Final Report

Integration of precision irrigation and non-water based measures to suppress common scab of potato

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1. Summary

This project aimed to increase potato quality through reduction of common scab whilst addressing environmental concerns over the volume of water currently used to control this disease. The project was initiated in 2006 as a LINK Collaborative Research grant (SA-LINK LK0989) to Central Science Laboratory (CSL), Cambridge University Farm (CUF) and Scottish Agricultural College (SAC) with industrial contribution from British Potato Council, Branston Ltd, QV Foods, Cobrey Farms and Wroot Water.

Data obtained from commercial potato fields have confirmed that the most important factor influencing potato common scab development is the soil in which the potatoes are grown. Novel molecular diagnostics have shown that the build up of pathogenic *Streptomyces* spp. on initiating tubers and the resulting common scab levels vary from field to field and appear to be inversely related to the total actinomycete activity of the soil during tuber initiation. Whether the total actinomycete populations directly inhibit multiplication of pathogenic *Streptomycete* spp., or merely indicate soil types with high general biotic activity, in which other organisms inhibit the pathogens, remains to be determined.

A number of *Streptomyces* species, in addition to *Streptomyces scabei*, were confirmed to be causal agents of common scab in commercial fields. Two of these, *S. acidiscabies* and *S. turgidiscabies* have so far been confirmed as new records for the UK. Under field conditions, the various species detected appeared to be widespread in commercial production in England and Scotland. In fact, different pathogenic *Streptomyces* species could often be detected on the same tuber and in the same common scab lesions.

The role of seed-borne *Streptomyces* in common scab development was confirmed to be minor in comparison with that of soil-borne inoculum. Nevertheless, seed with severe common scab was seen to contribute to the pathogenic *Streptomyces* populations detected on initiating tubers compared to seed with minor or no scab. The role of seed in spreading new pathogen species is therefore expected to be important. The susceptibility of different potato cultivars to common scab appeared to be affected by seasonal and site differences. Hence, the expected higher tolerance to common scab of cv. Estima compared with cvs. Maris Piper and Desiree was not observed across all commercial sites and seasons. During the course of field studies, no significant effects of non-water based control measures (soil amendments with sulphur or rapeseed meal) on common scab development were observed, despite earlier positive findings in other trials

These studies have confirmed that the most reliable control of common scab remains through careful application of irrigation water over the critical period when initiating tubers are susceptible to infection. Suppression of pathogenic *Streptomyces* populations in response to irrigation was further evident in the field trials conducted within this project, even under the high pathogen inoculum pressure usually found at the CUF experimental field site. Moreover, advances in molecular diagnostics have allowed quantification of pathogen populations in response to varied irrigation regimes, allowing optimisation of the amount and frequency of water required to provide adequate pathogen suppression and disease control. Two years of research at the disease-conducive site at CUF has increased understanding of the effects of precision irrigation on populations of *Streptomyces* on developing tubers. Results from this site showed that irrigation slowed the increase in both total actinomycete and pathogenic *Streptomyces* spp. between 1 and 3 weeks after tuber initiation compared with unirrigated plots. The ratio of pathogenic to non-pathogenic species was significantly higher in unirrigated

plots, or in plots irrigated for only 2 weeks after tuber initiation, than in plots irrigated over 4- and 6-week durations.

Further diagnostic advances, involving the use of pyrosequencing to study population dynamics of the whole bacterial microflora on initiating tubers, have raised the possibility that scab suppression by irrigation may be enacted at least partially by microbial taxa other than non-pathogenic actinomycetes. The largest increase in proportion of a single taxa in response to irrigation was observed in the genus *Pseudomonas*, levels of which were approximately 10 % of the sequences identified to genus in irrigated plots but less than 2% in unirrigated plots.

The studies conducted at CUF, despite being conducted under high inoculum pressure in soils with generally low total actinomycete populations, have indicated potential savings in water use and reduction in drainage losses through the use of precision irrigation regimes designed specifically for that site. Even in the scab conducive situation at CUF, optimising the frequency and critical period over which irrigation was found to suppress the pathogens on developing tubers could lead to potential water savings without affecting the degree of common scab control. Limiting the period over which irrigation was applied to 4, rather than 6, weeks after tuber initiation resulted in water savings of around 25%, irrespective of whether drip or sprinkler irrigation was used. By applying water at 4-6 day intervals rather than daily over the 4 week period, a further water saving of 13 to 22% was made. By shortening the period of irrigation from 6 to 4 weeks after tuber initiation, drainage water losses were reduced by 50% with daily irrigation and over the 4 week period were further reduced by 62% with drip and 41% with sprinkler by lengthening the irrigation interval to 4-6 days. Potential water conservation is expected to be even more significant at sites that are less disease conducive.

The findings from this project have been carefully considered and general practical advice for growers has been formulated to assist the goal of consistent common scab control with minimal water input.

2. Experimental Section

Introduction

Despite significant research into control of common scab, surveys have shown this disease to affect an average of 87% of potato crops assessed. Common scab can be largely eliminated through correctly timed irrigation to achieve uniform wetting of the ridge or bed around initiating potato tubers. In fact, the need to meet quality standards for common scab is a main driver for irrigation of potatoes, particularly for the pre-packed market. Even minor scab lesions can drastically reduce the value of crops. However, increasing restrictions on water abstraction and costs of irrigation have resulted from changes in legislation and policy on environmental protection, as summarised in the BPC report ‘Changes to water policy and their effect on the potato industry’ (Tompkins and Clayton, 2003).

This project was therefore initiated in 2006 as a LINK Collaborative Research grant (SA-LINK LK0989) to further investigate scab control resulting from pathogen suppression at the tuber surface, mainly by non-pathogenic *Streptomyces* spp. Studies on the mechanisms of common scab control have previously been hindered by a lack of methods to accurately differentiate and quantify pathogenic and non-pathogenic strains *in situ*. Developments in molecular diagnostics have been exploited in this project to investigate the population dynamics of *Streptomyces* on developing tubers and in the surrounding soil, making it possible to elucidate and optimize the mechanisms by which irrigation and alternative measures can be used to

control common scab at a practical, cost effective and environmentally acceptable level. The project was conducted by a Consortium of The Central Science Laboratory (CSL), Cambridge University Farm (CUF) and Scottish Agricultural College (SAC) with industrial contribution from Potato Council, Ltd., Branston Ltd, QV Foods Ltd., Cobrey Farms and Wroot Water Systems.

Aim of the project

The overall aim of the project was to increase potato quality through reduction of common scab and address environmental concerns over the volume of water currently used to control scab.

Specific project objectives were to:

(a) Optimize the methodology of real-time PCR assays and conventional microbiology in order to identify and quantify scab-forming and total streptomycetes and other antagonists;
(b) Quantify the effects of control measures *in vitro* (e.g. moisture level, pH and organic matter) on the populations of *Streptomyces* on initiating tubers and compare with scab incidence and severity;
(c) Optimize the effects of precision irrigation and other integrated control measures in field trials;
(d) Compare the population dynamics of *Streptomyces* in scab-conducive and suppressive soils in commercial crops;
(e) Correlate symptoms with species range; and
(f) Design practical control guidelines.

Material and methods

1. Molecular methods optimised for detection, identification and quantification of *Streptomyces* species

A real-time PCR assay for quantification of total actinomycete populations by amplification of the 16S rDNA had previously been developed (Cullen *et al*., 2000). Initial work on developing further molecular methods for quantification of *Streptomyces* species focused on optimisation of DNA extraction methods for soil and tubers, and development of a real-time PCR method for quantification of pathogenic *Streptomyces*.

DNA extraction

Prior to DNA extraction, whole tubers were either pulverised with a mallet (if the tuber was less than *c*. 3 cm in length, or a strip of peel was removed from stolon to rose end for extraction. Samples from 10 tubers were pooled in each extraction. Peel was macerated in polythene bags containing 15 ml extraction buffer (7.5 mM Na_2HPO_4, 3.5 mM NaH_2PO_4, 0.4 mM $Na_4P_2O_7$, pH 7.0). A 5 ml aliquot of the resulting suspension was centrifuged to pellet the debris, which was then resuspended in 1 ml of the remaining supernatant. The suspension was then treated with 3 mg lysozyme per ml at 37 ºC for 15 min before extraction using the Wizard DNA purification kit for food (Promega) and a Kingfisher ML magnetic automated DNA extraction instrument following the manufacturer's instructions. DNA was eluted in 200 µl sterile water and stored at –30 ºC. DNA was extracted from soil using the Ultra-Clean Mega Prep Soil DNA kit (MoBio).

Real-time PCR quantification of *Streptomyces*

A conventional PCR method for detection, but not quantification, of pathogenic *Streptomyces* by amplification of the *txtA* gene, encoding the thaxtomin phytotoxin, had previously been described (Wang and Lazarovits, 2004). A new real-time PCR assay was designed for quantitative detection of the *txtA* gene. For quantification of 16S rDNA, real-time PCR primers were 16S495F (agcagccgcggtaatacg), 16S556R (cgagctctttacgcccaataa) and the probe was 16S514T (agggcgcgagcgttgtccg). For quantification of *txtA* levels, real-time PCR primers were Txt-Q-F (cgacaccgtcgtgctcaac) and Txt-Q-R (gcgcagatggtcgaacaggta) and the probe was Txt-Q-P (cgtgatccagtactttccctcaggcga). Probes were labelled with FAM (5') and TAMRA (3'). Real-time PCR reactions were carried out in 25 µl volumes containing 1 x reaction buffer (Applied Biosystems), 3.5 mM $MgCl_2$, 200 µM each dNTP, 300 nM each primer, 100 nM probe and 0.625 U Amplitaq Gold DNA polymerase (Applied Biosystems). PCR conditions were 95ºC for 10 min followed by 45 cycles of 95ºC for 15 s and 60ºC for 1 min. Data was collected during the 60 ºC elongation step. Quantification was achieved by including in assays a dilution series of plasmid clones containing the target sequence for each assay at known concentrations. A standard curve of Ct value vs. concentration was constructed for each PCR reaction to allow calculation of either 16S rDNA or *txtA* present in samples.

Speciation of *Streptomyces* by PCR and DNA sequencing

The identity of *Streptomyces* species occurring in soil and tuber samples was determined by PCR amplification using sets of primers targeted to variable regions within the 16S rDNA (Wanner, 2006). This allowed identification of *S. scabiei/europaeiscabiei*, *S. stelliscabiei*, *S. bottropensis*, *S. acidiscabies*, *S. turgidiscabies*, *S. aurofaciens* and a currently unclassified group referred to as *Streptomyces* 'group X'. Identity of individual isolates was confirmed by sequencing variable regions of the 16S rDNA amplified using universal primers (Wiesburg *et al*., 1991).

Characterisation of total bacterial populations by pyrosequencing

DNA extracted from potato tubers harvested from irrigation trials was used in PCR reactions to amplify an approximately 450 bp region of the bacterial 16S rDNA using the eubacterial 16S 27f forward primers (5'-AGAGTTTGATCCTGGCTCAG-3') and the universal reverse primer 338r (5'-TGCTGCCTCCCGTAGGAGT-3'). These primers were selected to amplify a region of the 16S rDNA containing the γ variable region known to contain a relatively rich source of phylogenetic variability. Primers had been modified to incorporate linker sequences required for pyrosequencing library construction at the 5' end. Additionally, the reverse primer contained a 10 nt barcode, or multiplex identifier (MID) sequence (http://www.454.com/products-solutions/sequencing-services/faqs.asp) to allow multiplexing of samples in a single pyrosequencing run. Amplified products were quantified, purified subjected to pyrosequencing on a 454 GS FLX instrument. Resulting sequences were partitioned *in silico* according to MID sequence allowing analysis of individual samples. Sequences were assigned to phylogenetic taxa using the RDP classifier tool of the ribosome database project (Cole *et al*., 2009).

2. Greenhouse trials

a) Preliminary polytunnel experiment to evaluate the effect of soil type and moisture level on population changes of *Streptomyces* and development of common scab

In some soil types, common scab appears to develop only to a limited extent even when environmental conditions favour disease development. Such soils may be considered 'suppressive'. In order to examine whether suppressiveness is linked to *Streptomyces* populations in soil, a polytunnel experiment was established at SAC Aberdeen with the following objectives:

1. To evaluate the development of common scab symptoms in suppressive and non-suppressive soils under uniform conditions
2. To follow changes of pathogenic and total *Steptomyces* populations on developing tubers during crop development
3. To evaluate the effect of soil moisture on pathogenic and total *Steptomyces* populations and common scab development

Mini-tubers (18-20mm) of cultivar Maris Piper were planted in 25cm pots (25cm diameter at top and 25cm depth – soil capacity 7 kg) containing soil collected from 4 fields, two considered suppressive (QV and Elgin) and two non-suppressive (CUF and Forres). Half of the pots were watered sparingly (dry) during the tuber initiation phase, sustaining the plants at or close to wilting point and half were watered liberally (wet) on a daily basis to mimic normal irrigation practice. Thus the experiment comprised 4 soil types, 2 moisture levels, 4 sampling dates (2, 4 and 8 weeks after 50% emergence and final harvest) and 4 replicates. All pots received the same fertiliser and crop protection programmes. At each sampling date, all four replicates were removed and all tubers harvested. For the first three harvest dates, tubers were dispatched to CSL by courier and tested for pathogenic and total *Steptomyces* populations. At the last harvest, the tubers were assessed for incidence and severity of common scab before dispatch.

b) Effect of soil amendments on scab incidence and severity

Pot experiments were conducted under glasshouse conditions at CSL to confirm the pathogenicity of new *Streptomyces* isolates on potato and to assess the effect of soil amendment with sulphur and lime on symptoms caused by these isolates. Maris Piper minitubers were planted in sterile compost in sterilized 3 l pots. Five different compost treatments were tested: no amendment, amendment with 1 or 2 g sulphur per l or amendment with 1 or 2 g lime per l. When leaves emerged, pots were inoculated with 100 ml suspensions of *Streptomyces* containing 10^7 cfu/ml. Strains tested were YME11 (*Streptomyces* 'group X'), P6802 (*S. scabiei*), P6803 (*S. turgidiscabies*), P6809 and (*S. acidiscabies*). These strains had been isolated from symptomatic material collected during the course of the project. Inoculation with *S. scabiei* strain NCPPB2537 was done as a positive control, and inocula were tested on a minimum of four plants. Inoculation was repeated one week later. Plants were grown for 14 weeks after initial inoculation and tubers were harvested and assessed for scab levels. *Streptomyces* were reisolated from symptomatic tubers, and their identity confirmed by PCR using the method described above.

3. Field trials to assess the effect of sulphur and other non-water control measures on development of common scab

In a literature review of non-water control measures (Stead & Wale, 2004) identified a number of chemical control measures that had shown promise in reducing common scab. Over a number a number of years SAC had been testing chemical control options with moderate success (Hilton & Wale, 2008). Of those tested, sulphur (S) and rapeseed meal

had shown most promise. These products were tested over the three years of the project in field trials in Scotland and England.

The susceptible cultivar Maris Piper was used in all trials. Treatments were applied either in-furrow at the time of planting (S) or by broadcasting and incorporation before planting (rapeseed meal). S was supplied as 90% elemental sulphur and the rapeseed meal was supplied in dry powdery form after oil extraction. Trials were either of a randomised block design, usually unirrigated or incorporated into factorial or split plot experiments where irrigation was also a factor being investigated. Plot sizes were typically 6.25m long by 4 drills, with the centre two rows harvested. Tubers were assessed after harvest for incidence and severity of common scab. In some trials, tubers were sampled and *Streptomyces* populations on the tuber surface determined as described before. All husbandry and other crop protection measures were standard as per local practice.

4. Field trials to assess the effects of irrigation timing and frequency on scab incidence and severity

a) Field experiment 1 (2007)

Experiment 1 was at CUF and was a fully-randomized factorial design involving four irrigation treatments (rainfed only (Unirrigated); bi-daily drip irrigation for 8 weeks post-tuber initiation (Drip); micro-sprinkler irrigation for 8 weeks post-initiation (Sprinkler); late irrigation from the end of the scab control period 8 weeks post initiation (Late)), four levels of sulphur amendment (0; 50; 125; 250 kg S/ha) and three replicate blocks. The experiment was planted on 4 April 2007 using 35-40 mm Maris Piper SE1 seed at 30 cm spacing 12 cm deep into pre-formed ridges spaced at 76 cm. Plots were four rows wide and 5 m in length except in plots that were sampled for *Streptomyces*, which were eight rows wide. Sulphur amendments (micronised elemental sulphur (695 g S/l) suspension fertilizer, Omex) were sprayed onto ridges just prior to planting and incorporated into the ridge by raking following dibbing. Liquid fertilizer nitrogen was applied at a rate of 132 kg N/ha post-planting. Plant emergence was recorded every 2 days by counting the number of plants emerged in the two central harvest rows. Tuber initiation (TI) was determined by counting the number of plants in a two-plant sample that was dug every 1-2 days starting 14 days after 50 % plant emergence.

Irrigation was scheduled using the CUF Potato Irrigation Scheduling Model using weekly grid measurements of ground cover and meteorological data from a Delta-T Devices weather station located 200 m away. Drip irrigation was via John Deere Ro-drip ultra-low flow (1.25 l/m/min, 20 cm emitter spacing) tape (Wroot Water Ltd) installed in the ridge after planting at a depth of 25-30 mm and 20 mm to the right of the centre of the ridge to avoid the seed tuber. The micro-sprinklers (Dan Modular Small Swivel Yellow Anti-mist nozzles) were on 1 m risers and installed in every alternate furrow at 1 m spacing. They were adjusted to run at very low pressure (*c*. 0.5-0.6 bar) to reduce the risk of misting and drift into adjacent plots. Application amounts at each irrigation ranged from 1-2 mm (i.e. 2-4 mm/day) to account for an expanding crop canopy and variable atmospheric demand. Drip-irrigated plots were calibrated to receive the same total dose of irrigation each morning (5:00 h) and evening (19:00 h) as the sprinklers but the application took *c*. four times longer. Irrigation following the end of the 8-week scab control period was applied using a boom (RST Irrigation) and hose reel (Perrot SA, SH63/280) combination. Plots were differentially irrigated by turning nozzles on or off along the length of the boom. Nozzles were spaced at *c*. 0.5 m, so individual plots could be irrigated. Irrigation amounts are shown in Table 1. Daily spot readings of soil water content in the ridge mid-way between two adjacent plants at 15 cm depth were made with a Delta-T Devices Theta Probe ML2.

TABLE 1.RAINFALL AND AMOUNTS OF IRRIGATION (MM) APPLIED DURING SCAB PERIOD AND REST OF SEASON

		Irrigation regime			
	Rainfall	Unirrigated	Late	Drip	Sprinkler
TI + 8 weeks	169	0	0	143	143
Rest of season	88	0	61	61	61
Total	257	0	61	204	204

From Unirrigated and Sprinkler plots with no applied sulphur, samples of 10 tubers from five plants per plot were sent to CSL in perforated plastic bags after 1, 3, 5, 7, 9 and 16 weeks after TI for determination of *Streptomyces* populations. A sample of peel from apical to stolon end was taken and the peel samples from all 10 tubers used to extract the DNA, which was then amplified using PCR to permit quantitative determination of a 16S

rRNA gene sequence (common to all *Streptomyces* spp., including non-pathogenic species and other actinomycetes) and the t*xtA* gene sequence (common only to the pathogenic common scab-forming *Streptomyces* spp.). The results were expressed as copies of the 16S or *txtA* gene sequence per g of tuber peel. Two harvests of 12 plants were taken on 16 July and 13 September and the tubers assessed for common scab incidence and severity in the categories: 0, 0-1, 2-5, 6-25, 25-60 and 61-100 % surface area infected with scab. Tubers were also assessed for secondary growth defects at final harvest.

b) Field experiment 2 (2007)

Experiment 2 at Cobrey Farms was set in a commercial field of Maris Peer planted by machine on 23 March 2007. Plots comprised of a single 3-row bed of 12 m length and irrigation treatments were randomized with three replicates over four adjacent beds along 168 m of field, with buffer strips of drip-irrigated beds either side of the experiment to prevent overlapping by rainguns. There were 18 irrigation treatments including control unirrigated plots. The intention was to study the effects of irrigation method, timing and duration. There were two methods of applying irrigation: drip or sprinkler. Timings were either daily or weekly (the latter actually timed to fit in with the commercial irrigation on the rest of the field which was scheduled using the CUF Potato Irrigation Scheduling Model). Duration was all combinations of applying irrigation using drip or sprinkler or not at all for the first 4 weeks of scab control (starting at TI) and / or weeks four to eight. A total of 247 mm of rain fell from the onset of TI until the end of the planned programme 8 weeks later, with no irrigation deemed necessary in the first 7 days post-initiation and no irrigation applied after 4 weeks. Treatments that had been planned to receive irrigation between 4 and 8 weeks post-initiation were therefore abandoned and used to add extra replication (9 replicates for irrigated treatments, 18 replicates for control) for the 0-4 weeks duration treatments when examining disease levels at harvest.

