Final Report

Common scab control: reducing the irrigation water requirements and the effect of beneficial soil micro-organisms and biofumigation

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1. SUMMARY

1.1. Aims of the project

The aims of the project were a) to improve irrigation scheduling for varieties that are less susceptible to common scab than Maris Piper and for salad potato crops where irrigation is often continued for 8 weeks after tuber initiation (TI); b) gain an improved understanding of the mechanism by which irrigation reduces the population build-up of pathogenic *Streptomyces* on tubers; c) better understand the effect of beneficial soil micro-organisms and biofumigation on common scab; d) determine how soil structure within the ridge or bed influences the optimal irrigation regime for scab and e) quantify the effects of over-watering on root and crop growth, tuber health and quality and nitrogen use efficiency.

1.2. Methodology

Field and glasshouse experiments were set up by Cambridge University Farm (CUF) and Fera in 2011-2013 to improve understanding of how irrigation and biofumigation control the development of common scab. A range of irrigation regimes and different soils were used in the programme of experiments. The size and dynamics of the populations of pathogenic *Streptomyces* and their potential antagonists were determined using quantitative PCR from DNA extracted from the peel of potato tubers and compared with the incidence and severity of common scab on the surface of tubers. Assessments of other tuber disorders such as powdery scab, cracking and internal defects under the contrasting irrigation regimes were made. In some experiments where soils were maintained at or above field capacity, soil nitrogen uptake was measured to assess the effect of over-watering on nitrogen economy.

1.3. Key findings

1.3.1. General overview

Despite contrasting dry and wet periods between seasons during TI and the subsequent 4-week period, the incidence and severity of common scab over the duration of the project was very low compared with previous work with irrigation in the same fields. In 2011, very dry soils during the period between planting and

emergence resulted in low common scab severity. In 2012, perhaps not surprisingly given the quantity and frequency of rain that fell before and during the 4 weeks after TI, there was little development of common scab and no effect of irrigation regime. However, the severity of scab in unirrigated Maris Piper plots (< 1 % SA infected) was the lowest ever recorded at CUF and disease severity under rainfed conditions was lower than the most successful irrigation treatments operated over the last 20 years at CUF. Disease severity was again low in 2013 but unirrigated Maris Piper was worse than other irrigation treatments with irrigation regime having no effect in most other varieties.

On outside experimental sites in commercial fields of Maris Piper, maintaining a soil moisture deficit (SMD) of < 10 mm reduced common scab compared with allowing the SMD to increase to 25-35 mm the during scab control period, indicating that wet soils following TI are important in ensuring freedom from unacceptable scab infection in this variety.

In all pot experiments at Fera, soil moisture had no significant effect on common scab infection. However, differences in scab severity were detected in pots planted with soils dug up from commercial fields with different textures. Tubers grown in a loamy sand soil had consistently greater common scab severity than in sandy loam, sandy clay loam, peat or silt loam soils but there was no effect of moisture level on scab. Inoculating soil with *Streptomyces turgidiscabies* increased disease incidence and severity but irrigation regime again had no effect. Biofumigation with Caliente mustard had inconsistent effects on common scab. Tubers grown in pots using Caliente mustard biofumigated soils from two of the three fields sampled did not produce appreciable common scab. However, tubers grown in soil from one field that had been treated with biofumigant developed more common scab than in soil collected from the same field but where biofumigant had not been successfully grown or incorporated. Again, the soil moisture level maintained in the pot had no effect on common scab incidence or severity. Where the soil in pots was irradiated, in the absence of microorganisms other than the scab pathogen, irrigation level alone was shown to have no effect on populations of pathogenic *Streptomyces* or on development of disease, indicating that an intact soil microflora was required to mediate control by irrigation. However, in the presence of microorganisms, there were again few or no effects of irrigation on pathogen populations or scab severity.

1.3.2. Varietal differences in scab in response to irrigation regimes

Irrigation regimes which maintained soils at field capacity during the 4 weeks post-TI had very low severity of common scab even in the extremely susceptible variety Maris Piper (0.6-4.7 % surface area (SA) infected and < 1 % SA in all other varieties), irrespective of irrigation regime. Reducing the frequency of irrigation from daily applications to every 4-7 days at a trigger SMD of 15 mm resulted in similar scab severity in most varieties other than Maris Piper, providing evidence that soils could be maintained in a drier state for varieties less susceptible to scab than Maris Piper. The varieties Desiree, Estima, Flair, King Edward, Melody, Nectar, Sylvana and Venezia all have the potential to be irrigated less frequently and at higher SMDs than the maincrop Maris Piper and the salad variety Maris Peer. With varieties such as Bute, Jelly, Lanorma, Orchestra, Regina, Vales Sovereign and Volare, even more extreme irrigation practices could be employed (see 1.4 Practical recommendations).

1.3.3. Length of control period in salad potatoes

In the experiments examining the correct length of control period for salad potatoes, scab severity was low in both seasons but there was no indication that irrigation for 6 or 8 weeks was more successful with respect to control of scab or pack-out than irrigating for 4 weeks in low-susceptibility varieties such as Venezia or Regina and even in the susceptible variety Maris Peer, irrigation could be reduced to 6 weeks rather than the current recommendation of 8 weeks. This is despite the small tuber size of the salad crops grown, with many tubers in the 25-35 mm size grade. It is a widely-held view that tubers < 35 mm in diameter may be still at risk from common scab infection owing to them having unsuberized lenticels, but that did not appear to be the case in the experiments conducted in this project.

1.3.4. Delayed-start irrigation

Delaying the start of irrigation until 1 week after TI slightly increased the scab severity and reduced the packout in Maris Piper in every experiment where this regime was

tested but none of the effects were statistically significant. It did not matter whether the period of delayed-start irrigation was ended 3 or 4 weeks after TI or whether the regime applied irrigation daily or at 4-6 day intervals at larger SMDs. In all other varieties tested, scab was extremely low and the delayed start irrigation regime produced equally good scab suppression to irrigation regimes commencing at TI. This indicates that whilst populations of pathogenic *Streptomyces* may not be detectable on the surfaces of tubers until 2 weeks after TI (see 1.3.5 below), there may be a lag in altering the populations of antagonists following commencement of irrigation which may lead to increased disease in very susceptible varieties e.g. Maris Piper, but delayed-start irrigation could be used in less susceptible varieties to reduce the pressure on irrigation and shorten the time of scab control.

1.3.5. Time course of *Streptomyces* populations

The time course of populations of pathogenic *Streptomyces* (also referred to as *txtA Streptomyces*) was broadly similar to previous work, in that significant populations of pathogens could not be detected on the surface of Maris Piper tubers until at least 2 weeks after TI and this was delayed by a further week in crops maintained close to or at field capacity compared with drier or unirrigated soils. The rate of increase in pathogenic *Streptomyces* in Maris Piper in the week after detection was similar for all irrigation regimes but there were generally significantly more pathogenic *Streptomyces* 4 weeks after TI in unirrigated crops and crops allowed to reach 15 mm SMD than where crops were kept at field capacity. In the last season of the project, however, small quantities of pathogenic *Streptomyces* could be detected 1 week after TI even in very wet irrigation treatments. Overall, peak populations of pathogenic *Streptomyces* were lowest in 0 SMD treatments, with 15 SMD and delayed-start irrigation treatments having fewer pathogens than unirrigated treatments, irrespective of variety. However, in Desiree there was little evidence that irrigation regime had any consistent effect on the time course of development of *Streptomyces* populations.

Varieties with lower susceptibility to common scab and lower severity of infection in this project (e.g. Flair, Jelly, Melody, Sylvana and Vales Sovereign) in general had lower peak populations of pathogenic *Streptomyces* than Maris Piper, lending credence to the view that suppression of the build-up of pathogens plays a key role in

disease severity. However, there were some exceptions to this general pattern. In 2013, there were no significant differences in pathogenic *Streptomyces* populations on tubers 4 weeks after TI in eight varieties (excluding Maris Piper), but there were few differences in scab severity either. The very low severity of common scab observed in 2011 in all varieties, despite the dry season, was associated with undetectable populations of pathogenic *Streptomyces* on developing tubers in both field and pot experiments (which used the same soil). Whilst total *Streptomyces* populations also increased to a peak around 4 weeks after TI, the absolute populations were much lower than found in previous experiments at the same site, indicating inhibition of growth of *Streptomyces* of some kind.

1.3.6. Tuber cracking and internal defects

In irrigation experiments at CUF, external tuber cracking was more severe where soils were maintained at, or above, field capacity during TI than where a modest SMD (e.g. 15 mm) was used during the scab control period. Tuber cracking was exceptionally high in some varieties where soils were maintained above field capacity during the scab control period and maintaining soils with a small SMD (e.g. 10-15 mm) rather than field capacity reduced the cracking significantly, albeit not to the level of treatments which maintained drier soil. The most susceptible varieties to cracking from over-watering were Safari, Estima, Vales Sovereign (both linear and superficial cracking), Melody, Orchestra and Nectar. All of these varieties were worse than Maris Piper, which has been shown to be highly sensitive to cracking following over-watering in previous experiments at CUF. The maincrop varieties Desiree and Saturna and the two salad varieties Regina and Venezia appeared to be very insensitive to over-watering with respect to external cracking and internal defects such as internal rust spot and hollow heart.

1.3.7. Nitrogen uptake

Nitrogen uptake by plants was reduced by over-watering during the 3 weeks after TI, possibly as a consequence of nitrogen leaching below rooting depth and of denitrification. This reduced canopy size, canopy longevity and yield. Over-watering later in the season had relatively little effect on nitrogen uptake or crop performance,

indicating greater sensitivity of the rooting system to waterlogging early in the crop's life.

1.3.8. Effects of ridge cloddiness structure

An experiment in 2011 showed that the cloddiness of the ridge structure at planting had little effect on common scab incidence and severity and that irrigation regime was the over-riding factor in determining the level of control of scab. Eight trials in commercial fields examining the aggressiveness of declodding on common scab showed no effect of web pitch, star spacing, rotor or forward speed on scab incidence and severity, indicating that ped size distribution may have less effect on scab development than previously thought. This has been supported by similar data from Potato Council Project R459, "improving cultivation practices in potatoes".

1.3.9. Antagonists

Pyrosequencing of potential antagonists demonstrated differences in populations of the major bacterial phyla *Actinobacteria, Bacteroidetes, Proteobacteria and Acidobacteria* in soils used for experiments, although no clear correlation with common scab severity was found. The analysis of similarities (ANOSIM) which tested whether the potential antagonist microbial community composition was linked either to location (i.e. soil or field) or to populations of pathogenic *Streptomyces* showed that there was no significant correlation between community composition at the genus level and irrigation level. However, there was a weak correlation between community structure and location. There was no apparent link between community structure and levels of pathogenic *Streptomyces,* as assessed by quantitative real-time PCR using an assay for the *txtA* pathogenicity gene (which is common to pathogenic *Streptomyces*).

1.4. Practical recommendations

1.4.1. Varietal scheduling

Irrigation regimes for common scab could be adapted according to varietal susceptibility.

Maris Piper was clearly much more susceptible to scab than the other varieties examined and there was considerable evidence that other, less-susceptible varieties can be irrigated for shorter periods or at greater SMDs than Maris Piper. Table 1 shows a tentative grouping of varieties for common scab control using different soils and irrigation schedules.

Several pot experiments at Fera showed no effect of irrigation regime on common scab, even using soils from commercial fields. This indicates that changes in microbial activity in response to soil physical conditions (e.g. water content) may be very different in pots compared with field situations and the data from glasshouse studies need interpreting with care in relation to actual field experiences.

Table 1. Maximum soil moisture deficits (mm) for common scab control in different varietal scheduling groups used in the project

	Group	1. Sensitive	2. Intermediate	3. Insensitive
Soil texture	Varieties	Maris Piper Maris Peer	Desiree Estima Flair King Edward Melody Nectar Sylvana (Safari)† Venezia	Bute Jelly Lanorma Orchestra Regina Vales Sovereign Volare
Sand		9.8	14.6	18.8
Loamy Sand		12.0	17.9	23.1
Sandy Loam		13.4	20.0	25.8
Sandy Silt Loam		14.4	21.5	27.7
Silt Loam		16.3	24.3	31.4
Clay Loam/Clay‡		14.4	21.5	27.7

Notes:
Soil moisture deficit for top 25 cm of ridge
Stone-free ridge profile
†Safari: tentative
‡Excessively cloddy soils may need to be maintained at a smaller SMD

1.4.2. Delayed start irrigation

For all varieties other than Maris Piper, delaying start of irrigation until 1 week after initial TI would produce equally good control of common scab to commencing irrigation at TI.

Delayed-start irrigation timing should be based on initial TI as using the date of 50 % TI in variably-emerging fields could lead to more scab infection.

Since it was consistently observed that pathogenic *Streptomyces* did not populate the periderm of tubers in great numbers until 2 weeks after TI, it is likely that the crucial phase for wetting soils is around 1-2 weeks after TI rather than at initiation. However, in Maris Piper delaying irrigation until 1 week after TI resulted in poorer control of scab than starting at TI, irrespective of the SMD (0 or 15 mm) maintained during weeks 2-4 of the susceptible period. In all other maincrop varieties tested in the program, the delayed start irrigation regime produced similar control of scab to starting at TI, so this could be a practical recommendation for less susceptible varieties. Caution is needed however, as soils need to be wetted thoroughly at 7 days after TI and this may be more difficult than at TI as ridges may have dried out, hindering wetting. Additionally, emergence in most varieties in the experimental programme was short (e.g. 3-5 days from first to 50 % emergence) but in commercial fields emergence is generally more protracted (e.g. 4-7 days or more). Most varieties in the project tuberized 15-20 days after emergence (initial or 50 %), with only Bute and Nectar taking longer (24 days).

1.4.3. Duration of irrigation for salad varieties

A 6-week period for scab control is sufficient in susceptible varieties such as Maris Peer and probably a 4-5 week period in less susceptible varieties such as Regina or Venezia.

There was no indication that irrigation for 8 weeks was more successful in preventing scab than irrigating for 6 weeks. This would reduce the risk of over-watering, wastage of water and delaying skinset post desiccation.

1.4.4. Risk of over-watering

Over-watering during TI and the scab control phase should be avoided as this increases the incidence of tuber cracking and rotting diseases and reduces nitrogen uptake and promotes early senescence in some varieties.

Mid-and late over-watering (more typically resulting from intense or prolonged mid-summer rainfall events rather than irrigation) had relatively little effect on crop growth and quality, with the exception of varieties where wet soils encouraged tuber cracking centred on lenticels.

1.4.5. Soil structural conditions

Growers should not be producing overly-fine seedbeds as this does not improve control of common scab.

There was limited evidence that aggressive declodding operations (e.g. closed stars and narrow-pitch webs) or cultivation regimes which produced fine-structured ridges reduced common scab compared with the coarser-structured ridges (e.g. large web pitch) typically used for processing potatoes. This finding has also been supported by work from Potato Council Project R459 examining cultivation practices. Growers often persist in producing overly-fine ridges to avoid common scab, whereas in reality ridges need to be composed of very large clods or peds (e.g. 15-45 mm diameter) to be at an increased risk of common scab under irrigated conditions.

2. INTRODUCTION

Control of common scab to the standards required by supermarkets is generally better where soils are kept wet following TI than where irrigated at higher SMDs or where rainfall is not supplemented with irrigation. However, experiments have frequently demonstrated no significant difference between wet treatments and those maintaining a maximum SMD of 15mm, giving potential savings in water application and losses from drainage. There are good opportunities to reduce the irrigation requirements through better understanding of the interactions between seedbed conditions, soil water availability, variety and the management of soil microorganisms affecting pathogenic *Streptomyces* species. Work by staff at CUF, FERA and SAC (Elphinstone *et al.* 2009) identified that populations of pathogenic *Streptomyces* were maintained at lower levels in Vales Sovereign in moist and unirrigated soils than in the susceptible variety Maris Piper, suggesting that control strategies for scab could differ appreciably across varieties. However, these results were obtained from a limited range of soils and there is a requirement for more data on other soils so that better recommendations can be given to the industry. Formulation of a ranking order for scab susceptibility and optimized irrigation scheduling regimes for newly-introduced varieties would enable savings in irrigation since current recommendations are typically based on susceptible benchmark varieties.

Potato Council-sponsored work on common scab during 2007-2010 (Elphinstone *et al.* 2009; Thwaites & Stalham 2010) showed that our knowledge of the mechanism of control of populations of pathogenic *Streptomyces* species under contrasting soil water regimes is rudimentary and further work was needed to look at possible antagonists to *Streptomyces*. This project (R448) was instigated in 2011 and staff from FERA and CUF have jointly investigated the antagonist theory in the control of common scab in greater detail.

Whilst striving to achieve adequate soil wetness during scab control, many growers over-irrigate, either uniformly or in parts of fields owing to poor distribution of water from rainguns. Quantifying the losses from detrimental effects (e.g. growth cracking, lenticel eruption, internal rust spot, processing quality and tuber disease) of over-irrigating will help guide better timing and application practices throughout the industry, reduce losses of water and nitrogen and improve profitability.

The main aims of the project were to improve irrigation scheduling for control of common scab in different varieties by a) investigating the effects of different irrigation schedules and non-water strategies (e.g. ridge structure and biofumigants) in diverse soil types on the populations of groups of microorganisms linked to suppression of common scab and b) determine the extent to which scab control is due to these organisms and how much (if any) is due to the effect of different moisture levels on *Streptomyces* spp.

3. MATERIALS AND METHODS

3.1. 2011 experiments

There were three experiments at CUF and two with Greenvale AP Ltd in Norfolk investigating the effect of irrigation regime on common scab and the populations of *Streptomyces* and antagonists on tubers. Work at Fera concentrated on pot experiments designed to improve the understanding of the environmental factors affecting common scab and soil bacterial communities.

3.1.1. CUF and Greenvale AP experiments

3.1.1.1.1. Expt 1

Experiment 1 was a randomized split-plot design, with all combinations of three varieties (Estima, King Edward, Maris Piper), two degrees of cloddiness within ridges (Cloddy, Fine) and four irrigation regimes. The regimes were rainfed only (Unirr), irrigated with 3-5 mm to maintain 0 mm SMD at the start of each day from onset of TI for 4 weeks (0 SMD), irrigated with 15 mm at 15 mm SMD from TI for 4 weeks (15 SMD) and irrigated as 0 SMD between 1 and 3 weeks after TI (TI 1-3). There were three replicate blocks, with main plots comprised of cloddiness treatments and sub-plots were combinations of variety and irrigation treatments.

The experiment was conducted on a sandy clay loam soil (51% sand, 28% silt and 21% clay) with 7% stone, 3.3% organic matter content and a pH of 6.2. The experiment was planted on 11 April 2011 using 25-35 mm SE1 seed (Estima 18.4 g, King Edward 22.1 g, Maris Piper 23.4 g) at a within-row spacing of 25 cm in 76 cm rows. The Cloddy main-plots were created by using a set of Massey Ferguson discs

on ploughed soil that had been allowed to dry for 11 days and then were ridged using a fixed-body Cousins ridger. The Fine plots were created on the same day using a single pass of a Howard rotavator on the ploughed soil which was then ridged up. The seed was dibbed 12 cm deep into pre-formed ridges, which were raked after planting to re-form the original ridge. Plots were 6 m long and four rows wide. A concentrated (34.2% vol.) solution of ammonium nitrate was applied at a rate of 180 kg N/ha post-planting but pre-emergence. Herbicides and fungicides were applied as required to maintain the experiment.

Irrigation was scheduled using the CUF Potato Irrigation Scheduling Model based on meteorological data obtained from a Delta-T Devices weather station *c*. 200 m from the experiment. The different irrigation treatments were timed based on the mean SMD across all varieties and cloddiness treatments. Mini sprinklers (Dan Modular Small Swivel Yellow Anti-mist nozzles) on 1 m risers were installed at 1 m spacing between the two central harvest rows of each plot. They were adjusted to run at very low pressure (*c*. 0.55 bar) to reduce the risk of misting and drift into adjacent plots. The sprinkler systems were calibrated at the beginning of the season to determine application rates in mm/hour at 0.55 bar pressure in each plot. The mean application rate was 12 mm/hour. Irrigation for 0 SMD treatments was timed to start automatically at 05:00h, and the application rate adjusted between 3 and 5 mm/day according to evapotranspiration demand, to ensure that plots were returned to Field Capacity. Other irrigation treatments were watered between 08:00 and 18:00h. Irrigation commenced in 0 and 15 SMD treatments at the onset of TI to ensure that the respective trigger SMDs were reached at 50 % TI. The TI 1-3 treatment received its first irrigation 7 days after 50% TI. After the scab control period had been completed (4 weeks after TI), all irrigated treatments were watered with 15-20 mm whenever the SMD subsequently reached 20 mm. Irrigation amounts applied are detailed in Table 2.

Table 2. Expt 1: Rainfall, irrigation and drainage (mm) during the season

	Unirr	0 SMD	15 SMD	TI 1-3
Rainfall				
TI + 4 weeks	64	64	64	64
Emergence to harvest	180	180	180	180
Irrigation				
TI + 4 weeks	0	81	70	54
Emergence to harvest	0	247	207	181
Drainage				
TI + 4 weeks	0	55	34	28
Emergence to harvest	0	85	51	39

In each plot of two replicate blocks, soil water content was measured at 60-minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed at emergence in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm and logged using a Delta-T Devices DL2 logger.

Plant emergence was recorded every 1-2 days in each plot by counting the number of plants emerged in two harvest rows. Tuber initiation was determined by digging two plants per plot every 1-2 days from 12 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 was measured weekly after emergence until final harvest using a grid in two positions in each plot.

Samples of 10 tubers from five plants per plot were sent to Fera, in perforated plastic bags 1, 2, 3, and 4 weeks after TI for determination of populations of total *Actinomycetes* and pathogenic *Streptomyces*. These were assessed by quantitative real-time PCR, using assays for 16S rDNA and the *txtA* pathogenicity gene (which is common to pathogenic *Streptomyces*), respectively. Populations of potential antagonists were assessed from tubers sampled 4 weeks after TI (the date that peak pathogen populations were expected) from King Edward and Maris Piper Fine cloddiness treatments only.

A final harvest of 12 plants was taken on 12 September. The tubers were graded, counted and weighed in 10 mm increments. A representative sample of tubers weighing *c*. 500g was dried at 90°C for 48h to measure tuber dry matter concentration ([DM]). All remaining tubers were assessed for incidence and severity (% surface

area (SA) infected) of common scab in the categories of 0, 0-1, 2-5, 5-10% SA and then in 10% increments. The number of tubers with powdery scab (*Spongospora subterranea*) was recorded. Tubers were also assessed for type, incidence and severity of tuber cracking and growth defects at final harvest. The cracks were divided into two types: a) 3-8mm deep linear cracks traversing either along the longitudinal axis or from the apical end and b) superficial (1-3 mm deep) cracks emanating from a focus centred on a lenticel and usually with 2-4 cracks arising from the same point. The SA of the tuber covered by superficial cracks was scored in categories of 0, 0-1, 2-5, 5-10% SA and then in 10% increments.

3.1.1.1.2. Expt 2

Experiment 2 was a randomized block design, with all factorial combinations of three varieties (Maris Piper, Saturna, Vales Sovereign) and five irrigation regimes. The regimes were rainfed only (Unirr), irrigated with 15-25 mm at 25 mm SMD from emergence (25 SMD), irrigated with 4-6 mm daily to maintain soil over field capacity at the start of each day from onset of TI for 3 weeks (OW 0-3), as OW 0-3 but over-watered from 3-6 weeks after TI (OW 3-6) and as OW 0-3 but over-watered from 10-13 weeks after TI (OW 10-13). There were three replicate blocks.

The experiment was conducted adjacent to Expt 1 on a sandy clay loam soil (54% sand, 27% silt and 20% clay) with 6% stone and 3.6% organic matter content. The experiment was planted on 6 April 2011 using SE1 seed (Maris Piper 23.4 g, Saturna 46.5 g, Vales Sovereign 22.4 g) at a within-row spacing of 25 cm in 76 cm rows. The seed was dibbed 12 cm deep into ridges pre-formed using a Rumptstad rototiller, which were raked after planting to re-form the original ridge. Plots were 8 m long and four rows wide. A concentrated (34.2% vol.) solution of ammonium nitrate was applied at a rate of 180kg N/ha post-planting.

Irrigation scheduling and applications were as Expt 1. Irrigation for over-watered treatments was timed to start automatically at 05:00 and 19:00h and the application rate adjusted between 4 and 6mm/day (i.e. 2-3mm twice daily) according to evapotranspiration demand, to ensure that plots remained above field capacity during the day. A pre-dose of 20 mm was applied at the commencement of each over-watered period to ensure the soil was above field capacity. Once each over-watered

treatment was completed, it was irrigated according to the protocol in the 25 SMD treatment. Other treatments were irrigated between 08:00 and 18:00h. Irrigation amounts applied are detailed in Table 3.

Table 3. Expt 2: Rainfall, irrigation and drainage (mm) during the season

	Unirr	25 SMD	OW 0-3	OW 3-6	OW 10-13
Rainfall					
During OW period	-	-	30	60	23
Emergence to harvest	182	182	182	182	182
Irrigation					
During OW period	-	-	110	106	102
Emergence to harvest	-	182	278	243	247
Drainage					
During OW period	0	-	71	91	57
Emergence to harvest	0	17	98	94	90

In all Maris Piper plots of two replicate blocks, soil water content was measured at 60-minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed at emergence in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm and logged using a Delta-T Devices DL6 logger.

Emergence, TI, ground cover, common scab, powdery scab and cracking measurements were as detailed for Expt 1. Four sequential harvests of 12 plants from guarded areas were taken on 13 June, 3 July, 8 August and 13 September. Foliage from each plot was weighed, sub-sampled to *c*. 1kg and dried. Dried haulm and tuber samples were analysed for total N content at NRM Ltd.

Immediately after the crop was sampled at each sequential harvest, soil cores were taken with an auger (‘Dutch’ type, 55mmm external diameter) from the 15 plots growing Maris Piper. Cores were taken from between plants in either row 2 or row 3, at depths of 0-30, 30-60 and 60-90 cm from the soil surface, and from the furrow, between rows 2 and 3 at depths of 0-30 and 30-60 cm from the soil surface. The cores were placed in polythene bags and kept in a cool-box together with ice-packs

before being dispatched within 24 hours to NRM Ltd for analysis for soil mineral N (SMN).

3.1.1.1.3. Expt 3

Experiment 3 was a randomized block design, with all factorial combinations of four varieties (Jelly, Maris Piper, Sylvana, Vales Sovereign) and four irrigation regimes. The regimes were rainfed only (Unirr), irrigated with 4-6 mm daily to maintain soil at field capacity at the start of each day from onset of TI until desiccation (0 SMD), irrigated with 15 mm at 15 mm SMD from TI (15 SMD) and irrigated with 35 mm at 35 mm SMD from TI (35 SMD). There were three replicate blocks.

The experiment was conducted adjacent to Expt 2 on a sandy clay loam soil (52% sand, 27% silt and 21% clay) with 5% stone and 3.9% organic matter content. The experiment was planted on 7 April 2011 using 25-35 mm (*c*. 22 g) SE1 seed at a within-row spacing of 25 cm in 76 cm rows. The seed was dibbed 12 cm deep into ridges pre-formed using a Rumptstad rototiller, which were raked after planting to re-form the original ridge. Plots were 5 m long and eight rows wide with the inner rows used for harvest. A concentrated (34.2% vol.) solution of ammonium nitrate was applied at a rate of 180 kg N/ha post-planting.

Irrigation scheduling and application were as Expt 1, with three lines of sprinklers per plot. Irrigation for 0 SMD treatments was timed to start automatically at 05:00 and the application rate adjusted between 4 and 6 mm/day according to evapotranspiration demand, to ensure that plots were at field capacity at the start of the day. Other treatments were irrigated between 08:00 and 18:00 h. Irrigation amounts applied are detailed in Table 4.

Table 4. Expt 3: Total rainfall, irrigation and drainage (mm) during the season

	Unirr	0 SMD	15 SMD	35 SMD
Rainfall	166	166	166	166
Irrigation	0	408	235	140
Drainage	0	206	55	18

Emergence, TI, ground cover, common scab, powdery scab and cracking measurements were as detailed for Expt 1. The experiment was desiccated on 2

September with Reglone at a rate of 3 l/ha. A hand-harvested area of eight plants was dug on 20 September before the rest of the plot was lifted by machine on 21 September.

3.1.1.1.4. Expts 4 and 5

Experiments 4 and 5 were located in commercial fields of Maris Piper grown by Greenvale AP which were irrigated with solid-set sprinkler systems (Coverline, Wroot Water). In each field, a representative area of six lines of sprinklers was selected and a randomised pattern of plots laid out with six replicates of two treatments (irrigation withheld from TI for 4 weeks (Dry) and irrigated according to a CUF schedule from TI for 4 weeks (Wet)). Each 'plot' was located in the center of the over-lap patterns of the circular sprinklers. In Dry plots, once the system had been pressure-tested, the four sprinklers contributing to the irrigation had their heads changed for Nelson, restricted-angle sprinkler heads which allowed the plot, through adjustment of the rotating sector, to remain unirrigated even though the rest of the line was operating. Raingauges were positioned to measure the application rates at initial irrigations to perform a calibration and subsequently irrigation quantities were based on the time each line was operating. Dry plots were not protected from rainfall. Meteorological data for running the irrigation model was provided by the Met Office's MORECS service. The Wet plots were scheduled to be irrigated whenever the SMD reached 13 mm (Expt 4) or 14 mm (Expt 5), with the allowable SMDs based on soil texture. Emergence in each plot was determined by counting the number of plants emerged in 5 m of row every 1-3 days from initial emergence until > 90 % of plants had emerged. Starting at 14 days after initial emergence, TI was estimated every 2 days by counting the number of plants with tubers in a two-plant sample from each plot. Ground cover was estimated weekly as in Expt 1 on three replicates of each treatment.

Experiment 4 was located in Chapmans field, Raveningham, Norfolk on a clay loam (49% sand, 28% silt, 23% clay and 1.7% organic matter). It was planted 17 cm deep on 16 April with 35-45 mm E1 seed at a spacing of 32 cm. Experiment 5 was located in 18 Acres field, Somerleyton, Norfolk on a sandy clay loam (57 % sand, 24 % silt, 18 % clay and 1.4 % organic matter). It was planted 18 cm deep on 14 April with the

same seed at a spacing of 30 cm. Table 5 details the irrigation application and rainfall.

Table 5. Expts 4 and 5: Rainfall and irrigation (mm)

	Expt 4		Expt 5	
Irrigation treatment	Dry	Wet	Dry	Wet
Rainfall				
During scab period	76	76	54	54
Emergence to harvest	260	260	244	244
Irrigation				
During scab period	0	32	0	32
Emergence to harvest	70	102	52	84

Samples of 10 tubers from five plants per plot were sent to Fera, in perforated plastic bags from a harvest taken 4 weeks after TI for determination of populations of total *Actinomycetes*, pathogenic *Streptomyces* and populations of potential antagonists.

Both experiments were desiccated with split-dose Reglone, with Expt 4 desiccated on 19 August and 2 September and Expt 5 on 28 August and 5 September. A final harvest of 3 m of single row was taken on 7 September in Expt 4 and 14 September in Expt 5. Tubers were counted, graded, weighed and assessed for common scab and cracking as in Expt 1.

3.1.1.1.5. Expts 6 and 7

Experiment 6 was conducted on a clay loam soil (43 % sand, 37 % silt and 20 % clay) with 1.8 % OM content at Eversons, North Burlingham farmed by Greenseed International Ltd. The experiment was planted on 28 April using 35-45 mm Maris Piper seed at a within-row spacing of 20 cm in 91 cm rows. Treatments were all combinations of two degrees of aggressiveness of declodding (Coarse and Fine) created by altering the star spacing on the Grimme CS1500 destoner, three planter hood types (Cage, Round and Trapezoidal) and two planter hood pressures (Low and High). The different hood types were swapped on a Grimme GB215 planter. The experiment was a split-plot design with hood type as main-plots and destoner aggressiveness and hood pressure as sub-plots. There were three replicates and

plots were 30 m long and four rows wide, with the harvest area being confined to the middle 5 m of each plot.

Experiment 7 was conducted on a clay loam soil (39% sand, 42% silt and 20% clay) with 2.1% OM content at Goulders, Felmingham farmed by LF Papworth Ltd. The experiment was planted on 26 April using 45-50 mm Pentland Dell seed at a within-row spacing of 31 cm in 91 cm rows. It was a randomized block design with four replicates of factorial combinations of three destoning depths (Shallow, Normal and Deep) and two degrees of aggressiveness of declodding (Coarse and Fine) created by altering the star spacing on the Grimme CS1500 destoner. Plots were 30 m long and four rows wide, with the harvest area being confined to the middle 5 m of each plot.

3.1.2. Fera experiments

3.1.2.1.1. Expt 8

Experiment 8 tested the effect of moisture level on common scab in six field soils. Approximately 200kg of soil from six commercial potato fields (CUF, sandy clay loam; GVAP Somerleyton 18 Acres, sandy clay loam; GVAP Raveningham Chapmans, clay loam; G Shropshire R24, peat; Greenseed Galley Pit, loamy sand; and GVAP Taylor, silt loam) were supplied by CUF on 12 May 2011 to Fera. The samples were dug from potato ridges which had been planted but where plants had not yet emerged. The soils were immediately transferred to a cold store (4°C). On 15 June, the soils were placed in 7.5 l pots in a plant growth room that was set at 20°C (day), 16°C (night), 62% RH and 15 h full light. One minituber of *cv.* Maris Piper (Higgins Agriculture Ltd) was planted in each pot. The experimental design consisted of six soils, three watering regimes and four replicates. Treatments (soils x moisture) were randomised within blocks (replicates). Soils were watered to provide the following moisture regimes for mineral soils: Low, 16 to 20% moisture volumetric (*c*. 25 mm SMD); Medium, 25 to 30% moisture and High, 40 to 50% moisture (field capacity). For soils with high organic matter (Shropshire R6), the moisture levels were: Low, 40% (*c*. 25 mm SMD); Medium, 60% and High, 80% (field capacity). Moisture levels were assessed at least twice a week using a Delta-T Devices Theta Probe ML2 soil moisture probe and adjusted as required. Tuber initiation was estimated to be on 27 June, and 3 weeks after this date plants from blocks 1 and 4 were harvested and total

nucleic acid extracts prepared from whole tubers and soils. Tubers from blocks 2 and 3 were harvested on 3 November and assessed for common scab surface area (% severity).

3.1.2.1.2. Expt 9

Experiment 9 tested the effect of low and high soil moisture levels on common scab in soils artificially amended with *Streptomyces turgidiscabies*. Approximately 75 l of each of two soils supplied by CUF (CUF; and Greenseed Galley Pit as in Expt 8) were sterilised by autoclaving (120°C, 20 min) on two separate occasions. Soils were placed in 7.5 l pots in a plant growth room (conditions as above). A minituber of *cv.* Maris Piper was placed in each pot on 12 August. A 2 ml aliquot of liquid inoculum of *S. turgidiscabies* (isolate P6803) was added to the surface of each minituber in the inoculated treatments and covered with sterile soil. Sterile water was added to the non-inoculated treatments as controls. The experimental design consisted of two soils, two inoculum treatments (inoculated and non-inoculated), two watering regimes and four replicates. Treatments (inoculum x soils x moisture) were randomised within blocks (replicates). Soils were watered to provide the following moisture regimes: Low, 16 to 20 % moisture (*c*. 25 mm SMD) and High, 40 to 50 % moisture (field capacity). Tubers from blocks 1 and 2 were harvested at approximately 4 weeks after TI (7 October) and processed for whole nucleic acid extractions. Approximately 100 g soil was placed in labelled zip-lock polythene bags and stored at 4°C until required for DNA extractions. Tubers from blocks 3 and 4 were harvested on 17 January 2012 and assessed for common scab surface area (% severity).

3.1.2.1.3. Expt 10

Experiment 10 examined the effect of biofumigation treatment on common scab. Soil samples (approximately 75 l each treatment) were collected from three Branston sites that had been treated with hot (Caliente) biofumigant mustard prior to planting. At each field, soil from areas where mustard treatment had not been successful was collected as an untreated control. Soils were added to 7.5 l pots, then one minituber of Maris Piper was added to each pot on 28 June. Pots were placed in a plant growth

room as detailed for Expt 8. The experimental design consisted of six soils (three with biofumigant treatment, three without), two watering regimes and four replicates. Treatments (soils x moisture) were randomised within blocks (replicates). Soils were watered to provide the following moisture regimes for mineral soils: Low, 16 to 20 % moisture (*c*. 25 mm SMD) and Medium, 25 to 30 % moisture. Tubers from blocks 1 and 4 were harvested at 4 weeks after TI and total DNA extracted from whole tubers and soils. Tubers from blocks 2 and 3 were harvested on 3 November for common scab assessments.

3.1.2.1.4. Expt 11

Soil samples were delivered to Fera from CUF and from Branston's biofumigant trial fields (Table 6). Bacterial communities were analysed by 454 pyrosequencing an amplified portion of the conserved 16S rDNA from DNA extracted from each soil. Briefly, the 16S rDNA was amplified using the KAPA HiFi HotStart kit (Kapabiosystems, Woburn, USA) with the 454-16S fusion PCR primers:

16SFL: GCCTCCCTCGCGCCATCAG*NNNNNNNNCA****TGCTGCCTCCCGTAGGAGT***
16SRL: GCCTTGCCAGCCCGCTCAGTC**AGAGTTTGATCCTGGCTCAG**

Underlined sequences represent 454 sequencing primers and bold sequences are bacterial primers developed for the broad coverage of bacterial communities (Hamady *et al.* 2008). To be able to differentiate samples sequenced in multiplex, unique tags were added to the reverse primer indicated by *NNNNNNNN*. These were used to separate data from different samples after sequencing. Sequences of low quality were removed from the dataset, as were sequences shorter than 200bp. To obtain an overview of the taxonomic composition of bacteria present in the samples, the Naïve Bayesian rRNA classifier available from the Ribosomal Database Project (RDP, Wang *et al.* 2007; http://rdp.cme.msu.edu/) was used with a bootstrap value of 50 %.

Table 6. Expt 11: Soil samples analysed for the bacterial community structure

Sample name	Soil texture	Experiment
CUF	Sandy clay loam	Expt 8
Greenseed Galley Pit	Loamy sand	Expt 8
GVAP Raveningham Chapmans	Clay loam	Expt 8
GVAP Somerleyton 18 Acres	Sandy clay loam	Expt 8
GVAP Taylor	Silt loam	Expt 8
G Shropshire R24	Peat	Expt 8
Branston Mairs Treated	Sandy loam	Expt 10 - Treated
Branston Mairs Untreated	Sandy loam	Expt 10 - Untreated
Branston Hays Treated	Sandy loam	Expt 10 - Treated
Branston Hays Untreated	Sandy loam	Expt 10 - Untreated

3.2. 2012 experiments

There were three experiments at CUF and two with Greenvale AP Ltd in Norfolk investigating the effect of irrigation regime on common scab and the populations of *Streptomyces* and antagonists on tubers and other aspects of tuber quality.

At FERA, a controlled pot experiment was carried out in glasshouses investigating the effect of irrigation on common scab and the populations of *Streptomyces* and antagonists in different soils. There was also an experiment where soil was irradiated to kill microbes and then inoculated with pathogenic *Streptomyces*.

3.2.1. CUF and Greenvale AP experiments

3.2.1.1.1. Expt 12

Experiment 12 was a randomized block design, with all combinations of seven varieties (Bute, Desiree, Flair, Jelly, Maris Piper, Sylvana and Vales Sovereign) and five irrigation regimes. The irrigation regimes were rainfed only (Unirr), irrigated with 3-5 mm to maintain 0 mm SMD at the start of each day from onset of TI for 4 weeks (0 SMD), irrigated with 15 mm at 15 mm SMD throughout the season (15 SMD), irrigated with 35 mm at 35 mm SMD throughout the season (35 SMD) and irrigated as 0 SMD except no irrigation applied until 1 week after TI (TI 1-4). There were three replicate blocks.

The experiment was conducted on a sandy clay loam soil (56% sand, 25% silt and 19% clay) with 6% stone, 3.4% organic matter content and a pH of 6.5. The field was

cultivated with a Simba Solo at 18 and 36 cm on 12 August and 1 September 2011, respectively. It was then ploughed on 21 March and roto-ridged with a Rumptstad rototiller on 22 March. The experiment was planted on 19 April using 30-35 mm (Jelly 30-40 mm) SE seed (mean seed weight 26 g for 30-40 mm seed, Jelly 46 g) at a within-row spacing of 30 cm in 76 cm rows. The seed was dibbed 12 cm deep into pre-formed ridges, which were raked after planting to re-form the original ridge. Plots were 3.9 m long (7.8 m for Desiree and Maris Piper to permit additional *Streptomyces* sampling) and four rows wide. A concentrated (34.2 % vol.) solution of ammonium nitrate was applied at a rate of 200 kg N/ha post-planting. Herbicides and fungicides were applied as required to maintain the experiment.

Irrigation was scheduled and applied as detailed in Expt 1. Irrigation for 0 SMD and TI 1-4 treatments was timed to start automatically at 05:00h, and the application rate adjusted between 3 and 5 mm/day according to evapotranspiration demand, to ensure that plots were returned to Field Capacity. When daily demand exceeded 4 mm, irrigation was split into two equal doses, with the second at 19:00h. Other irrigation treatments were watered between 08:00 and 18:00h. Irrigation commenced in 0 SMD and 15 SMD treatments at the onset of TI to ensure that the respective trigger SMDs were reached at 50% TI. The TI 1-4 treatment received its first irrigation 7 days after 50% TI. Rainfall, irrigation and drainage amounts are detailed in Table 7.

Table 7. Expt 12: Rainfall, irrigation and drainage (mm) during the season (Maris Piper plots)

	Unirr	0 SMD	15 SMD	35 SMD	TI 1-4
Rainfall					
TI + 4 weeks	104	104	104	104	104
Emergence to harvest	299	299	299	299	299
Irrigation					
TI + 4 weeks	0	78	30	0	58
Emergence to harvest	0	271	162	105	245
Drainage					
TI + 4 weeks	12	91	38	12	67
Emergence to harvest	97	218	134	97	191

In each plot of Maris Piper in two replicate blocks, soil water content was measured at 60-minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed at

emergence in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm and logged using a Delta-T Devices DL2 logger.

