuber samples consisted of 400 tubers divided in to four sub-samples, each of 100 tubers. Each of the 100 tuber sub-samples were further

divided into two 50 tuber batches. One 50 tuber batch was stored at SASA at 4°C prior to processing for molecular diagnostic analyses. The other 50 tuber batch was sent to Sutton Bridge Crop Storage Research for surface disease assessment and were stored at 3°C at Sutton Bridge prior to assessment. Every consignment destined for export had a netted bag of 50-100 tubers prepared prior to shipping. A single battery operated TinyTag Plus 2 data logger (Fig. 2, Gemini Data Loggers Ltd., Chichester, UK) was placed within each export consignment in the netted bag with the tubers. The data loggers recorded temperature, relative humidity and dew point measurements during the export process.

On arrival in the importing country, the netted bag containing the sample of 50-100 tubers and the TinyTag Plus 2 data logger was to be returned by air courier to SASA. Returned samples were stored in a potato cold store in the Quarantine Unit at SASA at 4°C until analysis. Disease assessments of post-export samples were performed at SASA. The output from the data loggers was retrieved using Tinytag SWCD0040 Data Logger Software (Gemini Data Loggers Ltd.).

Fig. 2 TinyTag Plus 2 data logger

3.1.2 Year 2 export crops

Five export consignments were identified for use in year 2 of the project. Three of the consignments were destined for the North African market (NA2), the largest market for Scottish seed potatoes, with the remaining two destined for the Middle East (ME) and another market in North Africa (NA3). Five potato varieties were represented in the exported consignments (Table 3).

Table 3 Details of 2018-19 season seed potato consignments used in Year 2 of the study

Code	Variety	Export destination
NA2_2018A	Galactica	North Africa (NA2)
NA2_2018B	Slaney	North Africa (NA2)
NA2_2018C	Hermes	North Africa (NA2)
ME_2018	Winston	Middle East (ME)
NA3_2018	Kerr's Pink	North Africa (NA3)

Galactica is grown for the fresh produce market, has resistance to tuber blight and common scab but is particularly susceptible to foliar blight. Slaney is produced for the fresh and chipping markets and is susceptible to PLRV, powdery scab and tuber blight. Hermes is used in the chip and crisp processing markets, has resistance to powdery scab and is moderately susceptible to tuber and foliar blight. Kerr's Pink is suitable for pre-pack and general ware and is highly susceptible to skin spot (Table 4).

Table 4 Disease resistance ratings from the AHDB Potato variety database for the potato varieties used in year 2 of this study

Disease (Pathogen)	**Variety**				
	Galactica	**Slaney**	**Hermes**	**Winston**	**Kerr's Pink**
PVY O	3 [a]	7	7	7	6
PLRV	6	5	7	5	5
Common scab (*Streptomyces* scabies)	6	5	6	5	4
Blackleg *(Pectobacterium* spp.)	5	6	6	6	
Powdery scab *(Spongospora subterranea)*		4	8	4	
Tuber blight (*Phytophthora infestans*)	3	5	4	5	4
Foliar blight (*Phytophthora infestans*)	3	5	3	5	4
Dry rot (*Fusarium* spp.)					5
Skin spot (*Polyscytalum pustulans)*					2

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

Three 200 hundred tuber samples were collected from each crop prior to export. One 200 tuber sample was processed immediately (harvest), another was stored in a potato store at SASA to be processed when the exported tubers arrived back in Scotland (stored), and the final 200 tuber sample was exported (exported). Two data loggers were included with each consignment; one basic data logger (TinyTag) that recorded temperature, relative humidity and dew point measurements and one custom built data logger developed at the University of Warwick (Fig. 3), which in addition to temperature and humidity, was able to monitor light, movement, CO_2 and some indication of total VOC (volatile organic compounds). Both data loggers were placed in a netted bag along with a sample of the seed lot included with the consignment for export. The custom-built advanced data logger unit is approximately 22 cm x 11 cm x 7.5 cm and powered by 9 'type C' batteries. Seven units were produced for use in this project. On arrival in the

importing country, the netted bag containing the sample of 200 tubers was to be returned by air courier to SASA. Returned samples were stored in a potato cold store in the Quarantine Unit at SASA at 4°C until analysis. Disease assessments of all samples were performed at SASA.

Fig. 3. Advanced Logger Unit

3.1.3 Year 3 (2019-20) export crops

In the 2019-20 export season the variety Hermes was used for all export consignments monitored. Three crops were identified that were all destined for export to North Africa (NA2)(Table 5). A basic TinyTag data logger was included in each consignment.

Table 5 Details of 2019-20 season seed potato consignments used in this research

Code	Variety	Export destination
NA2_2019A	Hermes	North Africa (NA2)
NA2_2019B	Hermes	North Africa (NA2)
NA2_2019C	Hermes	North Africa (NA2)

3.2 Surface disease assessments

Tubers were rinsed with tap water and visually assessed for disease symptoms. The presence of the surface blemish diseases powdery scab, common scab, silver scurf, black scurf, black dot, skin spot and *Verticillium* were assessed as a proportion of the surface area exhibiting typical disease symptoms. The scores for percentage surface area affected were assigned values based on the categories in Table 6.

Table 6. Disease severity and scoring scale for assessing surface blemishes or internal rots

Surface area affected (%)	Score
0	0
0-2	1
>2-5	3.5
>5-10	7.5
>10-25	17.5
>25-50	37.5
>50	75

Internal rots were scored as soft rots, dry rots or gangrene-like symptoms. If symptoms typical of a rot disease were observed on the tuber surface, the tuber was cut in half through the symptomatic tissue. Disease symptoms were assessed as the proportion of the tuber flesh exhibiting necrotic areas of tissue. Percent infection scores were grouped in to the seven scoring categories as for the surface blemishes (Table 6). Disease incidence was calculated as the number of tubers with greater than 12.5% surface area affected by a specific disease as is the procedure for official tuber inspections within the UK. Variation in disease incidence attributed to the status of the tubers when scored (harvested, stored in the UK or post-export) was assessed using a generalized linear model that modelled binomial proportions following LOGIT transformation of the data using GenStat v14 (VSN International, 2011). Incidence of each disease was analysed separately as a proportion of the total number of tubers scored from each status category.

3.3 Molecular tests for plant pathogen diagnostics

Tuber samples were processed in bulks of ten tuber subsamples. Peel samples were collected from heel to rose end using a flame sterilised standard nylon handled potato peeler (Victorinox AG, Schmiedgasse, Switzerland) and pooled in an extraction bag (BioReba AG, Reinch, Switzerland). For the detection of *Pectobacterium atrosepticum*, a core sample from the heel end was collected using a sterile peeler. Peel and heel-end samples were placed in to separate extraction bags (BioReba AG) and 8 mL extraction buffer (10 mM Tris-HCl pH 8.5, 5 mM EDTA, 0.002% w/v sodium dodecyl sulphate, 200 mM NaCl) was added to each tissue sample prior to DNA extraction. Tissue was lysed using a Homex 6 homogenizer (BioReba AG) and the extract collected in sterile 2 mL Eppendorf tubes. DNA was extracted following the BioSprint 15 DNA Plant kit (Qiagen, Hilden, Germany) protocol using a KingFisherTM mL Purification system (Thermo Fisher Scientific, Paisley, UK).

Isolates of fungal, oomycete and bacterial pathogens were obtained from the SASA potato pathogen culture collection and sub-cultured on potato dextrose agar (Oxoid Thermo Fisher Scientific) plates at 18°C in the dark. *P. atrosepticum* 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 mins and the pellets stored at -20°C. Fungal and oomycete isolates were cultured on PDA plates for 14 days after which hyphae were removed from the surface using a sterile scalpel. Hyphae were fragmented using a TissueLyser II (Qiagen) for 60-90 seconds at 30 hertz. Pathogen DNA was extracted using the illustra Nucleon Phytopure DNA extraction kit (GE Healthcare, Buckinghamshire, UK) and quantified using a Nanodrop ND-1000 spectrophotometer. Ten-fold dilution series of each pathogen DNA were prepared from 10 ng μL^{-1} to 1 fg μL^{-1} to facilitate quantification of pathogen DNA in potato tissue samples.

DNA was quantified using a Nanodrop ND-1000 (Thermo Fisher Scientific) prior to use in polymerase chain reaction (PCR)-based molecular diagnostic assays. Primers designed to amplify the potato cytochrome oxidase (COX) gene were used as a positive control to confirm that amplifiable DNA had been extracted from tissue samples (Table 7). Quantitative PCR (qPCR) assays were performed to detect *P. pustulans* (Lees *et al*., 2009), *Colletotrichum coccodes* (Cullen *et al*., 2002), *Spongospora subterranea* (Qu *et al*., 2011), *Helminthosporium solani* (Cullen *et al*., 2001), *Botrytis cinerea* (Suarez *et al*. 2005), *Fusarium sulphureum* (Cullen *et al*., 2005), *Rhizoctonia solani* (Budge *et al*.,

2009), *Pythium ultimum* (Cullen *et al*., 2007) and *Pectobacterium atrosepticum* (Humphris *et al.*, 2015).

Detection of *B. cinerea*, *H. solani*, *R. solani* AG3*, P. ultimum*, *F. sulphureum*, *P. pustulans*, *C. coccodes, S. subterranea* and *P*. *atrosepticum* DNA in potato tuber tissue was performed using qPCR amplification with the Takyon™ Rox Probe Mastermix dTTP Blue system (EuroGentec, Liege, Belgium) following the manufacturer's protocol. DNA quality was assessed by amplification of the potato COX gene (Weller *et al*., 2000). Quantitative PCR conditions for *P. pustulans*, *C. coccodes*, *S. subterranea*, *H.* solani, *B. cinerea*, *R. solani*, *P. ultimum* and the potato COX gene were as follows: 50°C for 2 min followed by an activation phase of 3 min at 95°C and then 40 cycles of 95°C for 10 sec and 60°C for 1 min. Pathogen DNA levels were estimated using the qPCR amplification from standard curves of pathogen DNA using the CFX Manager software v3.1 (Bio-Rad laboratories, 2012). *F. sulphureum* DNA was amplified using the following programme: 50°C for 2 min followed by an activation phase of 3 min at 95°C and then 45 cycles of 95°C for 10 sec and 60°C for 1 min. *P. atrosepticum* DNA was amplified using the following programme: 50°C for 2 min followed by an activation phase of 3 min at 95°C and then 40 cycles of 95°C for 15 sec and 60°C for 1 min.

Table 7 Primers used for qPCR diagnostics of pathogens in seed potato 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]
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	**Eca F**	0.3 µM	ACA TTC AGG CTG ATA TTC CCC CTG CC
(Blackleg)	**Eca R**	0.3 µM	CGG CAT CAT AAA AAC ACG CC
	Eca P	0.1 µM	CCT GTG TAA TAT CCG AAA GGT GG [a]
Botrytis cinerea	**Bc3F**	0.3 µM	GCT GTA ATT TCA ATG TGC AGA ATC
(Grey mould)	**Bc3R**	0.3 µM	GGA GCA ACA ATT AAT CGC ATT TC
	Bc3P	0.1 µM	TCA CCT TGC AAT GAG TGG [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 [c]
Rhizoctonia solani AG3	**AG3_F**	0.25 µM	TCT ACA GGG ATT CCA GAT TAC GC
(Black scurf)	**AG3_R**	0.25 µM	TCA CGG ATC TTG GAA ATC AAC A
	AG3_P	0.08 µM	AGG AAC CGG TGC TGG TAT GGG GAC TC [c]
Pythium ultimum	**92F**	0.3 µM	TGT TTT CAT TTT TGG ACA CTG GA

(Watery wound rot)	**166R**	0.3 µM	TCC ATC ATA ACT TGC ATT ACA ACA GA
	116T	0.05 µM	CGG GAG TCA GCA GGA CGA AGG TTG [c]
Fusarium sulphureum	**Sulp63(F)**	0.9 µM	CAA ACC CCT GTG AAC ATA CCT YTA
(Dry rot)	**Sulp127(R)A**	0.9 µM	GCG GGC CGT TCC GA
	Sulp127(R)B	0.9 µM	CGG GCC GTT CCG TGA
	SulpRC(P)	0.1 µM	ACG GGC TGA TTC GCC GAG G GG [c]
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]

[a] Sequence modifications = 5' 6-FAM; quenchers = 3' BHQ-1, [b] 5' HEX; 3' TAMRA, [c] 5' FAM; 3' TAMRA

3.4 Transit mimic experiment

Using the data returned by the TinyTag Plus 2 data logger an experiment was set up to mimic the transit conditions to determine how post-export conditions affect tuber quality deterioration caused by *P*. *atrosepticum* infections in high and low health stocks. High health stocks were selected from commercial seed potato stocks that were destined for export to North Africa (NA2) (Table 8). All three high health stocks were of the variety Hermes and all had low levels of blackleg recorded during visual inspection of the growing crop. Low health stocks were selected from seed potato crops of three different varieties that are known to be susceptible to blackleg. The three seed potato crops had blackleg levels which exceeded those permitted for certified seed crops in Scotland and were either voluntarily withdrawn from the scheme by the grower or downgraded to a ware crop at inspection (Table 8). Stocks were stored in a potato store at SASA or Sutton Bridge Crop Storage Research at 3.5-4°C prior to use in the experiments.

Table 8 Potato stocks used in transit mimic experiments

Code	Variety	Field generation	Attained class	Health status	Blackleg status
High_2019A	Hermes	6	SE	High	0.01% in crop at second inspection
High _2019B	Hermes	4	E	High	0.78% in crop at third inspection
High _2019C	Hermes	5	E	High	0.11% in crop at second inspection
Low_2019A	N/A	5	None	Low	~2% in crop; withdrawn prior to inspection
Low _2019B	N/A	4	W	Low	1.57% in crop at second inspection
Low_2019C	N/A	3	None	Low	~7.5% in crop; withdrawn prior to inspection

P. *atrosepticum* levels were measured in a subsample of 100 tubers from each stock using molecular diagnostics and bacterial colony forming unit (CFU) counts. Each subsample of 100 tubers were further separated into bulks of 10 tubers and from each tuber, two heel end cores were removed. One heel end core from each of the 10 tubers was pooled in an extraction bag and stored at -20°C prior to DNA extraction and qPCR determination of *P*. *atrosepticum* DNA levels as described above (see section 3.3). The second heel end cores were used to determine the number of *P*. *atrosepticum* CFUs following the method of Hélias *et al*. (2012) with the following modifications. Heel end cores were placed in a disposable plastic container with a sealable lid (Greiner, Bio-one Ltd., Stonehouse, UK), 40 mL of extraction buffer (30 mM Na_2HPO_4 (anhydrous); 20 mM KH_2PO_4; de Tempe & Binnerts, 1979) added, and the containers incubated at 5°C in a MaxQ 6000 Incubated Shaker (Thermo Fisher Scientific, Paisley, UK) for 16-24 hours at 100 rpm. Following incubation, the liquid from each sample was decanted into centrifuge tubes and centrifuged at 10000 rpm at 4°C for 10 minutes. The supernatant was discarded, and the pellet resuspended in 1.5 mL Ringers solution (Sigma-Aldrich). The resuspended solution was diluted from 10^0 to 10^{-7} in Ringers solution, plated on to crystal violet pectate medium agar (CVPM) and incubated at 25°C for 48 hours. CVPM was prepared in two layers. The basal consisted of 1.2% (w/v) agar; 0.8% (w/v) peptone (BD-BioSciences, San Jose, CA, USA); 0.6% (w/v) tryptone (w/v; BD-BioSciences); 0.24% (w/v) yeast extract (BD-BioSciences); 68 mM NaCl; 15 mM L- asparagine; 10 mM sodium dodecyl sulphate mixed and adjusted to pH 7.2. The basal layer mix was autoclaved, cooled and 0.002% 2, 3, 5-triphenyltetrazolium and 5.12 ng μL^{-1} Polymyxin B sulphate added. Once the basal layer had been plated and allowed to set an over-layer was prepared. The over-layer consisted of 0.8% (w/v) agar; 3% (w/v) sodium

polypectate; 0.00015% (v/v) crystal violet (Sigma-Aldrich); 0.13% (w/v) $CaCl_2$; 24 mM $NaNO_3$; 17 mM tri sodium citrate ($C_6H_9Na_3O_9$) and after autoclaving 0.72 ng μL^{-1} Polymyxin B sulphate added prior to pouring the over-layer on top of the basal layer of the medium. After 48 hours plates were inspected for colonies demonstrating pectinolytic activity; these were subcultured on nutrient agar (Oxoid, Basingstoke, Hampshire, UK) and then confirmed as *P. atrosepticum* by qPCR. Loops of bacterial colonies were placed in 500 µL sterile distilled water and boiled for 5 minutes at 100°C. qPCR was done as described (Humphris *et al*., 2015) using 1 µL of the bacterial boil preparation as template.

