Project title: Field-grown salads: quantifying the risk of pathogen contamination through irrigation water

Project number: FV 292a

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Report: Final report (31 October 2008)

Previous reports: Annual reports for FV292 (May 31st 2007 & April 30th 2008)

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Date commenced: 1st April 2008

Key words: Leafy vegetables, food safety, microbial contamination, irrigation

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Grower Summary

Headlines

- FV292 and FV292a have shown that even at the high level of contamination (10^5 CFU g^{-1}) the numbers of human pathogens remaining on the growing leaves of spinach and iceberg lettuce after one week of strong, bright sunshine was normally below the level that could be measured (<10 CFU g^{-1}); was only sporadically detected by the second week; and was not detectable from all leaf samples after three weeks.
- Consideration must be given to the time interval between irrigating and harvesting when using a water source that is vulnerable to contamination although ideal crop growing conditions are conducive to the rapid decline of pathogens that can cause foodborne illness i.e. strong sunlight, dry and warm.
- Where soil has been contaminated by irrigation water, soil splash may contaminate leafy salads. However, significant contamination will only occur with the droplet sizes at the upper end of irrigation droplet size distribution.
- Design and maintenance of irrigation systems to minimise droplet sizes <3.6 mm would minimise risks of soil splash through irrigation

Background and commercial objectives

There is increased focus on the microbiological risks associated with the consumption of ready to eat produce within the retail sector. These concerns are being driven by two main factors, namely increased government (i.e. FSA and EU) scrutiny of agricultural food production practices and the legislative implementation of process controls. In addition, retailer's protocols are becoming more and more stringent to minimise the risk of 'bad press' and damaged brand resulting from food poisoning which is traced to retailer-specific produce.

Previous HDC-funded work (FV248) established that almost three quarters of salad crops were irrigated through direct abstraction of surface water. Such water sources are most vulnerable to contamination with faecal pathogens from agricultural activities such as livestock farming. In addition, the majority of salad crops (60-85%) were irrigated with overhead booms – directly applying water to the leaf surfaces. Sampling of water sources demonstrated that irrigation water quality was variable, at times exceeding the WHO guideline for coliform bacteria (although it should be noted that the WHO guideline is for potable water). Nevertheless, the lack of data obtained from scientifically-sound studies which describes the real risk of pathogens entering the food chain from contaminated irrigation water has led to a situation where *any theoretical risk*, no matter how significant, has to be minimised. Consequently, growers

are now being encouraged into investing in water disinfection systems (UV, ozonation etc) which are expensive to buy and to operate, if anything other than a potable water source is used for fresh produce irrigation.

Project FV292 determined the lengths of time that zoonotic agents (e.g. *Salmonella*, *E. coli* O157 and *Campylobacter*) can survive in the soil (Year 1) and on the surface of the produce (Year 2) and the role of UV light (a component of sunlight) and temperature on the persistence of pathogens.

The following questions (each being an experimental objective) were addressed in this report:

1. What is the persistence on the surface of crops of pathogens introduced through irrigation at different times in a growing season.
2. Quantify the relative risk of contamination by soil splash from irrigation systems

Summary of the project and main conclusions

Field trials were carried out at Harper Adams University College, Shropshire, to study persistence of added pathogens on spinach and lettuce crops in mid summer (July 2008). This work complements FV292 where the mid summer planting was missed in 2007 due to heavy rain.

Treatments (FV292a)

- 3 levels of pathogen* zero, low (1×10^2 CFU [colony forming units] ml^{-1}) and high (1×10^5 CFU ml^{-1})
- 2 crops: spinach and lettuce

*Pathogens were applied as a composite mix of *Salmonella enterica* serotype Enteriditis, *Campylobacter jejuni* and *E. coli* O157 (a non-pathogenic, non-toxin producing strain) each at a similar concentration.

Irrigation treatments using contaminated water were applied only at the start of each experiment. Crop foliage was sampled weekly and tested for numbers of pathogens initially. When pathogen numbers fell below the detection limit of the quantitative testing methods, the presence of the inoculated pathogens was detected using enrichment-based testing methods.

What is the persistence on the surface of crops of pathogens introduced through irrigation at different times in a growing season (Objective 1)?

In contrast to soil persistence studied in 2006 in FV292, the persistence of pathogens on the surface of lettuce and spinach was much shorter. All three bacteria studied declined rapidly to absence in 14-21 days when using the relevant horizontal laboratory food testing methods described by the Organisation for International Standards. In 2008, the persistence of pathogens was similar to the early and late season treatments in 2007 (FV292). This was unexpected as it was anticipated that persistence of pathogens would be even less in hot dry mid summer UK conditions. However, the level of light overall was less in mid summer 2008 than late season 2007. This lower light level was particularly marked at the start of the mid summer 2008 experiment.

When the results are considered for FV292 and FV292a, over all three experiments, all three bacteria studied declined rapidly to absence in 14-21 days.

Summary Objective 1:

- Moderate to high levels of leaf contamination through irrigation water will pose little or no risk after a maximum of three weeks in an average UK growing environment.
- Care is needed to prevent irrigation of crops with contaminated water within 3 weeks of harvest.
- Guidance for growers should be derived from work undertaken in a UK growing environment – it can be misleading and inappropriate to extrapolate international studies to the UK growing environment.

What is the risk of contamination by soil splash from irrigation systems (objective 2)?

FV292 demonstrated that applying contaminated irrigation water to crops and soils introduces a risk to ready to eat produce grown in that soil. The persistence of the bacterial contamination in the soil was related to the level of initial contamination, with the high level of contamination persisting longer than the low level in all cases. However, pathogen persistence was much greater in the soil than on the surface of the crops. The soil may act as a reservoir of pathogen and may pose a risk of re-contamination of the crop before harvest, particularly through soil splash during an irrigation event.

The experiments on soil splash demonstrated that significant levels of soil borne contamination could be distributed over ranges that would allow leafy salads to be contaminated in the field. The main factor influencing the extent of contamination was

drop size. The study of splash using irrigation nozzles showed that those commercial sprinkler nozzles which generated relatively large droplets could pose a risk. Studying drops of defined dimensions (3.6 – 4.8 mm) demonstrated that it was only those drops found at the upper end of irrigation droplet size distribution that were capable of significant soil splash. Taken as a whole the work has demonstrated the potential for soil splash as a route for contamination and that the irrigation (and rain) droplet sizes that pose greatest risk are at the upper end of distributions experienced in the field. Any measures that reduce the droplet size to below 3.6 mm will markedly reduce the extent of soil splash and hence the risk of contamination of leafy salads from contaminated soil.

An interesting observation from the droplet size work was that the soil type influenced the extent of contamination with a peaty soil having higher soil splash. It may be that larger volumes of surface soil were dispersed with the peaty soil due to the looser structure associated with the higher sand content. Further work would be needed to establish the cause of this response.

Summary Objective 2:

- Where soil has been contaminated by irrigation water, soil splash may contaminate leafy salads. However, significant contamination will only occur with the droplet sizes at the upper end of irrigation droplet size distribution.
- Design and maintenance of irrigation systems to minimise droplet sizes <3.6 mm would minimise risks of soil splash through irrigation

Financial benefits

This work is not well suited to estimating financial benefit. However, knowledge that prevents food illness associated with leafy salads can save the sector a lot of money. The *E. coli* O157 outbreak in the USA associated with spinach led to 1/3rd of the entire US spinach crop being lost.