Emergence, TI, ground cover, common scab, powdery scab and cracking measurements were as detailed in Expt 1. Samples of 10 tubers from five plants per plot were sent to Fera, in perforated plastic bags 1, 2, 3, and 4 weeks after TI for determination of populations of total *Actinomycetes* and pathogenic *Streptomyces*. These were assessed by quantitative real-time PCR, using assays for 16S rDNA and the *txtA* pathogenicity gene (which is common to pathogenic *Streptomyces*), respectively. Populations of potential antagonists were assessed from tubers sampled 4 weeks after TI (the date that peak pathogen populations were expected) from Desiree and Maris Piper treatments only.

A final harvest of 10 guarded plants was taken on 14 September (Bute, Desiree, Flair, Sylvana) and 19 September (Jelly, Maris Piper and Vales Sovereign). The tubers were graded, counted and weighed in 10 mm increments. A representative sample of tubers weighing *c*. 500 g was dried at 90°C for 48h to measure tuber dry matter concentration ([DM]). All remaining tubers were assessed for incidence and severity (% surface area (SA) infected) of common scab in the categories of 0, 0-1, 2-5, 5-10% SA and then in 10% increments. The number of tubers with powdery scab (*Spongospora subterranea*) was recorded. Tubers were also assessed for type, incidence and severity of tuber cracking and growth defects at final harvest. The cracks were divided into two types: a) > 3 mm deep linear cracks traversing either along the longitudinal axis or from the apical end and b) superficial (1-3 mm deep) cracks emanating from a focus centred on a lenticel and usually with 2-4 cracks arising from the same point. The SA of the tuber covered by superficial cracks was scored in categories of 0, 0-1, 2-5, 5-10% SA and then in 10% increments.

3.2.1.1.2. Expt 13

Experiment 13 was a randomized block design, with all factorial combinations of three varieties (Maris Piper, Markies and Saturna) and four irrigation regimes. The regimes were rainfed only (Unirr), irrigated with 25 mm at 25 mm SMD from emergence (25 SMD), irrigated with 4-8 mm daily to maintain soil over field capacity at the start of each day from onset of TI for 3 weeks (OW 0-3) and as OW 0-3 but over-watered from

10-13 weeks after TI (OW 10-13). Irrigation in OW 0-3 and OW 10-13 treatments outside the over-watering period was 25 mm whenever the SMD reached 25 mm. There were three replicate blocks.

The experiment was conducted adjacent to Expt 12 on a sandy loam soil (57% sand, 26% silt and 17% clay) with 6% stone, 3.0% organic matter content and a pH of 6.8. The experiment was planted on 12 April using SE1 seed (Maris Piper 35-40 mm 37 g, Markies 35-40 mm 44 g, Saturna 25-35 mm 25 g) at a within-row spacing of 30 cm in 76 cm rows. The seed was dibbed 12 cm deep into ridges pre-formed using a Rumptstad roto-tiller, which were raked after planting to re-form the original ridge. Plots were 9 m long and four rows wide. A concentrated (34.2 % vol.) solution of ammonium nitrate was applied at a rate of 200 kg N/ha post-planting.

Irrigation scheduling and application were as Expt 1. Irrigation for over-watered treatments was timed to start automatically at 05:00 and 19:00h and the application rate adjusted between 4 and 8 mm/day (i.e. 2-4 mm twice daily) according to evapotranspiration demand, to ensure that plots received *c*. twice the daily sum of ET and bare soil evaporation and remained above field capacity during the day. A pre-dose of 15 mm (OW 0-3) or 25 mm (OW 10-13) was applied at the start of each over-watered period to ensure the soil reached field capacity before treatments began. Once each over-watered treatment was completed, it was irrigated according to the protocol in the 25 SMD treatment. Other treatments were irrigated between 08:00 and 18:00 h. Rainfall, irrigation and drainage amounts are detailed in Table 8.

Table 8. Expt 13: Rainfall, irrigation and drainage (mm) during different periods (Maris Piper plots)

	Unirr	25 SMD	OW 0-3	OW 10-13
Rainfall				
During OW 0-3 period	75	75	75	75
During OW 10-13 period	42	42	42	42
Emergence to harvest	328	328	328	328
Irrigation				
During OW 0-3 period	0	25	126	25
During OW 10-13 period	0	75	75	148
Emergence to harvest	0	150	251	248
Drainage				
During OW 0-3 period	18	21	121	22
During OW 10-13 period	0	12	14	100
Emergence to harvest	90	133	239	215

In all Maris Piper plots of two replicate blocks, soil water content was measured at 60-minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed at emergence in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm and logged using a Delta-T Devices DL6 logger.

Emergence, TI, ground cover, common scab, powdery scab and cracking measurements were as detailed for Expt 1. Four sequential harvests of 10 plants from guarded areas were taken on 5 July, 27 July, 24 August and 24 September. Foliage from each plot was weighed, sub-sampled to *c*. 1 kg and dried. Dried haulm and tuber samples were analysed for total N content at NRM Ltd, Bracknell.

Immediately after the crop was sampled at each sequential harvest, soil cores were taken with an auger ('Dutch' type, 55 mm external diameter) from the 12 plots growing Maris Piper. Cores were taken from between plants in one of the two central harvest rows, at depths of 0-30, 30-60 and 60-90 cm from the soil surface, and from the furrow, between the two harvest rows at depths of 0-30 and 30-60 cm from the soil surface. The cores were placed in polythene bags and kept in a cool-box together with ice-packs before being dispatched within 24 hours to NRM Ltd for analysis for soil mineral N (SMN).

3.2.1.1.3. Expt 14

Experiment 14 was a randomized block design, with all factorial combinations of two varieties (Maris Peer and Venezia), two irrigation timings and two irrigation durations. The timings were: irrigated with 4-6 mm daily to maintain soil at field capacity at the start of each day from onset of TI (0 SMD) and irrigated with 15 mm at 15 mm SMD from TI (15 SMD). The three irrigation durations were from TI to 4 weeks after TI (4 wks), TI to 6 weeks after TI (6 wks) and TI to 8 weeks after TI (8 wks). Once irrigation was completed in each duration period, 25 mm of irrigation was scheduled to be applied whenever the SMD reached 25 mm but in practice, none was required. There were three replicate blocks.

The experiment was conducted adjacent to Expt 13 on a sandy loam soil (61 % sand, 23 % silt and 16 % clay) with 12 % stone, 3.3 % organic matter content and a pH of 6.8. The experiment was planted on 12 April using 35-40 mm (39-49 g) SE1 seed at a within-row spacing of 15 cm in 76 cm rows. The seed was dibbed by hand 12 cm

deep into ridges pre-formed using a Rumptstad rototiller, which were raked after planting to re-form the original ridge. Plots were 4.5 m long and four rows with the inner two rows used for harvest. A concentrated (34.2 % vol.) solution of ammonium nitrate was applied at a rate of 120 kg N/ha post-planting.

Irrigation scheduling and application were as Expt 1. Irrigation for 0 SMD treatments was timed to start automatically at 05:00 and the application rate adjusted between 3 and 5 mm/day according to evapotranspiration demand, to ensure that plots started at field capacity at the start of the day. The 15 SMD treatment was irrigated between 08:00 and 18:00 h. Rainfall, irrigation and drainage amounts are detailed in Table 9.

Table 9. Expt 14: Total rainfall, irrigation and drainage (mm) during the treatment periods

Timing	0 SMD			15 SMD		
Duration	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks
Rainfall	115	199	216	115	199	216
Irrigation	77	97	138	30	30	60
Drainage	96	160	173	51	103	103

Emergence, TI, ground cover, yield, common scab, powdery scab and cracking measurements were as detailed for Expt 1. The experiment was desiccated on 1 August with Reglone at a rate of 3 l/ha. A hand-harvested area of 20 plants was dug on 16 August.

Samples of 10 tubers from five plants per plot for the 0 SMD treatment were sent to Fera in perforated plastic bags 4, 6 and 8 weeks after TI for determination of populations of total *Actinomycetes* and pathogenic *Streptomyces* as in Expt 10. Populations of potential antagonists were assessed from tubers sampled 8 weeks after TI in 0 SMD treatments.

3.2.1.1.4. Expts 15 and 16

Experiments 15 and 16 were located in commercial fields of Maris Piper grown by Greenvale AP which were irrigated as detailed in Expts 4 and 5. The Wet plots in both experiments were scheduled to be irrigated whenever the SMD reached 13 mm, with the allowable SMDs based on soil texture.

Experiment 15 was located in Beacon Hill field, Raveningham, Norfolk on a sandy loam (69% sand, 19% silt, 13% clay and 1.9% organic matter). It was planted by machine 18 cm deep on 23 March with 30-40 mm E1 seed at a spacing of 27 cm. Experiment 16 was located in Ashby 9 field, Somerleyton, Norfolk on a sandy loam (63 % sand, 21 % silt, 16 % clay and 1.3 % organic matter). It was planted by machine 18 cm deep on 7 April with 40-50 mm E1 seed at a spacing of 38 cm. Table 10 details the irrigation application and rainfall. Only a single dose of 5 mm was applied to the Wet treatment in Expt 16, 2 days before the end of scab control.

Table 10. Expts 15 and 16: Rainfall and irrigation (mm)

	Expt 13		Expt 14	
Irrigation treatment	Dry	Wet	Dry	Wet
Rainfall				
During scab period	75	75	61	61
Emergence to harvest	285	285	250	250
Irrigation				
During scab period	0	23	0	5
Emergence to harvest	63	86	25	30

Samples of 10 tubers from five plants per plot were sent to Fera in perforated plastic bags from a harvest taken 4 weeks after TI for determination of populations of total *Actinomycetes*, pathogenic *Streptomyces* and populations of potential antagonists as detailed in Expt 1.

Experiment 15 was desiccated with split-dose Reglone on 15 and 20 September. Experiment 16 was harvested green-top without desiccation. A final harvest of 3 m of single row was taken on 17 September in Expt 15 and on 23 August in Expt 16. Tubers were counted, graded, weighed and assessed for common scab and cracking as in Expt 1.

3.2.2. Fera experiments

3.2.2.1.1. Expt 17

Experiment 17 examined the effect of irrigation on common scab in field soils under controlled conditions. Approximately 100 kg of each of four soils from potato fields (CUF, sandy loam; Worth, clay loam; Cold Harbour, loamy sand and LG21, peat) were supplied by CUF on 27 June 2012 to Fera, Sand Hutton. The soils were immediately

transferred to a cold store (4°C). On 5 July, the soils were placed in 7.5 l pots in a plant growth room that was set at 20°C (day), 16 °C (night), 62 % RH and 15 hours full light. One minituber of *cv*. Maris Piper (30-40 mm, TLC Potatoes Ltd) was planted in each pot on 5 July. The experimental design consisted of four soils, two watering regimes (high or low irrigation levels) and 10 replicates (four removed at first harvest and six removed at late harvest). Treatments (soils x moisture) were randomised within blocks (replicates). Soils were watered to provide the following moisture regimes for mineral soils: Low, 15% moisture (*c*. 25 mm SMD) and High, 25% moisture (near field capacity). For soils with high organic matter (LG21), the moisture levels were: Low, 40% (*c*. 25 mm SMD) and high, 60%. Moisture levels were assessed at least twice a week using a Delta-T Devices Theta Probe ML2 soil moisture probe and adjusted as required. Approximately 3 weeks after TI (TI was estimated to be on 13 August), plants from blocks 1 to 4 were harvested and total nucleic acid extracts prepared from whole tubers and soils. Tubers from blocks 1 to 6 were harvested on 31 October and assessed for common scab surface area (% severity).

3.2.2.1.2. Expt 18

In order to investigate whether water alone had an effect on populations of pathogenic *Streptomyces* and disease development, an experiment was devised to isolate this effect from the influence of other soil microbiota. Experiment 18 examined the effect of irrigation alone on common scab. Samples of the CUF soil (as above, Expt 17) were sealed in bags in 15 kg quantities and were sterilised by γ-irradiation (IPS Product Supplies Ltd) on 24 July. Soils were placed in 7.5 l pots in a plant growth room (conditions as described in Expt 15). One minituber of *cv*. Maris Piper was placed in each pot on 5 September. A 2 ml aliquot of liquid inoculum of either *S. scabiei* or *S. stelliscabiei* was added to the surface of each minituber in the inoculated treatments and covered with sterile soil. Sterile water was added to the non-inoculated treatments as controls. The experimental design consisted of one soil, three inoculum treatments (including the non-inoculated control), two watering regimes, and four replicates for harvest 1 (four weeks post TI) and six replicates for harvest 2 (at maturity). Treatments (inoculum x soils x moisture) were randomised within blocks (replicates). Soils were watered with sterile distilled water to provide the

following moisture regimes: Low, 16 % moisture (*c*. 25 mm SMD) and High, 25 % moisture (near field capacity). Tubers from blocks 1, 3, 5 and 7 were harvested at approximately 4 weeks after TI (31 October) and processed for whole nucleic acid extractions. Approximately 100 g soil was placed in labelled zip-lock polythene bags and stored at 4 °C until required for DNA extractions. Tubers from blocks 2, 4, 6, 8, 9 and 10 were harvested on 20 November 2012 and assessed for common scab surface area (% severity).

3.2.2.1.3. Measurement of microbial communities

The DNA extracted from tuber peels which was used for measurement of total *Actinomyces* and pathogenic *Streptomyces* was used in Expts 12, 14, 15 and 16 to produce amplicons of bacterial 16S rDNA for subsequent analysis by pyrosequencing. Sequence libraries were produced by amplifying 16S amplicons using fusion primers containing the 454 pyrosequencing A and B adapters along with a 10 bp 'MID' identifier to allow multiplexing of many samples in a single sequencing run. Sequences were run in ⅛ portions of a Titanium plate on a GS FLX+ sequencer, processed using shotgun filtering and sequence datasets for individual samples sorted according to MID using in-house scripts enabled in the Galaxy workflow environment. Sequences were individually classified using the Ribosome Database Project (RDP) pipeline to assign to the appropriate taxonomic level.

3.3. 2013 experiments

There were two experiments at CUF and two with Greenvale AP Ltd in Norfolk investigating the effect of irrigation regime on common scab and the populations of *Streptomyces* and antagonists on tubers in maincrop and salad varieties. At Fera the DNA extracted from tuber peelings from Expts 19-22 was used for soil microbial community analysis. In addition, three pot experiments were conducted examining a) microbial diversity, b) biofumigation and c) inoculation with *Streptomyces*.

3.3.1. CUF and Greenvale AP experiments

3.3.1.1.1. Expt 19

Experiment 19 was a randomized block design, with all combinations of eight varieties (Jelly, Lanorma, Maris Piper, Melody, Nectar, Orchestra, Safari and Volare) and five irrigation regimes. The regimes were rainfed only (Unirr), irrigated with 3-5 mm to maintain 0 mm SMD at the start of each day from onset of TI for 4 weeks (0 SMD), irrigated with 15 mm at 15 mm SMD throughout the season (15 SMD), irrigated with 35 mm at 35 mm SMD throughout the season (35 SMD) and irrigated as 15 SMD except no irrigation applied until 1 week after TI (TI 1-4). There were three replicate blocks.

The experiment was conducted on a sandy loam soil (74% sand, 13% silt and 13% clay) with 8-20% stone, 7.4% organic matter content and a pH of 6.7. The field was ploughed on 13 March and roto-ridged with a Rumptstad rototiller on 14 March. The experiment was planted on 19 April using 30-40 mm SE seed at a within-row spacing of 30 cm in 76 cm rows. The seed was dibbed by hand 12 cm deep into pre-formed ridges, which were raked after planting to re-form the original ridge. Plots were 3.9 m long and four rows wide (eight rows for Maris Piper and Melody to permit additional *Streptomyces* sampling). A concentrated (34.2% vol.) solution of ammonium nitrate was applied at a rate of 200 kg N/ha post-planting. Herbicides and fungicides were applied as required to maintain the experiment.

Irrigation was scheduled and applied as detailed in Expt 1. Irrigation commenced in 0 and 15 mm SMD treatments at the onset of TI to ensure that the respective trigger SMDs were reached at 50% TI. The TI 1-4 treatment received its first irrigation 7 days after 50% TI and the soil was wetted back up to field capacity in one single dose before reverting to irrigation as per the 15 SMD treatment. Rainfall, irrigation and drainage amounts are detailed in Table 11.

Table 11. Expt 19: Rainfall, irrigation and drainage (mm) during the season (Maris Piper plots)

	Irrigation regime				
	Unirr	0 SMD	15 SMD	35 SMD	TI 1-4
Rainfall					
TI + 4 weeks	23	23	23	23	23
Emergence to harvest	179	179	179	179	179
Irrigation					
TI + 4 weeks	0	109	85	70	78
Emergence to harvest	0	391	250	198	223
Drainage					
TI + 4 weeks	0	25	1	0	1
Emergence to harvest	0	162	37	30	31

In each plot of Maris Piper in two replicate blocks, soil water content was measured at 60-minute intervals using a Delta-T Devices Theta Probe ML2 permanently installed at emergence in the centre of the ridge mid-way between two adjacent plants at a depth of 15 cm and logged using a Delta-T Devices DL2 logger.

Emergence, TI, ground cover, common scab, powdery scab and cracking measurements were as detailed for Expt 1. Samples of 10 tubers from five plants per plot were sent to Fera in perforated plastic bags 1, 2, 3, and 4 weeks after TI for determination of populations of total *Actinomycetes* and pathogenic *Streptomyces*. These were assessed by quantitative real-time PCR, using assays for 16S rDNA and the *txtA* pathogenicity gene (which is common to pathogenic *Streptomyces*), respectively. Populations of potential antagonists were assessed from tubers sampled 4 weeks after TI (the date that peak pathogen populations were expected) from Maris Piper and Melody treatments only.

A final harvest of 10 guarded plants was taken when the canopies were almost completely senesced on 3 September (Melody, Nectar, Orchestra), 16 September (Lanorma, Safari), 25 September (Jelly) and 30 September (Maris Piper). Tubers were counted, graded, weighed and assessed for dry matter percentage, common and powdery scab, cracking and internal defects as detailed in Expt 1.

3.3.1.1.2. Expt 20

Experiment 20 was a randomized block design, with all factorial combinations of three varieties (Maris Peer, Regina and Venezia) and three irrigation durations. The irrigation durations were from TI to 4 weeks after TI (4 wks), TI to 6 weeks after TI (6 wks) and TI to 8 weeks after TI (8 wks). In all cases, 15 mm of irrigation was applied at 15 mm SMD commencing at the onset of TI. Once irrigation was completed in each duration period, no further irrigation was applied until desiccation. There were three replicate blocks.

The experiment was conducted adjacent to Expt 18 on a sandy loam soil (73% sand, 13% silt and 14% clay) with 8% stone, 8.8% organic matter content and a pH of 6.6. The experiment was planted on 3 May using 35-40 mm (40-50 g) SE1 seed at a within-row spacing of 15 cm in 76 cm rows. The seed was dibbed by hand 12 cm deep into ridges pre-formed using a Rumptstad rototiller, which were raked after planting to re-form the original ridge. Plots were 4.5 m long and four rows wide with the inner two rows used for harvest. A concentrated (34.2 % vol.) solution of ammonium nitrate was applied at a rate of 120 kg N/ha post-planting.

Irrigation scheduling and application were as Expt 1. Rainfall, irrigation and drainage amounts are detailed in Table 12.

Table 12. Expt 20: Total rainfall, irrigation and drainage (mm) during the treatment periods (mean for all varieties)

Duration	4 wks	6 wks	8 wks
Rainfall	16	49	10
Irrigation	105	130	170
Drainage	0	2	10

Emergence, TI, ground cover, yield, common scab, powdery scab and cracking measurements were as detailed for Expt 1. The experiment was desiccated on 23 August with Reglone at a rate of 2 l/ha. A hand-harvested area of 20 plants was dug on 5 September.

Samples of 10 tubers from five plants per plot for the 0 SMD treatment were sent to Fera in perforated plastic bags 4, 6 and 8 weeks after TI for determination of populations of total *Actinomycetes* and pathogenic *Streptomyces* as in Expt 1.

Populations of potential antagonists were assessed from tubers sampled 8 weeks after TI in 0 SMD treatments.

3.3.1.1.3. Expts 21 and 22

Experiments 21 and 22 were located in commercial fields of Maris Piper grown by Greenvale AP which were irrigated with solid-set sprinkler systems as detailed in Expts 4 and 5. The Wet plots in both experiments were scheduled to be irrigated whenever the SMD reached 13 mm, with the allowable SMDs based on soil texture.

Experiment 21 was located in Red House West field, Raveningham, Norfolk on a sandy loam (66% sand, 21% silt, 14% clay and 1.9% organic matter). It was planted by machine 18 cm deep on 10 April with 35-50 mm E1 seed at a spacing of 38 cm in 96.5 cm rows. Experiment 22 was located in Hales Hospital field, Hales, Norfolk on a sandy loam (66% sand, 20% silt, 15% clay and 2.1% organic matter). It was planted by machine 18 cm deep on 17 April with 35-50 mm E1 seed at a spacing of 38 cm in 96.5 cm rows. Table 13 details the irrigation application and rainfall.

Table 13. Expts 21 and 22: Rainfall and irrigation (mm)

	Expt 19		Expt 20	
Irrigation treatment	Dry	Wet	Dry	Wet
Rainfall				
During scab period	33	33	33	33
Emergence to harvest	108	108	75	75
Irrigation				
During scab period	0	48	0	40
Emergence to harvest	184	232	145	185

Samples of 10 tubers from five plants per plot were sent to Fera in perforated plastic bags from a harvest taken 4 weeks after TI for determination of populations of total *Actinomycetes*, pathogenic *Streptomyces* and populations of potential antagonists as detailed in Expt 1.

Both experiments were desiccated with split-dose Reglone on 31 August and 7 September. A final harvest of 3 m of single row was taken on 24 September. Tubers

were counted, graded, weighed and assessed for common scab and cracking as in Expt 1.

3.3.2. Fera experiments

3.3.2.1. *Methods for soil microbial community analysis used in 2013*

The DNA extracted from tuber peelings from Expts 19-22 was used for the soil microbial community analysis.

3.3.2.1.1. Bacterial communities

Bacterial community structure was assessed based on the V3-V5 region of the 16S rRNA gene (Klindworth *et al*. 2012). Amplification reactions of 20 µl were carried out using the Phusion High-Fidelity DNA Polymerase (New England Biolabs, UK) containing 4 µL of HF buffer, 0.3 µM of forward and reverse primers, 0.3 mM of dNTPs, 0.4 U Phusion DNA polymerase and 0.5 µL of template DNA. The final reaction volume was made up with nuclease-free water (Severn Biotech, UK). The primers used were 341f (5'-**CCATCTCATCCCTGCGTGTCTCCGAC***TCAG*NNNNCCTACGGGNGGCWGCAG-3') containing the 454 sequence adapter A (bold), a 454 amplicon sequencing specific 4-mer amplification key (italics) followed by a 10-mer barcode sequence to aid multiplexing (NNNN) and the amplification primer itself as well as 805r (5'-<u>CCTATCCCCTGTGTGCCTTGGCAGTCTCAG</u>*GACTACHVGGGTATCTAATCC*-3') with 454 sequence adapter B (underlined) followed by reverse amplification sequence (*italics*).

All PCRs were carried out on a C1000 thermal cycler (BioRad, UK). Amplification of the 16S rRNA gene fragment started with an initial single denaturing step at 98°C for 2 min, followed by 25 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. PCR products were checked on 1% agarose gels and visualised under UV light. PCR products were pooled to similar concentrations and the mix was purified on a 2% agarose gel. The band corresponding to an amplicon size of 420 bp was excised from the gel followed by DNA purification using the Qiaquick gel extraction kit (Qiagen, Germany).

Potato samples contained large numbers of potato chloroplasts using the above PCR protocol, which was confirmed using the RDP match tool (Cole *et al*. 2014) indicating that 9,503 of 16,962 chloroplast sequences could be matched to the primer 341f alone including *Solanum* spp. and *Quercus* spp.. A set of primers was developed to block such sequences and stop amplification by addition of 2'-3'-Dideoxycytosine attached to the blocking primers at the 3'-end. The blocking primers were designed to anneal in close proximity to the forward and reverse primers above and to mainly match *Solanum* spp.. Coverage was checked using the SILVA testProbe 3.0 and the RefNR 116 (Quast *et al.* 2013). Primers Pl_16SF4_block (5'-TGGGGAATTTTCCG CAATGGG-DDC-3') and Pl_16SR1_block (5'-ATTCGCTCCCCTAGCTTTCGT-DDC-3') were chosen for experimental tests.

In detail, Phusion High-Fidelity DNA Polymerase, HF buffer and dNTPs were added as described above, whereas 0.15 µM of forward, reverse primers and blocking primers were added. Except of an increase in amplification temperature to 59°C PCR conditions were kept as described above.

3.3.2.1.2. Fungal communities

An overview of present fungi was obtained based on the intergenic spacer amplified using the primers ITS1-F_KYO2f and ITS2_KYO2r (Toju *et al.* 2012) amended for 454 sequencing. Specifically, ITS1-F_KYO2f (5'-**CCATCTCATCCCTGCGTGTCTCCGAC** *TCAG*NNNNTAGAGGAAGTAAAAGTCGTAA-3') contained the 454 sequence adapter A (bold), a 454 amplicon sequencing specific 4-mer amplification key (italics) followed by a 10-mer barcode sequence to aid multiplexing (NNNN) and the amplification primer itself as well as ITS2_KYO2r (5'-<u>CCTATCCCCTGTGTGCCTTGGC AGTCTCAG</u>*TTYRCTRCGTTCTTCATC* -3') with 454 sequence adapter B (underlined) followed by reverse amplification sequence (*italics*).

An overview of barcode sequences used can be found in Table 14.

Table 14. Barcode sequences used for microbial community approach

MID	Sequence
L1	ACGAGTGCGT
L2	ACGCTCGACA
L3	AGACGCACTC
L4	AGCACTGTAG
L5	ATCAGACACG
L6	ATATCGCGAG
L7	CGTGTCTCTA
L8	CTCGCGTGTC
L9	TAGTATCAGC
L10	TCTCTATGCG
L11	TGATACGTCT
L12	TACTGAGCTA

Amplification reactions of 20 µl were carried out using the Phusion High-Fidelity DNA Polymerase (New England Biolabs, UK) containing 4 µL of HF buffer, 0.3 µM of forward and reverse primer, 0.3 mM of dNTPs, 0.4 U Phusion DNA polymerase and 0.5 µL of template DNA. The final reaction volume was made up with nuclease-free water (Severn Biotech, UK).

All PCRs were carried out on a C1000 thermal cycler (BioRad, UK). Amplification of ITS1 started with an initial single denaturing step at 98°C for 2 min, followed by 35 cycles of denaturation at 98°C for 20 s, annealing at 50°C for 45 sec and extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min.

PCR products were checked on 1% agarose gels and visualised under UV light. PCR products were pooled to similar concentrations and the mix was purified on a 2% agarose gel. The bands corresponding to 250-400 bp (ITS1) were excised from the gel followed by DNA purification using the Qiaquick gel extraction kit (Qiagen, Germany).

The preparation of amplicon libraries was carried out using Roche's recommendations. Amplicon sequencing was done by 454-GSFLX+ genome sequencer for bacterial and fungal communities. To assign taxonomy to bacterial sequences, the Naïve Bayesian rRNA classifier of the Ribosomal Database Project (RDP; http://rdp.cme.msu.edu/) was used applying a bootstrap value of 80%. All subsequent analyses were based on genus level data. Taxonomic classification of these sequences was done by QIIME (Quantitative Insights Into Microbial Ecology), an open source software package.

Statistical analyses were carried out using the subroutines multidimensional scaling (MDS) and analysis of similarities (ANOSIM) of the PRIMER 6.1.15 software suite (PRIMER-E, Ltd., UK). For ordination and ANOSIM Bray–Curtis similarities based on standardised and square root transformed data were used to minimise the effect of outliers or any dominance. Additionally, hierarchical agglomerative clustering of Bray–Curtis similarities was performed using the complete linkage method of the PRIMER software.

Principal component analysis (PCA) is an eigenanalysis-based statistical procedure that identifies the principal directions in which the data varies. The community data used for PCA were relative abundance and square root transformed and the analysis was performed from calculating out five principal components (PC) and a ordination biplot was drawn from PC1 and PC2.

3.3.2.2. ***Diversity amendment studies***

The choice of experimental approaches to increase our understanding of the role of microbial diversity in suppression of common scab under irrigated conditions is very challenging. For example, the addition of cultivated organisms to sterile soil is highly problematic due to low cultivability of intrinsic microorganisms. Also the application of antibiotics against potential antagonists is questionable since few specific antibiotics like Cephalosporins against gram-positive bacteria exist. Therefore, we chose to amend the soil diversity itself. This was achieved by applying a removal approach which uses dilution of the present diversity as described previously (Van Elsas *et al.* 2012; Wertz *et al.* 2006; Griffith *et al.* 2004). Soil from CUF was used as a model soil to link the experiments to field trials carried out at CUF.

3.3.2.2.1. Expt 23

To test whether microbial diversity *per se* had an effect on common scab severity, a pot experiment was set up using sterile soil. Soil from CUF was obtained in the summer of 2012 and was gamma-radiated as suggested by Trevors (1996). Specifically, soil was adjusted to similar water content and put into polythene sacs. Samples were frozen afterwards. An exposure to 20 kGy for 1 week was used for soil sterilisation (IPS Product Supplies Ltd, London, UK) which was equivalent to 2 Mrad.

In December 2012, 4 l of sterile soil was put into 24 x 5 l pots. The diversity amendment study was set up based on a method published by Griffith *et al.* (2001). A 25 g quantity of native CUF soil (non-sterilised) was incubated in 10 ml of ¼ strength Ringer solution (NaCL: 2.25 g/l, KCl 0.105 g/l, $CaCl_2.6H_2O$ 0.12 g/l, $NaHCO_3$ 0.05 g/l) and shaken in an incubator for 60 minutes. Following this, dilutions were carried out in ¼ strength Ringer solution in triplicate. Dilutions prepared ranged from 10^{-2} to 10^{-12}. Four litres of sterile soil were inoculated with 4 ml of respective soil community dilution in triplicate. The prepared pots were distributed using a random block design and kept in the dark at 16 °C and 55 % air humidity for stabilisation of the community (Griffith *et al.* 2004). The trial also included controls without addition of the native soil community. Soil moisture was kept between 5 and 10 % using sterile deionised water throughout the settling period. The soil was mixed monthly by hand. In May 2013 soil samples were taken to assess differences in bacterial diversity after the settling period. Amplifications were carried out as described before without blocking primers.

Streptomyces scabiei cultures (NCPPB 4086) were prepared as inoculum to study the effect of diversity changes on the pathogenicity of this particular species. We tested it for presence of thaxtomin which was positive. The strain was cultivated on YME agar plates (4 g/l yeast extract, 10 g/l malt extract, 4 g/l glucose, pH 7.2) of which single colonies were grown in liquid YME medium at 28 °C for 12 days. The cells were centrifuged at 10,000 g for 10 minutes after which the pellet was taken up in 50 ml sterile water. For enumeration of bacterial cells in suspension, a dilution series was prepared ranging from 10^{-1} to 10^{-5} in triplicate. An aliquot of 100 µl of each dilution was plated on a YME agar plate. Bacterial colonies were counted after 5 days growth at 28°C. The prepared bacterial solution contained 289,433 cells/ml.

In June 2013, the soil of all treatments was prepared to have a 11 cm deep cavity in which a potato tuber was planted. This was followed by the inoculation with 2 ml of *S. scabiei* cells reflecting 578,867 cells per tuber after which soil was distributed to cover the tuber. For the controls, three pots containing sterile soil received *S. scabiei* cells and three others received none acting as negative control. After 3 months of growth, potato tubers were harvested. The symptoms of common scab were assessed and peel was used for DNA extraction.

3.3.2.2.2. Expt 24

A controlled environment experiment was established using soils sourced from a field experiment conducted by Branston. This experiment provided soils from plots that had been subjected to biofumigation (using *Brassica juncea* cv. Caliente) or from plots in which brassicas had been grown previously. Soil treatments are given in Table 15.

Table 15. Treatments applied to soils used for pot experiments with biofumigated soils

Soil	Treatment
Hangar A	Caliente biofumigant
Hangar B	No biofumigant
Paddock 1	Caliente biofumigant
Paddock 2	No biofumigant
G7	No biofumigant, but brassicas in rotation
G4 A	No biofumigant but brassicas in rotation
G4 B	Biofumigation in 2011, wheat then potatoes as subsequent crops
G4 C	Brassicas in 2011, wheat then potatoes as subsequent crops

Soils were placed into 7.5 l plastic pots and each was planted with a single Maris Piper minituber. A total of 10 replicates per soil treatment were planted comprising six replicates for subsequent analysis and four sacrificial pots to assess plant development during the course of the experiment. Pots were kept in a plant growth room set at 20°C (daytime) and 16°C (night), 62% RH and 15 h full light. Pots were kept at low moisture levels (15-20% volumetric) to encourage development of scab symptoms. Tubers were harvested 96 days after planting and scab disease levels were assessed on all tubers by placing into categories according to % surface area affected. Categories were 0 %, 0-1%, 2-5%, 6-25%, 26-60% and 61-100%.

3.3.2.2.3. Expt 25: pot experiment with inoculated soils

A bin trial was set up at CUF in June 2013 with non-sterile soil to assess the effects of different bacterial communities on the pathogenicity of *S. scabiei* on potatoes. Bins of 80 l volume were filled with respective soil in triplicate. The soils tested were a) CUF soil used for Expt 19 and soil from another (sandier) area of the same field that that had produced tubers with very low severity of common scab in the control plots of a soil fungicide experiment in 2008, despite a lack of irrigation during TI. There were

two *S. scabiei* inoculum treatments: none and inoculated. The bins were positioned in a random block design in Osier field at CUF and Maris Piper tubers were planted 10 cm deep on 4 June. The treated pots were inoculated with around the seed tubers with *S. scabiei* cells grown as described previously accounting for 2,865,040 cells per tuber.

The tubers were harvested on 5 September, symptoms of common scab were assessed and peel was taken for DNA extraction. For peel samples, amplification of bacteria was carried out as described before using the method including blocking primers.

3.4. Statistical analysis

Variates were analysed by analysis of variance using the GenStat® Release 16.1 statistical package. Treatment means are stated to be significantly different only if the probability of differences occurring by chance were less than 5 % ($P < 0.05$). All error bars in figures are one standard error (S.E.) in length. The respective degrees of freedom (D.F.) are given in tables or figures where S.E.s are presented.

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 : non-pathogenic *Streptomyces* is decreased compared with dry soils, whether as a consequence of resource competition or antibiotic production by non-pathogenic *Streptomyces* is unclear (Adams & Lapwood 1978; Neeno-Eckwall *et al*. 2001). Since there were some plots where apparently no *txtA* DNA was observed, the ratio could not be calculated. A better approach was suggested by Rodger White of the Statistics Group at the Centre for Mathematics and Computational Biology, Rothamsted Research (personal communication), which involved analysing 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 the 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 S.E. bars have been positioned in figures so that they give an indication of the error variation between treatments.

4. RESULTS

4.1. 2011 experiments

4.1.1. CUF and Greenvale AP experiments

4.1.1.1. *Emergence, tuber initiation, ground cover and radiation absorption*

4.1.1.1.1. Expt 1

In Expt 1, 50% emergence was rapid and synchronous (11-13 May for all varieties). Tuber initiation occurred 14, 18 and 16 days after emergence in Estima, King Edward and Maris Piper, respectively. Ground covers in unirrigated Estima only reached 60-70% and canopies senesced by the middle of August but in King Edward and Maris Piper, close to complete canopy cover was achieved in the absence of irrigation and maintained until mid-August in Maris Piper but only the end of July in King Edward. For all varieties, maintaining a 0 mm SMD during TI did not advance the date of achieving full canopy cover compared with a 15 mm SMD or delaying irrigation until 1 week after TI. In Estima and King Edward, all irrigated regimes produced similar patterns of ground cover duration. In Maris Piper, however, maintaining an SMD of 0 mm throughout the scab control period advanced senescence compared with less frequent watering.

4.1.1.1.2. Expt 2

In Expt 2, Maris Piper and Saturna reached 50% emergence on 6 May whereas Vales Sovereign was 3 days later reaching 50% emergence. Tuber initiation occurred 16 days after emergence for all varieties. Ground cover expanded most rapidly in Saturna owing to the higher stem density *c.f*. Maris Piper and Vales Sovereign but canopy duration was limited in Saturna crops and senescence commenced in the third

week of July. In Saturna, whilst early over-watering (OW 0-3) advanced the date at which canopy closure occurred, it also advanced the onset and rate of senescence such that crops senesced around the same time as those which were unirrigated throughout. Later periods of over-watering actually delayed senescence slightly compared with the standard 25 SMD treatment. In Maris Piper, all crops reached complete cover within 1 week of each other (20-27 June). Unirrigated crops commenced senescence at the end of July but early over-watering (OW 0-3) started to senesce 1 week later and other irrigation regimes did not commence senescence for another week. Over-watering in mid-August (OW 10-13) delayed senescence in Maris Piper and in Vales Sovereign *c.f.* the 25 SMD treatment.

When measured at final harvest on 13 September, season-long integrated ground cover was significantly larger in Maris Piper and Vales Sovereign when compared with Saturna (Table 16*a*). Had the canopies of Maris Piper and Vales Sovereign been allowed to fully senesce, then this difference would have been slightly larger. The integrated ground covers of the unirrigated crops were significantly smaller than those of the other irrigation treatments whilst, numerically, the OW 3-6 and OW 10-13 had the most persistent canopies. The effects of the treatments on season-long radiation absorption were similar to those on integrated ground cover (Table 16*b*).

Table 16. Expt 2: effect of variety and irrigation on *(a)* season-long integrated ground cover (% days) and *(b)* radiation absorption (TJ/ha)

	Variety	Unirr	25	OW 0-3	OW 3-6	OW 10-13
(a)	Maris Piper	7847	9463	8775	9568	10496
	Saturna	6041	7256	6373	7895	7223
	Vales Sovereign	8312	8308	8695	9310	9029
	Mean	7400	8342	7947	8924	8916
		S.E. (28 D.F.): Variety, 228; Irrigation, 295 and Variety×Irrigation, 510				
(b)	Maris Piper	13.0	15.1	14.3	14.9	16.2
	Saturna	10.6	12.6	11.2	13.2	12.3
	Vales Sovereign	12.9	13.2	13.5	14.3	13.8
	Mean	12.2	13.6	13.0	14.1	14.1
		S.E. (28 D.F.): Variety, 0.31; Irrigation, 0.40 and Variety×Irrigation, 0.70				

4.1.1.1.3. Expt 3

In Expt 3, all varieties reached 50% emergence on 7 May except Vales Sovereign (9 May). Tuber initiation occurred 17 days after emergence for all varieties except Sylvana which initiated earlier at 15 days after emergence. Sylvana is a determinate

variety and had the least persistent ground cover duration. Unirrigated and 35 SMD treatments took longer to reach maximum canopy, which was 91% in Unirr and 95% in 35 SMD. The Unirr treatment also senesced earlier than the other irrigation regimes. Maintaining very wet soil did not alter ground cover significantly *c.f.* a more moderate 15 mm SMD. Jelly was slightly more determinate than Maris Piper and maintaining SMDs < 15 mm advanced the date of attaining full ground cover in both varieties compared to higher SMDs. In Jelly, all crops commenced senescence around the same time but the 35 mm SMD treatment was slower in senescing. In Maris Piper, unirrigated crops senesced considerably earlier than the frequent regimes (0 SMD and 15 SMD), whilst the 35 SMD regime maintained almost full ground cover until desiccation. In Vales Sovereign, maintaining soils at field capacity advanced senescence by *c.* 4 weeks *c.f.* maintaining a moderate or high SMD.

4.1.1.1.4. Expts 4 and 5

Emergence commenced in Expt 4 on 14 May and 50% emergence was measured on 16 May, whilst TI was on 3 June. Complete canopy cover in the experimental area was not reached until 27 July but ground cover was *c.* 98% from 6 July and the plots still had full ground cover at desiccation. In Expt 5, emergence commenced on 9 May and 50% emergence was measured on 13 May, whilst TI was on 31 May. Complete canopy cover was reached more rapidly in Expt 5 (6 July) than Expt 4 (22 June) in both treatments and the plots still had full ground cover at desiccation.

