Title	Protected lettuce: biology and control of bacterial leaf rot (petiole blackening)
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The results and conclusions in this report are based on a series of experiments conducted over a 16-month period. The conditions under which experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the result, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report presents a true and accurate record of the results obtained.

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Headline

A postal survey of UK lettuce growers suggests bacterial leaf rot, a sporadic problem in protected lettuce, has been gradually increasing since 1993 and is mainly seen in mild autumns, in maturing crops, close to harvest. *Pseudomonas* bacteria of the *fluorescens marginalis* complex are generally found associated with the disease. It is suggested that, 'soft', fast-growing plants become infected with *Pseudomonas* species, probably originating from irrigation water or the soil.

Background and expected deliverables

Petiole blackening caused by bacterial infection was widespread in English lettuce crops from November 2000 - March 2001 and over the same period in 2001/02. On some nurseries all the plantings during October were badly affected. This problem has become increasingly worse over the last 4 years. In many crops a high incidence of plants were affected (up to 50%), often with one or two petioles in the plant centre discoloured and a high proportion of plants were thus unmarketable. Up until the mid 1990s only isolated plants have been affected, usually with just one or two basal leaves which could be trimmed off and nearly always in winter crops, though occasionally at this level in some summer crops. Isolation from blackened petioles generally resulted in recovery of bacteria, most commonly *Pseudomonas* isolates *fluorescens/marginalis* complex, on opportunistic pathogen.

The expected deliverables from this project are:

- A greater understanding of an unpredictable disease through collation of growers' observations of the problem
- Identification of bacteria associated with leaf rot and conditions under which the disease symptoms develop

Summary of the project and main conclusions

Review of lettuce bacterial soft rot

Pseudomonas isolates of the *fluorescens/marginalis* complex and *Erwinia carotovora* are the bacterial groups most commonly associated with lettuce soft rot. Both are widespread soil-borne organisms and may be found on lettuce leaves in the absence of disease symptoms as part of the normal surface microbiological flora. Under favourable field or storage conditions, surface populations of these soft-rot bacteria may increase rapidly and cause decay of lettuce tissue.

In the UK, *Ps. marginalis* has been described as a cause of sudden wilt and butt rot in lettuce. But the dark discoloration of the midrib (petiole blackening) of one or more petioles (often on the inner leaves), with no apparent wilting or butt rot, has not been formally reported as a symptom of bacterial wilt or butt rot.

Isolations from such symptoms, which have been on the increase since 1993, in ADAS and other Plant Clinics have generally resulted in recovery of *Pseudomonas* isolates of the *fluorescens/marginalis* complex.

Figure A Lettuce bacterial soft rot

Work in New Zealand indicates that lettuce cultivars differ greatly in their susceptibility to a marginal leaf blight caused by *Ps. marginalis* and that mature plants were more susceptible than young plants. Work in the USA has found high numbers of fluorescent *Pseudomonas* isolates on the lower leaves of mature outdoor lettuce plants. The greatest populations of soft-rotting bacteria were recovered from plants where there was a high water table, following rain, and in mature crops.

Occurrence of bacterial leaf rot and predisposing factors – results of the 2002/03 grower survey

A questionnaire, including a colour photograph of the problem, was devised and sent to over 100 growers throughout England (see Appendix 1). Twenty-seven completed forms were returned and analysis of the information received suggested the following points:

- The disease is mainly seen in October – December maturing crops
- The problem has been gradually increasing since 1993
- Most varieties are susceptible, but large petiole varieties (e.g. Loreley) appear particularly prone
- Bacterial leaf rot mainly develops as a visible problem in the last 2 weeks before harvest
- Often only one or two leaves are affected, usually in the basal whorl
- 'Soft' or drawn plants were described as most affected
- Most growers irrigate with water from stored supplies in reservoirs (52%) or tanks (41%); no water treatments were being used, other than to adjust pH. Filters were occasionally used to remove grit and particles (NFT crops)

A suggested explanation and treatment

Why are the basal leaves most commonly affected?
As the basal leaves will have been present the longest, they will have been prone to the variation in weather/environmental conditions the most (the heart leaves are formed towards the end of the crop).
The basal leaves will also have had the most water applied to them. The heart leaves hardly any. So, as the plant grows, the basal leaves (in the autumn/winter) are in high humidity conditions for longer periods. There were many comments in the survey about wetness as a trigger (disease present in wet areas, 52%; under gutters, 26%; under nozzles, 22%). Only 15% of respondents noted the disease in dry areas.

A possible explanation
A common cause of bacterial leaf rot in lettuce is *Pseudomonas* of the *fluorescens/marginalis* complex. One possible explanation for the recent outbreaks is that these infect `soft`, fast-growing plants in mild autumns, in conditions remaining damp for long periods. Generally the disease is not seen at other times of the year, when these conditions do not occur. The disease was only rarely seen in autumn 2002, or autumn 2003. This may be because of colder weather. In colder weather, growth would not be so soft, and bacteria would not multiply so rapidly to the high populations (more likely to cause disease symptoms).

Infection routes
Bacteria are now known to be present in stored irrigation water, including *Pseudomonas* isolates as shown in this study, and these may survive and increase when deposited on lettuce leaves during irrigation.

Alternatively, bacterial contamination of leaves may occur in soil-water splashed onto them during irrigation. A further possibility is that bacteria may invade the butt and heart leaves via senescent leaves on wet soil.