Drip tape and sprinkler systems were the same as Expt 1. A single sprinkler line irrigated each plot and the pressure to each sprinkler line was adjusted (typically 0.6 bar) using a small tap to ensure minimal drift outside the plot. The drip tape was installed 6 days after 50 % emergence using a tractor-mounted tape installer. It proved impossible to install three rows of tape per bed as a) planting was very shallow (9 cm) and b) insufficient soil was available to cover the tape on the outside of the bed. Therefore, only two tapes were installed per bed, *c*. 75 mm inside the outer rows and at 25 mm depth. Pressure within the drip lines was *c*. 0.8 bar and by varying the length of each irrigation period according to the flow rates measured under drip emitters and from sprinkler nozzles, ensured that drip and sprinkler outputs were closely matched.

Plant emergence was recorded every 3-4 days by counting the number of plants emerged in all three rows of each bed in three random plots in each block. Tuber initiation was determined in the same plots by counting the number of plants in a two-plant sample that was dug every 2-3 days starting 14 days after 50 % plant emergence. From unirrigated, daily sprinkler-irrigated and weekly sprinkler-irrigated plots only, samples of 10 tubers from five plants per plot were sent to CSL after 1, 3, 5, 7, 9 and 11 weeks post-TI for determination of *Streptomyces* populations as Expt 1. A final harvest of 100 tubers was taken on 27 July and the tubers assessed for common scab incidence and severity in the categories: 0, 0-1, 2-5, 6-25, 25-60 and 61-100 % surface area infected with scab.

c) Field experiment 3 (2007)

Two trials were established at SCRI, Dundee and Mintlaw, Aberdeenshire using the cultivars Maris Piper and Maris Peer respectively to evaluate the interaction of irrigation and non-water control measures on the development of common scab. The trials were of a factorial design and three replicates. Two levels of irrigation were compared, unirrigated and drip irrigated for 4 weeks after tuber initiation to a schedule provided by CUF. Non-water control measures evaluated were sulphur (Tiger 90CR, 90% S) applied at 125 and 250 kg/ha and rapeseed meal applied at 1 t/ha. The sulphur treatments were applied in furrow at planting and the rapeseed meal was incorporated prior to planting. The non-water control measures were compared to an untreated control.

Soil was sampled from across each trial site and sent to CSL prior to trial establishment. Tuber samples were harvested from each trial as shown in Table 2 and sent to CSL for evaluation of total and pathogenic *Streptomyces* populations. Visual tuber disease assessments were made at both sites 5 weeks after tuber initiation and at final harvest.

TABLE 2. SCHEDULE OF *STREPTOMYCES* SAMPLING AT DUNDEE AND MINTLAW 2007. X = 3 REPLICATE SAMPLES.

	Mintlaw		Dundee	
Weeks after Ti	Untreated & unirrigated	Untreated & irrigated	Unirrigated & untreated	Unirrigated & 250 kg/ha S
1	X	X		
2	X	X	X	X
4	X	X		
8	X	X	X	X
Final harvest	X	X	X	X

d) Field experiment 4 (2008)

Further experiments took place in 2008 at CUF and SAC to examine more closely the effect of withholding irrigation at different times during the initial 4 weeks of tuber development. The Expt. 4 trial conducted at CUF examined the effects of irrigation duration, timing and method on the populations of *Streptomyces* on developing tubers and the incidence of common scab. The experiment was a fully randomized factorial design plus unirrigated control, involving three irrigation durations (irrigated for 2; 4; 6 weeks post-TI), two irrigation methods (Drip; Sprinkler) and two irrigation timings (Frequent; Infrequent). Irrigation was scheduled using the CUF Potato Irrigation Scheduling Model based on meteorological data obtained from a Delta-T Devices weather station 200 m from the experiment. Frequent irrigation for both methods was twice daily (7:00 and 19:00 h) with 1.8-5.5 mm/day, depending on evaporative demand. For Sprinkler irrigation, Infrequent timing was 15 mm every 4-6 days whenever the soil moisture deficit (SMD) reached 15 mm and for Drip 7.5 mm every 2-4 days at a trigger SMD of 7.5 mm. The control (Unirrigated) treatment was grown under a rainshelter that was covered when it was raining or rain was imminent. There were three replicate blocks with a single Unirrigated plot per block.

The experiment was planted on 1 April 2008 using 35-40 mm Maris Piper SE 1 seed at 30 cm spacing 12 cm deep into pre-formed ridges that were raked after planting to re-form the original ridge. Plots were 5 m in length and either eight rows (6.10 m) wide (Sprinkler) or four rows (Drip). Liquid fertilizer nitrogen was applied at a rate of 132 kg N/ha post-planting. Drip irrigation was via John Deere Ro-drip 16 mm low flow tape (5 l/h/m at 0.55 bar pressure, 20 cm emitter spacing, Wroot Water Ltd) installed on top of the ridge at first plant emergence and initially held in place with plastic pegs. It was then covered with *c*. 25 mm of soil at 50 % plant emergence. The sprinklers (Dan Modular Small Swivel Yellow Anti-mist nozzles) were on 1 m risers and installed in every alternate furrow at 1 m spacing. They were adjusted to run at very low pressure (*c*. 0.5-0.6 bar) to reduce the risk of misting and drift into adjacent plots. The drip and sprinkler systems were calibrated at the beginning of the season to determine flow rates at various pressures between 0.4 and 0.6 bar and charts created relating application rates in mm/hour to inlet

pressure and number of plots being irrigated simultaneously. Irrigation amounts applied are detailed in Table 3. The scheduled Infrequent treatments were sometimes delayed by rainfall, hence the slightly different amounts applied for Drip and Sprinkler irrigation. Following the end of the scab control period (6 weeks), irrigation was scheduled to maintain < 30 mm SMD in all treatments except Unirrigated, which remained uncovered from the end of the scab control period and therefore received natural rainfall.

TABLE 3. TOTAL RAINFALL AND IRRIGATION (MM) APPLIED DURING THE DIFFERENT DURATIONS OF SCAB CONTROL

		Method			
		Drip		Sprinkler	
Duration	Rainfall†	Frequent	Infrequent	Frequent	Infrequent
2 weeks	29	41	30	41	23
4 weeks	36	86	68	86	75
6 weeks	84	115	90	115	90

†Unirrigated plots were covered during most rainfall events but 8 mm of rain was not intercepted by the covers between weeks 4 and 6

In the unirrigated and frequent sprinkler 6-week treatments, soil water content was measured at 15 minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm. Weekly measurements of soil water content were taken using a mobile ML2 probe inserted in an equivalent position in every plot. Four replicate readings per plot were taken for these spot measurements. Other spot measurements of soil water content were made pre- and post-irrigation in selected treatments.

Plant emergence was recorded daily in every plot by counting the number of plants emerged in two harvest rows. Tuber initiation was determined by digging two plants per plot every day from 14 days after 50 % plant emergence and recording a plant as having initiated tubers if one or more stolons had swollen to twice their diameter at the tip. Ground cover for the irrigation scheduling model was measured weekly after emergence until final harvest using a grid.

Samples of 10 tubers from 5 plants per plot were sent to CSL in perforated plastic bags 1, 2, 3, 4, 6 and 14 weeks post-TI for determination of *Streptomyces* populations as in Expt 1. Tubers were sampled from Sprinkler and Unirrigated plots only. The sample programme is shown in Table 4. A final harvest of 12 plants was taken on 9 September and the tubers assessed for common scab incidence and severity in the categories: 0, 0-1, 2-5, 6-25, 26-60 and 61-100 % surface area infected with scab. Tubers were also assessed for secondary growth defects at final harvest.

TABLE 4. SCHEDULE OF *STREPTOMYCES* SAMPLING AT CUF. X = 3 REPLICATE SAMPLES. SPRINKLER AND UNIRRIGATED PLOTS ONLY

		Weeks after tuber initiation					
Timing	Duration	1	2	3	4	6	14
Unirrigated		X	X	X	X	X	X
Frequent	2 weeks		X	X	X	X	
Frequent	4 weeks			X	X	X	
Frequent	6 weeks	X	X	X	X	X	X
Infrequent	2 weeks		X	X	X	X	
Infrequent	4 weeks			X	X	X	
Infrequent	6 weeks	X	X	X	X	X	X

Expt 3 conducted at SAC generally followed the experiment at CUF, also examining the effects of irrigation duration and timing on the populations of *Streptomyces* on developing tubers and the incidence and severity of common scab. In addition, sulphur treatments

were included as a factor. The experiment was a fully randomized factorial design plus unirrigated control, involving three irrigation durations (irrigated for 2; 4; 6 weeks post-TI), two irrigation timings (Frequent; Infrequent) and two sulphur levels (0 and 150 kg/ha). Sprinkler irrigation was used throughout. Irrigation for the infrequent timings was scheduled using the CUF Potato Irrigation Scheduling Model based on meteorological data obtained weather station within the field. Applications were made weekly aiming to keep the SMD below 15mm. Frequent irrigation was applied once a day with 5-6 mm/day, depending on evaporative demand but SMD was kept below 5mm. Two Delta T Theta Probes were used to calculate SMD for the frequent irrigation. Sulphur treatments were applied in furrow at planting using Tiger 90CR (90% S). The control (Unirrigated) treatment was grown under conditions as free from natural rain as possible, particularly during and for 6 weeks after tuber initiation. To achieve this a rain-shelter was used to cover plots when rain was imminent. There were three replicate blocks with two unirrigated plots per block (with and without sulphur).

The experiment was planted on 23 May 2008 using 35-40 mm Maris Piper SE 1 seed at 30 cm spacing 12 cm deep using the SAC experimental plot planter into destoned beds. Plots were 5 m in length and either eight (5.52 m) wide or four rows wide. Based on soil analysis 555 kg/ha 12:24:18 was applied prior to planting and 329 kg/ha 34.5% nitrogen applied post-planting. The sprinklers (Dan Modular Small Swivel Yellow Anti-mist nozzles) were on 1 m risers and installed in every alternate furrow at 1 m spacing. They were adjusted to run at very low pressure (*c.* 0.5-0.6 bar) to reduce the risk of misting and drift into adjacent plots. The sprinkler system was calibrated at the beginning of the season to determine flow rates at various pressures between 0.4 and 0.6 bar and charts created relating application rates in mm/hour to inlet pressure and number of plots being irrigated simultaneously. Irrigation amounts applied are detailed in Table 5. Following the end of the scab control period (6 weeks), irrigation was scheduled to maintain < 30 mm SMD in all treatments except Unirrigated.

TABLE 5. TOTAL RAINFALL AND IRRIGATION (MM) APPLIED DURING THE DIFFERENT DURATIONS OF SCAB CONTROL

		Sprinkler	
Duration	Rainfall†	Frequent	Infrequent
2 weeks	46	39	0
4 weeks	18	53	30
6 weeks	36	5	0

†Unirrigated plots were covered during most rainfall events

In the Unirrigated, Frequent and Infrequent 6-week treatments, soil water content was measured at 15 minute intervals using Delta-T Devices Theta Probes ML2 permanently installed in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm.

Plant emergence was recorded regularly in every plot by counting the number of plants emerged in two harvest rows. Tuber initiation was determined by digging two plants per plot every day from 14 days after 50 % plant emergence and recording a plant as having initiated tubers if one or more stolons had swollen to twice their diameter at the tip. Ground cover for the irrigation scheduling model was measured weekly after emergence until final harvest using a grid.

Samples of 10 tubers from 5 plants per plot were sent to CSL in perforated plastic bags 1, 2, 3, 4, 6 and 16 weeks post-TI for determination of *Streptomyces* populations as in Expt 1. Tubers were sampled from Unirrigated, Frequently irrigated and sulphur treated plots only. The sample programme is shown in Table 6. A final harvest of 12 plants was taken on 21

October and the tubers assessed for common scab incidence and severity. The results were then assigned to the categories: 0, 0-1, 2-5, 6-25, 26-60 and 61-100 % surface area infected with scab. Tubers were also assessed for other diseases and defects at final harvest.

TABLE 6.SCHEDULE OF *STREPTOMYCES* SAMPLING AT CSL. X = 3 REPLICATE SAMPLES. SPRINKLER AND UNIRRIGATED PLOTS ONLY

			Weeks after tuber initiation					
Timing	Duration	Sulphur	1	2	3	4	6	16
Unirrigated	-	0	X	X	X	X	X	X
Unirrigated	-	150	X	X	X	X	X	X
Frequent	2 weeks	0			X	X	X	X
Frequent	2 weeks	150			X	X	X	X
Frequent	6 weeks	0	X	X	X	X	X	X
Frequent	6 weeks	150	X	X	X	X	X	X

e) Statistical analysis

Owing to the large populations of *Streptomyces* observed on the surface of tubers, the raw data relating to copies of DNA/g of peel were log-converted before conducting analysis of variance. One of the major hypotheses of control of common scab by irrigation is that in wet soils the ratio of pathogenic to non-pathogenic *Streptomyces* in decreased compared with dry soils. Since there were some plots where apparently no *txtA* DNA was observed, the ratio could not be calculated. Rodger White of the Statistics Group at the Centre suggested a better approach for Mathematics and Computational Biology, Rothamsted Research (personal communication) involving analysis of the population data for both strains together using a split-plot analysis. The main assumption is that the extracted DNA is assayed separately for 16S and *txtA*. At the split-plot level, differences on the log-transformed data are equivalent to ratios but it is also possible get an indication of whether or not there are interactions at the split-plot level for 16S and *txtA*. This is better than analysing the ratio of *txtA* : 16S directly. In order to deal with zero values, the data had 1 added to the population prior to log-transformation.

In figures presenting data on *Streptomyces* populations, it is difficult to represent standard errors (S.E.) in a meaningful way if y-axis scales are logarithmic. Therefore, the text explains whether there were significant differences in populations at a particular sampling and the error bars (1 S.E. in length) have been positioned in figures so that they give an indication of the error variation between treatments.

5. Effect of location, seed infection and variety on scab incidence and severity in commercial fields

a) 2007

Preliminary field experiments were conducted in 2007 on two commercial field sites at Branston Potatoes and QV Foods. Small field observation plots were installed to assess the relative importance of seed-borne inoculum, soil-borne inoculum, *Streptomyces* spp. and potato variety. Seed stocks of 3 varieties (Desiree, Estima and Maris Piper) were selected. For each variety, stocks with high and low common scab severity were sourced. An additional stock (variety Kondor), known to be infected with *S. acidiscabies*, was also included.

Soil samples at each site were analysed to determine the range and populations of pathogenic *Streptomyces* present in the fields before planting. Soil was sampled by collecting 1.5 – 2 kg by thoroughly mixing 100 sub-samples taken in a "W" pattern across the whole area to be planted. Each sub-sample (of 15-20 g) was taken either with a soil auger or spade to include a column of soil to a depth of 20 cm.

Each stock was planted in 3 replicated plots, each with 4 rows of 8 plants. The outer 2 rows were considered discard rows and the inner 2 rows were used for sampling. Husbandry followed normal commercial practices. 4 weeks after tuber initiation, plants 2 and 3 were lifted from rows 2 and 3 of each plot and all tubers were sent to CSL for PCR analysis after assessment for common scab symptoms using the following scale (based on Conn & Lazarovits 1999):

0 = no visible scab lesions
1 = trace to 1% surface area (SA) of tuber covered with scab lesions
2 = 2 to 5 % SA covered
3 = 6 % to 25 % SA covered
4 = 26 % to 60 % SA covered
5 = 61 % to 100 % SA covered

At harvest, all tubers from plants 5, 6 and 7 of rows 2 and 3 were lifted and separated according to the severity of scab symptoms using the above scale. At least 10 tubers per plot with scab symptoms were analysed by PCR to determine the amounts and type of pathogenic *Streptomyces* species present.

All soil and tuber samples were tested following the DNA extraction and PCR methods described in Section 1.

b) 2008

Further investigations were conducted to compare the importance of factors influencing common scab development under commercial potato production conditions. These included soil- and seed-borne inoculum, the variety of potato and the relative populations of pathogenic *Streptomyces* spp. and competing actinomycete populations on developing progeny tubers.

In February 2008, 27 seed sources of 2 varieties (Maris Piper and Estima) were analysed and 2 seed sources of each variety were selected which varied in terms of the populations and species of pathogenic *Streptomyces* present on the seed before planting. Soils were also sampled before planting (as described above) from a range of potential field sites producing potatoes for QV Foods, Branston Potatoes and Cobrey Farms and a total of 7 sites were again selected which varied in the populations and species of pathogenic *Streptomyces* detected in the soil before planting. DNA extractions and PCR analyses of soil and potato tubers were conducted as described above.

The two selected seed stocks (Estima and Maris Piper) were planted at each of the 7 selected sites in four replicated plots, each with four rows of 15 plants. The outer two rows were considered discard rows and the inner two rows were used for sampling. Husbandry followed normal commercial practice at each site.

Four plants per plot were harvested at each of 1, 3 and 5 weeks after the mean tuber initiation date. Ten randomly chosen tubers per replicated plot were then sampled for PCR

analysis at CSL. At final harvest, the remaining eight test plants from each replicated plot were harvested for scab severity scoring and PCR analysis at CSL.

All DNA extractions and PCR analyses of soil and potato tubers were conducted as described in Section 1.

Results

1. Molecular methods optimised for detection, identification and quantification of *Streptomyces* species

Milestones achieved:

- ***DNA extraction optimised for real time PCR analysis of plant and soil samples.***
- ***Sensitivity and specificity of real time PCR assays compared with conventional isolation and identification methods.***

A real-time PCR assay to detect and quantify pathogenic *Streptomyces* spp. was developed at CSL using the thaxtomin A gene (*txtA*) sequence. Extraction methods were optimised for detection of the pathogen in potato and soil matrices. A 16S rRNA gene sequence (Cullen *et al*., 2000) was also used in real-time PCR to quantify total actinomycete populations. Specificity of the *txtA* assay was determined by testing the ability of the primers to amplify non-pathogenic *Streptomyces* spp., which did not possess the *txtA* gene, as verified by conventional PCR. No amplification was observed from any of these species. Using the *txtA* real-time PCR assay, detection of 100 copies of the *txtA* gene per g potato peel was achieved.
Streptomyces spp. were isolated from symptomatic tubers by plating extracts onto selective medium (Conn *et al*., 1998). Pathogenicity of these isolates was determined by inoculating radish seedlings grown *in vitro*. Cultures were also assayed for presence of the *txtA* gene using the real-time PCR test. Positive results were only obtained from those isolates that induced necrotic symptoms typical of *Streptomyces*, demonstrating that the assay correctly identified pathogenic *Streptomyces*.

The identity of *Streptomyces* spp. present in samples was determined using a range of conventional PCR assays (Wanner, 2006). This method was used to determine which species were present in seed, soil and on isolation plates. Using this method it was established that symptomatic tubers did not necessarily contain a single pathogenic species, and that it was common for multiple species to be present in a single infected tuber.

2. Greenhouse trials

Milestones achieved:

- ***Effects of water status and other factors on populations dynamics quantified in vitro.***
- ***Combined effects for maximum suppression of scab-forming Streptomyces spp. determined.***

a) Preliminary polytunnel experiment to evaluate the effect of soil type and moisture level on population changes of *Streptomyces* and development of common scab.

