ANNUAL REPORT

Keeping it clean

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

FV 292

Year 1 (2006-07)

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

Project number: **FV 292**

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Report: **Annual report (May 31st 2007)**

Previous reports: **-**

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Date commenced: **1st April 2006**

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

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CONTENTS

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Grower Summary

Headline

- Human pathogen survival is decreased with high light levels, warm temperatures and dry soil with organisms less likely to persist in peaty soils compared to mineral soils.
- *E. coli* O157 is more persistent in trial plots than either *Salmonella enteriditis* or *Campylobacter jejuni*

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 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 their produce.

Previous HDC funded work (FV248) established that almost three quarters of salad crops were irrigated through direct abstraction of surface water, the most vulnerable to contamination with faecal pathogens from agricultural activities. 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 drinking 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.

This project demonstrates the true risk to salad vegetables from irrigation water introducing pathogens to the soil (year 1) or to the surface of the produce (year 2) and the interaction with UV (sunlight) and temperature on the seasonal persistence of the pathogens.

The following questions (each being an experimental objective) were addressed in Yr 1:

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1. What is the persistence in the soil of pathogens introduced through irrigation at different times in a growing season?
2. Does persistence in the soil of pathogens introduced through irrigation differ with soil type?

Summary of the project and main conclusions

Treatments

- 2 soils: mineral, peat
- 3 levels of pathogen* zero, low (1×10^2 cfu ml^{-1}) and high (1×10^5 cfu ml^{-1})
- 3 experiments: early, mid and late season timing

*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 each experiment (Experiment 1 – 10 May 2006, Experiment 2 – 05 July 2006, and Experiment 3 – 06 September 2006). Surface soil was sampled weekly and tested for pathogens.

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

Season had a marked effect on persistence of introduced bacteria. The hot, dry and high sunlight conditions of experiment 2 led to a marked reduction in the levels of bacteria recovered from the surface of the soil, with *Campylobacter* declining most rapidly. All three pathogens were at the level of enumeration after 2 weeks. In contrast the cooler, wetter and lower light levels experienced in experiment 1 and experiment 3 were associated with a greater persistence of pathogens. This was particularly marked in experiment 3 where *E. coli* O157, *Salmonella* and *Campylobacter* were still present in the surface of all plots after 6 weeks. A similar response was observed in experiment 1 after 5 weeks for *E. coli* O157 and *Salmonella*.

Findings of Objective 1:

- Persistence of pathogen is related to initial level of contamination
- Pathogens are killed by high light levels, warm temperatures and dry soil.
- Pathogens persist in cool, damp overcast conditions.
- Overall, *E. coli* O157 was the most persistent pathogen with presence detected in more of the plots by the end of each experiment than either *Salmonella enteriditis* or *Campylobacter jejuni*

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Does persistence in the soil of pathogens introduced through irrigation differ with soil type (Objective 2)?

Soil type had a significant effect on the persistence of introduced zoonoses particularly at the high level. There was a more rapid decline in all three pathogens in the peaty soil with the higher organic matter content compared to the mineral soil in experiments 1 and 3. It is suggested that the higher organic matter of the peaty soil was associated with a higher indigenous bacterial population i.e. it was a more biologically active soil, and that antagonistic interactions with indigenous microbial populations were influencing soil survival.

Findings of Objective 2:

- Soil type has an impact with higher organic matter being associated with reduced persistence of pathogen

Clearly there is a hypothetical risk of infecting crops by soil contamination – either through rain /irrigation splash or harvesting - and this work would suggest that the risk is greatest at the start and end of the season. Nevertheless, this work needs to be seen as a preliminary study giving only indications of risk. True risk to the consumer is through the crop – not the soil – and this data will be reported in year 2.

Financial benefits

The benefit to the industry of this work is to provide independent scientific study of the real risk of pathogens entering the supply chain through irrigation of salad vegetable crops in a UK growing environment. As such it is impossible to put a financial benefit on the work after year 1.

Action Points for growers

These are preliminary results – full action points will be produced after consideration of the results from year 2, where plants are included in the trial thus giving a measure of the risk to finished product.

Nevertheless, two clear action points can be drawn from this work

1. Minimise the level of contamination of irrigation water.
2. Minimise soil contamination of crops.

Both of these action points can be achieved by following the clear guidance set out in the HDC DVD ‘Keeping it clean!’ and the Assured Produce generic protocol guidance notes on Microbial Food Safety. A number of additional references are included in this latter document which may provide further background information.

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1 Science Section

1.0 Introduction

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 food production practices and the implementation of process controls; and also retailers seeking to minimise the risk of 'bad press' and damaged brand resulting from food poisoning traced to their produce

Previous HDC funded work (FV248) established that almost three quarters of salad crops were irrigated through direct abstraction of surface water, the most vulnerable to contamination with faecal pathogens from agricultural activities. 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 drinking water).

It is not commercially viable, using available technology, to remove microbial contamination from those products consumed raw, such as salad vegetables. The only widely-accepted approach to minimise the risk of microbial contamination of produce is to monitor and regulate the potential sources of contamination e.g. Irrigation water, manure, worker hygiene.

There is a body of data on the persistence of faecal pathogens on hands and well-proven best-practice hand wash procedures to limit the spread of faecal pathogens. Similarly there is considerable data on pathogen reduction through the composting process. However, there is a very conspicuous lack of data on the persistence of pathogens introduced to cropping areas through contaminated irrigation water.

It is clear from published HPA and FSA data that there is only a relatively low incidence of food poisoning associated with consumption of fresh produce grown in the UK. Tyrrel (2004) suggested that one reason for the low level of observed food illness correlated with lower quality irrigation water applied to salad crops could be due to pathogen die-off between irrigation and consumption. Another more plausible explanation is that microbiological testing identifies only whether a potential pathogen is present in water. Pathogens evolve to become suited to particular hosts and thus those encountered in agricultural environments are more likely to have the ability to infect livestock rather than humans. Thus, even if "pathogens" are present in irrigation water they may not represent a credible threat to human health.

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

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date on persistence of pathogens between irrigation and harvest has not been reported in the literature for UK (or European) growing conditions.

A number of recent studies in the USA, in response to a relatively high rate of food illness outbreaks associated with fresh produce, have looked at routes of transfer for faecal pathogens onto salad vegetable and other crops. Field work carried out in Georgia, USA has demonstrated that *E. coli* O157:H7 applied through irrigation can persist in soils for up to 200 days and on the surface of lettuce and coriander for 77 and 177 days after contamination respectively (Islam *et al*., 2004a & 2005). The same workers have also reported persistence of *Salmonella enterica* Typhimurium as persisting on leaves of lettuce for 63 days and parsley for 231 days; and in soils for 161 days (Islam *et al*. 2004b). Relative to *Salmonella* and *E. coli* O157:H7, *Campylobacter* is much less persistent in the environment. *Campylobacter* causes ten times more foodborne illness in the UK than *Salmonella* and *Campylobacter* combined (Adak *et al*., 2005). A Californian field study reported that *C. jejuni* only survives on Spinach leaves for 5 days at 10°C although soil persistence was five times longer (Brandl *et al*. 2004). However, the direct relevance of these data to UK production systems is limited. The role of 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), although the exact mechanism of this influence is unknown. This proposal aims to address this knowledge gap.