Action Points for growers

When the data from FV292a is considered along with that from FV292, a number of clear action points can be drawn from this work

- Minimise the numbers of contaminating bacteria in irrigation water, using a recognised risk assessment approach.
- Monitor microbiologically irrigation water quality at the point of application and trend results.
- Take particular care to use potable water within three weeks of harvest of leafy salad crops where water is applied directly on to the harvestable leaf.
- Maintain free draining surface soils through bed preparation to limit the accumulation of contaminated water near the soil surface.
- Minimise soil contamination of crops through choice of irrigation system.
- Manage irrigation systems to minimise droplet sizes <3.6 mm to minimise risks of soil splash through irrigation

Science Section

Introduction

Whilst there is a body of work on the persistence of faecal pathogens in soil incorporated manures and slurries in UK conditions (e.g. Hutchison et al. 2005), work to date on persistence of pathogens between irrigation and harvest has not been reported previously in the literature for UK (or European) growing conditions. FV292 has showed that in early and late season salad crops human pathogens introduced to soil at moderate and high levels through irrigation survived longer than an average cropping cycle, i.e. 5-6 weeks. In year 2, the same pathogens declined on the surface of Lettuce and Spinach so that they were no longer present at harvest. However, this conclusion is drawn from an incomplete data set.

Field work on leafy salads and herbs carried out in USA has demonstrated that pathogens such as *E. coli* O157:H7 (Islam et al., 2004a & 2005), *Salmonella enterica* Typhimurium (Islam et al. 2004b) can last for up to 231 days in the soil and 177 days on leaf surfaces. These reports are discussed at length in FV292 reports. However it is worth noting that these are worst case scenarios involving 'over wintered' crops and the direct relevance of these data to UK production systems is limited. The UK climate differs markedly from that in Georgia, USA and soil microflora, temperature and sunlight are known to influence degradation rates of these pathogens (Palacios et al., 2001; Brandl et al., 2004; Stine et al., 2005). It is important that a full data set is collected for pathogen decline on leaf surfaces and the mid summer experiment for year 2 needs to be completed to give robust data to support risk assessment decisions by UK growers.

A number of workers have studied the dispersal of plant pathogens such as botrytis by water droplets (e.g. Ntahimpera et al., 1999). However, to our knowledge, no studies have been reported on dispersal of human pathogens via this route. It is common sense that applying water to the leaf surface through overhead irrigation establishes a direct route for contamination. A few authors comment on the need to avoid soil splash to minimise the transfer of soil located faecal pathogens (e.g. Heaton & Jones, 2007) but do not support the statement with data to guide growers as to the size of droplets that may cause the greatest or least risk of dispersal and we have been unable to find any work in the literature that provides this data.

Overall aim of the project

To quantify the seasonal persistence of pathogens introduced to soil and produce surfaces directly and indirectly through contaminated irrigation water.

Specific objectives

- Establish the persistence on the surface of crops of pathogens introduced through irrigation in mid-summer. (Repeat of experiment 2, Year 2, FV292)

- Quantify the relative risk of contamination by soil splash from irrigation systems

Materials and Methods

Crop experiments

Treatments

- 1 experiment: mid summer (*identified as experiment 6 to follow FV292*)
- 2 crops: Spinach, Wholehead lettuce
- 3 levels of pathogen* zero, low (1×10^{2} CFU ml^{-1}) and high (1×10^{5} CFU ml^{-1})
- 3 replications for each treatment

*Pathogens were applied as a composite mix of *Salmonella*, *Campylobacter* and *E. coli* each at a similar concentration.

Irrigation treatments were applied using designated watering cans at one event at the start of the experiment (Experiment 6 – 09 July 2008). Leaf tissue was sampled for pathogen recovery initially and at weekly intervals; soil was sampled at the end of the experiment.

Plot preparation

The experiment s were carried out at Harper Adams University College, Shropshire in Birds Nest Field. The soil was a deep permeable sandy loam over loamy sand at 60 cm depth classified as Wick series (Beard, 1988). The trial area was under oats drilled the previous autumn. These were sprayed off and the land ploughed and power harrowed before a bed former was used. Iceberg lettuce transplants (*Lactuca sativa* cv Robinson) and spinach seed (*Spinacia oleracea* cv Toscane F1) were provided by PDM Ltd. Lettuce were transplanted by hand 1 week before treatments and spinach was drilled using a spider drill 10 days before treatments commenced. Plots were randomly distributed along a 1.4m wide bed. Each plot was 4 m long with a guard plot of 3 m between treatment plots. The lettuce and spinach beds were separated by 6 m of topped rye grass to prevent the risk of soil splash contaminating the adjacent crops. A sonic bird scarer was placed in the centre of the trial and cotton thread was suspended

across the plots to deter birds. Electrified rabbit fencing surrounded the experimental area.

Water for irrigation treatments was sourced from the irrigation lagoon filled from a borehole at Harper Adams University College.

Environmental measurements

A portable weather station (Mini-Met; Skye Instruments, Llandrindod Wells, UK) was installed in the buffer strip surrounding the field plots. Precipitation was continuously collected in a rain gauge (Skye Instruments). All other parameters were recorded every 10 minutes. Soil temperature was recorded at a depth of 5 cm. Air temperature and air humidity at 20cm above ground, daylight hours and the intensity of solar radiation were recorded for the duration of the experiments at 35 cm above ground. All data were saved on a DataHog 2 device (Skye Instruments) which was downloaded weekly.

Microorganisms used and their culture conditions

The human pathogens studied were a *Salmonella* Enteriditis, a *Campylobacter jejuni* and an *E. coli* O157 (which did not contain the genes for verocytoxin). The zoonotic agents used for these studies were all isolated originally from UK livestock. Because there are differences between human and livestock isolates of these pathogens, it is far more likely that agricultural environments would be contaminated with livestock rather than human isolates of pathogens. The organisms used were *Salmonella enterica* serotype Enteriditis (strain S8167/99), *Campylobacter jejuni* (strain 20001424) and a non-verotoxin-producing *Escherichia coli* O157 (strain 20001383). *E. coli* O157 and *Salmonella* were propagated in Buffered Peptone Water (Oxoid, Basingstoke, UK), *Campylobacter* was grown in modified Exeter Broth (mEB; Nutrient Broth (Oxoid) supplemented with 1% (v/v) water-lysed fresh horse blood, 250 mg l^{-1} of sodium metabisulphate, 250 mg l^{-1} of sodium pyruvate and 250 mg l^{-1} of ferrous sulphate). No media supplements were inhibitory to the bacteria used. Cultures were grown without agitation or aeration at 37°C (*E. coli* and *Salmonella*) or 42°C (*Campylobacter*). *Campylobacter* incubators were filled using a custom formulated mixture of 10% (v/v) carbon dioxide, 5% (v/v) oxygen, and 85% (v/v) nitrogen (British Oxygen Company, Guilford, UK). *Campylobacter* media was equilibrated in the modified atmosphere for 6 hours before use for bacterial propagation.