Pathogen development and symptoms

Lettuce differs from many other plants in that it does not form an abscission layer to cut off senescing leaves. The lower leaves remain attached to the plant and generally shrivel up. However, if the environment around a plant base remains permanently humid, as when a soil is kept wet, infection by *Pseudomonas* species through this tissue could result with direct access to the vascular tissue of the plant. It is possible that there might then be invasion from the butt into the main veins of one or more leaves, leading to the discolouration of midribs seen in winter crops in some years i.e. bacterial soft rot of leaves may be one symptom of a disease that can also result in vascular wilt and butt rot, as seen in outdoor crops.

A grower suggested treatment

Given that chlorine is used to control bacteria in drinking water, one grower has recently installed chlorination treatment for lettuce irrigation water, with a view to reducing bacterial soft rot.

Bacteria associated with leaf rot and present in irrigation water

Pseudomonas isolates of the *fluorescens/marginalis* complex were recovered from dark-discoloured midribs from soil-grown crops and from a watery rot of leaf lamina near the midrib of an NFT crop. *Pseudomonas* isolates were also recovered from water samples taken from irrigation lines, especially in late September/early October, and from mature lettuce heads.

Pathogenicity of *Pseudomonas* isolates to lettuce

Pathogenicity tests were conducted using an isolate of *Ps. marginalis* originally obtained from lettuce, purchased from the National Collection of Plant Pathogenic Bacteria (NCPPB 2380). Plots of cvs Barney, Josephine and Wynona were deliberately grown in humid conditions, with frequent overhead irrigation and relatively poor ventilation in a polythene tunnel. Crops planted between 5 and 23 September (5 planting dates), were spray-inoculated with *Ps. marginalis* or water (control) around 4 weeks after planting. A severe attack of *Rhizoctonia* bottom rot, with secondary bacterial rot, developed in most plots. Blackening of individual petioles on plants was rarely seen.

In further experiments, potted plants and detached leaves of cv. Wynona were stab-inoculated in the mid-rib of basal leaves with an isolate of *Ps. marginalis* obtained from an NFT lettuce crop in September 2003 (AR03/150), or with water (control). Plants were incubated in a cool glasshouse and in a controlled environment cabinet maintained at 23°C, and the detached leaves in the CE cabinet. No midrib or other leaf discolouration or soft rot developed after inoculation.

Trials on control of bacterial leaf rot

Due to the lack of natural outbreaks of the disease in commercial crops in winter 2002/03 and 2003/04, and the lack of typical symptoms when plants were inoculated with *Ps. marginalis*, practical work on control of the problem, planned in outline for years 2 and 3, has been deferred.

Financial benefits

Industry consultation indicated widespread losses in winter 2000/2001, with individual losses of 40-50% of the winter crops. The value of the winter lettuce crop in 1999/2000 was £9.24 million (Basic Horticultural Statistics), so just a 2% loss (as observed in surveys) represents £184,800. The increased understanding of the disease as a result of this project to date could help growers to minimise the problem and thereby reduce losses and maintain continuity of supply.

Action points for growers

1. Thoroughly remove leaf debris from the house at the end of cropping, especially after an outbreak of bacterial leaf rot

2. Ensure there are no poorly drained areas in the greenhouse

3. Ensure balanced nutrition in order to prevent premature senescence of lower leaves

4. Do no plant peat blocks too deeply, in order to minimise contact of lower leaves with the soil.

5. Take care to avoid growth checks that allow leaves to wilt and lie on the soil surface.

6. Ventilate crops as much as possible to reduce humidity around lower leaves

7. Do not overwater.

8. Avoid overhead irrigation late in crop production, or drips onto the soil that lead to water-splash of soil onto plants

9. Be aware that a high water table, especially in the final 2-3 weeks of crop growth, is likely to increase humidity around the base of the crops and thereby make it more at risk from bacterial soft rot.

10. Be aware that large petiole varieties appear to be particularly prone.

Science section

1. Introduction and review of lettuce bacterial soft rot

The two bacterial groups most commonly associated with soft rotting of lettuce in the UK are *Pseudomonas* isolates of the fluorescens/marginalis complex and *Erwinia carotovora.* They are both widespread soil-borne organisms. The bacteria are known to occur commonly on healthy leaf plants, as part of the normal surface microbiological flora, as well as in soil. *E. carotovora* usually occurs secondarily to physical damage or attack by botrytis or rhizoctonia and results in a rapid slimy rot. Unlike *E. carotovora,* fluorescent *Pseudomonas* species do not require wounded tissue to establish in the plant (Cleary, 1960).

A marginal leaf blight of lettuce caused by *Ps. marginalis* was described in the USA in 1918, and became known as Kansas lettuce disease. The symptoms were narrow, black lesions along the margins of the outermost leaves (i.e. a different symptom to that studied in this project). Work in New Zealand showed that lettuce varieties differ considerably in their susceptibility to marginal leaf blight (Miller 1980). Plants inoculated when young (2-4 weeks old) remained healthy, displaying apparent resistance to attack by *Ps. marginalis,* whereas mature plants inoculated on their outermost leaves and maintained at 23°C, developed black lesions normally associated with marginal leaf blight. Varieties found to be especially susceptible were Great Lakes, Webbs wonderful and Imperial; varieties less susceptible were Triumph, Buttercrunch and Cos. In Germany, *Ps. marginalis* was described as a cause of a wilt in lettuce for the first time in 1972; a photograph within the report shows petiole discolouration similar to that being investigated in this project (Kohn, 1973).