This project aims to quantify the true risk to salad vegetables from irrigation water introducing pathogens to the soil (year 1) or to the surface of the produce (year 2) and the interaction with UV (sunlight) and temperature on the seasonal persistence of the pathogen.

1.1 Overall aim of the project

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

1.2 Specific objectives

- Establish the persistence in the soil of pathogens introduced through irrigation at different times in a growing season. (Year 1)

- Evaluate the effect of soil type on persistence in the soil of pathogens introduced through irrigation. (Year 1)

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- Establish the persistence on the surface of crops of pathogens introduced through irrigation at different times in a growing season. (Year 2)

- Evaluate the effect of rainfall on the persistence of pathogens introduced on the surface of crops through irrigation. (Year 2)

2 Materials and Methods

Year 1 – Soil experiments

Treatments

- 3 experiments: early, mid and late season timing
- 2 soils: mineral, peat
- 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 each experiment (Experiment 1 – 10 May 2006, Experiment 2 – 05 July 2006, and Experiment 3 – 06 September 2006). Surface soil was sampled weekly and tested for pathogens.

2.0 Plot preparation

Two soil types sourced from Harper Adams University College farm: 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 1. For this report they are defined as peaty soil and mineral soil.

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Table 1: Physicochemical profile of the (pre-irrigated) soil used for these studies

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

Columns of the two defined soils were sunk into an open field environment using large plastic containers to prevent the migration of pathogens into the experimental soils. This system has been established previously at Harper Adams University College to study Potato Cyst Nematodes. The containers were spaced at 2 metres to minimise contamination of adjacent treatments by rain splash, additionally, ridged plastic cells were placed on the soil surface surrounding the buckets. Treatments were randomised within the design. New buckets were sunk into the ground for each of the three experiments.

Water for irrigation treatments was sourced from the irrigation lagoon filled from a bore hole at Harper Adams University College and tested for microbiological quality.

2.1 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.

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2.2 Microorganisms used and their culture conditions

The pathogens studied will be a *Salmonella* Enteriditis, a *Campylobacter jejuni* and an E. coli O157 (which does not contain the genes for verocytoxin).

The zoonotic agents used for these studies were all isolated originally from UK livestock[2]. 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^{o}C (*E. coli* and *Salmonella*) or 42^{o}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.

2.3 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 0.2 m^2 field plot was 2 litres applied using a 5 litre watering can. The contaminated water was applied as a single application 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. 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. Three six-week trials were run between May and October which is typical for the lettuce growing season in the UK. No crops were grown and samples were taken only from the soil.

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2.4 Sample collection from field plots and transit to the laboratory

Samples for analysis were collected from each replicated field plot each week for up to 6 weeks (a time period typically used for growing lettuces). 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. Samples were kept cool (<15^{o}C) for transport from the farm site to the laboratory. All microbiological testing commenced within 4h of sample collection.

2.5 Microbiological testing methods

Bacteria were initially enumerated from all of the samples taken. For the field samples, 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^{o}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^{o}C in Exeter broth was undertaken before plating onto mCCDA. Campylobacters were incubated under microaerophilic conditions at 42^{o}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^{o}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 [3,4].

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2.6 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). D-values (the number of days required for a 1-log decline in bacterial numbers) were calculated from data generated during the first 28 days. Groups of D-values were compared using one-way ANOVA with Tukey's post-hoc test (SPSS).

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3 Results

3.0 Environmental conditions

As was expected, the three experiments experienced different environmental conditions. Experiment 1 experienced overall an increasing soil temperature and light level and moderate rainfall for the first three weeks (Appendix - Figure I). In contrast, experiment 2 experienced high soil temperatures and light levels but virtually no rain (Appendix - Figure II). Experiment 3 experienced low soil temperatures and light levels and moderate rainfall over the whole experiment (Appendix - Figure III). The overall environmental conditions are summarised in Table 2.

Table 2: A summary of the environmental conditions experienced for each of the consecutive experiments.

Measured environmental parameter (units)	Experiment 1	Experiment 2	Experiment 3
Accumulated rainfall (mm)	82.0	6.0	106.8
Average daily soil temperature at 5 cm (°C)	16.5	23.8	14.6
Average daily accumulated sunlight (Wm^{-2})	5436.8	6506.6	2422.8

3.1 Pathogen persistence

In the first two experiments, large numbers of zoonotic agents were cultured for the high treatments and when these cultures were applied to the soils, there appeared to be an interaction between the bacteria and the soil. We speculate that the water drained leaving behind bacteria which had adhered to some of the soil components. Thus there was not an even distribution of bacteria and, in effect, some of the added cultures were concentrated in the upper layers of the soil. Although bacteria captured in this manner are more prone to UV irradiation, the upper limits of detection for the laboratory tests was $3x10^5$ bacteria per gram of soil. Numbers of bacteria above this limit could not be readily distinguished and were thus reported as >300000 CFU g^{-1}. In recognition of the problem, a more extensive range of dilutions was used for the third experiment allowing the exceptionally high numbers of bacteria that we encountered, to be accurately counted.

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3.1.1 Salmonella enteriditis

Control treatments demonstrated that there was no contamination of *Salmonella* in either the peaty soil or irrigation water used in the experiments. There was low level of contamination in one plot of the mineral soil initially in experiment 2 and after 2 weeks in experiment 3 (Figure 2). There was a more marked difference in the numbers of *Salmonella* isolated from high and low treatments applied to the peaty soil - despite using identical volumes of cultures from the same culture vessel for both soil types.

a) Peaty soil

Although higher levels of pathogen were recovered from the high treatments over the course of each experiment in both the high and low levels of inoculation the level of recoverable pathogen had declined so that it could no longer be enumerated after 3 weeks for both levels in experiments 1 and 3 (Figure 1). The rate of decline was most rapid in experiment 2 with the low level of treatment reaching the limit of enumeration by week 1 and the high treatment by week 2. By the end of each experiment *Salmonella* was still being detected (but not enumerated) in a few of the high treatment plots. The majority of plots inoculated with low levels of contamination did not contain detectable traces of *Salmonella* (Table 3). Experiment 3, which was run during periods of relatively low and decreasing hours of daylight with moderate rainfall, demonstrated that *Salmonella* added at high enough numbers could survive for at least 6 weeks in the peaty soil type.

b) Mineral soil

The decline of *Salmonella* in mineral soil was indistinguishable from the decline observed in peaty soils during the first two experiments. Furthermore, at the highest level of inoculation, the zoonotic agent persisted at the soil surface and could still be enumerated for 6 weeks after initial inoculation. There was an overall decline in the *Salmonella* population during this 6 week period however (Figure 2).