Plot inoculation

Cultures of bacterial pathogens were introduced into irrigation water sourced from a farm borehole typical of that used by commercial salad growers in the UK. Bacteria

were distributed through the water by gentle agitation taking care not to excessively oxygenate the liquid. The pathogens were applied at levels commonly found in the environment rather than artificially high 'spiked' levels. Initial levels of each individual bacterial pathogen in the contaminated waters were either 1×10^5 CFU ml^{-1} (high application) or 1×10^2 CFU ml^{-1} (low application). Negative control plots were watered with borehole water which did not contain any zoonotic agents. The mass of water used to irrigate each 5.6 m^2 field plot was 28 litres applied using a 5 litre watering can to give the equivalent of 5 mm overhead irrigation. The contaminated water was applied as a single treatment at the beginning of each experiment. All subsequent irrigation of all plots was according to standard commercial practices using fresh borehole water that did not contain pathogens through solid set irrigation. After each irrigation event, the water was left undisturbed to soak into the soil. Three replicate field plots were generated for each treatment and control. Declines in the numbers of each of the zoonotic agents were followed over a 6 week period or until no zoonotic agents could be detected.

Sample collection from field plots and transit to the laboratory

Samples for analysis were collected from each replicated field plot each week over a 6 week period or until no zoonotic agents could be detected. Each sample comprised a minimum of 10g of leaf material collected across the plot and collected using sanitised metal scissors. Soil samples were taken the week after no zoonotic agents were detected on the crops. Each sample comprised a minimum of 25 combined sub-samples taken from diverse areas of the plot and collected to a depth of 5cm using sanitised metal spoons. Soil and crop samples were kept cool (<15°C) for transport from the farm site to the laboratory. All microbiological testing commenced within 4h of sample collection.

Microbiological testing methods for leaf samples

Bacteria were initially enumerated from all of the samples taken. After bacterial numbers declined below the threshold for reliable enumeration, a switch to simple presence/absence detection using enrichment was made.

Enumeration of *Campylobacter* was by suspending 10g of sample in 10 volumes of mEB which had been pre-warmed to 42°C and pre-equilibrated in an atmosphere containing 10% CO_2. Decimal dilutions of suspended sample were undertaken in mEB before plating onto dried modified charcoal cefoperazone desoxycholate agar (mCCDA, Oxoid). For determination of the presence of *Campylobacter*, enrichment for 24h at 42°C in Exeter broth was undertaken before plating onto mCCDA. Campylobacters were incubated under microaerophilic conditions at 42°C. Confirmation of presumptive campylobacters for both tests was by corkscrew motility after microscopic examination of a loopful of bacteria in MRD and positive testing for

Oxidase activity.

Numbers of *E. coli* O157 were determined by suspending either 10g (field experiments) or 1g (laboratory drainage experiments) of sample in Modified Tryptone Soya Broth (mTSB, Oxoid) undertaking decimal dilutions in mTSB and plating onto Modified Sorbitol MacConkey Agar (CT SMAC, Oxoid), supplemented with 2.5 μg ml^{-1} potassium tellurite and 0.2 μg ml^{-1}cefixamine). Presence of *E. coli* O157 was by enrichment in mTSB for 48h at 42°C. Confirmation of presumptive *E. coli* O157 for field samples was by agglutination with latex-mounted anti-O157 polyclonal antibody (Oxoid, Dryspot). Isolates from laboratory drainage experiments were not confirmed.

Salmonella numbers were determined by initial suspension and decimal dilution of the sample in 9 volumes of Rappaport Vassiliadis Soya enrichment broth (RVS, Oxoid). Plating was onto xylose lysine deoxycholate agar (XLDA, Oxoid). Presence of *Salmonella* was by pre-enrichment in BPW at 37°C for 16 h. Enrichment was by transfer of 0.1 ml of the pre-enriched sample into 10 ml RVS medium and incubation at 42°C for 24h. Detection was by streaking onto XLDA. Confirmation for both *Salmonella* tests was by lack of oxidase activity and biochemical profiling (API20E; bioMérieux, Marcy l'Etoile, France) following the manufacturer's instructions.

Up to 5 presumptive colonies per plate were confirmed. Colony counts were converted to CFU g^{-1} waste according to the criteria specified by the International Standards Organisation.

Data Analysis

Log averages and associated standard deviations from each set of three replicates were calculated for each sample. R^2-values were determined by the least squares method and coefficients of variation (CV) calculated by dividing the means by the SD for each sample time. Groups of CVs were compared using the Mann-Whitney U-test for non-parametric data ($P<0.05$; SPSS 11.5, SPSS Inc., Chicago, USA).

	E. coli O157	*Salmonella*	*Campylobacter*
Initial diluent	TSB, 80µg ml^{-1} novobiocin	TSB, 40µg ml^{-1} novobiocin	PSW (boiled to remove O_2)
Cellulose nitrate filter pore size	0.45µm	0.45µm	0.1µm
Resuscitation media	TSB, 40µg ml^{-1} novobiocin	TSB, 20µg ml^{-1} novobiocin; 1% (w/v) Iodine	BFEB microaerophilic conditions
Resuscitation time (h)	6	16	24
Counting media	CHROMagar O157 (Becton Dickson 264105)	Rambach Agar	CCDA microaerophilic conditions
Incubation conditions prior to counting	16h at 41°C	28h at 37°C	28h at 37°C
Colour/morphology of presumptive positive colonies	Purple	Pinkish red	Grey/moist/flat
Confirmation by	Latex agglutination (Oxoid DR120M)	API 20E (BioMérieux)	API Campylobacter (BioMérieux)

Table 1. Selective media and organism-specific variations used for enumeration of bacterial zoonotic agents. All media sourced from Oxoid, (Basingstoke, UK) unless otherwise specified. TSB is Tryptone Soya Broth, PSW is Peptone Salt Water, BFEB is Blood Free Enrichment broth, CCDA is Campylobacter agar with charcoal and deoxycholate.

Soil splash experiments

A novel approach was taken to study soil splash where target soil inoculated with bacterial marker (*E.coli* K12) was exposed to a) varying droplet size and b) various irrigation nozzles. Selective media (i.e. MacConkey agar supplemented with nalidixic acid) was used to identify the extent of bacteria splashed from the soil surface onto the test surface, giving a measure of colonies introduced (colony number) and the amount of bacteria splashed onto the test surface (colony area).

Microorganisms used and their culture conditions

Escherichia coli K12 isolate EQ1, resistant to 40 µg ml^{-1} nalidixic acid, was stored in Protect beads (Technical Services Consultants, Heywood, UK) at -70°C, and resuscitated by removing one bead, inoculated by streaking on a Columbia Blood Agar (Oxoid, Basingstoke, UK) plate to obtain isolated colonies. Luria Bertani Broth supplemented with 40 µg ml^{-1} nalidixic acid (LBN; 30 ml) was equilibrated to 37°C, then inoculated with one colony of *E. coli* K12 EQ1 and incubated at 37°C overnight without shaking.

Estimation of the percentage areas of the selective media colonised by splash-transferred marker bacteria.