The remaining 500 tubers of each stock were divided in to ten tuber samples and placed into orange netted bags. The bagged tubers were subsequently split in to two groups to test the effects of two varying transit conditions. The first condition was designed to mimic a model transit situation where tubers were kept at standard UK storage conditions (held at 3.5°C) prior to transit. Transit conditions were set to mimic those recorded by the data loggers at 5°C for four weeks (max temperature = 5.3°C; min = 2.8°C), the time it typically takes Scottish seed potatoes to reach Egypt, the biggest importer of the product, followed by further storage at standard UK storage conditions (3.5°C) for four weeks. The second experimental condition followed the same pre-transit storage and transit conditions as the first treatment but differed after the four weeks of transit mimicking conditions ended, with tubers held under a rising temperature to a final holding temperature of approximately 20°C (max. temperature = 22.8°C; min = 5.3°C) for four weeks. This was to mimic conditions of storage without suitable refrigeration. The two experimental conditions were programmed in two identical Memmert Peltier-cooled incubator IPP750plus (Memmert Gmbh + Co. KG, Scwbach, Germany). A subsample of 50 tubers from a total of five netted bags were collected at the start of the experiment, after four weeks of the transit mimicking conditions, and then at one week, two weeks and four weeks post transit conditions after being held at one of the two temperature regimes. Tubers were visually assessed for symptoms of bacterial soft rot and black leg and heel end core samples for subsequent DNA extraction of qPCR of *P. atrosepticum* DNA as described previously.

3.5 Statistical analyses

A two-step statistical model was used to assess variation in pathogen DNA levels between tubers that had been evaluated after either harvest, storage or export treatments. The first step used a generalized linear mixed model to assess variation in the incidence of pathogen detection in tubers sampled from the different treatments. Positive or negative qPCR results were converted in to a binary scale and the generalized linear mixed model used a binomial distribution with the Logit function to assess variation in pathogen incidence using treatment as the fixed model and stock, stock interacting with bulk sample replicates and stock interacting with treatment as the random model. The second step assessed differences in pathogen DNA levels between the qPCR samples that were positive for each tested pathogen in each specific stock. Pathogen DNA levels were log transformed and variation assessed using a restricted maximum likelihood model (REML) where the fixed model was treatments and the random model was the bulk sample replicates. Least significant difference testing was used to determine significant difference between treatments at $P<0.05$.

To assess variation in *P. atrosepticum* CFU between bulked tuber samples from high and low health stocks prior to use in the transit mimic experiment a generalized linear mixed model was first used to determine differences in the incidence of pathogen CFU identified in tubers sampled from the different health status stocks. Positive or negative

CFU results were converted to a binary scale and the generalized linear mixed model used a binomial distribution with the Logit function to assess variation in CFU incidence using health as the fixed model and stock as the random model. A second step analysis was used to assess differences in *P*. *atrosepticum* CFU numbers between the samples that were positive for *P*. *atrosepticum* in each specific stock. *P*. *atrosepticum* CFU numbers were log transformed and a REML model, where the fixed model was health and the random model was stocks, was used to assess variation. Variation in *P*. *atrosepticum* DNA levels were assessed using the same two models used for the CFU analysis to first assess differences in pathogen incidence as determined by qPCR detection and then to test for variation in bacterial DNA levels between positive samples. *P*. *atrosepticum* DNA levels were log transformed prior to analysis with the REML model. Correlation between the plate CFU and qPCR detection methods was assessed using the Pearson correlation coefficient in MS Excel. Only samples that gave a positive detection result for *P*. *atrosepticum* were included in the analysis. *P*. *atrosepticum* DNA levels (pg) and CFU numbers were log transformed prior to analysis.

To analyse the effects on *P*. *atrosepticum* DNA levels observed during the transit mimic experiment, DNA levels were log transformed and variation assessed using REML where data from each time point was analysed separately. Within the REML analysis the fixed model was health of the stock and the random model was stock for time point 1; whereas, for time point 2 onwards the fixed model was health and treatment as well as the interaction term between these factors and stock was the random model. Least significant difference testing was used to determine significant difference between treatments at $P<0.05$.

4. RESULTS

Effect of transit on seed potato quality during export season year 1 (2017-18)

Of the eight seed potato stocks exported in 2017-18, samples from three consignments were returned for analysis. Two stocks from southeast Asia (SEA1) and one from the Middle East (ME) returned tubers that could be assessed. For crop SEA1_2017A (Fig. 4A), surface disease assessments, showed no significant differences in the incidence of silver scurf (p=0.264), black dot (p=0.196) or powdery scab (p=0.264) between tubers stored in the UK and those exported. There was a significant difference in silver scurf incidence in crop SEA1_2017B with more disease observed on tubers after export than those stored in the UK (p=0.011) whereas the incidence of black dot (p=0.196) and powdery scab (p=0.264) was not significantly different between the two sample points (Fig. 4B). No disease symptoms exceeded the threshold, based on disease tolerance for export requirements, for disease incidence in crop ME_2017 (data not shown).

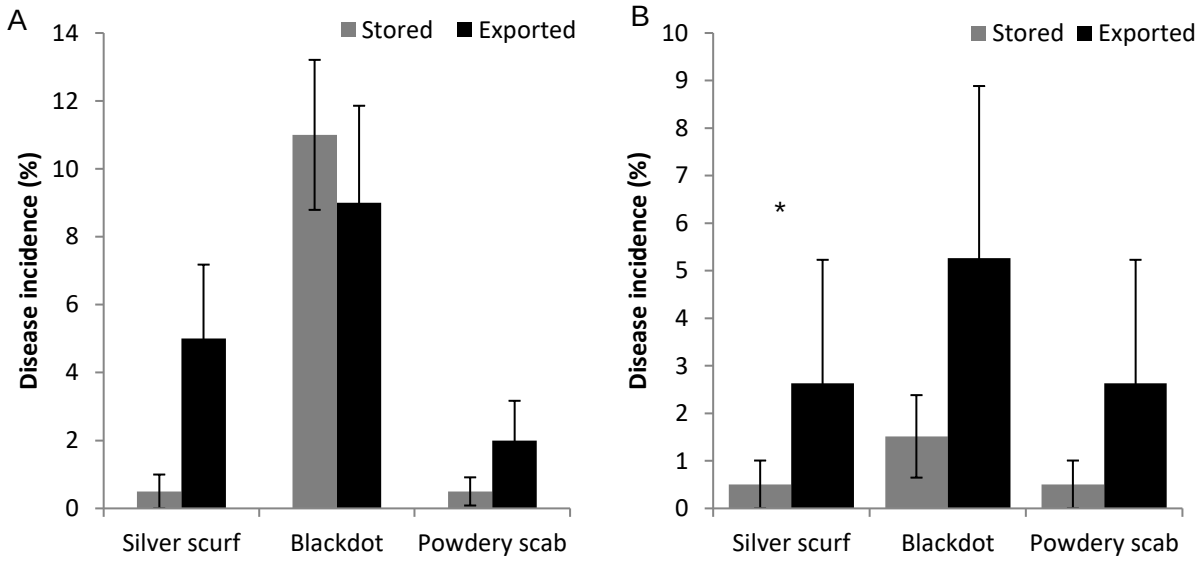

Fig. 4 Variation in disease incidence, as assessed by visual examination, on seed potato tubers stored in the UK or exported to SEA1 in 2017-18 (A) SEA1_2017A, (B) SEA1_2017B. * p <0.05

Molecular determination for latent infection of exported potatoes in year 1 (2017-18)

Significant differences in the incidence of pathogen detection were observed in 2017-18 for *C*. *coccodes* (P = 0.007), *H*. *solani* (P <0.001) and *S*. *subterranea* (P = 0.018). In all cases more positive results were recorded in the samples assessed after storage in the UK compared to those exported. For crop SEA1_2017B, higher levels of *P*. *atrosepticum* DNA (P = 0.040) were found in exported tubers compared to those kept in storage in the UK (Fig. 6A). Significantly higher levels of *B*. *cinerea* DNA (P = 0.044) were detected in stored tubers in SEA1_2017B compared to exported tubers (Fig. 6B). There were not enough positive samples for meaningful statistical analyses for *R*. *solani*, *F*. *sulphureum*, *P*. *pustulans* or *P*. *ultimum* in any of the stocks from 2017-18 nor for *H*. *solani* and *B*. *cinerea* in Israel_2017 or *C*. *coccodes* in SEA1_2017B or ME_2017. No significant differences were observed in pathogen DNA levels between treatments for any other pathogen by stock combination (Fig. 5-7).

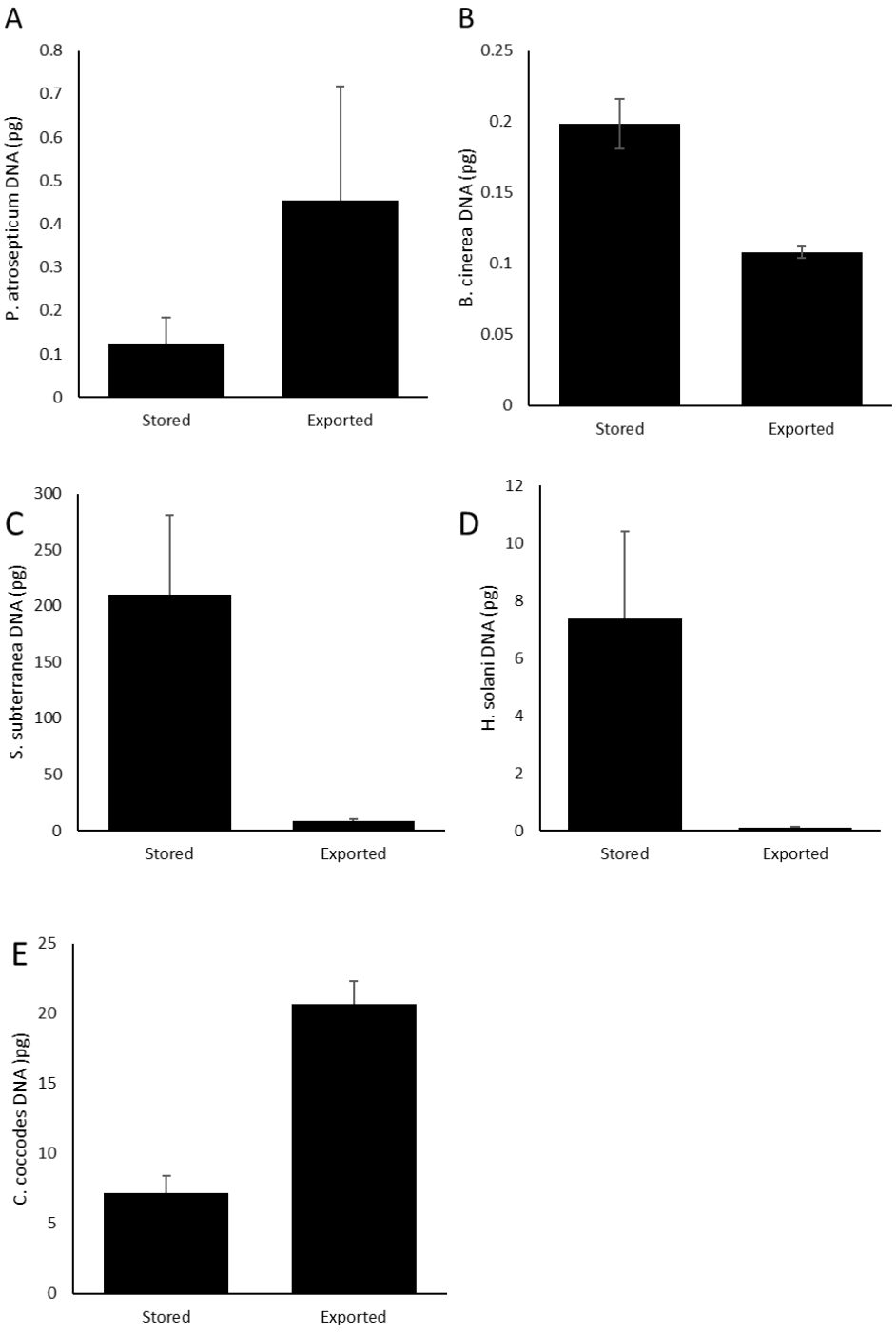

Fig. 5 Pathogen DNA levels in seed potato tubers of stock SEA1_2017A following export during the 2017-18 season. (A) *P*. *atrosepticum*, (B) *B*. *cinerea*, (C) *S*. *subterranea*, (D) *H*. *solani* and (E) *C*. *coccodes*. DNA levels were quantified using qPCR. * = $P < 0.05$ indicates significant difference between stored and exported samples. Error bars indicate ±1 SE.