Table 3 Number of plots (n=3) with* Salmonella *detected at end of trial

	Experiment 1 +5 weeks		***Experiment 2*** +3 weeks		***Experiment 3*** +6 weeks	
	Peaty	Mineral	Peaty	Mineral	Peaty	Mineral
Low treatment	**0/3**	1/3	1/3	1/3	1/3	2/3
High treatment	2/3	3/3	1/3	**0/3**	3/3	3/3

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Figure 1. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of Salmonella enteriditis applied to the surface of peaty soil: a) Experiment 1; b) Experiment 2; c) Experiment 3. (Bars represent +/- SD n=3).

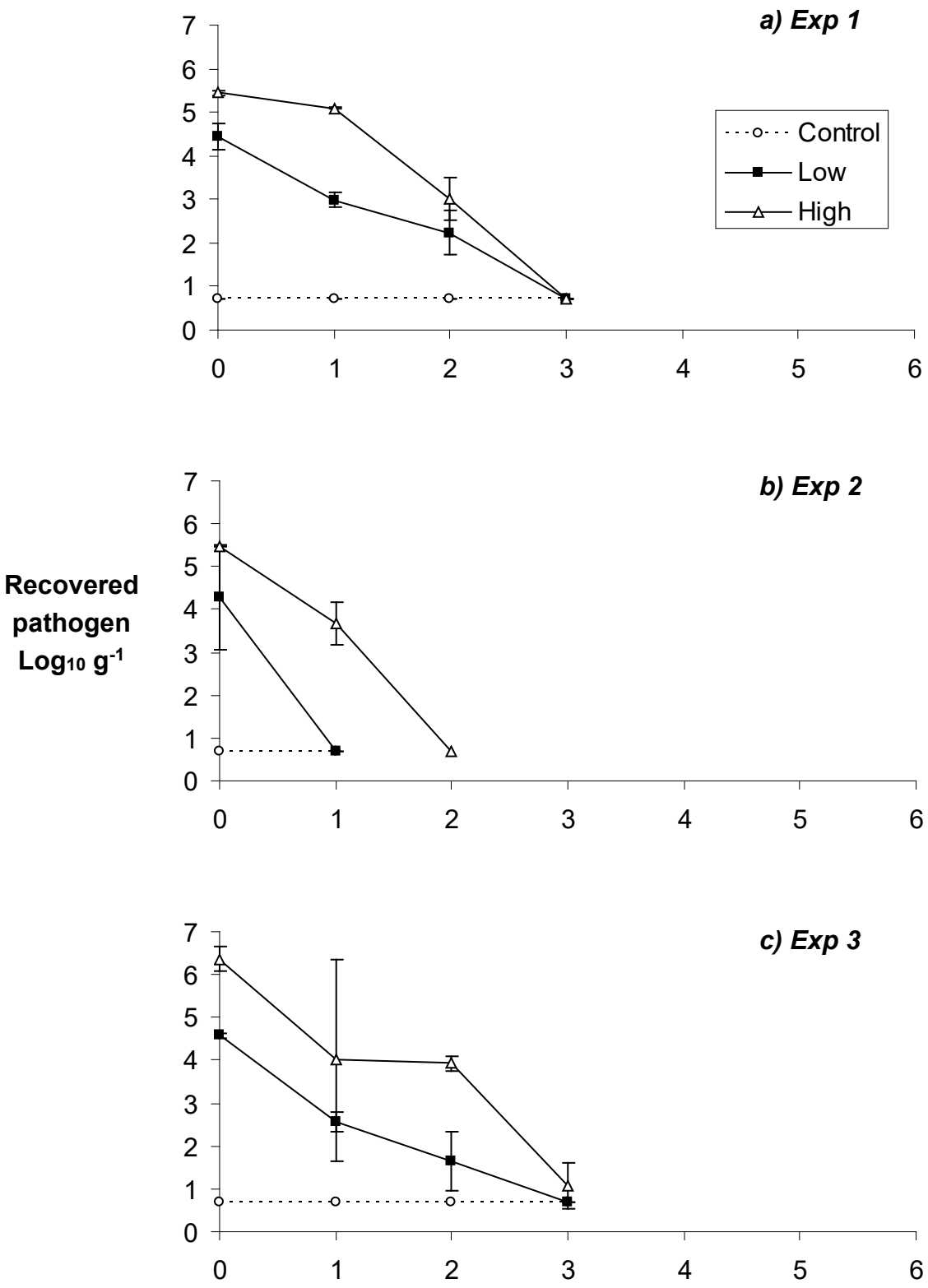

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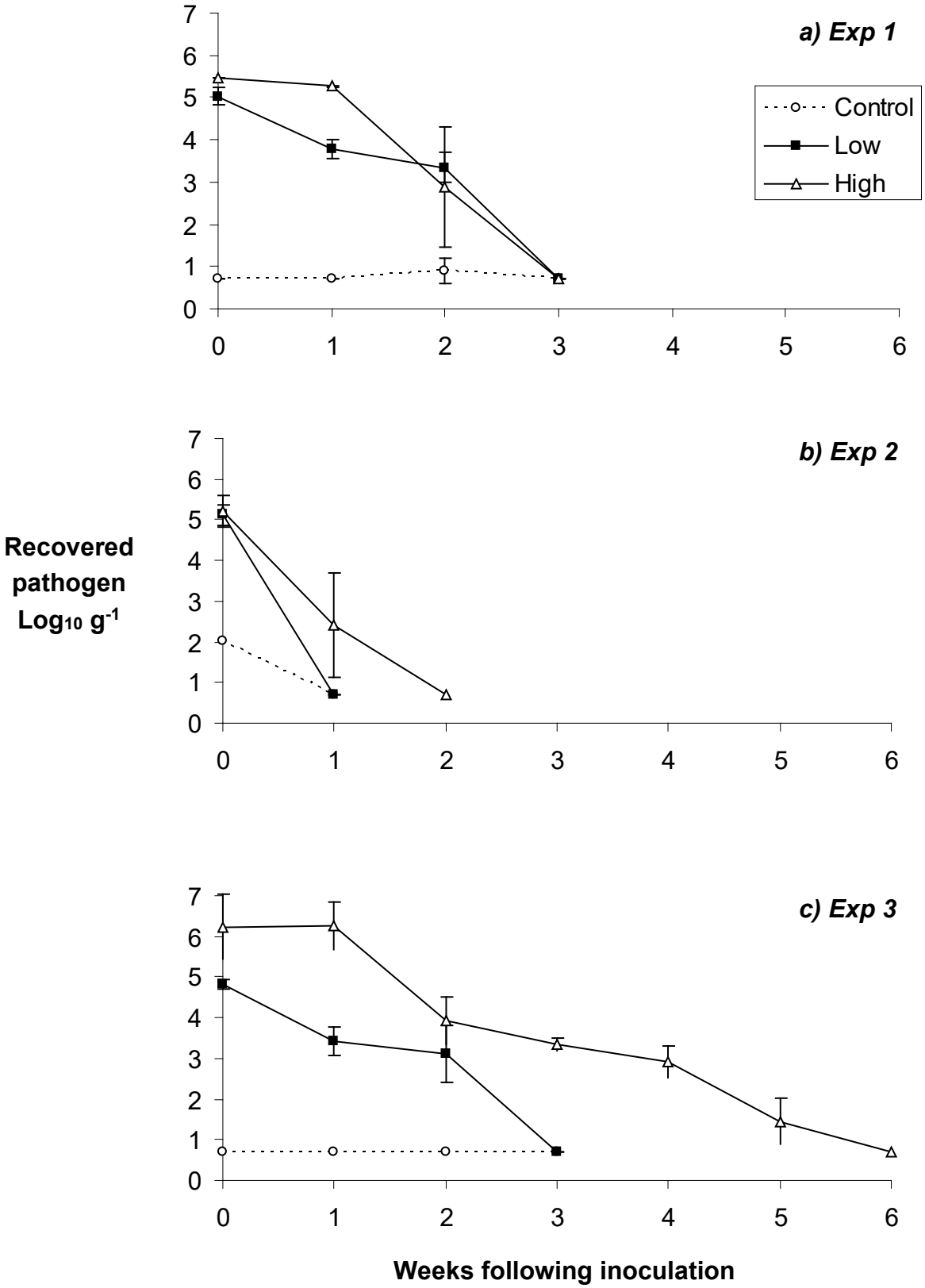
Figure 2. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of* Salmonella enteriditis *applied to the surface of mineral soil: a) Experiment 1; b) Experiment 2; c) Experiment 3.(Bars represent +/- SD n=3).