The selective media were incubated at 37°C ± 1°C for 48h before being photographed using a Nikon D90 digital camera (Nikon, Kingston upon Thames, UK) at maximum resolution (4288 x 2488 pixels). The photographs were imported into Adobe Photoshop Elements (version 6.0; Adobe Systems, Stockley Park, UK) and the Magic Wand tool used to identify and select regions of the picture of similar colour. For the identification of total strip area, the Wand tolerance was set to 10 and a section of media that was clear of bacterial growth clicked. If required, further areas of the media were shift-clicked until the area was selected. The Histogram function was used to determine the total number of pixels that were selected. The total area of the media colonised with marker bacteria was determined using a Wand tolerance of 25 and selection of areas of the media containing colonies (Appendix, Figure ii). The percentage of the media that was colonised was calculated as: total area colonised/ (total area colonised + total uncolonised area)
The numbers of individual colonies on the surface of the media were counted using traditional counting methods.

Soil types studied

Two soil types sourced from Harper Adams University College farm were used for the soil splash experiments: both were defined as silty loams but with differing organic matter contents. A representative sample of each soil type was sent to an external testing laboratory (Eurofins Ltd, Wolverhampton) for physical and chemical

characterisation and results are presented in Table 2. For this report they are defined as peaty soil and mineral soil.

Table 2. Physicochemical profile of the soil studied

Analysis	Peaty Soil Mean result (n=2)	Mineral Soil Mean result (n=2)
Total Nitrogen (% m/m)	0.48	0.16
Organic matter (% m/m)	8.98	1.81
Particle size distribution:		
2000-600 μm – Coarse Sand	0.48	0.16
600-212 μm – Med Sand	8.98	1.81
212-63 μm – Fine Sand	6.5	7
63-20 μm – Coarse Silt	32	60.5
20-2 μm – Fine Silt	27	18
<2 μm – Clay	5	2.5

Irrigation nozzles

A range of irrigation nozzles were studied to establish the potential for soil splash. This study was not a definitive comparison of all available nozzles – but an indication of the range of droplet sizes that are available and the potential for soil splash. Nozzles were attached to a single 1 m riser of a solid set irrigation system. The nozzle was run initially to observe the spread of water and the best location for the inoculated tray of soil. A plastic beaker was placed over the top to prevent any water spreading and the beaker was removed for 10 seconds and replaced. The sprinklers were connected one at a time to the irrigation ring main and operated at approximately 2 bar. Seed trays of inoculated soil were placed under the falling irrigation droplets at the edge of the irrigation throw with Petri dishes supported at height of 10 cm over the soil. By placing the trays at the edge of the throw it enabled study of the largest drops produced as they would have the greatest mass and hence velocity. The Petri dishes were collected after each experiment and cultured as described previously.

The nozzles studied were (with descriptive text from the product specifications):

1. Naan Dan 427B AG (green)

Rotating plastic impact sprinkler (full or part circle), recommended for field vegetables and greenhouses, giving a range of droplet size from small to large.

2. Naan Dan 501 (black)

Low volume, low angle sprinkler. Recommended for orchards and greenhouse use, giving fine droplet size.

3. Mini Compact micro Sprinkler Series 800 (black)

Recommended for orchards, greenhouses and nurseries, giving a medium droplet size.

4. Vibrospin sprinkler medium (blue)

Self cleaning nozzle with rotating anvil for even distribution, recommended for plug plants.

5. Eindor Ser 809 Bridge Mister (blue)

Produces mist in very tiny droplets. Recommended to maintain a constant humidity; to reduce high temperatures in hot climates by evaporation, and for irrigation in protected structures.

Droplet size (rain splash)

The effect of droplet size on distribution of bacteria in the surface soil was studied following the method of Jenkinson and Parry (1994). Uniform drops of water were produced from 3 different Pasteur pipettes. The medium drops were from an unaltered pipette, the large drops from a pipette which had been cut 4.3 cm from the end; and the small drops from a pipette drawn out in a Bunsen flame. The pipettes were attached to a 50ml syringe with plastic tubing. Incident drop diameters were determined by measuring the weight of 50 drops. Assuming the drops to be spherical, the mean drop volume was calculated from which a mean drop diameter was estimated (Table 3).

Table 3. Average drop volume and diameter estimated for the three drop sizes studied.

Drop size	Average drop volume (ml)	Average drop diameter (mm)
Small	0.025	3.6
Medium	0.040	4.2
Large	0.056	4.8

Trays of soil (30 x 20 cm), filled either with peaty soil or mineral soil were inoculated with bacterial marker (*E.coli* K12). Perspex strips (45 cm) were coated with 2-3 mm layer of selective medium and were oriented in a Cartesian X, Y, Z layout away from the point of impact on the soil. The number of drops required for 2 ml were then released at a height of 6 m to allow drops to reach terminal velocity. The experiment was repeated three times for each droplet size x soil combination.

Results

Pathogen persistence on crop tissue

Environmental conditions

The crop experienced warm temperatures and sporadic rainfall, with a heavy rain event in the final week of the trial. However, the light levels were lower than would be expected due to significant cloud cover for much of the trial (Appendix – Figure i). As a consequence, the average daily accumulated sunlight was lower than experienced for the early season experiment in 2007 (Table 4) and only 71% of light level experienced mid season in 2006 (6506.6 Wm^{-2}).

Table 4: A summary of the environmental conditions experienced for crop experiments in 2008 with 2007 averages for comparison.

Measured environmental parameter (units)	*Early season*	*Late season*	*Mid season*
	2007	2007	2008
Accumulated rainfall (mm)*	39.8	21.6	54.4
Average daily temperature (°C)**	12.8	13.3	16.5
Average daily accumulated sunlight (Wm^{-2})	5276.6	2535.6	4651.4

* Crops were irrigated in addition to rainfall

** Temperature measured at 20 cm height

Salmonella enterica serotype Enteriditis

No Salmonella was detected on any control treatment.

a) Lettuce

The level of Salmonella initially recovered from the lettuce was higher in the high treatment than the low treatment (Figure 1). After 1 week the numbers of *Salmonella* recovered were reduced by approximately 4 log for both rates of application. After 2 weeks the levels of salmonella recovered were below the limit of enumeration (<10 CFU g^{-1}) for both treatments and was absent from the leaves of all three plots of the high treatment but *Salmonella* was recovered from one of three plots of the low treatment. No *Salmonella* were recovered after 3 weeks (Table 5).

b) Spinach

The recovery of *Salmonella* from spinach leaves followed an identical pattern to lettuce (Figure 2, Table 5).