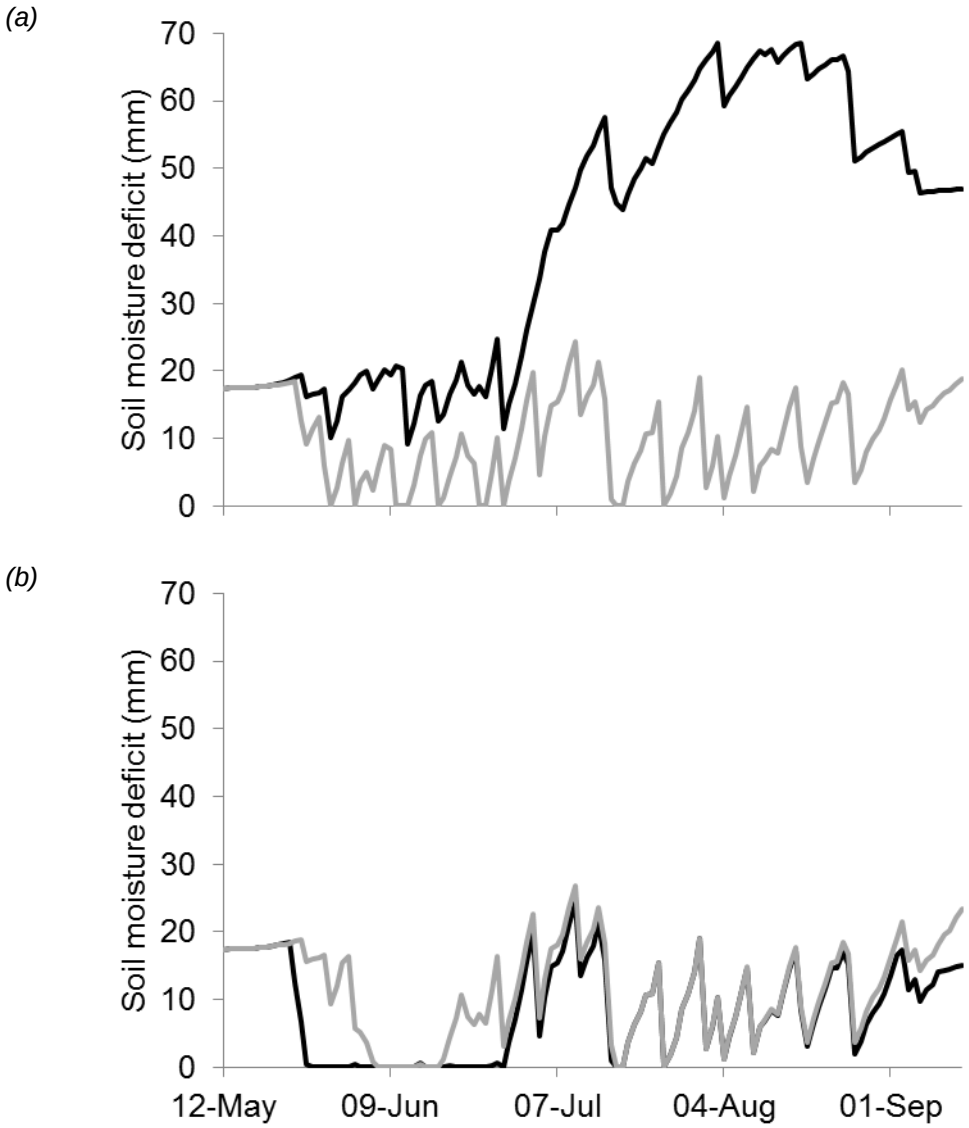
4.1.1.2. ***Modelled SMDs***

The 2011 season was characterized by high ET in May, variable and slightly higher than average ET in June and July with only 1 day over 5 mm *c.f.* 9 in 2010. However, June's evenly-spread rainfall receipts matched ET demand more closely than any other season with above-average ET in June since 1993. August was similar to 2010 in being dull with low ET demand but rainfall was only one-third as much as in 2010. There was a much higher than average proportion of the days where ET demand was between 3 and 4 mm/day which produced rapid crop growth rates without excessive heat or water stress.

4.1.1.2.1. Expt 1

In Expt 1, all treatments other than Unirr were maintained below a 25 mm SMD throughout the season (Figure 1). The maximum SMD reached in Unirr was *c*. 69 mm at the beginning of August. In the TI 1-3 treatment, the maximum SMD reached in the first and last weeks of the 4-week control period was 16 mm but it was maintained at zero during the middle 2 weeks.

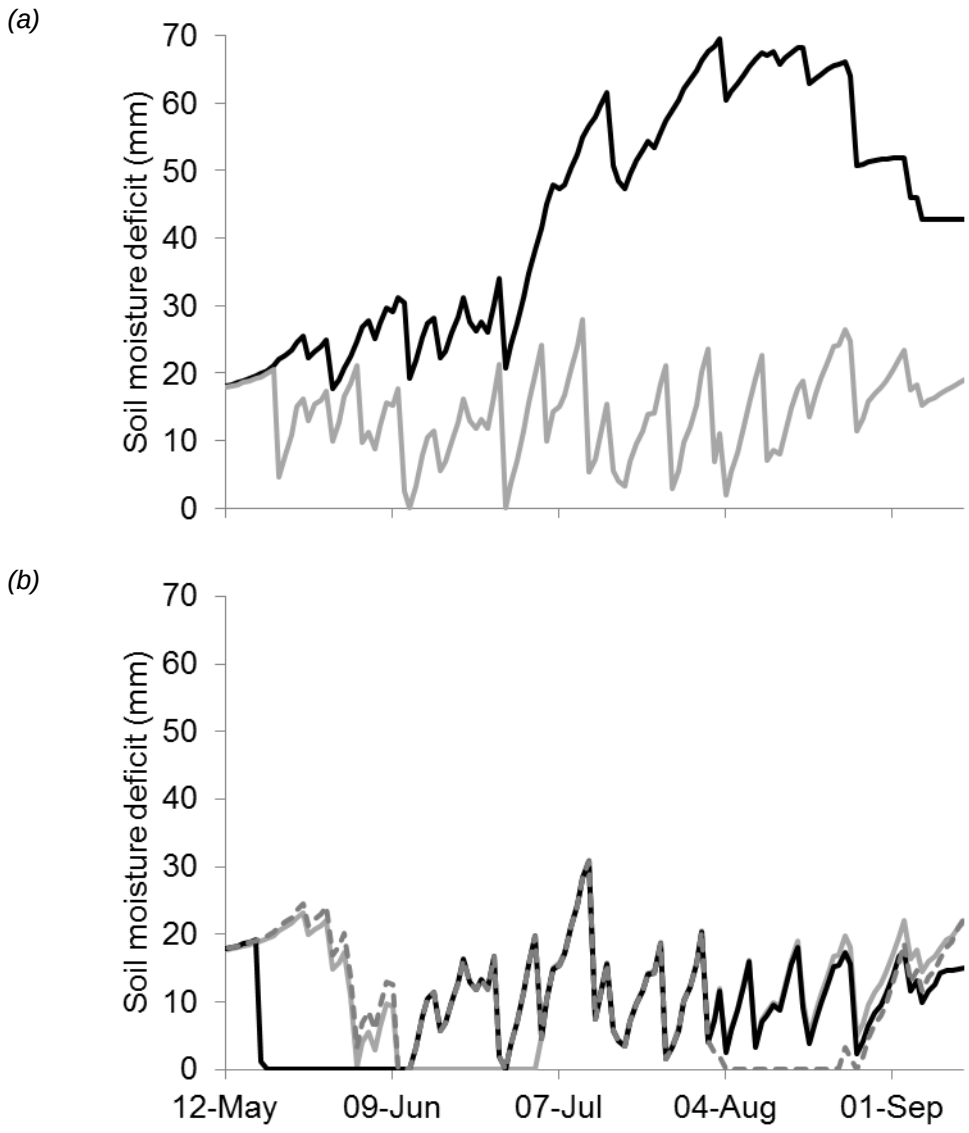
Figure 1. Expt 1: Modelled soil moisture deficits in Maris Piper plots. *(a)* Unirr, —; 15 SMD —; *(b)* 0 SMD, —; TI 1-3, —.



4.1.1.2.2. Expts 2 and 3

In Expts 2 and 3, peak SMDs again reached *c*. 69 mm at the beginning of August in unirrigated plots. Otherwise, SMDs were maintained close to the designed treatment protocols (Expt 2, Figure 2). Drainage was considerable under the crops maintained at field capacity or in a periodic over-watered status (Table 2; Table 3; Table 4).

Figure 2. Expt 2: Modelled soil moisture deficits in Maris Piper plots. *(a)* Unirr, —; 25 SMD —; *(b)* OW 0-3, —; OW 3-6, —, OW 10-13, – –.



4.1.1.2.3. Expts 4 and 5

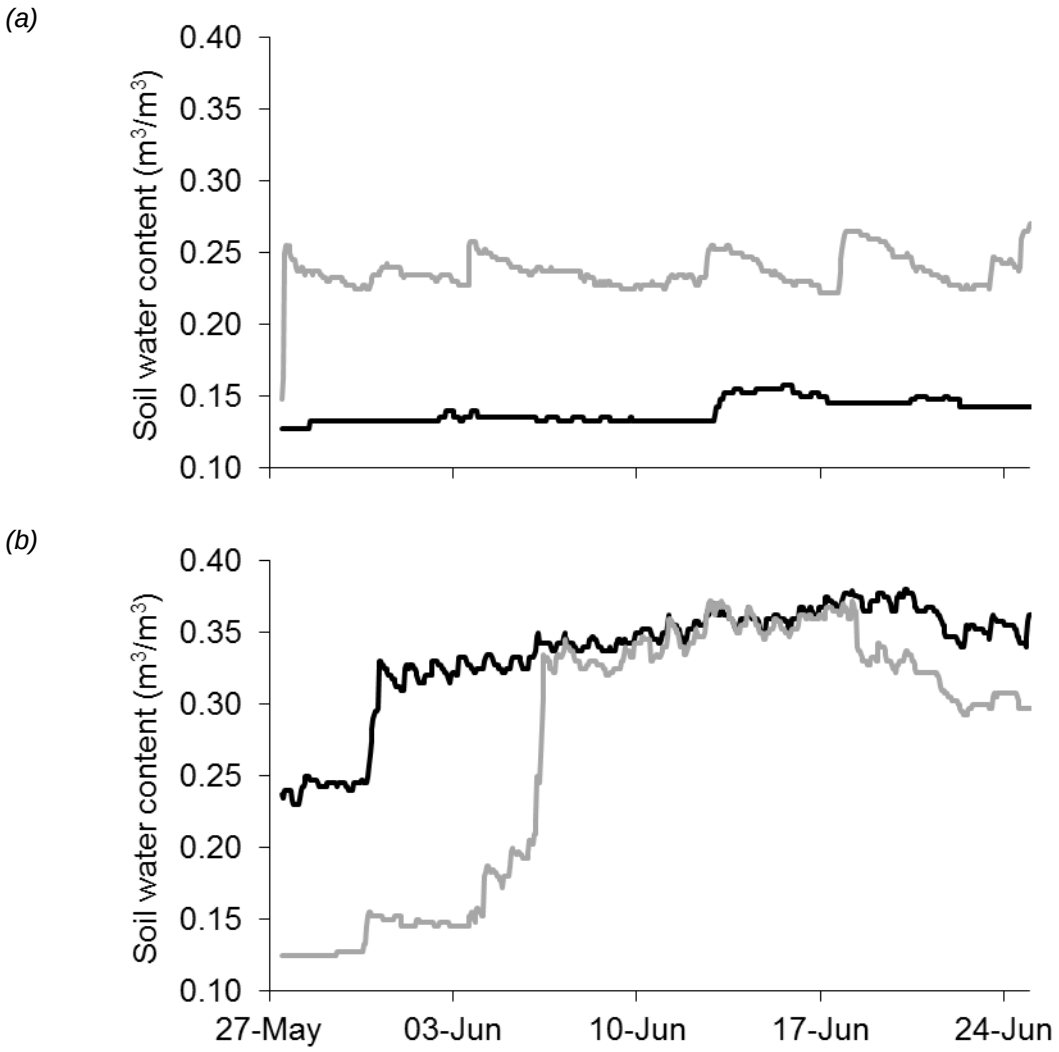
In Expt 4, average SMDs during the 4 weeks post-TI were 3 mm (maximum 9 mm) in Wet treatments and 18 mm (maximum 27 mm) in the Dry. In Expt 5, average SMDs during the 4 weeks post-TI were 10 mm (maximum 15 mm) in Wet treatments and 29 mm (maximum 42 mm) in Dry.

4.1.1.3. *Measured soil water content*

4.1.1.3.1. Expt 1

Soil in the ridge was very dry (0.13 m^3/m^3, 13% vol.) in unirrigated plots in Expt 1 for the first 2 weeks after TI (Figure 3*a*). There was some rain which wetted the ridge in the third week after TI but water content remained at *c*. 0.14 m^3/m^3. The 15 SMD treatment reached 0.27 m^3/m^3 after each irrigation and never decreased below 0.23 m^3/m^3 in the ridge in the 4 weeks after TI (Figure 3*a*). Soil in the 0 SMD treatments wet up quickly at the beginning of TI and reached the field capacity value of 0.35 m^3/m^3 after 2 weeks (Figure 3*b*). The soil water content continued to increase slightly over the next two weeks. Ridges in the delayed start treatment were slower in wetting up after irrigation commenced 1 week after TI but reached field capacity within 3 days (Figure 3*b*). On cessation of irrigation 3 weeks after TI, the soil water content decreased to 0.30 m^3/m^3.

Figure 3. Expt 1: Soil water content measured in the ridge during the scab control period in Maris Piper plots. *(a)* Unirr, —; 15 SMD —; *(b)* 0 SMD, —; TI 1-3, —.

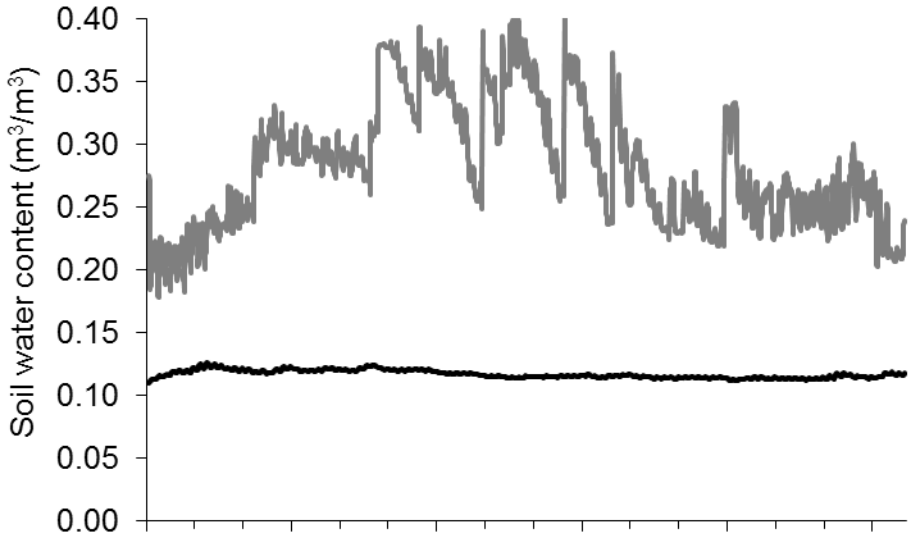


4.1.1.3.2. Expt 2

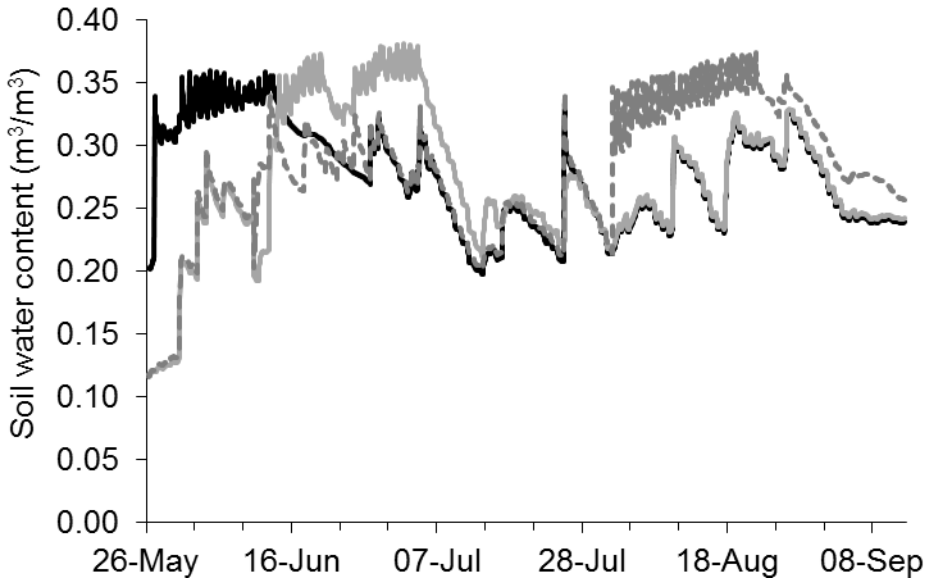
Soil in the ridge was also very dry (< 0.13 m^3/m^3) in unirrigated plots in Expt 2 for all of the season (Figure 4*a*). The 25 SMD treatment went above field capacity in the ridge at most irrigation events after each irrigation and never decreased below 0.22 m^3/m^3 at its driest (Figure 4*a*). Ridge soil in the different over-watered periods was maintained slightly above the field capacity value of 0.35 m^3/m^3 for the majority of each 3-week period (Figure 4*b*).

Figure 4. Expt 2: Soil water content measured in the ridge in Maris Piper plots. *(a)* Unirr, —; 15 SMD, —; *(b)* OW 0-3, —; OW 3-6, —, OW 10-13, – –.

(a)



(b)



4.1.1.4. *Common scab*

4.1.1.4.1. Expt 1

Incidence of common scab was *c*. 99% for all varieties but many tubers were infected with < 1% SA, particularly in Estima and King Edward (Table 17). Tubers with > 5% SA are generally regarded as unsuitable for packing and a typical minimum acceptable packout is 70%, i.e. ≤ 30% rejectable tubers. When using this criterion to judge scab incidence, Estima tubers were virtually all packable, whilst in King Edward only crops maintained at 0 SMD for a period (0 SMD and TI 1-3) had 100 % packable

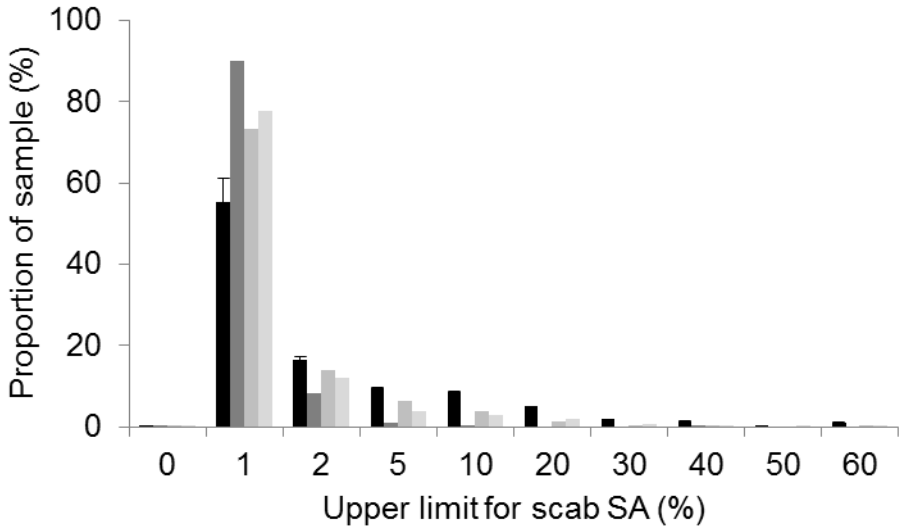
tubers (Table 17). The severity of common scab in Estima and King Edward was very low (0.47-0.78% SA). In Maris Piper, the overall severity of infection was extremely low compared with previous seasons. Typically, in previous seasons, the most effective irrigation regimes resulted in *c*. 2-3 % SA infected, whilst unirrigated crops ranged from 6-28 % SA infected. The unirrigated crops in 2011 had only 4.0-4.7 % SA infected, whilst the best treatments for control of scab produced tubers with only 0.6-0.7 % SA, similar to those found in the much more resistant varieties Estima and King Edward (Table 17). These treatments produced the lowest common scab found in Maris Piper in any irrigation experiment at CUF since comparable experiments began in 1992. In Maris Piper, withholding irrigation until 1 week after TI and stopping at 3 weeks increased the severity of scab significantly compared with maintaining the soil at field capacity for 4 weeks after TI but the results on control of scab were similar to those obtained with a more commercial regime of 15 mm at an SMD of 15 mm (Table 17). However, 16 mm less irrigation was applied in TI 1-3 treatments compared with the 15 SMD treatment. The distribution of scab on the surface of the tuber was different for the different irrigation regimes in Maris Piper, with there being more tubers in all categories > 5% SA in the unirrigated treatment than those receiving irrigation (Figure 5).

Table 17. Expt 1: Common scab incidence and severity

Variety	Cloddiness	Irrigation regime	Incidence < 5 % surface area		Severity
			%	Ang. trans.†	% SA affected
Estima	Cloddy	Unirr	99.3	87.3	0.58
		0 SMD	100.0	90.0	0.53
		15 SMD	100.0	90.0	0.47
		TI 1-3	100.0	90.0	0.51
	Fine	Unirr	100.0	90.0	0.51
		0 SMD	100.0	90.0	0.57
		15 SMD	100.0	90.0	0.50
		TI 1-3	100.0	90.0	0.52
King Edward	Cloddy	Unirr	99.6	87.9	0.78
		0 SMD	100.0	90.0	0.51
		15 SMD	98.8	85.1	0.75
		TI 1-3	100.0	90.0	0.52
	Fine	Unirr	99.3	86.1	0.64
		0 SMD	100.0	90.0	0.58
		15 SMD	99.1	86.7	0.62
		TI 1-3	99.9	89.0	0.63
Maris Piper	Cloddy	Unirr	79.1	65.2	4.74
		0 SMD	99.1	84.7	0.74
		15 SMD	93.3	75.7	1.80
		TI 1-3	95.6	80.2	1.60
	Fine	Unirr	83.2	66.9	4.01
		0 SMD	99.7	88.1	0.61
		15 SMD	94.1	76.2	1.67
		TI 1-3	92.2	74.2	1.74
S.E. (44 D.F.)			-	2.99	0.722
S.E. Same Cloddiness			-	3.03	0.698

†Angularly transformed data for statistical analysis

Figure 5. Expt 1: Distribution of common scab in different irrigation treatments in Maris Piper (mean of both cloddiness treatments). Unirrigated, ■; 0, ■; 15, ■; TI 1-3, ■. Bars represent 1 S.E.



The cloddiness of soil within the ridge had no effect on common scab even where soils were unirrigated. The incidence of powdery scab was high (*c*. 24-25 % in Estima and King Edward, 6 % in Maris Piper), and it was observed in all plots except one and was similar across all irrigation regimes. However, there was a significantly lower incidence of powdery scab in Fine (11 ± 2.4 %) than Cloddy (26 %) ridges.

4.1.1.4.2. Expt 2

As in other experiments at CUF in 2011, maintaining soils at field capacity or above soon after TI in Maris Piper resulted in much less severe common scab and reduction in the loss due to scabbed tubers with > 5% SA infected compared with rainfed crops (Table 18). In contrast with Expt 1, where a maximum SMD of 15 mm after TI produced an acceptably low scab severity (*c*. 1.7 % SA), albeit not as good as irrigating to field capacity every day, allowing a 25 mm SMD to accumulate before irrigating resulted in similar severity of scab (> 4 % SA) as the unirrigated plots in Expt 1. Common scab infection was lower in Saturna and Vales Sovereign than Maris Piper and unaffected by a wide range of SMD's following TI (Table 18). Powdery scab was observed in every plot except four and the mean incidence was 7, 12 and 2 % in Maris Piper, Saturna and Vales Sovereign, respectively, with no evidence for any effect of irrigation regime.

Table 18. Expt 2: Common scab incidence and severity

Variety	Irrigation regime	Incidence < 5 % surface area		Severity
		%	Ang. trans.†	% SA affected
Maris Piper	Unirr	62.9	52.9	8.12
	25 SMD	82.6	66.3	4.21
	OW 0-3	97.9	81.9	0.80
	OW 3-6	76.2	60.9	4.87
	OW 10-13	72.0	58.6	5.11
Saturna	Unirr	100.0	90.0	0.55
	25 SMD	100.0	90.0	0.57
	OW 0-3	99.8	88.4	0.63
	OW 3-6	99.4	87.5	0.64
	OW 10-13	100.0	90.0	0.59
Vales Sovereign	Unirr	99.3	87.2	0.66
	25 SMD	100.0	90.0	0.80
	OW 0-3	100.0	90.0	0.65
	OW 3-6	99.4	87.4	0.67
	OW 10-13	96.8	84.0	1.01
S.E. (28 D.F.)		-	2.97	0.951

†Angularly transformed data for statistical analysis

4.1.1.4.3. Expt 3

The incidence and severity of common scab was relatively low in Expt 3 consistent with Expts 1 and 2. In Maris Piper, as in Expt 1, maintaining the SMD ≤ 15 mm gave good control of common scab, similar to 0 SMD in this case (Table 19). There was no effect of a wide range of irrigation regimes on common scab in Jelly, Sylvana or Vales Sovereign, where scab severity was low. The maximum SMD reached in the 4 weeks after TI in the 35 and Unirr treatments in Maris Piper was 28-30 mm and therefore higher than the 25 SMD treatment in Expt 2 and this was reflected in the relative scab severities of these treatments.

The incidence of powdery scab was very high in Jelly (and unrelated to irrigation regime) and the disease was absent in Sylvana (Table 19). Powdery scab was present at a low incidence (≥ 3 %) on Maris Piper and Vales Sovereign seed but not detected on Sylvana and Jelly stocks.

Table 19. Expt 3: Common scab incidence and severity and powdery scab incidence

		Incidence < 5 % surface area		Severity	Powdery scab	
Variety	Irrigation regime	%	Ang. trans.†	% SA affected	Incidence (%)	Ang. trans.†
Jelly	Unirr	98.4	85.8	0.80	20.7	27.1
	0 SMD	97.0	81.8	1.37	32.1	34.5
	15 SMD	98.4	85.8	1.15	43.2	41.1
	35 SMD	100.0	90.0	1.02	38.0	38.1
Maris Piper	Unirr	70.5	58.1	6.59	10.6	19.0
	0 SMD	100.0	90.0	1.31	10.0	18.4
	15 SMD	96.7	81.5	1.42	10.2	18.6
	35 SMD	74.0	61.0	5.08	3.2	10.3
Sylvana	Unirr	98.9	86.5	0.83	0.0	0.0
	0 SMD	97.7	84.9	1.58	0.0	0.0
	15 SMD	98.9	86.5	1.15	0.0	0.0
	35 SMD	100.0	90.0	1.06	0.0	0.0
Vales	Unirr	100.0	90.0	0.78	2.2	8.5
Sovereign	0 SMD	98.9	86.5	1.15	0.0	0.0
	15 SMD	100.0	90.0	0.86	0.0	0.0
	35 SMD	100.0	90.0	0.64	1.7	7.5
S.E. (30 D.F.)		-	4.04	0.694	-	6.87

†Angularly transformed data for statistical analysis

4.1.1.4.4. Expts 4 and 5

In Expt 4, the severity of common scab was increased in soil kept dry following TI (6.2 ± 1.08% SA) compared with Wet (2.1% SA) and the proportion of tubers with < 5% SA infected was lower in Dry (68 ± 7.7%) than Wet (93%). In Expt 5, the differences in common scab between Dry and Wet treatments were smaller than in Expt 4 and not significant. Dry plots had 3.4 ± 0.27% SA infected and an 85 ± 1.5 % packout compared with 2.8% SA and 88%, respectively for Wet. There was a trace (< 1% incidence) infection of powdery scab in Expt 5 but no infection was observed in Expt 4.

4.1.1.4.5. Expts 6-7

Despite quite considerable differences in the soil ped-size distribution within ridges created using different destoning aggressiveness, there was no effect of cloddiness on common scab incidence or severity, even though the experiments were unirrigated. In

Expt 6, tubers in fine-structured ridges (mean of all ridge profiles and pressures) had, on average,1.55 ± 0.089% SA infected with scab, whilst tubers in cloddy ridges had 1.65 %. In Expt 7, tubers in fine-structured ridges had, on average, 2.99 ± 0.244% SA infected with scab, whilst tubers in cloddy ridges had 3.07% (mean of all destoning depths).

4.1.1.5. *Streptomyces populations*

4.1.1.5.1. Expt 1

Difficulty was experienced with quantification of streptomycete populations by real time PCR and this was attributed of the inhibition of the PCR reaction. The PCR was repeated several times but similar results were produced. Overall populations of 16S *Streptomyces* were much lower than in experiments conducted in the previous four years and only four samples out of 288 from Expt 1 showed detectable *txtA*-*Streptomyces.* The *txtA*-positive samples were random and not associated with a common treatment. The threshold for detection of DNA with the PCR technique used was 100 copies/g of peel, but in reality, no detectable pathogenic *Streptomyces* were found in 2011, which had only previously been observed in soils maintained very wet in 2009 and 2010. It is not clear why such inhibition occurred but it may have been that the very dry conditions within the ridge between planting and emergence at CUF resulted in low populations of *Streptomyces* at TI. The lack of detectable *txtA Streptomyces* was, however, consistent with the very low severity of common scab observed. The time courses of populations of total 16S *Streptomyces* are shown in Figure 6. There was no significant effect of any treatment at weeks 1 and 2 after TI but at week 3, when averaged over variety and cloddiness treatments, unirrigated plots had significantly greater populations than irrigated regimes. By 4 weeks after TI, populations of 16S *Streptomyces* under different irrigation regimes had separated further as 15 SMD treatments had a more 16S than the treatment maintained at 0 mm SMD, with unirrigated plots having greater populations than either. The treatment means for irrigation, variety and cloddiness at week 4 are presented in Table 20.

Figure 6. Expt 1: Total 16S *Streptomyces* populations. *(a)* Estima Cloddy; *(b)* Estima Fine; *(c)* King Edward Cloddy; *(d)* King Edward Fine; *(e)* Maris Piper Cloddy; *(f)* Maris Piper Fine. ■, 0 SMD; □, 15 SMD; ▲, TI 1-3; △, Unirr. Bars represent S.E.

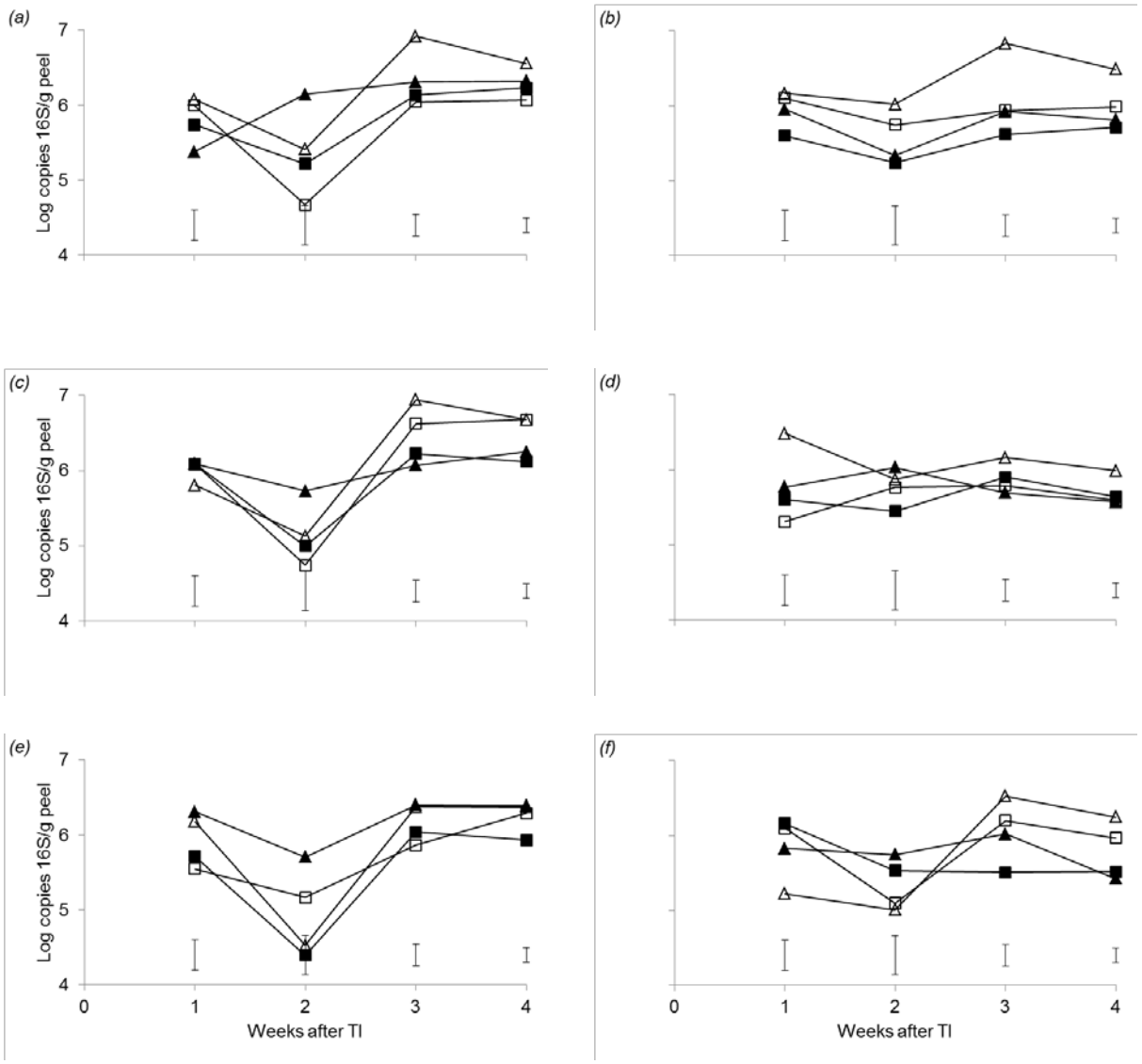


Table 20. Expt 1: Populations of 16S *Streptomyces* (log copies/g peel) at 4 weeks post tuber initiation. Main effects of treatments shown only

Variety	Irrigation regime	Cloddiness	16 S population
Estima			6.24
King Edward			6.23
Maris Piper			6.06
S.E. (44 D.F.)			0.071
	Unirr		6.39
	0 SMD		5.86
	15 SMD		6.10
	TI 1-3		5.96
	S.E. (44 D.F.)		0.082
		Cloddy	6.39
		Fine	5.86
		S.E. (3 D.F.)	0.104

It had been planned to use pyrosequencing to characterise populations of potential antagonists in samples extracted from tubers harvested 4 weeks post-TI in Expt 1. This was not conducted since the scab severity and *txtA Streptomyces* populations were so low.

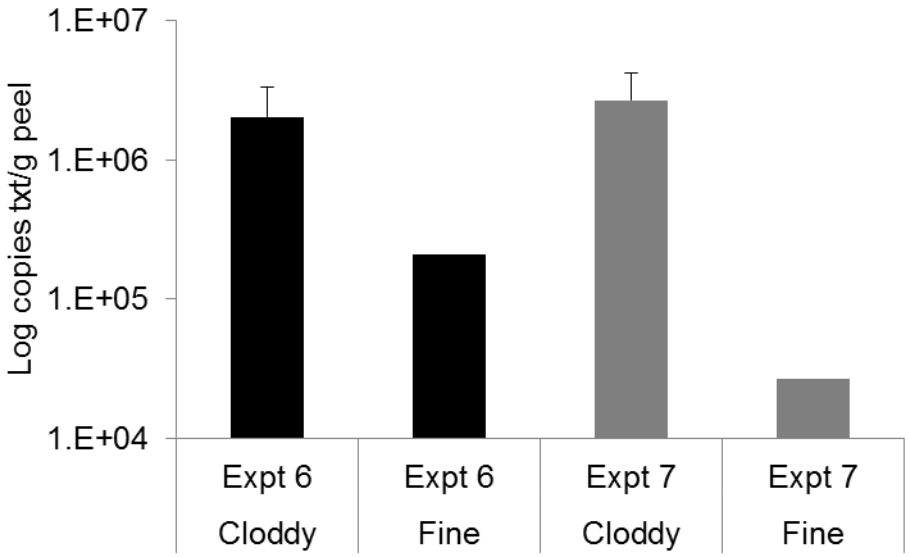
4.1.1.5.2. Expts 4 and 5

In Expt 4, there was no effect of irrigation regime on pathogenic *txtA Streptomyces* populations on tubers sampled 4 weeks after TI (mean 2.41 x 10^6 ± 2.03 x 10^6 copies/g of peel) or in Expt 5 (mean 2.77 x 10^6 ± 1.88 x 10^6 copies/g of peel) but the directional difference between Dry and Wet treatments mirrored the scab severity (i.e. Dry less than Wet).

4.1.1.5.3. Expts 6 and 7

Both cultivation experiments showed significantly lower populations of *txtA Streptomyces* on tubers growing in finely-structured ridges compared with coarse ridges despite there being no significant effect on common scab severity (Figure 7). There were no differences in 16S populations on tubers from the different cultivation treatments in either experiment.

Figure 7. Expts 6 and 7: *txt Streptomyces* populations on tubers at 4 weeks after tuber initiation. Expt 6: mean of Round ridge profile and Low pressure. Expt 7: mean of Normal depth.



4.1.1.6. *Tuber cracking*

4.1.1.6.1. Expt 1

Estima had more linear cracking than Maris Piper or King Edward (which both had a very low incidence) and unirrigated plots had less cracking than irrigated plots (Table 21). In Estima and Maris Piper, plots maintained at field capacity during the entire scab control period had more cracking than following a restricted period at field capacity (TI 1-3) or where the maximum SMD was 15 mm. In King Edward, the three irrigated regimes had a similar incidence of linear cracking. Cloddy soils had a higher incidence of linear cracking than fine. There was no significant effect of irrigation regime on superficial cracking, which was present at a very low incidence (mean 0.7 %).

Table 21. Expt 1: Tuber cracking incidence and severity

			Linear cracks		Superficial cracks
Variety	Cloddiness	Irrigation regime	Incidence (%)	Ang. trans.†	Incidence (%)
Estima	Cloddy	Unirr	1.2	3.7	0.6
		0 SMD	14.5	22.3	0.4
		15 SMD	5.8	13.7	1.0
		TI 1-3	5.2	12.8	0.7
	Fine	Unirr	0.6	2.6	0.9
		0 SMD	11.1	18.7	0.8
		15 SMD	0.0	0.0	0.4
		TI 1-3	1.5	4.1	0.5
King Edward	Cloddy	Unirr	0.4	2.0	0.3
		0 SMD	2.5	8.9	0.5
		15 SMD	2.8	7.2	0.7
		TI 1-3	2.1	6.3	0.5
	Fine	Unirr	0.0	0.0	1.0
		0 SMD	1.3	6.5	0.5
		15 SMD	1.6	5.8	0.8
		TI 1-3	1.8	7.7	0.6
Maris Piper	Cloddy	Unirr	0.5	2.3	1.0
		0 SMD	2.0	8.1	0.8
		15 SMD	0.4	2.0	0.7
		TI 1-3	0.5	2.4	0.9
	Fine	Unirr	0.0	0.0	0.9
		0 SMD	0.9	4.4	1.0
		15 SMD	0.4	2.0	0.6
		TI 1-3	0.4	2.1	0.7
S.E. (44 D.F.)			-	1.25	-
S.E. Same Cloddiness			-	1.30	-

†Angularly transformed data for statistical analysis

4.1.1.6.2. Expt 2

On average, 2.4% of tubers were affected by linear cracking which was generally, but not significantly, higher in over-watered Vales Sovereign than in the same treatments involving Maris Piper and Saturna (Table 22). Superficial cracking was much greater in Vales Sovereign than the other two varieties and despite all irrigated treatments having a higher numerical incidence than unirrigated in Maris Piper and Vales Sovereign, the differences were not significant (Table 22). The superficial cracking was almost universally confined to < 5 % SA and the severity was very low but higher in Vales Sovereign than Maris Piper and Saturna and unrelated to irrigation regime.

In Vales Sovereign, *c*. 25-33% of tubers were badly misshapen with secondary growth in the late over-watered OW 10-13 treatment. The misshapen tubers mostly, but not universally, had hollow heart and also had a high severity of superficial cracking. There were no significant misshapes from other irrigation regimes in Vales Sovereign. There was an increased incidence of hollow heart in unirrigated Saturna (6 %) *c.f*. other irrigation regimes (< 1 %) in the same variety. There was no internal rust spot observed in any plots.

Table 22. Expt 2: Tuber cracking incidence and severity

		Linear cracks		Superficial cracks			
Variety	Irrigation regime	Incidence (%)	Ang. trans.†	Incidence (%)	Ang. trans.†	Incidence < 5 % SA affected	Severity (% SA affected)
Maris Piper	Unirr	0.6	2.6	0.0	0.0	100.0	0.00
	25 SMD	1.2	5.0	1.1	4.2	100.0	0.00
	OW 0-3	1.5	5.8	0.7	2.8	100.0	0.00
	OW 3-6	0.8	4.3	2.6	7.5	100.0	0.01
	OW 10-13	2.0	4.7	1.6	5.9	100.0	0.01
Saturna	Unirr	2.1	6.8	0.7	2.8	99.7	0.03
	25 SMD	1.1	4.8	0.0	0.0	100.0	0.00
	OW 0-3	2.3	8.5	0.2	1.6	100.0	0.00
	OW 3-6	2.0	8.1	0.7	2.8	100.0	0.01
	OW 10-13	1.0	4.6	1.0	4.6	100.0	0.01
Vales	Unirr	1.4	3.9	3.4	8.7	100.0	0.02
Sovereign	25 SMD	1.4	3.9	13.4	19.9	100.0	0.12
	OW 0-3	4.7	12.1	13.6	20.6	100.0	0.10
	OW 3-6	3.0	5.9	5.1	10.7	100.0	0.04
	OW 10-13	10.4	17.0	9.8	18.1	98.8	0.21
S.E. (28 D.F.)		-	3.61	-	3.40	-	0.038

†Angularly transformed data for statistical analysis

4.1.1.6.3. Expt 3

The incidence of linear cracking in Jelly, Maris Piper and Sylvana was greatest where soils were kept at field capacity throughout the season, although Sylvana had linear cracks in all irrigated treatments and the incidence was < 5 % for any treatment (Table 23). The incidence of superficial cracking was greatest in the 0 SMD treatments (mean 44 %) and decreased as the soils were maintained drier except in Vales Sovereign where the 15 SMD treatment was similar to the 0 SMD treatment.

Table 23. Expt 3: Tuber cracking incidence and severity

		Linear cracks		Superficial cracks			
Variety	Irrigation regime	Incidence (%)	Ang. trans.†	Incidence (%)	Ang. trans.†	Incidence < 5 % SA affected	Severity (% SA affected)
Jelly	Unirr	0.0	0.0	3.0	8.2	100.0	0.01
	0 SMD	3.0	8.2	23.4	28.6	100.0	0.17
	15 SMD	0.0	0.0	4.5	10.0	100.0	0.02
	35 SMD	0.0	0.0	4.4	9.6	100.0	0.02
Maris Piper	Unirr	0.0	0.0	6.4	11.7	100.0	0.03
	0 SMD	3.3	6.1	49.8	44.9	100.0	0.36
	15 SMD	1.1	3.5	25.9	29.8	100.0	0.14
	35 SMD	0.0	0.0	10.5	18.5	100.0	0.07
Sylvana	Unirr	0.0	0.0	8.6	16..6	100.0	0.07
	0 SMD	4.8	10.2	56.0	48.5	100.0	0.50
	15 SMD	2.7	7.7	23.5	28.6	98..9	0.20
	35 SMD	1.2	3.7	22.2	28.1	100.0	0.18
Vales Sovereign	Unirr	1.0	3.4	16.6	23.1	100.0	0.11
	0 SMD	3.0	8.2	46.6	42.4	100.0	0.44
	15 SMD	1.6	4.2	54.1	48.1	94.0	1.26
	35 SMD	1.4	3.9	24.6	28.6	100.0	0.12
S.E. (30 D.F.)		-	2.23	-	5.23	-	0.243

†Angularly transformed data for statistical analysis

4.1.1.6.4. Expts 4-7

There was no superficial cracking in Expt 4 and the incidence of linear cracking was < 0.5 % and the same for both Dry and Wet treatments. There was also no superficial cracking in Expt 5 and whilst the incidence of linear cracking was slightly higher at 1.7 %, it was not affected by irrigation treatments. There was no cracking in either Expt 6 or Expt 7.