In the UK, a bacterial wilt disease of lettuce was associated with infection by *Pseudomonas* species, probably *Ps. marginalis* (Cleary, 1960). Symptoms were leaf wilting, a change in leaf colour from shiny, light green to a duller, deep green, dark discolouration of the vascular system in the stem, followed by a basal stem rot and plant collapse. The disease known as 'butt rot' caused by *Ps. marginalis* was subsequently described (Anon, 1986) and appears to be the same, or a very similar problem. Symptoms are most often seen in autumn and winter crops as plants approach maturity. Affected plants wilt suddenly and generally do not recover. The tissue in the crown of the stem becomes replaced by an olive-green to brown jelly-like rot. Sometimes the rot forms a cavity in the stem and the head may break away easily when pulled. Cultivars prone to `glassiness` (e.g. the now superseded cultivars Hamlet, Pallas, Ravel and Nordine) are more susceptible to butt rot. Over the last 10 years, ADAS and other Plant Clinics have tested occasional samples with dark brown or black discoloration of the lower surface of leaf midribs. Isolates from these tissues have generally resulted in recovery of *Pseudomonas* isolates of the *fluorescens/marginalis* complex.

In the USA, researchers have investigated bacterial populations on basal leaves of field-grown lettuce and on soil from under lettuce plants (Pieczarka & Lorbeer, 1974). They hypothesised that soil-borne *Erwinia* species and fluorescent *Pseudomonas* became established on healthy lettuce leaves and then, under favourable field or storage conditions, surface populations increased rapidly to cause decay of lettuce tissues. They found that the greatest number of bacteria was associated with older healthy leaves in contact with the soil, senescing leaves and soil from under mature (6-8 week old) plants. Bacterial populations on lower leaves increased greatly between weeks 4 and 8. Bacterial populations on lower leaves were lower for plants grown on ridged bed (10 cm high, 17 cm wide) where conditions were drier because of better air circulation. The restricted air movement and resulting high humidities under maturing plants contributed to moist conditions which, with available substrate from senescing leaves, was favourable for bacterial growth.

Additionally a rapid rise in bacterial numbers on young leaves was observed after rain, possibly originating from infested organic matter being splashed onto the leaves. When pathogenicity tests were conducted, results were variable and a low percentage of soft-rotting bacteria and fluorescent *Pseudomonas* isolates produced expanding lesions when stab inoculated into lettuce. i.e. not all soft-rotting *Erwinia* or fluorescent *Pseudomonas* bacteria readily cause rot of lettuce tissue, or do so only under certain, specific conditions.

The development of bacterial leaf rot in lettuce is further complicated by the fact that there is evidence that *Ps. marginalis* can colonise plant tissue without causing disease symptoms; it is possible that simply damaging tissue allows latent infections to become aggressive and cause tissue rotting (van Outryve *et al*., 1989).

Winfree *et al*., (1958) described a bacterial rot of field-grown lettuce in Florida. The disease first appeared as a sudden wilting of lower leaves. Soon the outer leaves turned slimy and plants died within 48 hours. Close examination revealed that wilting was preceded by vascular discolouration of the stem, gradually extending into the midrib and lateral veins (i.e. midribs turn brown), and then the stem butt gradually broke down into a jelly rot. *Erwinia* was found associated with all phases of the disease, although occasionally a *Pseudomonas* species was isolated in addition to *Erwinia*. In experimental work they found that his disease was favoured by a high water table, and was not reduced by soil sterilisation with chloropicrin.

An HDC project to study the biology and control of lettuce bacterial rot (petiole blackening) in England was devised. In this first year of the project, work was done to:

- Collate growers' observations and comments on occurrence of the problem.
- Examine plants with soft rot symptoms and stored water supplies on lettuce nurseries for the occurrence of bacteria.
- Conduct pathogenicity tests with *Pseudomonas* isolates to try and reproduce leaf petiole blackening symptoms.

2. Occurrence of bacterial leaf rot and predisposing factors – results of the 2002/03 grower survey

A survey of protected lettuce growers was conducted in winter 2002/03 in response to increasing concern over the occurrence of bacterial soft rot. The questionnaire sent to over 100 growers is shown in Appendix 1. The objective was to collect information from across the country about the occurrence and possible causes of this problem. Twenty-seven growers replied. The detailed information received, including grower comments and ideas, was analysed, and the following picture is suggested as to why and how bacterial soft rot occurs. The full results of the survey are tabulated in Appendix 2.

Summary of key points

<u>When it occurs</u>

- The disease is mainly seen in October, November and December maturing crops (48-56% of respondents had seen it in these months) which is planting dates of mid-September to mid-October. i.e. it affects crops that are planted in decreasing day length and are also maturing in decreasing day length. Although it is seen at other times of the year, it occurs not nearly as much.

<u>An increasing problem</u>

- Occurrence of bacterial soft rot has gradually been increasing since 1993.

<u>Varieties affected</u>

- From the survey it appears that most varieties are susceptible, but large-petiole varieties (e.g. Loreley) appear to be particularly prone.

<u>Age of plants</u>

- Bacterial soft rot is mainly seen at harvest but it can become visible up to two weeks before, or occasionally earlier.

- It is never seen on young plants – possibly this indicates the disease is slow to develop and there is insufficient time for symptom development on young plants. Or, consistent with previous studies (Miller, 1980), young plants are less susceptible than older ones.

<u>Which leaves are affected?</u>

- Mainly up to five leaves are affected – often only one or two, rarely more than six. This tends to suggest the problem is not readily systemic, otherwise one would expect commonly to see many leaves with the problem, not just one or two.

- Bacterial soft rot usually occurs in the basal whorl of leaves, up to the 2nd whorl from base. Never in the heart (youngest leaves).