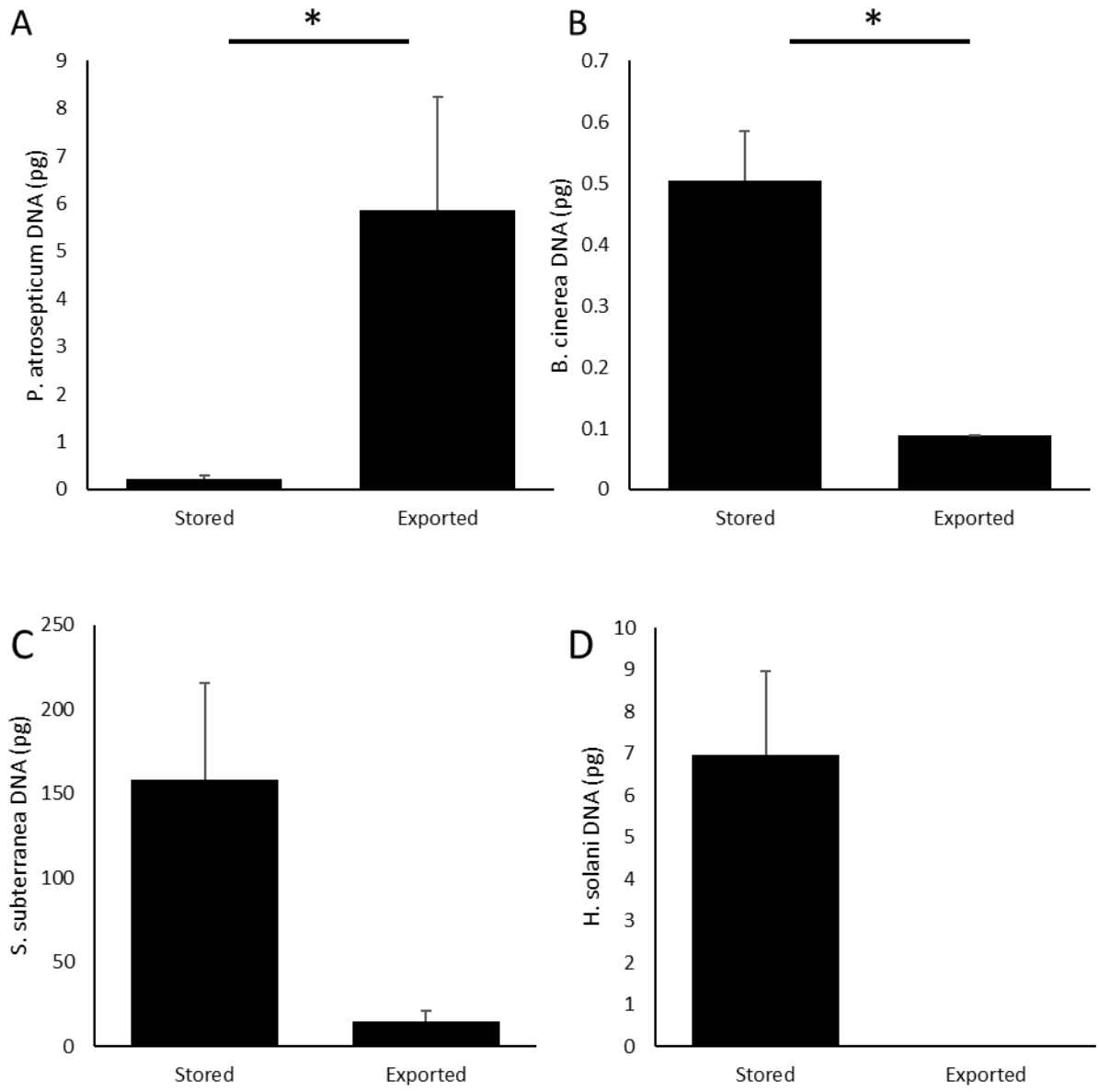

Fig. 6 Pathogen DNA levels in seed potato tubers of stock SEA1_2017B following export during the 2017-18 season. (A) *P*. *atrosepticum*, (B) *B*. *cinerea*, (C) *S*. *subterranea* and (D) *H*. *solani*. DNA levels were quantified using qPCR. * = $P < 0.05$ indicates significant difference between stored and exported samples. Error bars indicate ±1 SE.

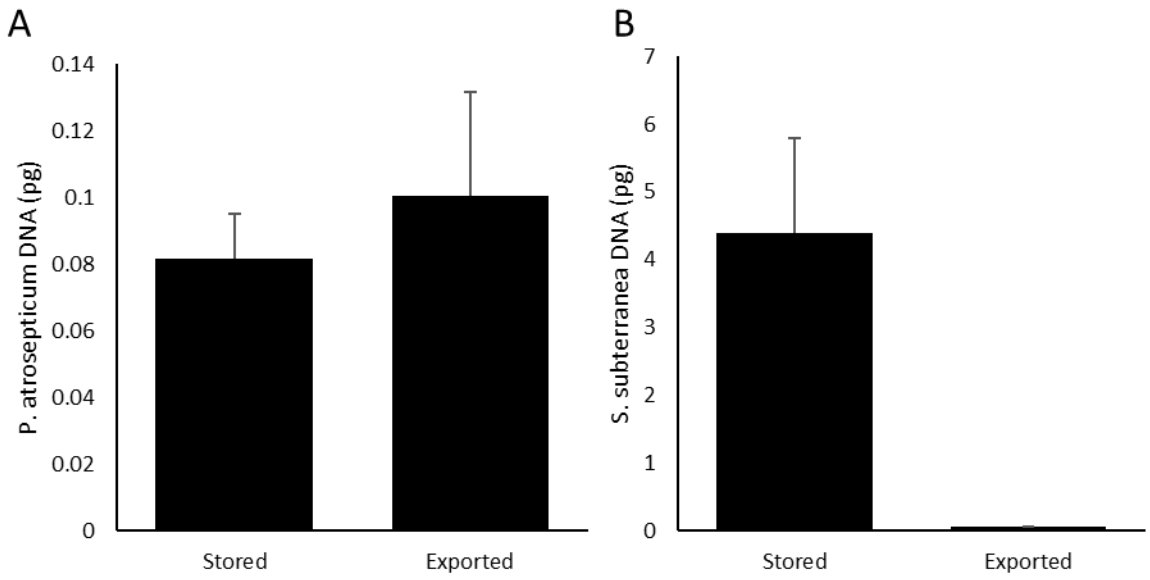

Fig. 7 Pathogen DNA levels in seed potato tubers of stock ME_2017 following export to ME during the 2017-18 season. (A) *P. atrosepticum* and (B) *S. subterranea*. DNA levels were quantified using qPCR. * = $P < 0.05$ indicates significant difference between stored and exported samples. Error bars indicate ±1 SE.

Transit conditions during export of seed potato stocks in year 1 (2017-18)

TinyTag data loggers were returned for five shipments in total. In addition to SEA1_2017A, SEA2_2017B and ME_2017, data loggers were returned for SEA2_2017A and SEA2_2017C. In the 2017-18 export season, during a 48 day transit, SEA1_2017A (Fig. 8A) was kept at an average temperature of 5.9°C (max. = 6.4°C ; min = 5.6°C) at 100% relative humidity and an average dew point of 5.9°C (max = 6.4°C; min = 5.6°C) whereas SEA2_2017B (Fig. 8B) was kept at an average temperature of 6.0°C (max = 7.3°C; min.= 5.2°C) at 99.9% relative humidity (max = 100%; min = 98.1%) with an average dew point measurement of 6.0°C (max = 7.3°C; min = 5.2°C) during a 35 day exportation to the same country.

ME_2017 (Fig. 8C) was kept at an average temperature of 6.7°C (max = 8.0°C; min 6.4°C), at 99.9% relative humidity (max = 100%; min = 99.0%) with a dew point of 6.7°C (max = 7.9°C; min = 6.4°C) during a 22 day exportation to ME. SEA2_2017A was shipped for 60 days and was kept at an average temperature of 9.1°C (max = 14.3°C; min 8.1°C), at 99.3% relative humidity (max = 100%; min = 83.2%) with a dew point of 9.0°C (max = 12.2°C; min = 7.5°C) during export. Whereas, SEA2_2017C was in transit for 42 days and kept at an average temperature of 8.0°C (max = 9.3°C; min 6.9°C), at 99.9% relative humidity (max = 100%; min = 97.2%) with a dew point of 8.0°C (max = 9.3°C; min = 6.9°C) (Fig. 9).

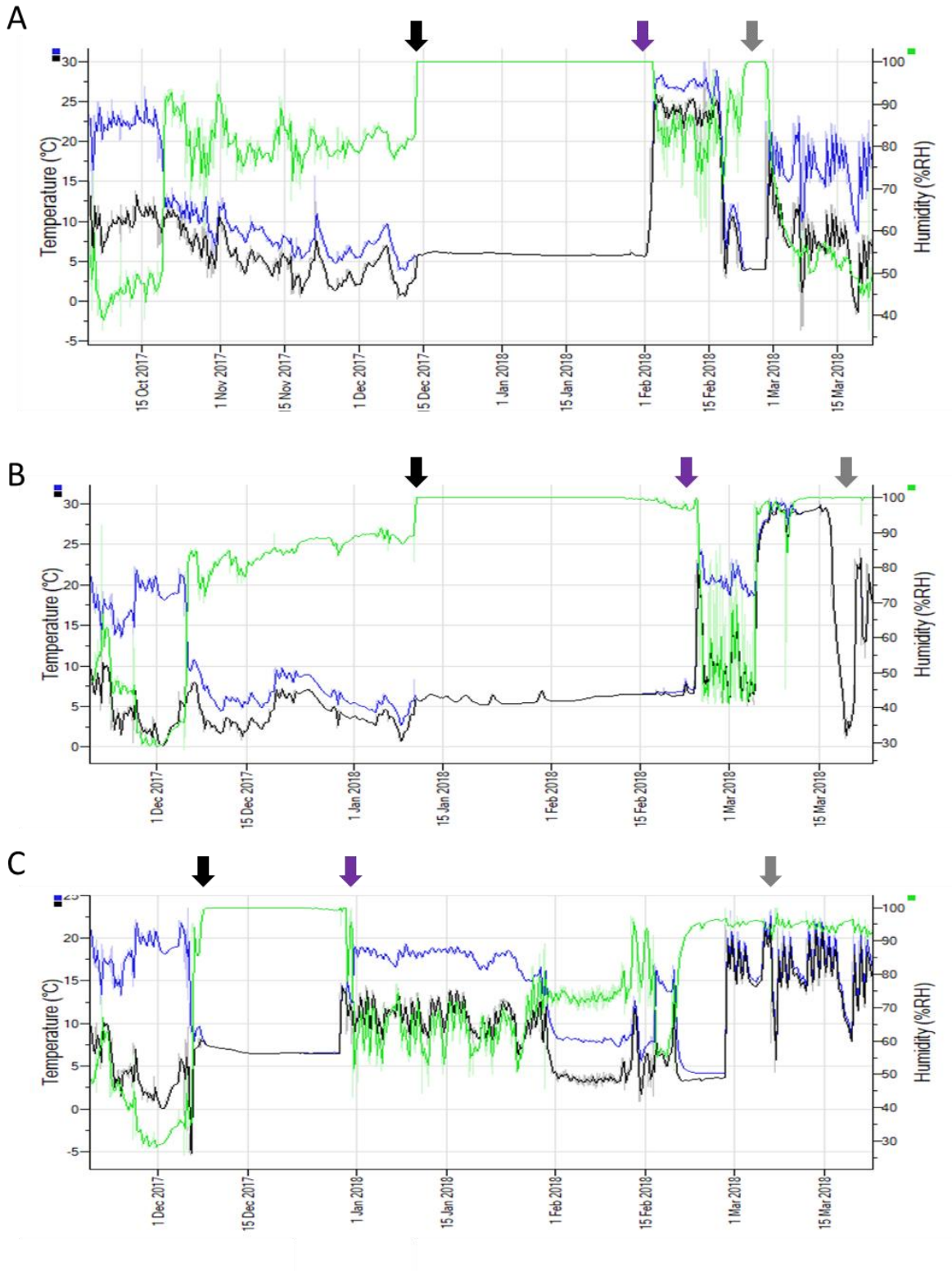

Fig. 8 Data logger output traces from seed potato stocks in the 2017-18 export season. (A) SEA1_2017A, (B) SEA1_2017B, (C) ME_2017. Data loggers collected temperature (blue line), relative humidity (green line) and dew point (black line) measurements during the export process. Black arrow is the date the shipment was exported, purple arrow is the date the shipment arrived at its destination and the dark grey arrow is the date the data logger and tubers arrived back in the UK for processing.

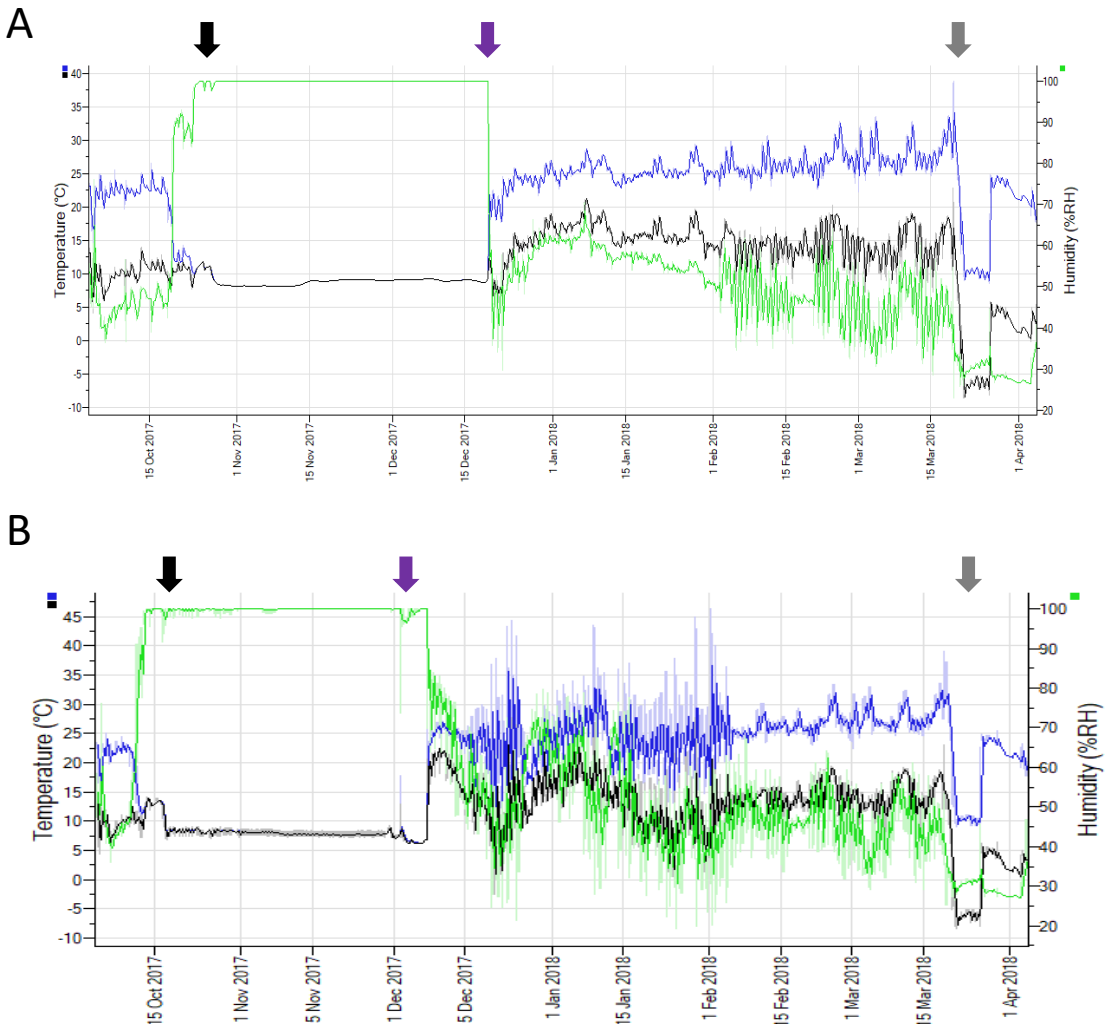

Fig. 9 Data logger output traces from seed potato stocks in the 2017-18 export season with no returned tubers for analysis. (A) SEA2_2017A, (B) SEA2_2017C. Data loggers collected temperature (blue line), relative humidity (green line) and dew point (black line) measurements during the export process. Black arrow is the date the shipment was exported, purple arrow is the date the shipment arrived at its destination and the dark grey arrow is the date the data logger arrived back in the UK for processing.