4.1.1.7. *Number of tubers and tuber yield*

4.1.1.7.1. Expt 1

In Expt 1, the number of tubers > 10 mm was increased by maintaining a 0 mm SMD throughout the 4 weeks following TI *c.f.* a 15 mm SMD or a shorter period at field capacity (Table 24), resulting from an increase in the number of tubers/stem (data not shown). This repeats previous findings of 2009 that very wet soils at TI can produce increased numbers of viable tubers which is contradictory to most previous work at CUF (Stalham 2010). In cloddy soils, fewer tubers were produced in unirrigated than

all other irrigated treatments but in fine soils, the unirrigated treatment only produced significantly fewer tubers than the very wet 0 SMD treatment (Table 24). Total and ware (> 40 mm) fresh weight yields and dry weight yields were similar for crops receiving irrigation but were much lower where unirrigated (Table 24). Tuber [DM] was also greater where crops were unirrigated but other irrigation regimes were similar. Maris Piper produced higher yields than Estima and King Edward, which had similar yields. Partially as a consequence of the high tuber [DM], tuber dry weight yields in Maris Piper were very high.

Table 24. Expt 1: Number of tubers, yield and dry matter concentration at final harvest

Variety	Cloddiness	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Yield > 40 mm (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Estima	Cloddy	Unirr	277	46.0	44.9	21.9	10.0
		0 SMD	363	62.5	60.9	19.6	12.2
		15 SMD	286	65.3	64.3	21.3	13.9
		TI 1-3	293	62.1	60.7	20.0	12.4
	Fine	Unirr	244	39.3	38.3	22.6	8.9
		0 SMD	316	60.0	58.5	20.5	12.3
		15 SMD	300	67.4	66.4	21.9	14.8
		TI 1-3	283	66.7	65.6	20.8	13.8
King	Cloddy	Unirr	439	38.1	33.6	25.1	9.5
Edward		0 SMD	617	63.6	58.5	23.2	14.8
		15 SMD	502	62.5	59.6	22.8	14.3
		TI 1-3	542	60.8	56.5	23.4	14.3
	Fine	Unirr	452	41.4	37.1	23.5	9.7
		0 SMD	582	60.1	56.7	22.9	13.8
		15 SMD	580	57.8	53.6	22.9	13.2
		TI 1-3	483	58.3	55.3	23.3	13.6
Maris Piper	Cloddy	Unirr	334	42.4	40.2	26.5	11.2
		0 SMD	541	73.0	70.3	26.1	19.1
		15 SMD	420	73.5	71.6	25.3	18.7
		TI 1-3	411	67.9	65.9	25.4	17.3
	Fine	Unirr	409	49.3	46.1	27.5	13.5
		0 SMD	484	72.5	69.2	25.1	18.2
		15 SMD	395	71.7	69.7	25.0	18.0
		TI 1-3	416	76.0	73.6	25.4	19.1
S.E. (44 D.F.)			30.7	3.87	3.96	0.81	1.05
S.E. Same Cloddiness			29.0	3.57	3.59	0.84	1.01

4.1.1.7.2. Expt 2

The final harvest results are presented in Table 25. Similar to Expt 1, the number of tubers > 10 mm was increased where crops were maintained over field capacity

during the 3 weeks after TI compared with other irrigation regimes (Table 25). The relative increase averaged across all varieties was *c*. 20 %. Tuber fresh and dry weight yields were large in Maris Piper and Vales Sovereign and whilst irrigation increased yields in all varieties, none of the over-watering regimes affected yield compared with the standard 25 SMD irrigation regime (Table 25). The largest yield loss caused by not irrigating was observed in Vales Sovereign (22.4 t/ha), with Saturna showing the smallest difference (16.5 t/ha). Tuber [DM] tended to be greatest in unirrigated crops but was only significantly different from the mid and late over-watered treatments (Table 25).

Table 25. Expt 2: Number of tubers, yield and dry matter concentration at final harvest

Variety	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Maris Piper	Unirr	435	49.8	26.1	12.9
	25 SMD	456	67.6	25.6	17.3
	OW 0-3	523	69.0	24.9	17.2
	OW 3-6	419	64.5	25.1	16.2
	OW 10-13	454	69.7	25.0	17.4
Saturna	Unirr	580	38.3	26.4	10.1
	25 SMD	605	58.3	25.9	15.1
	OW 0-3	668	54.1	25.8	13.9
	OW 3-6	592	56.4	24.8	14.0
	OW 10-13	586	50.2	25.9	13.0
Vales Sovereign	Unirr	244	55.2	23.4	13.0
	25 SMD	280	75.0	21.9	16.3
	OW 0-3	390	81.4	22.7	18.5
	OW 3-6	286	75.3	20.2	15.1
	OW 10-13	280	78.7	20.5	16.0
S.E. (28 D.F.)		36.3	7.00	0.68	1.57

4.1.1.7.3. Expt 3

Yields were generally high and there was no significant difference in irrigating at a 0 or 15 mm SMD on fresh weight or dry weight yield (Table 26). All varieties produced similar yields when soil was kept wet. Allowing the SMD to increase to 35 mm before irrigating reduced yield by an average of 10.6 t/ha across all varieties but Sylvana showed the greatest reduction in yield by increasing the SMD, whilst Jelly was the least sensitive to change in soil dryness. As in Expt 1, keeping soils at field capacity during TI and shortly afterwards significantly increased the number of tubers retained compared with drier regimes. Tuber [DM] generally increased as the SMD for

commencing irrigation increased, except in Sylvana where unirrigated crops had a similar [DM] to fully-irrigated crops.

Table 26. Expt 3: Number of tubers, yield and dry matter concentration at final harvest

Variety	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Jelly	Unirr	234	55.5	21.3	13.2
	0 SMD	273	73.4	19.4	14.2
	15 SMD	247	70.9	20.2	14.3
	35 SMD	236	61.2	21.9	13.3
Maris Piper	Unirr	462	49.5	25.3	12.5
	0 SMD	547	71.4	21.6	15.4
	15 SMD	481	69.5	22.5	15.7
	35 SMD	499	61.9	24.1	14.9
Sylvana	Unirr	210	49.1	19.5	9.6
	0 SMD	258	73.8	18.9	14.0
	15 SMD	225	73.5	19.9	14.6
	35 SMD	217	61.6	20.5	12.6
Vales Sovereign	Unirr	267	56.9	23.8	13.6
	0 SMD	315	77.2	20.5	15.7
	15 SMD	273	76.5	21.0	16.1
	35 SMD	276	66.2	20.6	13.6
S.E. (30 D.F.)		22.2	3.29	0.74	1.00

4.1.1.7.4. Expts 4-7

There was no significant difference in total yield (55.2 ± 0.92 t/ha), number of tubers (382 000 ± 17 700 /ha) or tuber [DM] (23.7 ± 0.19 %) between Dry and Wet treatments in Expt 4. In Expt 5, which was allowed to grow for longer before desiccation, total yield (71.7 ± 1.86 t/ha), number of tubers (393 000 ± 19 300 /ha) and tuber [DM] (23.1 ± 0.33 %) were again similar between Dry and Wet. There was no effect of ridge cloddiness on the yield, number of tubers or tuber [DM] in Expts 6 or 7 (data not shown).

4.1.1.8. *Radiation use efficiency, total DM yield, soil mineral nitrogen and nitrogen uptake*

4.1.1.8.1. Expt 2

All three varieties had the same season-long radiation use efficiency (RUE) although, numerically, the efficiency of Vales Sovereign was larger than that of Saturna (Table

27*a*). The unirrigated treatment had the smallest RUE but differences between treatments that received some irrigation during the growing season were relatively small. The overall mean RUE (1.35 t DM/TJ) was similar to values found in other experiments at CUF and elsewhere in previous seasons. Total DM yield is a function of radiation absorption and the efficiency with which the crop converts the absorbed radiation to DM. Total DM yield was significantly affected by both variety (mainly as a consequence of varietal effects on integrated ground cover and radiation absorption) and also irrigation regime (a combination of its effects on RUE and on the amount of radiation absorbed). There was nearly a two-fold difference between the smallest DM yield (unirrigated Saturna) and the largest (Vales Sovereign OW 0-3, Table 27*b*). When averaged over the five irrigation treatments, Saturna had the smallest total N uptake and Vales Sovereign had the largest. There was no good evidence to suggest that periods of over-watering reduced DM accumulation since all the over-water treatments had similar DM yields to the crops maintained at an SMD of *c*. 25 mm throughout the season.

Table 27. Expt 2: Effect of variety and irrigation on *(a)* season-long average radiation used efficiency (t DM/TJ); *(b)* total DM yield (t/ha) and (*c*) maximum total N uptake (kg N/ha)

	Variety	Unirr	25 SMD	OW 0-3	OW 3-6	OW 10-13
(*a*)	Maris Piper	1.19	1.34	1.49	1.40	1.35
	Saturna	1.14	1.34	1.38	1.29	1.31
	Vales Sovereign	1.32	1.44	1.52	1.31	1.48
	Mean	1.21	1.37	1.46	1.33	1.38
		S.E. (28 D.F.): Variety, 0.034; Irrigation, 0.043 and Variety×Irrigation, 0.075				
(*b*)	Maris Piper	14.7	19.6	19.4	19.8	20.5
	Saturna	11.2	16.5	15.7	15.7	14.3
	Vales Sovereign	15.3	18.3	20.8	17.6	19.4
	Mean	13.7	18.1	18.6	17.7	18.1
		S.E. (28 D.F.): Variety, 0.86; Irrigation, 1.11 and Variety×Irrigation, 1.91				
(*c*)	Maris Piper	217	289	238	277	321
	Saturna	186	235	219	253	223
	Vales Sovereign	277	307	250	318	339
	Mean	227	277	236	283	294
		S.E. (28 D.F.): Variety, 13.2; Irrigation, 17.1 and Variety×Irrigation, 29.6				

Crop N uptake was smallest in the early over-watered (OW 0-3) and the unirrigated crops (Table 27*c*). However, there was relatively little difference in total N uptakes of the 25 SMD, OW 3-6 and OW 10-13 treatments. The CUF N model has shown that

total DM yield and tuber FW yield are, to a certain extent, related to N uptake by the crop. These data may appear to be contrary to this since, in the case of the early over-watered Vales Sovereign (OW 0-3), the largest DM yields were obtained with the smallest N uptake. However, this treatment also had the slowest rate of tuber N uptake and thus depletion of N reserves in the canopy was sufficiently slow to ensure the canopy was persistent enough to give large yields. These data emphasize the need for both total N uptake and the rate of tuber N uptake to be known in order to accurately predict likely canopy persistence. The effects of the irrigation treatments on the combined amount of N in the crop-soil system are shown in Table 28 and Table 29. Care is needed in interpreting these data since they have relatively large S.E.s.

At the first sampling (*c*. 39 DAE), the unirrigated plots contained most N and the early over-watered (OW 0-3) the least. The small amount of N in the OW 0-3 treatment was a consequence of restricted N uptake and also small amounts of SMN (Table 28 and Table 29). This decrease in SMN may be a consequence of N leaching or loss of N by denitrification since drainage below 90 cm was estimated to be 71 mm in the period that the OW 0-3 treatments were being imposed. At the second crop and soil sampling (58 DAE), the overall amount of crop and soil N in the unirrigated plots had decreased by *c.* 200 kg N/ha and this was due to a decrease in SMN of *c*. 320 kg N/ha (Table 29). This decline in SMN is unlikely to be due to N leaching or denitrification since there was little drainage and probably due to the immobilization of N by the soil micro-flora. The amount of N in OW 0-3 was reasonably consistent between the first and second sampling but there was some evidence of a decrease in N for the later over-watering treatments. With the exception of the unirrigated treatment, and once the size of the standard errors were taken into account, there was probably little change in amount of crop-soil N between the second and third samplings. Numerically, the amount of N increased slightly between the third and fourth samplings, particularly in the plots maintained at 25 mm SMD but, overall, there was probably little difference in the amount of N in the crop-soil system between the second and fourth harvests. These data suggest that after *c*. 58 DAE mineralization of N may slow (or inputs to the crop-soil system were balanced by losses). The restricted N uptake of the unirrigated crop resulted in the largest residues of SMN remaining in the soil in the autumn and these could be at risk of leaching.

Table 28. Expt 2: Combined quantity of N (kg N/ha) in crop and soil (0-90 cm) in Maris Piper plots on four occasions

Irrigation regime	14 June	3 July	8 August	13 September
Unirr	702	493	347	365
25 SMD	377	308	269	366
OW 0-3	206	218	295	285
OW 3-6	447	239	248	318
OW 10-13	422	355	318	362
Mean	431	323	295	339
S.E. (8 D.F.)	44.0	23.5	21.7	35.6

When averaged over all the irrigation treatments, the quantity of SMN was largest at the first harvest and then tended to decrease as the season progressed (Table 29). The quantity of SMN was consistently largest in the unirrigated plots and at early harvests, smallest in the over-watered plots OW 0-3 and OW 3-6 but this was also related to the timing of the over-water treatments. Despite having a very low amount of SMN on 14 June, the OW 0-3 treatment had similar amount of SMN at final harvest to other over-watered treatments and only marginally less than '25'. The seasonal drainage totals for Unirr, 25 SMD, OW 0-3, OW 3-6 and OW 10-13 were 0, 17, 98, 94 and 90 mm, respectively (Table 3).

Table 29. Expt 2: Effect of irrigation treatments on soil mineral nitrogen (kg N/ha, 0-90 cm) under Maris Piper plots on four occasions during the season

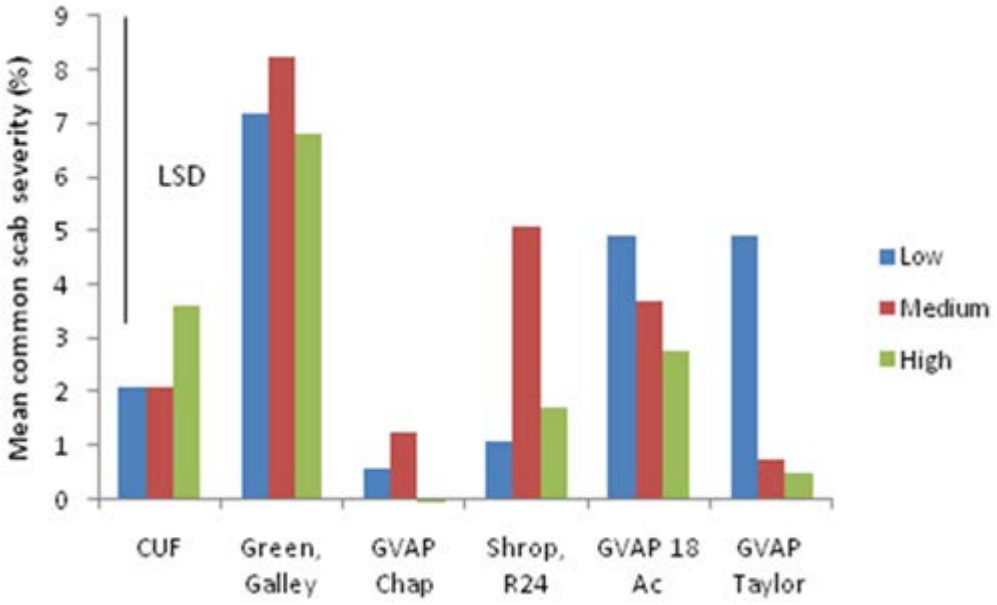
Irrigation regime	14 June	3 July	8 August	13 September
Unirr	587	262	123	148
25 SMD	258	90	30	77
OW 0-3	59	29	41	48
OW 3-6	344	33	22	41
OW 10-13	303	112	29	42
Mean	310	105	49	71
S.E. (8 D.F.)	45.5	19.2	14.4	33.3

4.1.2. Fera experiments

4.1.2.1.1. Expt 8

Significant differences in common scab severity (P=0.002) were detected between the field soils after harvest (Figure 8). Tubers grown in soil from Greenseed Galley Pit had consistently greater common scab severity than in soils from the other sites. There was no effect of moisture level on common scab.

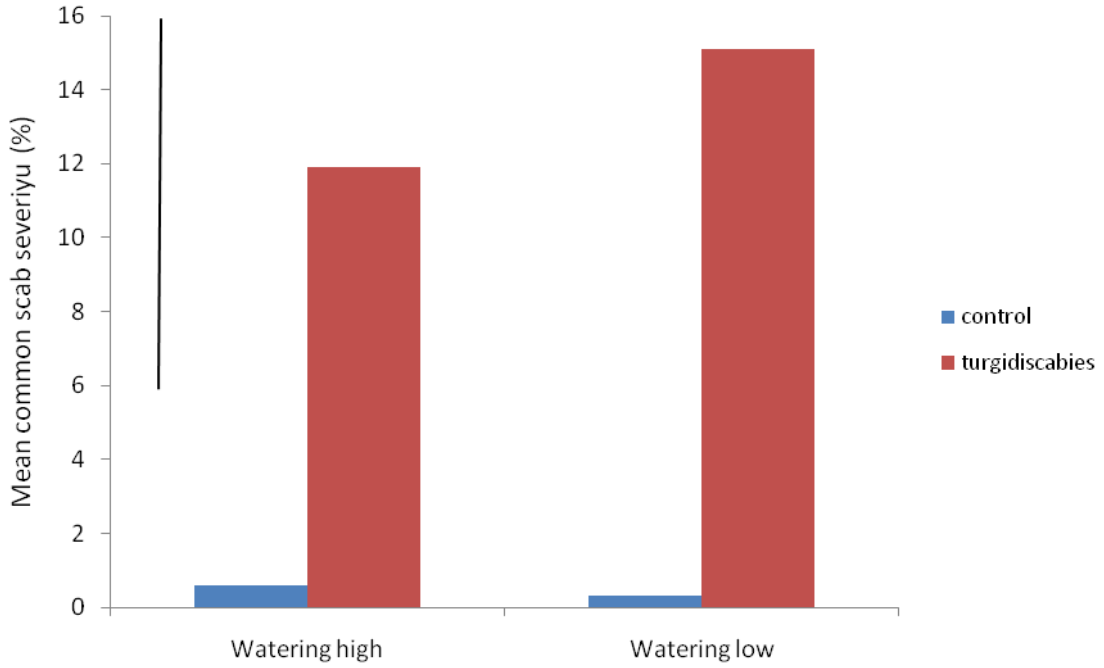
Figure 8. Expt 8: Effect of moisture level on common scab in tubers grown in pots using soils from six fields.



4.1.2.1.2. Expt 9

Streptomyces turgidiscabies was shown to be effective in producing common scab (Figure 9). However, more severe common scab might have been expected based on the results of previous work at Fera in pots. No thaxtomin was detected in DNA extracts from tubers so it is possible that the isolate of *Streptomyce*s used had lost the ability to produce the toxin during culture or storage. Moisture level did not affect common scab.

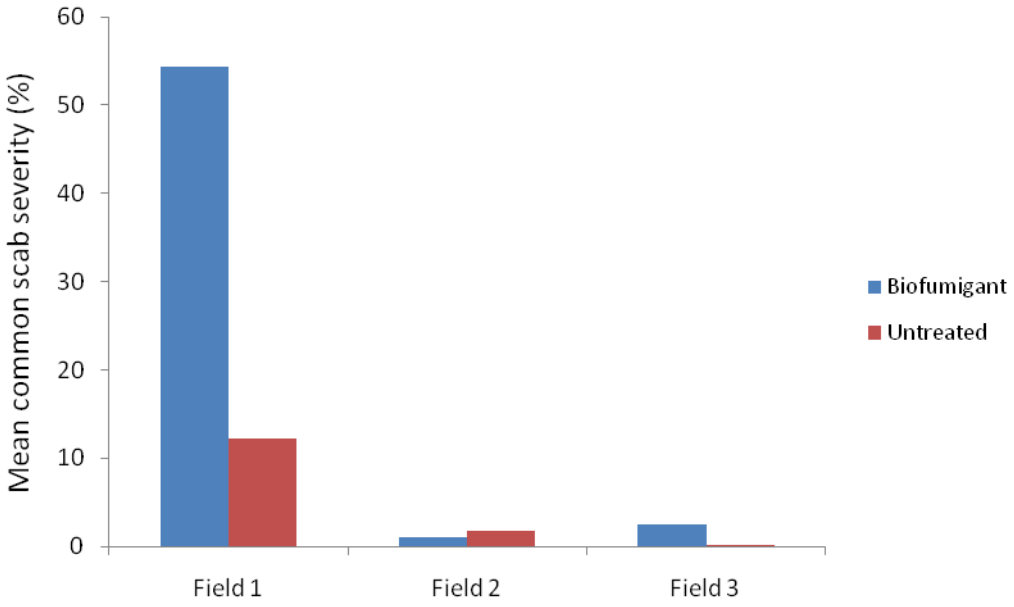
Figure 9. Expt 9: Effect of low and high soil moisture level on common scab in tubers subjected to artificial inoculation by *Streptomyces turgidiscabies.* The vertical bar is the least significant difference. Mean of two soil types.



4.1.2.1.3. Expt 10

Tubers grown in soils from two of the three fields sampled did not produce appreciable common scab (Figure 10). However, tubers grown in soil from Field 1 that had been treated with biofumigant developed more common scab than in soil collected from the same field but where biofumigant had not been successfully grown or incorporated. Moisture level had no effect on common scab levels.

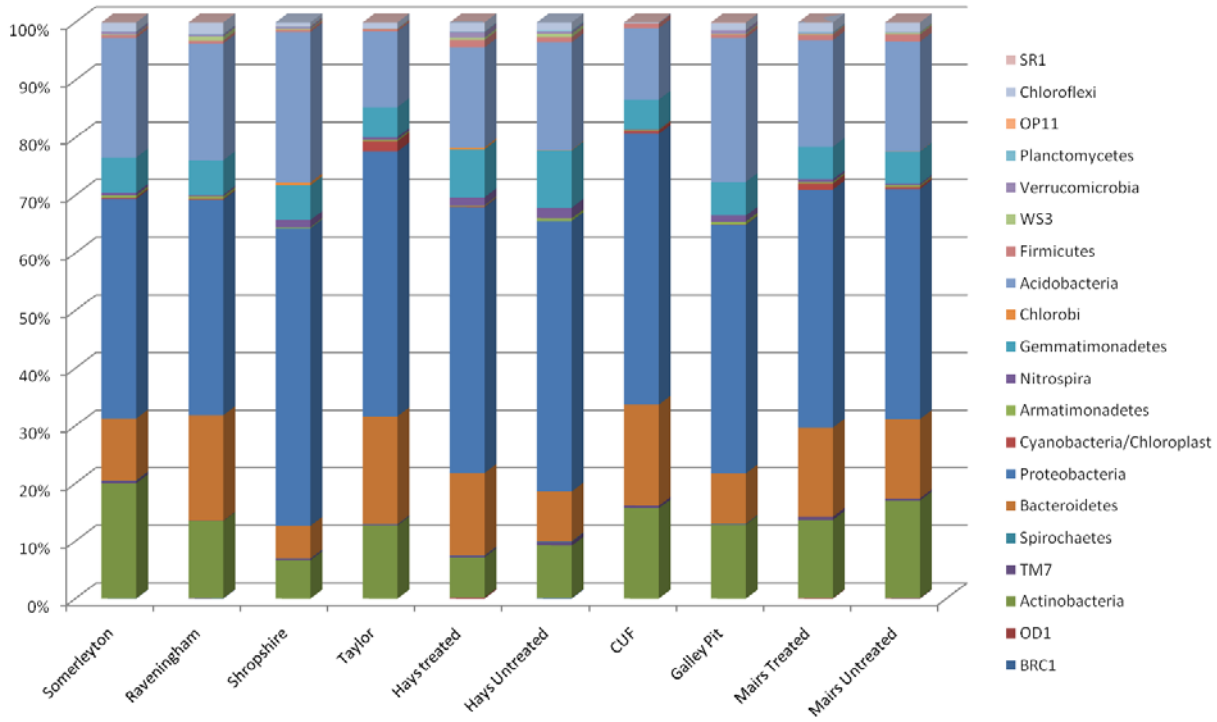
Figure 10. Expt 10: Effect of biofumigant treatment on common scab levels. SED = 3.69.



4.1.2.1.4. Expt 11

Sequencing yielded a total of *c.*19,000 sequences from the 10 soil DNA samples after filtering for quality and length. Sequences were classified to phylum level by comparison with public databases and results are presented in Figure 11. Differences in populations of the major bacterial phyla *Actinobateria, Bacteroidetes, Proteobacteria* and *Acidobacteria* could be observed, although no clear correlation with scab levels was found (data not shown).

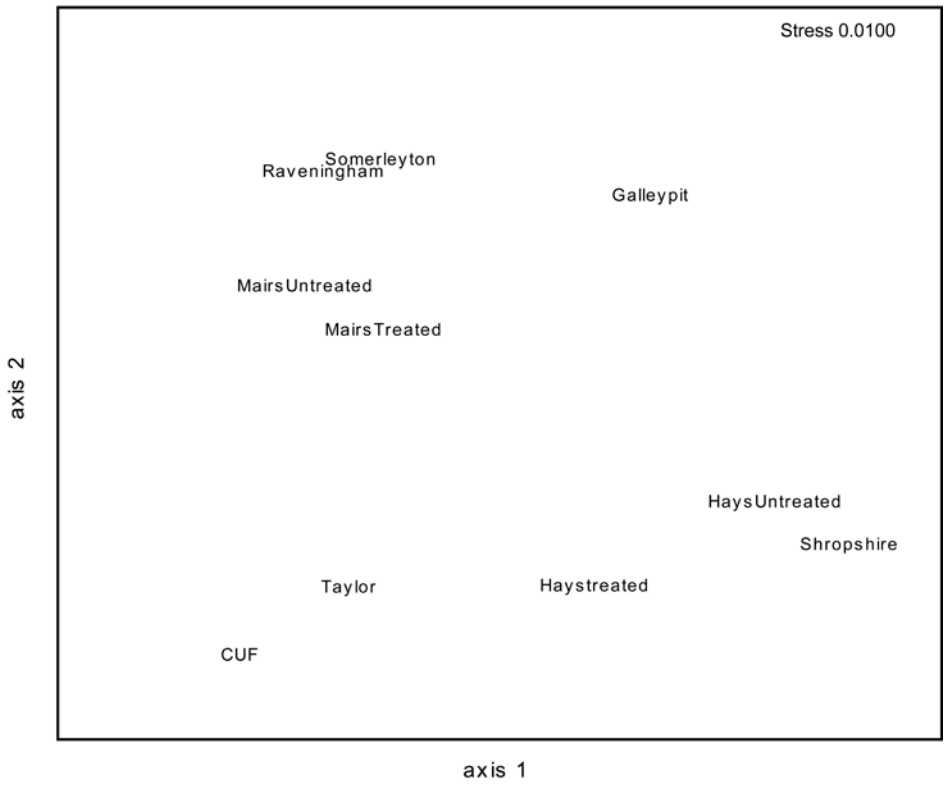
Figure 11. Overview of the bacterial phyla* found in analysed soil samples.



*TM7, WS3, BRC1 (NKB19), SR1, OP11, and OD1 describe candidate bacterial phyla for which no known cultivable representative has yet been found.

In order to compare total soil microflora a non-metric multidimensional scaling (nMDS) analysis was undertaken; an ordination plot is shown in Figure 12. Using nMDS analysis showed that the bacterial communities of some soils selected for use in pot trials were similar in their composition. Examples include the soil communities from GVAP Somerleyton 18 Acres and GVAP Raveningham Chapmans or from CUF and GVAP Taylor. In contrast, the soil community from G Shropshire R24 appeared more similar to the Branston Hays soil whereas the Greenseed Galley Pit-derived communities appeared dissimilar from any other soils analysed. Interestingly, the communities from Branston soils subjected to biofumigation treatment were very similar to communities in the untreated counterparts, which indicates a strong influence of the soil biota and abiotic characteristics over the effect of any biofumigation treatment.

Figure 12. Non-metric Multidimensional Scaling biplot of bacterial communities from potato grown in different soils under different irrigation and fumigant treatment. Sample names correspond to soils listed in Table 6.



4.2. 2012 experiments

4.2.1. CUF and Greenvale AP experiments

4.2.1.1. *Emergence, tuber initiation and ground cover*

4.2.1.1.1. Expt 12

In Expt 12, 50% emergence was reasonably synchronous for most varieties (35-39 days after planting), but the number of days taken between 10 and 90% emergence was shorter in Desiree, Jelly and Maris Piper (5-6 days) than in Bute, Flair and Sylvana (10-14 days). Initial emergence was later in Vales Sovereign than other varieties, 50 % emergence was only attained at 50 days after planting and nearly 40% of plants failed to emerge. As a consequence, there was a large variation in the size of adjacent plants in this variety, making the mean date of TI difficult to estimate so that irrigation scheduling was problematic. Owing to the variation in emergence in Vales Sovereign, frequent irrigation timings (0 SMD and TI 1-4) were started at 10% TI rather than 50%. Tuber initiation (50%) occurred 17, 15, 18, 18 and 15 days after

50% emergence in Desiree, Flair, Jelly, Maris Piper and Sylvana, respectively. Bute initiated tubers much later (24 days after emergence) than other varieties.

All crops reached full (> 99 %) ground cover, even with no irrigation, with the exception of Vales Sovereign, where the patchiness of crop emergence made it difficult to measure a representative area of the plot. Maintaining soils at field capacity for each plant in Vales Sovereign was impossible and it was clear that this variety was over-watered in the 0 SMD and TI 1-4 treatments and this had adverse effects on ground cover duration (Table 30). Similar effects of over-watering on ground cover duration have been observed in experiments during 2009-2011. Ground covers in Bute were similar across irrigation regimes but early scab control irrigation (0 SMD) was slower in reaching full ground cover and Unirr senesced earlier than 15 SMD and TI 1-4. In Desiree, ground cover was sustained longer in the 0 SMD treatment than 35 SMD or Unirrig (Table 30). In Flair, treatments that were irrigated frequently during scab control (0 SMD and TI 1-4) maintained their canopies longer than less frequently-watered treatments. With Jelly and Maris Piper, Unirrig and 35 SMD treatments suffered premature senescence compared with more frequently-irrigated treatments, but in Maris Piper maintaining soil at field capacity from TI caused canopies to senesce earlier than 15 SMD and TI 1-4 treatments. Other than Bute, Sylvana had the shortest-lived canopies and the irrigation treatments followed a similar pattern in terms of onset of senescence as Maris Piper (Table 30).

Table 30. Expt 12: Effect of variety and irrigation on season-long integrated ground cover (% days)

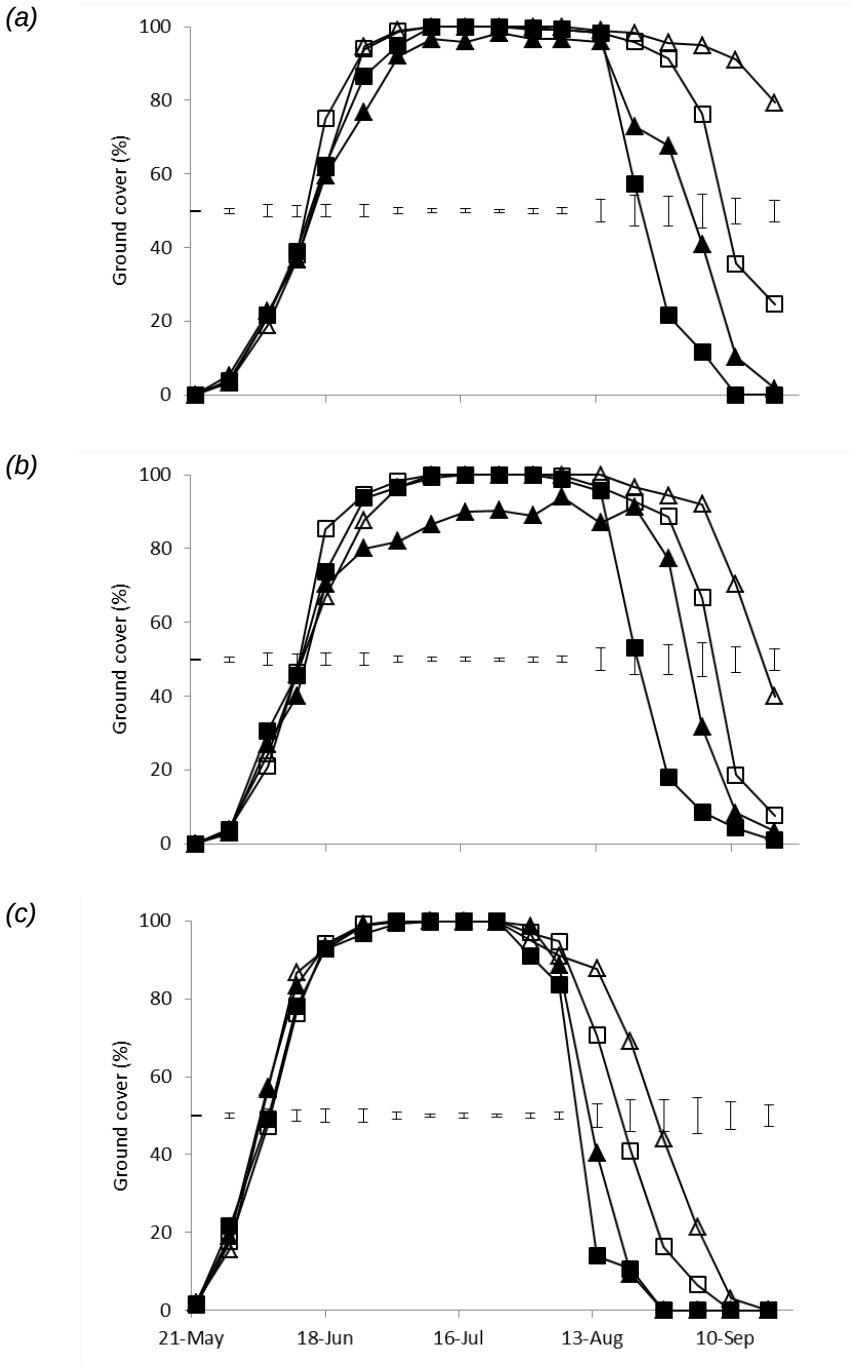
	Irrigation regime				
	Unirr	0 SMD	15 SMD	35 SMD	TI 1-4
Bute	5838	6020	6403	5980	6291
Desiree	6648	8003	7614	7098	7520
Flair	5535	6498	5544	5558	6692
Jelly	6291	8596	8638	7679	9228
Maris Piper	6930	8302	8783	8234	8818
Sylvana	5626	6120	6979	6347	6606
Vales Sovereign	5931	4657	7254	6662	6396
S.E. (68 D.F.)			386.2		
Mean	6114	6885	7316	6794	7364
S.E. (68 D.F.)			146.0		

4.2.1.1.2. Expt 13

Emergence was *c*. 17 days later in 2012 than the same experiment in 2011. Saturna emerged rapidly reaching 50 % emergence 32 days after planting, with Maris Piper and Markies emerging 9 days later. The interval from 10-90% emergence was 7 days in Markies and Saturna and only 4 days in Maris Piper. Tuber initiation occurred 21 days after 50% emergence in Saturna and 19 days after emergence in Maris Piper and Markies. Tuber initiation was very short in all varieties (4-5 days from 10-90% plants tuberized).

The effect of irrigation regime and variety on ground cover development is shown in Figure 13. Ground cover increased similarly for all irrigation treatments within each variety but ground cover expansion slowed in the Markies OW 0-3 treatment at *c*. 80% ground cover and maximum canopy cover did not exceed 90%, whereas it reached > 98% in all other treatment combinations (Figure 13*b*). There was also some evidence that the OW 0-3 treatment in Maris Piper slowed ground cover development around about the same time but the effect was not significantly different from other irrigation regimes (Figure 13*a*). Canopies in unirrigated crops senesced earliest (beginning of August) and at the most rapid rate of all irrigation treatments. However, early over-watered crops closely followed the senescence of unirrigated crops, with the late OW 10-13 treatment being last to commence senescence and the only plots with ground cover still remaining in Maris Piper and Markies at final harvest on 24 September (Figure 13).

Figure 13. Expt 13: Effect of irrigation regime on ground cover. *(a)* Maris Piper, *(b)* Markies, *(c)* Saturna. Unirr, ■; 25 SMD, □; OW 0-3, ▲; OW 10-13, △. S.E. based on 22 D.F.



The canopies of Markies and Maris Piper were more persistent than those of Saturna and this result is consistent with results from Expt 2. The integrated ground covers of unirrigated crops were significantly smaller than 25 SMD and OW 10-13 treatments but similar to early over-watered OW 0-3 (Table 31*a*). Integrated ground cover for OW 10-13 was numerically greater than the 25 SMD treatment, suggesting that

maintaining a smaller SMD in mid-August prolonged canopy duration. The effects of the irrigation treatments on season-long radiation absorption were slightly different to those on integrated ground cover, as radiation receipts were higher in June than in July and August, so the more advanced Saturna canopies absorbed more radiation during this period than Maris Piper or Markies which partially reduced the effect of shorter-lived canopies in Saturna (Table 31*b*). There were few significant differences between irrigation regimes in terms of radiation absorption but the OW 0-3 treatment absorbed less radiation in Maris Piper and Markies than other irrigation regimes, whereas in Saturna early over-watering did not compromise radiation absorption (Table 31*b*). When compared with similar treatments in 2011, radiation absorption was *c*. 1 TJ/ha less in 2012.

Table 31. Expt 13: Effect of variety and irrigation on (*a*) season-long integrated ground cover (% days) and (*b*) radiation absorption (TJ/ha)

	Variety	Unirrigated	25 SMD	OW 0-3	OW 10-13
(*a*)	Markies	7160	8550	7365	9314
	Maris Piper	6978	8841	7471	9842
	Saturna	6514	7410	6882	8112
	Mean	6884	8267	7239	9090
		S.E. (22 D.F.): Irrigation, 124 and Variety × Irrigation 215			
(*b*)	Markies	11.52	13.49	11.64	14.37
	Maris Piper	11.22	13.78	11.83	14.90
	Saturna	10.57	11.94	11.13	12.92
	Mean	11.10	13.07	11.53	14.07
		S.E. (22 D.F.): Irrigation, 0.173 and Variety × Irrigation 0.299			

4.2.1.1.3. Expt 14

Emergence commenced in Maris Peer 34 days after planting and 50% emergence was reached by 39 days after planting. In Venezia, emergence was slightly later (40 and 43 days after planting for first and 50% emergence, respectively). The time taken for emergence to increase from 10 to 90% was 6 days in Maris Peer and 5 days in Venezia. The TI period was brief, with 50 % of plants tuberized 20 and 19 days after emergence in Maris Peer and Venezia, respectively, and 80 % of tubers (10-90 %) initiated over a 2-3 day period in both varieties. Ground cover development was rapid and all plots reached > 96 % ground cover by 7 weeks after emergence. However, senescence commenced early partially due to *Alternaria* infection and by desiccation (at the end of the 8-week control period) ground covers were only *c*. 10 %.

Desiccation was applied ahead of schedule owing to infection with *Phytopthora infestans*.

4.2.1.1.4. Expts 15 and 16

In Expt 15, emergence commenced on 11 May and 50% emergence was measured on 15 May, whilst TI was on 1 June. In Expt 16, emergence commenced on 15 May and 50% emergence was measured on 20 May, whilst TI was on 7 June. Full ground cover was achieved 52 days after emergence in Expt 15 and 47 days after emergence in Expt 16. Ground cover remained > 90% until final harvest at both sites.

4.2.1.2. ***Modelled SMDs***

The 2012 season was the wettest recorded at CUF since detailed meteorological records have been collected at Cambridge in 1981. There was also 4% less radiation in the May-September period than average. Consequently, evapotranspiration was lower than average, and combined with the rainfall, there was little need to irrigate crops for long periods unless specified by frequent irrigation treatments for scab control. June was twice as wet as the long-term average and July nearly 2.7 times wetter.

4.2.1.2.1. Expt 12

Soils were brought back to field capacity by 25 mm of rain at the onset of TI in the earliest-emerging varieties. Deficits in the unirrigated Maris Piper reached a maximum of 23 mm in the third week after TI and averaged 11 mm in the 4 weeks after TI compared with 17 mm in 2011. The SMD in the ridge (0-25 cm) averaged only 3 mm (maximum 14 mm) compared with 14 mm in Expt 1 in 2011. The TI 1-4 treatment reached a maximum of 18 mm SMD before the soil was brought back to field capacity. The trigger SMD in 15 SMD treatments was only reached on two occasions during scab control. Despite the wet early start to the season, the maximum SMD in Unirr reached 66 mm at the end of August, a similar peak but much later than in similar experiments in 2009-2011. The trigger SMD in the 35 SMD treatment was only reached on three occasions (in August) during the season. Drainage was substantial in frequently-irrigated treatments (Table 7).

4.2.1.2.2. Expt 13

Peak SMDs reached *c*. 65 mm at the end of August in unirrigated plots, otherwise, SMDs were maintained close to the designed treatment protocols. Drainage was considerable under the crops maintained at field capacity or in a period of over-watering (Table 8).

4.2.1.2.3. Expt 14

Soil moisture deficits were maintained at field capacity in 0 SMD treatments but little irrigation (20 mm) was required between 4 and 6 weeks after TI. No irrigation was triggered in the 15 mm 6 week duration treatment between 4 and 6 weeks after TI and only two irrigations were triggered in each of the 0-4 week and 6-8 week periods after TI.