<u>Why are the basal leaves most commonly affected?</u>

- As the basal leaves have been there the longest this may indicate the following:
  - They will have been prone to the variation in weather/environmental conditions the most (the heart leaves are formed towards the end of the crop)

  - The basal leaves will have had the most water applied to them. The heart leaves hardly any. So, as the plant grows, the basal leaves (in the autumn/winter) are in a “high” humidity conditions for longer periods. There were many comments in the survey about wetness as a trigger (disease present wet areas, 52%; under gutters, 26%; under nozzles, 22%). Only 15% of respondents noted the disease in dry areas.

- They are in closest proximity to the soil surface and therefore more prone to splash of soil-contaminated water onto them.

Type of plant affected

- The type of plants most commonly affected were described as soft/drawn (41%), large petioles (44%) or having wet leaves (26%). These all indicate high humidity conditions (warm, dull), creating soft growth. Soft growth is likely to be more susceptible to bacterial infection because of thinner plant cell walls, or cuticle and possible growth cracks. The disease was not seen so much where there was plenty of air movement or dry conditions.

Irrigation water

- Water used for irrigation is primarily obtained from stored supplies in reservoirs (52%) or tanks (41%).

- Currently, no growers treat the irrigation water, other than to adjust pH (e.g. NFT crops) or use a sand filter.

Other production factors

- Many of the factors examined in the survey (soil type, nutritional levels, glasshouse temperature, pesticides, spraying operations) did not appear to correlate with occurrence of bacterial soft rot. The key factors which appeared to influence bacterial soft rot were time of year, age of plants, `wetness`, and type of growth (see above).

A suggested explanation

- A common cause of bacterial leaf rot in lettuce is *Pseudomonas* species, an opportunistic pathogen. One possible explanation for the recent outbreaks is that these bacteria infect `soft`, fast-growing plants in mild autumns, in conditions remaining damp for long periods. Generally the disease is not seen at other times of the year, when these conditions do not occur. The disease was only rarely seen in autumn 2002, or autumn 2003. This may be because of colder weather. In colder weather, growth would not be so soft, and bacteria would not multiply so rapidly to high populations (necessary to cause disease symptoms).

Possible infection routes

- Bacteria are now known to be present in stored irrigation water, including *Pseudomonas* isolates as shown in this study, and these may survive and increase when deposited on lettuce leaves during irrigation.
- Alternatively, bacterial contamination of leaves may occur in soil-water splashed onto them during irrigation or from drips. A further possibility is that bacteria may invade the butt and heart leaves via senescent leaves on wet soil.

Pathogen development and symptoms

- Lettuce differs from many other plants in that it does not form an abscission layer to cut off senescing leaves. The lower leaves remain attached to the plant and generally shrivel up. However, if the environment around a plant base remains permanently humid, as when a soil is kept wet, infection by *Pseudomonas* species through this tissue could result, with direct access to the vascular tissue of the plant. It is possible that there might then be invasion from the butt into the main veins of one or more leaves, leading to the discolouration of midribs seen in winter crops in some years i.e. bacterial soft rot of leaves may be one symptom of a disease that can also result in vascular wilt and butt rot as seen in outdoor crops.

A grower suggested treatment

Given that chlorine is used to control bacteria in drinking water, one grower has recently installed chlorination treatment for lettuce irrigation water, with a view to reducing bacterial soft rot.

3. Bacteria associated with leaf rot and present in irrigation water

In February-March 2001, dark-brown or black discolouration of the underside of occasional midribs of lower interior leaves occurred in several successive crops of lettuce on a nursery in Essex. There was no evidence of spread to the periphery of leaves or to the underlying leaves. Samples were tested on two occasions and *Pseudomonas* isolates of the *fluorescens/marginalis* complex were isolated each time. Young lettuce plants were inoculated with the bacteria by the CSL but no leaf midrib browning symptoms developed.

Over 50 visits were made to protected lettuce crops by the authors during the period October-December 2002, and again in 2003, and the specific symptom of petiole browning was not seen in any of them.

A bacterial soft rot occurred in an NFT crop of lettuce (cv. Benjamin) in Hertfordshire in September – October 2003. Initial symptoms were vascular staining in the stem base and a soft, watery rot of leaf lamina around the midrib of one or two leaves near the plant base. Later, there was a more extensive soft, dark-brown rot of the stem base and complete collapse of plants. Botrytis was present occasionally on plants but the majority showed no evidence of grey mould and no fungi were recovered from affected leaves. Isolation from the soft rotting leaf tissue did however result in recovery of a Group IV *Pseudomonas* of the *fluorescens/marginalis* (AR 03/150). No *Erwinia* species were isolated.

Irrigation water supplies were collected at monthly intervals from August to October on two nurseries, taking the samples early in the morning from the overhead spray nozzles before the irrigation was used that day. Additionally, the water supply at the trial site was tested in October. The samples were tested by Direct Laboratories, Wolverhampton, for total bacteria (at two temperatures) and total *Pseudomonas* number. Bacteria were recovered in all samples (Table 3.1). *Pseudomonas* numbers were relatively low at all times, being greatest in late September/early October. Examination of lettuce leaves from a crop on a different commercial nursery, taken in early September, showed *Pseudomonas* isolates on the leaf surface. This result is not unexpected. Previous studies in the USA (Pieczarka & Lorbeer, 1974), recorded levels of 4.9×10^4 cells/cm^2 of green fluorescent *Pseudomonas* on lower leaves of 6-8 week old outdoor lettuce.