Effect of transit on seed potato quality during export season year 2 (2018-19)

Three of the five consignments exported to non-EU destinations returned tuber and data loggers for analysis. All three of these consignments were returned from NA2 and had passed the country specific standards enabling them to be exported from Scotland. The consignment sent to NA3 (NA3_2018) was rejected at the destination for failing to meet country-specific requirements. However, the tubers were not returned to SASA for analysis so this crop could not be used in the experiment. The data loggers associated with NA3_2018 were returned by ship haulage. Unfortunately, neither the tuber samples nor the data loggers were returned from ME (ME_2018).

Disease levels were negligible on NA2_2018A cv. Galactica tubers with no surface disease observed on the harvested tubers, those stored at SASA nor those exported to NA2. No internal rot symptoms were recorded on the harvested sample, whilst two tubers from both the stored and exported samples displayed very low (≤3.5%) levels of dry rot. However, none of the disease levels scored on the cv. Galactica tubers from NA2_2018A exceeded the threshold for disease incidence. Trace levels of black scurf, common scab and powdery scab were observed on tubers from NA2_2018B. However, only black dot showed symptom levels that exceeded the incidence threshold. In this crop black dot incidence was significantly higher ($p<0.01$) in stored and exported tubers compared to those scored after harvest but there was no difference in incidence between tubers stored in the UK and those exported (Fig. 10A). Crop NA2_2018C presented very low incidence of common scab symptoms but there were no significant differences in common scab incidence ($p=0.453$) between tubers scored at harvest, after storage in the UK or after export (Fig. 10B). No evidence of skin spot, soft rot, wet rot, or gangrene-like symptoms were observed on any of the tubers irrespective of shipment status.

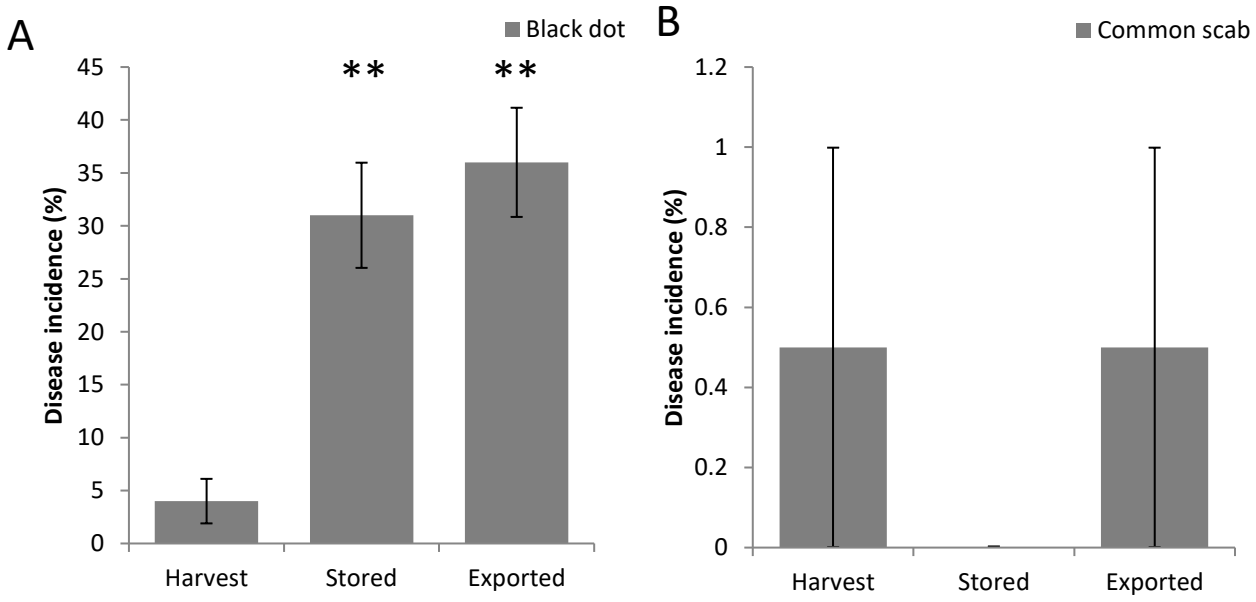

Fig. 10 Variation in disease incidence, as assessed by visual examination, on seed potato tubers scored after harvest, storage in the UK or exported to north Africa in 2018-19 (A) NA2_2018B, (B) NA2_2018C. ** p <0.01

Molecular determination for latent infection of exported potatoes in year 2 (2018-19)

There were no significant differences in pathogen incidence between the treatments in 2018-19 except for *S*. *subterranea* (P = 0.005) which had more positive samples from tubers stored in the UK (45.7%) compared to those assessed at harvest (21.9%) or exported (11.3%). *P*. *atrosepticum* DNA levels differed between treatments in NA2_2018A (Fig. 11A; P = 0.002) and NA2_2018B (Fig. 12A; P < 0.001). In both stocks *P*. *atrosepticum* DNA levels were higher in exported tubers compared to those assessed at harvest or after storage in the UK. *B*. *cinerea* DNA was significantly different between treatments in NA2_2018A (Fig. 11B; P = 0.003) with higher DNA levels measured in stored tubers and tubers assessed at harvest compared to exported tubers. In NA2_2018B *S*. *subterranea* DNA levels were higher in stored tubers compared to those that were exported (Fig. 12C). Significant differences in *F*. *sulphureum* DNA levels were observed between treatments for NA2_2018A (Fig. 11D; P < 0.001) and NA2_2018B (Fig. 12D; P < 0.001). Higher levels of *F*. *sulphureum* DNA were recorded in tubers processed after harvest compared to those tested after storage or export in NA2_2018A, whereas in NA2_2018B *F*. *sulphureum* DNA was higher in tubers assessed after storage compared to those measured after harvest or export. *H*. *solani* levels were higher in tubers assessed after storage in the UK in NA2_2018C compared to the other two treatments (Fig. 13G; P = 0.01). There were not enough positive samples for meaningful statistical analyses for *C*. *coccodes* or *P*. *ultimum* in any of the stocks from 2018-19 nor for *H*. *solani* in NA2_2018A and NA2_2018B or *P*. *pustulans* and *R*. *solani* in NA2_2018A. No significant differences were observed in pathogen DNA levels between treatments for any other pathogen by stock combination (Fig. 11-13).

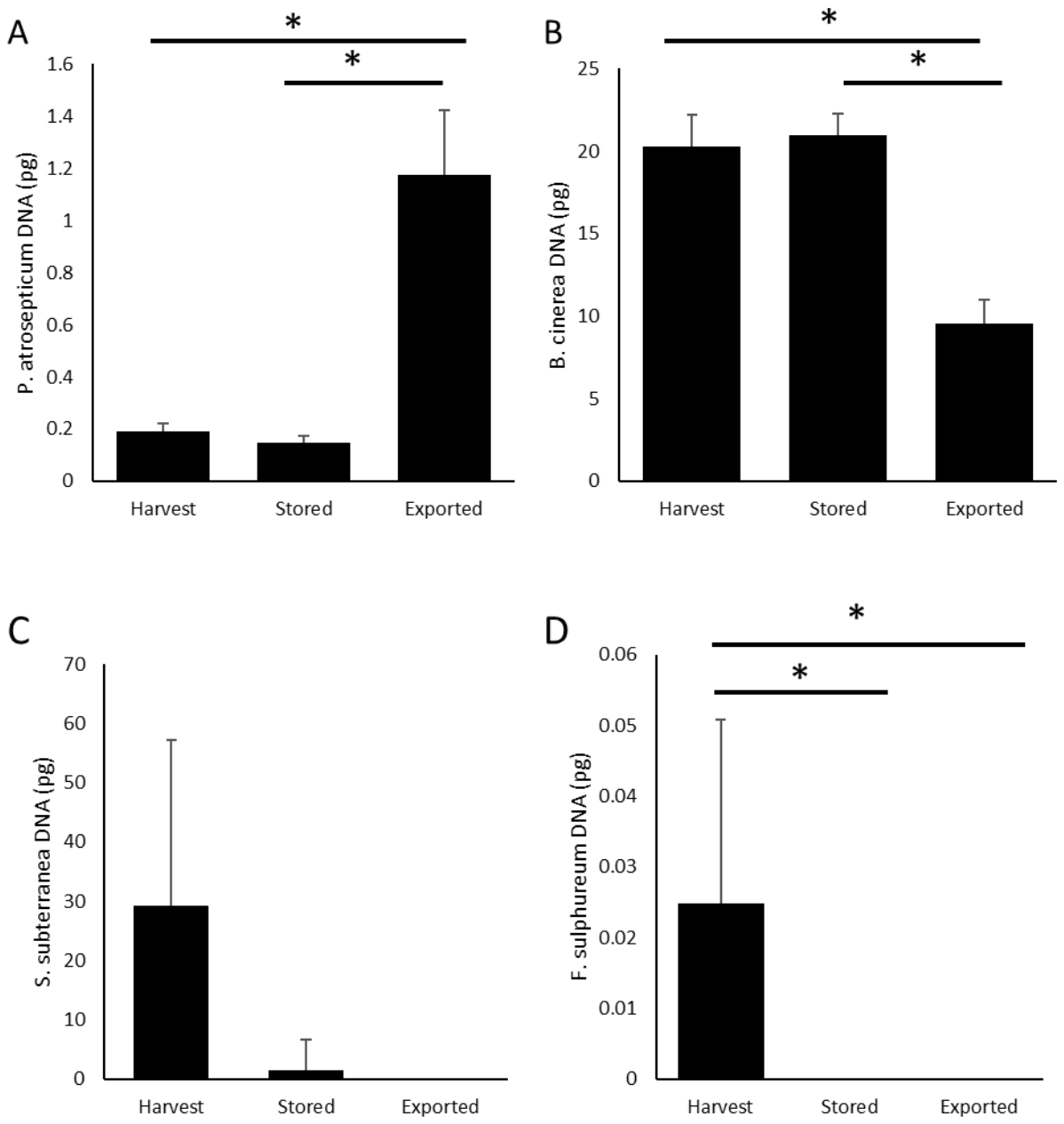

Fig. 11 Pathogen DNA levels in seed potato tubers of crop NA2_20178A following export during the 2018-19 season. (A) *P. atrosepticum*, (B) *B. cinerea*, (C) *S. subterranea* and (D) *F. sulphureum*. DNA levels were quantified using qPCR. * = P < 0.05 indicates significant difference between stored, exported and post-harvest samples. Error bars indicate ±1 SE.

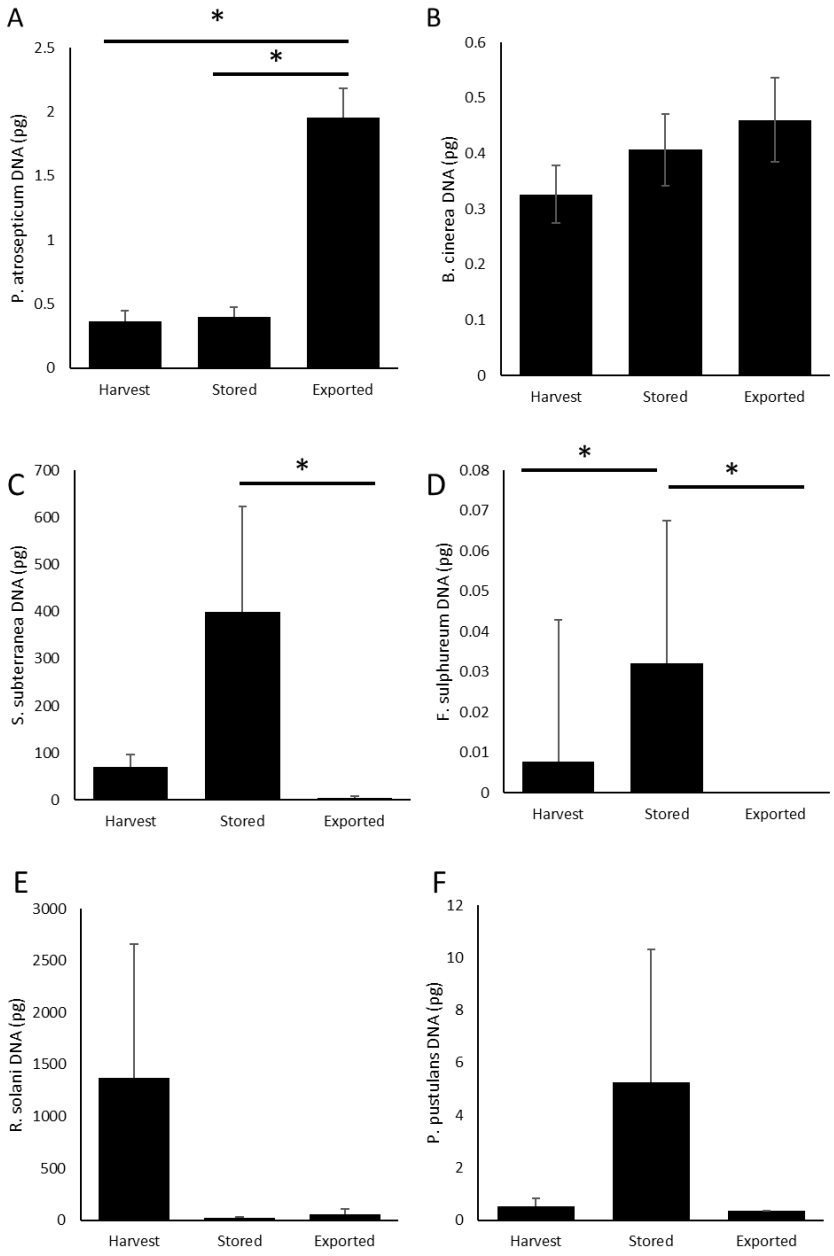

Fig. 12 Pathogen DNA levels in seed potato tubers of stock NA2_20178B following export during the 2018-19 season. (A) *P. atrosepticum*, (B) *B. cinerea*, (C) *S. subterranea*, (D) *F. sulphureum*, (E) *R. solani* and (F) *P. pustulans*. DNA levels were quantified using qPCR. * = $P < 0.05$ indicates significant difference between stored, exported and post-harvest samples. Error bars indicate ±1 SE.