Despite conditions being conducive to development of common scab, symptom developemnt under dry conditions was limited (Table 7)

TABLE 7: INCIDENCE AND SEVERITY OF COMMON SCAB ON CV. MARIS PIPER GROWN FROM MINITUBERS IN SOILS SHOWING EITHER SUPPRESSIVENESS OR NON-SUPPRESSIVE AND EITHER MAINTAINED WET OR DRY

Incidence (% tubers infected)		Weeks after 50% emergence			
Field Name	Treatment	2	4	8	14
Forres	Wet	0.0	6.0	1.1	22.9
Forres	Dry	0.0	39.9	57.1	70.0
Elgin	Wet	1.6	6.7	0.0	2.0
Elgin	Dry	0.0	1.4	0.0	3.5
CUF	Wet	0.0	0.0	0.0	2.4
CUF	Dry	0.0	0.0	0.0	2.1
QV	Wet	*	*	*	*
QV	Dry	*	0.0	0.0	5.6

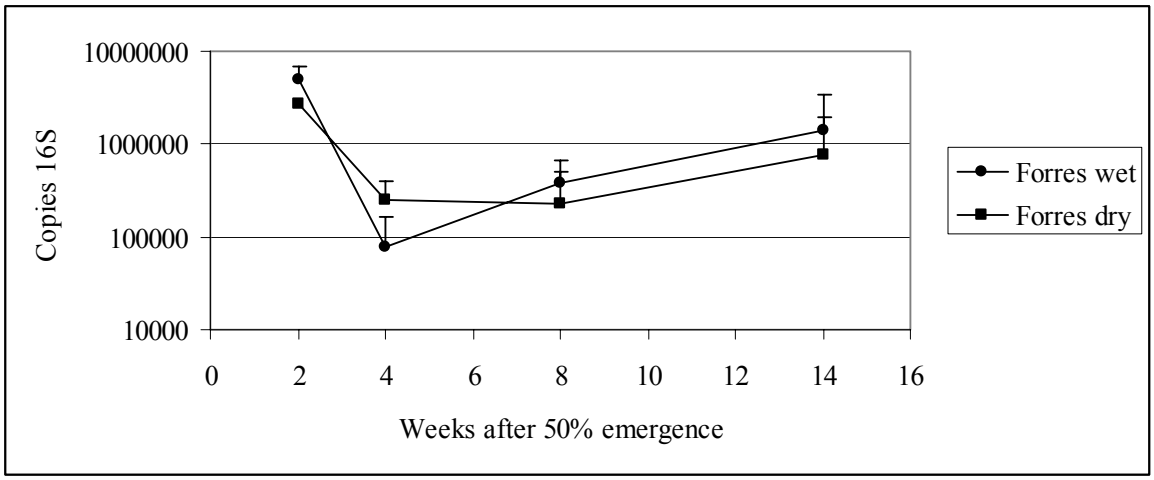
Severity (% surface area)		Weeks after 50% emergence			
Field Name	Treatment	2	4	8	14
Forres	Wet	0	0.06	0.01	0.51
Forres	Dry	0	1.7	4.3	5.5
Elgin	Wet	0.02	0.07	0	0.05
Elgin	Dry	0	0.015	0	0.11
CUF	Wet	0.0	0.0	0.0	0.03
CUF	Dry	0.0	0.0	0.0	0.025
QV	Wet	*	*	*	*
QV	Dry	*	0.0	0.0	0.03

Analysis of data for Forres site alone indicated significant differences between wet and dry and between sampling dates and their interaction for both incidence and severity. LSD's wet/dry - incidence = 14.8, severity = 1.15; sampling date – incidence = 20.9, severity = 14.8
* indicates absence of tubers

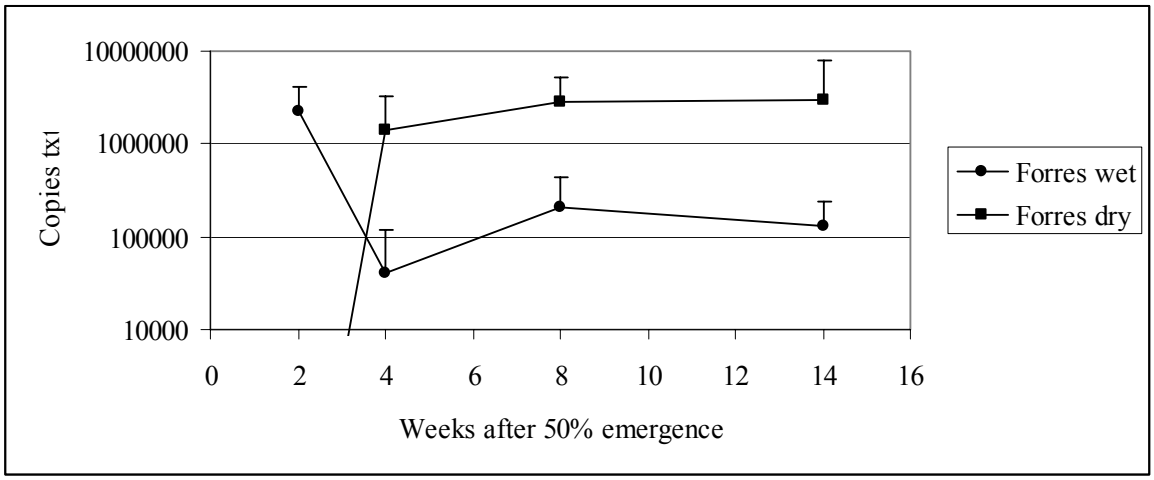
Common scab developed to any extent only in the lightest soil, a sandy loam (Forres). From 4 weeks after 50% emergence, incidence and severity was significantly greater with dry conditions. Determination of total *Streptomyces* (as copies of 16S per g tuber) on the tuber surface with time after 50% emergence indicated that there were no significant differences in numbers between wet and dry conditions (Fig. 1a). Numbers fell from 2 to 4 weeks after 50% emergence and then increased subsequently.

By contrast numbers of pathogenic *Streptomyces* (as copies of *txtA* per g tuber) were significantly different throughout the period of growth (Fig. 1b). At 2 weeks there were significantly fewer pathogenic *Streptomyces* present in the dry conditions. However, by 4 weeks the reverse was evident and the number remained significantly higher in dry conditions thereafter. The rise in numbers of pathogenic *Streptomyces* detected on the tuber surface corresponded with levels of common scab observed visually. The ratio of 16S to *txtA* (Fig. 1c) similarly indicated a large difference between the wet and dry soils. 16S and *txtA* values were highly variable in other soils.

a. Copies of 16S (total *Streptomyces* spp.)



b. Copies of txt (pathogenic *Streptomyces* spp.)



c. Ratio of txt to 16S

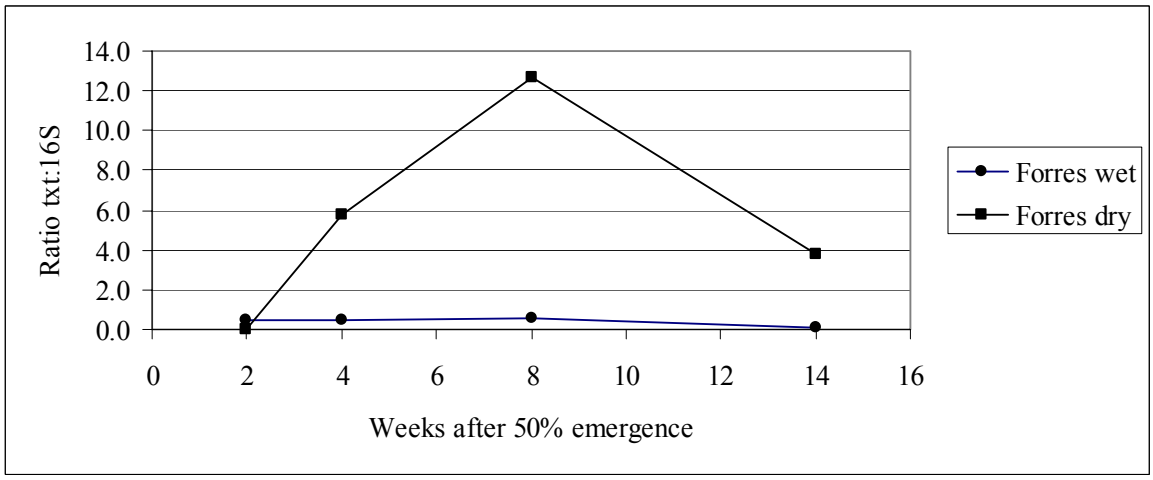


FIGURE 1: NUMBER OF TOTAL AND PATHOGENIC *STREPTOMYCES* PRESENT ON TUBER SURFACES AT DIFFERENT TIMES AFTER 50% EMERGENCE IN A SINGLE SOIL TYPE WHEN MINITUBERS OF CV. MARIS PIPER WERE GROWN UNDER WET OR DRY CONDITIONS. BARS INDICATE SD.

The results for the Forres soils indicate a substantial rise in pathogenic *Streptomyces* around the time of tuber initiation (2 weeks after 50% emergence was probably just before and 4 weeks just after tuber initiation). In consequence, the ratio of *txtA* to 16S remained relatively level throughout the period of growth.

b) Effect of soil amendments on scab incidence and severity.

A greenhouse trial was conducted to explore the effect of sulphur or lime at 2 different concentrations on scab incidence and severity caused by different pathogenic *Streptomyces* species. This trial confirmed pathogenicity on potato of *S. turgidiscabies* and *S. acidiscabies* isolates from the UK but no significant difference in disease incidence or severity was observed between lime or sulphur treatments.

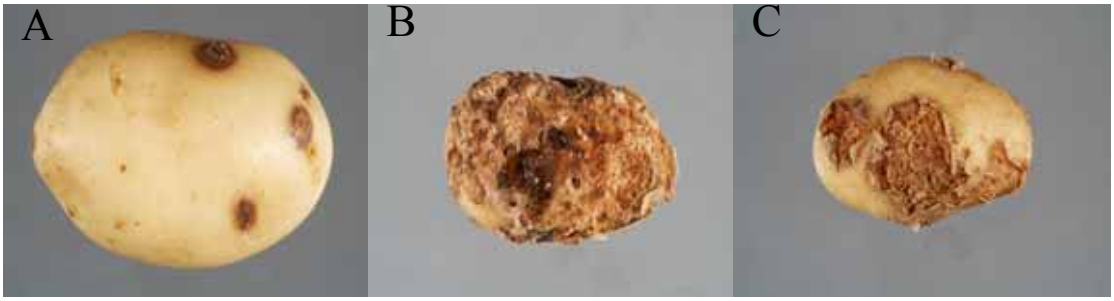


FIG. 2. SYMPTOMS OBTAINED IN GLASSHOUSE TRIALS OF TUBERS INOCULATED WITH *STREPTOMYCES* SPP. ISOLATED FROM THE UK. A: *S. ACIDISCABIES*, B: *S. SCABIEI*, C: *S. TURGIDISCABIES*

3. Field trials to assess the effect of sulphur and other non-water control measures on development of common scab.

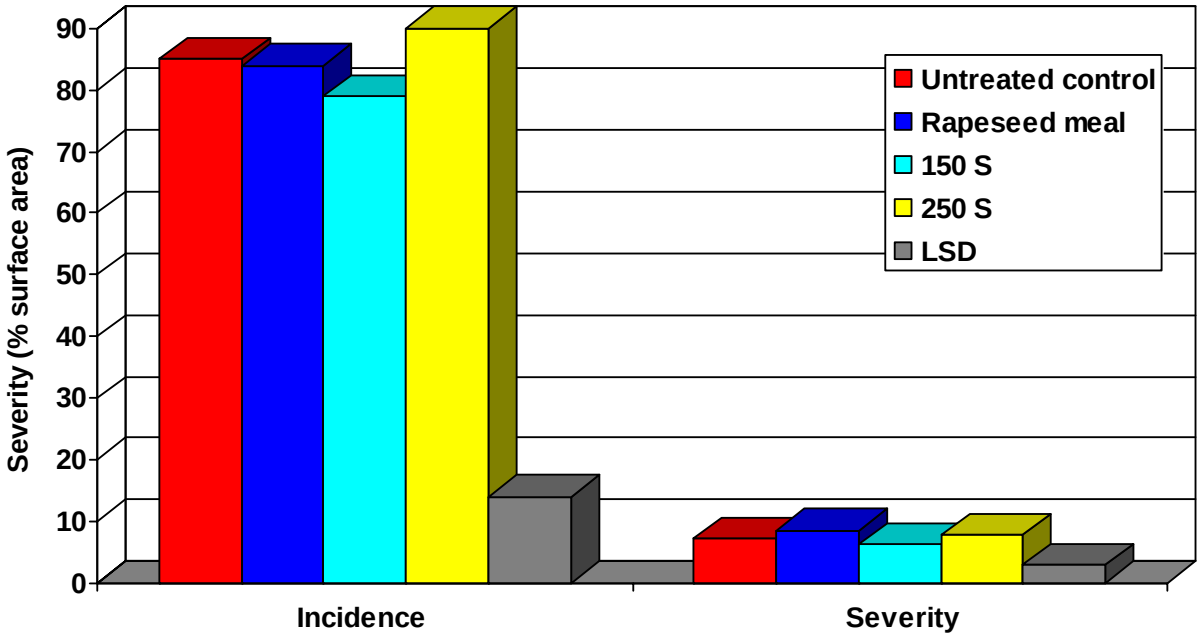
Milestones achieved:

- ***Field trials established for evaluation of potential common scab control measures.***
- ***Samples from field trials analysed and most appropriate control measures selected.***

a) 2006 Dundee

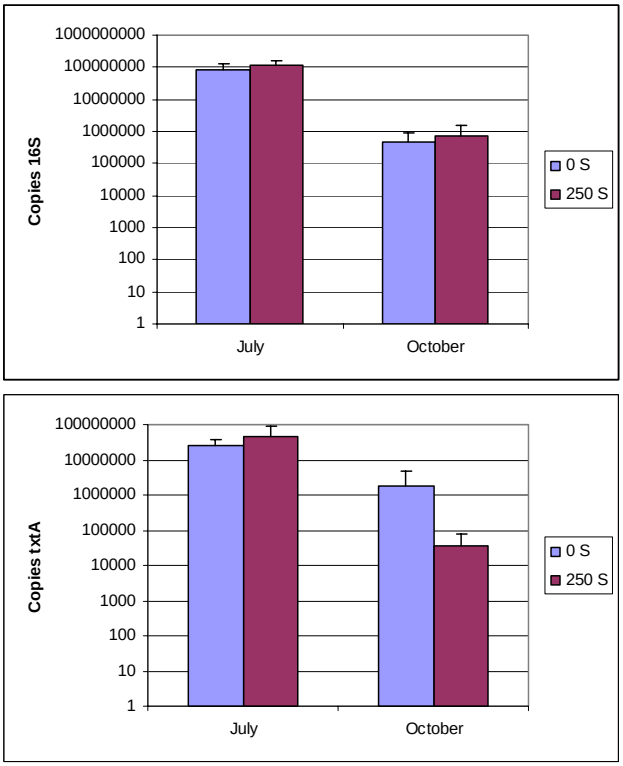
In a trial evaluating a number of potential chemical control measures for common scab control, an untreated control was compared with S applied at 150 and 250 kg/ha and rapeseed meal at 1000 kg/ha. No significant differences were observed between incidence and severity of common scab (Fig. 3).

FIG. 3: EFFECT OF S APPLIED IN FURROW AT PLANTING AND RAPESEED MEAL INCORPORATED INTO SOIL PRE-PLANTING ON INCIDENCE AND SEVERITY OF COMMON SCAB, CV. MARIS PIPER. DUNDEE 2006.



The trial was planted on 16 May 2006 and 50% emergence was 9 June. Samples of daughter tubers were taken from this trial in July, 2 weeks after tuber initiation and at final harvest and analysed for pathogenic and total *Streptomyces* levels on tuber surfaces. The results are shown in Fig. 4. In July there were no significant differences between the untreated control and 250g S. However, by harvest 250kg/ha S had reduced pathogenic *Streptomyces* significantly compared to the untreated control.

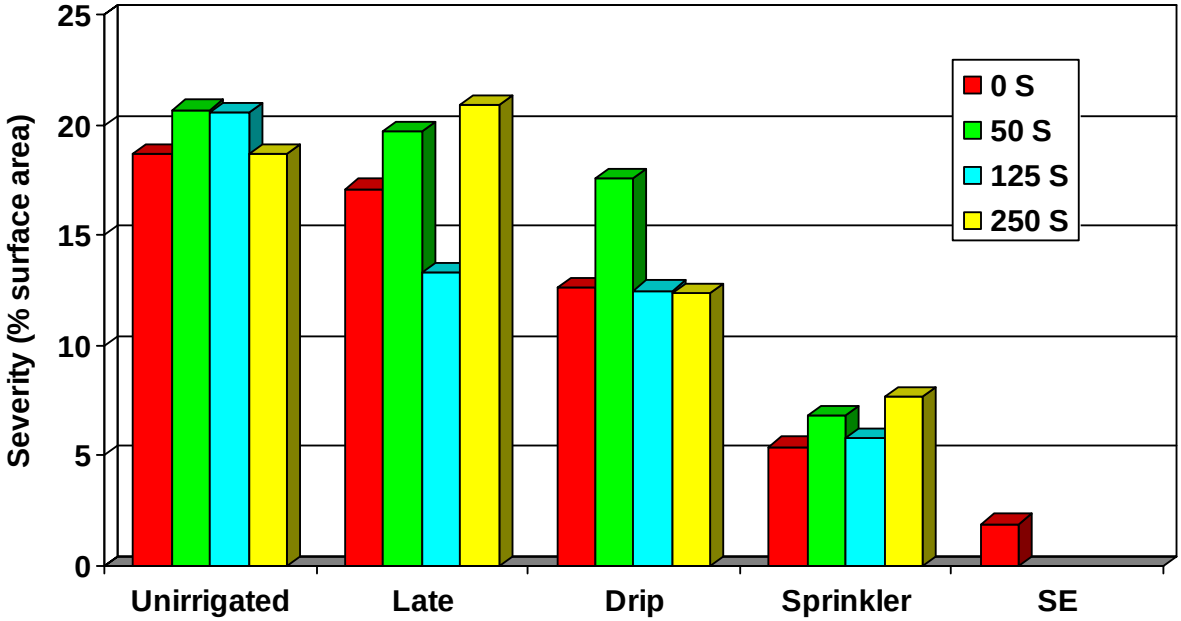
FIG. 4: PATHOGENIC (TXTA) AND NON-PATHOGENIC (16S) *STREPTOMYCES* PRESENT ON TUBERS CV. MARIS PIPER, 2 WEEKS AFTER TUBER INITIATION AND AT FINAL HARVEST (DUNDEE 2006)



b) 2007 CUF

As part of a trial investigating the effect of different irrigation treatments on the control of common scab, S was applied at 0, 50, 125 and 250 kg of product per ha. The severity of common scab is shown in Fig. 5. No significant effect of sulphur was found whether unirrigated or irrigation was applied by drip, sprinkler or at a sub-optimal time for common scab control.

FIG. 5: EFFECT OF S APPLIED IN FURROW AT PLANTING AT DIFFERENT DOSES ON INCIDENCE AND SEVERITY OF COMMON SCAB, CV. MARIS PIPER. CUF 2007.



c) 2007 Dundee and Mintlaw

The 2006 trial at Dundee was repeated in 2007 at two locations except that irrigation was also applied as a factor. Irrigation was applied by drip and scheduled by CUF. The Mintlaw site was planted on 2 May and reached 50% emergence on 29 May. The equivalent dates at the Dundee site were 9 May and 3 June. 2007 was a particularly wet summer with above average rainfall in June, July and August and the need for irrigation was limited at either site. Monthly average rainfall at Dundee was: May – 58.1 mm, June – 104.6 mm, July – 117.3 mm, Aug – 76.2 mm, Sep – 19.7 mm. A single irrigation event of 12.9 mm was made on 10 July. At Mintlaw, monthly average rainfall was less but only two irrigation events were required: 14 mm on 22 June and 15 mm on 11 July. Incidence and severity were low at harvest with over 95% tubers exhibiting less than 5% surface area infected. The results, expressed as an index are shown in Fig. 6. The index was calculated as:

$$\frac{\text{Sum tubers with 0, 0-1, 2-5, 6-25, 26-60, 61-100\% surface area multiplied by 0, 1, 2, 3, 4, 5 respectively}}{\text{Total number tubers}}$$

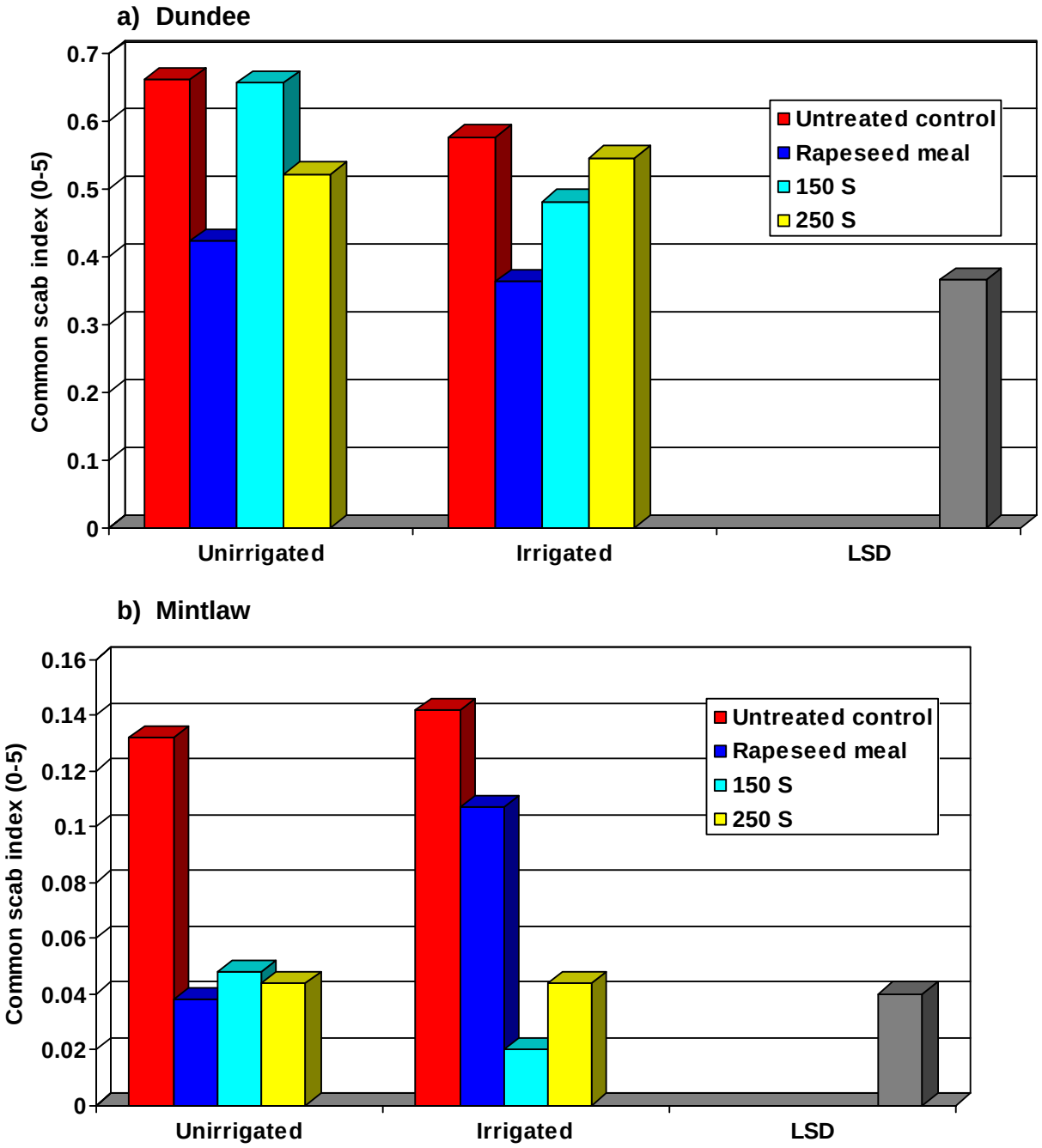


FIG. 6: EFFECT OF S APPLIED IN FURROW AT PLANTING OR RAPESEED MEAL INCORPORATED INTO SOIL PRE-PLANTING AND THEIR INTERACTION WITH IRRIGATION ON COMMON SCAB INDEX AT FINAL HARVEST. CV. MARIS PIPER. A) DUNDEE 2007, B) MINTLAW 2007.