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3.1.2 E. coli O157

A low level of contamination was observed in the controls - week 1 in the peaty soil and initially (week 0) in the mineral soil in experiment 2. The numbers of *E. coli* O157 recovered at the start of each experiment was greater for the high treatment for all experiments with, as observed for *Salmonella*, a greater difference between the low and high treatments when inoculation was into the peaty soil.

a) Peaty soil

E. coli O157 numbers for the low treatment had fallen below the detection threshold within 2 weeks for experiments 1 and 3. Experiment 2 had a more rapid decline, and the numbers of *E. coli* O157 in the low treatment had fallen below the enumeration threshold in less than one week (Figure 3). By the end of experiments 1 and 2, *E. coli* O157 was absent in two of the low treatment three plots. In contrast, *E. coli* was present in two of three low treatment plots by the end of experiment 3 (Table 4). As expected, the high treatment was more persistent and at the end of all three experiments *E. coli* was still detected in all three plots (Table 4).

b) Mineral soil

In general, *E. coli* O157 was more persistent in the mineral soil than peaty soil. A similar pattern of decline was observed for both soils in experiment 2. In contrast to the peaty soil data, the numbers of *E. coli* O157 present in the low treatment plots declined to <5 cfu g^{-1} after 4 weeks in experiment 1 and 3 weeks in experiment 3 (Figure 4). At the higher level of inoculation extended persistence was observed. A pronounced tailing of the survival curve was observed during experiment 3 where similar levels, about $1x10^3$ cfu g^{-1}, of *E. coli* O157 were recovered from weeks 3 to 6; when the experiment was ended. *E. coli* O157 was still detected in the mineral soil in all three high treatment plots at the end of all three experiments (Table 4). Interestingly, no *E. coli* O157 was detected in the low treatment plots in experiment 2, but contaminated plots were detected at the end of experiments 1 and 3.

Table 4. Number of plots (n=3) with E. coli O157 detected at end of trial

	Experiment 1 +5 weeks		***Experiment 2*** +3 weeks		***Experiment 3*** +6 weeks	
	Peaty	Mineral	Peaty	Mineral	Peaty	Mineral
Low treatment	1/3	3/3	1/3	**0/3**	2/3	2/3
High treatment	3/3	3/3	3/3	3/3	3/3	3/3

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Figure 3. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of E. coli 0157 applied to the surface of peaty soil: a) Experiment 1; b) Experiment 2; c) Experiment 3.(Bars represent +/- SD n=3).

