PROJECT CP 19c - The effects of the growing environment on the multiplication and survival of human pathogens on vegetable crops

Headline

The growth of lettuce under enhanced UV radiation (plastic tunnels) can reduce the number of bacteria on leaves which has positive implications for the risk management of human pathogens on salad crops.

Removal of outer leaves reduces potential levels of contaminants.

Background and expected deliverables

Outbreaks of human disease attributed to the consumption of contaminated fruit and vegetables continue to be a significant problem (Jones and Heaton 2007). Last year (2006) both Canada and Mexico threatened to ban imports of salad vegetables from the US because of wide spread publicity over contamination of spinach with *Escherichia coli* 0157. Recent major outbreaks are shown in table 1. Whilst there have not been major outbreaks linked to UK grown produce, growers here are keen to pursue the topic and maintain best practice to ensure they are keeping their crops clean.

Table 1 Outbreaks of food poisoning from fresh produce 2004-2006

Causal organism	**Crop**	**Number**	**Country of cases**	**Source**
2006				
Escherichia coli 0157:H7	Spinach	199	USA	Irrigation water
Salmonella Typhimurium	Tomatoes	185	USA and Canada	?
S. Newport	Tomatoes	106	USA	?
E. coli 0157:H7	Lettuce	77	USA	?
E. coli 0157:H7	Lettuce	87	USA	?
4 outbreaks of norovirus	Raspberries	43	Sweden	Chinese imports
2005				
E. coli O157:H7	Lettuce	120	Sweden	Stream water
Norovirus	Raspeberries	500	Denmark	Polish imports
Norovirus	Raspeberries	5	France	Unknown imports
S. Typhimurium DT104	Lettuce	96	UK	Spanish imports:
		56	Finland	Wastewater
S. Javiana and	Tomatoes	561	USA and	Post harvest
S. Braenderup			Canada	washing
2004				
S. Thompson	Rocket	12	Norway	Italian imports
S. Newport	Iceberg lettuc	386	UK	Unknown source

Hepatitis A	Spring onions	400	USA	Poor hygiene durin harvest

In this project we have investigated the ways in which food poisoning bacteria attach to and persist on the intact leaves of growing crop plants, focusing on:

1. UV radiation, which is a major factor in controlling bacteria.
2. Interactions between *Salmonella* and microbes normally found on the leaf.

The expected deliverables include:

1. Better understanding of how food poisoning bacteria attach to intact leaves of lettuce on the growing plant. This is important because most studies of attachment of bacteria to lettuce have been with cut or damaged plants.
2. Whether growth under enhanced UV radiation is useful in reducing the number of food poisoning bacteria on lettuce.

Summary of the project and main conclusions

<u>Faecal coliform contamination of lettuce at point of sale in different retail outlets</u>

Faecal coliforms are bacteria that are found in large numbers in the faeces of humans and warm blooded animals. This is in contrast to disease-causing bacteria, such as *Salmonella* and *E. coli* 0157:H7, which are present in low numbers and are difficult and expensive to detect. The presence of faecal coliforms in food or aquatic environments is used as an indicator (surrogate) for faecal pollution and the probable presence of enteropathogens.

In this study point of sale samples were initially taken over a 12-month period, from March 2004 - February 2005, from an organic cooperative, a major supermarket chain and a local greengrocer. This data set was then extended to include samples from a second supermarket, a market, a convenience store and organic samples from a supermarket. This extended work was completed between Feb-July 2005 and ensured that at least 55 samples were taken from each outlet. Across the whole sampling period the following total number of samples were taken from each point of sale - Supermarket 91; Greengrocer - 73; Organic- 63; Convenience store - 55; Market-60; Supermarket - 60; Supermarket organic- 60

At each sampling point, three separate heads of lettuce were randomly selected. Samples were rinsed under running water to remove obvious soil contamination and a representative sub-sample from these 3 heads, including both inner and outer leaves was weighed (25g), mixed with sterile buffer (1:10 dilution) and then examined for faecal coliforms.

The results in Figure 1 show mean values across the whole sampling period for each point of sale. These results show relatively low levels of faecal coliform contamination with the highest levels recorded from the organic cooperative. Although these results are encouraging, it indicates that even washed produce at point of sale has signs of faecal contamination.

Figure 1: Faecal coliform numbers on lettuce samples taken over a 12-month period.