There was no significant effect of irrigation or chemical treatment in the Dundee trial but at Mintlaw there was a significant reduction in index between the untreated control and all chemical treatments in the unirrigated plots and between the untreated control and the S treatments in the irrigated plots. The level of common scab was low in both trials but notably at the Mintlaw site.

d) Oldmeldrum 2008

The trial examining the method and frequency of irrigation in Scotland (see page 34) also included S at 0 and 150kg/ha as an additional factor. Overall, there was no significant effect of S on incidence or severity of common scab at the final harvest. There was no interaction between S treatment and irrigation method or timing (Fig. 7).

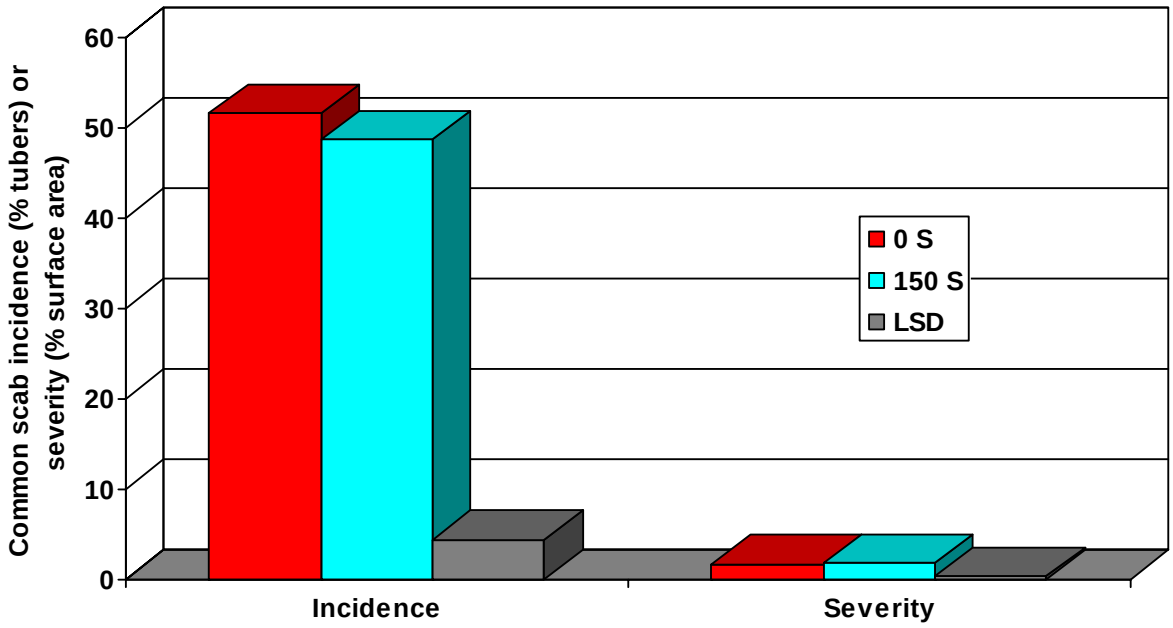


FIG. 7: EFFECT OF S APPLIED IN FURROW AT PLANTING ON INCIDENCE AND SEVERITY OF COMMON SCAB AT FINAL HARVEST. CV. MARIS PIPER. OLDMELDRUM 2008.

The level of tuber contamination by pathogenic (*txtA*) and total (16S) *Streptomyces* was determined 1, 2, 3, 4, 6 and 16 weeks (final harvest) after tuber initiation (TI) on the unirrigated and 6weeks daily irrigated plots. The changes in population and the ratio between *txtA* and 16S are shown in Fig. 8.

Daily irrigation suppressed total *Streptomyces* populations compared to the untreated control. However, there was no significant effect of S on total populations (Fig. 8a). By contrast, populations of pathogenic *Streptomyces* were significantly reduced in the unirrigated plots (except for week 2) (Fig. 8b). In the irrigated plots, except for week 6, the pathogenic *Streptomyces* levels were low or undetectable. In consequence, differences in the ratio of *txtA*:16S were only apparent in unirrigated plots where S suppressed pathogenic *Streptomyces* (Fig. 8c).

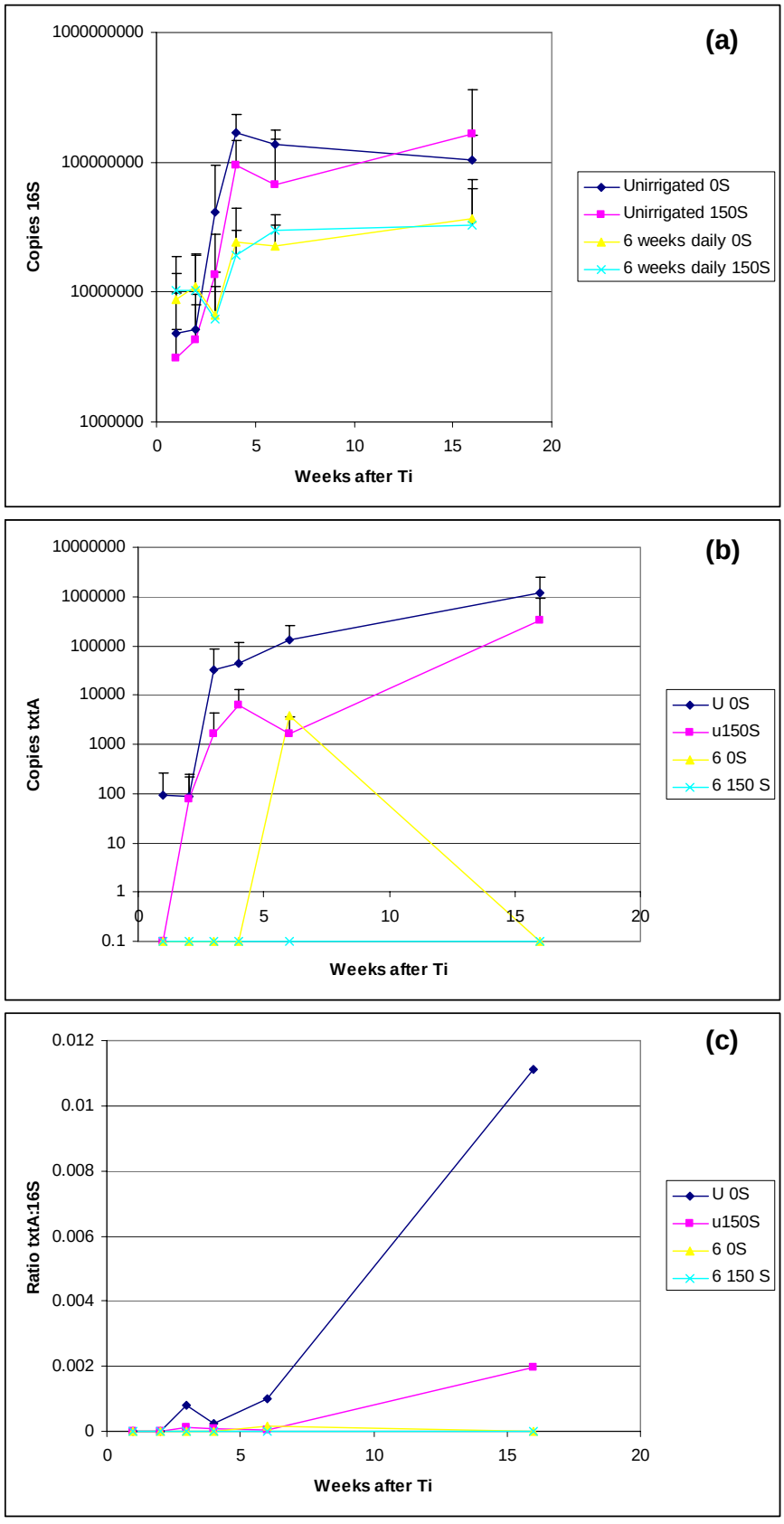


FIG. 8: PATHOGENIC (TXTA – A.) AND TOTAL (16S – B.) *STREPTOMYCES* AND THEIR RATIO (C.) PRESENT ON TUBERS CV. MARIS PIPER, AT TIMES AFTER TUBER INITIATION FOR UNIRRIGATED AND 6 WEEKS DAILY IRRIGATION WHERE EITHER 0 OR 150 KG/HA S WAS APPLIED. OLDMELDRUM, 2008.

4. Field trials to assess the effects of irrigation timing and frequency on scab incidence and severity.

Milestones achieved:

- ***Field trials established for evaluation of potential common scab control measures.***
- ***Samples from field trials analysed and most appropriate control measures selected.***

a) Field Experiment 1 (2007)

CUF

An initial soil sample prior to planting showed that there were six common species of *Streptomyces* present: *S. scabiei* / *europaescabiei*, *S. stelliscabiei*, *S. bottropensis*, *S. turgidiscabies*, *S. acidiscabies* and a 'Group x' (a pathogenic isolate of unknown species in which the *nec1* gene is absent). The only common group missing was *S. aurofaciens*. All other commercial and experimental sites in the programme showed a similar mixed profile, with the exception being two silty soils which had no 'Group x' present. Of particular interest was the almost universal presence of *S. acidiscabies*, which has been reported to be acid tolerant and less responsive to irrigation than *S. scabiei*. It was not thought that *S. acidiscabies* existed in the UK until the project recorded its presence in 2006.

Plots reached 50 % emergence on 7 May with first to 95 % emergence taking 8 days. First to 95 % tuberization took 3 days with 50 % of plants tuberized on 23 May. There was a sudden increase in both total (16S) and pathogenic (*txtA*) populations on developing tubers between 1 and 3 weeks after TI followed by a decrease so that populations of 16S were reasonably constant between 5 and 16 weeks after initiation (Fig 2. a*, b*). There was a very large increase in *txtA* population in Unirrigated plots between 1 and 3 weeks after initiation followed by a plateau and a slower increase in *txtA* populations in Sprinkler-irrigated plots such that peak populations were reached around 7 weeks after initiation (Fig. 2. *b*). However, the error variation was so great between plots and replication too limited to detect any significant differences in *txtA* populations between Unirrigated and Sprinkler plots. Between 5 and 9 weeks after initiation, the ratio of *txtA* : 16S populations was greater in Unirrigated plots than in Sprinkler-irrigated plots but only significantly so at 5 weeks (Fig 2. *c*). The split-plot analysis of 16S and *txtA* showed that there was an interaction between 16S and *txtA* at 5, 7 and 9 weeks post-TI that was not apparent at the other sample dates i.e. there was a different relationship between 16S and *txtA* during the middle period of scab control than at the beginning. It appeared that maintaining wet soils during the first 6-8 weeks after TI decreased the ratio of *txtA*: 16S through slowing the multiplication of *txtA* and reducing the populations of general *Streptomyces* spp.

The incidence of common scab was very high (90-100 %) at both an early harvest 8 weeks (16 July) and final harvest 16 weeks after initiation (13 September). Between the two harvests, surface area infected with scab increased by *c*. 5 % area across all irrigation regimes (data not shown). Plots that were sprinkler-irrigated twice daily had the lowest incidence surface area infected with scab, with Unirrigated plots having significantly higher scab infection at final harvest (Fig. 9.). Applying Late irrigation from the end of the control period (8 weeks post-initiation) did not affect subsequent scab proliferation. Drip irrigation improved scab severity compared with Unirrigated and Late but was disappointing compared with Sprinkler irrigation. Sulphur amendments had no effect on scab severity (Table 8).

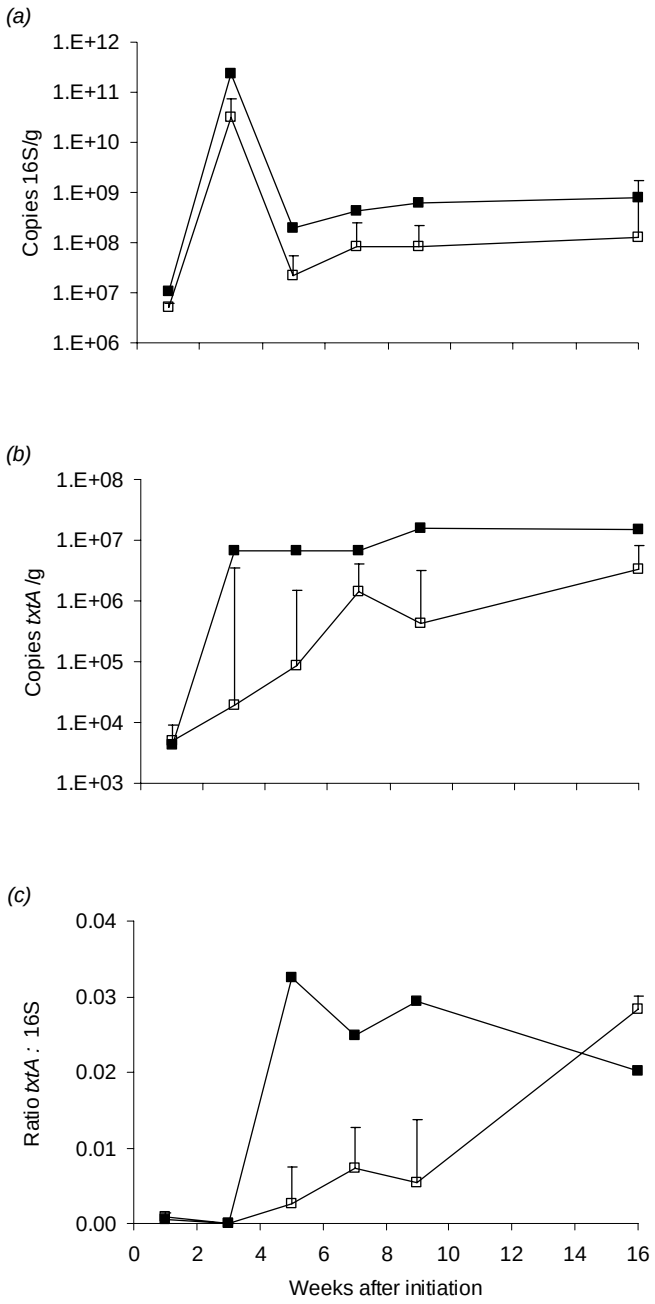


FIG. 9: CHANGES IN *STREPTOMYCES* AND ACTINOMYCETE POPULATIONS ON TUBERS IN FIELD EXPERIMENT 1. *(A)* TOTAL ACTINOMYCETES (COPIES 16S RRNA PER G TUBER TISSUE); *(B)* PATHOGENIC *STREPTOMYCES* SPP. (COPIES *TXTA* PER G TUBER TISSUE); *(C)* RATIO *TXTA* : 16S RRNA. UNIRRIGATED, ■; SPRINKLER, □. S.E BASED ON 3 D.F.

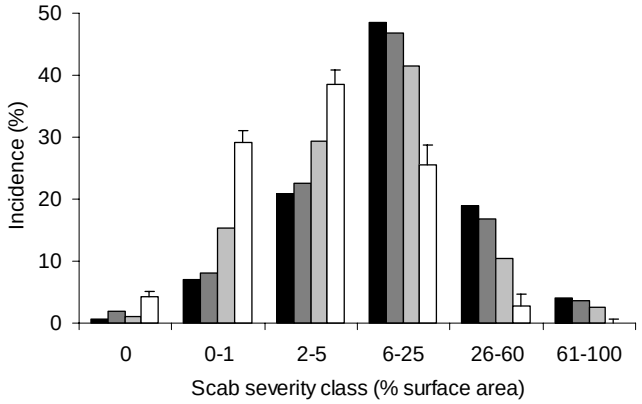


FIG. 10: EFFECT OF IRRIGATION REGIME ON COMMON SCAB SEVERITY 16 WEEKS AFTER TUBER INITIATION IN FIELD EXPERIMENT 1. UNIRRIGATED, ■; LATE, ■; DRIP, ■; SPRINKLER, □. S.E BASED ON 30 D.F.

TABLE 8: EFFECT OF IRRIGATION REGIME AND SULPHUR AMENDMENT ON THE MEAN SURFACE AREA (%) INFECTED WITH COMMON SCAB 16 WEEKS AFTER TUBER INITIATION IN EXPT 1

	Sulphur level (kg S/ha)			
Irrigation regime	0	50	125	250
Unirrigated	18.7	20.7	20.6	18.7
Late	17.1	19.7	13.3	20.9
Drip	12.6	17.6	12.5	12.4
Sprinkler	5.4	6.8	5.8	7.7
S.E. (30 D.F.)	1.90			

Daily spot readings of soil water content in the ridge at 15 cm depth showed that Sprinkler-irrigated plots were being maintained slightly wetter (0.33 m^3/m^3) than Field Capacity (0.30 m^3/m^3) during the first 4 weeks post-initiation, whereas the CUF Irrigation Scheduling Model predicted they were slightly in deficit (mean SMD 1.4 mm, range 0-4.7 mm). The model assumes that water drains away within 4 hours from the ridge profile but cannot account for bi-daily irrigation events, which means that the soil may have spent longer above Field Capacity, particularly during the night. It was noticeable that the Sprinkler-irrigated plots were uniformly wet across the ridge both pre- and post-irrigation whereas soil in the side of the ridge opposite the drip tape was 0.03-0.04 m^3/m^3 drier than the centre of the ridge when irrigation commenced, albeit soil was close to saturation (0.36 m^3/m^3) underneath the drip tape immediately after irrigation.

There was a massive increase in growth cracking in Sprinkler-irrigated plots (26.0 % incidence) *c.f.* Unirrigated (1.4 % ± 1.71 %) but surprisingly little in Drip-irrigated plots (3.5 %) when compared with previous experiments with frequently applied drip irrigation.

b) Field Experiment 2 (2007)

CUF

At Cobrey Farms, plots reached 50 % emergence on 25 April and 50 % of plants had tuberized on 13 May but there was considerable variation in plant development since emergence was more protracted than in Expt 1 (7 days from first to 50 % plant emergence). Despite applying 57 mm of irrigation to daily sprinkler-irrigated plots in the first 4 weeks of scab control and 44 mm in the weekly sprinkler-irrigated plots, there were no significant effects of irrigation regime on the populations of 16S or *txtA Streptomyces* spp. detected on progeny tubers (Fig. 11.). Populations of *txtA* on tubers were at their peak *c*. 9 weeks after initiation when total *Streptomyces* populations had decreased to the lowest level recorded.

No consistent effects of irrigation were observed on the ratios of pathogenic to total *Streptomyces* populations.

There was no significant effect of irrigation regime on final incidence or severity of common scab (Fig. 12.). As in Expt 1, the incidence of scab was very high (*c*. 99 %) but the scab was less severe than in Expt 1 in the Unirrigated treatments. Given the excessive rainfall, there was a disappointing level of common scab on the commercial Maris Peer crop in Expt 2. Irrigation was scheduled to commence on the day before 50 % of plants had tuberized, however in hindsight, given the variability of emergence, irrigation should probably have commenced 2-3 days earlier but the irrigation system had not been fully connected.