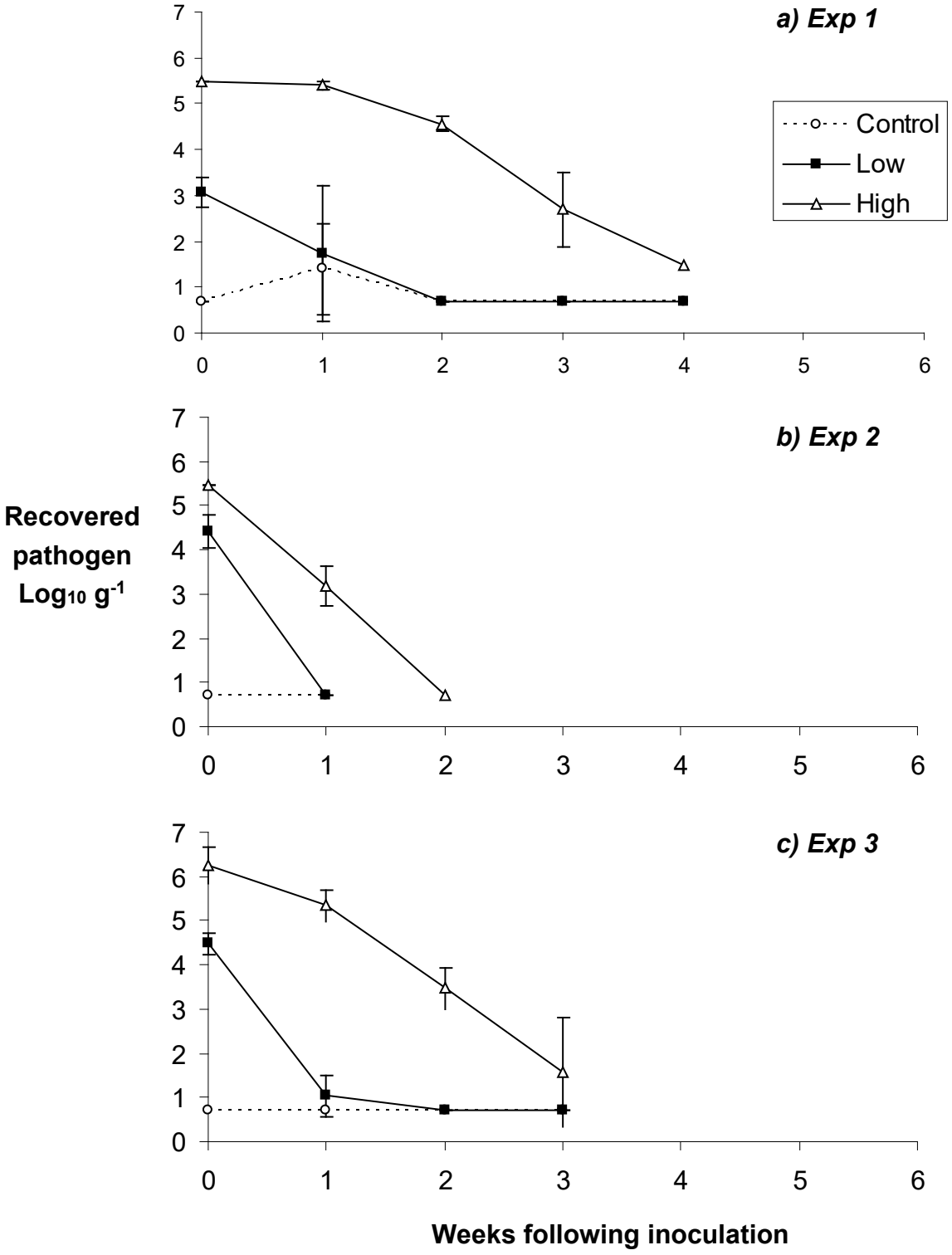

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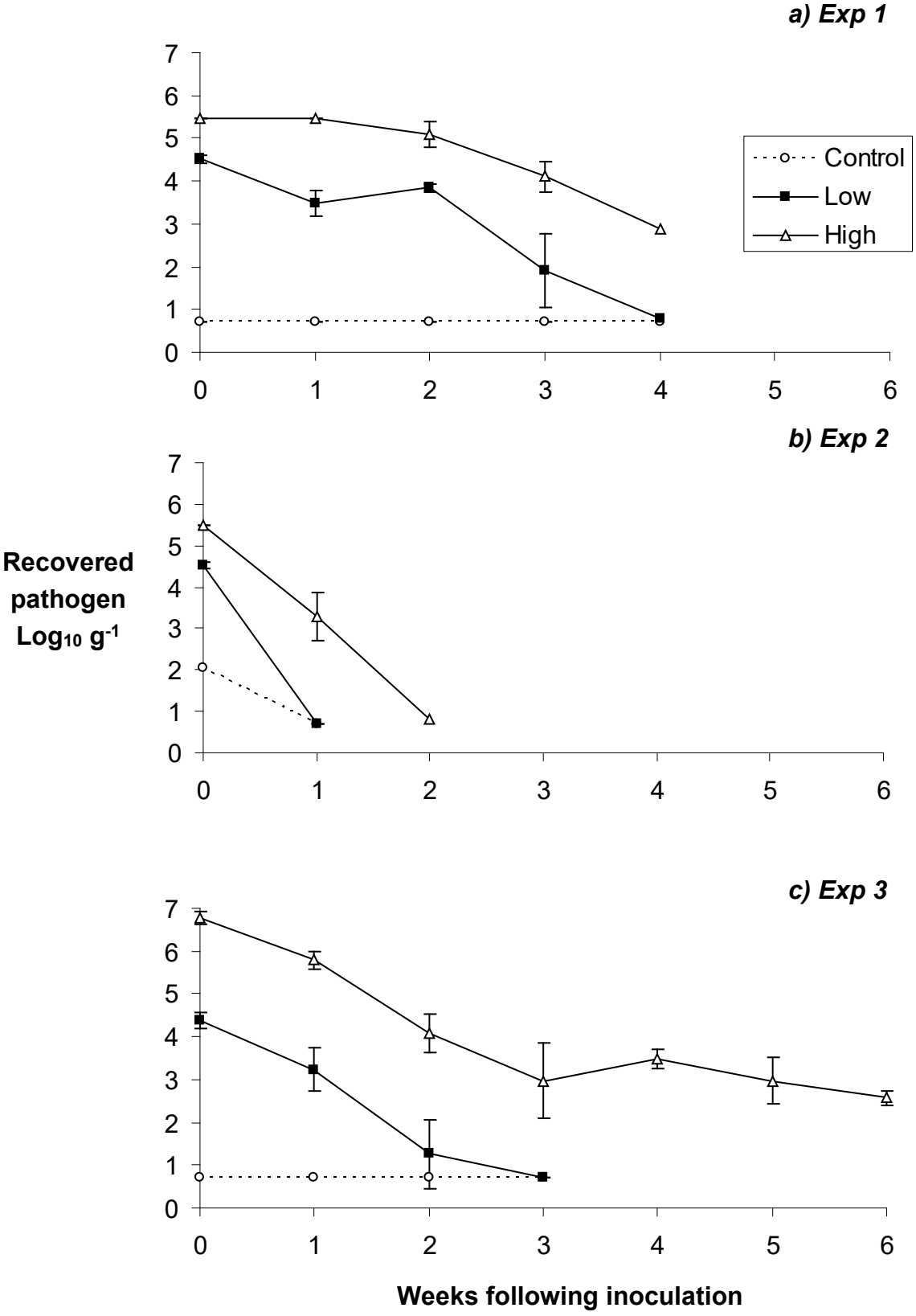
Figure 4. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of E. coli 0157 applied to the surface of mineral soil: a) Experiment 1; b) Experiment 2; c) Experiment 3.(Bars represent +/- SD n=3).



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3.1.3 Campylobacter jejuni

No *Campylobacter* was recovered from the soil in experiment 1, although microscopic inspection of the culture prior to application revealed viable cells with characteristic energetic corkscrew motility. No contamination of the controls was detected in the experiments.

a) Peaty soil

In experiment 2 for both soil types, the numbers of *C. jejuni* were <5 cfu g^{-1} after 1 week. No *Campylobacter* was detected after 3 weeks (Table 5). *Campylobacter* was more persistent during the lower light level and moderate rainfall conditions experienced during experiment 3. Although the levels of *Campylobacter* had declined to close to the level of enumeration after 3 weeks at the high treatment (Figure 5) *Campylobacter* was still detectable in all three plots after a further 3 weeks (Table 5).

b) Mineral soil

The results from experiment 2 were identical to those observed in the peaty soil. However, in experiment 3, as with the other pathogens studied, *Campylobacter* was markedly more persistent in the mineral soil. Although numbers of *Campylobacter* in the soil declined slowly at the high treatment in experiment 3; Campylobacter's were still present at $1x10^2$ CFU g^{-1} by the end of the experiment. By the end of experiment 3 *Campylobacter* was present in all three plots of both the high and low treatments (Table 5)

Table 5. Number of plots (n=3) with Campylobacter jejuni detected at end of trial

	Experiment 1		***Experiment 2***		***Experiment 3***	
	+5 weeks		+3 weeks		+6 weeks	
	Peaty	Mineral	Peaty	Mineral	Peaty	Mineral
Low treatment	-	-	**0/3**	**0/3**	1/3	3/3
High treatment	-	-	**0/3**	**0/3**	3/3	3/3

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Figure 5. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of Campylobacter jejuni applied to the surface of peaty soil: Experiment 3.(Bars represent +/- SD n=3).