The Commission Regulation on Microbiological Criteria for Foodstuffs (2073/2005/EC) state that for fresh produce to be rated as: -

- *<u>Satisfactory</u>, E. coli (faecal coliforms) numbers must be less than or equal to 100 colony forming units (CFU) in a 25g sample, in 5 of 5 samples taken.*
- *<u>Acceptable quality</u>, 2 of 5 samples must yield between 100-1000 CFU and the remaining 3/5 less than or equal to 100 CFU.*
- *<u>Unsatisfactory</u> results are obtained when 1/5 is greater than 1000 CFU or more than 2 samples yield 100-1000 CFU.*

The current guidelines for microbial quality of fresh produce, expect that 5 samples are taken at each sampling point, and 25g of each is assessed separately.

Other regulations based on faecal coliform numbers include the EU Bathing Water Quality Directive, which states that bathing waters must not exceed values of 2000 faecal coliforms per 100ml in 95% of samples, and the WHO standards for irrigation water, which state that irrigation water should not exceed 1000 faecal coliforms per 100ml.

The effects of contrasting UV growth environments on bacterial contamination of growing lettuce

This work was done at the field site in Cawood, East Yorkshire (STC). Lettuce (*Lactuca sativa* cv. Rex) was propagated under plastics (UV-transparent and standard horticultural), in a glasshouse (UV-opaque treatment) and in a field plot. These conditions provided extremes of UV exposure and represent common growing conditions used in the cultivation of salad crops.
Representative plants from each treatment were tested for bacterial contamination (total viable count).

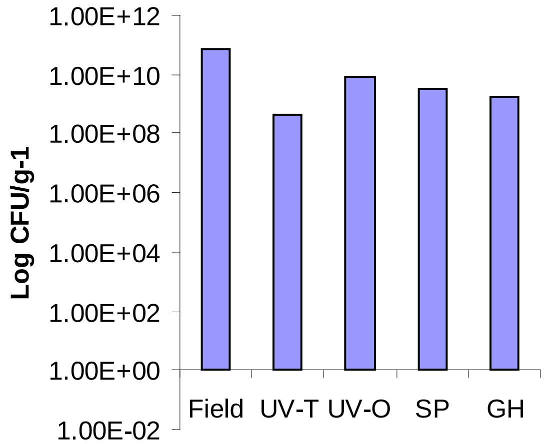
Lettuces Grown Under UV-Transparent Spectral Plastic

The results (fig 2) show that the highest bacterial numbers were found in lettuce grown in the open field, followed by growth in UV opaque, standard horticultural plastic, glasshouse and UV transparent poly tunnels. Therefore, UV appears to reduce the level of bacterial contamination on lettuce grown under cover but any UV effects in the field, which has the highest UV levels, are probably negated by increased exposure to contamination from the environment.

Figure 2: Bacterial count (colony forming units/g-1 fresh weight) of control field samples

Field samples were propagated under a range of conditions: open field (field), ultraviolet transparent plastic (UV-T), ultraviolet-opaque plastic (UV-O), standard horticultural plastic (SP) and glasshouse (GH).

The mean background counts at day 35 were significantly lower under UV-T.



However, over the sampling period, no significant differences were shown overall. This data does not imply that UV-T can be used to reduce microbes, but rather that the natural leaf microflora is limited by the UV-T and this may impact on the survival of enteropathogenic bacteria introduced to the leaf. This may happen because:
a) immigrant bacteria are less likely to attach well to the leaf surface if bacterial aggregates are not present,
b) there is likely to be reduced protection from the 'biofilm' of natural leaf bacteria which protect the immigrant bacteria.
Overall, bacteria contamination of lettuce crops grown in the field or under cover is much greater than found in lettuce at point of sale.