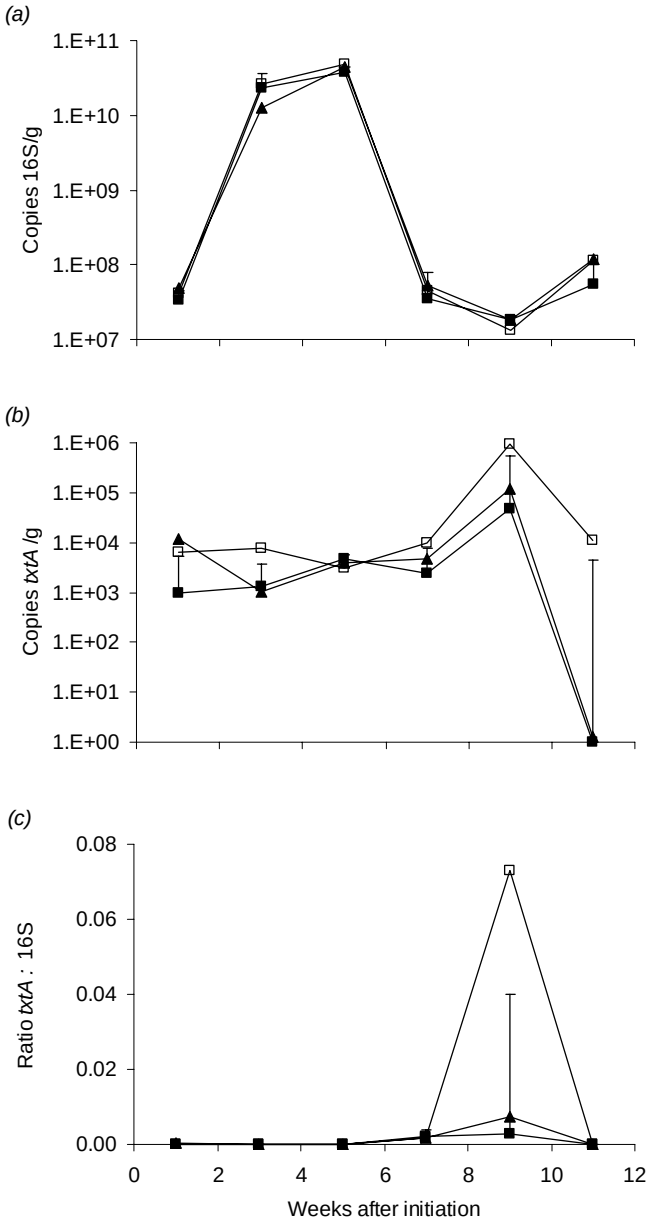


FIG. 11: CHANGES IN *STREPTOMYCES* POPULATIONS ON TUBERS IN EXPT 2. *(A)* 16SRRNA; *(B) TXTA*; *(C)* RATIO *TXTA* : 16S. UNIRRIGATED, ■; 7-DAY SPRINKLER, □; DAILY SPRINKLER, ▲. S.E BASED ON 4 D.F.

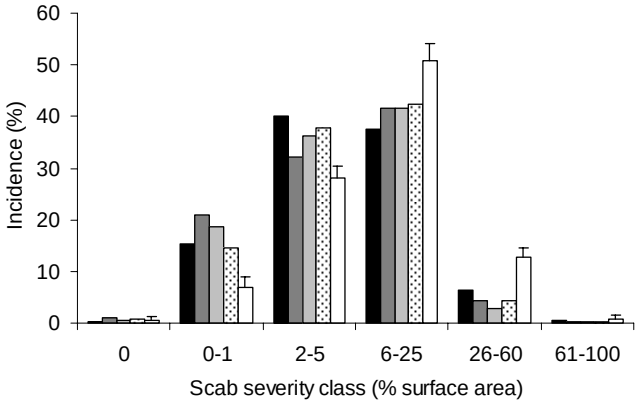


FIG. 12: EFFECT OF IRRIGATION REGIME ON COMMON SCAB SEVERITY 11 WEEKS AFTER TUBER INITIATION IN EXPT 2. UNIRRIGATED, ■; WEEKLY DRIP, ■; WEEKLY SPRINKLER, ■; DAILY DRIP, ▒; DAILY SPRINKLER, □. S.E BASED ON46 D.F.

TABLE 9: EFFECT OF IRRIGATION REGIME ON THE MEAN SURFACE AREA (%) INFECTED WITH COMMON SCAB 11 WEEKS AFTER TUBER INITIATION IN EXPT 2

	Unirrigated	Weekly drip	Weekly sprinkler	Daily drip	Daily sprinkler
	10.0	9.3	8.9	9.6	14.5
S.E. (46 D.F.)			1.48		

c) Field Experiment 3 (2007)

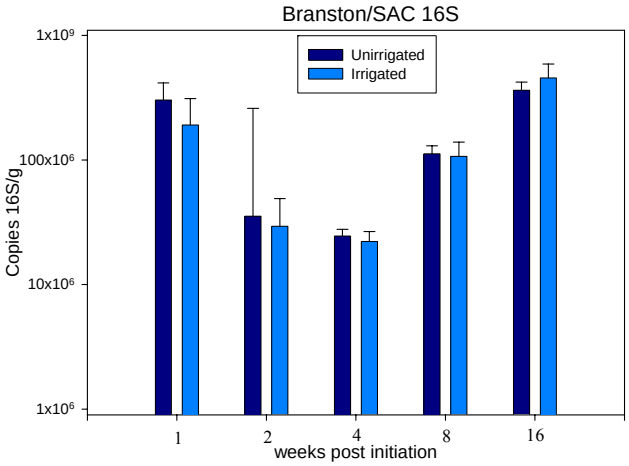
The analysis of *Streptomyces* species in soil samples from both sites showed that *S. scabiei/eropaeiscabiei*, *S. stelliscabiei*, *S. bottropensis*, *S. acidiscabies* and *S. turgidiscabies* were present prior to planting at both sites.

During 2007 the rainfall at both the Dundee and Mintlaw sites was above average, particularly during the period after tuber initiation. As a result only one irrigation event was required at Dundee, on 10 July 16 days after tuber initiation when 12.9mm was applied. At Mintlaw there were two irrigation events on 22 June (14mm applied) and 11 July (15mm applied), 3 and 22 days after tuber initiation.

At the Mintlaw site 16S populations were high (>10^8) one week after tuber initiation. The numbers fell two and four weeks after TI but rose again 8 weeks after TI and at the final harvest 16 weeks after TI. There were no significant differences between unirrigated and irrigated plots at any sampling date (Fig. 13a). *TxtA* populations were detected 1, 2 and 4 weeks after tuber initiation but not thereafter. There were no significant differences between irrigated and unirrigated treatments in weeks 1 and 4 after TI but at week 2 *txtA* populations were only detected on unirrigated plots (Fig. 13b).

The level of common scab on Maris Peer at Mintlaw was low with only 7% tubers exhibiting symptoms and the severity at trace levels. There were no significant differences between irrigated and unirrigated treatments, which reflects the absence of difference in 16S or *txtA* populations on most assessment dates.

a) 16S populations



b) txtA populations

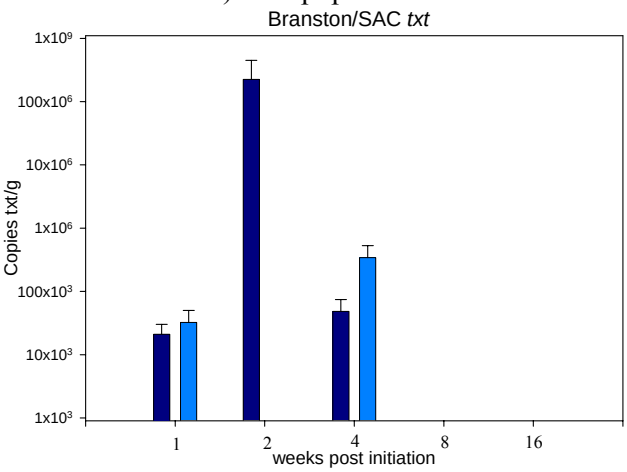


FIG. 13: CHANGES IN A) 16S POPULATIONS B) TXTA POPULATIONS ON TUBERS IN EXPT 3, MINTLAW.

Levels of common scab were also low at Dundee with no significant differences between irrigated and unirrigated treatments. On unirrigated plots incidence was 31.5% and severity 0.5%. Comparable figures for irrigation were 28.7% and 0.4%. These also reflect the absence of any significant differences in 16S and *txtA* populations (see section on effect of sulphur and other non-water control measures on development of common scab).

d. Field experiment 4 (2008)

(i) CUF

Crop emergence was fairly synchronous across all plots, with 5-95 % plant emergence taking 8 days and 50 % plant emergence was recorded on 10 May. The date that 50 % of plants tuberized was 1 June (5-95 % TI was 6 days) so that the time interval from emergence to TI (22 days) was longer than in Expt 1 (16 days).

An initial soil sample prior to planting showed that there were undetectable levels of most pathogenic *Streptomyces* present. The only detectable pathogenic species was the 'Group x'. This contrasted with Expt 1 when six species were detected in the soil pre-planting.

General observations were that peak and mean total actinomycete populations (16S) on the surface of the tuber were *c*. 100 times lower than in Expt 1. The actinomycete populations were similar across all treatments 1 week after TI and increased from 1 to 3 weeks after TI in unirrigated plots but remained constant over the same period in irrigated plots (Fig. 14.). Between 3 and 4 weeks after TI, irrigated crops showed a large decrease in the total actinomycete populations, whereas in plots where irrigation stopped at 2 weeks after TI, populations were maintained for the next 3 weeks. The results were similar for frequent and infrequent timing. There was a large decrease in the total actinomycete population in control plots between 4 and 6 week after TI, such that all treatments were similar at the end of the scab control period, however, there was a significantly higher population at final harvest in unirrigated plots compared with 6-week irrigation (Fig. 14).

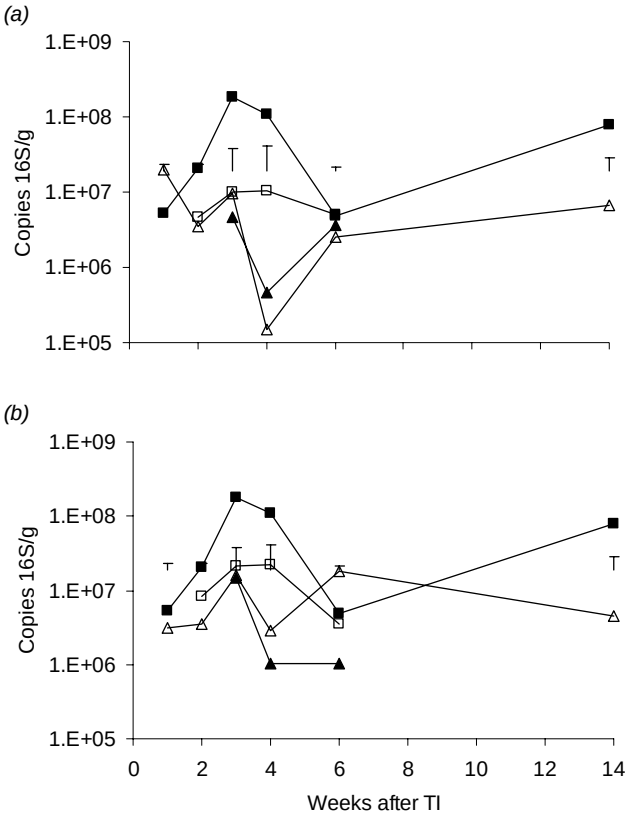


FIG. 14: CHANGES IN 16S POPULATIONS ON TUBERS IN EXPT 3. (A) FREQUENT; (B) INFREQUENT IRRIGATION. UNIRRIGATED, ■; 2-WEEK, □; 4-WEEK, ▲; 6-WEEK, △. S.E. BASED ON 4-12 D.F.

Peak and mean values of pathogenic *Streptomyces* (*txtA*) populations were much lower (x 100) than in Expt 1 in irrigated treatments but were similar in unirrigated plots across both seasons. There was a similar time course in *txtA* to 16S but there was a much larger relative change from the values recorded at 1 week after TI (Fig. 15.). Unirrigated control plots had the highest pathogenic *Streptomyces* populations throughout, with 2-week duration irrigation having higher populations between 4 and 6 weeks after TI than 4- and 6-week duration. There was apparently no pathogenic *Streptomyces* found in Frequent 4- and 6-week duration 4 weeks after TI and only a small amount in Infrequent. It is unlikely than no DNA was present but rather that the test is too insensitive (100 copies/g) to detect the very small amounts of DNA present in the peel samples.

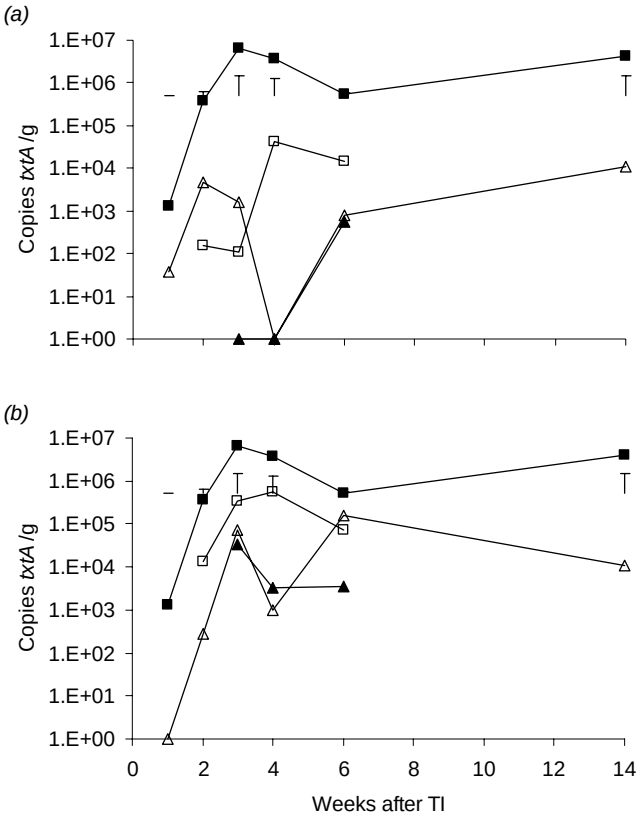


FIG. 15: CHANGES IN *TXTA* POPULATIONS ON TUBERS IN EXPT 3. *(A)* FREQUENT; *(B)* INFREQUENT IRRIGATION. UNIRRIGATED, ■; 2-WEEK, □; 4-WEEK, ▲; 6-WEEK, △. S.E. BASED ON 4-12 D.F.

An initial hypothesis of the project was that irrigation controls the population of pathogenic *Streptomyces* spp. (*txtA*) by encouraging the population of antibiotic-producing *Streptomyces* spp. through maintaining suitable edaphic conditions for their proliferation. These antibiotic-producing species may a) kill pathogenic species or b) the increased populations of actinomycetes compete strongly for resources on the surface of the developing tuber which suppresses the multiplication of pathogenic *Streptomyces* spp. If this hypothesis were true, then the ratio of *txtA* : 16S should be lower in irrigated than dry conditions. The ratio in Expt 3 was much lower (x 1000) than in Expt 1 in irrigated treatments and slightly lower (x 10) in unirrigated plots. Fig. 16 shows the ratio of *txtA* : 16S. The more powerful split-plot analysis statistical technique showed a number of important findings. Firstly, the populations of *txtA* were significantly lower than 16S throughout the sampling period. Secondly, relative differences in the populations of 16S under different irrigation regimes were generally smaller than the relative differences in *txtA*. Therefore, at all sampling dates, there was a reduction in *txtA* in irrigated plots compared with unirrigated control plots whereas differences in 16S were relatively smaller or not significant. Thirdly, 3 weeks after TI, Frequent irrigation reduced *txtA* populations more than Infrequent. Lastly, 4 weeks after TI, the *txtA* populations were much lower in crops that had been irrigated since TI than in crops where irrigation ceased at 2 weeks after TI.

Therefore, there was clear evidence in both Expt 1 and Expt 3 that maintaining wet soils for 6-8 weeks after TI decreased the populations of pathogenic species in the total *Streptomyces* population. However, the difference in *txtA* populations between unirrigated and irrigated was greater where bi-daily irrigation was applied than where the irrigation

took place every 4-6 days. This difference in *Streptomyces* populations between irrigation frequencies, however, did not manifest itself in terms of scab incidence (see below).

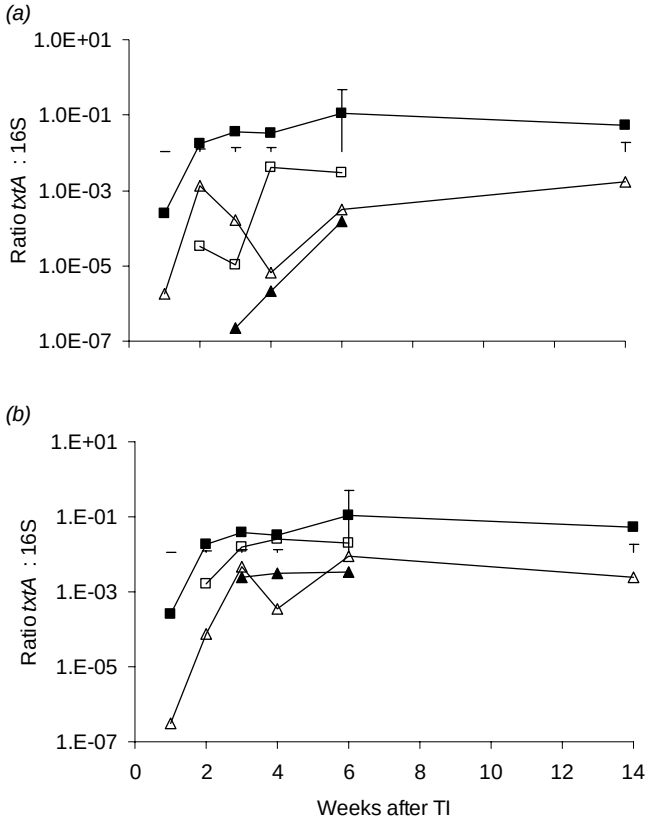


FIG. 16. CHANGES IN *TXTA* : 16S RATIO ON TUBERS IN EXPT 3. *(A)* FREQUENT; *(B)* INFREQUENT IRRIGATION. UNIRRIGATED, ■; 2-WEEK, □; 4-WEEK, ▲; 6-WEEK, △. S.E. BASED ON 4-12 D.F.

There was a very high incidence of common scab in all treatments but there were significant differences between irrigation methods in the proportion of tubers with < 5 % surface area affected by scab (Unirrigated 29 %, Drip 58 %, Sprinkler 77 %, S.E. = 3.3 %). Irrigation reduced scab *c.f.* no irrigation but Drip irrigation had more scab than Sprinkler irrigation, although the difference was smaller than in Expt 1. There was no significant effect of irrigation frequency, with Infrequent sprinkler irrigation applied every 4-6 days being as good as Frequent bi-daily irrigation (Table 10). There was also no effect of duration of irrigation after TI, with ceasing irrigation after 2 weeks being as good as 4 and 6 weeks (Table 11). It should be noted, however, that irrigation was applied to the infrequent timings on the last day of the 2 week period which would have kept the soil sufficiently wet to avoid scab for a further 4-5 days.

TABLE 10: EFFECT OF IRRIGATION METHOD AND FREQUENCY ON SCAB SEVERITY (% SA) IN EXPT 3. DATA ARE MEANS OF ALL IRRIGATION DURATIONS

	Frequency	
	Bi-daily	Infrequent
Drip	8.23	9.85
Sprinkler	4.88	5.76
Unirrigated	20.22	
S.E. (S.E. for Unirrigated) (24 D.F.)	1.622 (2.293)	

TABLE 11: EFFECT OF IRRIGATION DURATION ON SCAB SEVERITY (% SA). DATA ARE MEANS OF BOTH IRRIGATION FREQUENCIES

	Method	
Duration	Drip	Sprinkler
2 weeks	8.20	6.95
4 weeks	8.87	4.59
6 weeks	10.06	4.42
Unirrigated	20.22	
S.E. (S.E. for Unirrigated))(24 D.F.)	1.622 (2.293)	

There was a significant increase in the incidence of growth cracking in Frequent Sprinkler plots (6.6 % incidence) *c.f.* the mean of all other treatments (2.2 %, S.E. = 0.99 %) but the incidence was much lower than in Expt 1 (26.0 %) in the same treatments. Soils were kept slightly drier in frequently irrigated plots in Expt 3 than in Expt 1.

Readings of soil water content in the ridge at 15 cm depth made with Theta Probes installed at emergence and logged every 15 minutes were converted to SMDs within the ridge profile. These showed that Frequent Sprinkler plots irrigated for 6 weeks post-Ti were maintained at a mean SMD of 1.3 mm during the scab control period with two short spells above Field Capacity (Fig. 17). The maximum SMD was 5.8 mm, with previous work showing that the threshold for common scab on soils at CUF is typically *c*. 10 mm. For commercial scheduling, where larger doses of irrigation typically have to be applied, a target SMD of 12.9 mm would be recommended. The Unirrigated plots exceeded the target for scab infection *c*. 7 days after TI and progressively dried, reaching a maximum SMD of 26 mm 5 weeks after TI. The CUF Irrigation Scheduling Model predicted that SMDs in the top 25 cm were within 2 mm of Field Capacity in the most frequently irrigated plots, with SMDs in the unirrigated plots reaching a maximum of over 40 mm. It was noticeable that the sprinkler-irrigated plots were uniformly wet across the ridge whereas from spot readings with a Theta Probe, soil in the flanks of the ridge in drip-irrigated plots was 0.04-0.05 m^3/m^3 drier than the centre of the ridge, albeit the soil was above Field Capacity underneath the drip tape following irrigation. This greater variation in soil water content across the ridge probably explained the poorer scab control in the drip-irrigated plots.