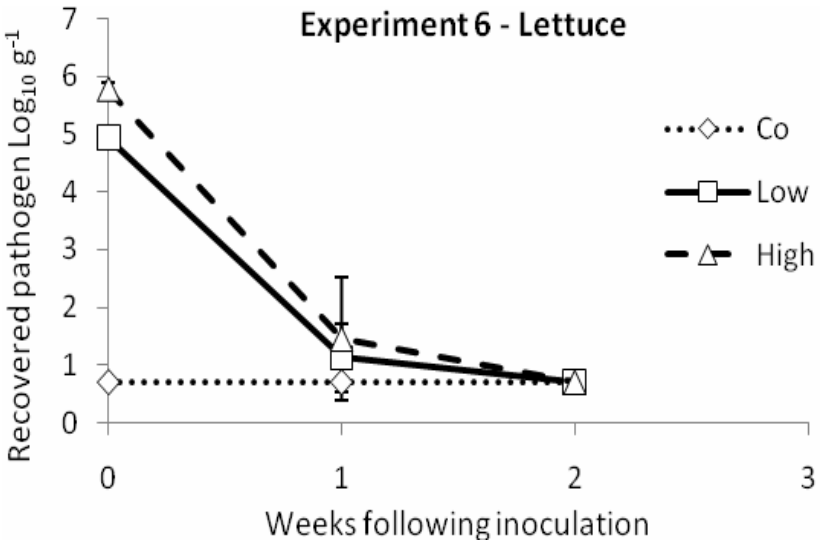
c) Soil

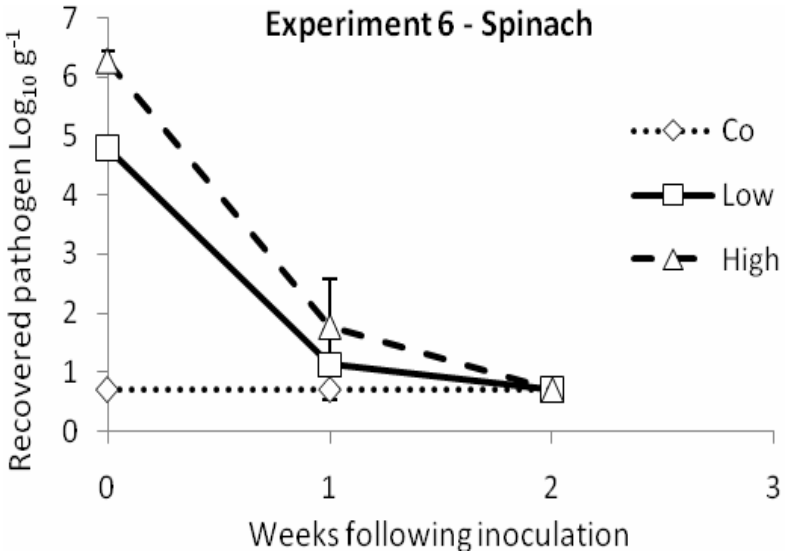
No *Salmonella* were detected in the surface soil samples taken after three weeks

Table 5. Number of plots (n=3) where *Salmonella* Enteriditis was detected on leaf tissue and surface soil following inoculation with Low and High levels of *Salmonella* Enteriditis applied to the crop through overhead irrigation.

	Lettuce		Spinach	
Week	Low	High	Low	High
+1	3	3	3	3
+2	1	0	1	0
+3	0	0	0	0
+3 (Soil)	0	0	0	0

Figure 1. Recovered pathogen from leaf surface following inoculation with Control, Low and High levels of *Salmonella* Enteriditis applied to the crop through overhead irrigation: a) Lettuce, b) Spinach Experiment 6 (Bars represent +/- SD n=3).





E. coli O157

No *E. coli* O157 were detected in the control treatments in Experiment 6.

a) Lettuce

The initial recovery of *E. coli* from lettuce leaves was greater from the high treatment with approximately 1 Log higher level of recovery (Figure 3). This difference was still present after 1 week, although relative levels had reduced by approximately 2.5 Log. After 2 weeks the level of recovery from both treatments was below the level of enumeration (<10 CFU g^{-1}) but *E. coli* were present in two of three plots for both the high and low treatments (Table 6). After three weeks no *E. coli* were detected on the leaves of lettuce.

b) Spinach

The levels of *E. coli* recovered from spinach leaves declined more slowly than observed with lettuce leaves (Figure 4). After 1 week levels had declined by approximately 1.5 log for the high treatment and 1 Log for the low treatment. The levels declined sharply in the following week and after 2 weeks the level of recovery from both treatments was below the level of enumeration (<10 CFU g^{-1}) but *E. coli* was present in all three plots for the low treatments and one plot of the heigh treatment (Table 6). After three weeks no *E. coli* was detected on the leaves of Spinach.

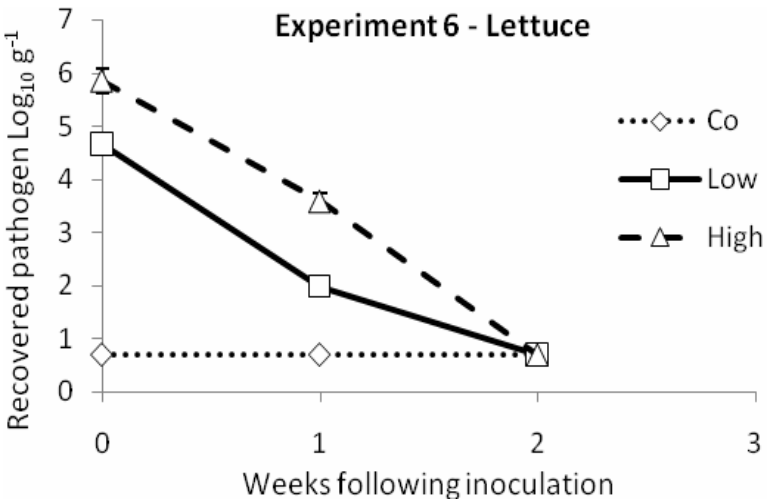
c) Soil

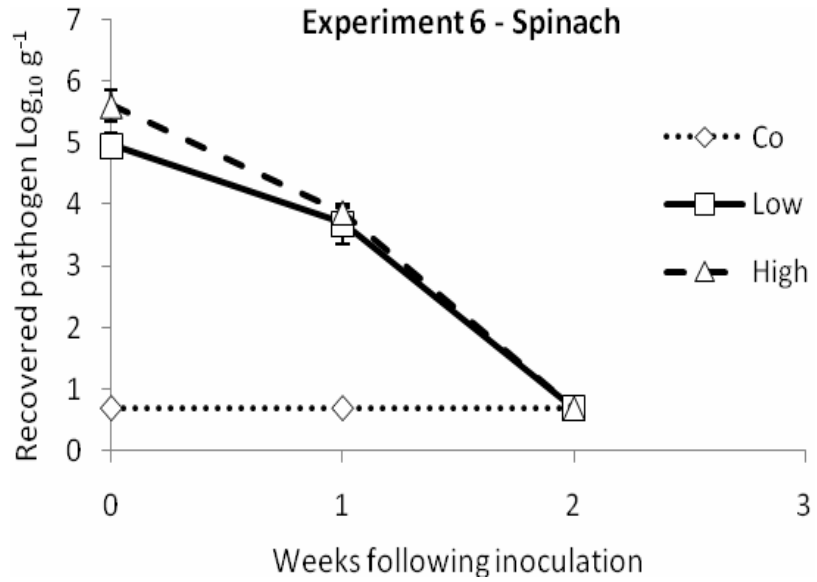
E. coli O157 was not detected in the surface soil samples taken after three weeks.

Table 6. Number of plots (n=3) where *E. coli* O157 was detected on leaf tissue and surface soil following inoculation with Low and High levels of *E. coli* O157 applied to the crop through overhead irrigation.

	Lettuce		Spinach	
Week	Low	High	Low	High
+1	3	3	3	3
+2	2	2	3	1
+3	0	0	0	0
+3 (Soil)	0	0	0	0

Figure 3. Recovered pathogen from leaf surface following inoculation with Control, Low and High levels of *E. coli* O157 applied to the crop through overhead irrigation: a) Lettuce; b) Spinach (Bars represent +/- SD n=3).