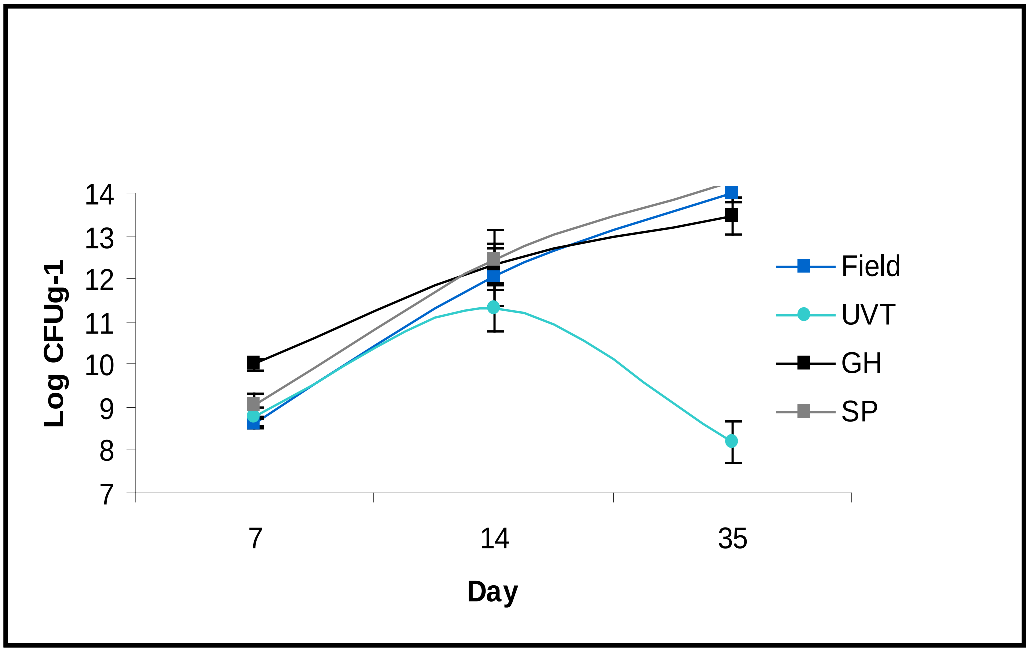
The field trial at STC also showed that microbial loads are higher on field crops when wrapper leaves are not removed and suggest that the bulk of the microbial load is contained on these leaves rather than the heart leaf.

<u>Effect of growth in different UV regimes on the persistence of bacteria on lettuce in the field</u>

Lettuce plants were grown in the same UV conditions at in the previous experiment and were harvested at days 0, 7, 14 and 35 (day 35 represented commercial harvest).
The results (figure 3) show that at harvest, counts on lettuce exposed to UV (under UV-T) were significantly lower than in the other treatments. The differences between populations under UV-T and the open field crop suggest that other factors, for example, irrigation strategies and contamination from the environment, may also have an impact on the number of contaminating bacteria.

Figure 3: Total viable count epiphytic microorganisms (Log colony forming units/g-1 fresh weight) isolated from lettuce samples, days 7-35.

Growing environments were open field (field), ultraviolet-transparent plastic (UV-T), glasshouse (GH) and standard horticultural plastic (SP).



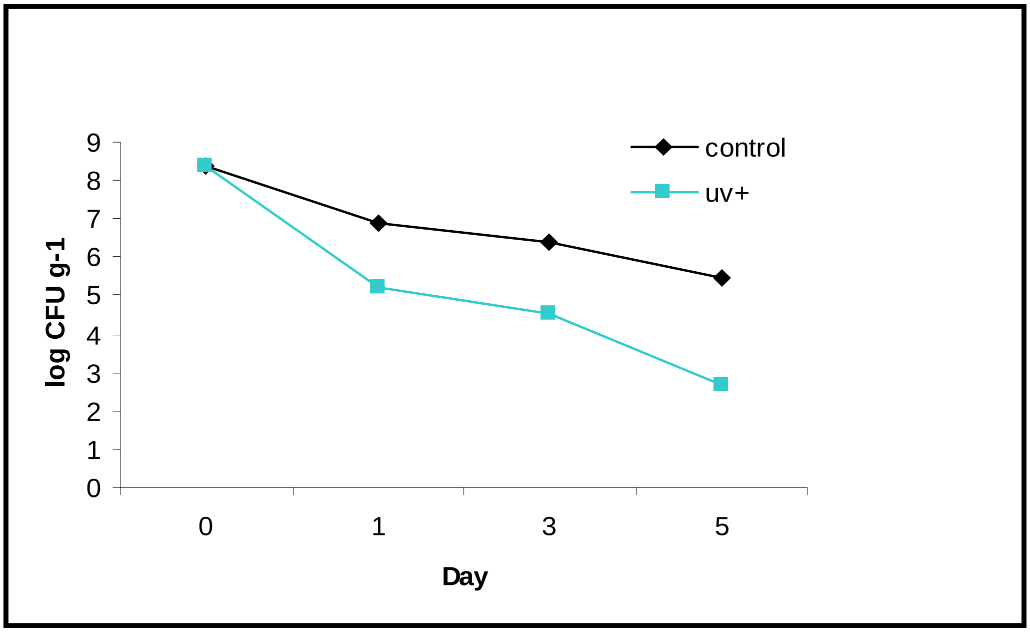
NB: Although it was demonstrated that UV had some effect in altering bacterial numbers on lettuce at STC, there were problems doing the bacteriological analyses at the field site and so work was done in the more controlled conditions at Lancaster University.