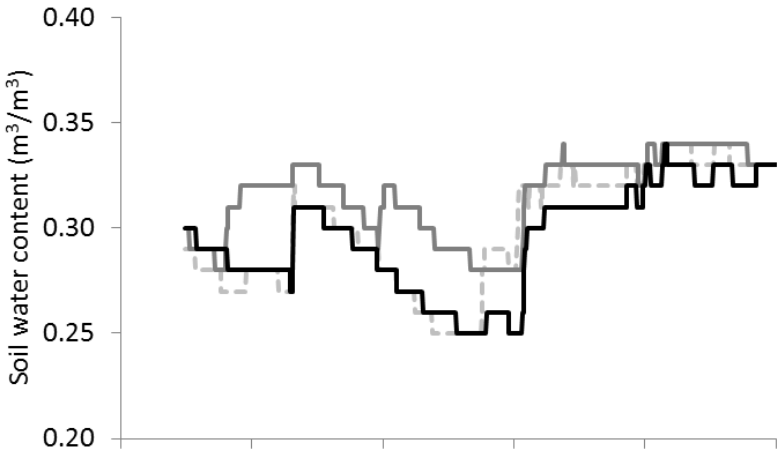
4.2.1.3. ***Measured soil water content***

4.2.1.3.1. Expt 12

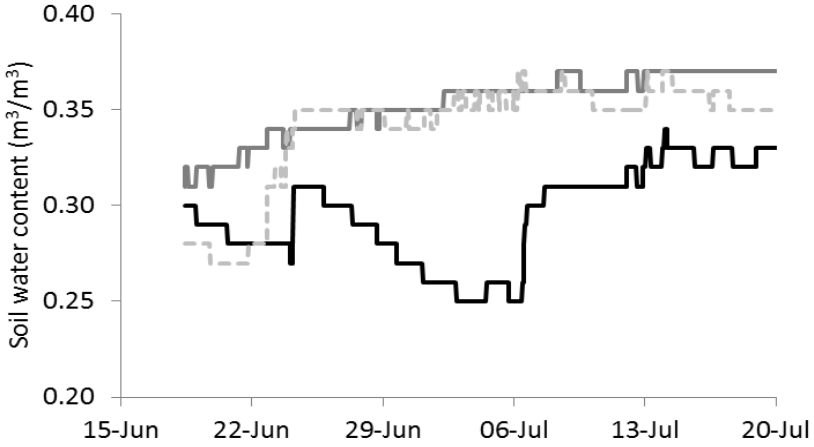
Soil moisture probe data in Maris Piper plots indicated that 0 SMD and TI 1-4 treatments were maintained slightly above field capacity (0.34 m^3/m^3) in the centre of the ridge from 1 week after TI. The 15 SMD treatment reached a minimum soil water content of 0.27 m^3/m^3 and the unirrigated 0.25 m^3/m^3 (Figure 14).

Figure 14. Expt 12: Soil water content measured in the ridge during the scab control period in Maris Piper plots. *(a)* Unirr, —; 15 SMD, —; 35 SMD, – –; *(b)* Unirr, —; 0 SMD, —, TI 1-4, – –.

(a)



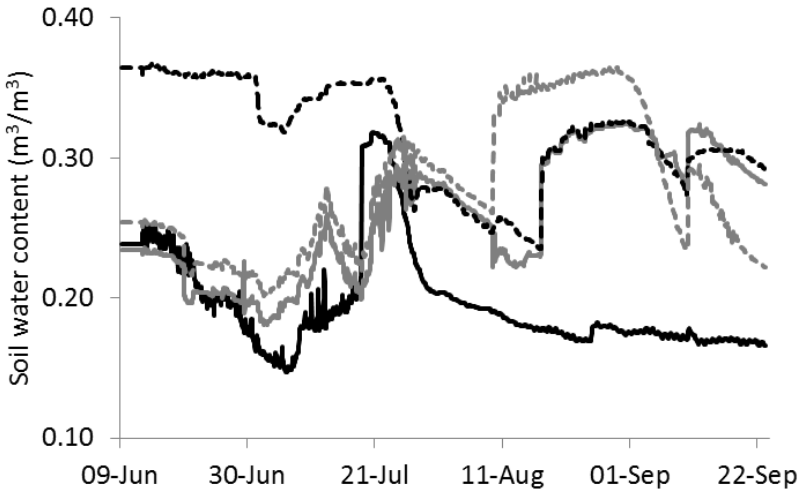
(b)



4.2.1.3.2. Expt 13

The measured soil water content in the ridge was maintained close at 0.36 m^3/m^3 during the early over-watering phase and at 0.35 m^3/m^3 during the late period (field capacity value 0.34 m^3/m^3), although there was a gradual increase in the water content during the second period whilst the ridge wetted up more quickly in the early period (Figure 15).

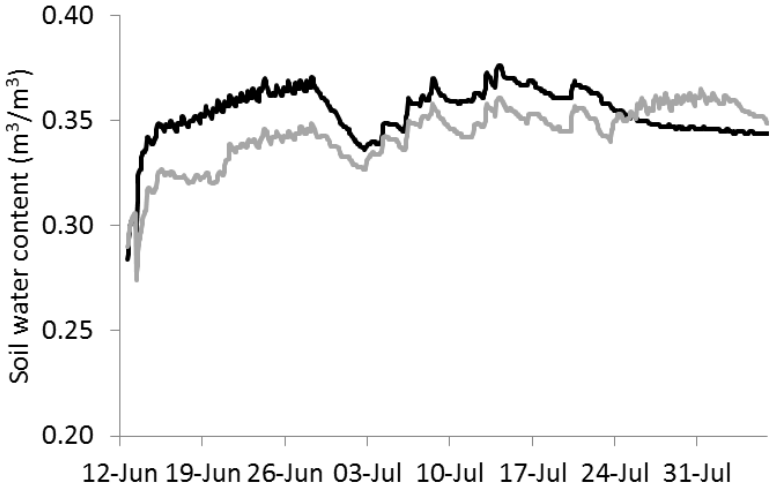
Figure 15. Expt 13: Soil water content measured in the ridge throughout the season in Maris Piper plots. Unirr, —; 25 SMD, —; OW 0-3, – –; OW 10-13, – –.



4.2.1.3.3. Expt 14

The measured soil water content in the ridge remained greater in 0 SMD than in 15 SMD treatments until the last 2 weeks of the season (Figure 16).

Figure 16. Expt 14: Soil water content measured in the ridge throughout the season in Maris Peer 8 week plots. 0 SMD, —; 15 SMD, —.



4.2.1.3.4. Expts 15 and 16

In Expt 15, average SMDs during the 4 weeks post-TI were 3 mm (maximum 9 mm) in Wet treatments and 18 mm (maximum 27 mm) in the Dry. In Expt 16, average SMDs

during the 4 weeks post-TI were 10 mm (maximum 15 mm) in Wet treatments and 29 mm (maximum 42 mm) in Dry.

4.2.1.4. *Common scab*

4.2.1.4.1. Expt 12

All tubers examined were visually infected with common scab, albeit with < 1% SA. Tubers with > 5% SA are generally regarded as unsuitable for packing and a typical minimum acceptable packout is 70 %, i.e. ≤ 30 % rejectable tubers. When using this criterion to judge scab incidence, the overall severity of infection was extremely low compared with previous seasons, even compared with 2011. Typically, the most effective irrigation regimes in the very susceptible variety Maris Piper at CUF in previous experiments in 1992-2010 resulted in *c*. 2-3% SA infected, whilst unirrigated crops ranged from 6-28 % SA infected. The unirrigated crops in 2011 had only 4-5% SA infected, whilst the best treatments for control of scab produced tubers with only 0.6-0.7% SA, similar to those found in the much more resistant varieties Estima and King Edward. However, in 2012 unirrigated Maris Piper had < 1% SA infected, the lowest recorded at CUF and similar to the other irrigation regimes (Table 32). In contrast with 2011, when the soils were very dry following planting and emergence (which is a possible explanation for the low severity of common scab observed), in 2012 the soils in unirrigated treatments were much wetter than 2011 particularly in the ridge zone (Figure 15). There was no significant effect of irrigation regime on scab severity, even in soils which were maintained at field capacity from TI (Table 32). There was significantly more severe scab on Sylvana than other varieties but the lesions constituted atypically enlarged lenticels, from which DNA of pathogenic *Streptomyces* could be extracted, although this is not conclusive evidence that the lesions were common scab.

Table 32. Expt 12: Common scab incidence and severity

Variety	Irrigation regime	Incidence < 5 % surface area		Severity
		%	Ang. trans.	% SA affected
Bute	Unirr	100.0	90.0	0.63
	0 SMD	100.0	90.0	0.52
	15 SMD	99.6	88.0	0.55
	TI 1-4	99.5	87.7	0.55
Desiree	Unirr	99.6	88.0	0.53
	0 SMD	100.0	90.0	0.65
	15 SMD	100.0	90.0	0.51
	TI 1-4	100.0	90.0	0.56
Flair	Unirr	100.0	90.0	0.50
	0 SMD	97.4	82.4	1.13
	15 SMD	100.0	90.0	0.62
	TI 1-4	99.2	87.0	0.90
Jelly	Unirr	100.0	90.0	0.51
	0 SMD	99.6	87.8	0.66
	15 SMD	100.0	90.0	0.63
	TI 1-4	97.9	82.0	1.06
Maris Piper	Unirr	98.2	83.9	0.99
	0 SMD	98.8	85.0	0.97
	15 SMD	98.8	85.0	0.88
	TI 1-4	99.6	88.0	0.73
Sylvana	Unirr	100.0	90.0	0.55
	0 SMD	98.6	84.5	1.15
	15 SMD	96.1	81.1	1.40
	TI 1-4	92.6	80.6	1.87
Vales Sovereign	Unirr	100.0	90.0	0.83
	0 SMD	99.2	87.0	0.85
	15 SMD	100.0	90.0	0.73
	TI 1-4	96.8	84.0	0.95
S.E. (54 D.F.)†		-	2.87	0.104

†Irrigation treatment '35' was not assessed for common scab

4.2.1.4.2. Expt 14

All tubers in this experiment had < 5% SA infected with scab. Despite the generally very low severity of scab, Maris Peer irrigated for 8 weeks had a slightly higher scab severity than when irrigated for 4 weeks in both 0 SMD and 15 SMD timing treatments. This appeared to be an anomaly as it could not be explained by differences in tuber size but there were tubers with 2-5% SA infected with scab in these treatments which were not apparent in 4 and 6 week durations. There was no

effect of duration of the control period in Venezia but Venezia had significantly less scab than Maris Peer (Table 33).

Table 33. Expt 14: Common scab incidence and severity

Variety	Timing	Duration	Severity % SA affected
Maris Peer	0 SMD	4 wks	0.59
		6 wks	0.69
		8 wks	0.79
	15 SMD	4 wks	0.57
		6 wks	0.59
		8 wks	0.71
Venezia	0 SMD	4 wks	0.53
		6 wks	0.53
		8 wks	0.53
	15 SMD	4 wks	0.51
		6 wks	0.53
		8 wks	0.55
S.E. (22 D.F.)			0.034

4.2.1.4.3. Expts 15 and 16

In Expts 15 and 16, there was no effect of irrigation regime on common scab. More than 99% of tubers had < 5% surface area infected and the mean severity was very low in both Expt 15 (0.87 ± 0.093% SA) and Expt 16 (0.98 ± 0.076% SA).

4.2.1.4.4. Commercial trials

Despite quite large differences in the ped size produced within ridges from different destoning practices, there were no effects on common scab severity (Table 34) or greening or cracking incidence (data not shown). This indicated that ped size distribution may have less effect on scab development than previously thought. However, all sites had considerable rainfall during the scab control period and disease severity was very low.

Table 34. Effect of soil tilth on common scab severity (% SA infected) in eight commercial trials

Grower-Field	Fine tilth	Coarse tilth	S.E.	Sig. Diff.
Papworth Felmingham	2.92	2.28	1.140	None
Papworth Sco Marlers	0.73	1.21	0.209	None
GVAP Black Barn 1	0.90	0.99	0.170	None
GVAP Norton L	0.73	0.70	0.145	None
GVAP Norton H	0.73	0.87	0.169	None
Greenseed Banns	0.56	0.56	0.024	None
E J Andrews 300A Cross	0.69	0.60	0.099	None
Stevenson Harriets	0.58	0.61	0.169	None

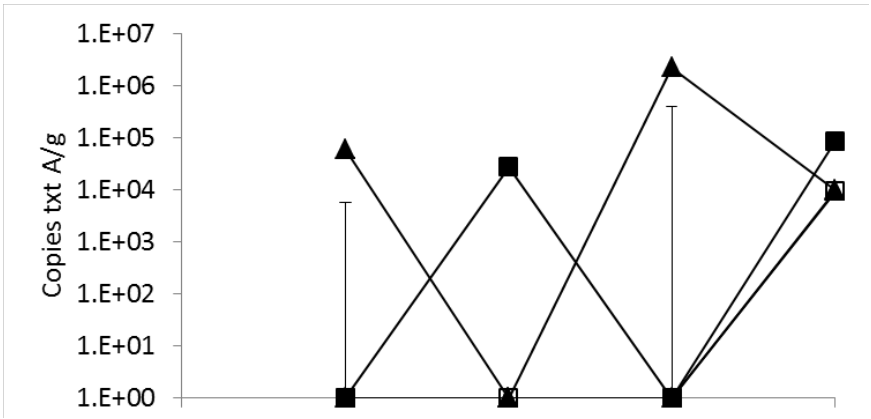
4.2.1.5. *Streptomyces populations*

4.2.1.5.1. Expt 12

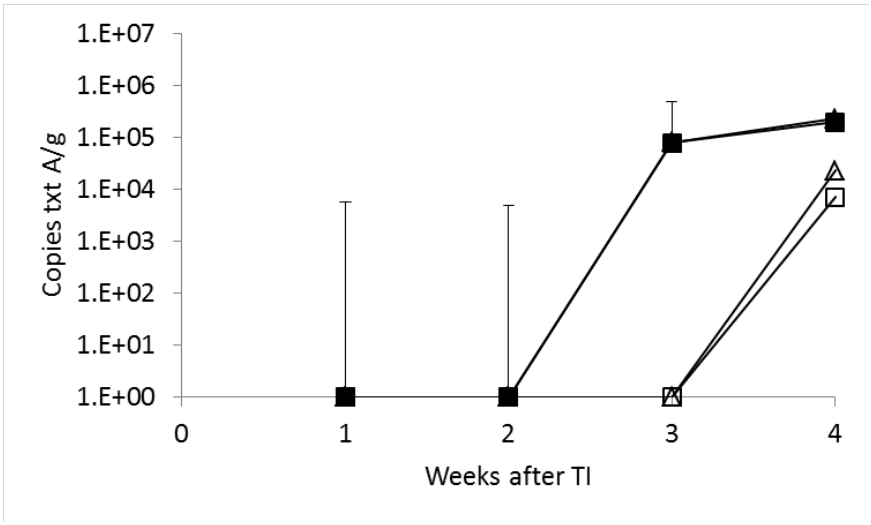
In Maris Piper, there were no pathogenic *Streptomyces* detected until 2 weeks after TI, a feature found in previous seasons (Figure 17*b*). In the driest treatments monitored (Unirr, 15 SMD), the populations of pathogenic *Streptomyces* increased between 2 and 3 weeks after TI and did not increase significantly thereafter. In treatments kept at field capacity from 1 week after TI, the increase in pathogenic *Streptomyces* was delayed until between 3 and 4 weeks after TI. In Desiree, there was the same pattern as Maris Piper in 0 SMD and TI 1-4 treatments in the time lag after TI before pathogenic Streptomyces were detected but there were odd fluctuations in their populations in 15 SMD and Unirr treatments (Figure 17*a*).

Figure 17. Expt 12: Timecourse of pathogenic *Streptomyces* populations. *(a)* Desiree and *(b)* Maris Piper. Unirr, ■; 0 SMD, □; 15 SMD, ▲; TI 1-4, △. Bars represent 1 S.E.

(a)

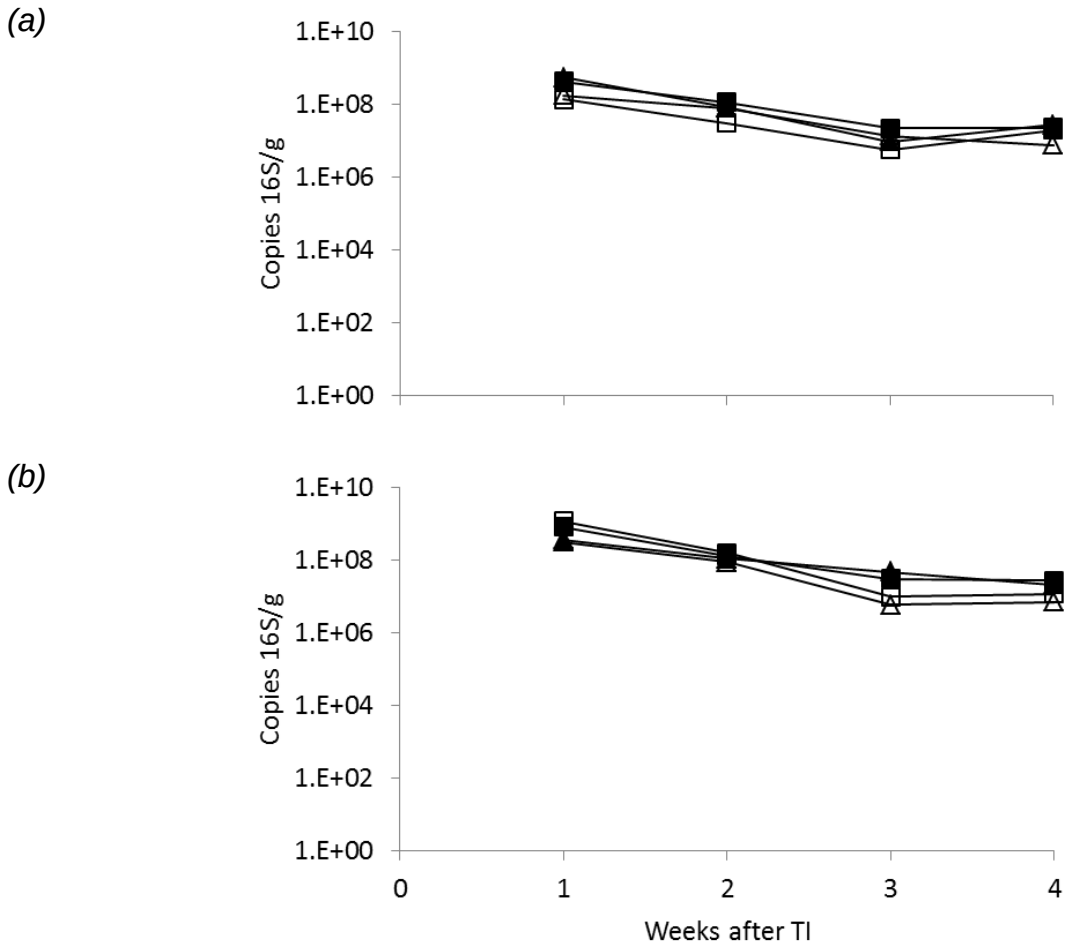


(b)



The populations of 16S *Streptomyces* decreased over the 4 weeks following TI and were largely similar between varieties and across irrigation regimes, except at week 3, when 16S populations were numerically lower in 0 SMD and TI 1-4 than in Unirr and 15 SMD in Maris Piper and lower in 0 SMD than in Unirr in Desiree (Figure 18).

Figure 18. Expt 12: Timecourse of 16S *Streptomyces* populations. *(a)* Desiree and *(b)* Maris Piper. Unirr, ■; 0 SMD, □; 15 SMD, ▲; TI 1-4, △. Bars represent 1 S.E.



Samples of tubers from all varieties and all irrigation treatments apart from the 35 SMD were assessed for the population of pathogenic *Streptomyces* at 4 weeks after TI. Overall, there was a greater population of pathogenic *Streptomyces* on Maris Piper tubers but similar populations for other varieties except for Bute which had intermediate populations (Table 35). Averaged across varieties, there were fewer pathogenic *Streptomyces* on the surface of tubers in 0 SMD and TI 1-4 treatments than in Unirr or 15 SMD (Table 35).

Table 35. Expt 12: Populations of *txtA Streptomyces* on tubers (x10^3 copies/g) 4 weeks after TI. Main effects of treatments shown only

Variety	Irrigation regime	*txtA* population
Bute		77.2
Desiree		29.4
Flair		14.6
Jelly		27.0
Maris Piper		115.5
Sylvana		19.1
Vales Sovereign		36.1
	S.E. (54 D.F.)	23.1
	Unirr	99.0
	0 SMD	7.9
	15 SMD	61.3
	TI 1-4	13.9
	S.E. (54 D.F.)	14.9

4.2.1.5.2. Expt 14

There were no pathogenic *Streptomyces* detectable in the first 6 weeks after TI and at the final sampling 8 weeks after TI, there were no differences between treatments and, on average, there were 41000 copies/g in Maris Peer but there were many plots with tubers with no detectable *txtA*, which supports the very low severity of scab observed.

4.2.1.5.3. Expts 15 and 16

In Expt 15, there were significantly fewer pathogenic *Streptomyces* on tubers 4 weeks after TI in irrigated (4200 ± 5304 copies/g) compared with unirrigated (23675) but the populations were all low. In Expt 16, the population of *txtA* was not significantly different between irrigated and unirrigated treatments (mean 66138 ± 34896 copies/g).

4.2.1.6. *Tuber cracking*

4.2.1.6.1. Expt 12

The incidence of linear cracking was much greater in soils maintained at field capacity from TI than other irrigation regimes and was severe in Bute, Maris Piper, Sylvana and

Vales Sovereign (Table 36). However, in Bute, Sylvana and Vales Sovereign, there was a similar incidence of linear cracking in 0 SMD and TI 1-4 treatments. Linear cracking in unirrigated, 15 SMD and 35 SMD treatments was largely similar within varieties. Desiree had zero incidence of linear cracking but it was also low in Flair and Jelly (Table 36).

The incidence of superficial cracking was severe and numerically greatest in 0 SMD and TI 1-4 treatments but even plots scheduled at 15 mm SMD had a high incidence in Bute, Sylvana and Vales Sovereign (Table 36). Desiree had no superficial cracking and the incidence was low in Flair and Jelly. With the exception of Vales Sovereign, the surface area affected by superficial cracking was, on average, low (< 3% SA) but reached 8% SA in Vales Sovereign TI 1-4 (Table 36).

Table 36. Expt 12: Tuber cracking incidence and severity

		Linear cracking		Superficial cracking		
Variety	Irrigation regime	%	Ang. trans.†	%	Ang. trans.†	Severity % SA affected
Bute	Unirr	5.4	10.6	10.5	15.6	0.16
	0 SMD	31.1	33.4	41.0	39.8	1.74
	15 SMD	12.2	19.9	46.5	42.8	1.48
	35 SMD	6.9	14.7	18.2	24.8	0.25
	TI 1-4	19.2	25.8	63.7	53.4	2.86
Desiree	Unirr	0.0	0.0	0.0	0.0	0.00
	0 SMD	0.0	0.0	0.0	0.0	0.00
	15 SMD	0.0	0.0	0.0	0.0	0.00
	35 SMD	0.0	0.0	0.0	0.0	0.00
	TI 1-4	0.0	0.0	0.0	0.0	0.00
Flair	Unirr	1.2	3.6	0.4	2.1	0.01
	0 SMD	7.4	15.7	1.4	5.7	0.01
	15 SMD	1.4	5.5	1.6	5.7	0.03
	35 SMD	0.7	2.8	2.7	7.7	0.01
	TI 1-4	0.4	2.0	2.5	6.9	0.02
Jelly	Unirr	1.2	5.1	0.0	0.0	0.00
	0 SMD	5.1	12.6	6.9	12.5	0.16
	15 SMD	0.0	0.0	0.0	0.0	0.00
	35 SMD	0.7	2.8	0.5	2.4	0.01
	TI 1-4	0.8	3.0	2.0	6.3	0.03
Maris Piper	Unirr	0.5	2.3	0.0	0.0	0.00
	0 SMD	33.0	35.0	51.9	46.1	1.50
	15 SMD	0.4	2.0	8.6	13.7	0.10
	35 SMD	0.9	3.2	5.5	12.8	0.03
	TI 1-4	8.7	15.1	33.8	35.5	0.49
Sylvana	Unirr	4.7	9.5	12.5	16.1	0.15
	0 SMD	26.1	30.6	19.8	22.0	0.69
	15 SMD	4.3	11.6	15.8	19.0	0.30
	35 SMD	1.7	6.2	8.9	14.3	0.09
	TI 1-4	19.9	26.4	24.4	24.7	0.27
Vales Sovereign	Unirr	4.7	12.2	12.2	16.9	0.40
	0 SMD	37.8	37.8	63.6	53.2	3.32
	15 SMD	3.7	10.9	47.6	43.6	0.91
	35 SMD	3.8	10.9	22.0	27.8	0.26
	TI 1-4	41.4	39.9	74.0	60.1	8.20
S.E. (68 D.F.)†		-	3.24	-	4.75	0.468

†Angularly transformed data for statistical analysis

4.2.1.6.2. Expt 13

Early over-watering increased the incidence of both linear and superficial cracking in Maris Piper and Markies but late over-watering had little effect on cracking compared with a more normal regime of irrigating to a maximum SMD of 25 mm (Table 37). As with Desiree in Expt 12, Saturna did not show any cracking.

Table 37. Expt 13: Tuber cracking incidence and severity

		Linear cracking		Superficial cracking		
Variety	Irrigation regime	%	Ang. trans.†	%	Ang. trans.†	Severity % SA affected
Maris Piper	Unirr	0.6	2.5	0.0	0.0	0.00
	25 SMD	0.7	2.4	7.0	13.4	0.07
	OW 0-3	47.4	43.4	60.4	52.9	2.50
	OW 10-13	1.4	4.0	20.0	22.6	0.26
Markies	Unirr	0.0	0.0	0.0	0.0	0.00
	25 SMD	1.0	3.4	3.0	8.9	0.03
	OW 0-3	26.4	29.9	13.6	17.8	0.16
	OW 10-13	2.6	7.3	5.1	11.9	0.06
Saturna	Unirr	0.0	0.0	0.0	0.0	0.00
	25 SMD	0.0	0.0	0.0	0.0	0.00
	OW 0-3	0.0	0.0	0.0	0.0	0.00
	OW 10-13	0.0	0.0	0.0	0.0	0.00
S.E. (68 D.F.)†		-	2.99	-	3.26	0.003

†Angularly transformed data for statistical analysis

4.2.1.6.3. Expt 14

There was no cracking observed in Expt 14.

4.2.1.6.4. Expts 15 and 16

There was no difference between the two irrigation regimes in Expt 15, either in linear (1.5 ± 0.61% incidence) or superficial (0.8 ± 0.44%) cracking. In Expt 16, early watering for scab control increased the incidence of linear cracking from 5.3 ± 1.03% in unirrigated plots to 19.3% and from 3.3% to 8.3% for superficial cracking.

4.2.1.7. *Number of tubers and tuber yield*

4.2.1.7.1. Expt 12

In Expt 12, the number of tubers > 10 mm was increased by maintaining a 0 mm SMD throughout the 4 weeks following TI compared with a 15 mm SMD or a shorter period at field capacity (Table 38), manifested by an increase in the number of tubers/stem. The mean number of tubers/stem in the 0 SMD treatment was 4.58 ± 0.134 compared with the mean of all other irrigation treatments of 3.83, an increase of 20 % across varieties. The increase resulted from significantly more tubers in the 30-40 and 40-50 mm size grades compared with other irrigated treatments rather than a bigger proportion of tubers < 30 mm. This repeats the findings of 2009-2011 that very wet soils at TI can produce increased numbers of viable tubers which is contradictory to most previous work at CUF prior to this period. The observation that the tuber population was increased in the 0 but not the TI 1-4 treatment indicated that the effect resulted from conditions in the first week after TI.

Total and ware (> 40 mm) fresh weight yields and dry weight yields were similar for crops receiving irrigation but were *c*. 11 t/ha lower where unirrigated (Table 38). In Desiree, Flair, Jelly and Maris Piper, yields were numerically (but not significantly) greatest where SMD's were maintained close to field capacity but in Bute and Sylvana, maintaining the SMD at a maximum of 15 mm produced significantly higher yields than those kept at field capacity from TI.

Tuber dry matter concentration ([DM]) was greater where crops were unirrigated but other irrigation regimes were not significantly different (Table 38). Bute had a very low tuber [DM] at final harvest (17.1%) with Maris Piper having the highest [DM] (25.2%). Desiree, Flair and Jelly had the same [DM] at 23.0 %, with Sylvana and Vales Sovereign at 21.4 %.

Tuber dry matter (DM) yield followed a slightly different pattern to fresh weight (FW) yield. Whilst unirrigated crops generally had the lowest DM yield and the 35 SMD treatment the next lowest, in Bute, Sylvana and Vales Sovereign, maintaining the soil at field capacity for long periods reduced yield compared with slightly drier soils such that applying *c*. 271 mm of irrigation had no significant effect on yield compared with unirrigated crops. In Desiree and Flair, the wettest treatments had the highest tuber

DM yield, whilst in Jelly and Maris Piper there was no difference in tuber DM yield across 0 SMD, TI 1-4 and 15 SMD irrigation regimes (Table 38).

Table 38. Expt 12: Number of tubers, yield and dry matter concentration at final harvest

Variety	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Yield > 40 mm (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Bute	Unirr	484	52.4	49.1	18.2	9.5
	0 SMD	684	57.1	50.0	17.3	9.9
	15 SMD	502	65.4	62.8	17.1	11.2
	35 SMD	550	58.8	55.2	16.9	10.0
	TI 1-4	449	58.3	56.3	16.2	9.5
Desiree	Unirr	487	49.5	45.3	23.7	11.7
	0 SMD	503	63.1	59.4	23.1	14.6
	15 SMD	484	57.9	54.1	22.1	12.8
	35 SMD	470	53.2	49.7	22.2	11.9
	TI 1-4	506	63.4	60.1	23.7	14.9
Flair	Unirr	570	42.4	35.8	22.4	9.5
	0 SMD	572	53.4	48.7	23.5	12.6
	15 SMD	550	48.8	44.2	22.4	10.9
	35 SMD	522	46.0	42.3	22.6	10.4
	TI 1-4	550	56.2	51.7	23.9	13.4
Jelly	Unirr	309	46.3	45.4	24.2	11.2
	0 SMD	436	69.1	67.0	22.3	15.3
	15 SMD	363	61.7	60.5	24.1	14.9
	35 SMD	300	56.9	56.3	22.3	12.8
	TI 1-4	413	73.7	72.1	22.1	16.2
Maris Piper	Unirr	505	46.3	41.5	25.5	11.8
	0 SMD	585	65.8	61.3	24.7	16.2
	15 SMD	462	63.2	60.5	26.2	16.5
	35 SMD	470	60.5	58.1	24.6	14.8
	TI 1-4	522	65.1	62.1	25.1	16.1
Sylvana	Unirr	284	54.3	53.4	21.8	11.6
	0 SMD	328	49.0	46.2	22.1	10.8
	15 SMD	279	65.7	64.9	21.0	13.8
	35 SMD	316	59.3	58.5	20.7	12.2
	TI 1-4	316	61.0	59.8	21.4	13.2
Vales Sovereign	Unirr	251	46.4	44.9	23.4	10.8
	0 SMD	273	45.7	44.4	21.1	9.6
	15 SMD	290	61.4	59.6	22.3	13.7
	35 SMD	331	63.6	61.6	19.9	12.7
	TI 1-4	262	46.4	44.2	20.2	9.4
S.E. (68 D.F.)		39.3	4.36	4.33	0.83	0.97

4.2.1.7.2. Expt 13

Similar to Expt 12, the number of tubers > 10 mm was increased where crops were maintained above field capacity during the 3 weeks after TI compared with other irrigation regimes (Table 39). The relative increase compared with irrigating at 25 mm SMD (25 SMD and OW 10-13) averaged across all varieties was *c*. 19 % (20 % in 2011). The effect of early over-watering was slightly different to Expt 12 in that there was a significant increase in number of tubers across all grades < 50 mm in OW 0-3 compared with other irrigated treatments (data not shown).

Over-watering during the three weeks after TI reduced fresh and dry weight yields considerably compared to maintaining a maximum SMD of 25 mm (Table 39). The proportional reductions in yields were similar across varieties (21-27%), which is similar to previous work conducted on early over-watering at CUF. Yields of the OW 0-3 were the same as the unirrigated. Although not significant, there was a trend across all varieties for the late over-watered plots to have a higher numerical yield than the 25 SMD treatment despite the SMD for the 25 SMD not exceeding 27 mm throughout the season and there being only two days where ET rates exceeded 5 mm during the late over-water period. Normally, a maximum 25 mm SMD would ensure little restriction to growth owing to water shortage but the excessive rainfall during the period of root expansion probably restricted both rooting depth and density. This would restrict the uptake of water (and tuber bulking rate) on hot days more than would be expected. Ground covers in the late over-watered treatment commenced senescence later and persisted longer than the 25 SMD, but this did not translate into more radiation absorption.

Table 39. Expt 13: Number of tubers, yield and dry matter concentration at final harvest

Variety	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Maris Piper	Unirr	640	52.8	25.5	13.5
	25 SMD	681	73.6	23.8	17.5
	OW 0-3	837	55.8	24.6	13.8
	OW 10-13	601	77.7	23.9	18.6
Markies	Unirr	529	51.9	26.5	13.7
	25 SMD	615	70.0	24.7	17.2
	OW 0-3	664	50.6	25.6	13.0
	OW 10-13	580	74.4	25.5	19.0
Saturna	Unirr	801	47.4	27.2	12.9
	25 SMD	799	64.5	25.6	16.4
	OW 0-3	901	50.9	25.3	12.9
	OW 10-13	766	66.0	26.0	17.2
Mean	Unirr	657	50.7	26.4	13.3
	25 SMD	699	69.3	24.7	17.1
	OW 0-3	801	52.5	25.2	13.2
	OW 10-13	649	72.7	25.1	18.2
S.E. Variety*Irrigation (22 D.F.)		48.6	3.30	0.43	0.85
S.E. Irrigation (22 D.F.)		28.0	1.90	0.25	0.49

4.2.1.7.3. Expt 14

There were more tubers produced in both varieties where irrigation was maintained at field capacity in the week after TI than where the SMD was allowed to increase to 15 mm and this was associated with an increased number of tubers < 25 mm in diameter (Table 40). There was no effect of irrigation timing on yield or tuber [DM] (Table 40).

Table 40. Expt 14: Number of tubers, yield and dry matter concentration at final harvest

Variety	Timing	No. tubers < 25 mm (000/ha)	Total no. tubers (000/ha)	No. tubers per stem	Total yield (t/ha)	Tuber [DM] (%)
Maris Peer	0 SMD	785	1688	4.50	18.9	20.4
	15 SMD	639	1656	4.38	20.8	20.8
Venezia	0 SMD	523	1561	3.58	23.1	18.9
	15 SMD	380	1439	3.45	22.9	18.8
S.E. (22 D.F.)		42.1	34.8	0.085	0.69	0.15

4.2.1.7.4. Expts 15 and 16

There was no significant difference in total yield (55.2 ±0.92 t/ha), number of tubers (382 000 ±17 700 /ha) or tuber [DM] (23.7 ± 0.19 %) between Dry and Wet treatments in Expt 15. In Expt 16, which was allowed to grow for longer before desiccation, total yield (71.7 ± 1.86 t/ha), number of tubers (393 000 ± 19 300 /ha) and tuber [DM] (23.1 ± 0.33%) were again similar between Dry and Wet.

4.2.1.8. *Radiation use efficiency, total DM yield, soil mineral nitrogen (SMN) and nitrogen (N) uptake*

4.2.1.8.1. Expt 13

As was found in 2011, all three varieties had similar radiation use efficiencies (Table 41*a*). However, unlike 2011, the unirrigated crops in 2012 had similar RUE to those maintained at a soil moisture of 25 mm and is this probably a consequence of the wet spring and summer keeping the SMD in this treatment below a limiting value for much of the season. The RUE of the early over-water treatment was significantly lower than the other treatments and suggests that yield formation in this treatment was reduced due to excess water. In 2011, the mean RUE for all treatments was 1.35 t DM/TJ whereas in 2012 it was 1.46 t DM/TJ suggesting that although the crops in 2012 were growing in generally duller conditions, this would not have resulted in a *pro-rata* decrease in yield. When averaged over all irrigation treatments Maris Piper and Markies had similar total DM yields (18.6 t/ha) whereas the yield of Saturna was significantly smaller (17.0 t/ha). Early over-watering (OW 0-3) resulted in the smallest DM yield (15.1 t/ha) and this was a numerically smaller total DM yield than measured in unirrigated crops. The late over-water treatment (OW 10-13) had the largest total DM yield. In 2011, there was no evidence that periods of over-watering were associated with a decrease in DM yield. However, the 2012 data suggest that the combination of excessive spring rainfall and early over-watering significantly reduced DM yields. In 2011, total N uptake averaged 263 kg N/ha whereas in 2012 it averaged only 204 kg N/ha. The 2012 data show that, when averaged over the irrigation treatments there was no significant difference in the N uptake of the three varieties. Total N uptake was significantly reduced in the early over-watering (OW 0-3) treatment whilst the N uptakes of the unirrigated crops were similar to those crops

maintained at an SMD of 25 mm. The late over-watering (OW 10-13) treatment had the largest total N uptake (tuber + haulm determined from CUF N Model) as was found in Expt 2 in 2011.

Table 41. Expt 13: Effect of variety and irrigation on *(a)* season-long average radiation use efficiency (t DM/TJ); *(b)* total DM yield (t/ha) and *(c)* maximum total N uptake (kg N/ha)

	Variety	Unirr	25 SMD	OW 0-3	OW 10-13	Mean
(*a*)	Markies	1.37	1.48	1.32	1.59	1.44
	Maris Piper	1.51	1.53	1.33	1.56	1.48
	Saturna	1.46	1.54	1.38	1.48	1.47
	Mean	1.45	1.51	1.34	1.54	1.46
		S.E. (22 D.F.): Variety, 0.028; Irrigation, 0.032 and Variety × Irrigation 0.056				
(*b*)	Markies	16.3	20.3	14.9	22.7	18.5
	Maris Piper	15.8	20.8	15.9	22.4	18.7
	Saturna	14.8	18.8	14.5	19.9	17.0
	Mean	15.6	20.0	15.1	21.7	18.1
		S.E. (22 D.F.): Variety, 0.46; Irrigation, 0.53 and Variety × Irrigation 0.92				
(*c*)	Markies	186	210	139	239	194
	Maris Piper	213	216	142	277	212
	Saturna	199	225	162	238	206
	Mean	199	217	148	251	204
		S.E. (22 D.F.): Variety, 7.7; Irrigation, 8.9 and Variety × Irrigation 15.4				

The effects of irrigation regime on SMN were measured on three occasions during the growing season on plots growing Maris Piper (Table 42). In general, the amounts of SMN were smaller than those recorded in 2011 but the temporal variation and the effects of the irrigation treatments were similar in both seasons. For example, there was more SMN at the initial sample than at others, and at the final sampling, the amount of SMN was largest in the unirrigated treatments and smallest in the late over-watering treatments. In general, the combined quantities of SMN and crop N were also smaller than found in 2011 but changes over time and the effect of the treatments were similar. For example on 26 September 2012, the average amount of N in Unirr and 25 SMD treatment was 284 kg N/ha whilst in 2011 the average for these two treatment was 366 kg N/ha. It is not known if the 80 kg N/ha difference between the two seasons was a consequence of leaching of N or was due to less N mineralisation due to differences in soil texture and organic matter. When averaged over the three sampling dates, the combined quantity of N in the 2012 OW 0-3 treatment was 146 kg N/ha compared with 251 kg N/ha in 2011.

Table 42. Expt 13: Effect of irrigation treatments on soil mineral nitrogen (SMN, kg N/ha, 0-90 cm) and on total crop N plus SMN (kg N/ha) in Maris Piper on three occasions during the growing season

	4 July		27 July		26 September	
	SMN	Crop + SMN	SMN	Crop + SMN	SMN	Crop + SMN
Unirr	70	299	26	232	62	274
25 SMD	48	256	22	194	43	294
OW 0-3	22	111	26	126	42	201
OW 10-13	61	241	21	197	38	313
Mean	51	227	24	187	46	270
S.E. (18 D.F.)	18.3	24.4	2.6	20.1	6.9	17.1

4.2.1.9. *Microbial population analysis*

4.2.1.9.1. Expts 12, 14, 15 and 16

The number of sequences obtained from each sample subjected to pyrosequencing is presented in Table 43. These sequences were assigned to taxa and used to generate a representation of the bacterial community present in each sample. Proportions of bacterial taxa in each sample are presented at phylum and genus level in Figure 19 and Figure 20, respectively. Bacterial phyla were variable between samples but tended to be dominated by the *Proteobacteria* and *Bacteroidetes*. Greater variability was apparent at the genus level, although significant levels of *Flavobacteria* and *Caulobacter* were common to all samples.

Table 43. Expts 12, 14, 15, 16: Number of 16S rDNA sequences obtained from each sample subjected to pyrosequencing analysis

Sample	Experiment	Number of sequences
Desiree, Unirrigated, Week 4	12	53,132
Desiree, 0 SMD, Week 4	12	21,207
Maris Piper, 0 SMD, Week 4	12	19,363
Maris Piper, Unirrigated, Week 4	12	41,786
Maris Peer, 0 SMD, Week 4	14	25,150
Maris Peer, 0 SMD, Week 8	14	29,886
Greenvale, Raveningham, Wet	15	17,066
Greenvale, Raveningham, Restricted	15	22,348
Greenvale, Somerleyton, Wet	16	26,775
Greenvale, Somerleyton, Restricted	16	7,462

Figure 19. Proportional plot of bacterial phyla present in each sample derived from 16S pyrosequencing data.