Table 3.1. Occurrence of bacteria in lettuce irrigation water supplies and on lettuce plant heads-2003

Location	Sample date	Sample point	Total bacteria 22°C	Total bacteria 37°C	Total *Pseudomonas* spp. (cfu/ml)
Nursery 1 (JC)	27-8-03	Spray line	680	<1	<1
	24-9-03	Spray line	39	<1	50
	23-10-03	Spray line	81	1	<1
Nursery 2 (IH)	27-8-03	Spray line	4	<1	<1
	24-9-03	Spray line	7	<1	22
	23-10-03	Spray line	26	<1	<1
Arthur Rickwood	13-10-03	Spray line	2900	960	110
On mature heads					
Harlow	4-9-03	Leaves			3900 cfu/g

4. Pathogenicity of *Pseudomonas* isolates to lettuce

Introduction

Due to the lack of bacterial soft rot with petiole discolouration in commercial crops of protected lettuce during the period of this project, attempts were made to reproduce the problem experimentally. One fully replicated trial was undertaken at ADAS Arthur Rickwood. Additionally, potted plants and detached leaves were inoculated with *Pseudomonas* isolates obtained from lettuce.

Materials and methods

Experiment 1: Effect of planting date and inoculation with *Pseudomonas marginalis* (isolate 2380 NCPPB) on bacterial leaf rot on lettuce-greenhouse trial

Lettuce was grown in a polythene tunnel from 5 September to 30 November 2003. The soil had been fallow for 3 years, and prior to that had grown several crops of lettuce for experimental work on control of *Rhizoctonia* and botrytis basal rots. Plants were grown to a commercial standard (spacing, irrigation, nutrition) as advised by David Stokes, with the exception that minimum ventilation was applied (partial opening of side vents only), and the crop was given frequent irrigation in order to provide high humidity and leaf wetness, conditions believed to favour development of bacterial soft rot. Aliette and Fubol Gold were applied for control of downy mildew; Amistar was applied shortly after planting to protect against *Rhizoctonia* bottom rot.

The experiment consisted of five planting dates (5, 9, 16, 19 and 23 September 2003) using block-raised plants of cvs Barney (planting 1), Josephine (plantings 2-4) and Wyona (planting 5). There were four replications arranged as a two-way factorial in randomised blocks with planting date as factor 1 (main plots) and inoculation with *Pseudomonas* (factor 2) as sub-plots. Each plot consisted of 30 plants in a 6x5 arrangement. One half of each plot (15 plants), selected at random, was inoculated 25-28 days after planting by spraying to the point of run-off with a cell suspension (10^7 cfu/ml) of *Ps. marginalis* isolate 2380 (ex NCPPB) originally isolated from lettuce. Plants were harvested as they matured, on 20 and 28 October and 5, 14 and 28 November. At harvest, plants were assessed for basal rot on a 0-3 index as follows:

0 – No rot
1 – Rot on lowest whorl of leaves
2 – Rot on second lowest whorl of leaves
3 – Rot penetration into third whorl of leaves, or further into the heart.

Additionally, occurrence of leaf petiole dicolouration or soft rot was recorded and leaf margin rot. After trimming off all affected leaves, the remaining head weight was recorded. Results were examined by ANOVA for mean head weight, number of marketable plants and number of plants with basal rot, following transformation of data where necessary. Where there were a large number of zeroes in data sets (e.g. mean incidence of bacterial leaf rot) results were examined by Friedman`s test, comparing medians.

Experiment 2: Pathogenicity of two *Pseudomonas* isolates to pot-grown lettuce

Pot grown lettuce, cv. Wyona, were stab-inoculated in the midrib on three basal leaves (one basal whorl, one second whorl and one third whorl) with i) water ii) *Ps. marginalis* AR 03/150 iii) *Ps. marginalis* (NCPPB isolate 2380). Inoculated plants were incubated at high humidity, by covering them with a polythene bag, in an illuminated CE cabinet at 23°C, and in a cool glasshouse. There were 3 inoculated plants per treatment. Plants were examined for leaf rot after 5 and 21 days.

Experiment 3: Pathogenicity of two *Pseudomonas* isolates to detached lettuce leaves
Outer, healthy leaves from mature-grown winter lettuce were laid in plastic trays and inoculated as described above. Leaves were incubated at high humidity at 23°C for 7 days in an illuminated CE cabinet and then assessed for bacterial soft rot.

Results and discussion

Experiment 1: Greenhouse crop trial
A severe attack of *Rhizoctonia* bottom rot affected many of the plants at all harvests, especially from the second planting date onwards. There was bacterial soft rot associated with the fungal infection, resulting in a soft, slimy basal rot. On occasional plants, there was a bacterial rot of one or two petioles with no evidence of infection by *Rhizoctonia*. A leaf margin rot of basal leaves in contact with the soil surface was found to be due to *Rhizoctonia*.

It is interesting that a highly pathogenic inoculum of *Rhizoctonia* persisted in the soil from previous experiments where the soil had been deliberately inoculated with the fungus. The poor control given by Amistar in this trial may have been due to the high inoculum of *Rhizoctonia* in the soil and/or that the conditions believed conducive to bacterial soft rot (wet, poor ventilation) that were deliberately used, are conditions which also tend to favour *Rhizoctonia* basal rot.

Mean plant head weight after trimming differed between planting dates, generally reducing with the later planting dates. Inoculation with *Pseudomonas* had no effect on trimmed head weight, the incidence of plants with basal rot (*Rhizoctonia* and/or bacterial) or the number of marketable plants (more than 170 g after trimming). The mean incidence of plants with bacterial leaf rot (i.e. midrib browning without any obvious *Rhizoctonia* infection) was low at all assessments (Table 4.1). Examination of median values showed no statistically significant differences when inoculated vs uninoculated were compared at each planting date, or when different planting dates were compared for the inoculated and uninoculated sets of plants (data not shown).