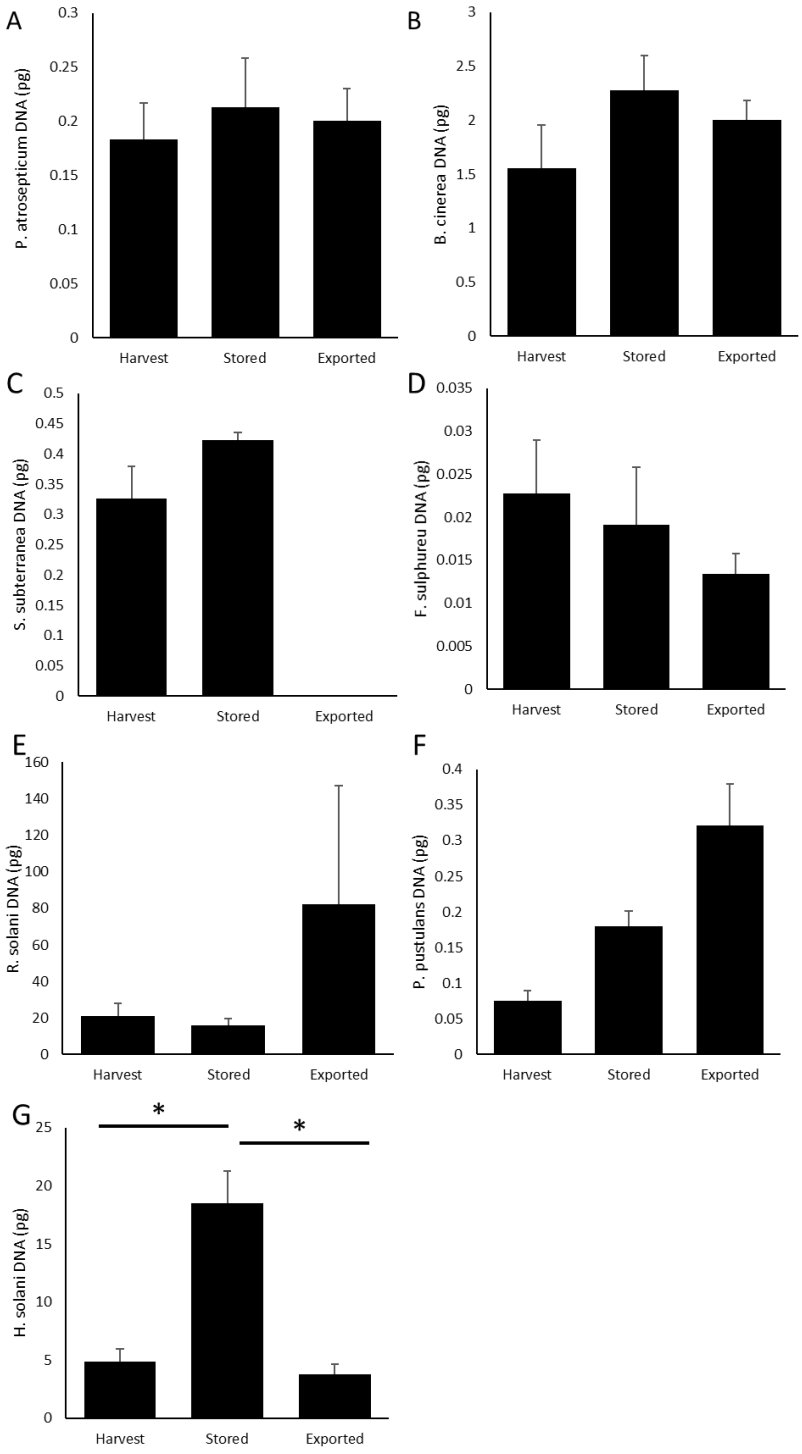

Fig. 13 Pathogen DNA levels in seed potato tubers of stock NA2_20178C following export during the 2018-19 season. (A) *P. atrosepticum*, (B) *B. cinerea*, (C) *S. subterranea*, (D) *F. sulphureum*, (E) *R. solani*, (F) *P. pustulans* and (G) *H. solani*. DNA levels were quantified using qPCR. * = $P < 0.05$ indicates significant difference between stored, exported and post-harvest samples. Error bars indicate ±1 SE.

Transit conditions during export of seed potato stocks in year 2 (2018-19)

Data loggers and sensor units were returned with four of the selected export consignments, including NA2_2018A, NA2_2018B, NA2_2018C and NA3_2018. During the 2018-19 export season the three stocks all took 27 days to be exported to North Africa (NA2). Data was returned from the TinyTag data loggers associated with each consignment. NA2_2018A was exported at a mean temperature of temperature of 6.7°C (max. = 10.2°C; min = 5.8°C) at 81.2% average relative humidity (max = 99.5%; min = 73.5%) and an average dew point of 3.6°C (max = 9.8°C; min = 1.9°C). During transit, NA2_2019B was stored at an average temperature of 7.4°C (max. = 11.1°C ; min = 5. 2°C), at a mean of 96.6% relative humidity (max = 100%; min = 79.4%) and an average dew point of 6.9°C (max = 11.1°C; min = 2.3°C). NA2_2018C was held at an average temperature of 6.4°C (max = 10.9°C; min = 5. 5°C) during export at an average relative humidity of 84.6% (max = 100%; min = 60.9%) and an average dew point of 3.9°C (max = 10.2°C; min = -1.1°C) throughout transit (Fig. 14). The stock sent to the North Africa (NA3) took 16 days to reach its destination. NA3_2018 (Fig. 15) was exported at a mean temperature of temperature of 7.3°C (max. = 20.0°C; min = 6.0°C) at 95.5% average relative humidity (max = 100%; min = 37.8%) and an average dew point of 6.4°C (max = 10.6°C; min = -1.3°C). The data logger for NA3_2018 was not returned by air courier but instead was shipped back by sea on the boat used to export the tubers.

Additionally, a custom-built unit was produced in an attempt to record additional information about the environment. The unit was designed to measure a range of environmental factors, such as temperature, humidity, VOC (volatile organic compounds) and carbon dioxide. The unit was brick sized and contained a main circuit board, sensors, SD card and was battery powered. This phase of the project was seriously affected by three issues, these were:

1. Power consumption: This was a major issue with the unit and there were difficulties in reducing the power consumption for long term recording. A number of different battery solutions were tested; however, the unit was only able to function for 6 weeks before the batteries failed.
2. Unit losses: A significant number of units were misplaced when they arrived at their destination. It appeared that those receiving the potatoes and units either disposed of the tracker or kept it for other purposes. This was a risk not fully appreciated at the beginning of the project even although the main objectives of the project had been communicated with the customers in importing countries prior to the project starting.
3. Environment: An unexpected situation to the unit designers was the extreme humidity level inside the storage container. As a result, there was water condensate on the electronics and inside the sensors. It is likely that this caused short circuits etc. on the main circuit board and badly affected those sensors that need optical paths to work.

The custom data logger activity was ended early in the project due to funding limitations. Figure 16 shows some example outputs from the logger through the transport process. The initial phases of the output are associated with the logger being loaded into the consignment for export and then being transported to the port etc. As can be seen, there is significant activity through the initial phases of the transport, but once arriving on the boat, all the parameters stabilise, with the unit output stable until the power failed. The effect of humidity on the CO_2 measurement optics can also be observed, it is shown as zero output between day 8 and day 22. If such logging systems are to be used in the

future, an alternative form of CO_2 measurement (for example electrochemical) may well be more suited to this environment. Another option may be to seal the sensor boards and electronics to make them more robust.

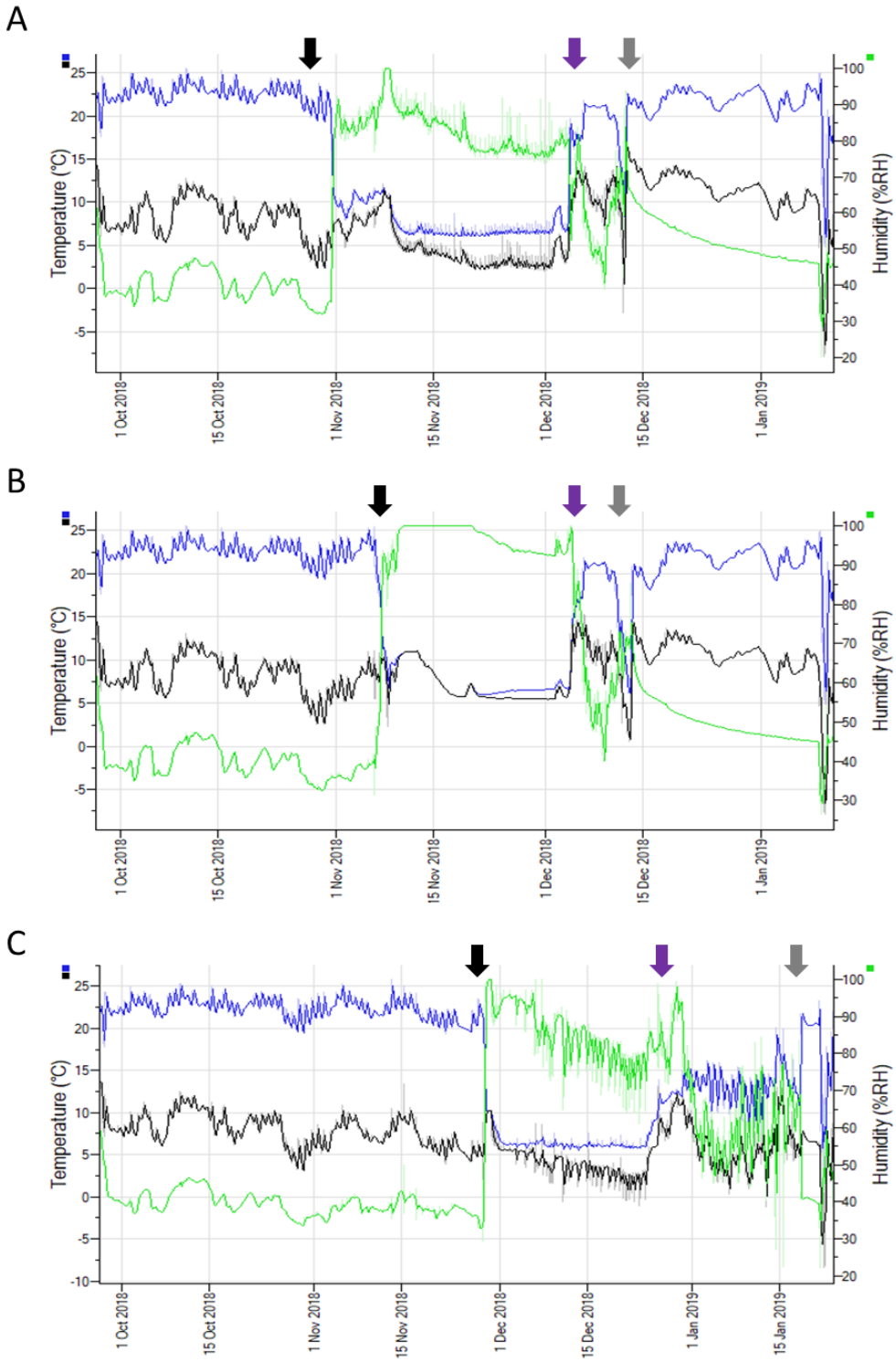

Fig. 14 Data logger output traces from seed potato stocks in the 2018-19 export season. (A) NA2_2018A, (B) NA2_2018B, (C) NA2_2018C. Data loggers collected temperature (blue line), relative humidity (green line) and dew point (black line) measurements during the export process. Black arrow is the date the shipment was exported, purple arrow is the date the shipment arrived at its destination and the dark grey arrow is the date the data logger and tubers arrived back in the UK for processing.

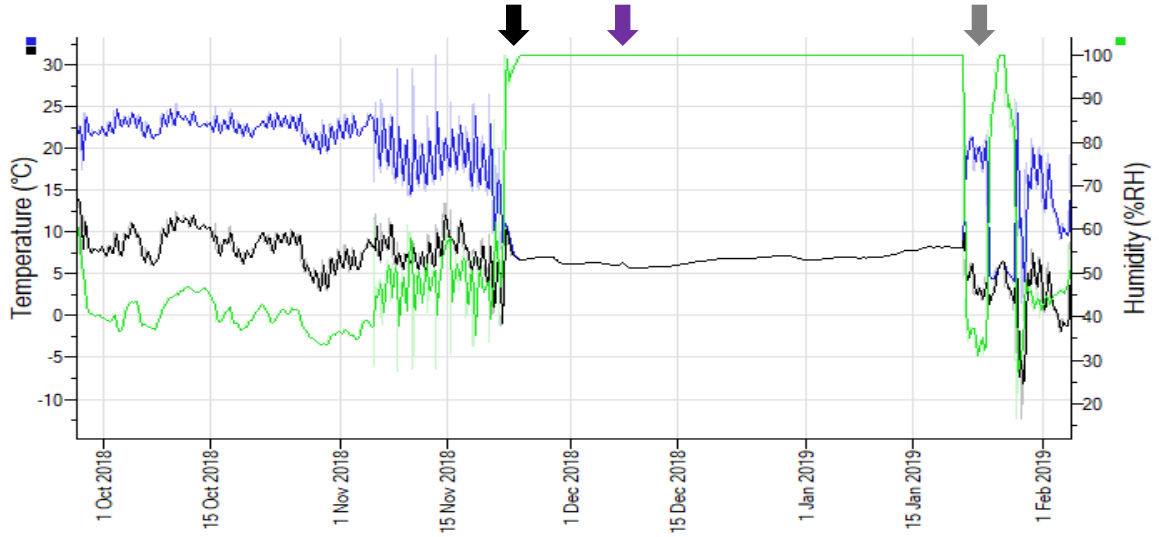

Fig. 15 Data logger output traces from seed potato stock NA3_2018 in the 2018-19 export season. Data loggers collected temperature (blue line), relative humidity (green line) and dew point (black line) measurements during the export process. Black arrow is the date the shipment was exported, purple arrow is the date the shipment arrived at its destination and the dark grey arrow is the date the data logger arrived back in the UK.

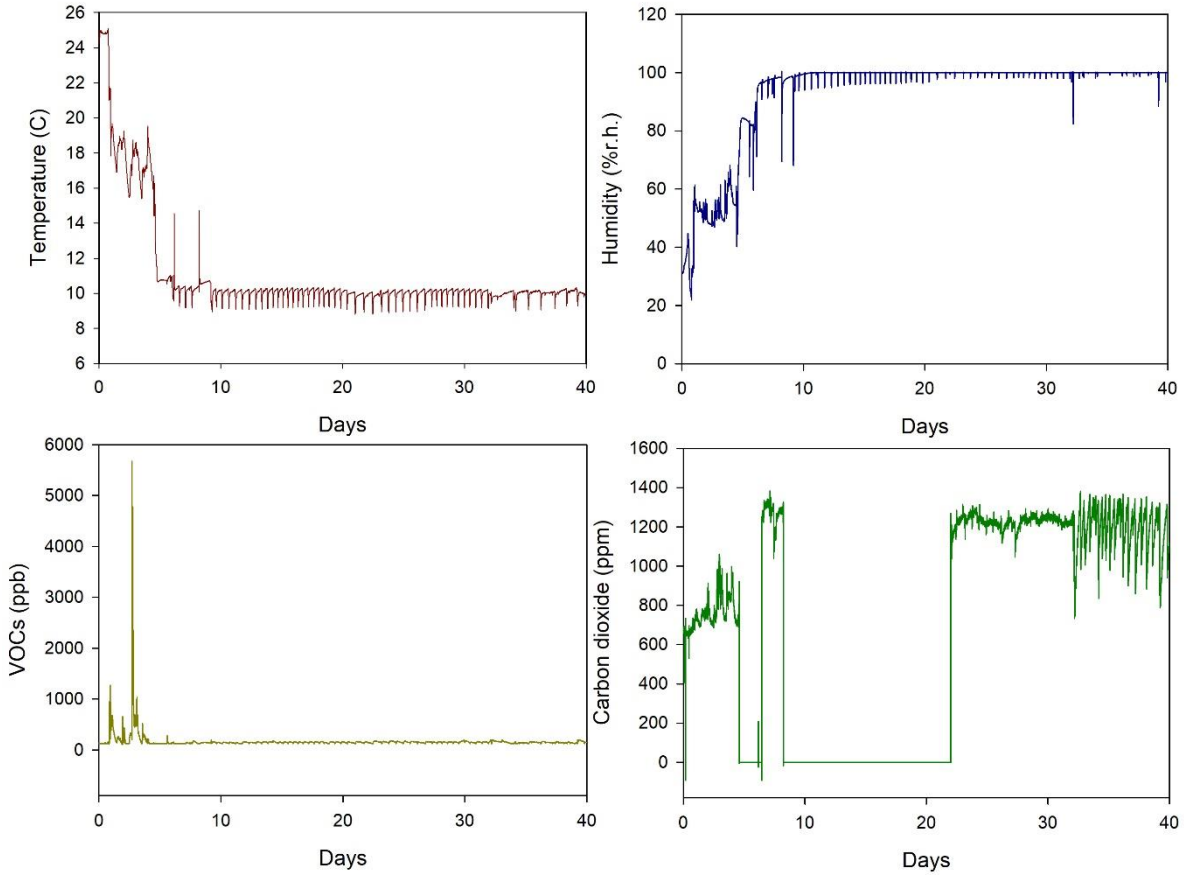

Fig. 16: Example output from the custom logger units. In addition to temperature and relative humidity, the custom-built units also measured VOC and carbon dioxide.