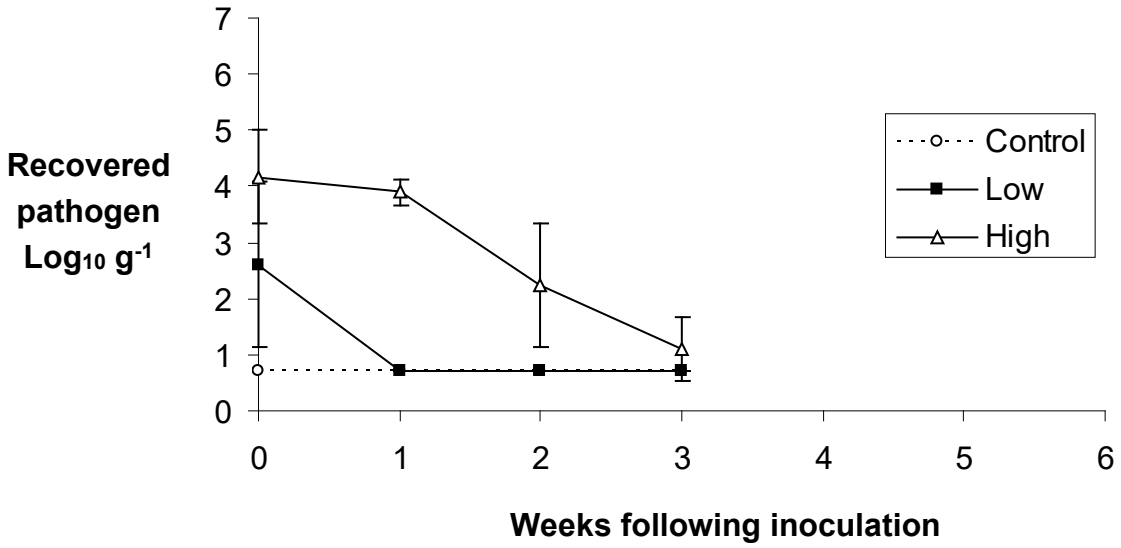
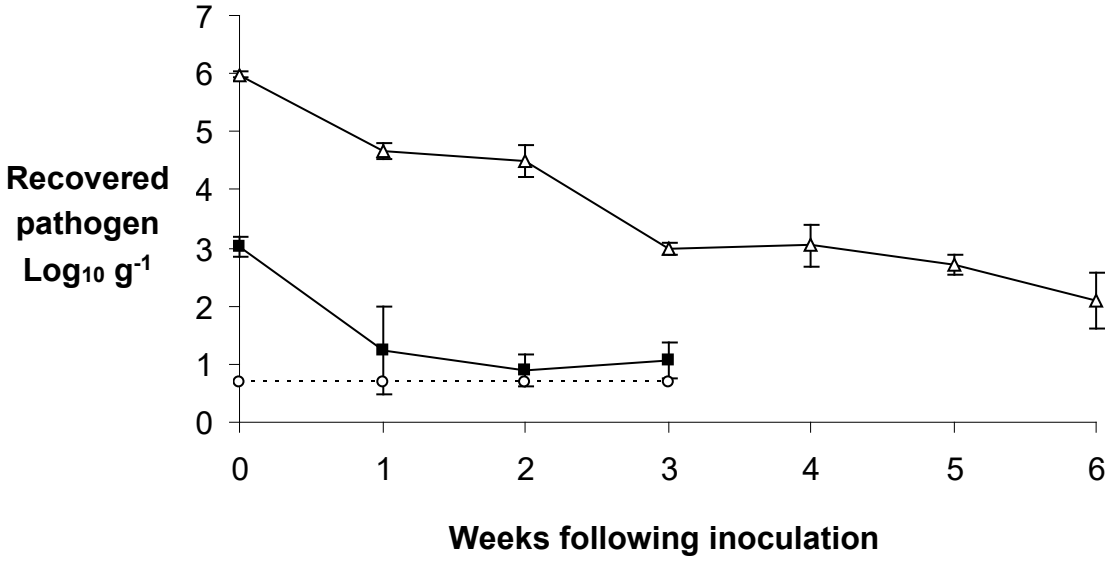


Figure 6. Recovered pathogen from the soil surface following inoculation with Control, Low and High levels of Campylobacter jejuni applied to the surface of mineral soil: Experiment 3.(Bars represent +/- SD n=3).



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4 Discussion

The weather conditions during the three experiments provided contrasting conditions typical of early, mid and late season field production: experiment 1 started as cold and wet conditions and developed to be warm and dry with increasing sunlight levels; experiment 2 was hot and dry with high levels of sunlight; experiment 3 was wet with reducing light levels and temperatures as it progressed.

It was notable that the initial levels of pathogen recovered from soil were higher than expected. Plots of soil were contaminated with water containing approximately 1×10^2 and $1x10^5$ CFU ml^{-1} for the low and high treatments respectively, but the levels recovered were in the region of 2 logs higher for the low treatments and 1-2 logs higher for the high treatments (where not limited by the upper level of detection – see section 4.1). This accumulation of target bacteria could be explained by some binding of bacteria to soil components, with water draining from the surface to leave a higher concentration of bacteria. The consequence of this accumulation of bacteria at the surface is not clear as, although there is a greater concentration of bacteria posing a risk of contamination to growing crops, the surface of the soil is exposed to high levels of UV from sunlight, periods of drying out in warm conditions and a marked diurnal temperature cycle. All of these factors are likely to be conducive with rapid reductions in bacterial numbers. The distribution of bacterial zoonotic agents will be further studied in a small pot trial in year 2.

Does season have an effect on pathogen persistence?

Season had a marked effect on persistence of introduced bacteria. The hot, dry and high sunlight conditions of experiment 2 were associated with the fastest observed reduction in bacterial numbers recovered from the surface of the soil. *Campylobacter* declined most rapidly. All three potential human pathogens were at or below the threshold for reliable enumeration after 2 weeks. In contrast the cooler, wetter and lower light levels experienced in experiment 1 and experiment 3 were associated with a greater and more prolonged persistence of pathogens. Persistence was particularly marked in experiment 3 where *E. coli* O157, *Salmonella* and *Campylobacter* were still present in the surface of all plots after 6 weeks. A similar response was observed in experiment 1 after 5 weeks for *E. coli* O157 and *Salmonella*.

Field work carried out in Georgia, USA has shown that following application through irrigation water in October *E. coli* O157:H7 can persist in soils for up to 200 days (Islam *et al*., 2004a & 2005) and *Salmonella enterica* Typhimurium for 161 days (Islam *et al*. 2004b). The same workers reported that 6 weeks after the application of contaminated irrigation water, comparable to the high treatment studied in experiment 3, *E. coli* and

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Salmonella were being recovered from soil at levels of $1x10^3$ and $1x10^2$ CFU g^{-1} respectively. These levels are in general agreement with the data from experiment 3 in the mineral soil with low organic matter; although climactic conditions in GA are markedly different from those encountered in the UK.

It is clear that under commercial production systems there is the possibility that pathogens introduced to the soil at high levels through irrigation water can persist for the duration of a lettuce crop, particularly with the environmental conditions commonly experienced at the very start and end of the season. This can be viewed as a worst case scenario.

Under conditions where there is the greatest requirement for irrigation i.e. hot and dry with strong sunlight the decline of introduced pathogens is much more rapid and a moderate level of contamination would be undetectable after approximately 3 weeks. We have so far been unable to find literature reporting pathogen persistence in soil from field experiments in summer conditions. Thus the results of this study are novel, and can be used to properly inform regulators as to the realistic risks of contamination of fresh produce with contaminated irrigation water.