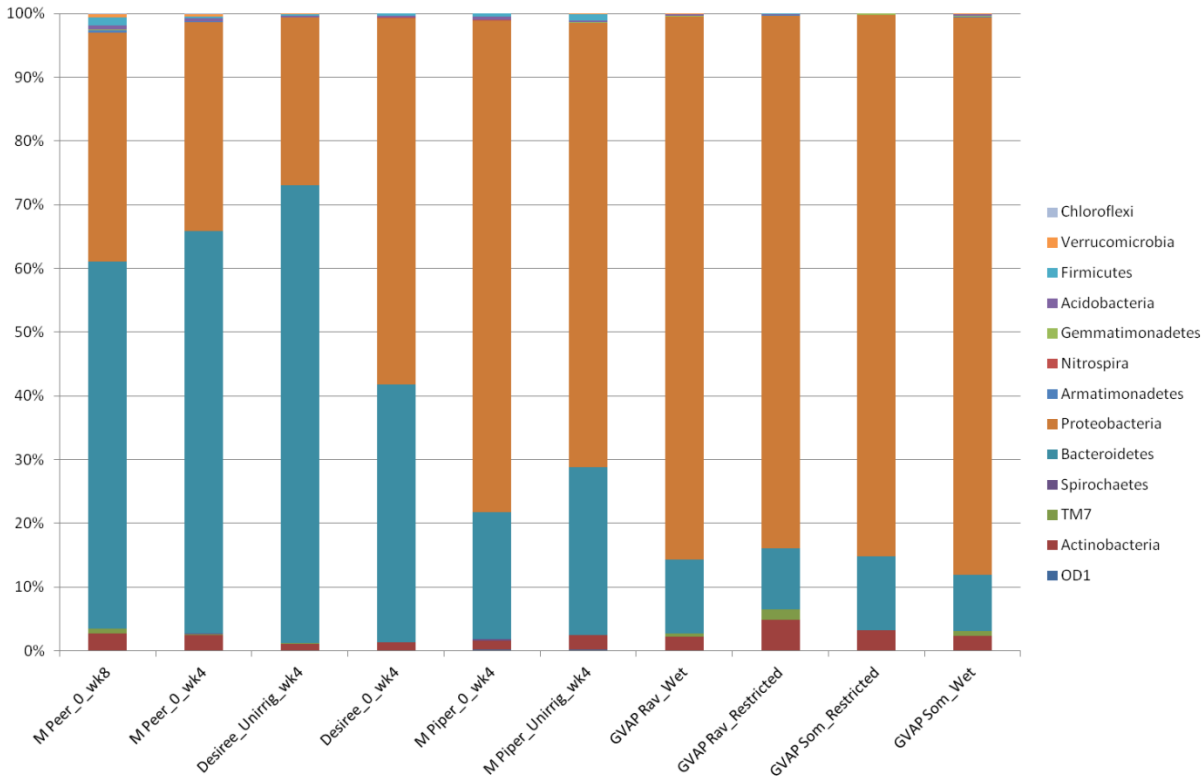
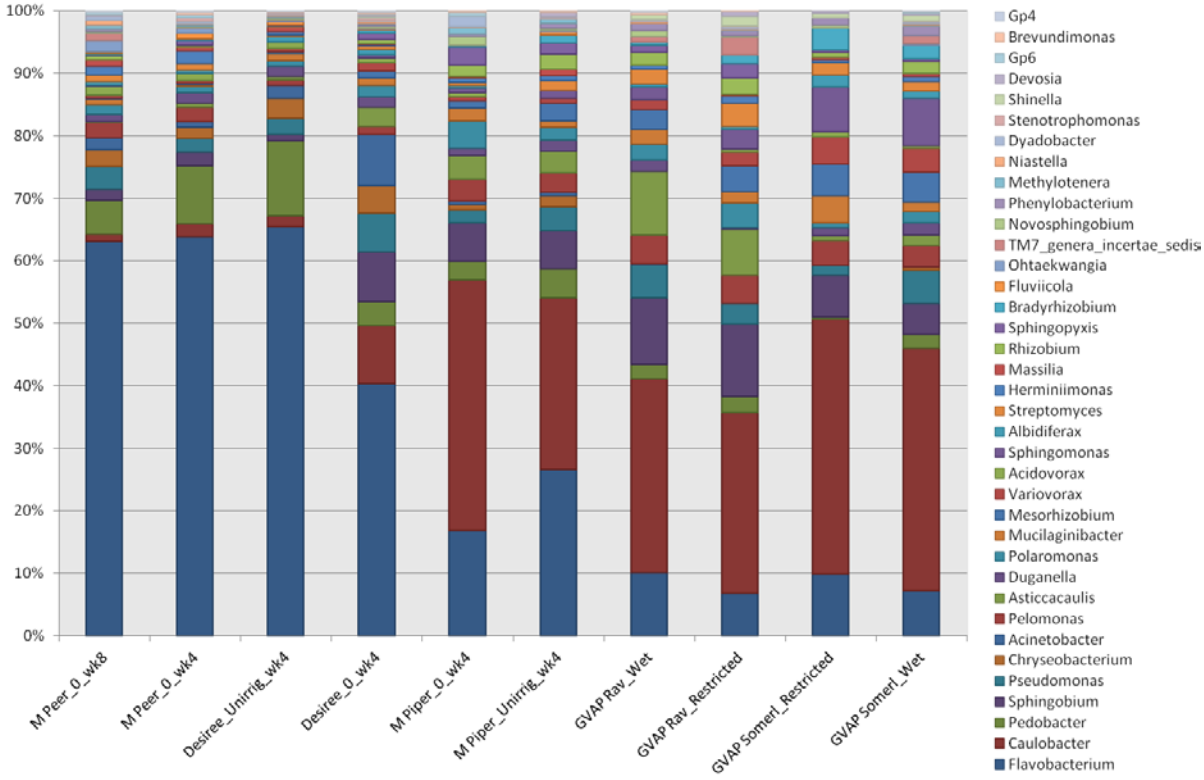


Figure 20. Proportional plot of bacterial genera present in each sample derived from 16S pyrosequencing data.



In order to test whether microbial community composition was linked either to location (i.e. an effect of soil type and local conditions) or to levels of pathogenic *Streptomyces*, an analysis of similarities (ANOSIM) approach was taken to ordinate community profiles and correlate to location, irrigation level and quantity of *txtA* gene as measured by real-time PCR. Ordination plots showing location and *txtA* levels related to community composition are given in Figure 21 and Figure 22, respectively. There was no significant correlation between community composition at the genus level and irrigation level. However, there was a weak correlation between community structure and location (R=0.496, p=0.019) and a stronger correlation between community structure and variety (R=0.756, p=0.002). There was no apparent link between community structure and levels of pathogenic *Streptomyces* as measured by *txtA* real-time PCR.

Figure 21. Anosim analysis of bacterial community structure at the genus level compared with location.

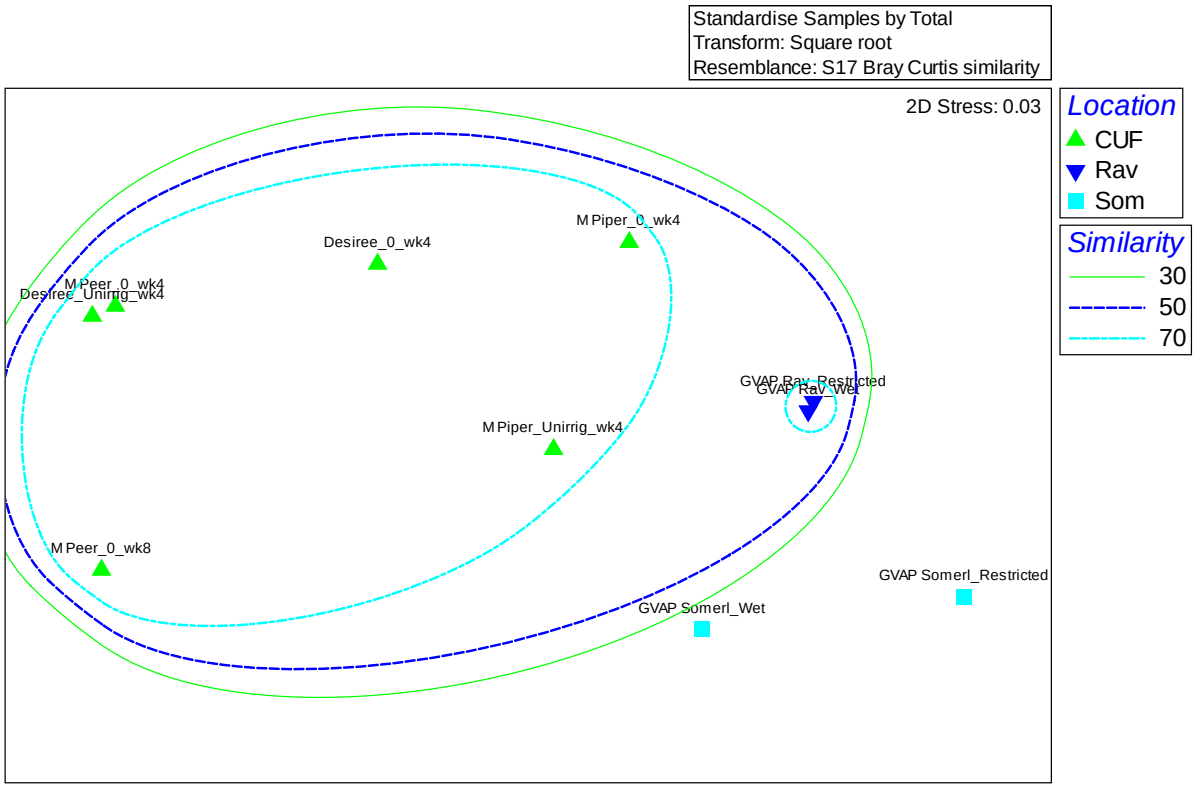
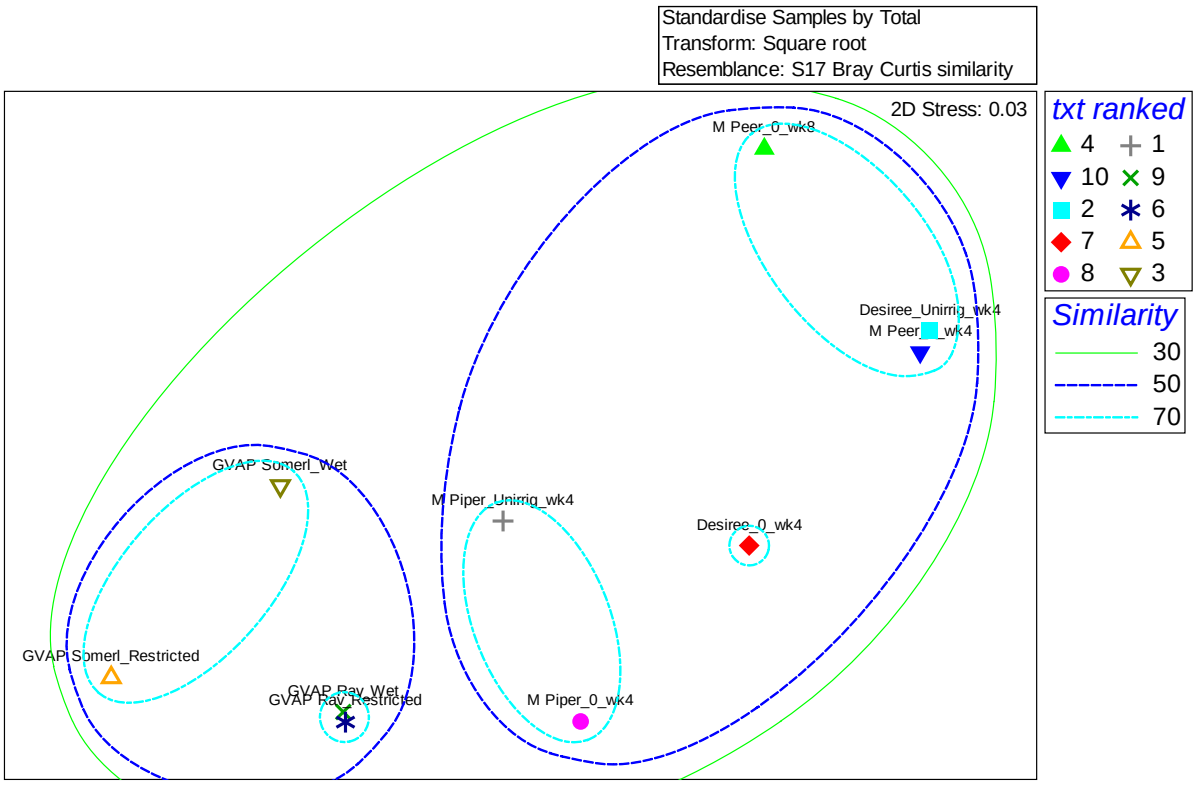


Figure 22. Anosim analysis of bacterial community structure at the genus level compared with *txtA* level. Levels of *txtA* are shown ranked from 1 (low) to 10 (high).



4.2.2. Fera experiments

4.2.2.1.1. Expt 17

Experiment 17 tested the effect of irrigation on scab level in field soils under controlled conditions. Quantities of *txtA Streptomyces* differed between soils and irrigation treatments (Figure 23 and Figure 24). Low moisture soils had higher *txtA* than high moisture soils (P=0.044). Also, there were considerable differences in *txtA* populations between soils (P<0.001). This was largely due to differences between the soil from Coldharbour (CH) and the others. There was a significant interaction effect because one soil, LG21, was unaffected by moisture. For the peaty LG21 soil, populations of *txtA Streptomyces* were similar between irrigation regimes but scab severity was higher in soil kept dry.

Figure 23. Log plot showing quantity of *txtA* gene present on the surface of potato tubers grown under controlled conditions in four soils under low and high irrigation. LSD $_{(P=0.05)}$ = 0.80 (63 D.F.).

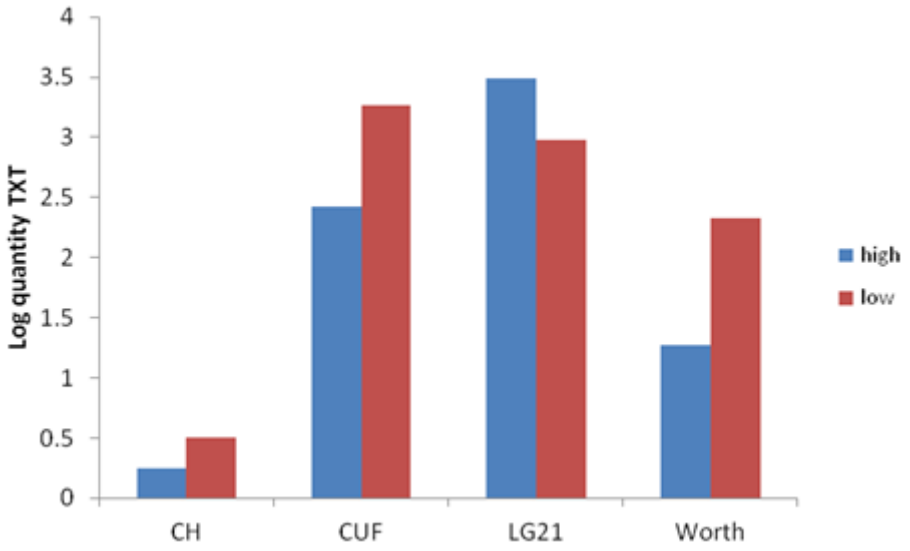
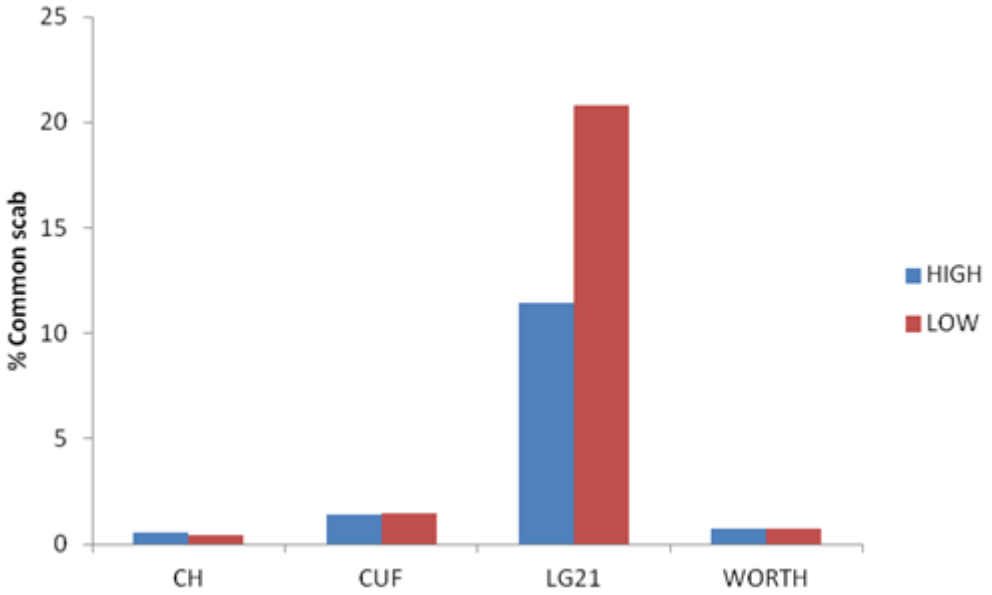


Figure 24. Percentage cover of common scab symptoms on the surface of potato tubers grown under controlled conditions in four soils under low and high irrigation. LSD $_{(P=0.05)}$ = 7.53 (47 D.F.).



4.2.2.1.2. Expt 18

No significant difference in scab level or *Streptomyces* population numbers was observable between irrigation treatments (Figure 25 and Figure 26).

Figure 25. Percentage coverage of common scab symptoms on the surface of tubers grown in sterile soil inoculated with either *Streptomyces scabiei*, *S. turgidiscabies* or nothing ('nil') under low and high irrigation conditions. Vertical bars are standard errors.

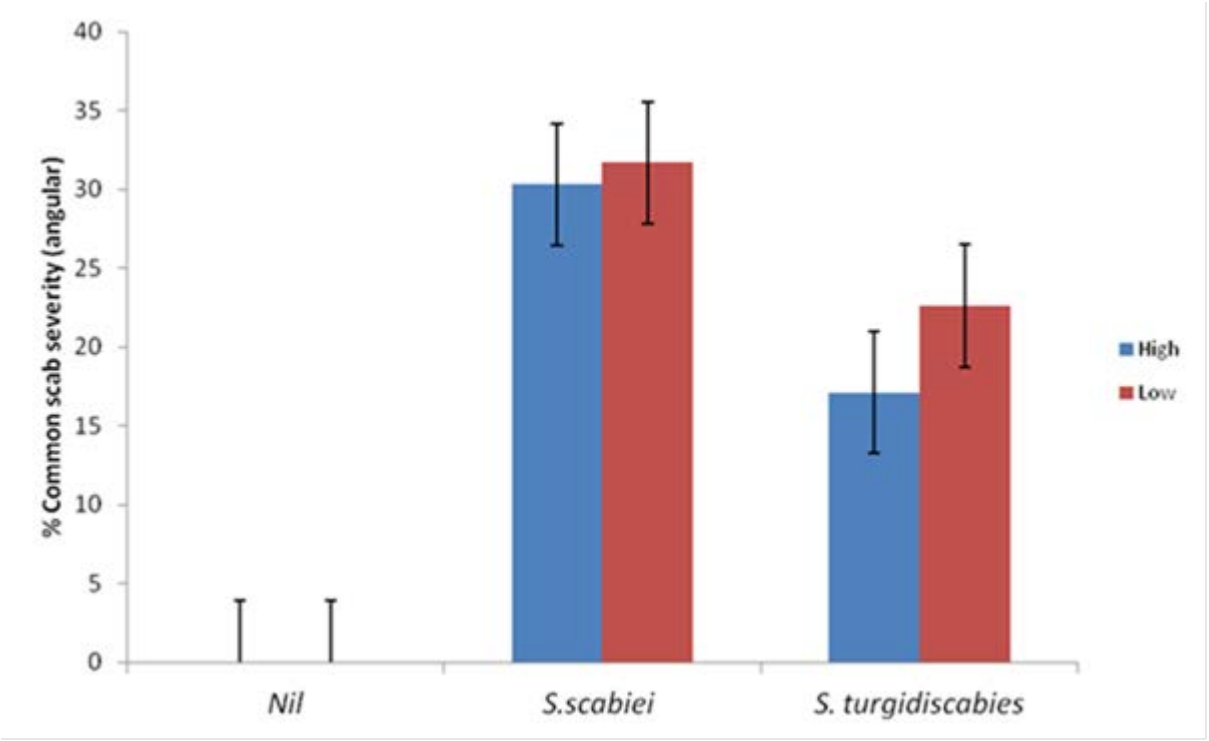
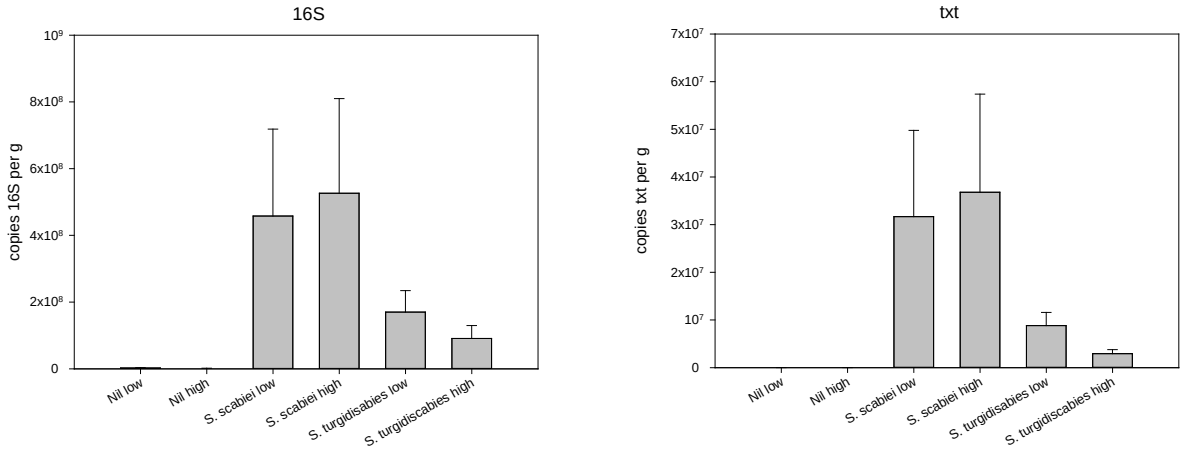


Figure 26. Levels of total actinomycetes (16S) and total pathogenic *Streptomyces* (*txtA*) on the surface of tubers grown in sterile soil inoculated with either *Streptomyces scabiei*, *S. turgidiscabies* or nothing ('nil') under low and high irrigation conditions. Vertical bars are standard errors.



4.3. 2013 experiments

4.3.1. CUF and Greenvale AP experiments

4.3.1.1. *Emergence, tuber initiation and ground cover*

4.3.1.1.1. Expt 19

In Expt 19, 50% emergence was reasonably synchronous for most varieties (32-36 days after planting), but the number of days taken between 10 and 90% emergence was shorter in Volare (5 days) than other varieties (8-10 days). Tuber initiation (50%) occurred between 19-22 days after 50% emergence in most varieties but was longer in Jelly (25 days) and shorter in Orchestra (16 days).

All crops reached full (> 98%) ground cover, even with no irrigation. Ground covers in Jelly were maintained slightly longer in the driest two treatments (Unirr, 35 SMD) than in other irrigated treatments (Table 44). In Lanorma, ground cover was sustained longer in irrigated treatments than Unirrig (Table 44). In Maris Piper, unirrigated crops senesced earlier than other treatments including 35 SMD. Melody was relatively short-lived and there were only small responses in ground cover duration to different irrigation regimes. In Nectar, the canopies of the two driest irrigation regimes lived longest (Table 44). With the exception of unirrigated Volare, Orchestra had the shortest-lived canopies with little effect of irrigation regime on prolonging canopy

duration. In Safari, unirrigated crops struggled to reach full ground cover and began to senesce earlier than irrigated treatments with a consequential large loss in leaf area duration (Table 44). Along with Orchestra, Volare had the shortest-lived canopies. The unirrigated treatment in this variety was particularly short-lived, with little effect across the other irrigation regimes (Table 44).

Table 44. Expt 19: Effect of variety and irrigation on season-long integrated ground cover (% days)

	Irrigation regime				
	Unirr	0 SMD	15 SMD	35 SMD	TI 1-4
Jelly	9457	9207	8909	9599	9151
Lanorma	7875	8260	8178	8398	8640
Maris Piper	8757	9450	9067	9938	9399
Melody	7037	7205	7301	7541	7684
Nectar	8498	8363	7777	8659	7852
Orchestra	6211	6531	6225	6636	6704
Safari	6972	8057	8468	7821	8379
Volare	4527	6573	6393	6638	7049
S.E. (78 D.F.)			291.2		
Mean	7417	7956	7790	8154	8107
S.E. (78 D.F.)			103.0		

4.3.1.1.2. Expt 20

Emergence commenced 25-28 days after planting and 50% emergence was reached by 30, 33 and 32 days after planting in Maris Peer, Regina and Venezia, respectively. The time taken for emergence to increase from 10 to 90% was 7 days in Maris Peer, 8 days in Regina and 6 days in Venezia. The TI period was brief, with 50% of plants tuberized 15 days after emergence in Maris Peer and 14 days after emergence in Regina and Venezia, and 80% of tubers (10-90%) initiated over a 3-day period in all varieties. Ground cover development was rapid and all plots reached 100% ground cover by 4 weeks after emergence. At desiccation, 4 wks and 6 wks treatments had *c*. 24% ground cover, whilst 8 wks treatments had *c*. 59%.

4.3.1.1.3. Expts 21 and 22

In Expt 21, emergence commenced on 10 May and 50 % emergence was recorded on 15 May, whilst TI was on 2 June. In Expt 22, emergence commenced on 20 May and 50 % emergence was recorded on 27 May, whilst TI was on 14 June. Full ground

cover was achieved by 53 days after emergence in both experiments. Ground cover remained > 99 % until final harvest at both sites.

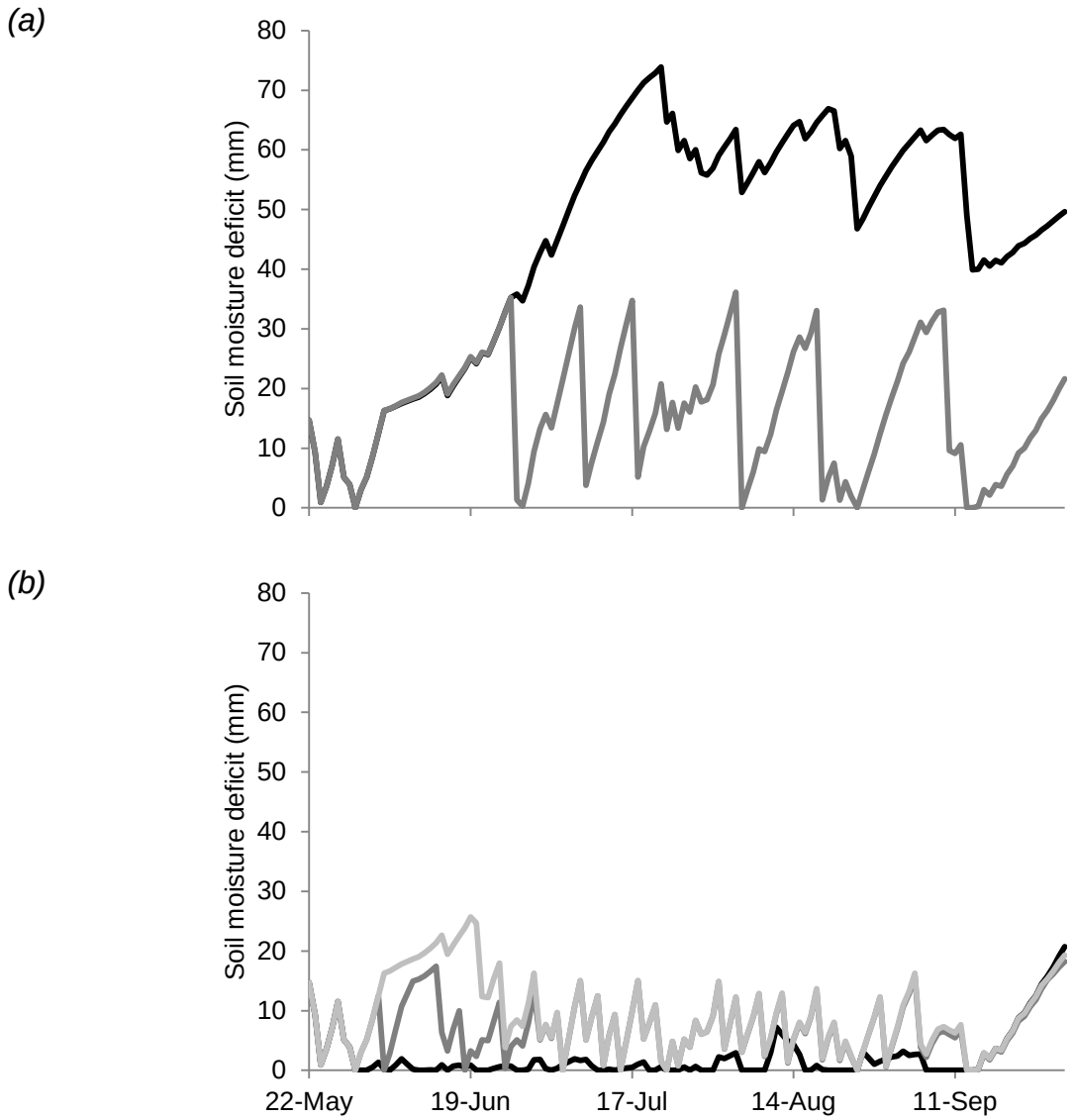
4.3.1.2. *Modelled SMDs*

The 2013 season was very dry in June and July and evapotranspiration was higher than the long-term average values for these months. Consequently, demand for irrigation during scab control was high.

4.3.1.2.1. Expt 19

The treatments were kept close to their intended SMDs, with the 0 SMD treatment being maintained < 4 mm SMD throughout the season and the 15 SMD and 35 SMD treatments reaching their trigger deficits on a high number of occasions compared with the same treatments in Expts 1 and 12 in 2011-2012 (Figure 27). The SMD in Unirr plots was 20 mm at the onset of TI, increasing to 58 mm 4 weeks later. The TI 1-4 treatment reached a maximum of 26 mm SMD before the soil was brought back to field capacity one week after TI. The trigger SMD in 15 mm SMD treatments was reached on seven occasions during scab control and the 35 SMD on two occasions. The maximum SMD reached in Unirr was 74 mm on 22 July. Drainage was substantial in frequently-irrigated treatments (Table 11).

Figure 27. Expt 19: Modelled soil moisture deficits in Maris Piper plots. *(a)* Unirr, —; 35 SMD, —; *(b)* 0 SMD, —; 15 SMD, — TI 1-4, —.



4.3.1.2.2. Expt 20

Soil moisture deficits were maintained < 15 mm until the point when the treatment was timed to end. Irrigation was applied fairly consistently every 3-5 days throughout the longest period of control (8 wks), with the exception of a rainy period in the last week of July when there was a gap of 10 days. When irrigation was ceased in the 4 wks treatment, its SMD increased to 46 mm in the following 4 weeks, whilst in the 6 wks treatment it increased to 33 mm.

4.3.1.2.3. Expts 21 and 22

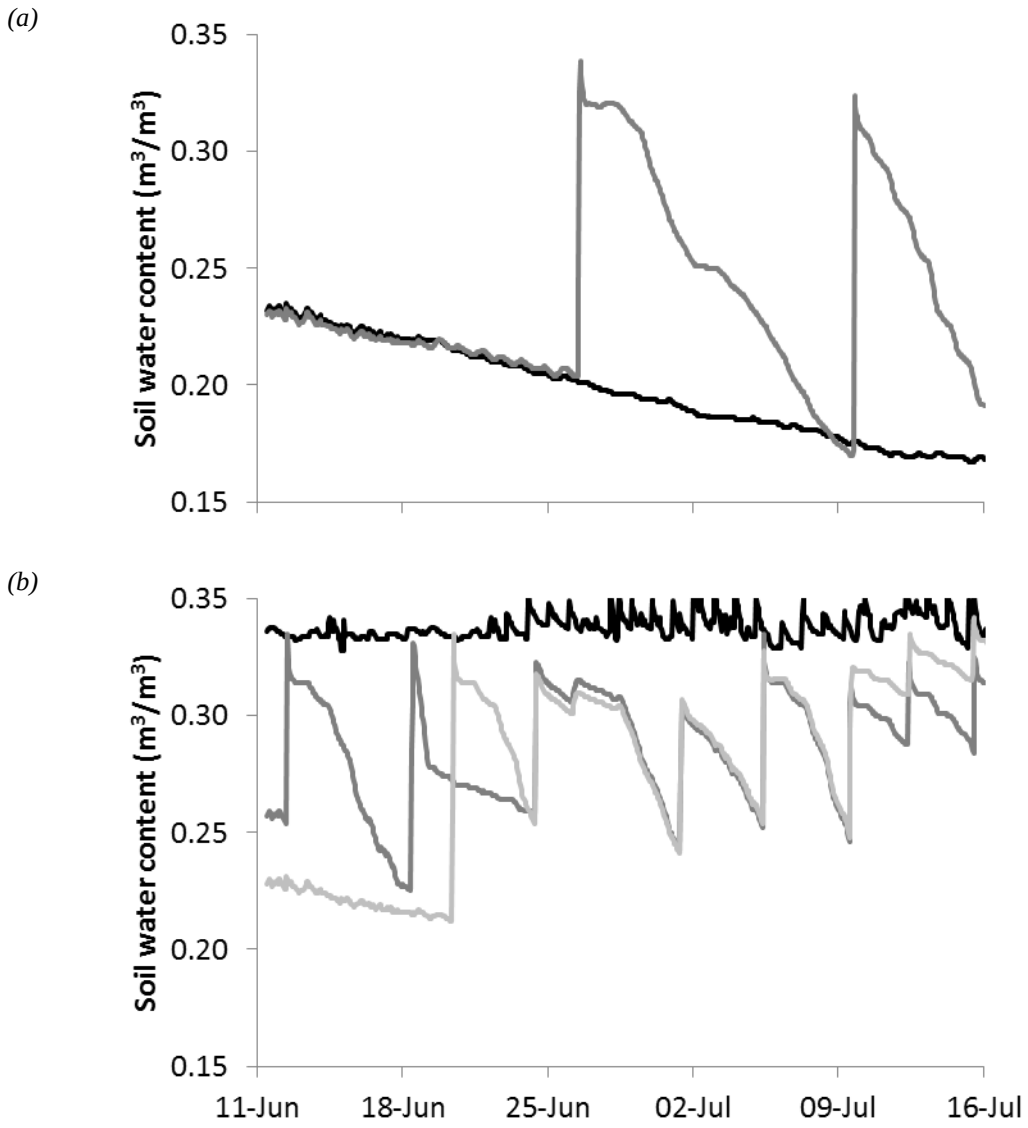
In Expt 21, average SMDs during the 4 weeks post-TI were 4 mm (maximum 11 mm) in Wet treatments and 18 mm (maximum 29 mm) in the Dry. In Expt 22, average SMDs during the 4 weeks post-TI were 8 mm (maximum 19 mm) in Wet treatments and 17 mm (maximum 39 mm) in Dry.

4.3.1.3. *Measured soil water content*

4.3.1.3.1. Expt 19

Soil moisture probe data in Maris Piper and Melody plots indicated that the 0 SMD treatment was maintained at 0.33-0.35 m^3/m^3, close to field capacity (0.34 m^3/m^3) in the centre of the ridge from TI for 4 weeks (Figure 28*b*). With the exception of the period immediately following irrigation, the 15 SMD treatment was maintained at a water content of 0.24-0.28 m^3/m^3 during the scab control period (Figure 28*b*), The delayed start TI 1-4 treatment reached 0.21 m^3/m^3 7 days after TI but thereafter closely followed the 15 SMD treatment (Figure 28*b*). The unirrigated decreased from 0.23 to 0.17 m^3/m^3 over the same period, whilst the 35 SMD treatment reached a minimum of 0.18-0.21 m^3/m^3 on each of the two occasions it was irrigated during scab control (Figure 28*a*).

Figure 28. Expt 19: Measured soil water content in Maris Piper and Melody plots. *(a)* Unirr, —; 35, —; *(b)* 0, —; 15, — TI 1-4, —.



4.3.1.3.2. Expt 20

Most of the soil moisture probe data were lost owing to the logger malfunctioning from being waterlogged.

4.3.1.4. *Common scab*

4.3.1.4.1. Expt 19

All tubers examined were infected with common scab, albeit with < 1% SA. Tubers with > 5% SA are generally regarded as unsuitable for packing and a typical minimum

acceptable packout is 70%, i.e. ≤ 30% rejectable tubers. Typically, the most effective irrigation regimes in the very susceptible variety Maris Piper at CUF in previous experiments in 1992-2010 resulted in *c*. 2-3% SA infected, whilst unirrigated crops ranged from 6-28% SA infected. In Expt 19, in Maris Piper, the most susceptible variety, unirrigated crops had only 4.3 % SA infected (comparable with 2011 but higher than the 1.0% SA in 2012). Maris Piper was the worst variety affected by common scab but its best irrigation treatments produced tubers with only *c*. 1% SA infected, with more than 98% of tubers being packable (Table 45). As found in 2011-2012, the delayed start TI 1-4 was numerically (but not statistically) worse in terms of scab severity and packout than the 15 SMD treatment. In all other varieties tested, scab was extremely low (< 1% SA and > 98% of tubers packable; Table 45). However, Safari generally had slightly worse scab than all other varieties except Maris Piper (Table 45). The odd treatment to stand out was Melody kept at field capacity during the control period which had moderate scab and rejectable packout infection despite the wet soil (Table 45). In this treatment, almost half the tubers had growth cracks and more severe scab infection was associated with tubers which were cracked, indicating a possible alternative invasion route for pathogenic *Streptomyces*.

Table 45. Expt 19: Common scab incidence and severity

Variety	Irrigation regime	Incidence < 5 % surface area		Severity
		%	Ang. trans.	% SA affected
Jelly	Unirr	100.0	90.0	0.54
	0 SMD	100.0	90.0	0.52
	15 SMD	99.3	87.3	0.57
	35 SMD	100.0	90.0	0.65
	TI 1-4	100.0	90.0	0.57
Lanorma	Unirr	100.0	90.0	0.88
	0 SMD	100.0	90.0	0.63
	15 SMD	99.3	87.3	0.61
	35 SMD	100.0	90.0	0.57
	TI 1-4	100.0	90.0	0.58
Maris Piper	Unirr	78.8	61.4	4.32
	0 SMD	100.0	90.0	0.78
	15 SMD	98.7	84.6	1.12
	35 SMD	86.7	69.4	2.75
	TI 1-4	91.3	73.0	2.22
Melody	Unirr	100.0	90.0	0.61
	0 SMD	63.3	52.9	8.16
	15 SMD	98.7	84.6	1.65
	35 SMD	99.3	87.3	1.13
	TI 1-4	100.0	90.0	1.00
Nectar	Unirr	100.0	90.0	0.89
	0 SMD	98.7	86.2	1.13
	15 SMD	99.3	87.3	0.77
	35 SMD	99.3	87.3	0.96
	TI 1-4	99.3	87.3	0.67
Orchestra	Unirr	100.0	90.0	0.70
	0 SMD	100.0	90.0	0.55
	15 SMD	100.0	90.0	0.57
	35 SMD	100.0	90.0	0.61
	TI 1-4	100.0	90.0	0.57
Safari	Unirr	93.5	78.8	2.11
	0 SMD	100.0	90.0	1.02
	15 SMD	96.7	81.4	1.08
	35 SMD	96.7	81.7	1.59
	TI 1-4	98.0	85.3	0.99
Volare	Unirr	100.0	90.0	0.65
	0 SMD	100.0	90.0	0.55
	15 SMD	100.0	90.0	0.52
	35 SMD	100.0	90.0	0.56
	TI 1-4	100.0	90.0	0.59
S.E. (78 D.F.)		-	2.44	0.602

4.3.1.4.2. Expt 20

Most tubers in this experiment had < 5% SA infected with scab but there were more scabby tubers than Expt 13 in 2012. Despite the generally very low severity of scab, Maris Peer irrigated for 4 weeks had a slightly higher scab severity than when irrigated for 6 weeks but 6 weeks was equally as good as 8 weeks (Table 46). There was no effect of duration of the control period in Regina and the scab severity was very low, with some tubers exhibiting no symptoms whatsoever (Table 46). In Venezia, scab tended to decrease with increase in duration although differences were not significant. In Maris Peer, 4 week duration had significantly more severe scab than 6 wks and numerically more than 8 wks (Table 46).

Table 46. Expt 20: effect of irrigation duration on common scab incidence and severity

Variety	Duration	Incidence <5 % SA (%)	Incidence <5 % SA (ANG)	Severity % SA affected
Maris Peer	4 wks	96.0	78.4	1.25
	6 wks	98.7	84.6	0.77
	8 wks	97.3	82.6	0.97
Regina	4 wks	100.0	90.0	0.57
	6 wks	100.0	90.0	0.54
	8 wks	100.0	90.0	0.57
Venezia	4 wks	98.0	81.9	0.97
	6 wks	98.0	83.4	0.83
	8 wks	100.0	90.0	0.63
S.E. (16 D.F.)		-	1.92	0.130

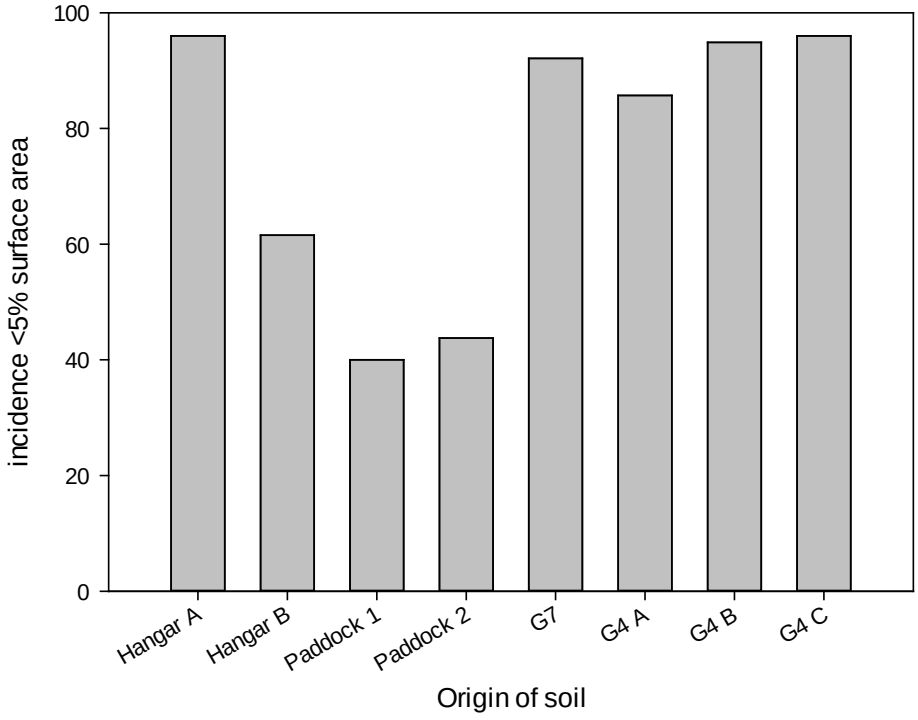
4.3.1.4.3. Expts 21 and 22

In Expt 21, scab was more severe in unirrigated (3.73 ± 0.416% SA) than in irrigated treatments (1.10% SA). Around 97% of tubers in irrigated plots were infected below the packing threshold of >5% SA, whereas only 77% would have made the packing grade in unirrigated. Similarly, in Expt 22 scab was more severe in unirrigated (4.11 ± 0.771% SA) than in irrigated (1.58% SA), with 93% of tubers packable in irrigated and only 75 % in unirrigated.