Campylobacter jejuni

No contamination was observed in the control treatments.

a) Lettuce

The level of campylobacter initially recovered from the lettuce was higher in the high treatment than the low treatment (Figure 5). After 1 week the level of campylobacter recovered was reduced by approximately 2 Log for both rates of application. After 2 weeks campylobacter was no longer detected on lettuce leaves sampled for both treatments (Table 7).

b) Spinach

The initial recovery of campylobacter from spinach leaves was similar for both treatments with approximately $5x10^4$ Log CFU g^{-1} (Figure 6). After 1 week, levels had reduced to approximately $1x10^3$ Log CFU g^{-1}. After 2 weeks the level of recovery from both treatments was below the level of enumeration (<10 CFU g^{-1}) but campylobacters were present in one of three plots for both the high and low treatments (Table 7). After three weeks no campylobacters were detected on the leaves of lettuce.

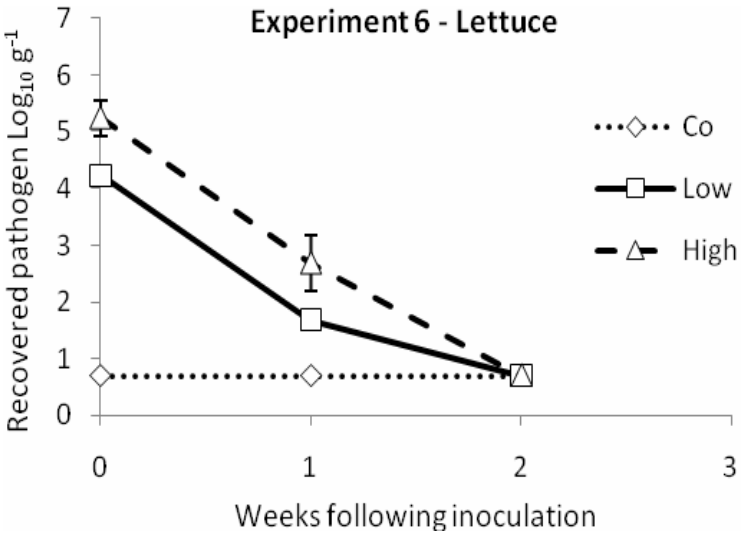
c) Soil

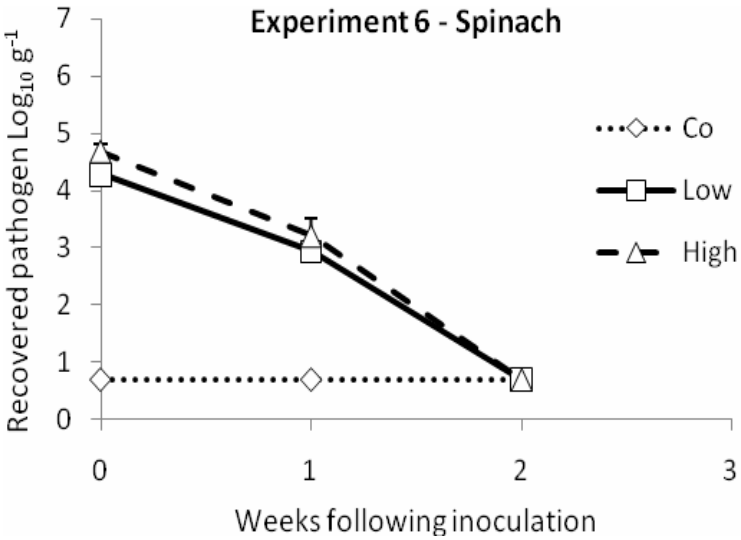
Campylobacter jejuni was not detected in any of the surface soil samples taken from the treated plots at the end of the experiment.

Table 7. Number of plots (n=3) where *Campylobacter jejuni* was detected on leaf tissue and surface soil following inoculation with Low and High levels of *Campylobacter jejuni* applied to the crop through overhead irrigation.

	Lettuce		Spinach	
Week	Low	High	Low	High
+1	3	3	3	3
+2	0	0	1	1
+3	0	0	0	0
+3 (Soil)	0	0	0	0

Figure 5. Recovered pathogen from leaf surface following inoculation with Control, Low and High levels of *Campylobacter jejuni* applied to the crop through overhead irrigation: a) Lettuce; b) Spinach (Bars represent +/- SD n=3).





Soil splash experiments

Irrigation nozzle

The description of the droplet size from the nozzles given in the technical specifications was not useful in distinguishing droplet size distribution. Consequently the droplet size was visually assessed on an arbitrary scale. Quantifying the distribution of droplet size is possible but was not undertaken in this preliminary study. Further work may consider this area.

The nozzles categorised as fine or mist gave no soil splash on to the selective media plates after 10 seconds exposure to irrigation at the edge of the irrigation throw (Table 8). However, the larger droplet sizes observed at the edge of the irrigation throw with nozzles 1 and 2 splashed contaminated soil up on to the plates. There was no difference between nozzle 1 and 2 for the number of colonies ($p=0.37$) but a significantly greater area of the plate was contaminated with nozzle 1 compared to nozzle 2 ($p=0.02$). This suggests that the larger observed droplets dispersed a greater amount of contaminated material from the soil on to the selective medium.

Table 8. Arbitrary droplet size, water flow, radius of spread, average colonies counted and average area of plate contaminated for the nozzles studied.

	Droplet size at edge of irrigation throw*	Volume of water (m^3 h^{-1})	Radius of spread (cm)	Average No. of colonies (n=3)	Average area of plate contaminated (%) (n=3)
1	Large-medium	0.68	745	6.3	20.4
2	Medium	0.26	560	8.7	10.6
3	Fine	0.12	330	0	0
4	Fine	0.08	245	0	0
5	Mist	0.04	100	0	0

* This is an arbitrary scale based on visual assessment of the sprinklers in operation.

Droplet size

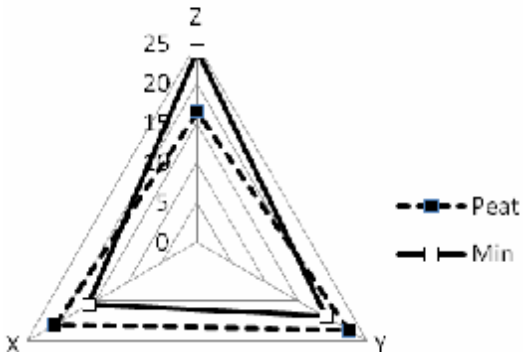
The data represents the extent of distribution of droplets capable of dispersing bacterial contamination within a 45 cm half sphere with the droplet impact as the centre. Figures 7 & 8 represent the overall distribution of colonies as either the average percentage of selective media covered (Figure 7 a-c) or the average number of colonies (Figure 8 a-c). It is clear that as the drop size got smaller the extent of contamination decreased along all three dimensions. There was no significant effect of orientation, i.e. the same pattern of response was observed for the X axis as for the Y and/or Z axes. As a consequence the values were averaged for all three axes. The area of contaminated selective medium ($p<0.001$) and the number of colonies increased significantly ($p<0.001$) with drop size (Table 9). Both measures of contamination were approximately 5 times greater with an average droplet diameter of 4.8 mm compared to 3.6 mm. Overall, soil had a significant ($p<0.01$) effect on the average number of colonies with 45.7 colonies splashed up from the mineral soil compared to 24.4 from the peaty soil. However, the interaction between drop size and soil was not significant.