Effect of transit on seed potato quality during export season year 3 (2019-20)

One of the three consignments exported to NA2 (North Africa) returned tuber and data loggers for analysis. Trace levels of common scab were observed on tubers of stock NA2_2019A assessed after harvest and following UK storage. However, only two tubers that had been stored in the UK had levels of this disease that met the 12.5% surface area threshold (data not shown). No common scab symptoms were observed on any tubers assessed following export. No rot diseases were observed on any tubers from this stock.

Molecular determination for latent infection of exported potatoes in year 3 (2019-20)

qPCR analysis of these tubers showed that there was a significant effect of treatment on *P*. *atrosepticum* detection (P = 0.044) with no bacteria detected in tubers sampled after harvest or following storage in the UK. *P*. *atrosepticum* was detected in some of the bulked samples following export to NA2, however, due to the lack of detection in the post-harvest and UK storage samples no statistical analysis of changes in bacterial DNA levels between the treatments could be made (Fig. 17).

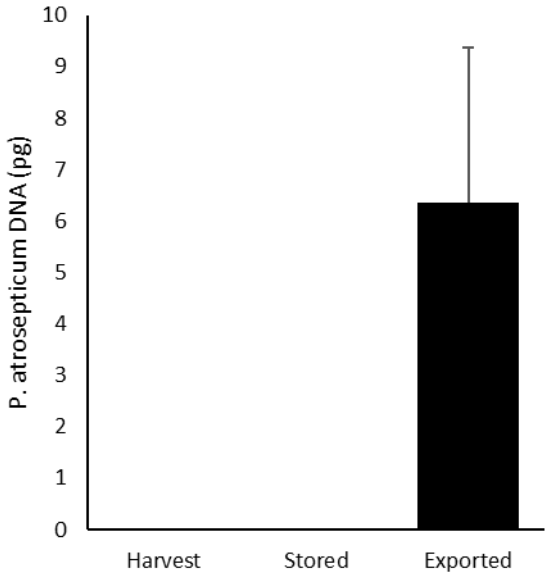

Fig. 17 *Pectobacterium atrosepticum* DNA levels in seed potato tubers following export to North Africa (NA2_2019A) during the 2018-19 season. DNA levels were quantified using qPCR. Error bars indicate ±1 SE.

Transit conditions during export of seed potato stocks in year 3 (2019-20)

In the 2019-20 season NA2_2019A was in transit for 33 days during exportation at an average temperature of 5.1°C (max. = 7.8°C; min = 2.1°C) at 99.4% relative humidity (max = 100%; min = 75.5%) and an average dew point of 5.0°C (max = 7.8°C; min = 1.3°C) during transit (Fig. 18).

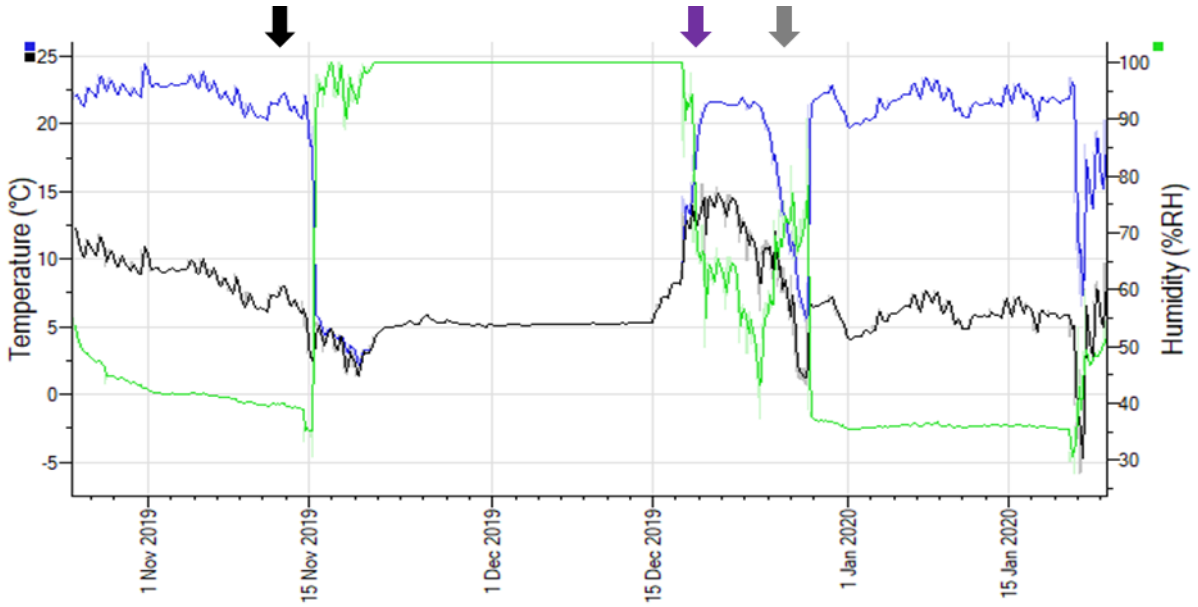

Fig. 18 Data logger output traces from seed potato stocks in the 2019-20 export season. NA2_2019A. Data loggers collected temperature (blue line), relative humidity (green line) and dew point (black line) measurements during the export process. Black arrow is the date the shipment was exported, purple arrow is the date the shipment arrived at its destination and the dark grey arrow is the date the data logger and tubers arrived back in the UK for processing.

Transit mimic experiment

Preliminary testing to determine the *P. atrosepticum* status of the high and low health stocks showed clear differences in the number of bacterial CFUs and levels of pathogen DNA between the high and low health stocks. No *P. atrosepticum* CFUs were found in stock High_2019A or High_2019C but one bulk from stock High_2019B had 540 CFU mL^{-1}. The low health stocks had varying numbers of bulks testing positive for *P. atrosepticum* CFUs with mean CFU counts of approximately 1.1 x 10^4, 1.5 x 10^3 and 728 CFU mL^{-1} for stock Low_2019A, Low_2019B and Low_2019C, respectively (Fig. 19A). There was a significantly higher number of bulk samples that yielded *P. atrosepticum* CFUs from the low health stocks compared to those from high health (P=0.007). No significant differences in the number of CFUs detected were observed between those samples positive for *P. atrosepticum* CFUs between the two health statuses (P=0.759). No *P. atrosepticum* DNA was detected in any bulks from High_2019A or High_2019B but two bulks from High_2019C showed very low levels of bacterial DNA. The incidence of *P. atrosepticum* DNA detected by qPCR was significantly different between high and low health stocks (P=0.047) with more bulk samples testing positive for the bacterium from the low health stocks. However, even though it appeared that much higher levels of *P. atrosepticum* were found in the low health stocks (Fig. 19B), no significant difference in *P. atrosepticum* DNA levels were observed between the positive samples recorded in the high and low health stocks (P=0.143).

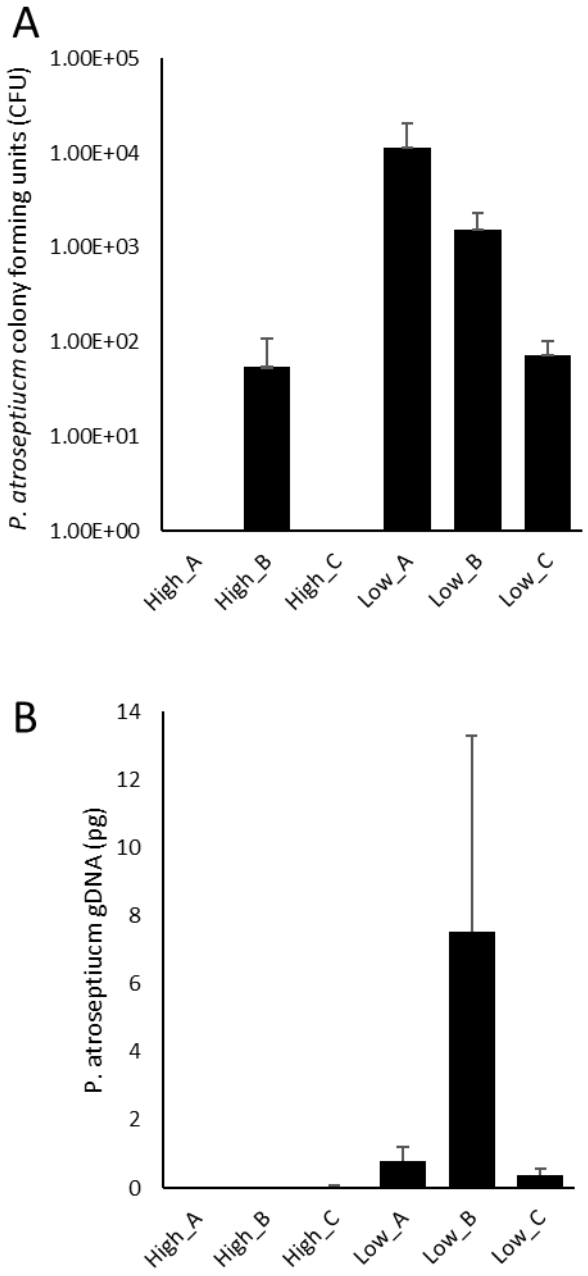

Fig. 19 Assessment of *P*. *atrosepticum* levels in high and low health seed potato stocks used in transit mimic experiment. (A) Colony forming unit counts (log 10) and (B) DNA levels (pg) were quantified using qPCR. Error bars indicate ±1 SE.

To compare the more traditional plate assay and the qPCR analysis for *P*. *atrosepticum* detection, a correlation analysis was performed. Only samples that returned a positive result from either the plate test or the qPCR assay were included in the analysis resulting in 25/60 samples being used. Of the 25 samples seven were positive for *P*. *atrosepticum* CFUs in the plate assay with no detection by qPCR whereas 11 samples were positive by qPCR with no CFUs counted in the plate assay. Seven samples were positive for *P*. *atrosepticum* by both methods. The Pearson correlation coefficient between the two assays showed a moderate (r = 0.590) but statistically significant (P=0.002), positive correlation between *P*. *atrosepticum* CFU counts and qPCR determination of *P*. *atrosepticum* DNA (Fig. 20).

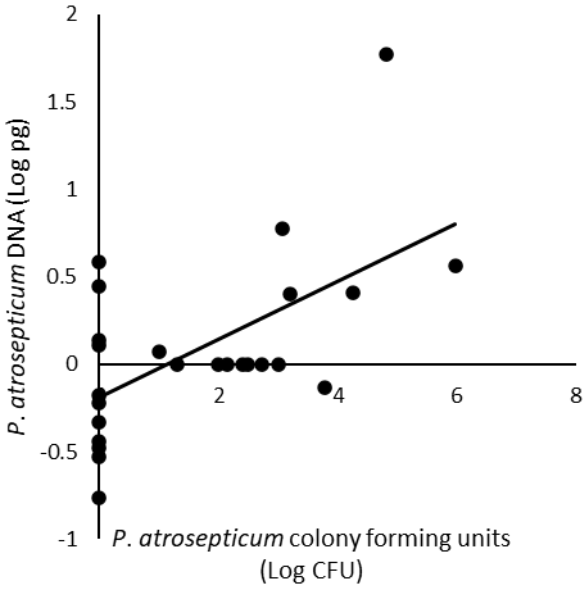

Fig. 20 Correlation between *P. atrosepticum* detection and quantification by DNA analysis using qPCR and assessing colony forming unit numbers using the crystal violet pectate medium agar plate method.

Very few symptoms of bacterial soft rot or blackleg were seen in any of the tubers regardless of stock health status or transit conditions. In fact, only two tubers from a single replicate of stock Low_2019A showed typical bacterial soft rot symptoms in this experiment. Despite the rare observations of bacterial rot symptoms qPCR analysis indicated that latent infections were present in bulk samples from both the high and low health stocks. Levels of *P. atrosepticum* DNA varied between stocks, treatments and over time. Of particular note, was the high level of heterogeneity of detection of *P. atrosepticum* between samples. *P. atrosepticum* DNA was detected in all bulk samples for each interaction (stock, health and treatment) at time points 1-3. However, at time points 4 and 5 the number of bulk samples for specific stock, health and treatment combinations became more variable (Fig. 21). At time point 4 one bulk sample from Low_2019A_control, Low_2019A_high and High_2019A_high had no detectable *P. atrosepticum* DNA whereas three bulk samples from High_2019B_control and High_2019B_high did not amplify bacterial DNA. At time point 5 one bulk sample from Low_2019C_high, two bulk samples from High_2019_control and four bulk samples from both Low_2019A_control and High_2019B_high had no detectable *P. atrosepticum* DNA.