4.3.1.4.4. Expt 24

There was no consistent relationship between biofumigant treatment and the proportion of packable tubers with < 5% SA infected with common scab (Figure 29) although the proportion of rejectable tubers in Hangar A soil, which had been treated with a biofumigant, was lower than in untreated soil from the same field (Hangar B). In soils from 'Paddock' and 'G4' plots biofumigation did not affect scab incidence and packout.

Figure 29. Expt 24: Percentage of harvested tubers with < 5 % surface area coverage of scab symptoms from pot experiments established using soil from field plots subjected to various biofumigation treatments.



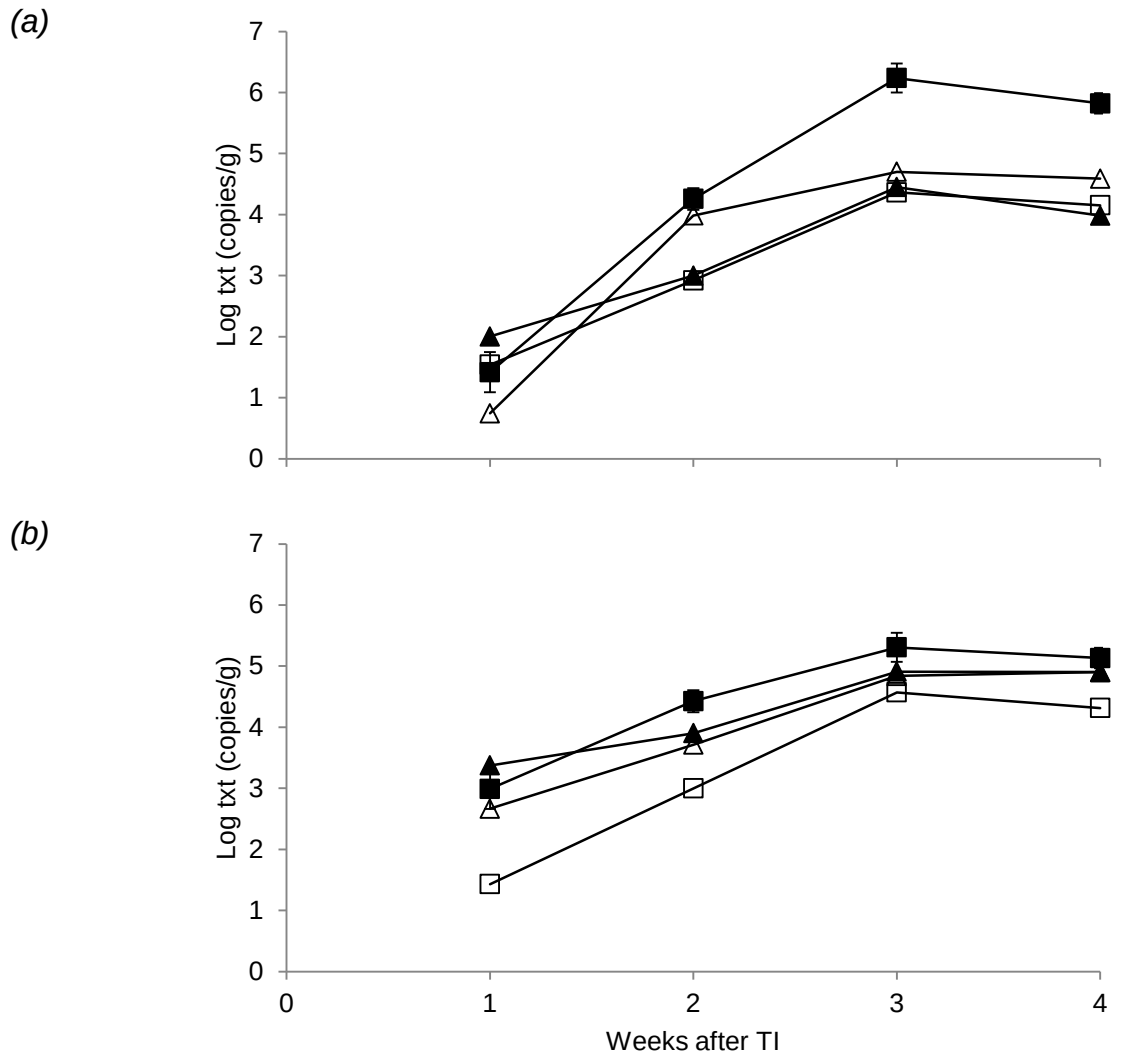
4.3.1.5. *Streptomyces populations*

4.3.1.5.1. Expt 19

Unlike previous seasons where there were no pathogenic *Streptomyces* detected until 2 weeks after TI, there were considerable *txtA* populations at week 1 in both varieties in 2013 (Figure 30). In Maris Piper, the 0 SMD and 15 SMD treatments were similar throughout the sampling period but in the delayed-start TI 1-4, more pathogens were found at week 2 than in 0 SMD or 15 SMD treatments. By week 3, however, all irrigated plots in Maris Piper had similar *txtA* populations (Figure 30*a*). In Melody, the

0 SMD had significantly lower *txtA* populations than the other irrigation regimes, particularly up to 2 weeks after TI (Figure 30*b*). When averaged over both varieties, unirrigated crops had lower populations of *txtA* than other irrigation regimes at weeks 3 and 4 after TI. Peak populations occurred around week 3 and were similar between varieties except that Unirr Maris Piper had significantly more *txtA* than Unirr Melody (Figure 30).

Figure 30. Expt 19: Timecourse of pathogenic *Streptomyces* populations. *(a)* Maris Piper and *(b)* Melody. Unirr, ■; 0 SMD, □; 15 SMD, ▲; TI 1-4, △. Bars represent 1 S.E.



Samples of tubers from all varieties and all irrigation treatments apart from 35 SMD were assessed for the population of pathogenic *Streptomyces* at 4 weeks after TI. Overall, unlike Expt 1 (2011) and Expt 12 (2012), there was no effect of variety on the population of *txtA*, even between the scab-susceptible Maris Piper and the most resistant varieties (Table 47). There were, however, more pathogenic *Streptomyces*

on tubers at the end of the control period in the Unirr treatment than in other irrigation regimes, with 0 SMD having fewer than 15 SMD or TI 1-4 (Table 47).

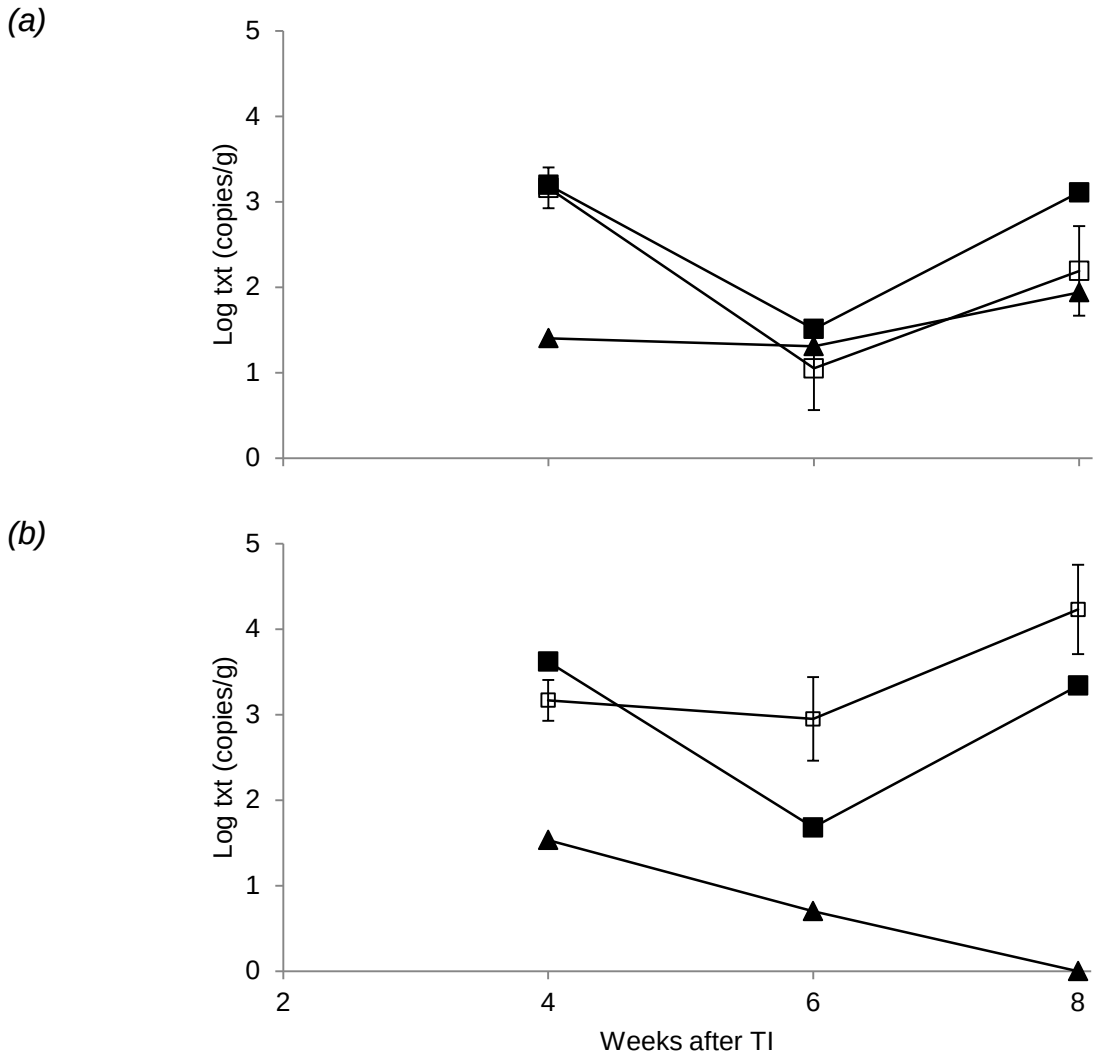
Table 47. Expt 19: Populations of *txtA Streptomyces* 4 weeks after TI in (log copies/g peel)

	Irrigation regime			
Variety	Unirr	0 SMD	15 SMD	TI 1-4
Jelly	5.16	3.65	4.59	4.08
Lanorma	5.93	6.37	4.78	4.45
Maris Piper	5.89	4.23	4.68	4.67
Melody	5.25	4.37	5.08	5.07
Nectar	5.16	4.17	4.68	4.63
Orchestra	5.66	3.87	5.15	4.95
Safari	5.60	4.66	4.96	4.74
Volare	5.74	4.63	4.55	4.58
S.E. (62 D.F)		0.310		
Mean	5.55	4.16	4.81	4.65
S.E. (62 D.F.)		0.110		

4.3.1.5.2. Expt 20

There was an anomaly in the data from the Salad Expt which could not be explained even after checking the data. At the first sampling, 4 weeks after TI, all irrigation treatments within a variety should have had similar *txtA* populations but the 8 wks treatment in both varieties had significantly lower *txtA* populations than 4 wks or 6 wks treatments (Figure 31). In Maris Peer, there were no significant differences in pathogenic *txtA* at 6 or 8 weeks after TI (Figure 31*a*), but in Venezia there were fewer *txtA* 8 weeks after TI in the 8 wks treatment than in the 4 or 6 wks treatments (Figure 31*b*).

Figure 31. Expt 20: Timecourse of pathogenic *Streptomyces* populations. *(a)* Maris Peer and *(b)* Venezia. 4 wks, ■; 6 wks, □; 8 wks, ▲. Bars represent 1 S.E.



4.3.1.5.3. Expts 21 and 22

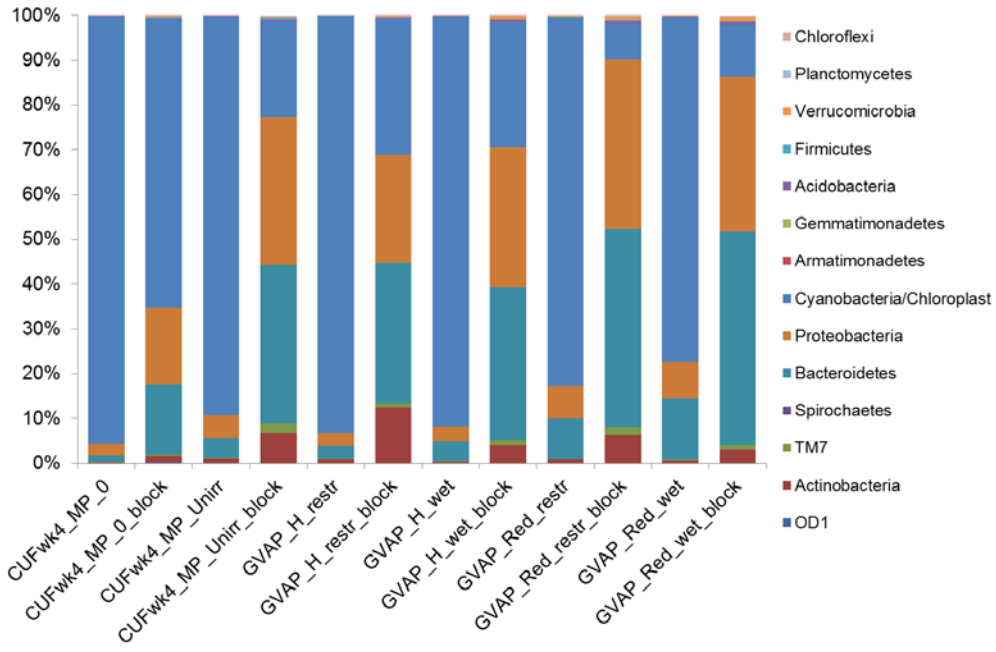
In Expt 21, there were significantly fewer pathogenic *txtA* on tubers 4 weeks after TI in irrigated (3.11 ± 0.631 log copies/g) compared with unirrigated (5.43). In Expt 22, the difference between irrigation regimes was smaller than in Expt 3 but there were significantly fewer pathogenic *txtA* on tubers 4 weeks after TI in irrigated (4.99 ± 0.254 log copies/g) compared with unirrigated (5.89).

4.3.1.6. *Antagonist populations*

4.3.1.6.1. Methodological improvement for bacterial antagonist analysis

Improvements in the coverage of bacteria within the 16S rRNA gene pyrosequencing data set were achieved using the chloroplast blocking primers. On the phylum level chloroplasts accounted on average for 88% of sequences ranging from 77-93% in the tested samples without the use of blocking primers, which was reduced to an average of 27% with blocking primers ranging from 8-64 % in the tested samples (Figure 32).

Figure 32. Proportional plot of bacterial phyla present in each sample in 2013 derived from 16S pyrosequencing data with chloroplast blocking primers ("block") or without.



No effects on detection (presence/absence) were observed for the following genera:

Arthrobacter, Streptomyces, Aeromicrobium, TM7_genera_incertae_sedis, Niastella, Pedobacter, Mucilaginibacter, Fluviicola, Flavobacterium, Chryseobacterium, Acidovorax, Variovorax, Polaromonas, Duganella, Herminiimonas, Massilia, Methylophilus, Rhizobium, Phyllobacterium, Mesorhizobium, Devosia, Asticcacaulis, Caulobacter, Sphingomonas, Sphingopyxis, Sphingobium, Cellvibrio, Pseudomonas, Lysobacter, Arenimonas, Dyadobacter, Chitinophaga, Shinella, Halomonas, Pseudoxanthomonas, Opitutus, Lentzea, Phycicoccus, Agromyces, Undibacterium, Stenotrophomonas, Aeromonas, Leadbetterella, Herbaspirillum, Microvirga, Amycolatopsis, Microbacterium, Janthinobacterium, Kaistia, Solirubrobacter,

Lechevalieria, Pseudonocardia, Demequina, Leifsonia, Actinoplanes, Phaselicystis, Uliginosibacterium, Hyphomicrobium, Catellibacterium, Amaricoccus, Perlucidibaca, Legionella and *Dokdonella.*

However, differences were observed with some genera solely found in the approach using blocking primers. These were:

Oxalicibacterium, Renibacterium, Pimelobacter, Sediminibacterium, Luteimonas, Aciditerrimonas, Flavisolibacter, Aminobacter, Janibacter, Blastococcus, Longispora, Angustibacter, Turneriella, Niabella, Planobacteriumm, Peredibacter, Myxococcus, Paucibacter, Ottowia, Geminicoccus, Agromonas, Labrys, Martelella, Sphingosinicella, Erythromicrobium, Rugamonas, Aquicella, Rahnella, Obesumbacterium, Aspromonas, Armatimonas/Armatimonadetes_gp1 and *Gemmata.*

The blocking primer approach did not capture some genera present at low level in the tested samples. These were genera *BRC1_genera_incertae_sedis, Umezawaea, Kitasatospora, Nonomuraea, Nocardia, Catellatospora, Allocatelliglobosispora, Kineosporia, Adhaeribacter, Sorangium, Piscinibacter, Chitinimonas, Achromobacter, Limnohabitans, Beijerinckia, Pseudochrobactrum, Roseomonas, Paracoccus, Yersinia, Steroidobacter, Gp22, Gp1, Tissierella, Cohnella, Sphaerobacter, Parachlamydia, Glycomyces, Algoriphagus, Hymenobacter, Vampirovibrio, Naxibacter, Gp5, Gp10,* and *Planctomyces*. Additionally, the genera *Burkholderia* and *Nitrospira* were only found without blocking primers.

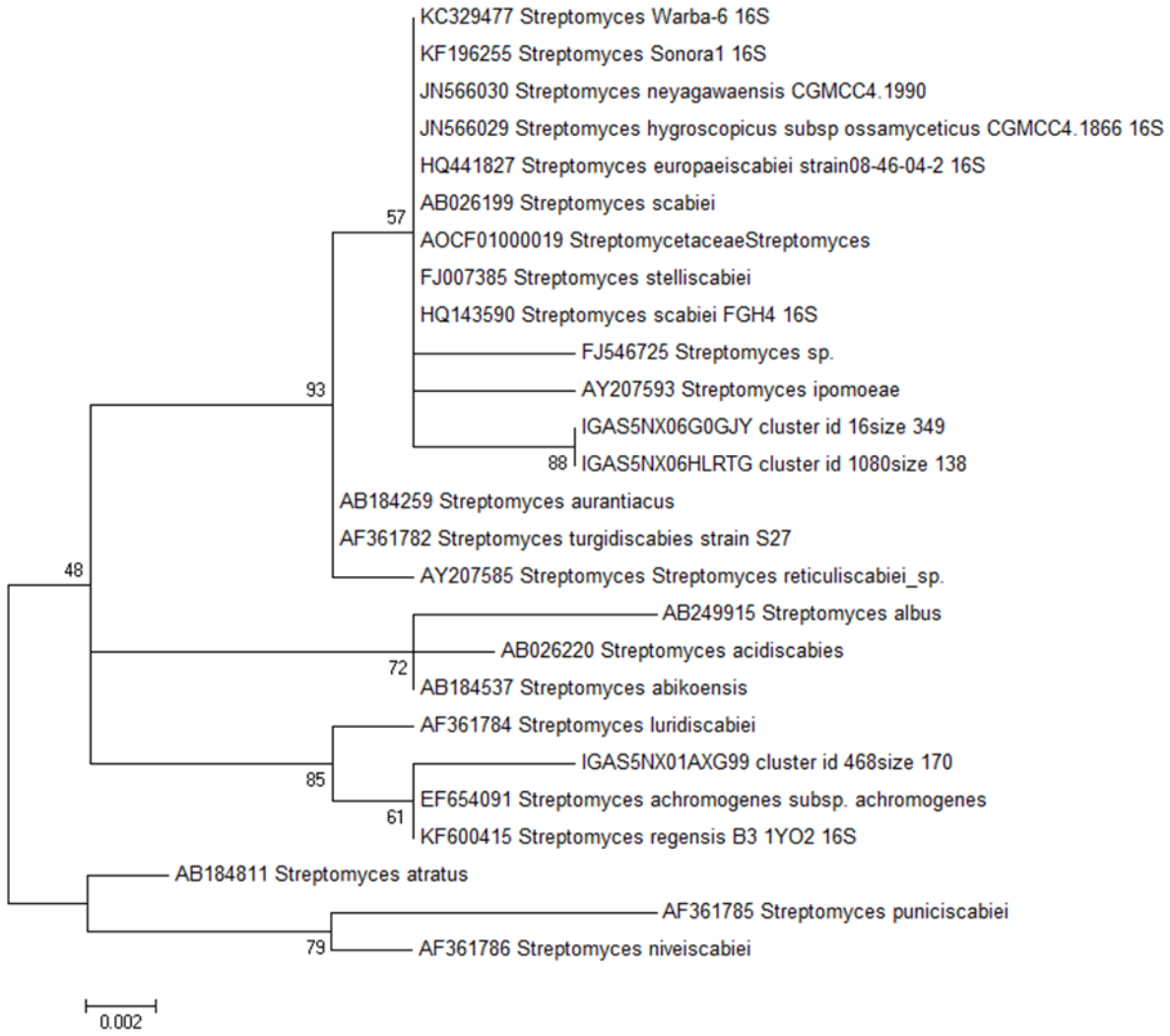
Slight variation of relative abundance was detected for *Nocardioides, Brevundimonas, Rhizobacter, Gp6, Ohtaekwangia, Albidiferax, Novosphingobium, Phenylobacterium, Bradyrhizobium, Luteolibacter, Herpetosiphon, Ferruginibacter, Pelomonas, Acinetobacter, Buttiauxella, Gp4, Paenibacillus, Kribbella, Bosea, Bacillus, Prosthecobacter, Subdivision3_genera_incertae_sedis, Terrabacter, Lacibacter, Rhodanobacter, Exiguobacterium, Spartobacteria_genera_incertae_sedis, Rhodococcus, Marmoricola, Alkanindiges, Gemmatimonas, Promicromonospora, Cytophaga, Hydrogenophaga, Shewanella, Gp16, OD1_genera_incertae_sedis, Cellulomonas, Rhodobacter, Ilumatobacter, Mycobacterium, Serratia, Terrimonas, Dongia, Streptacidiphilus, Emticicia, Sphingobacterium, Bdellovibrio, Georgfuchsia, Luteibacter, Gp25, Gp7* and *Pasteuria*.

It appeared that better detection was achieved with blocking primers for the genera *Nubsella, Methylotenera, Blastobacter, Vasilyevaea, Epilithonimonas, Kofleria, Armatimonadetes_gp5, Allokutzneria, Arcicella, Aquabacterium, Porphyrobacter* and *Verrucomicrobium*.

4.3.1.6.2. Detection of different *Streptomyces* species using antagonist sequences

It was evaluated if future detection of different species of *Streptomyces* would be valuable for identification of plant pathogenic species based on the present antagonist sequences. Within the sequences obtained from 16S rRNA gene sequencing three different taxa were assigned to the genus Streptomyces. A phylogenetic tree was constructed including sequences obtained by pyrosequencing referred to IDs starting with “IGAS5NX0…”. Based on the phylogenetic tree distinguished taxa were related to *S. achromogenes* and *S. scabiei* (Figure 33).

Figure 33. Molecular Phylogenetic analysis by Maximum Likelihood method based on the Jukes-Cantor model. Accession numbers and species names for representative sequences are shown. Operational Taxonomic Units related to Streptomyces derived from pyrosequencing are indicated by IDs starting with "IGAS5NX0…" Evolutionary analyses were conducted in MEGA6.

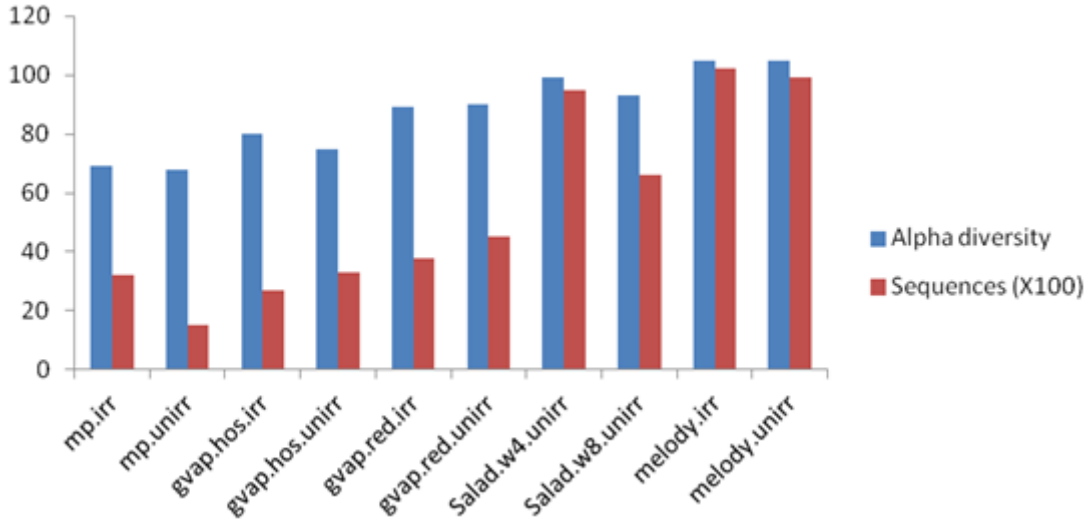


4.3.1.6.3. Bacterial community profile

The following comparative analyses are based on bacterial community profiles obtained using the blocking primer approach excluding any sequences classified as chloroplast.

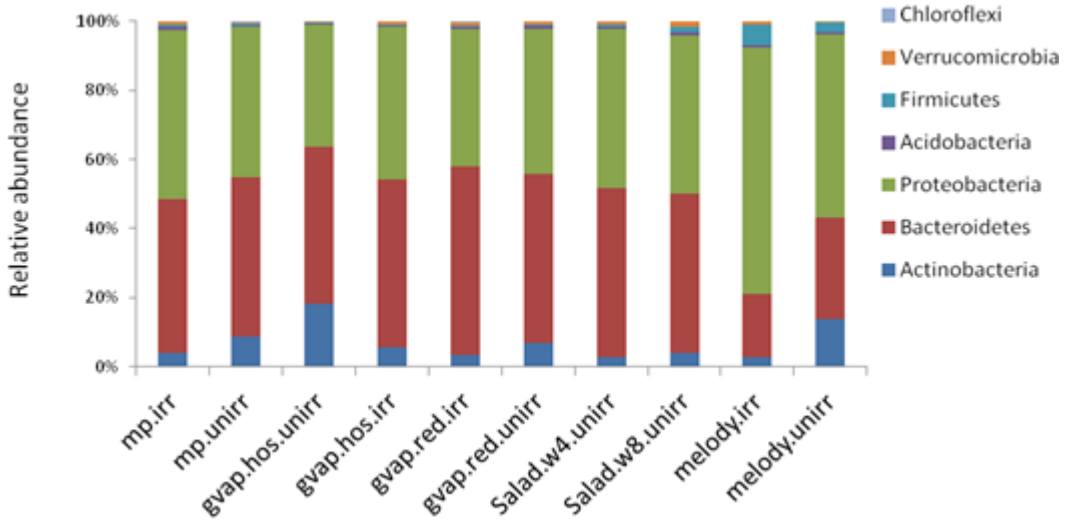
In Figure 34, a comparison was made between the total number of sequences obtained for each sample with the genera richness (alpha diversity) observed. Generally richness increased with an increase in the number of sequence, though in a different ratio across different samples.

Figure 34. Total number of sequences versus genus richness across all the samples. Potato varieties and locations are indicated as follows: Maris Piper from CUF (mp), Maris Piper from GVAP Hales Hospital (gvap.hos) and Maris Piper from GVAP Redhouse West (gvap.red). Irrigation regimes were either irrigated (irr) or unirrigated (unirr). Data from Expts 19-22.



Relative abundance of the bacterial community at phylum level (Figure 35) revealed *Proteobacteria* (35-48 %) and *Bacteroidetes* (45-53 %) were the top abundant phyla among all the samples irrespective of the irrigation treatment excepting the variety Melody (described later).

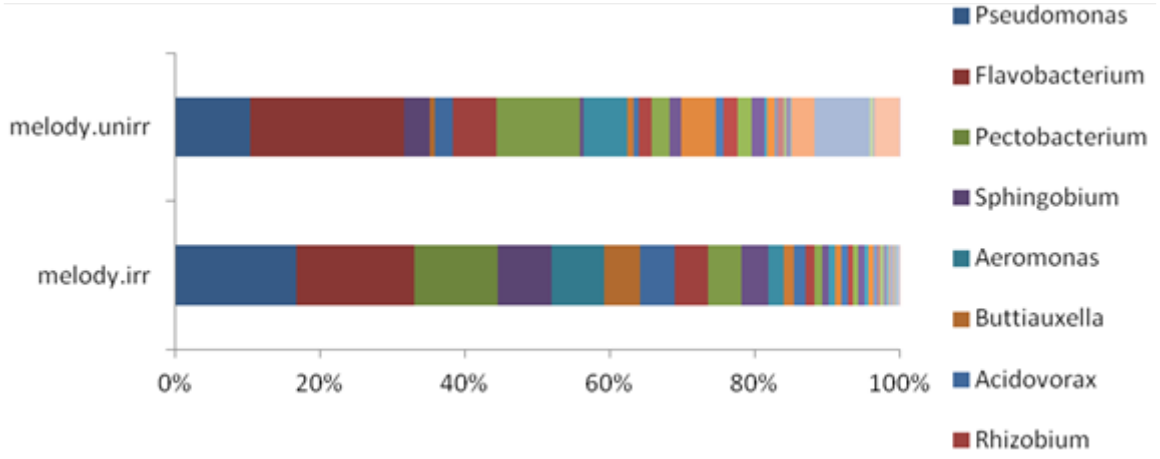
Figure 35. Relative abundance of bacterial community at phylum level in potato peel of different potato varieties from different locations in Expts 19-22. Potato varieties and locations are indicated as follows: Maris Piper from CUF (mp), Maris Piper from GVAP Hales Hospital (gvap.hos) and Maris Piper from GVAP Redhouse West (gvap.red). Irrigation regimes were either irrigated (irr) or unirrigated (unirr).



Other abundant phyla found were *Actinobacteria* (5-15%), *Firmicutes* (0.5-1%) and *Verrucomicrobia* (0.5-1%) but with large sample-to-sample variation. *Flavobacterium* (30-40%) from the phyla *Bacteroidetes* were the most abundant genera found across all samples. *Alphaproteobacteria* (10-18%) and the *Gammaproteobacteria* (5-40%) were the dominant classes of the phylum *Proteobacteria* with *Pseudomonas sp.* (5-15%) and *Rhizobium sp.* (3-5%) being the most abundant genera within these classes, respectively.

The bacterial community associated with the variety Melody were an exception since they were dominated by *Proteobacteria* in both irrigated (70%) and unirrigated (51%) samples (Figure 36). The irrigated sample was dominated by the bacterial genera *Pseudomonas* (15%) *Flavobacterium* (15%), *Pectobacterium* (10%), and *Sphingobium* (6%) whereas the unirrigated sample was dominated by the genera *Flavobacterium* (16%), *Pedobacter* (9%), *Pseudomonas* (8%) and *Streptomyces* (6%).

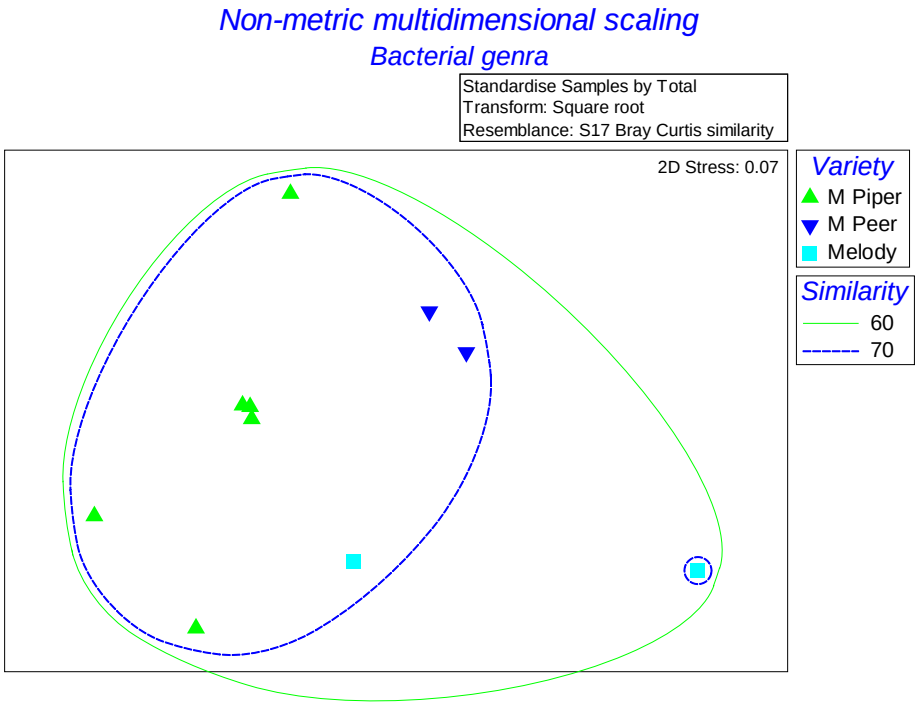
Figure 36. Expt 19: Relative abundance of bacterial genera associated with the variety Melody.



4.3.1.6.4. Multivariate analysis

NMDS (nonmetric-Multi Dimensional Scaling) was used to visualize similarities between potato associated bacterial communities at genus level. From the plot of nMDS (Figure 37), bacterial communities appeared very similar across different samples and no separation was observed among samples with similarity level as high as 60, which suggest overall community composition among samples was very similar.

Figure 37. nMDS plot based on Bray-Curtis similarities of relative abundances of bacterial genera of different potato varieties. Data from Expts 19 and 20.



Factors which could potentially have influenced community composition in this experiment include, SMD, variety, sampling location, scab level and scab severity. To reveal the significance of correlations between these experimental factors and the bacterial community composition, analysis of similarities (ANOSIM) was carried out (Table 48).

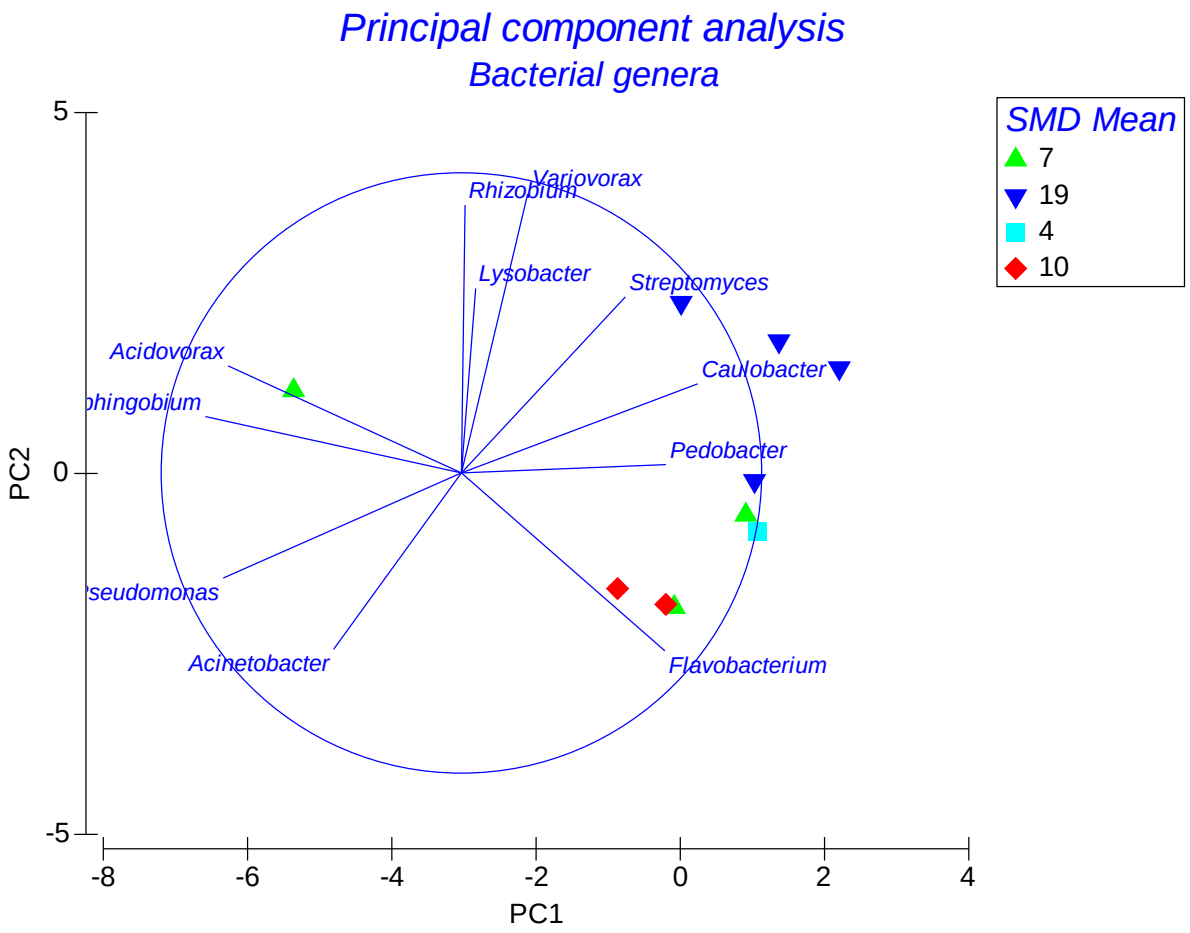
Table 48. Results of ANOSIM relating bacterial composition and different experimental factors

Factor	R	P
Variety	0.622	0.6 %
Location	-0.206	81.7 %
SMD (mean)	0.411	3.1 %
Scab level	0.269	10.9 %
Scab severity	0.395	1.8 %

From Table 48, no significant correlation was observed between community composition and several experimental factors: location, scab level and scab severity. However correlation was observed between the community profile and the SMD data (R=0.411, P=3.1 %) and a strong correlation was observed between community structure and different potato varieties (R=0.622, P=0.6 %), which suggests different potato varieties are responsible for influencing up bacterial communities on the potato peel or in the soil.

From the PCA biplot (Figure 38), it can be observed that bacterial genera *Streptomyces, Caulobacter, Lysobacter* were positively correlated with higher SMD (drier soil), whereas the genera *Pseudomonas, Acinetobacter, Sphingobium, and Acidovorax* were negatively correlated with higher SMD. The bacterial genus *Flavobacterium* was found to be positively correlated with the variety Maris Peer at medium SMD.

Figure 38. Ordination biplots generated by principal component analysis (PCA) by plotting principal component 1 against principal component 2 of bacterial communities based on the genus level in different potato varieties having different SMD. Data taken from Expts 19-22.



4.3.1.6.5. Fungal community analysis

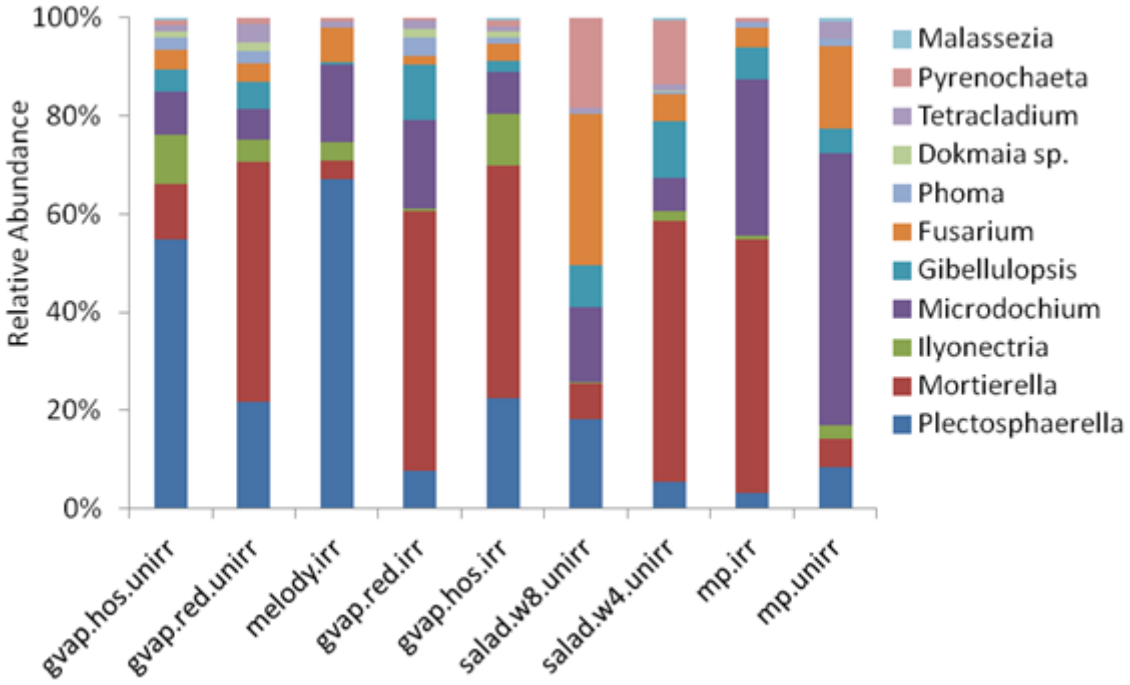
Total number of sequences obtained for ITS analysis is described in Table 49. A considerable amount of sequences were identified as originating from potato itself and were discarded prior to the analysis.