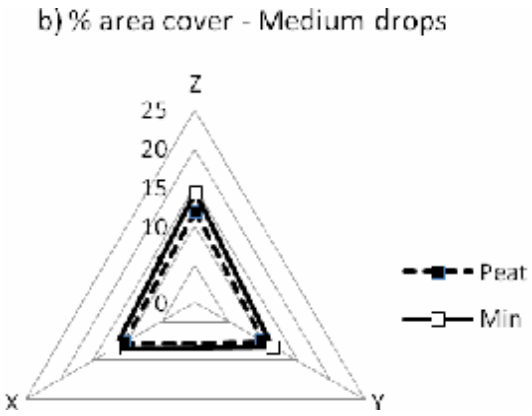
Table 9. Area cover and colony number averaged over all three dimensions.

Drop size (mm)	Area cover (%) (n=9)	Colony number (n=9)
3.6	4.13	11.2
4.2	11.72	33.6
4.8	19.69	60.3
$LSD_{(0.05)}$	*3.50*	*15.3*

There was a marked skew in the data along the x-axis for colony counts with the large and medium drops that was present in one of the reps. This may be the result of a draft of air deflecting drops in that run.

Figure 7. Percentage of media cover by colonies after 3 days.





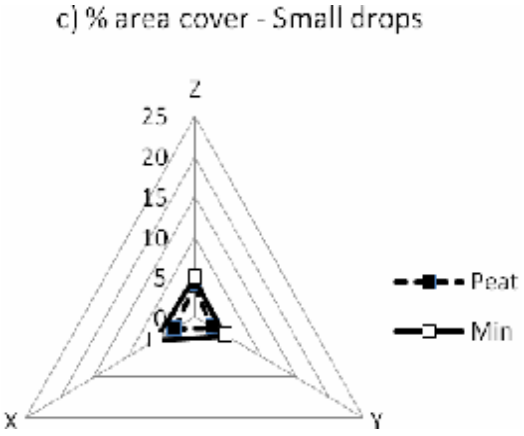
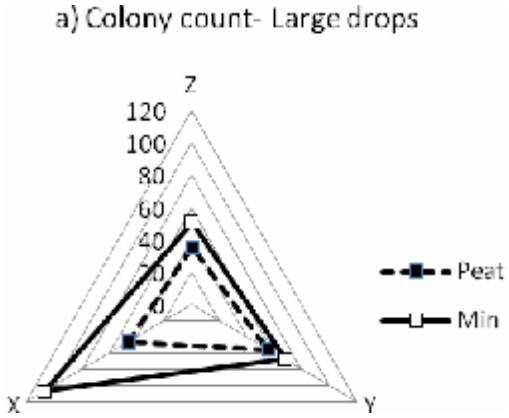
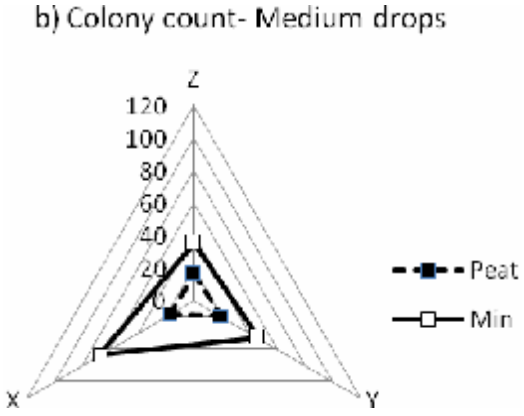
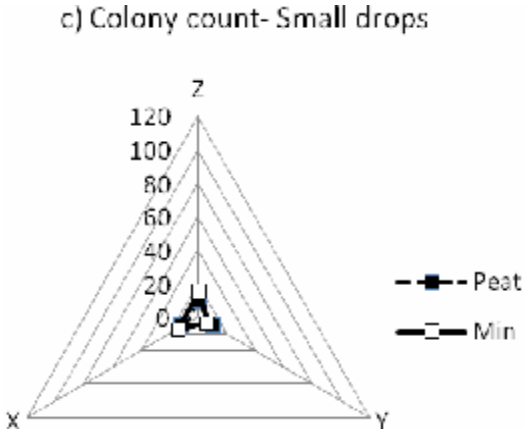


Figure 8. Number of colonies (CFU) after 3 days.







Discussion

What is the persistence on the surface of crops of pathogens introduced through irrigation at different times in a growing season?

As with FV292, there were differences between the concentrations of zoonotic agents applied in the water as compared with the concentrations recovered from leaf tissue. The treatments were applied to small plants and it is likely that the available bacterial binding sites on the leaf surface were being saturated or nearly saturated by the low treatments.

It is interesting to note that the persistence of pathogens were greater in the mid-summer treatments in FV292a (2008) compared to FV292 (2007) presented in table 10, below. This was unexpected as it was anticipated that persistence of pathogens would be even less in hot dry mid summer UK conditions. However, the level of light overall was significantly less in mid summer 2008 than late season 2007. This lower light level was particularly marked at the start of the mid summer 2008 experiment. When the results are considered for FV292 and FV292a, over all three experiments, all three bacteria studied declined rapidly to absence in 14-21 days. This does not agree with the data reported from the USA. Field trials carried out in Georgia, USA have demonstrated that *E. coli* O157:H7 applied through irrigation can persist on the surface of lettuce for 77 days after contamination (Islam *et al*., 2004a). The same workers have also reported persistence of *Salmonella enterica* Typhimurium as persisting on leaves of lettuce for 63 days (Islam *et al*., 2004b), i.e. pathogens are persisting for 3 to 4 times longer in the US work. Both the US work and the work reported here took place in a field environment, with young plant material being inoculated with similar levels of pathogen. However, climate and season would have been very different between the two growing sites. The University of Georgia Horticulture Farm is located in Tifton, GA (32.0852 lat, -84.1830 lon), roughly on the same latitude as northern Morocco. The US work took place from October, over winter and, although met data was not presented in the papers, the temperature ranges over winter in Georgia are moderate, rarely approaching freezing with a range that is not too different to the UK in spring, but with higher night temperatures. In marked contrast the work at Harper Adams took place from early May to the end of September, meaning that crops will have experienced higher temperatures than the US crops and higher levels of sunlight, and hence UV; the US work would have received day lengths less than 11 hours whereas the UK work received day lengths exceeding 13-14 hours. The US work was undertaken under conditions that are not representative of those experienced by UK-grown crops.

The climate has a marked effect on leaf borne pathogens – pathogens on dry leaves exposed to high levels of UV rapidly degrade The US data is a worse case scenario in conditions similar to North Africa in the winter (with greater rainfall!), not leafy salad production conditions in the UK.

Conclusion

- High levels of leaf contamination (10^5 CFU g^{-1}) of baby leaf spinach and wholehead lettuce pose minimal risk after a maximum of three weeks in a UK growing environment.
- Guidance for growers should be derived from work undertaken in a UK growing environment – it can be misleading to extrapolate international studies to the UK growing environment.