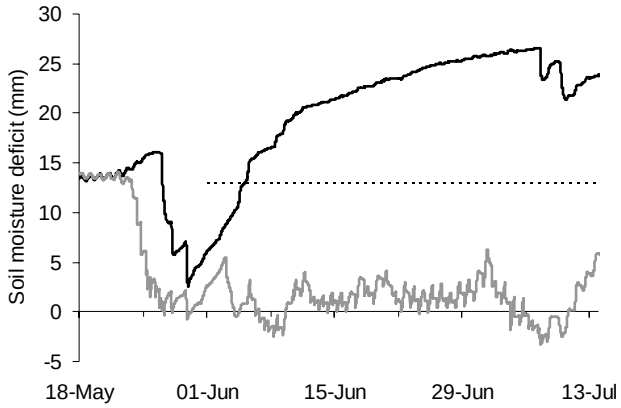


FIG. 17: SOIL MOISTURE DEFICITS IN RIDGE PROFILE MEASURED USING PERMANENTLY-INSTALLED THETA PROBES IN EXPT 3. CONTROL, ▬; FREQUENT SPRINKLER 6 WEEKS, ▬. DASHED LINE SHOWS SCAB CONTROL PERIOD.

(ii) SAC

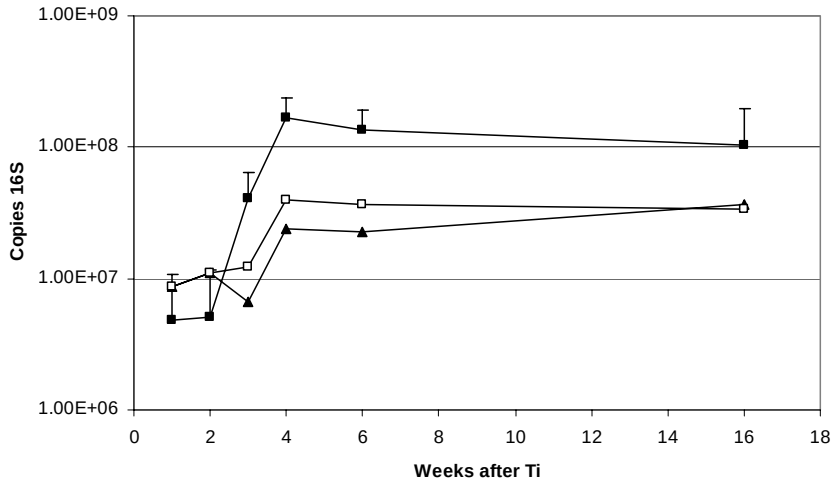
Crop emergence was fairly synchronous across all plots and 50 % plant emergence was recorded around 11 June. The date that 50 % of plants tuberised was 5 July so that the time interval from emergence to TI was 24 days.

An initial soil sample prior to planting showed that there were undetectable levels of most pathogenic *Streptomyces* present. The only detectable pathogenic species was the 'Group x'. This contrasted with Expt 1 when six species were detected in the soil pre-planting.

Levels of 16S populations rose in unirrigated plots during the first 4 weeks after tuber initiation and subsequently levelled off (Fig. 11a). A similar pattern was evident with frequent irrigation for 6 weeks but the increase was less than unirrigated plots. The frequent irrigation for the first 2 weeks after tuber initiation resulted in an increase in 16S populations similar to that of 6 weeks frequent irrigation, the increase continuing for two weeks after irrigation had stopped. 16S populations were broadly similar to that found at the comparable CUF trial.

Pathogenic *Streptomyces* populations (*txtA*) were undetectable on 6 week frequent irrigation plots at all sampling dates except week 6 where relatively low numbers were detected (Fig. 18b). This contrasted with the unirrigated plots where pathogenic populations were detected from week 1 and with a rapid rise in numbers after week 2, which was sustained at all sampling dates. Frequent irrigation for 2 weeks limited pathogenic populations during the period of irrigation but thereafter the population rose to close to that of the untreated control (Fig. 11b). Apart from week 4, all three treatments had significantly different *txtA* populations. The pattern of development of *txtA* populations on unirrigated plots was similar to that found at CUF, albeit at lower levels. Suppression of txtA populations at the SAC site was much greater than at the CUF site.

a) 16S populations



b) *txtA* populations

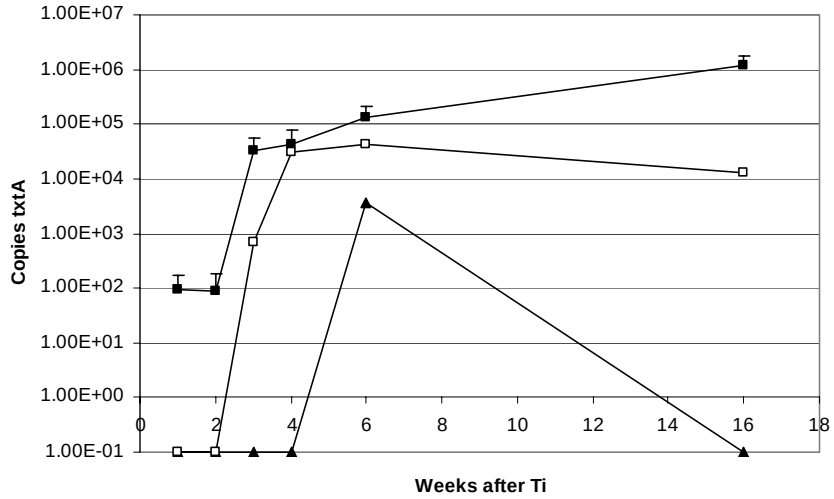


FIG. 18: CHANGES IN A) 16S AND B) TXTA POPULATIONS ON TUBERS IN EXPT 3 SAC. FREQUENT IRRIGATION. UNIRRIGATED, ■; 2-WEEK, □; 6-WEEK, ▲. S.E. INDICATED FOR EACH SAMPLING DATE

The ratio of *txtA* to 16S populations showed large differences between irrigated plots and unirrigated during the period of irrigation but that once stopped the benefit of irrigation in suppressing pathogenic populations ceased (Fig.19).

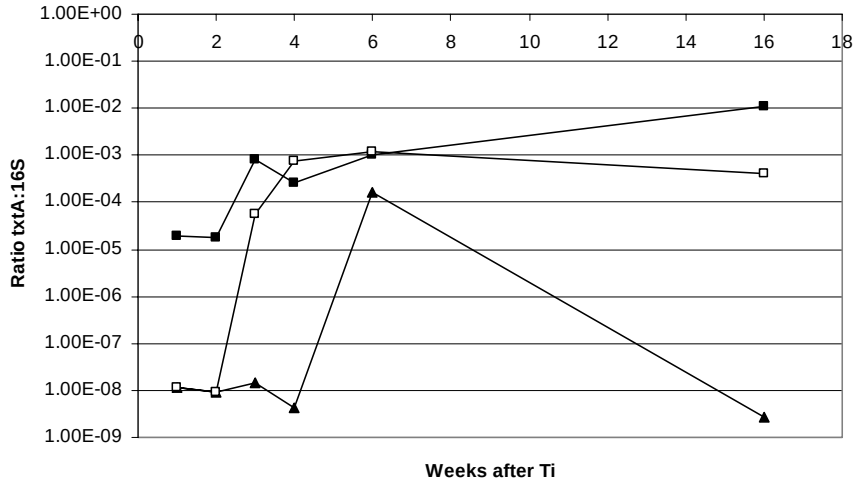


FIG. 19. CHANGES IN *TXTA* : 16S RATIO ON TUBERS IN EXPT 3 SAC. FREQUENT IRRIGATION. UNIRRIGATED, ■; 2-WEEK, □; 6-WEEK, ▲.

At final harvest, levels of common scab were low compared to the CUF site. However, the patterns of 16S and *txtA* changes reflected the severity of common scab that were present at final harvest. All frequent irrigation treatments significantly reduced common scab compared to unirrigated (Table 12). There was no significant difference between durations of irrigation with frequent irrigation, suggesting that the first two weeks of irrigation in suppressing pathogenic populations was of greatest importance. Infrequent irrigation, irrespective of duration, failed to reduce the level of common scab significantly. The effect of irrigation in increasing tubers free from common scab for both frequent and infrequent irrigation is shown in Fig. 20.

TABLE 12: EFFECT OF IRRIGATION DURATION AND TIMING ON COMMON SCAB SEVERITY (% SA).

Duration of irrigation	Frequency of irrigation	Common scab severity
2 weeks	Frequent	1.1
2 weeks	Infrequent	2.6
4 weeks	Frequent	1.3
4 weeks	Infrequent	1.4
6 weeks	Frequent	0.9
6 weeks	Infrequent	1.8
Unirrigated	-	3
LSD (41 df)		1.6

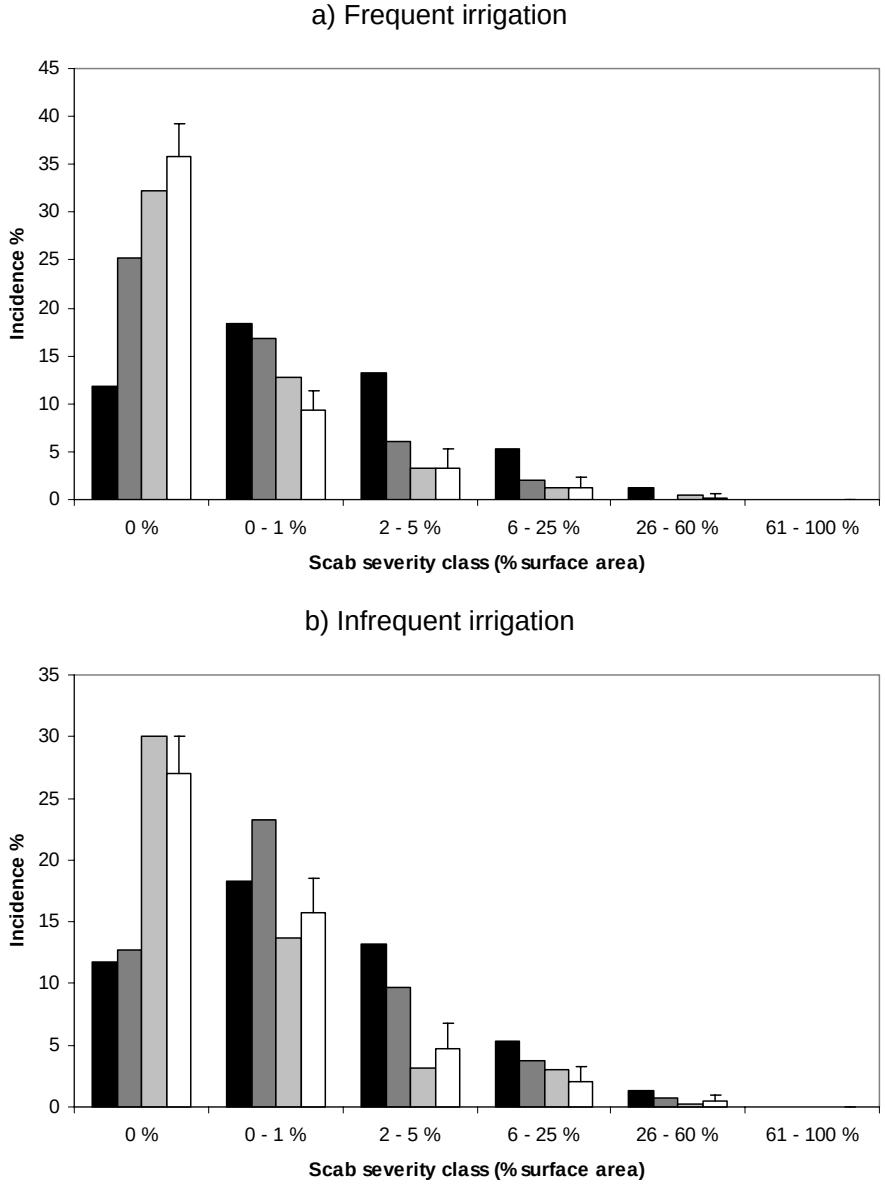


FIG. 20: EFFECT OF A) FREQUENT AND B) INFREQUENT IRRIGATION ON COMMON SCAB INCIDENCE 16 WEEKS AFTER TUBER INITIATION IN EXPT 3 SAC. UNIRRIGATED, ■; 2 WEEKS, ■; 4 WEEKS, ■; 6 WEEKS, □. S.E BASED ON 41 D.F.

Continuous measurement of soil moisture in the trial reflected the level of common scab found with different irrigation frequencies. Whilst the soil moisture deficit in unirrigated plots rose steadily from tuber initiation, it was constrained in the 6 weeks frequent irrigation plots to below 10mm (Fig. 21). In the infrequent 6-week irrigated plots, soil moisture deficit rose above 10mm on around 25% of days during the six weeks following 50% tuber initiation.

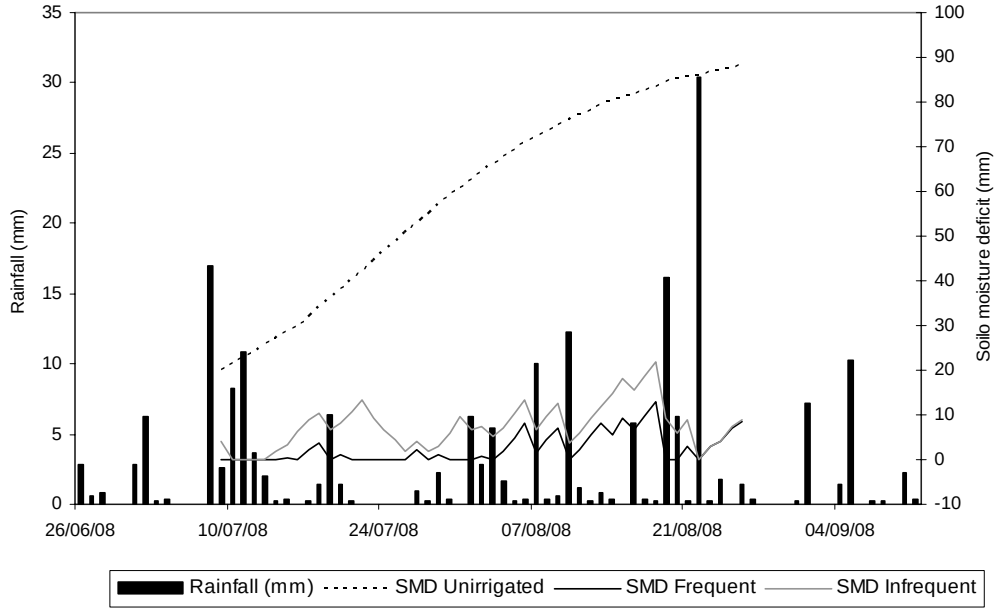


FIG. 21: SOIL MOISTURE DEFICITS IN RIDGE PROFILE MEASURED USING PERMANENTLY-INSTALLED THETA PROBES IN EXPT 3 SAC.

(iii) Characterisation of total bacterial populations by pyrosequencing.

DNA extracted from samples obtained from irrigation trials at CUF in 2008 was used in experiments to amplify 16S rDNA sequences from total bacterial populations. Samples were taken from four experimental treatments; unirrigated and frequently irrigated plots from 1 week and 6 weeks post tuber initiation. Three replicates per treatment were sampled, making a total of 12 samples, which were analysed in multiplex using 12 sets of barcoded primers. PCR amplification yielded the expected *c*.350 bp amplicon comprising a variable portion of the bacterial 16S rDNA. Pyrosequencing yielded a total of 57735 DNA sequences, at an average of approximately 4800 per sample. Of these, approximately 13000 (22.5%) could confidently be assigned to genus, enabling comparisons to be made between differences in the proportions of the major soil bacteria found between irrigation treatments. The major genera of soil bacteria present were *Streptomyces*, *Flavobacterium*, *Pedobacter*, *Arcicella*, *Acinetobacter*, *Pseudomonas* and *Variovorax*. Relative proportions of these are shown in Fig.22. The relatively low levels of these genera in samples taken one week after tuber initiation is a reflection of the large number of chloroplast 16S sequences in these samples. From the sequence data available it was not possible to conclude with confidence that these were bacterial (e.g. cyanobacterial) in origin and, since levels of these sequences were very low in samples taken six weeks after tuber initiation regardless of irrigation regime, these were not thought to be of significance. Levels of *Streptomyces* 16S were surprisingly low in all samples, although they were highest in unirrigated plots at six weeks post tuber initiation (37 sequences from a total of *c.* 3000). The greatest difference in bacterial populations between irrigated and unirrigated plots was observed in *Pseudomonas*, and levels of this bacterium increased to approximately 10% of the total identifiable 16S when irrigated compared with less than 2% in unirrigated plots.

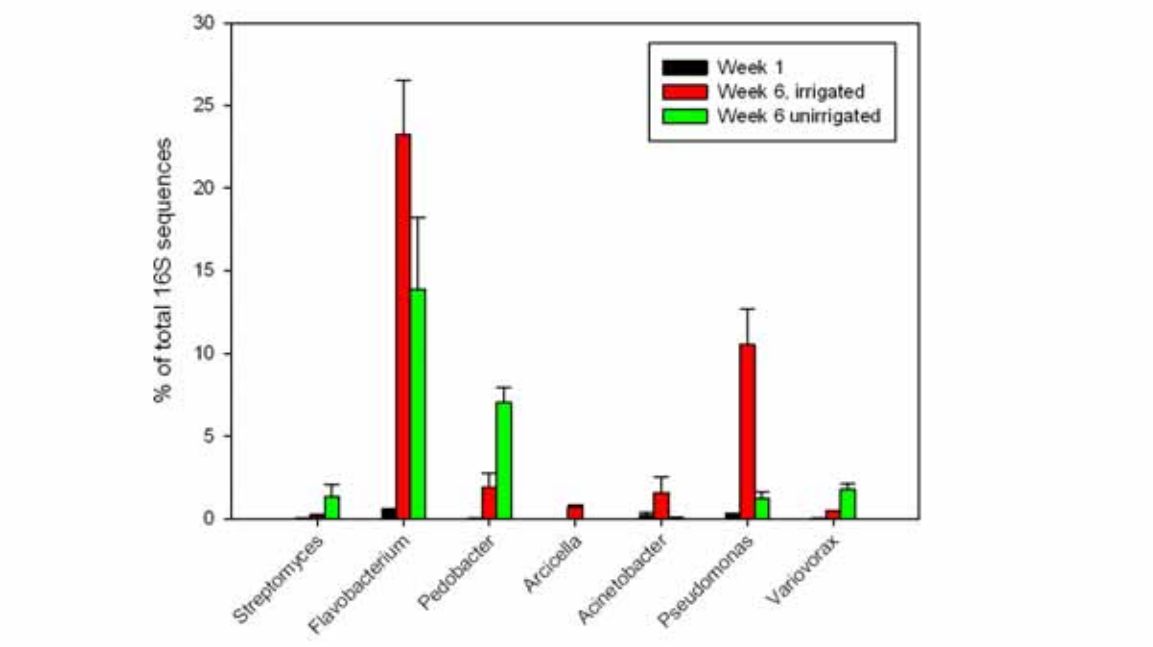


FIG. 22: RELATIVE LEVELS OF MAJOR SOIL BACTERIA PRESENT IN IRRIGATION TRIALS AT CUF IN 2008.

4. Effect of location, seed infection and variety on scab incidence and severity in commercial fields

Milestones achieved:

- ***Tuber and soil samples collected from commercial potato fields.***
- ***Samples analysed and population dynamics quantified.***

a) 2007

Scab incidence was much more influenced by location than by any other factor (Fig. 23.). At the QV site, scab severity was consistently low, irrespective of cultivar or seed source. Marketable yields were high with more than 90% of harvested tubers having less than 5% coverage with common scab symptoms, with the exception of the Kondor crop with 85%. In contrast, marketable yields of the susceptible cultivars Desiree and Maris Piper were significantly reduced by common scab at the Branston site. Although differences in levels of pathogenic *Streptomyces* spp. were measured on seed stocks with high and low scab severity prior to planting (Fig. 24.), this did not apparently affect the severity of common scab observed on the harvested crop.

PCR analysis showed that the proportion of total actinomycetes to pathogenic streptomycetes on progeny tubers was greater at the QV site than at the Branston site (Fig. 25). At the QV site, there were 100-10,000 fold more total actinomycetes detected than pathogenic streptomycetes. At the Branston site, this difference was only 10-100 fold.