Experiments 2 and 3
No soft rot developed following inoculation of potted lettuce plants or detached lettuce leaves.

Table 4.1 Effect of planting date and inoculation with *Pseudomonas marginalis* on basal rot of lettuce - 2003

Planting date	Mean number of plants with rhizoctonia basal rot (out of 15 plants			Mean incidence of bacterial petiole blackening (out of 15 plants)*	
	Uninoculated	Inoculated	Duncan Suffix	Uninoculated	Inoculated
5 September	10.0	7.5	a	1.0	0.8
9 September	14.3	14.5	b	0.5	1.0
16 September	13.5	14.5	bc	0.8	0.3
19September	12.8	13.8	bc	0.0	0.3
23 September	11.0	11.8	c	1.5	1.8

Results examined by Friedman's comparison of medians; no statistically significant differences between treatments were found.

Statistical analysis of the effect of block, planting date, and inoculation with *Pseudomonas* on mean number of plants with *Rhizoctonia* basal rot and mean severity (below) indicated that planting date had a significant effect, with least disease in the earliest planting (the shortest duration crop).

Variate	Significance*	SED	Df
Block	0.470	1.112	27
Planting date	<0.001	1.243	27
Inoculation	0.900	0.786	27
Planting date x inoculation	0.590	1.758	27

*Where the significance level is 0.05 or less, there are statistically significant differences between treatments of the specified variate at the 5% level of probability.

Table 4.2 Effect of planting date and inoculation with *Pseudomonas marginalis* on *Rhizoctonia* basal rot of lettuce - 2003

Planting date	Mean disease severity score (index 0-3)	
	Uninoculated	Inoculated
5 September	1.5	1.1
9 September	2.6	2.7
16 September	2.2	2.4
19September	2.2	2.6
23 September	2.1	2.1

* Severe Rhizoctonia substantially reduced the trimmed head weights

Statistical analysis of the effect of block, planting date, and inoculation with *Pseudomonas* on mean disease severity score of *Rhizoctonia* basal rot

Variate	Significance*	SED	Df
Block	0.029	0.223	27
Planting date	<0.001	0.253	27
Inoculation	0.611	0.160	27
Planting date x inoculation	0.522	0.358	27

*Where the significance level is 0.05 or less, there are statistically significant differences between treatments of the specified variate at the 5% level of probability.

The severe attack of *Rhizoctonia* in plantings 2-5 substantially reduced the trimmed head weights. Again, planting date was the only factor that significantly affected the trimmed head weight (Table 4.3) or the number of marketable plants (Table 4.4).

Table 4.3 Effect of planting date and inoculation with *Pseudomonas marginalis* on head weight of lettuce – 2003

Plant date	Harvest date	Mean trimmed head weight (g)	
		Uninoculated	Inoculated
5 September	20 October	139.6	147.2
9 September	28 October	56.9	55.5
16 September	5 November	86.2	80.2
19 September	14 November	79.3	60.2
23 September	28 November	43.9	43.2

Statistical analysis of the effect of block, planting date, and inoculation with *Pseudomonas* on mean trimmed head weight

Variate	Significance*	SED	Df
Block	0.075	14.02	12
Planting date	<0.001	15.68	12
Inoculation	0.310	3.72	15
Planting date x inoculation	0.285	6.74	15

*Where the significance level is 0.05 or less, there are statistically significant differences between treatments of the specified variate at the 5% level of probability. Results were also analysed as Log10 (plant weight+1) as the original data were squewed; this also showed a statistically significant effect from planting date and not from other variates.

Table 4.4 Effect of planting date and inoculation with *Pseudomonas marginalis* on mean number of marketable plants – 2003

Plant date	Harvest date	Mean number trimmed heads (of 15) more than 170 g	
		Uninoculated	Inoculated
5 September	20 October	5.3	5.5
9 September	28 October	0.5	0.3
16 September	5 November	0.8	1.3
19 September	14 November	0.3	0.5
23 September	28 November	0.3	0.3

Statistical analysis of the effect of block, planting date, and inoculation with *Pseudomonas* on mean number of marketable plants (of 15)

Variate	Significance*	SED	Df
Block	0.130	0.479	12
Planting date	<0.001	0.535	12
Inoculation	0.613	0.290	15
Planting date x inoculation	0.938	0.705	15

*Where the significance level is 0.05 or less, there are statistically significant differences between treatments of the specified variate at the 5% level of probability.

Conclusions and discussion

1. *Pseudomonas* isolates of the *fluorescens/marginalis* complex, an opportunistic bacterial plant pathogen cause wilt and butt rot in lettuce. This bacterium is also associated with, and probably cause, dark-brown discolouration of midribs of inner leaves.

2. *Pseudomonas* isolates of the *fluorescens/marginalis* complex commonly occur in soil. They are also found as part of the natural microbiological flora on the surface of lettuce leaves. *Pseudomonas* isolates (type not characterised) can also occur in irrigation water.

3. A UK grower survey indicates that incidence of bacterial soft rot (petiole blackening) is most common in large-vein varieties, in the autumn/winter period, and in the last two weeks before harvest.

4. The same survey indicates that bacterial soft rot (petiole blackening) is most common in `soft` or drawn plants, and that wetness is a trigger, the disease was reported by growers to be worse in wet areas, under gutters and under nozzles, for example.