Table 10. Summary of data combined from FV292 and FV292a showing persistence of pathogens on the surface of crops measured as weeks to pathogen recovery below the level of enumeration i.e. <10 $CFUg^{-1}$ and (weeks to absence of pathogen).

a) *Salmonella* Enteriditis

	Early season 2007		Mid Season 2008		Late season 2007	
	Low	High	Low	High	Low	High
Lettuce	1 (3)	1 (3)	1 (3)	1 (2)	1 (2)	2 (2)
Spinach	1 (3)	1 (3)	1 (3)	1 (2)	2 (2)	2 (3)

b) E. coli O157

	Early season 2007		Mid Season 2008		Late season 2007	
	Low	High	Low	High	Low	High
Lettuce	1 (2)	2 (2)	2 (3)	2 (3)	1 (2)	1 (3)
Spinach	1 (2)	1 (2)	2 (3)	2 (3)	2 (3)	1 (3)

c) Campylobacter jejuni

	Early season 2007		Mid Season 2008		Late season 2007	
	Low	High	Low	High	Low	High
Lettuce	1 (2)	1 (2)	2 (2)	2 (2)	1 (3)	1 (3)
Spinach	1 (2)	1 (2)	2 (3)	2 (3)	1 (3)	1 (3)

What is the risk of contamination by soil splash from irrigation systems?

FV292 demonstrated that applying contaminated irrigation water to crops and soils introduces a risk to ready to eat produce grown in that soil. The persistence of the bacterial contamination in the soil was related to the level of initial contamination, with the high level of contamination persisting longer than the low level in all cases. However, pathogen persistence was much greater in the soil than on the surface of the crops. The soil may act as a reservoir of pathogen and may pose a risk of re-contamination of the crop before harvest, particularly through soil splash during an irrigation event.

The experiments on soil splash demonstrated that significant levels of soil borne contamination could be distributed over ranges that would allow leafy salads to be contaminated in the field. The main factor influencing the extent of contamination was drop size. The study of splash using irrigation nozzles showed that commercial sprinkler nozzles that generated relatively large droplets could pose a risk. However it was not possible in this preliminary study to quantify the droplet size distribution from the sprinklers. It is generally accepted that sprinklers generate droplets over the range of 0.5 – 4 mm (Kay, 1983; Kincaid *et al*., 1996). The largest drops will be found at the edge of the irrigation throw as they have the greatest energy and are thrown the furthest (Kay, 1983).

Studying drops of defined dimensions (3.6 – 4.8 mm) demonstrated that it is only those drops found at the upper end of irrigation droplet size distribution that are capable of significant soil splash. Whilst it is difficult to get specific data on rain drop size distribution due to the large number of factors influencing it, the general consensus is that the great majority of rain drops are < 4 mm (e.g. Williams *et al*., 2000), although larger drops are associated with thunder storms. Taken as a whole the work has demonstrated the potential for soil splash as a route for contamination, but that the irrigation (and rain) droplet sizes that pose greatest risk are at the upper end of distributions experienced in the field. Any measures that reduce the droplet size at least

to <3.6 mm will markedly reduce the extent of soil splash and hence the risk of contamination of leafy salads from contaminated soil.

An interesting observation from the droplet size work was that soil type influenced the extent of contamination. Peaty soil was associated with fewer, larger colonies than mineral soil for droplets >4 mm. Previous studies in FV292 had suggested that the peaty soil was more-freely draining at the surface – explained by the higher organic matter and greater sand content.
It may be that larger volumes of surface soil were dispersed with the peaty soil due to the looser structure associated with the higher sand content. Further work would be needed to establish the cause of this response.

Conclusion

- Where soil has been contaminated by irrigation water, soil splash may contaminate leafy salads. However, significant contamination will only occur with the droplet sizes at the upper end of irrigation droplet size distribution.
- Design and maintenance of irrigation systems to minimise droplet sizes <3.6 mm would minimise risks of soil splash through irrigation

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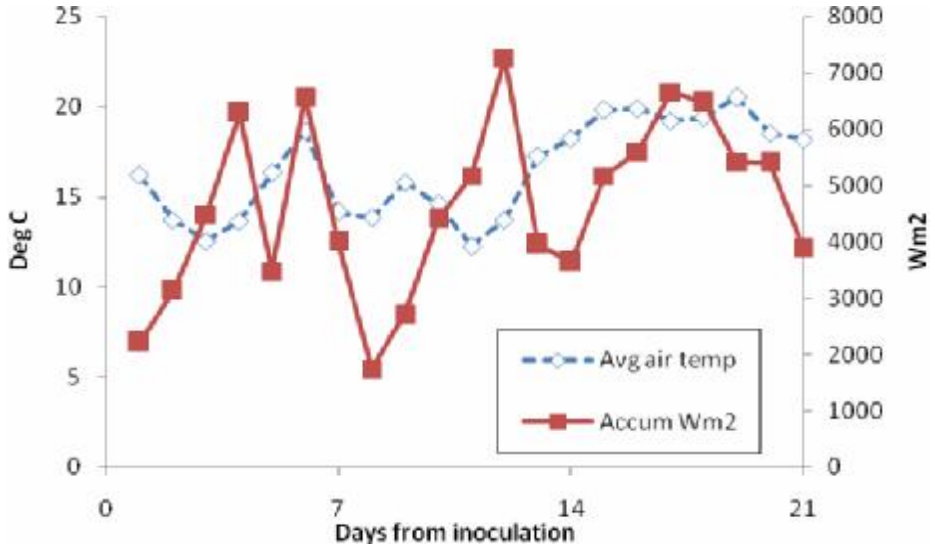
Appendix

Figure I. Weather data measured at the experimental site after the inoculation of soils (day 0 = 10 July 2008): a) Daily average air temperature (20 cm) and accumulated sun light; b) Daily rainfall. Experiment 4, Year 2.

a.



b.

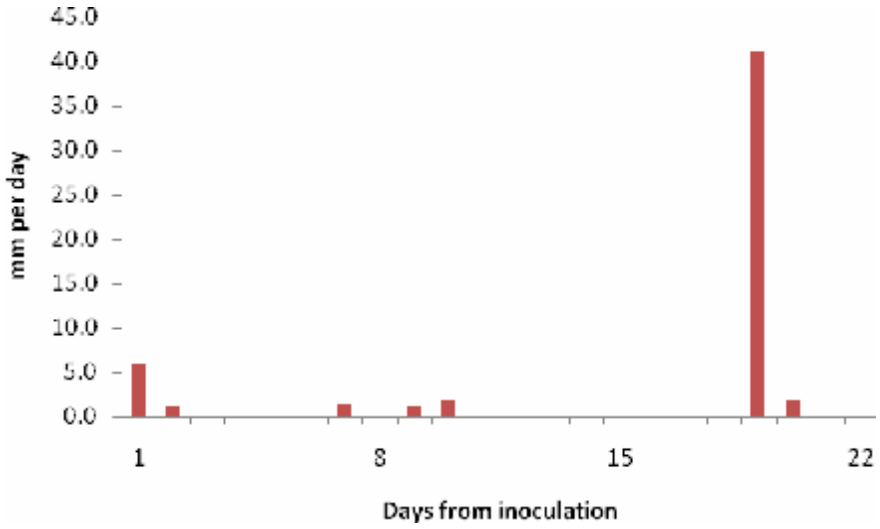


Figure ii). Calculating the area of pixels and hence surface of the selective media

colonised by *E.coli* K12.