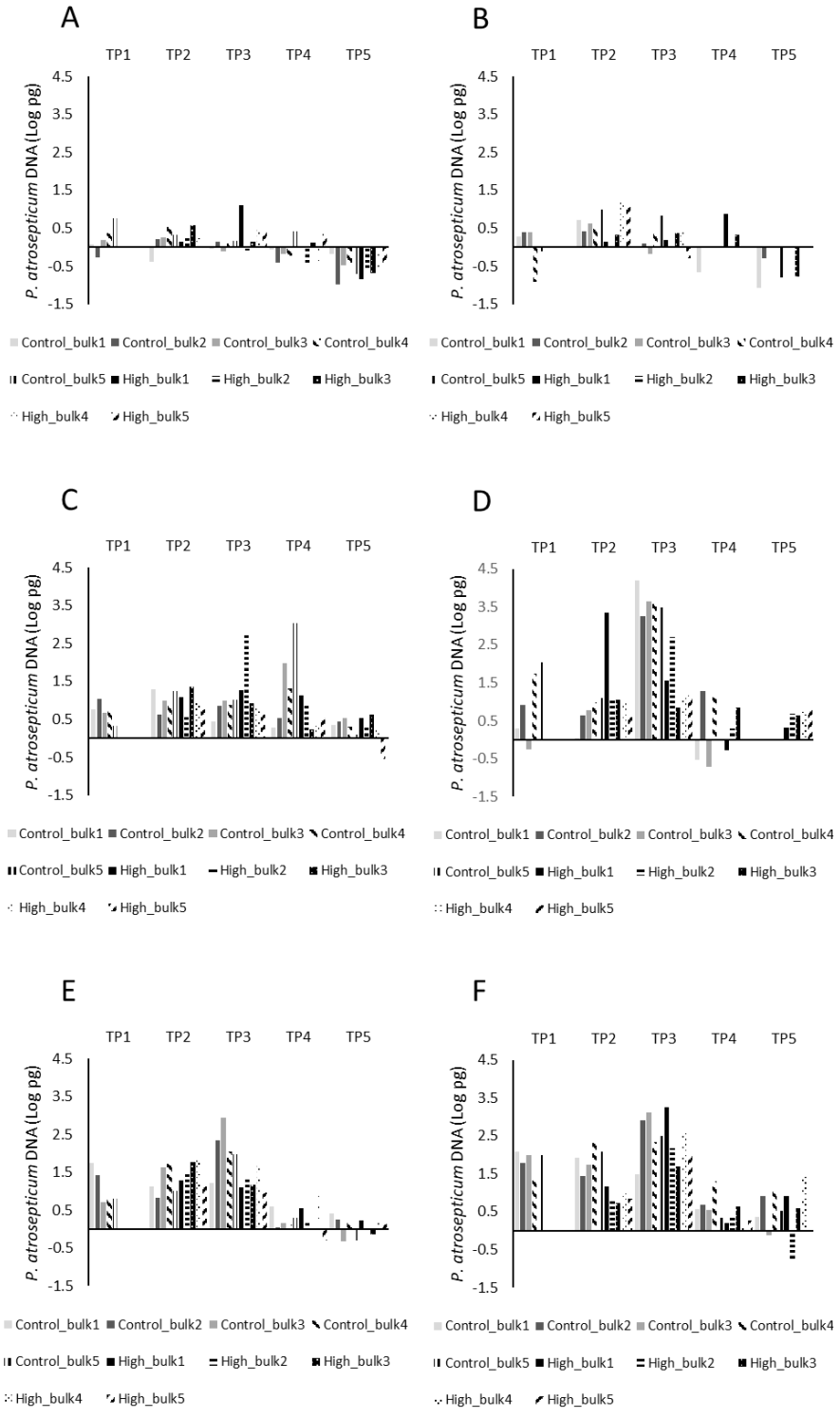

Fig. 21 Quantification of *Pectobacterium atrosepticum* DNA levels in potato tubers in a transit mimic experiment. Tubers from high and low health stocks were stored in conditions that mimic transit during export and then exposed to either standard potato storage conditions (control) or elevated temperatures (high). DNA levels (Log pg) were quantified from five bulked samples of ten tubers (bulk) using qPCR from tubers sampled prior to transit mimic conditions (TP1), after four weeks transit mimic conditions (TP2), following one week of storage (TP3), two weeks of storage (TP4) or four weeks of storage (TP5) at one of the two treatment conditions. Error bars indicate ±1 SE. (a) High_2019A, (B) High_2019B, (C) High_2019C, (D) Low_2019A, (E) Low_2019B, (F) Low_2019C.

Analysis comparing the effects of tuber health (high/low) and the two-post transit mimic storage treatments (control/high temperature) at each individual time point highlighted some variation in *P*. *atrosepticum* DNA levels that could be attributed to the tested factors (Fig. 22). At time point 1, prior to storing tubers in transit mimic conditions, there was a significant effect of the health of the stocks on *P*. *atrosepticum* DNA levels (P = 0.046) with higher levels of *P*. *atrosepticum* DNA detected in the low health tubers. Time point 2, sampled after the transit mimic conditions had ended, showed a significant effect of stock health on *P*. *atrosepticum* DNA levels (P = 0.046) with more bacterial DNA present in tubers from low health stocks. There was no significant effect of treatment (P=0.87) and no interaction between tuber health and treatment (P=0.89) on *P*. *atrosepticum* DNA levels. At time point 3, one-week post transit mimic conditions after storage at standard potato store temperatures (control) or at 20°C (high), a significant effect of tuber stock health (P = 0.012), treatment (P=0.010) and an interaction between health and treatment (P<0.001) was observed. At this time point, low health tubers under the control treatment conditions had significantly higher levels of *P*. *atrosepticum* DNA. No effect of tuber stock health (P=0.91), treatment (P=0.35) or health and treatment interaction (P=0.90) was observed at time point 4, two-weeks post transit mimic conditions after storage at standard potato store temperatures (control) or at 20°C (high). Similarly, at time point 5 four-weeks post transit mimic conditions after storage at standard potato store temperatures (control) or at 20°C (high) no effect of tuber stock health (P=0.12), treatment (P=0.82) or health and treatment interaction (P=0.38) was observed.

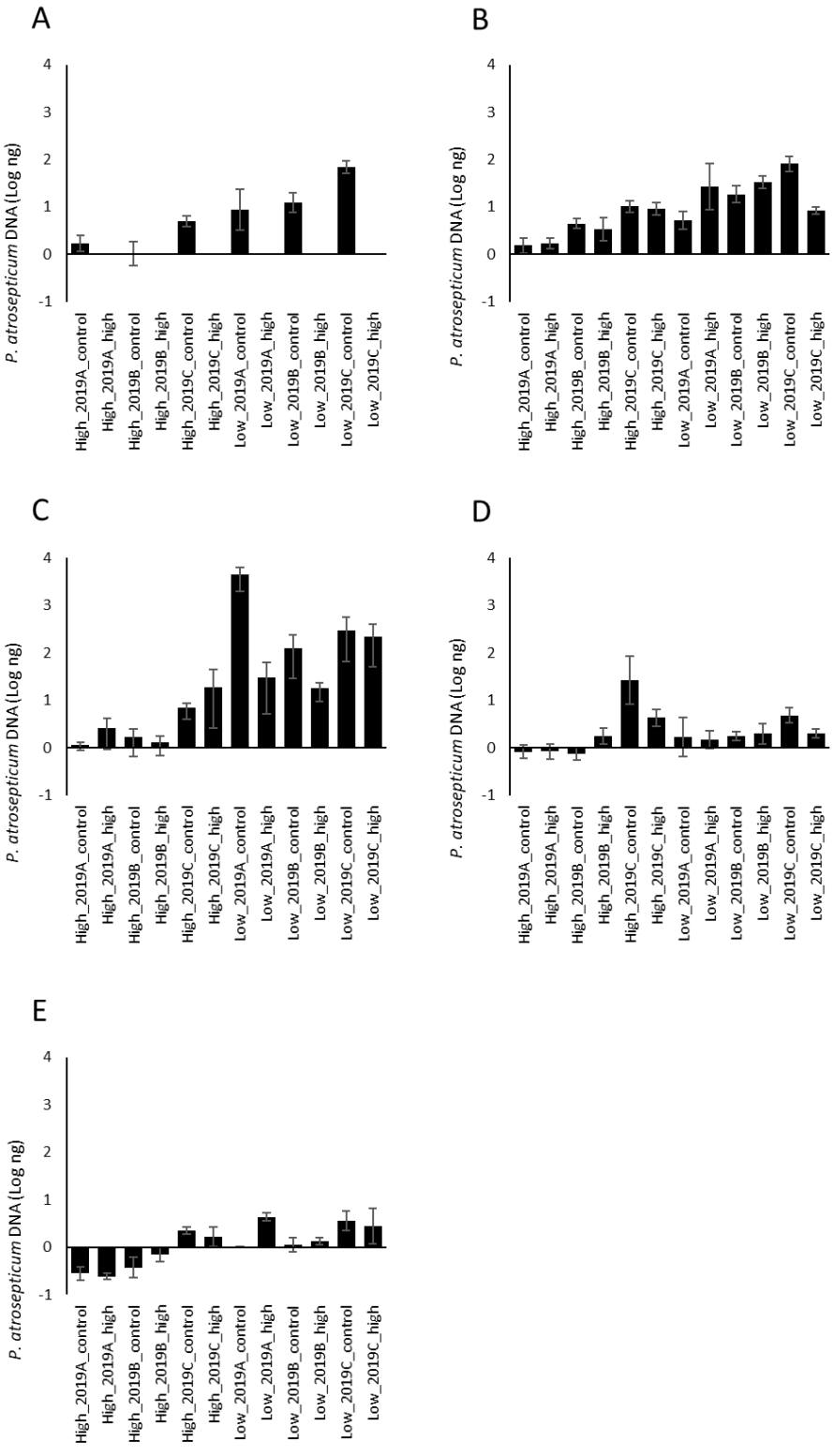

Fig. 22 Quantification of *Pectobacterium atrosepticum* DNA levels in potato tubers in a transit mimic experiment. Tubers from high and low health stocks were stored in conditions that mimic transit during export and then exposed to either standard potato storage conditions (control) or elevated temperatures (high). DNA levels (Log pg) were quantified using qPCR from tubers sampled prior to transit mimic conditions (A), after four weeks transit mimic conditions (B), following one week of storage (C), two weeks of storage (D) or four weeks of storage (E) at one of the two treatment conditions. Error bars indicate ±1 SE

5. DISCUSSION

As a vegetatively propagated crop, maintaining the health of seed potatoes is essential to protect the value of future harvests. Certification schemes are typically used to ensure that disease is kept to a minimum in crops destined for the seed market with stocks that do not meet the required tolerances either declining in value or if thresholds for specific diseases are exceeded, the stocks are no longer acceptable for use as seed crops and will not be certified as a result (Anon, 2016). A number of important potato diseases can remain asymptomatic after harvest with symptoms only developing during storage or transit to an importing country. These latent infections can be particularly problematic to the seed potato industry. Scottish seed potatoes are exported across the world as Scotland is recognised as a high health region for growing seed potatoes, supported by the absence of important diseases such as ring rot, brown rot and *Dickeya* spp. Seed potato crops are inspected throughout the growing season to determine the grade and ensure the health of the crop, and tubers are inspected after harvest to approve marketing of the crop. During the majority of tuber inspections, seed lots are usually found to fall within the tolerances for export, however, during transit, and on rare occasions, tuber quality can deteriorate resulting in a rejection of the consignment, or part of the consignment for failing to meet the phytosanitary requirements of the importing country (Fig. 1B) and economic losses for the seed potato producer. Latent infections of tubers with potato pathogens are thought to be the reason why these rejections occur, but very little is understood about the specific events that lead to these losses. This project aimed to investigate the effect of transit on potential latent infection by a number of important potato pathogens on seed potato health.

To assess the effect of commercially relevant diseases associated with the export process, disease incidence, based on the thresholds applied at official tuber inspections was used. Very limited expression of disease symptoms was recorded in any of the seed potato stocks used in this study, indicative of the high health status typically associated with Scottish seed potatoes. However, some variation in disease incidence was observed for some diseases between the crops tested. There was a small but statistically significant increase in silver scurf in crop SEA1_2017B whereas NA2_2018B showed significant differences in black dot incidence between tubers assessed after harvest and those stored in the UK or exported. Country specific tolerances are listed by a number of major importing nations of Scottish seed potatoes for black dot (https://www.gov.scot/publications/potato-exports-guide/) and it is known that black dot and silver scurf symptoms develop during storage (Errampalli *et al*., 2001; Hardy *et al*., 1997; Johnson *et al*., 2018; Lees and Hilton, 2003; Wustman, 2007) which could explain the observations seen in this crop. Development of this disease in storage can be restricted by maintaining tubers at low temperatures (<3.5°C) after curing for less than two weeks (Peters *et al*., 2016; Fiers *et al*., 2012). In both 2017-18 and 2018-19 tubers were typically stored at temperatures >5°C during transit whereas the UK potato store was set at 4°C. These storage temperatures may have influenced the development of black dot on tubers in these experiments. No rot symptoms were seen in the tubers from any stock scored after harvest, storage or export in any of the three years of the study. Together these data demonstrate that the risk of quality deteriorating due to increased symptom expression is limited if tubers are maintained at acceptable storage conditions during transit.

The observed increase in disease incidence was not supported by increased DNA levels of *H. solani* in exported samples compared to those stored in the UK in either 2017-18 or 2018-19. Overall higher DNA levels of a number of pathogens were identified in tubers stored in the UK compared to those that had been exported. Potential issues

with pathogen spread within the storage facilities could explain why pathogens such as *H*. *solani* and *B*. *cinerea* which are readily transmitted by air-borne spores during storage (Abbas *et al*., 2013; Errampalli *et al*., 2001; Hardy *et al*., 1997; Secor and Gudmestad, 1999) but does not explain why levels of *S*. *subterranea*, *C*. *coccodes*, *P*. *pustulans*, *P*. *ultimum*, *F*. *sulphureum* or *R*. *solani* were higher in stored tubers.

As observed for silver scurf in 2017-18 the increase in black dot incidence in NA2_2018B was not associated with an increase in pathogen DNA. Indeed, pathogen DNA levels in the 2018-19 crops were overall quite variable between samples collected after harvest, storage in the UK or export abroad. For the surface blemish diseases, dry rot and grey mould pathogens, no clear pattern of changes in pathogen DNA levels could be observed in tuber peels collected from the three different sampling points. The number of tubers sampled from each of the seed lots for export may explain the variation in pathogen DNA loads observed between samples, as it may be that too few tubers were used in the analysis to accurately assay pathogen loads in these samples. Using an appropriate sample size is important to minimise processing requirements and associated costs without losing detection power (Priou *et al*., 2001). A sample size of 200 tubers per 25 tonne seed stock is regularly used to detect quarantine bacterial pathogens in seed potatoes in the EU (Janse, 1988; EPPO, 1990) and this sample size should provide a 95% probability to detect at least one infected tuber in the seed stock, assuming 1.5% of tubers are infected (Czajkowski *et al*., 2015). Therefore, for this project, a 200-tuber sample was deemed a valid sample size to assess disease incidence and pathogen DNA levels in this work. However, the results from the molecular diagnostics suggests that the sampling technique may not be suitable to accurately quantify the selected surface blemish pathogens in tuber peel tissue.

As pectinolytic bacteria are typically found at higher levels in heel end tuber tissue (Helias *et al*., 2000; De Boer, 2002; Czajkowski *et al*., 2009), *P*. *atrosepticum* DNA levels were assayed in heel end tissue which provided more consistent results across the crops used in the three years of the trial. Higher levels of *P*. *atrosepticum* DNA was found in exported tubers from SEA1_2017A, SEA1_2017B and ME_2017 compared to tubers stored in the UK. Similarly, elevated levels of *P*. *atrosepticum* DNA were found in exported tubers NA2_2018A and NA2_2018B compared to tubers assessed after harvest or storage in the UK. *P*. *atrosepticum* DNA was only detected in tubers that had undergone export in sample NA2_2019A. These data suggest that transit of seed potatoes during export can lead to increases in *P*. *atrosepticum* levels in tubers which could directly impact tuber quality if tubers are not stored under suitable conditions after they arrive at the final destination. No symptoms of *P*. *atrosepticum* were observed on any of the tubers assessed from any of the seed stocks, highlighting that the risk of latent infection with this bacterium could lead to deterioration of tuber quality if not managed (Czajkowski *et al*., 2011).