Table 49. Number of sequences per sample in Expts 19-22

Sample	Sequences
gvap.hos.unirr	1527
gvap.red.unirr	625
melody.irr	671
gvap.red.irr	1430
gvap.hos.irr	287
salad.w8.unirr	591
salad.w4.unirr	935
melody.unirr	945
mp.irr	483
mp.unirr	412

Comparing relative abundance of fungal community at phylum level revealed *Ascomycota* (28-88%) as the most abundant phylum across all the samples with *Sordariomycetes* being the most abundant class. The next most abundant phylum was *Zygomycota* (2-43%) with *Mortierella* being the only fungal genus represented within it. Relative abundance of different fungal genera revealed *Plectosphaerella* (2-57%)*, Mortierella* (2-43%)*, Microdochium* (4-26%) were most abundant among samples with sample-to-sample variability (Figure 39). It is reported that *Mortierella* was previously isolated from the potato soil and identified as growth inhibitor of *Streptomyces turgidiscabiei* (Tagawa *et al.* 2010). Other fungal genera, *Fusarium*, *Plectosphaerella,* were reported to be associated with common scab and atypical blemishes of potato tuber (Fiers *et al*. 2010).

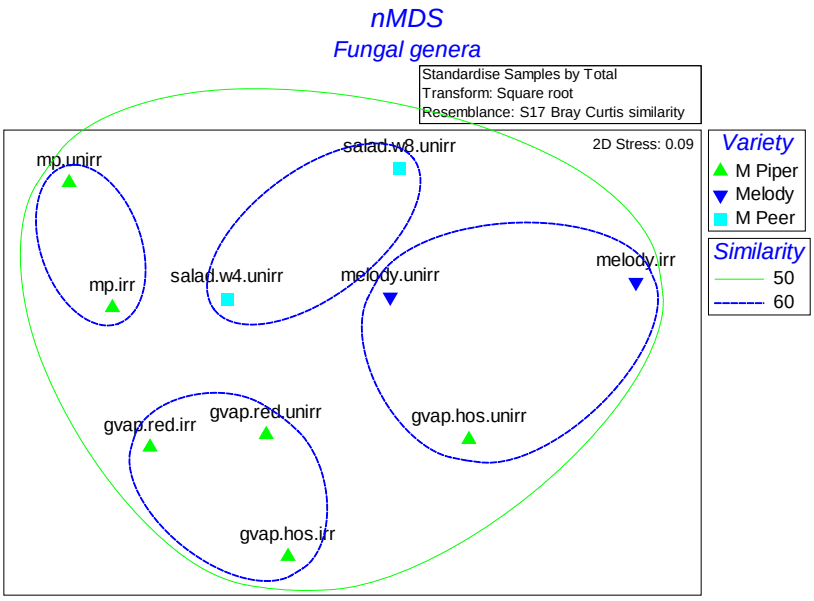
Figure 39. Relative abundance of fungal genera across different samples from Expts 19-22. Potato varieties and locations are indicated as follows: Maris Piper from CUF (mp), Maris Piper from GVAP Hales Hospital (gvap.hos) and Maris Piper from GVAP Redhouse West (gvap.red). Irrigation regimes were either irrigated (irr) or unirrigated (unirr).



4.3.1.6.6. Multivariate analysis

From the nMDS (non-matric multidimensional scaling), it was evident that fungal communities were similar at similarity level 50 across different samples (Figure 40). At similarity level 60 fungal communities of variety Maris Piper from CUF were clustered together, however the same variety (Maris Piper) from GVAP Redhouse formed a different cluster along with the irrigated sample collected from GVAP Hospital. Fungal community of the Maris Peer salad potatoes formed an independent cluster, but communities from the variety Melody and unirrigated samples of GVAP Hospital were grouped together. So at this point it is difficult to make any conclusion of the samples clustering based on the fungal community composition among varieties, location or treatment level.

Figure 40. nMDS plot based on Bray-Curtis similarities of square root transformed of relative abundances data of fungal genera of different potato varieties collected from different locations in Expt 19-22. Potato varieties and locations are indicated as follows: Maris Piper from CUF (mp), Maris Piper from GVAP Hales Hospital (gvap.hos) and Maris Piper from GVAP Redhouse West (gvap.red). Irrigation regimes were either irrigated (irr) or unirrigated (unirr).



To identify any correlations that may have existed between fungal community and the experimental factors (including SMD, variety, sampling location, scab level and scab severity), data were subjected to ANOSIM test. An additional experimental variable (thaxtomin gene level, *txtA* was included in the fungal community analysis to explore any effect it might have on shaping up fungal community among samples. A significant correlation was only observed between fungal community and the experimental factor scab level (R=0.457, P= 1.7 %; Table 50).

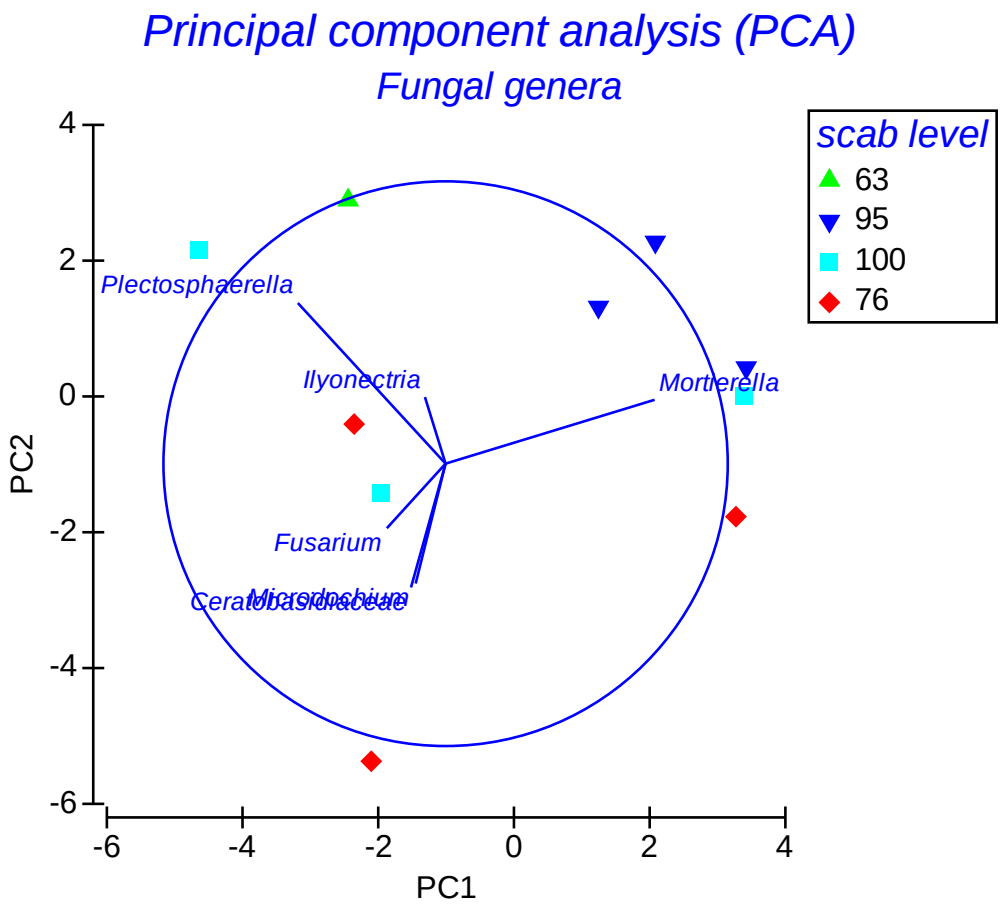
Table 50. Results of ANOSIM relating fungal composition and different experimental factors

Factor	R	P
Variety	0.298	7.3 %
Location	0.163	20.6 %
SMD (mean)	-0.145	73 %
Scab level	0.457	1.7 %
Scab severity	-0.049	58.9 %
	-0.01	52.3%
TxtA		

In order to identify the effects of different experimental factors on fungal community, a PCA was done on fungal genera data. Analysis revealed fungal genera

Plectospharella, Fusarium and Mortierella were correlated with higher scab level, whereas fungal genera *Ilyonectria, Microdochium, and Ceratobasidium* were found to be correlated with low scab level (Figure 41).

Figure 41. Ordination Biplots generated by principal component analysis of fungal community based on the genus level. Data from Expts 19-22.



4.3.1.7. *Tuber cracking, internal rust spot and hollow heart*

4.3.1.7.1. Expt 19

There was almost no superficial cracking but the incidence of linear cracking was increased significantly in frequently irrigated (0 SMD) treatments, particularly in Melody, Orchestra and Safari, which were worse affected than Maris Piper (Table 51). There was almost no internal rust spot and the only variety to show significant symptoms was Maris Piper. Unirrigated plots of Maris Piper had a greater incidence of rust spot (13 ± 1.40 % incidence) than irrigated plots (5 %). Safari was the only

variety to exhibit significant symptoms of hollow heart and the incidence in 0 SMD, 15 SMD and TI 1-4 treatments was greater (5.6 ± 0.85 %) than in Unirr or 35 SMD (0.4 %).

4.3.1.7.2. Expt 20

There was no cracking observed in Expt 20.

4.3.1.7.3. Expts 21 and 22

The incidence of linear cracking was very low and there was no difference between the two irrigation regimes in Expt 21 (mean 1.33 ± 1.135 %) or in Expt 22 (mean 0.33 ± 0.365 %).

Table 51. Expt 19: Tuber cracking incidence

		Linear cracking	
Variety	Irrigation regime	%	Ang. trans.†
Jelly	Unirr	5.3	10.9
	0 SMD	4.7	12.4
	15 SMD	4.7	10.2
	35 SMD	3.3	8.2
	TI 1-4	4.7	12.0
Lanorma	Unirr	5.8	13.4
	0 SMD	11.0	15.5
	15 SMD	2.8	7.9
	35 SMD	6.0	11.2
	TI 1-4	9.3	17.5
Maris Piper	Unirr	0.0	0.0
	0 SMD	10.0	17.8
	15 SMD	3.3	10.4
	35 SMD	1.3	3.9
	TI 1-4	2.0	8.1
Melody	Unirr	2.7	7.4
	0 SMD	44.0	41.5
	15 SMD	11.4	19.3
	35 SMD	0.0	0.0
	TI 1-4	5.4	11.0
Nectar	Unirr	4.7	12.4
	0 SMD	14.0	21.8
	15 SMD	6.0	14.1
	35 SMD	7.3	14.9
	TI 1-4	11.3	19.2
Orchestra	Unirr	0.0	0.0
	0 SMD	22.7	27.9
	15 SMD	2.7	7.7
	35 SMD	7.3	14.8
	TI 1-4	10.0	18.2
Safari	Unirr	3.7	8.8
	0 SMD	52.2	46.3
	15 SMD	14.8	18.2
	35 SMD	2.5	7.4
	TI 1-4	7.4	15.7
Volare	Unirr	1.3	3.9
	0 SMD	6.7	14.7
	15 SMD	3.3	8.6
	35 SMD	2.0	6.6
	TI 1-4	3.3	8.2
S.E. (78 D.F.)		-	4.08

†Angularly transformed data for statistical analysis

4.3.1.8. *Number of tubers and tuber yield*

4.3.1.8.1. Expt 19

In Expt 19, the number of tubers > 10 mm was increased by maintaining a 0 mm SMD throughout the 4 weeks following TI compared with a 15 mm SMD or a shorter period at field capacity (Table 52), and this resulted from an increase in the number of tubers/stem. The mean number of tubers/stem in the 0 SMD treatment was 5.69 ± 0.129 compared with the mean of all other irrigation treatments of 4.66, an increase of 22% across varieties, and therefore similar to the magnitude observed in Expt 12 in 2012. The increase resulted from significantly more tubers in the 30-40 and 40-50 mm size grades compared with other irrigated treatments rather than a bigger proportion of tubers < 30 mm. This repeats the findings of 2009-2012 that very wet soils at TI can produce increased numbers of viable tubers although previous work at CUF prior to this period found little effect of irrigation on tuber populations. The observation that the tuber population was increased in the 0 SMD but not the TI 1-4 treatment indicated that the effect resulted from conditions in the first week after TI which precedes initial application of irrigation in some experiments.

Total and ware (> 40 mm) fresh weight yields and dry weight yields were very large, particularly in varieties with very low tuber [DM], and the irrigated yields in Jelly, Nectar and Volare were the largest ever recorded in experiments at CUF (Table 52). When comparing the irrigation response across varieties, the variety with the lowest unirrigated yield as a proportion of the maximum yield in irrigated plots was Volare (33 %) since this variety lost canopy cover very quickly once the SMD increased above 50 mm. Most other varieties produced 55-60 % of the fully-irrigated yield without irrigation, the exception being Safari which had a significantly greater proportional unirrigated : irrigated yield (65 %). Compared with 0 SMD or 15 SMD irrigation treatments, the restricted 35 SMD treatment caused little reduction in FW yield in Jelly, Lanorma, Nectar and Orchestra but FW yields in Maris Piper, Melody, Safari and Volare were significantly reduced by allowing the soils to dry out to an SMD of 35 mm (Table 52).

Table 52. Expt 19: Number of tubers, yield and dry matter concentration at final harvest

Variety	Irrigation regime	Total no. of tubers (000/ha)	Total yield (t/ha)	Yield > 40 mm (t/ha)	Tuber [DM] (%)	Tuber DM yield (t/ha)
Jelly	Unirr	343	61.6	60.0	22.0	13.5
	0 SMD	498	103.9	101.6	19.7	20.3
	15 SMD	414	110.2	108.1	19.5	21.5
	35 SMD	450	110.9	108.8	18.9	20.9
	TI 1-4	431	95.1	93.2	20.7	19.7
Lanorma	Unirr	268	56.9	55.9	21.5	12.2
	0 SMD	401	93.6	91.9	19.7	18.2
	15 SMD	373	97.5	95.8	18.5	18.0
	35 SMD	306	97.3	96.6	20.4	19.9
	TI 1-4	387	90.3	88.7	19.8	17.6
Maris Piper	Unirr	430	52.0	49.4	23.3	12.1
	0 SMD	589	90.5	88.0	22.6	20.5
	15 SMD	544	90.8	88.7	22.9	20.9
	35 SMD	475	81.4	79.1	21.2	17.2
	TI 1-4	538	97.9	95.9	22.8	22.3
Melody	Unirr	442	54.7	52.2	21.0	11.4
	0 SMD	580	90.3	87.0	18.9	17.0
	15 SMD	503	100.6	95.6	19.0	19.0
	35 SMD	411	81.6	80.1	19.8	16.2
	TI 1-4	468	99.0	97.3	19.1	19.0
Nectar	Unirr	558	60.1	55.3	21.7	13.0
	0 SMD	754	107.9	102.0	18.7	20.0
	15 SMD	665	109.0	104.8	19.1	20.8
	35 SMD	672	100.3	94.8	19.3	19.4
	TI 1-4	624	104.4	100.0	19.7	20.4
Orchestra	Unirr	487	49.3	45.3	18.0	8.9
	0 SMD	591	86.0	82.6	16.7	14.4
	15 SMD	536	80.8	78.1	17.2	13.8
	35 SMD	518	85.6	83.2	15.8	13.7
	TI 1-4	512	92.0	89.5	15.1	13.9
Safari	Unirr	265	61.5	60.2	20.2	12.4
	0 SMD	322	89.5	88.7	20.5	18.3
	15 SMD	236	100.0	99.1	19.4	19.3
	35 SMD	239	75.1	74.2	20.9	15.7
	TI 1-4	229	92.3	91.8	20.6	19.0
Volare	Unirr	535	41.5	37.7	16.7	6.9
	0 SMD	736	125.2	121.4	14.7	18.4
	15 SMD	658	110.8	107.4	15.2	16.7
	35 SMD	612	96.0	93.4	15.6	14.9
	TI 1-4	595	123.6	120.3	15.1	18.7
S.E. (78 D.F.)		43.84	6.59	6.67	0.65	1.21

Tuber dry matter concentration ([DM]) was greater where crops were unirrigated than where irrigated but irrigation regimes were not significantly different and, on average, the difference between irrigated and unirrigated [DM] was only 1.6 % (Table 52). Volare had an exceptionally low tuber [DM] at final harvest (mean 15.5 %) with Maris Piper having the highest [DM] (22.6 %). Tuber dry matter (DM) yield followed a slightly different pattern to fresh weight (FW) yield since there were large differences in tuber [DM]. The largest tuber DM yields were obtained in Jelly, Maris Piper and Nectar (Table 52).

4.3.1.8.2. Expt 20

Yields were reduced by ceasing irrigation sooner after the end of the scab control period (Table 53). The number of tubers was unaffected by the duration of irrigation.

Table 53. Expt 20: Number of tubers, yield and dry matter concentration at final harvest

Variety	Duration	Total no. tubers (000/ha)	Total yield (t/ha)	Tuber [DM] (%)
Maris Peer	4 wks	805	27.3	22.8
	6 wks	917	34.5	21.6
	8 wks	1022	34.0	22.0
Regina	4 wks	1060	26.1	20.9
	6 wks	1295	30.6	21.9
	8 wks	1204	35.2	20.2
Venezia	4 wks	1156	31.3	20.1
	6 wks	1134	34.0	20.3
	8 wks	1226	38.2	20.4
S.E. (16 D.F.)		79.6	1.46	0.59
	4 wks	1007	28.2	21.2
	6 wks	1115	33.0	21.3
	8 wks	1151	35.8	20.9
S.E. (16 D.F.)		45.9	0.84	0.34

4.3.1.8.3. Expts 21 and 22

There was no significant difference in total yield (55.2 ± 0.92 t/ha), number of tubers (382 000 ± 17 700 /ha) or tuber [DM] (23.7 ± 0.19 %) between Dry and Wet treatments in Expt 21. In Expt 22, which was allowed to grow for longer before desiccation, total

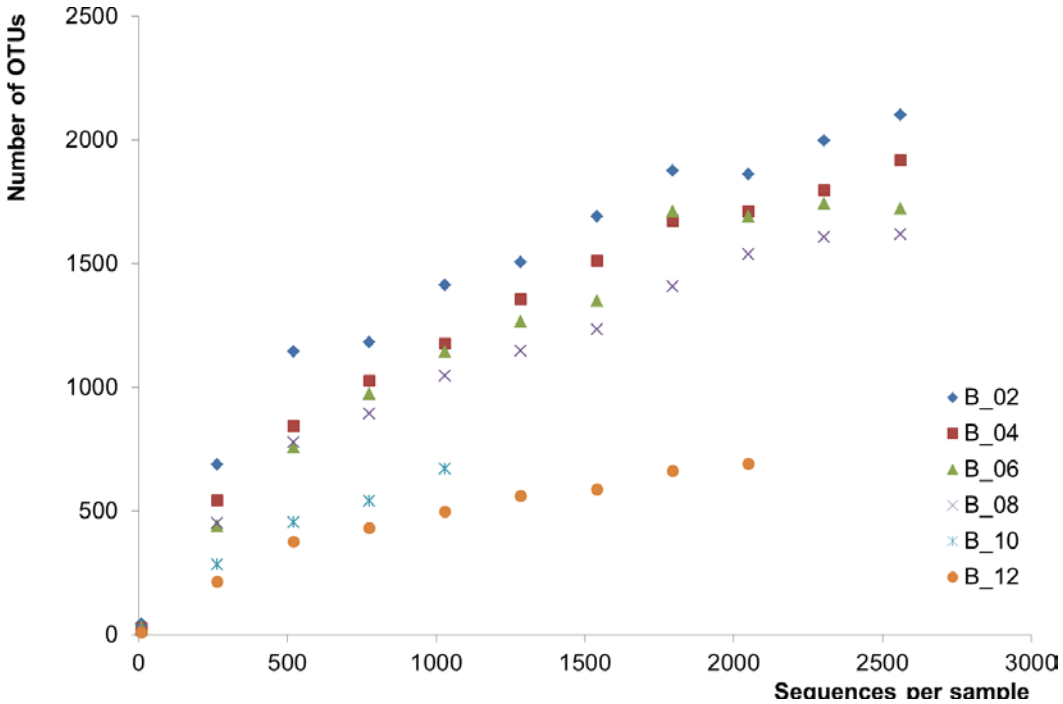
yield (71.7 ± 1.86 t/ha), number of tubers (393 000 ± 19 300 /ha) and tuber [DM] (23.1 ± 0.33 %) were again similar between Dry and Wet treatments.

4.3.2. Fera Experiments

4.3.2.1.1. Expt 23

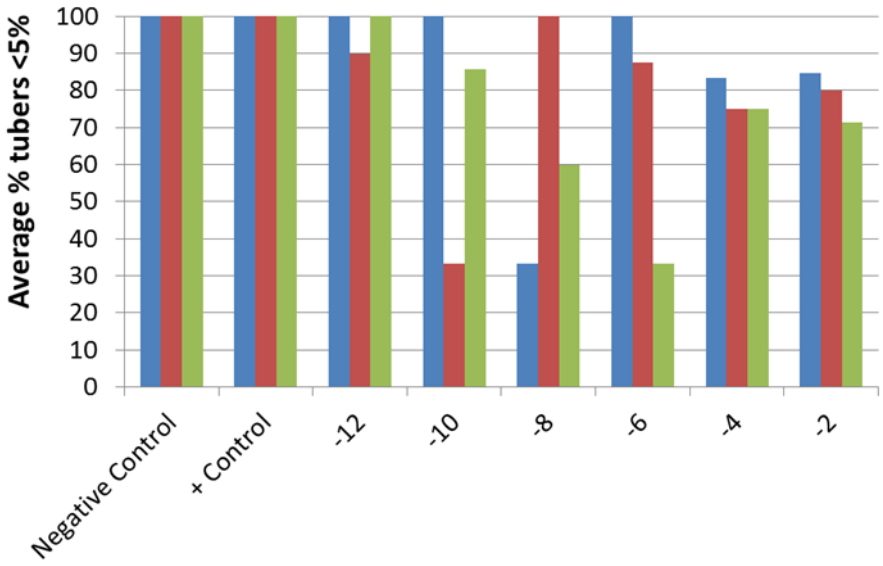
Figure 42 presents the number of operational taxonomic units (OTUs), which are clusters of similar sequences, isolated from the CUF soil.

Figure 42. Alpha-diversity of soil samples taken prior to tuber planting of replicate B including dilutions from 10^{-02} (B_02) to dilution 10^{-12} (B_12). Controls are not shown since no bacteria could be amplified from these DNA extracts.



Furthermore, the scab incidence was measured as described previously. Interestingly, positive controls and highly diluted samples showed hardly any scab symptoms and high variability between replicates was observed (Figure 43).

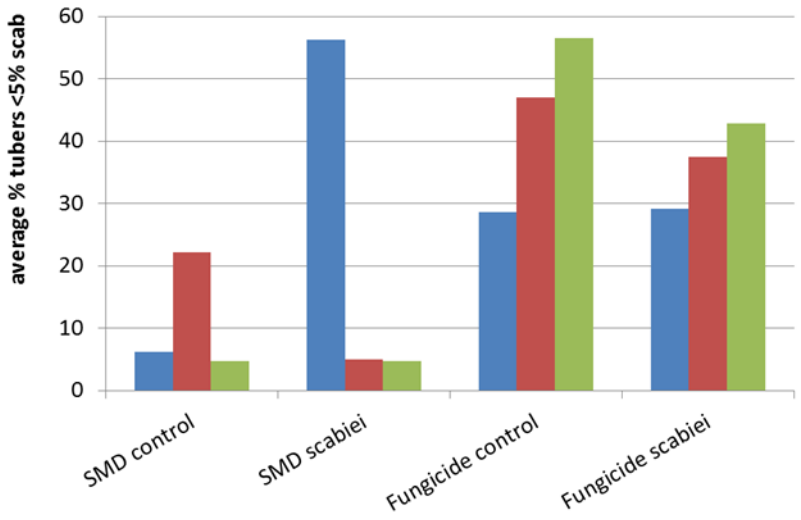
Figure 43. Percentage of harvested tubers showing less than 5 % surface area coverage of scab symptoms from the diversity amendment pot experiment established using CUF soil in Expt 23. The values on the x-axis represent dilution factors (x 10^{-12} etc.). Bars of same colour are same replicate.



4.3.2.1.2. Expt 25

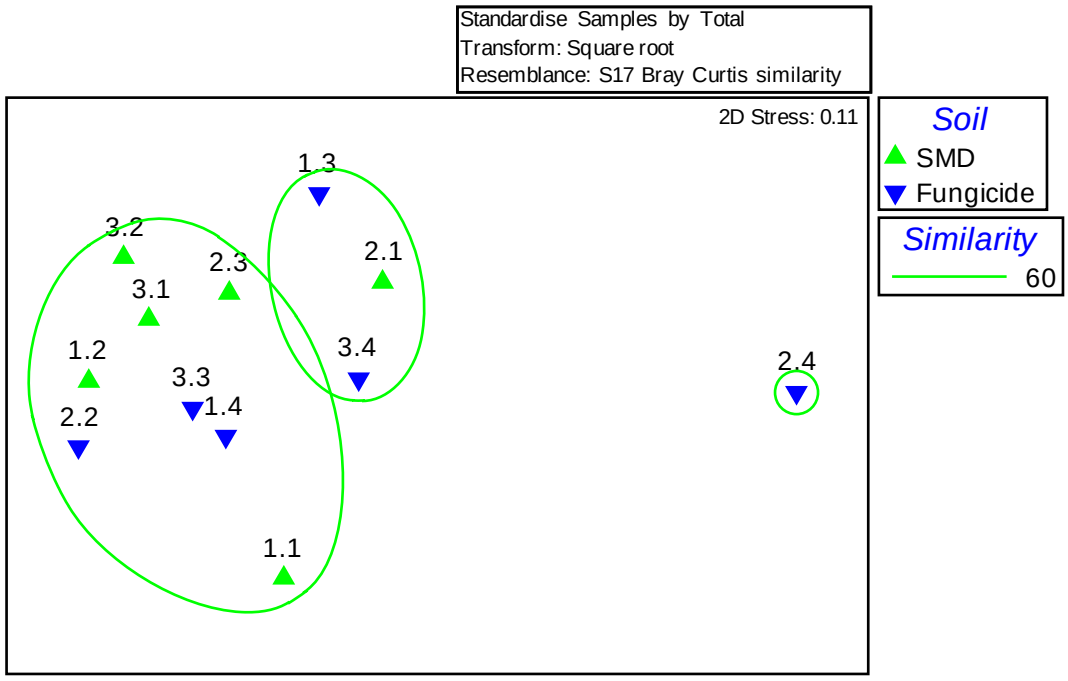
There was a higher incidence of common scab in the soil from the area of the field which had produced very low incidence of common scab in 2008 than in the soil from Expt 19 (where historically common scab has been more severe). The treatments receiving *S. scabiei* cells did not result in higher severity of common scab except for one replicate in the soils from Expt 19. However, two replicates of the fungicide treated control mesocosms displayed higher scab severity (Figure 44). Regarding the composition of the bacterial community, an effect by the addition of this bacterium was confirmed when the bacterial community profiles were compared (ANOSIM: R= 0.281, p=0.006).

Figure 44. Expt 25: Percentage of harvested tubers showing less than 5 % surface area coverage of scab symptoms from the mesocosm experiment established using CUF soil types (Expt 19, SMD), "Fungicide" reflects CUF soil from the sandy area of the soil fungicide experiment in 2008. Bars of same colour are same replicate.



No statistically significant difference was observed for bacterial communities associated with potato peel grown in the different soils (Figure 45).

Figure 45. Expt 25: nMDS plot based on Bray-Curtis similarities of square root transformed of relative abundances data of bacterial species associated with Maris Piper peel from soil from Expt 19 (SMD) or from the area where the soil fungicide experiment was located (fungicide).



5. DISCUSSION AND CONCLUSIONS

5.1. Scab incidence and severity

Typically, the most effective irrigation regimes in the very susceptible variety Maris Piper at CUF in experiments in 1992-2010 resulted in *c.* 2-3% SA infected, whilst unirrigated crops ranged from 6-28% SA infected. In experiments at CUF, common scab was much less severe in this project (2011-13) compared with 2007-2010, particularly in unirrigated crops.

In the first year of the project (2011), soils at CUF were very dry following planting in April and a subsequent dry May but there was 50-60 mm of rainfall during the 4 weeks after TI. However, soil moisture monitoring equipment installed within ridges indicated that rainfall did not penetrate to 15 cm in unirrigated plots during the control period. The average SMD during the first 2 weeks of the susceptible period for scab in the unirrigated crops was *c*. 21 mm, which would normally be expected to produce Maris Piper tubers with more severe common scab than the observed 4-8% SA.

In contrast to 2011, at CUF in 2012 soils returned to field capacity 10 days after emergence and remained at field capacity until TI. Soils only dried out to an SMD of 25 mm in the unirrigated plots during the third week after TI and this could explain the lower severity of scab in unwatered plots compared with 2011. Soil moisture monitoring equipment indicated that soil water content remained above 25% (0.25 m^3/m^3) in unirrigated plots during the susceptible phase which would be regarded as more than adequate to avoid significant common scab in most seasons.

At CUF in 2013, unirrigated plots of Maris Piper showed that this variety was much more susceptible to scab than the other eight varieties tested. There was no difference in scab control in these less sensitive varieties across a range of irrigation treatments (0 SMD, 15 SMD, TI 1-4 and even the restricted 35 SMD treatment). The findings from 2011-2013 give greater confidence in being able to schedule varieties differently to Maris Piper in terms of the maximum permissible SMD during the scab control period. With Bute, Jelly, Lanorma, Regina, Vales Sovereign and Volare, in particular, higher SMDs (up to 35 mm) could be allowed before scab becomes a significant issue in terms of packout.

5.2. Irrigation timing

Since it has been consistently observed in experiments at CUF prior to and during this project that pathogenic *Streptomyces* generally do not populate the periderm of tubers in great numbers until 2 weeks after TI, it is likely that the crucial phase for wetting soils is around 1-2 weeks after TI rather than at TI. One of the hypotheses to test was to only start applying irrigation 1 week after TI to effectively match the population dynamics of pathogenic *Streptomyces*. However, in Maris Piper delaying irrigation until 1 week after TI resulted in poorer control of scab than starting at TI, irrespective of the SMD (0 or 15 mm) maintained during weeks 2-4 of the susceptible period. In all other maincrop varieties tested in the program, the delayed start irrigation regime produced similar control of scab to starting at TI.

Testing the hypothesis of whether salad crops remain susceptible to common scab infection until 6 or 8 weeks after TI showed that more resistant varieties such as Regina may be able to be irrigated for a shorter period (e.g. 4 weeks) than currently recommended but even in Maris Peer and Venezia, a 6-week irrigation duration was equally effective as an 8-week period, meaning that current recommendation may be maintaining soils wet for too long, increasing the risk of over-watering, wastage of water and delaying skinset post desiccation.

5.3. *Streptomyces* population dynamics

Despite the variable patterns of rainfall between seasons and the lack of irrigation effects on common scab, the timecourse and differences in populations of pathogenic *Streptomyces* between very wet and unirrigated soils match those found in previous work (particularly the lag period after TI when the populations begin to increase) and these need to be related to antagonist populations to better understand the method of disease control. The difficulty experienced with quantification of *Streptomyces* populations by real time PCR in both field and pot experiments, due to apparent inhibition in soils in 2011, is interesting. Populations of *txtA Streptomyces* have only previously been undetectable in soils maintained in a very wet status, not in soils kept drier. It is not clear why such inhibition occurred but it may have been that the very dry conditions within the ridge between planting and emergence at CUF resulted in

low populations of *Streptomyces* at TI. The lack of detectable pathogenic *Streptomyces* in 2011 was, however, consistent with the very low severity of common scab observed in both field and pot soils.

There was no evidence in any of the controlled environment pot experiments at Fera that soil moisture treatments affected common scab. This was supported by quantitative PCR data, similar to the findings in field experiments at CUF where little scab developed. In pot trials using soil containing native microflora (i.e. unsterilized), the similarity in scab severities between irrigation treatments may indicate that the soil moisture levels achieved did not contrast sufficiently between treatments. In the trial using sterile soil inoculated with *S. turgidiscabies*, scab severity did not appear to be dependent on moisture level, although experimental variation was too great to draw conclusions.

5.4. Ridge soil structure

Excessively cloddy ridges have often been implicated in poor control of common scab with irrigation but the results from different declodding aggressiveness treatments in commercial fields in 2012 did not show any benefit in reducing scab by producing a very fine tilth soil compared with a larger mean ped size. This suggests that soils are being excessively cultivated with little benefit to control of scab, although the low severity of scab in 2012 did not provide a conclusive test of this. The fineness of soil within the ridge at CUF in 2011 also had no effect on common scab even where soils were unirrigated and irrigation regime was far more important in controlling the severity of the disease than the ped size distribution in the ridge. Further work in the Potato Council Soil Cultivation Project R459 has also shown no difference in scab between fine and moderately cloddy ridges (produced by more or less aggressive destoning, respectively).

5.5. Additional observations

The very large increase in the number of retained tubers in all varieties in irrigation regimes that maintained soils at or above field capacity during the 3-4 weeks after TI is very interesting. Analysis of a considerable body of data from CUF (*c*. 80+

experiments) has previously indicated that conventional irrigation for scab control was not associated with an increase in the number of tubers (Firman *et al.* 2007). More recent work has concentrated on maintaining some treatments at, or above, field capacity with daily or bi-daily irrigation rather than a small SMD and the magnitude of the effects have been consistent. The effects on grading may be very important when the increases in number of tubers are *c*. 20 % and tubers grow sufficiently large to be marketable but in all three maincrop experiments (Expts 1, 12 and 19), more tubers were produced in the 30-50 mm size fraction in 0 SMD *c.f.* other irrigation regimes. In salad crops, there were more tubers produced in all grades < 50 mm in crops kept very wet during the scab control period rather than an increase in tubers < 25 mm. It is important to recognize that the effect only occurred when soils were kept very wet from TI rather than 1 week later, as this indicates that the trigger for increased production of tubers occurs at, or very soon after, TI.

Tuber cracking was increased where soils were maintained at or above field capacity and the effect was increased with duration of the treatment, particularly for superficial cracking. Linear cracking tended to be worse when watering in excess of field capacity began at the start of TI. In susceptible varieties, slight adjustments in irrigation scheduling (e.g. 15 mm SMD for scab control) can reduce the proportion of outgrades caused by cracking without compromising control of common scab or total yield. The magnitude of the effects of over-watering on cracking was dependent on variety. Over the course of the project, no cracking was observed in Desiree or Saturna or the salad varieties Venezia and Regina and only limited cracking occurred in Flair and Jelly. However, Bute, Maris Piper, Melody, Orchestra, Safari, Sylvana and Vales Sovereign proved very susceptible to linear and/or superficial cracking with early over-watering.

Early season over-watering was very detrimental to yield in Expt 13 (2012) but there was no negative effect of over-watering in August (10-13 weeks after TI) compared with scheduling irrigation at a maximum SMD of 25 mm and even a slight positive effect on yield occurred in 2012. Overall crop N uptake was improved by avoiding over-watering in the 3 weeks after TI, possibly as a consequence of avoiding N leaching below rooting depth and denitrification. The amount of soil N was reduced to very low amounts following the early over-watering period. Late over-watering in August increased maximum N uptake, prolonged canopy duration and produced

higher yields in Maris Piper and Markies in 2012 which could be explained by poor root growth early in the season which compromised late-season water and N uptake. Maintaining a 0 mm SMD during TI did not advance the date of achieving full canopy cover compared with a 15 mm SMD or delaying irrigation until 1 week after TI. However, in Maris Piper and particularly Vales Sovereign, keeping the SMD at 0 mm throughout the scab control period appeared to advance senescence compared with less frequent watering. Likewise, in Saturna early over-watering also advanced the onset and rate of senescence such that crops were dead around the same time as those which were droughted.

5.6. Microbial communities

The nMDS analysis showed that the bacterial communities of some of the soils used in experiments were similar in their composition but there was a large dissimilarity in the community makeup between other soils. It is not yet known what these data mean in terms of potential suppression of common scab.

Significant variation in bacterial community composition was observed between different locations and soil types and a weak correlation was apparent between community composition and the location of trials. A stronger correlation was observed between community composition and variety. Since DNA used for characterisation of communities was extracted from the tuber surface, communities analysed would have been in very close association with the potato tissue, and our data suggest that variety has a significant influence upon the microbial community present. The nature of any influence this microbial community exerts upon populations of pathogenic *Streptomyces* is unknown, although in our controlled environment experiments the soil microbial community was intrinsically involved in water-mediated control of common scab since, when the microbial community component was removed from the pathosystem, water level no longer influenced populations of *Streptomyces* or scab level.

Despite the low incidence and severity of common scab, the time course and differences in populations of pathogenic *Streptomyces* between wet and unirrigated soils match those found in previous work but the preliminary data on antagonist populations seem confusing when related to the method of disease control. There

appears to be only limited evidence that decreases in pathogenic *Streptomyces* are a consequence of alterations in one or more major genera in the bacterial or fungal populations. There is clearly a complicated relationship between the changes in antagonistic bacterial and fungal genera and pathogenic *Streptomyces* populations despite the large differences in pathogen populations observed between unirrigated and very wet irrigation regimes. However, the project successfully amended the previously used approach of bacterial community profiling from peel by using blocking primers specific to chloroplast sequences thus obtaining much better coverage of the actual bacterial community associated with potatoes.

It could be shown that different *Streptomyces* species were present in the samples of tuber peel analysed. However, no congruence between 16S rRNA gene phylogeny and pathogenicity on potato for *Streptomyces* spp. could be found but rather high distribution across *Streptomyces* diversity as shown in Bramwell *et al.* (1998). Specific regions in the 16S rRNA gene can be used to discriminate between the pathogenic streptomycetes (Wanner *et al.* 2006), but none of these were used for antagonist profiles suggesting that *Streptomyces* species-specific assays would be useful for future studies.

Bacterial communities associated with potato skin were found to be strongly linked to the specific variety they were residing on. A weaker, but significant, correlation with SMD suggests linkages to soil moisture. This indicates that not just the interaction of soil microorganisms with irrigation but also the crop itself plays a large role in shaping the microorganisms in its surroundings. This relationship requires more attention to identify specific antagonist-scab interactions.

After successful amendment of the intrinsic soil biodiversity, no direct link between common scab incidence and microbial diversity *per se* was observed. However, it needs to be noted that a dilution of 10-12 was not sufficient to remove the full diversity as anticipated which is likely due to the very high levels of diversity in the soil. Furthermore, it is assumed that the addition of *Streptomyces scabiei* cells did not result in high common scab incidence due to low soil moisture. Nevertheless scab symptoms occurred, most likely caused by native common scab causing organisms present in the inoculum which were most successful in lower dilutions. This in turn indicates the presence of antagonists of lower abundance in the higher diversity treatments.

A large pot experiment conducted by Fera at CUF using soil from two areas of the same field with documented histories of low and high common scab severity in the same season using the same seed produced interesting results that support previous work at CUF. They showed that the 'low scab' soil from 2008 had a higher scab incidence in 2013 than the 'high scab' soil from 2008, which indicates that soil microbial communities can rapidly alter in response to soil conditions.

5.7. Practical recommendations

A varietal scheduling group table has been produced for the varieties included in the project (Table 1). It shows the varieties grouped in sensitivity groups and the optimum irrigation scheduling regime (SMD) for different soil types. For example, on sand soils, irrigation in Maris Piper would be best scheduled with 10 mm doses whenever the SMD reached 10 mm in the top 25 cm of the ridge profile during the 4 weeks after TI, whereas larger doses (15-16 mm) could be applied less frequently on the most water-retentive silt loam soils, where the allowable SMD would be around 16 mm. As a contrast, the maincrop varieties in Group 3 (Insensitive) could be allowed to develop a 19 mm SMD on sands or 31 mm on silty soils, more suited to 20 mm and 25 mm applications, respectively. When irrigating with hosereels and rainguns, it is best to over-apply water slightly to ensure the ridge profile is re-wetted as thoroughly as possible. With boom and sprinkler irrigation, the irrigation amount can be more closely matched to the target SMD to avoid over-watering.

With the exception of Maris Piper, in all maincrop varieties tested in the program the delayed start irrigation regime produced similar control of scab to starting at TI, so this could be a practical recommendation for less susceptible varieties. Caution is needed, however, as soils need to be wetted thoroughly at 7 days after TI and this may be more difficult than at TI as ridges may have dried out, hindering wetting. Provided sufficient irrigation capacity was available to wet soils up quickly, varieties in Groups 2 (Intermediate) and 3 (Insensitive) could receive their first scab control irrigation 1 week after initial TI without reducing the level of control of scab.

A key part of successful control of common scab with irrigation is to ensure the susceptible period for the crop is as short as possible. This starts with uniform emergence which should lead to a short period of TI across the majority of plants in a

field. Emergence in most varieties in the experimental program was brief (e.g. 3-5 days from first to 50 % emergence) and in commercial fields is generally longer (e.g. 4-7 days). Most varieties in the project tuberized 15-20 days after emergence (initial or 50%) with only Bute and Nectar taking longer (24 days). Delayed-start irrigation timing should be based on initial TI (or emergence) as using the date of 50 % TI in variably-emerging fields could lead to more scab infection.

Tuber cracking was increased very considerably in some varieties where soils were maintained at or above field capacity for different period. Linear cracking tended to be worse when watering in excess of field capacity began at the start of TI whereas superficial cracking centred on lenticels was increased the longer the period(s) of excessive soil moisture during the season. A risk table has been formulated for the varieties included in the project (Table 54).

Table 54. Risk table for external tuber cracking resulting from over-watering. Varieties in High risk group are ranked according to risk

	Group	
1. High risk	**2. Moderate risk**	**3. Low risk**
Safari **Estima** **Vales Sovereign** **Melody** **Orchestra** **Nectar** **Maris Piper** **Lanorma** **Bute** **Sylvana**	**Flair** **Jelly** **King Edward** **Maris Peer**	**Desiree** **Regina** **Venezia**

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