5. Different researchers have failed to reproduce typical symptoms of bacterial soft rot by inoculation with *Pseudomonas* isolates, or have only occasionally recorded soft rotting. These results may indicate that a very specific, and possibly narrow range of conditions (e.g. plant type, leaf wetness duration, high bacterial numbers) may be required for *Pseudomonas* to infect and develop in lettuce. Alternatively, *Pseudomonas* isolates may readily lose their virulence in culture.

6. A possible explanation for the development of leaf petiole blackening in lettuce, associated with infection by *Pseudomonas,* is that the leaves are contaminated with the bacterium from stored water, applied through the irrigation lines or possibly water-splash of soil. If subsequently the environmental conditions create soft growth, the plants are believed to be more susceptible to infection by the bacteria, resulting in petiole blackening. Based on this theory, treatment of stored water with chlorine before irrigation should control the problem.

Technology transfer

1. On-going discussion of results with growers during consultancy work and crop sampling for the project.

2. Interim report summarising results of the grower questionnaire sent to all growers of protected lettuce (May 2003)

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## Appendix 1:		HDC Grower Survey Form

1. **Grower site**

Your name/ nursery	
Address	
Post Code	
Telephone	
Fax	
E-mail	

Disease occurrence

2. **Have you ever seen bacterial soft rot (petiole blackening) on your nursery?**

Yes	No

Lettuce bacterial soft rot *(Pseudomonas* sp.)

Please continue and complete this form even if you have never had the problem.

3. **If seen within the last 12 months, tick in which month it usually occurred**

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec

4. **If bacterial soft rot was observed over 12 months ago, tick which year**

’02	’01	’00	’99	’98	’97	’96	’95	’94	’93

5. **Approximately what proportion of the crop is usually unmarketable because of the problem?**

1-5%	6-10%	11-20%	21-40%	41-60%	61-80%	81-100%	Variable

6. **Which varieties are affected?** (please tick)

Short day	Nil	A little	A lot
Emerald			
Hiliary			
Josephine			
Loreley			
Montel			
Patrick			
Wynona			
Others			

Long day	Nil	A little	A lot
Alexandria			
Atlantis			
Barney			
Benjamin			
Charles			
Clare			
Trinity			
Voyager			
Others			

Please comment on other major varieties grown in previous years

………

Type of plant and plant part affected

7. When do you first see the disease?

At harvest	1 week before	2 weeks before	Earlier

8. Approximately how many leaves are affected on a plant?

1	2	3	4	5	6	7	8

9. Which leaves are affected?

Basal	1st whorl (layer) up	2nd whorl up	3rd whorl up	4th whorl up	Heart leaves

(Tick more than one box if you wish)

Weather and plant growth

10. What is the general weather when the problem occurs? **(Please tick all appropriate boxes)**

	Warm	Cold	Wet	Dry	Windy	Don't know
Day						
Night						

11. Do you consider sudden weather changes start/stop the problem?

	Problem starts	Problem stops
Sudden change in weather from warm to cold		
Sudden change in weather from cold to warm		

12. What type of plants are affected?

Soft/ drawn	Sturdy/ compact	Large petioles	Small petioles	Wet leaves	Other

---

Glasshouse or tunnel crops with bacterial soft rot

13. Which area of crop is usually affected? (Please tick all appropriate boxes)

Growing structure	Wet area	Dry area	Under gutter	Under nozzles	Close to wall	Close to spray path	Far from spray path	Other
Glasshouse crops								
Tunnel crops								

Same place in a glasshouse/ tunnel in different years	Different areas in different years	Don't know

---

Soil, nutrition and irrigation

14. Soil type

Sandy	Loam	Peat	Clay	Other

15. Soil nutrient levels with affected crop (please tick)

Potassium (ppm)	Please tick
200-300	
301-400	
401-500	

Nitrogen (ppm)	Please tick
0-25	
26-50	
51-75	

Conductivity	Please tick
2100-2200	
2200-2300	
2300-2400	

501-600	
601-700	
701-800	
700 +	

76-100	
100 +	

2400-2500	
2500-2600	
2600-2700	
2700-2800	
2800 +	

16. General irrigation policy

A lot of water early	Last watering at rosette	Any later watering?	Comments

17. Irrigation layout

1 line/10½ bay	2 lines	Other

18. Water supply

Mains	Borehole	Reservoir	Other

19. Water treatment

PH adjusted	Chlorine dose	Reciclean	Sand filter	Other	None

20. Growing house temperature

Frost protection /	2°C night / 4°C day	Other	Comment

½°C			

21. Fungicides

Main fungicides used on the growing crop (please tick)	
Aliette	
Favour 600 SC	
Fubol Gold	
Filex/Proplant	
Octave	
Rovral WP	
Scala	
Unicrop Thianosan	
Other:	

22. Insecticides

Main insecticides used on the growing crop (please tick)	
Aphox	
Decis	
Nicotine	
Toppel 10	
Other:	

23. Other diseases

Does it occur with other diseases? (please tick)	
Botrytis	
Rhizoctonia basal rot	
Sclerotinia	
Phoma leaf spot/basal rot	
Pythium leaf or butt rot	
Downy mildew	
Ring spot	
Other:	

Spray application

24. Type of sprayer

Knapsack	Motorised knapsack	Hand held lance from trolley sprayer	Gantry	ULV

25.

Spray pressure (psi or bars)	

26.

Distance between spray paths	

Comments

27. If you wish to add your comments or ideas about this problem, please write in the space below

....
....
....
....
....