The risk of latent infection on seed potatoes by pectinolytic bacteria is highlighted by studies showing that seed stocks are the most important factor in determining the prevalence of blackleg in Switzerland and southern Germany (de Werra *et al*., 2020). Given the strict tolerances for blackleg in crops destined for use as seed potatoes and in the seed potato tubers themselves, levels of blackleg in seed potatoes tends to be minimal. Therefore, tubers infected with *P*. *atrosepticum* without showing any symptoms of disease provide a major risk to disease transmission to subsequent field crops. Other factors including location and local environmental conditions can also affect disease prevalence (Rossman *et al*., 2020) but the health of the seed stock appears to be most significantly associated with the development of blackleg symptoms (de Werra *et al*., 2020). These findings indicate that latent tuber infections by pectinolytic bacteria are a potential major source of primary infections in subsequent

field crops; however, the presence of *P*. *atrosepticum* in a tuber does not necessarily mean that blackleg or soft rot will develop. It is generally accepted that a minimum threshold of approximately 10^3 viable bacterial cells is required for disease to develop (Pérombelon, 2000), although, typically higher numbers of bacteria on tubers at planting tend to increase disease incidence and severity in the field (Bain *et al*., 1990; Moh *et al*., 2012; Toth *et al*., 2003). However, if environmental conditions at planting and during the growing season are particularly conducive to disease development then lower numbers of viable *P*. *atrosepticum* on tubers can result in high disease incidence (van der Wolf *et al*., 2017). Maintaining levels of *P*. *atrosepticum* as low as possible during the export process with strict temperature control and ventilation is therefore of great importance to prevent the increased risk of subsequent in-field blackleg development.

Transit conditions during the 2017/18, 2018/19 and 2019-20 export seasons were very consistent which, together with the low levels of disease observed on tubers after harvest, would explain why very little deterioration in tuber quality was recorded on any of the crops assessed. Given the observed transit conditions it is difficult to ascertain why levels of *P*. *atrosepticum* DNA increased in a number of the tested stocks after they had been exported. It is generally accepted that bacterial development tends to be slower at lower temperatures (Bartz and Kelman, 1984; Du Raan *et al*., 2016; Kushalappa and Zulfiqar, 2001; Smadja *et al*., 2004) but tubers stored at lower temperatures are more prone to wound injuries which can lead to increased bacterial contamination (Bartz and Kelman, 1984). Warmer tuber temperatures can increase the severity of disease (Bartz and Kelman, 1984; Moh *et al*., 2012) but no symptoms of bacterial rot disease were observed in any of the exported stocks. Increased wet incubation time, which can include factors such a surface moisture caused by condensation formed after tuber respiration during storage and transit, can also increase severity of bacterial soft rots (Kushalappa and Zulfiqar, 2001; Pringle, 1996). One factor not measured in the trials reported here is the effect of motion during export on tuber health. Rotating tumblers used to remove excess soil adhering to tubers during the washing procedure for potatoes can increase the incidence of soft rots caused by *Pectobacterium* spp. and the bacterial concentrations associated with the infected tubers (Wicks et al., 2007) suggesting that mechanical agitation and abrasion of tuber skin caused by soil particles in the tumbler can influence changes in *Pectobacterium* spp. Therefore, it is possible that movement of tubers within containers during transit may pose an increased risk to tuber health during the export process.

Potato storage conditions have often been considered a major risk factor associated with the deterioration of tuber quality due to bacterial rot disease (Elphinstone *et al*., 2018). *Pectobacterium* spp. levels can decrease immediately after being put into storage but over time the levels of bacteria tend to increase (Pringle *et al*., 1991). Typically, increases in storage temperature result in more disease caused by *Pectobacterium* spp. (Kushalappa and Zulfiqar, 2001), although Pringle *et al*. (1991) found no correlation between store temperature and relative humidity, and the changes in bacterial levels. This may be a reflection of the standard of potato storage facilities available 30 years ago and it does not accurately reflect the specification of modern potato stores particularly with respect to ventilation that can help prevent water films forming on tuber surfaces which influences bacterial rot development (Pringle, 1996; Pringle and Robinson, 1996). Mathematical modelling suggested that temperature, initial inoculum load and relative humidity are all factors that can significantly contribute to increasing pathogen population densities and soft rot disease in potatoes (Moh *et al*., 2012). Given that the output from the data loggers showed that conditions remained highly controlled and within the desired parameters during the transit period, the cause of these increases in *P*. *atrosepticum* DNA is unclear. One explanation may be offered by what happened to the tubers after transit. Outside of the periods of controlled storage

conditions, temperature, humidity and dew point readings were much more variable. Understanding what happened to the tubers during these periods post transit will be essential to devise strategies that will help protect Scottish seed potatoes from deterioration in tuber quality during the export process.

To assess the effect that variable post-transit conditions and latent infection may have on tuber health, a controlled environment experiment was devised to mimic the conditions recorded during export followed by a period of storage at two different temperatures. As the output from the data loggers received across the three export seasons of the trial suggested that the largest variation in parameters was related to an increase in temperature on arrival at the destination, the potential risk from elevated holding temperatures after transit was assessed using six tuber stocks designated as either high or low health stocks, based on the health of the growing crop. Preliminary *P*. *atrosepticum* diagnostic analysis of the low and health tuber stocks, using qPCR and assessing colony forming unit numbers using the CVPM agar plate method, confirmed bacterial incidence was higher in bulked samples from the low health stock. However, there did not appear to be a statistically significant difference in *P*. *atrosepticum* quantification between samples from the high and low health stocks despite both methods indicating higher levels of bacterial DNA or CFUs in bulks from the low health stocks (Fig. 19). Quantification of *P*. *atrosepticum* levels in bulked tuber samples by qPCR and assessing colony forming unit numbers using the CVPM agar plate method appeared to show a moderate correlation between the two methods (Fig. 20). It should be noted however, that more samples were judged positive for *P*. *atrosepticum* by the qPCR methods than the CVPM agar plate method, suggesting that qPCR is a more sensitive method for detection of this organism. Unfortunately, false negative results were found by both methods which is surprising considering that the tissue used for both assays was collected from tuber cores taken adjacent to another at the heel end of each tuber that comprised each bulk sample. With pectinolytic bacteria typically detected at higher concentrations at the heel end of potato tubers (Helias *et al*., 2000; De Boer, 2002; Czajkowski *et al*., 2009) the variation in detection of *P*. *atrosepticum* incidence between the qPCR and CVPM agar plate method was not expected. It is possible that the distribution of *P*. *atrosepticum* at the heel end of a tuber may well vary between contaminated tubers leading to the variation between the two methods for detecting *P*. *atrosepticum* infected tuber samples.

The transit mimic experiment confirmed that the health status of stocks significantly influenced *P*. *atrosepticum* DNA levels. This was particularly significant in samples analysed after the mimicked export treatment and after one week of storage at either standard potato store conditions or at an elevated storage temperature of 20°C. Curiously, higher *P*. *atrosepticum* DNA levels were noted in low health tubers after storage at the standard store conditions (control treatment) rather than at the elevated storage temperature. However, neither stock health status nor post-transit storage treatment had an effect on *P*. *atrosepticum* DNA levels (Fig. 22). The effect of tuber stock health on *P*. *atrosepticum* DNA levels follows what would be predicted based on the known epidemiology of blackleg and bacterial soft rot diseases. Seed-borne inoculum is a major route for both in-field blackleg and in-store soft rots caused by *P*. *atrosepticum* (de Werra *et al*., 2020; Pérombelon, 2000; Bain *et al*., 1990; Moh *et al*., 2012; Toth *et al*., 2003). This is why maintenance of high seed health is essential for a successful seed potato industry and underlies why many countries use a seed certification scheme to ensure health standards of seed potatoes are maintained (Czajkowski *et al*., 2011; Dehem-Schmutz *et al*., 2010). In Scotland, the seed potato certification scheme (SPCS) stipulates the tolerances for tuber diseases permitted for marketing of stocks destined to enter the seed supply chain, with stricter tolerances applied for the higher-grade crops. During the growing season, crops are inspected at

least twice for symptoms of virus, fungal and bacterial infection. Given the risk that blackleg poses to the Scottish seed potato industry, policy has evolved within the SPCS with the addition of risk-based inspections to ensure the health of the crop seen at inspection is maintained for the duration of the growing season and crop desiccation. Measures to protect against virus spread are also included in the SPCS, with a mandatory post-harvest test for crops deemed to be a risk to other crops in the vicinity. After harvest, tubers are then further inspected before they can be marketed and enter the seed potato supply chain. These policies have maintained the strong reputation of the Scottish seed potato industry as can be evidenced by the very low level of rejections of seed potato crops after they have been exported out of Scotland (Fig. 1B). However, the small number of rejections that do occur is concerning and suggests a number of factors influence the health of the seed crop from planting, through the growing season, harvest, storage and finally transit.

This risk of latent infections leading to crop losses after export means that asymptomatic pathogen infections pose a risk to the seed potato export industry. As a number of important potato diseases can develop during periods of storage (Adams and Griffith, 1978; Adams *et al*., 1980; Bojanowski *et al*., 2013; Carnegie *et al*., 1990; Croke and Logan, 1982; Hardy *et al*., 1997; Hide *et al*., 1994; Hide and Boorer, 1991; Hide and Cayley, 1987; Hide and Adams, 1980; Lees and Hilton, 2003; Lennard *et al*., 1980; Perombelon, 2000; Peters *et al*., 2016; Pringle and Robinson, 1996; Secor and Gudmestad, 1999) and as reported here, pathogen levels, as measured by quantification of DNA levels in tubers, can increase during transit; there is always a risk that the health status of seed potato consignments decline during export and the health of the seed lot is no longer comparable to the official tuber inspection prior to export. This can prove potentially problematic when assessing disease levels on tubers destined for very early season export markets such as Egypt, the largest non-EU destination for Scottish seed potatoes, as well as newly emerging markets such as Pakistan and Vietnam. Phytosanitary requirements for these export markets are usually based on the Scottish minimum tolerances for seed potato exports (https://www.gov.scot/publications/potato-exports-guide/supplementary-information/minimum-tolerances/) with a number of country specific requirements to reduce risks associated with specific disease threats. Visual inspection of tubers can only identify symptomatic infections and will inevitably miss latent colonisation by pathogens that can lead to disease; however, based on the evidence from this project disease levels did not significantly change during the export process suggesting that, in Scotland, visual inspections are fit for purpose. If symptoms of disease permitted within the specific tolerances is observed at inspection, tubers are permitted to be exported to these early markets; however, if latent infection by pathogens not permitted within the importing country's phytosanitary requirements develop during transit, the consignment could be denied entry by the importing country. Egypt, an important market for seed potatoes from Scotland, has specific requirements for black dot (Hide *et al*., 1994; Hide and Boorer, 1991; Lees and Hilton, 2003; Peters *et al*., 2016) and silver scurf (Hide and Adams, 1980; Hide *et al*., 1994; Hardy *et al*., 1997; Lennard *et al*., 1980) both of which develop during storage. Pakistan also has specific requirements for silver scurf, whereas Vietnam has a nil tolerance for dry rot (Bojanowski *et al*., 2013; Carnegie *et al*., 1990), Gangrene (Adams and Griffith, 1978; Adams *et al*., 1980; Croke and Logan, 1982) and skin spot (Hide and Boorer, 1991; Hide and Cayley, 1987). There is a risk of latent infection by these pathogens and therefore being able to evaluate the risk of latent infections is likely to become important if the Scottish seed potato industry is to satisfy demand in early markets in the future. The use of diagnostic tools may provide more sensitive tools to detect pathogens (Budge *et al*.,2009; Cullen *et al*., 2001; Cullen *et al*., 2002; Cullen *et al*., 2005; Cullen *et al*., 2007; Humphris *et al*., 2015; Lees *et al*., 2009;

Lees *et al*., 2002; Qu *et al*., 2011; Suarez *et al*., 2005), however, positive detection of a pathogen does not provide any direct correlation with the risk of disease occurring. DNA-based molecular diagnostics are very sensitive but do not discern between viable and non-viable pathogen material. If the DNA is present the diagnostic assay can detect it, provided the level is within the detection threshold of the assay. Assays for determining pathogen viability are available, often using selective media after isolating the pathogen (Helias *et al*., 2012; Bannon, 1974). These assays tend to be more time consuming than modern molecular diagnostics and can be less sensitive. However, despite improvements in modern diagnostic tools, there are still questions pertaining to the biological relevance of a positive result from a molecular test to actual threat of disease occurring in the future. Research to determine how diagnostic methods can be used to help predict the level of disease risk a stock of seed potatoes present would be of great use to the industry to help protect against loses from latent infections.

Although the relationship between the results of the molecular diagnostics and disease incidence observations do not provide any clear evidence on the effect of transit on influencing disease development and loss of tuber quality, the findings from this project indicate that latent infections may be present on seed potatoes that have passed internal inspection criteria and deemed acceptable for export. However, if suitable storage conditions during transit and on arrival at the export destination are maintained the risk that these latent infections could develop into disease is minimal. Molecular diagnostics show some promise to assess tuber health, however, as the presence of pathogens does not necessarily relate to disease development, visual inspection of a seed export consignment assesses a representative sample from the crop and is a robust method to assess tuber health. Molecular diagnostics generally test a 200 tuber sample for the qPCR-based testing for multiple pathogens compared to the minimum 300 kg of tubers required for visual inspections of a 25 tonne consignment of seed potatoes. Determining how exported seed potatoes are handled immediately after arrival at the export destination prior to inspections will provide critical information to help protect UK exporters from commercial loses due to quality rejections after export.

6. CONCLUSIONS

Despite the presence of latent infections of high health Scottish seed potatoes that enter the export market the data suggests that when conditions during export are maintained to those prescribed by industry standards the potential risk of loss of tuber quality is limited. However, even when transit conditions are controlled, some effects on tuber quality can be observed particularly for surface blemish diseases such as silver scurf or black dot which are known to increase during prolonged storage.

Quantitative PCR analysis of pathogen DNA did not show a clear association between pathogen DNA levels and changes in disease incidence between tubers assessed at harvest, after storage in the UK or post-export. However, molecular diagnostics did indicate that a range of pathogens responsible for surface blemish and rot diseases could be detected on tubers in the absence of disease symptoms. Of particular note was the effect of storage and export on *Pectobacterium atrosepticum* DNA levels. This bacterium appeared to increase during storage and export with levels typically highest in the tubers that had been exported. Confirmation of latent infection on seed potatoes indicates the potential threat that crops destined for export could be faced with if conditions during transit and subsequent delivery to, and handling and storage by, the end customer are not controlled.

With the largest fluctuations in temperature, relative humidity and dew point recorded by most of the data loggers after the consignment had arrived at the export destination, information on the location of the tubers and the logger in the importing country will help determine whether or not any of these factors influence the transition of pathogen development from latent to symptomatic. Further refinement of the custom-built sensor may help provide additional insights into the environmental changes that occur after transit which may prove useful in further protecting seed potato consignments from post-export rejections.

Seed health appears to be a critical factor in determining the risk of pathogen levels increasing during transit. Therefore, maintaining the highest levels of seed health for tubers destined for the export market is of the utmost importance. The current seed potato certification scheme protects tuber health from diseases through the use of inspections of the growing crop and tubers prior to export. Despite the presence of latent pathogen infections, the current surveillance and inspection procedures outlined in the seed potato certification scheme is a robust and reliable method to maintain the high health status of Scottish seed potatoes destined for the export market.

7. REFERENCES

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