Does soil type have an effect on pathogen persistence?

Soil type had a significant effect on the persistence of introduced zoonotic agents particularly at the high level of contamination. There was a more rapid decline in all three pathogens in the peaty soil with the higher organic matter content when compared to the mineral soil in experiments 1 and 3. It has been stated that the key factor effecting pathogen survival in soils is moisture (e.g. Jamieson *et al.* 2002) and limited soil moisture reduces persistence of enteric pathogens including *Salmonella typhimurium* and *E. coli* in dry soils (Chandler and Craven, 1980). However, the trial plots were not irrigated in this work so any differences in persistence related to moisture would be down to water retention by the soils. Although both soils were silty soils, of the two soils the peaty soil would be expected to retain more water as it had a higher organic matter content (9% versus 2%) suggesting that the pathogens should have had the greater survival in this soil, contrary to observation.

An alternative explanation, fitting the observed response, is that the higher organic matter of the peaty soil was associated with a higher indigenous microbial population i.e. a more biologically active soil, and that antagonistic interactions with indigenous microbial populations, and predation by amoebic organisms were influencing soil survival. Support for this comes from work showing that *E. coli* O157 (Jiang *et al.* 2002) and *Salmonella enterica* Newport (You *et al.* 2006) both persisted longer in autoclaved (sterilised) soils compared to unautoclaved soils. Further soil studies are needed to

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establish the role of soil type on pathogen survival and whether it is of practical importance in field production of ready to eat crops.

What is the risk of introducing a reservoir of viable pathogen that may contaminate produce at harvest?

The experiments demonstrated that applying contaminated irrigation water to soils introduces a risk to ready to eat produce grown in that soil. The persistence of the bacterial contamination was related to the level of initial contamination, with the high level of contamination persisting longer than the low level in all cases. In general, *E. coli* O157 was the more persistent bacteria studied, both in terms of the time taken to reduce to limit of enumeration and prolonged persistence by the end of the experiments. Clearly there is at least a hypothetical risk of infecting crops by soil contamination – either through rain/irrigation splash or harvesting - and this work would suggest that the risk is greatest at the start and end of the season. Nevertheless, this work needs to be seen as a preliminary study giving indications of potential risk. True risk to the consumer is however through consumption of a contaminated crop and not the soil used to cultivate produce. Data outlining true risk will be determined and reported during year 2 of this study.

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5 References

Adak G. K., S. M. Meakins, H. Yip, B. A. Lopman and S. J. O'Brien. (2005) Disease risks from foods, England and Wales, 1996-2000. *Emerg. Infect. Dis.* 11(2):365-372.

Brandl, M.T., Haxo, A.F., Bates, A.H. and Mandrell, R.E. (2004) Comparison of survival of *Campylobacter jejuni* in the phyllosphere with that in the rhizosphere of Spinach and Radish plants *Appl. Env. Microbiol*. 70(2):1182-1189

Chandler, D.S. and Craven J.A. (1980) Relationship of soil moisture to survival of *Eschericia coli* and *Salmonella typhimurium* in soils. Australian Journal of Agricultural Research 31(3):547-555.

Hutchison, M. L., Walters, L. D., Moore, A., Thomas D. J. I. and Avery, S. M. (2005). Fate of pathogens present in livestock wastes spread to fescue plots. *Appl. Env. Microbiol.* 71:696-696.

Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P. and Jiang, X. (2004a). Fate of enterohemorrhagic *Escherichia coli* 0157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *Journal of Food Protection* 67(7):1365-1370

Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P. and Jiang, X. (2004b). Persistence of *Salmonella enterica* Serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease* 1(1):27-35

Islam, M., Doyle, M.P., Phatak, S.C., Millner, P. and Jiang, X. (2005). Survival of *Escherichia coli* 0157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. *Food Microbiology* 22(1):63-70

Jiang, X., Morgan, J. and Doyle, M.P. (2002) Fate of *Eschericia coli* O157:H7 in manure-amended soil. *Appl. Env. Microbiol.* 68(5):2605-2609

Palacios, M.P., Lupiola, P., Tejedor, M.T., Del-Nero, E., Pardo, A. and Pita, L. (2001) Climatic effects on *Salmonella* survival in plant and soil irrigated with artificially inoculated wastewater: preliminary results. *Water Science and Technology* 43(12)103-108

Stine, S.W., Song, I.H., Choi, C.Y. and Gerba, C.P. (2005) Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection*. 68(5):913-918

Tyrrel S (2004) FV 248 Assuring the microbiological quality of water used to irrigate salad crops: an assessment of the options available. *HDC report*.

You, Y., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D. and Dou, Z. (2006) Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl. Env. Microbiol.* 72(9):5777-5783

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6 Appendix

Figure I. Weather data measured at the experimental site after the inoculation of soils (day 0 = 10 May 2006): a) Daily average soil temperature (5 cm) and accumulated sun light; b) Daily rainfall. Experiment 1, Year 1.