Short-term laboratory experiments with watercress

The microbial quality of watercress grown in irrigation water was used as a test of the microbial quality of that water. In this experiment, watercress was exposed to different levels of UVB radiation in short term experiments in the laboratory to determine whether UV affected the numbers of natural contaminating bacteria of the leaves.

The results (figure 4) showed that bacterial numbers declined by 5 log over 3 days with increased UVB.

Figure 4. Total bacterial viable count (colony forming units/g^{-1} fresh weight) on controls and watercress exposed to increased UV-B (UV+), under controlled conditions, days 0-3.

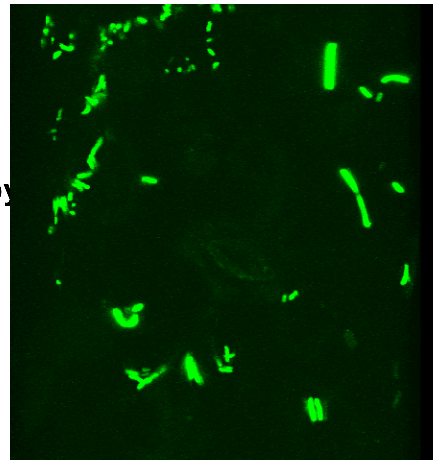


1. Which part of a lettuce leaf is most contaminated?

This work has mainly involved the use of a confocal microscope to determine the location of bacteria on the leaf surface. For this, the bacteria need to be made visible under the microscope using a stain. Initially, *Salmonella* expressing a green fluorescing protein (gfp) was used while techniques were optimised. However, as this is a genetically modified bacterium it could not be used in the field, so the work was done in the laboratory.

The results show that *Salmonella* congregate around the stomata of the leaf and attach preferentially to damaged surfaces. Damaged tissues leak nutrients that attract bacteria such as *Erwinia* and *Pantoea*, which in turn attract colonisation and growth by disease-causing *E. coli* and *Salmonella*. Some bacteria migrate into the internal tissues of crop leaves after entry via stomata. This work is ongoing and we are optimising methods using other types of stain to differentiate the naturally occurring bacteria on the leaves from introduced *Salmonella*.

GFP *E. coli* on lettuce observed
using confocal laser scanning microscopy



2. Does incorporation into biofilms on the leaf surface or association with naturally occurring bacteria prolong *Salmonella* survival in the field?

New techniques are currently being developed to enable us to determine whether introduced bacteria can integrate in the biofilm community (the naturally-occurring bacteria adapted to attachment and growth on leaves).

Preliminary results show that *Salmonella* preferentially attaches to bacteria already present on the leaf surface, rather than to the leaf itself. Therefore, paradoxically, a less colonised leaf may offer fewer opportunities for *Salmonella* to establish. Previously we had assumed that the smaller the number of indigenous bacteria on the leaf, the more space was available for colonisation by *Salmonella*.

Other work at Lancaster University has shown that *Salmonella* not only 'prefers' attachment to other bacteria when colonising a surface, it is also more metabolically active when in a biofilm with other bacteria. *Salmonella* is not only more metabolically active when attached to lettuce, the genes necessary for causing disease in humans are switched on when the bacterium attaches to lettuce. This means that lettuce containing *Salmonella* might have a low infective dose and goes some way to explaining the extent of the outbreaks listed in table 1.

3. What are the effects of UV radiation on *Salmonella* when suspended in water and when attached to a biofilm on a leaf surface?

Our most recent studies show that *Salmonella* survives for much longer when attached to a leaf surface in a biofilm than when unattached in water. Therefore, survival studies for *Salmonella* need to take this into account.

4. Are enteropathogens internalised inside plants?

Reports that pathogenic bacteria, such as *Salmonella* and *E. coli* 0157, can become internalised within healthy plants have caused consternation.

We investigated this phenomenon using water cress, but the results indicate that bacteria are only able to reach the internal tissues if the root systems are damaged in some way.
Internalisation does not seem to be an issue in the field but may still be relevant in processing conditions when the natural barriers of the crop are compromised.

Financial benefits
There are no financial benefits to be gained from growers from this work.

Action points for growers
Growth of lettuce under UV transparent plastic reduced the overall level of bacterial colonisation of the crop and has the potential to reduce the level of bacterial pathogens transferred to the crop from the environment**.**

References

Readily available publication on the background to this project on the Internet: -

Jones, K. and Heaton, J.C. Microbial contamination of fruit and vegetables. Health Protection Matters (issue 7, spring 2007), 28-31. (Available on the Health Protection Agency web site)

Heaton, J.C. and Jones, K. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. Journal of Applied Microbiology (2007). This article is in press, and a pre-publication version is available online – http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2672.2007.03587.x