28. Area of glasshouse and tunnel lettuce grown (m^2 or ha)

Glasshouse	Tunnel

Thank you for your help in completing this form. A summary of report will be sent to all respondents in 2003.

Please return this form to the HDC in the reply-paid envelope.

Appendix 2: Results of HDC grower survey, winter 2002/03

Table 1. Which month does it normally occur?

Month	January	February	March	April	May	June
No. of Respondents	8	4	3	3	3	2
% of Respondents	29.6	14.8	11.1	11.1	11.1	7.4

Month	July	August	September	October	November	December
No. of Respondents	1	3	9	13	15	15
% of Respondents	3.7	11.1	33.3	48.1	55.6	55.6

Table 2. Which year did it occur if over 12 months ago?

Year	2002	2001	2000	1999	1998
No. of Respondents	15	20	15	8	6
% of Respondents	55.6	74.1	55.6	29.6	22.2

Year	1997	1996	1995	1994	1993
No. of Respondents	5	3	3	2	2
% of Respondents	18.5	11.1	11.1	7.4	7.4

Table 3. Variety susceptibility

Variety	Number of growers reporting this variety is :-	
	Affected	**Not affected or little affected**
Short day		
Emerald	2	1
Hillary	3	1
Josephine	0	1
Loreley	7	7
Montel	1	2
Patrick	1	0
Wynona	3	5
Others	1	2
Long day		
Alexandria	2	2
Atlantis	0	0
Barney	2	2
Benjamin	0	1
Charles	0	0
Clare	0	1
Trinity	1	2
Voyager	1	1
Others	1	0

Table 4. When is the disease first seen?

Period first noticed	Number of respondents
At harvest	14
1 week before harvest	3
2 weeks before harvest	3
Earlier	5

Table 5. How many leaves are affected per plant?

Number of leaves	Number reporting that many leaves affected
One	8
Two	8
Three	8
Four	5
Five	6
Six	3
Seven	1
Eight	1

Table 6. Which leaves are usually affected?

Leaf type	Number reporting leaf-type affected
Basal	11
1st Whorl	16
2nd Whorl	13
3rd Whorl	7
4th Whorl	2

Table 7. What are the general weather conditions observed when the disease occurs?

Weather type	Time of Day	
	Day (no. of respondents)	Night (no. of respondents)
Warm	5	9
Cold	6	6
Wet	17	14
Dry	0	0
Windy	0	0
I don't know	7	6

Table 8. What is the affect of weather changes?

What does the disease do?	Sudden change: cold to warm	Sudden change: warm to cold
Starts	5	10
Stops	2	4

Table 9. What types of plant are affected?

Type of plant	Number reporting
Soft/drawn	11
Sturdy and compact	4
Large petioles	12
Small petioles	3
Wet leaves	7
Other	1

Table 10. Where are diseased plants located in the crop?

Location	Building type	
	Glasshouse (number reporting)	Polytunnel (number reporting)
Wet	14	2
Dry	4	1
Under gutter	7	1
Under nozzles	6	1
Close to wall	6	1
Close to spray path	3	1
Far from spray path	4	1
Other	2	2

Table 11. Does the location of the disease change?

Where occurring?	Number of respondents
Same place in different years	3
Different areas in different years	9
Don't know	11

Table 12. Does soil type affect disease?

Soil type	Number of respondents	
	All	Those reporting disease
Sandy	9	9
Loam	16	15
Peat	1	1
Clay	6	5
Other	1	0

Table 13. Does irrigation policy affect disease?

Irrigation policy	Number of respondents	
	All	Those reporting disease
Lots of water early	20	18
Last watering at rosette	15	14
Any last watering	5	5

Table 14. Does irrigation layout affect disease?

Irrigation layout	Number of respondents	
	All	Those reporting disease
1 line	14	13
2 lines	8	8
Other	1	1

Table 15. Does the source of irrigation water affect disease?

Water Source	Number of respondents	
	All	Those reporting disease
Mains	11	10
Borehole	3	3
Reservoir	14	13
Other	0	0

Table 16. Does water treatment affect disease?

Water treatment	Number of respondents	
	All	Those reporting disease
pH Adjusted	1	1
Chlorine dose	0	0
Reciclean	0	0
Sand Filter	4	4
Other	0	0
None	19	19

Table 17. What affect does Growing House temperature have?

Temperature	Number of respondents	
	All	Those reporting disease
Frost protection	13	12
2 deg C at night	11	10
Other	2	2

Table 18. Does the fungicide programme affect disease?

Fungicide	Number of respondents	
	All	Those reporting disease
Aliette	15	13
Favour 600 SC	9	8
Fubol Gold	12	12
Filex/Proplant	18	17
Octave	4	4
Rovral WP	20	19
Scala	5	5
Unicrop Thianosan	12	10
Other	1	1

Table 19. Insecticide programme

Insecticide	Number of respondents	
	All	Those reporting disease
Aphox	20	19
Decis	4	4
Nicotine	6	6
Toppel 10	18	17
Other	0	0

Table 20. Other diseases

	Number of respondents	
	All	**Those reporting disease**
Botrytis	7	7
Rhizoctonia basal rot	13	13
Sclerotinia	9	9
Phoma leaf spot/basal rot	2	2
Pythium leaf or butt rot	3	3
Downy Mildew	2	2
Ring Spot	3	3
Other	0	0

Table 21. Type of sprayer

Fungicide	**Number of respondents**	
	All	**Those reporting disease**
Knapsack	2	2
Motorised knapsack	3	2
Handheld lance/trolley	19	18
Gantry	3	3
ULV	1	1