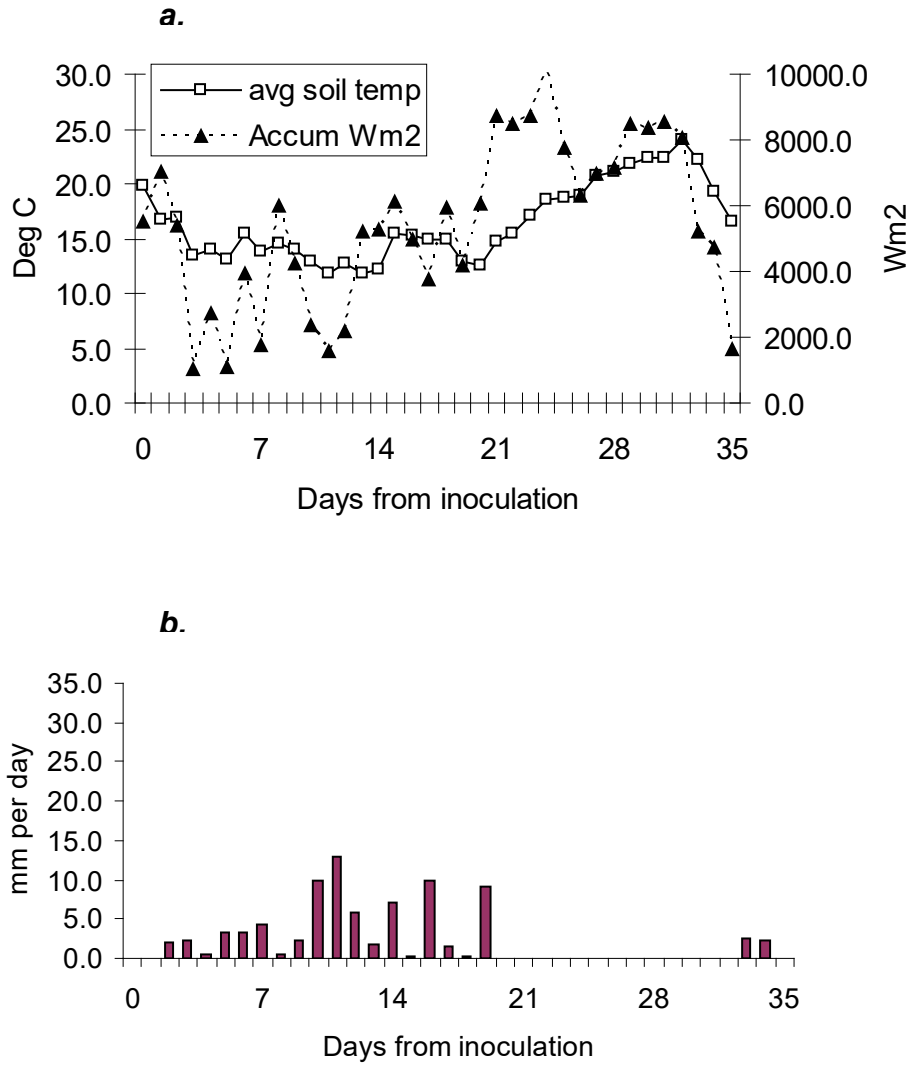

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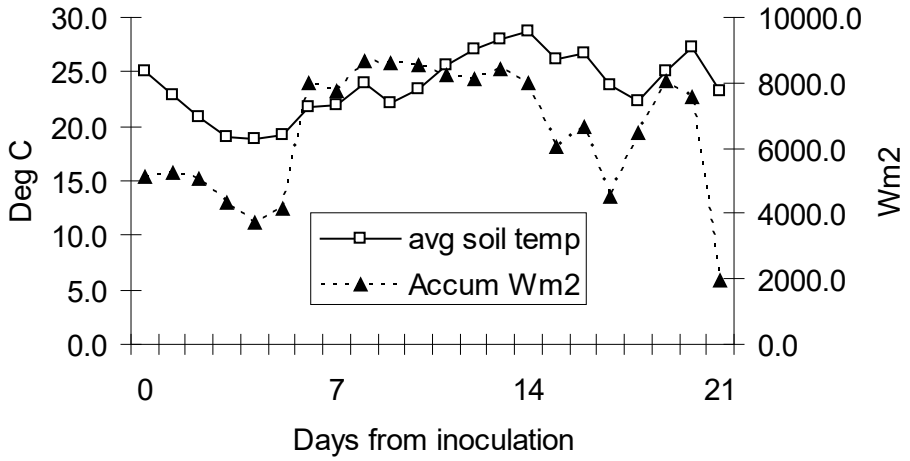
Figure II. Weather data measured at the experimental site after the inoculation of soils (day 0 = 5 July 2006) : a) Daily average soil temperature (5 cm) and accumulated sun light; b) Daily rainfall. Experiment 2, Year 1.

a.



b.

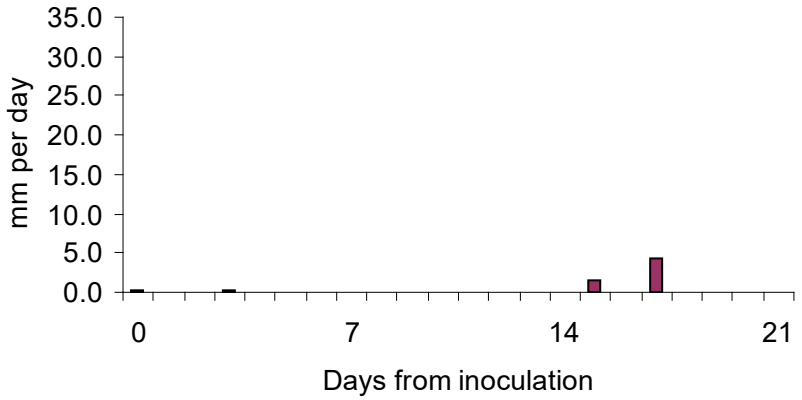


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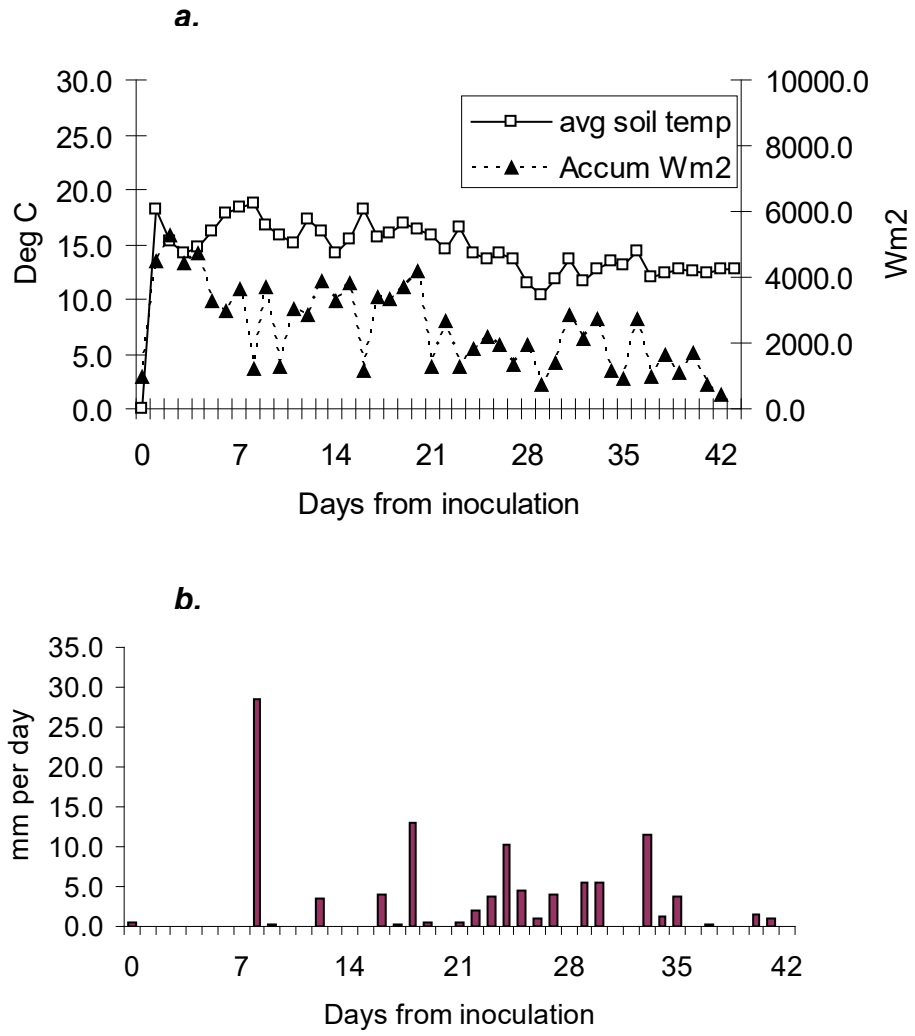
Figure III. Weather data measured at the experimental site after the inoculation of soils (day 0 = 6 September 2006): a) Daily average soil temperature (5 cm) and accumulated sun light; b) Daily rainfall. Experiment 3, Year 1.



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