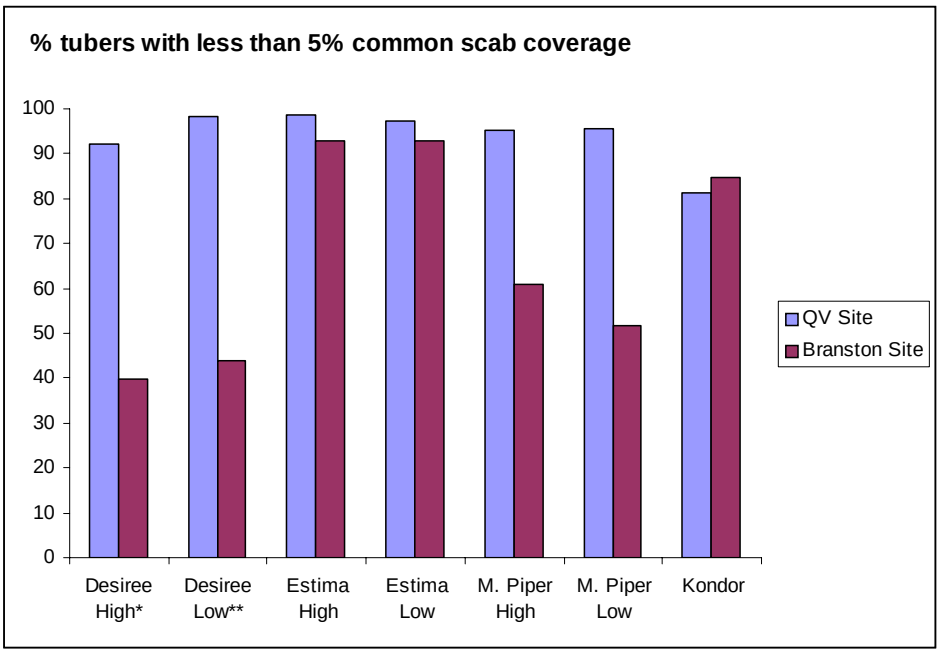


FIG. 23: MARKETABLE YIELD OBTAINED IN FIELD PLOTS GROWN FROM THE SAME SEED STOCKS AT DIFFERENT LOCATIONS (*HIGH COMMON SCAB SEVERITY ON SEED; **LOW COMMON SCAB SEVERITY ON SEED).

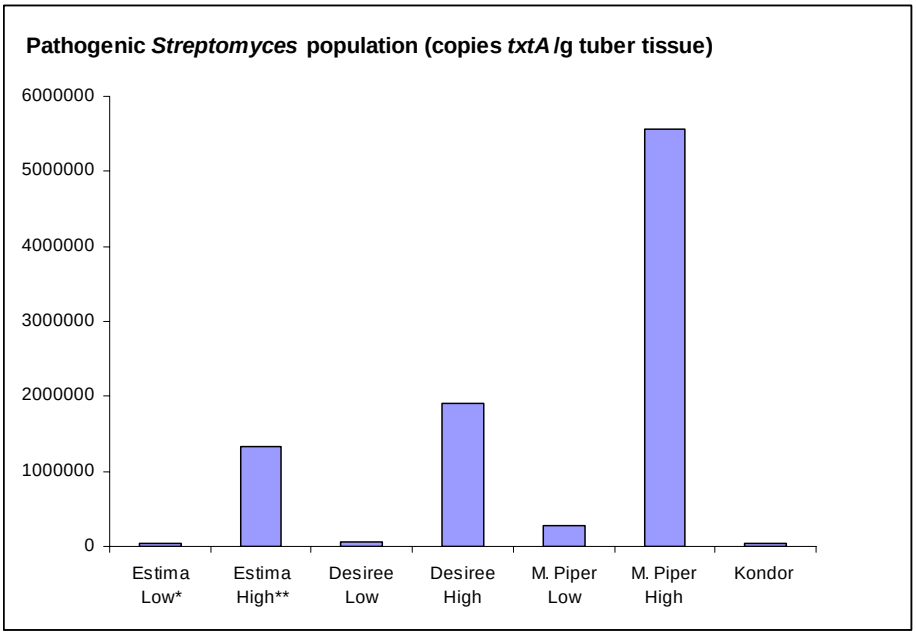


FIG 24: COMPARISON OF PATHOGENIC *STREPTOMYCES* POPULATIONS ON SEED TUBERS WITH LOW* AND HIGH** COMMON SCAB SEVERITY BEFORE PLANTING.

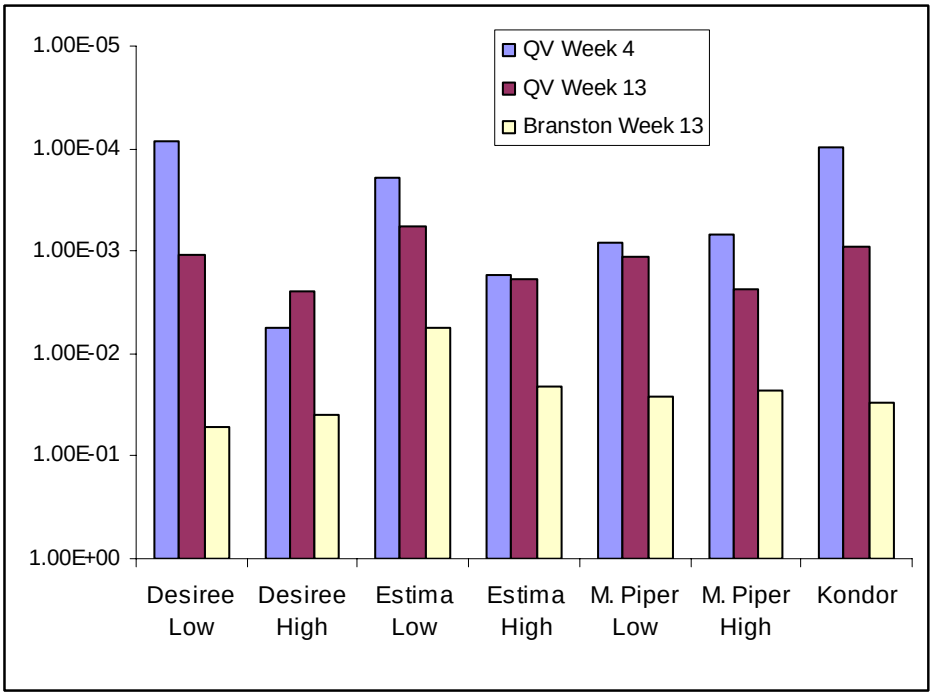


FIG. 25: RATIO OF PATHOGENIC TO TOTAL ACTINOMYCETES ON INITIATING (WEEK 4) AND HARVESTED (WEEK 13) TUBERS AT DIFFERENT LOCATIONS.

b) 2008

The marketable yield of harvested tubers (with 5% or less surface area affected by common scab) ranged from 20 to 98% and, as in the previous year, depended more on the location at which the potatoes were grown than on the variety or seed source (Fig. 26.).

Populations of total actinomycetes and pathogenic *Streptomyces* spp. were quantified (using real time PCR) on developing progeny tubers at intervals (1, 3 and 5 weeks) after tuber initiation and were compared with the incidence of common scab at harvest. Populations of pathogenic *Streptomyces* spp. were not high enough to quantify on seed or in the soil at any of the locations prior to planting. Pathogen populations increased over the tuber initiation period, becoming detectable at some but not all sites by 3 weeks after imitation (Fig. 27.).

At some sites (QV site 1 and Cobrey sites 1 and 2), the pathogen was rarely found in detectable numbers on tubers sampled at any time during initiation or at harvest. These sites also yielded tubers with the lowest scab incidence. Low incidence of common scab, below commercially important levels, again appeared to be correlated with high total actinomycete populations on initiating tubers (Fig. 28.) and the ratio of pathogenic *Streptomyces* spp. to total actinomycete populations (Fig. 29.).

A significant increase in total actinomycete populations was measured during the tuber initiation period in those locations where harvested potatoes were least affected by common scab. The lowest levels of common scab were observed where the ratio of pathogenic to non-pathogenic species exceeded 1 in 10,000. The sites where scab incidence was lowest (Cobrey sites 1 and 2) also had significantly higher levels of total actinomycetes detected in the first week after tuber initiation.

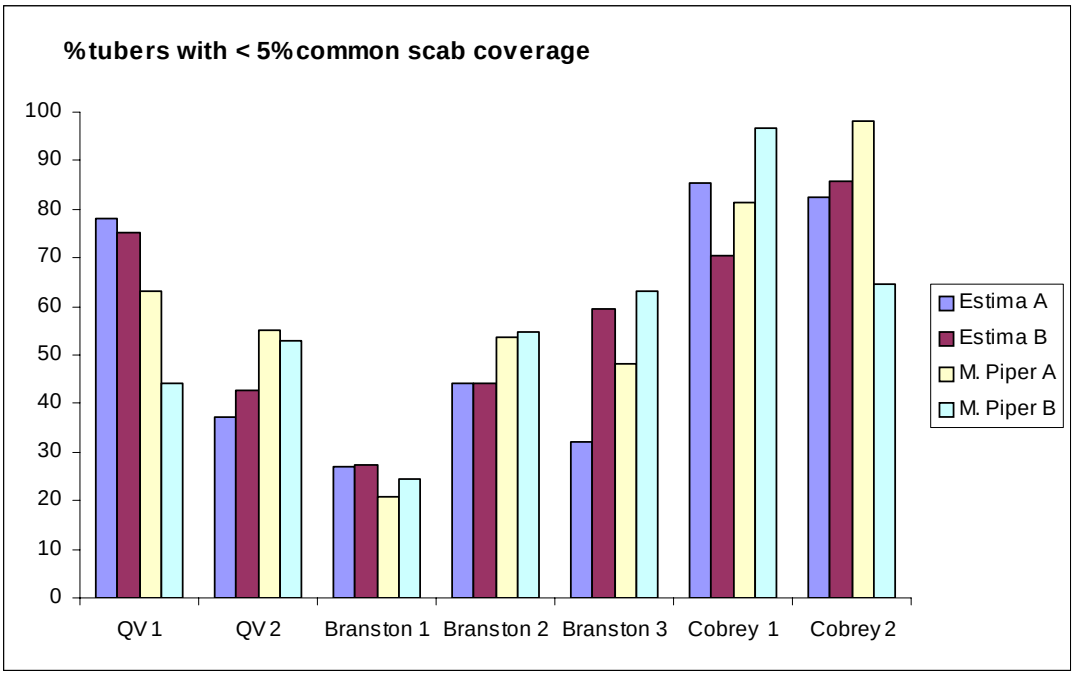


FIG. 26: MARKETABLE YIELD OBTAINED IN FIELD OBSERVATION PLOTS GROWN FROM 2 DIFFERENT SEED SOURCES (A AND B) OF EACH OF 2 VARIETIES (ESTIMA AND MARIS PIPER) AT 7 DIFFERENT LOCATIONS.

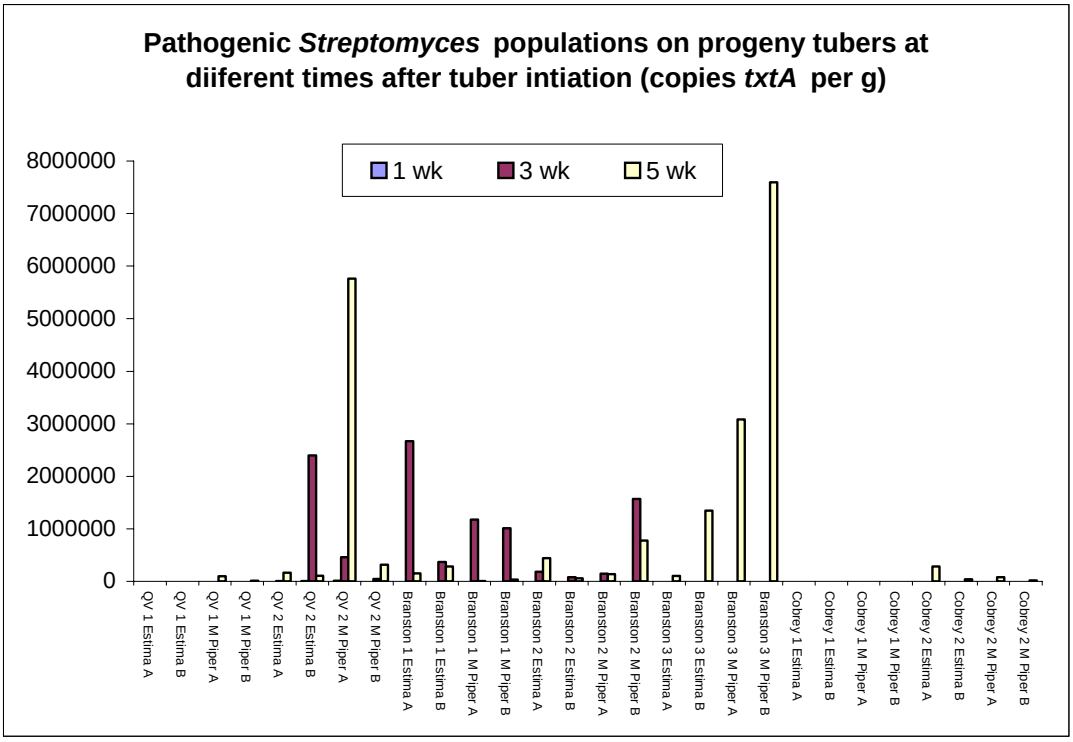


FIG. 27: PATHOGENIC *STREPTOMYCES* POPULATIONS DETECTED ON PROGENY TUBERS AT DIFFERENT TIMES AFTER TUBER INITIATION IN CROPS PRODUCED FROM DIFFERENT SEED SOURCES (A AND B) OF EACH OF 2 VARIETIES (ESTIMA AND MARIS PIPER) AT 7 DIFFERENT LOCATIONS.

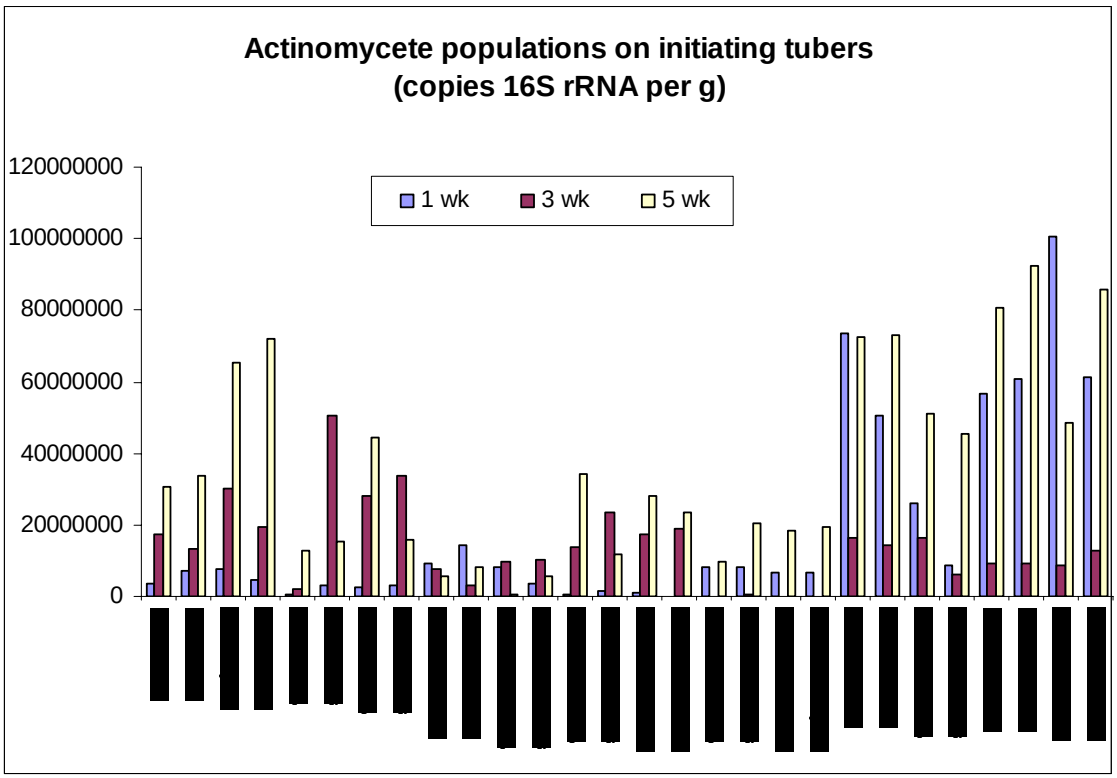


FIG. 28: TOTAL ACTINOMYCETE POPULATIONS DETECTED ON PROGENY TUBERS AT DIFFERENT TIMES AFTER TUBER INITIATION IN CROPS PRODUCED FROM DIFFERENT SEED SOURCES (A AND B) OF EACH OF 2 VARIETIES (ESTIMA AND MARIS PIPER) AT 7 DIFFERENT LOCATIONS.

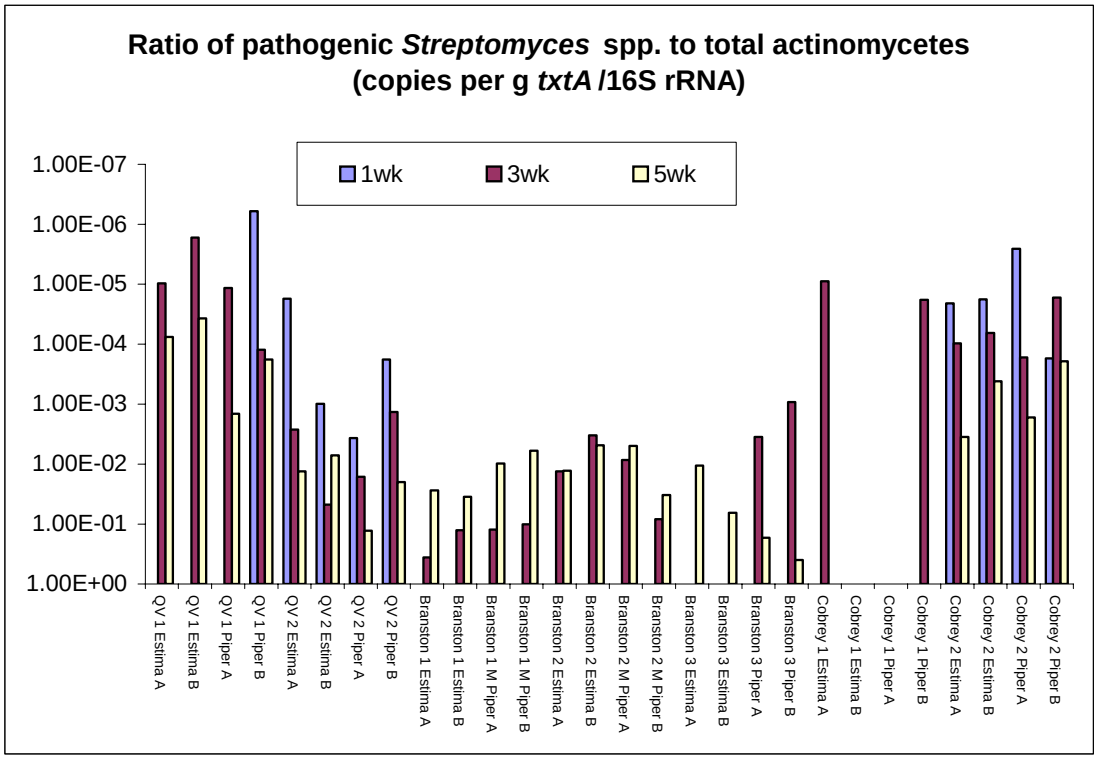


FIG. 29: RATIO OF PATHOGENIC *STREPTOMYCES* SPP. TO TOTAL ACTINOMYCETES ON PROGENY TUBERS A DIFFERENT TIMES AFTER TUBER INITIATION IN CROPS PRODUCED FROM DIFFERENT SEED SOURCES (A AND B) OF 2 VARIETIES (ESTIMA AND MARIS PIPER) AT 7 DIFFERENT LOCATIONS.

c) *Streptomyces* species identified

TABLE 13: *STREPTOMYCES* SPECIES DETECTED BY PCR IN SOIL, SEED AND HARVESTED POTATO SAMPLES.

Year	Sample	Source	*S. scabiei/ europaeiscabiei*	*S. stelliscabiei*	'group x'	*S. bottropensis*	*S. acidiscabies*	*S. turgidiscabies*
2007	Soil	QV Worth F54	+	+	(+)	+	(+)	+
		QV Worth F58	-	+	-	+	+	+
		QV Worth 43/4 N	-	+	+	+	+	+
		QV Worth 43/4 S	-	+	-	+	+	+
		BSW1	+	+	+	+	+	+
		BSW2	+	+	+	+	+	+
		BSW3	+	+	+	+	+	+
		BSW4	+	+	(+)	+	+	+
		BSW5	+	+	+	+	+	+
		BSW6	+	+	+	+	+	+
		Cobrey – Spread	+	+	+	+	+	+
		Bank	+	+	+	+	+	+
		Barker North Field	+	+	+	+	+	+
		Barker South Field	+	+	+	+	+	+
		Barker College	+	+	+	+	+	+
		Barker 10 acre	-	+	+	+	-	-
		Arms L8	+	+	+	+	+	+
		Arms L10	+	+	+	+	+	+
		CUF						
2007	Seed	Estima-L	-	+	-	+	+	-
		Estima-H	-	+	-	+	-	-
		Desiree-L	-	+	-	+	-	-
		Desiree-H	-	+	-	+	+	+
		M. Piper-L	-	+	-	+	-	-
		M. Piper-H	-	+	-	+	+	+
		Kondor	-	+	-	+	-	-
2008	Soil	QV1	-	+	+	+	+	+
		QV2	-	+	+	+	+	+
		QV3	-	+	+	+	+	+
		QV4	-	+	+	+	+	+
		QV5	-	+	+	+	+	+
		QV6	-	+	+	+	+	+
		QV7	-	-	-	-	-	-
		BR3	-	-	-	-	-	-
		BR4	-	+	+	+	-	+
		BR5	-	+	+	+	-	+
		BR6	-	+	-	-	-	-
		BR7	-	-	-	-	-	-
		BR8	-	-	-	-	-	-
		BR9	-	-	-	-	-	-
		BR10	-	-	-	-	-	-
		BR11	-	-	+	+	-	-
		BR12	-	-	-	-	-	-
		BR13	-	-	-	+	-	+
		BR14	-	-	+	+	+	+
		BR15	-	+	+	+	-	+
		BR16	-	-	-	-	-	-
		Cobrey 1	-	-	+	-	-	-
		Cobrey 2	-	-	+	-	-	-
		SAC26	-	+	+	+	+	-
		SAC27	-	+	+	+	+	-
		CUF1	-	-	+	-	-	-
		CUF2	-	-	+	-	-	-
		CUF3	-	-	-	-	-	-
2008	Seed	SAC1	-	+	+	+	-	-
		SAC2	-	+	+	-	-	-
		SAC3	-	-	+	+	-	+
		SAC4	-	-	+	+	-	-
		SAC5	-	-	+	+	-	-
		SAC6	-	+	+	+	-	-
		SAC7	-	+	+	+	-	-
		SAC8	-	+	+	-	-	-
		SAC9	-	-	+	+	-	-
		SAC10	-	+	+	+	-	+
		SAC11	-	+	+	+	-	-
		SAC12	-	+	+	+	-	+
		SAC13	-	-	+	-	-	-
		SAC14	-	+	+	+	-	-
		SAC15	-	-	+	-	-	+
		SAC16	-	-	+	+	-	-
		SAC17	+	-	+	+	-	+
		SAC18	+	+	+	+	-	-
		SAC19	+	+	+	+	+	+
		SAC20	-	+	+	+	-	-
		SAC21	-	+	+	+	-	-
		SAC22	+	+	+	+	+	-
		SAC23	+	+	+	-	-	-
		SAC24	+	+	+	-	-	-
		SAC25	-	+	+	+	-	-
		BR1	-	+	+	-	+	+
		BR2	-	+	+	+	+	+

PCR analysis with primers specific for detection of known scab-forming *Streptomyces* species detected a number of species in soil and on harvested potatoes with common scab symptoms (Table 13). No one species was associated with any particular disease symptom and more than one species could be isolated from single scab lesions. In addition to *S. scabiei*, a further two species (*S. turgidiscabies* and *S. acidiscabies*) have been isolated from potato common scab lesions and have been shown to induce common scab following inoculation of healthy potato and radish plants. The identification of these species has been confirmed according to their 16S rRNA gene sequences. These findings will be published as new UK disease records. Isolation, proof of pathogenicity and significance of the other *Streptomyces* species detected by PCR (*S. stelliscabiei*, *S. bottropensis* and *Streptomyces* sp. group X) remain to be determined.

5. Control guidelines

Milestones achieved:

- ***Recommendations for practical control measures agreed.***

Optimum methods of timing and targeting for precision irrigation of potato fields have been investigated and reviewed by Stalham *et al*. (1999), including the optimisation of common scab control. New information arising from this current project has been used to refine the recommendations with the aim to minimise water usage. The following practical common scab control guidelines were agreed and proposed to form the basis of an information sheet for recommendation to the potato industry:

- It is important to ensure soils are wet at tuber initiation for adequate control of common scab since populations of pathogenic *Streptomyces* (quantified according to the number of copies of the *txtA* gene detected per g of tuber sampled) on the surface of the tuber increase rapidly after tuber initiation and the increase is faster in dry soils than in wet. The crucial period appears to be the first 4 weeks after tuber initiation since pathogen populations either a) stabilize after 4-6 weeks so that differences between unirrigated and irrigated are maintained or b) become similar in irrigated and unirrigated as there is a progressive increase in *txtA* population in irrigated soils during the period when tubers are susceptible to infection.

- Soil structure is crucial in maintaining the films of water round tubers to suppress *Streptomyces* populations. Clods leave air voids that are bad for scab as these voids drain rapidly. However, over-cultivation to create a fine ridge structure can lead to 'siltation' of fine particles to the base of the ridge, which impedes drainage.

- Previous work at CUF has shown that irrigating for only 2 weeks after tuber initiation resulted in worse common scab than maintaining wet soil for 3 or more weeks. The observation that there was no significant difference in common scab severity with either 2-, 4- or 6-week irrigation in one experiment in the current project should be treated with caution, since modelled soil moisture deficits showed that the 2-week treatments remained sufficiently wet to avoid common scab for 4-5 days after the last irrigation was applied, despite the pathogenic *Streptomyces* populations increasing after the irrigation was stopped. Stopping irrigation after 2 weeks can often allow scab to infect the apical end of the tuber.

- Contrasting with previous work at CUF, there appeared to be no effect of frequency of irrigation on common scab severity when comparing daily irrigation with 4-6 day intervals and therefore current commercial practice appears frequent enough, which offers potential savings in water application and drainage losses. Depending on soil

texture and structure and evaporative demand, the irrigation interval can vary from 3-8 days in the absence of rain.

- The period between 50 % plant emergence and 50 % tuber initiation depends on variety but is relatively conservative for most varieties, typically being 19 ± 3 days with Cara at 25 ± 3 days, unless the seed is physiologically old, so emergence can be used to predict TI. In reality, using the date of initial emergence to predict TI is safer as this will ensure that irrigation commences when the first plants are tuberizing.

- The critical scab control period can be short (3 weeks from tuber initiation) where crop emergence occurs over a short period. The typical emergence period from initial to 50 % plants emerged is 3 days in experimental plots, whilst in commercial fields it can range from 2-14 days (or longer). A good target is 4 days. It takes a further 2 days from 50 % to 95 % emergence, so a target for initial to 95 % emergence would be 6 days. The scab control period needs to be lengthened beyond 3 weeks where the time taken to reach 95 % emergence is greater than 6 days.

- It is recognised from experimental work at CUF that as the rate of nitrogen fertilizer applied at planting increases, the onset of tuber bulking is delayed. Importantly, the effect is only significant when nitrogen rates are in excess of the optimum required for yield and often, but not always, results from a delay in emergence of up to 5 days at high nitrogen rates and a prolonging of the period taken to reach 95 % emergence (e.g. 20 days). *Rhizoctonia* infection can also prolong the period of emergence and tuberization. This variation needs to be measured to ensure the scab control period is of sufficient length.

- Ridges may be dry following the initial irrigation and a repeat application may need to be made within 3-4 days. Where soils are hydrophobic or capped, pre-irrigating 1-2 days before the onset of tuber initiation can help the subsequent irrigation be more effective in wetting the ridge or bed but allowing a longer time gap between this irrigation and the next will have little effect on wetting.

- The allowable soil moisture deficit during the critical scab control period can be increased from low values e.g. 9 mm (sand) - 18 mm (silt loam) to a value suitable for preventing yield loss (e.g. 30-50 mm) immediately the control period is over. There is no evidence that frequent watering during the control period impedes root growth thereby leaving the crop more susceptible to drought later in the season, unless the soil is kept above Field Capacity for substantial periods during the scab control phase.

- Maintaining soils close to or over Field Capacity during scab control or prolonging the control period unnecessarily will encourage lenticel eruption and increase the risk of powdery scab infection and spread of blackleg.

Discussion

Factors affecting scab incidence and severity

Results obtained from commercial potato fields during the current project have confirmed that the most important factor influencing potato common scab development is the soil in which the potatoes are grown. Antagonistic, competitive and enzymatic interactions in soil and on developing tubers are known to affect suppression of pathogenic *Streptomyces* spp. by other microorganisms, including by non-pathogenic *Streptomyces* spp. (Liu *et al*., 1995; Schmiedeknecht *et al.*, 1998; Neeno-Eckwall *et al*., 2001; Agbessi *et al*., 2003; Sessitch *et al*., 2004; Wiggins and Kinkel, 2005). Novel molecular diagnostics applied in this research have confirmed that the build up of pathogenic *Streptomyces* spp. on initiating tubers and the resulting common scab levels vary from field to field and appear to be inversely related to the total actinomycete activity of the soil during tuber initiation. Similar results were obtained independently in recent research in the USA using similar technology (Wanner, 2007). Whether the total actinomycete populations directly inhibit multiplication of pathogenic *Streptomycete* spp., or merely indicate soil types with high general biotic activity, in which other organisms inhibit the pathogens, remains to be determined. Initial pyrosequencing results presented above have indicated that *Pseudomonas* spp. may also play a role.

Prediction of the likelihood of disease development from *Streptomycete* populations in soil pre-planting is likely to be unreliable. Whilst recent glasshouse studies in Australia (Wiechel *et al*., 2007) have shown a clear relationship between soilborne inoculum levels and common scab incidence and severity, the field situation is likely to be more complicated. In the studies reported here, pathogenic *Streptomyces* populations were often undetectable in the soil before planting. Their ability to increase in population prior to or during tuber initiation, under the challenge of natural competition and other factors, is likely to be a key to common scab development.

The role of seed-borne *Streptomyces* in common scab development was confirmed to be minor in comparison with that of soil-borne inoculum. Nevertheless, seed with severe common scab was seen to contribute to the pathogenic *Streptomyces* populations detected on initiating tubers compared to seed with minor or no scab. The role of seed in spreading new pathogen species is therefore expected to be important.

A number of *Streptomyces* species, in addition to *Streptomyces scabei*, were confirmed to be causal agents of common scab in commercial fields. Two of these, *S. acidiscabies* and *S. turgidiscabies* have so far been confirmed as new records for the UK (Thwaites *et al*., in preparation). In glasshouse experiments, these organisms did not respond differently to pH modification of the growing medium. Under field conditions, the various species detected appeared to be widespread in commercial production in England and Scotland and not independently limited in different geographical locations, as inferred by survey work in the USA (Wanner, 2006). In fact, different pathogenic *Streptomyces* species could often be detected on the same tuber and in the same common scab lesions.

The susceptibility of different potato cultivars to common scab appeared to be affected by seasonal and site differences. Hence, the expected higher tolerance to common scab of cv. Estima compared with cvs. Maris Piper and Desiree was not observed across all commercial sites and seasons. New data from the USA (Wanner, 2007) suggests that different potato cultivars can influence microbial population diversity around initiating tubers, which in turn can influence common scab development. Changes in soil microflora and their population

dynamics in different locations and seasons may therefore influence cultivar susceptibility to common scab.

The success of measures to control common scab is likely to depend as much on their influence on the size and diversity of the competing microbial population surrounding initiating tubers, as on any direct effects on the pathogen populations themselves. Hence, efficacy of control is likely to vary with season and location and their affect on the prevailing soil microflora. Possibly for this reason, non-water-based control measures have tended to vary in reliability from report to report.

During the course of two field trials described above, sulphur appeared to have an effect in reducing the pathogenic population of *Streptomyces*. This was most marked in the 2008 trial where the effect occurred from 1 week after tuber initiation. Despite this effect, in general, S applications resulted in little reduction of final levels of common scab at harvest. The inconsistency of response to S has been recorded by other researchers (Stead & Wale, 2004). Where tested, the effect of rapeseed meal on common scab was also inconsistent. There was no evidence in the trials where S was examined in interaction with irrigation that the control of common scab was enhanced or that, through its application, water use could be reduced. Therefore, it was concluded from these studies that the non-water chemical control measures tested were not sufficiently or reliably effective for the control of common scab. In contrast, field studies in Canada and Australia have demonstrated positive effects of organic soil amendments on common scab reduction (Soltani *et al*., 2002; Crump *et al*., 2006). The reproducibility of such treatments is likely to vary with location and soil microflora. Furthermore, the sustainability of promising new chemical seed treatments for common scab control, such as the fungicide fludioxinil (Syngenta Crop Protection), is likely to depend upon its effect on the wider microbial population in the soil as well as any direct effect on pathogenic *Streptomyces* spp.

Effect of irrigation on scab control

These studies have confirmed that the most reliable way to control common scab remains through careful application of irrigation water over the critical period when initiating tubers are susceptible to infection. Suppression of common scab pathogens through irrigation has been thought for some time to be mediated by competition with other soil microorganisms (Adams and Lapwood, 1978) including antagonistic non-pathogenic *Streptomyces* spp. (Lorang *et al*., 1989; Sessitch *et al*., 2004; Wiggins and Kinkel, 2005). In wet soils, high populations of non scab-forming *Streptomyces* spp. have been shown to occur in the rhizosphere and on the surface of developing tubers, providing a level of protection against scab-forming species (Liu *et al*., 1995; Lorang *et al*., 1995). The period of susceptibility (i.e. until the lenticels on the latest-forming tubers become suberized) for most crops is relatively short (3-6 weeks from the onset of tuberisation) during which it is necessary to maintain moist or wet soil, or a thin film of water, around each tuber to prevent infection.

At the CUF experimental site there appeared to be no effect of frequency of irrigation on common scab severity when comparing daily with 4-6 day intervals. Current commercial practice therefore appeared sufficiently frequent, offering potential savings in both water application and drainage losses. Surprisingly, in 2008, irrigating for only 2 weeks after tuber initiation was as good as irrigating for 4 or 6 weeks. However, modelled soil moisture deficits showed that the 2-week treatments remained sufficiently wet to avoid common scab for 4-5 days after the last irrigation was applied, despite the pathogenic *Streptomyces* populations increasing immediately after the irrigation was stopped. The greater variation in moisture across ridge with drip *c.f.* sprinkler irrigation was associated with poorer scab control in both seasons at CUF and must be of concern since the same quantity of irrigation water applied as

for sprinkler resulted on worse scab control. It should be noted, however, that the soils at CUF are stony and have only a moderate hydraulic conductivity whilst wet and therefore suffer poor lateral movement from point-source emitters. Better water distribution, and therefore better scab control, could be expected on soils more suited to drip irrigation.

Further diagnostic advances, involving the use of pyrosequencing to study population dynamics of the whole bacterial microflora on initiating tubers, have raised the possibility that scab suppression by irrigation may be enacted at least partially by microbial taxa other than non-pathogenic actinomycetes. One preliminary pyrosequencing experiment conducted on a subset of soil samples from the 2008 irrigation trials at CUF identified the major bacterial taxa responding to irrigation treatments. At this site, the actinomycetes (including pathogenic *Streptomyces* spp.) represented only a small proportion of the total numbers of bacteria identified. Interestingly, at 6 weeks post tuber initiation, the largest increase in proportion of a single taxa in response to irrigation was observed in the genus *Pseudomonas*, levels of which were approximately 10 % of the sequences identified to genus in irrigated plots but less than 2% in unirrigated plots. While this does not establish a definite causal relationship between pseudomonads and scab suppression, it implicates *Pseudomonas* as a natural competitor of pathogenic *Streptomyces* in irrigated soils. Indeed strains of *Pseudomonas* are known to be effective biological control agents in soil (Haas and Défago, 2005).

Possibilities to reduce water consumption during scab control

The studies conducted at CUF, despite being conducted under high inoculum pressure in soils with generally low actinomycete populations, have indicated potential savings in water use and reduction in drainage losses through the use of precision irrigation regimes designed specifically for that site. Potential water conservation is expected to be even more significant at sites that are less disease conducive. It may even be possible to identify those soils for potato cropping where irrigation during the critical scab control period is not required at all. For example, under high rainfall conditions at the commercial sites in 2008 at Cobrey Farms, the unirrigated site had less severe common scab than the site that was irrigated during the critical scab control period. A number of site and seasonal specific factors are expected to influence the amount and duration of water required for scab control. Optimising the use of water for a particular site will therefore require prior information on soil types and structures, natural bacterial biodiversity and cultural practices (including cultivation methods, cultivar susceptibility, irrigation systems, fertiliser and pesticide applications) in addition to careful monitoring of soil moisture deficits and distribution of irrigation water applied. These issues have been carefully considered and general practical advice for growers has been formulated in Section 5 of the above results of this report, to assist the goal of consistent common scab control with minimal water input. .

Water savings, compared with current practices, are not expected across all potato production. Soil structure is expected to play an important role. For example, in soils with cloddy structure or coarse non-structured soils (e.g. sands), it may be difficult to maintain sufficient soil moisture over the entire surface area of the tuber without irrigating frequently. Additionally, soils with poor structure or fine-structured soils composed predominantly of clay particles can slump, consolidate or cap following rainfall or irrigation and make them hydrophobic with respect to water infiltration. Growers often respond to such circumstances by over-watering in their anxiety to avoid common scab, in many cases leading to waterlogging in some parts of the soil profile whilst still not completely wetting the targeted area. This leads to a waste of an increasingly scarce and valuable resource.

Knowledge of the level of natural suppression of pathogenic *Streptomyces* spp. in different soils available to a grower will assist decision support. For example, common scab susceptible

cultivars may be confined to less scab-conducive soils, where the amount of irrigation for control should be less than that required in more disease-conducive soils. Such knowledge will only be acquired through laboratory analysis of the initiating tubers and would be refined by repeating such analysis over a range of seasons. The understanding and optimisation of requirements for scab control is therefore likely to require ongoing location-specific investigation.

Even in the scab conducive situation at CUF, optimising the frequency and critical period over which irrigation was found to suppress the pathogens on developing tubers could lead to potential water savings without affecting the degree of common scab control. Table 14 charts the actual amounts of water (in mm) applied in each irrigation treatment in 2008. Limiting the period over which irrigation was applied to 4, rather than 6, weeks after tuber initiation resulted in a saving of around 25%, irrespective of whether the water was applied by drip or sprinker. Furthermore, by applying water at 4-6 day intervals rather than daily, a further water saving of 22% or 13% was made when using dripper or sprinkler over the 4 week period. Additional savings in run-off drainage water are shown in Table 15. By shortening the period of irrigation from 6 to 4 weeks after tuber initiation, drainage water losses were reduced by 50% with daily irrigation and over the 4 week period were further reduced by 62% with drip and 41% with sprinkler by lengthening the irrigation interval to 4-6 days.

TABLE 14: AMOUNTS OF WATER APPLIED (MM) DURING CUF IRRIGATION TRIALS IN 2008

Irrigation period (weeks after initiation)	Drip		Sprinkler	
	Daily	4-6 day interval	Daily	4-6 day interval
2	41	30	41	23
4	86	68	86	75
6	115	90	115	90

TABLE 15: AMOUNTS OF DRAINAGE WATER WASTED (MM) DURING CUF IRRIGATION TRIALS IN 2008

Irrigation period (weeks after initiation)	Drip		Sprinkler	
	Daily	4-6 day interval	Daily	4-6 day interval
2	41	30	41	23
4	86	68	86	75
6	115	90	115	90

Further research and development needs

- Many of the findings of this project are likely to be site specific and there is therefore a need to test optimised control measures and the principals on which they have been based over a wider range of locations. In particular, the potential for further reducing water inputs at sites where disease pressure is lower than that experienced at CUF merit further investigation.
- The pyrosequencing studies performed in 2009 were not original planned as part of the project and were therefore financially constrained and conducted as preliminary investigations only. Very interesting results, on the potential involvement of competing *Pseudomonas* spp. and other possible bacterial antagonists in the suppression of scab-forming streptomycetes, justify continuation of more detailed investigation using this technique. DNA extracted from a wide range of field sites is already stored at CSL and would be available and suitable for such further investigation which enables parallel analysis of several thousand DNA sequences from complex populations, and would shed valuable light on population dynamics and inform the design of future studies and optimised control measures.

- Further investigation into the mode of action of the fungicide fludioxinil (Syngenta Crop Protection) as a seed treatment against common scab is justified following favourable reports of its activity in field trials at SAC and CUF. In particular, a need to ensure sustainability of disease control through investigation of its activity on beneficial, in addition to pathogenic, microorganisms is recommended.

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4. Project deliverables

1. New molecular diagnostic methods including real-time PCR assays for detection and quantification of pathogenic *Streptomyces* species and conventional PCR assays for their identification have been developed and/or validated. Tests are currently available through the CSL diagnostic services. The details of these tests will be published in a plant pathology journal along with the other results from the project and will be freely available to other laboratories wishing to apply them.
2. Practical guidelines on maximizing common scab control with minimum water input will be disseminated via Potato Council Ltd. to the potato industry.