

VG317

**Integrated management of bacterial head
rot of broccoli**

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Dullahide, Sue Heisswolf, David Lyons**
Queensland Department of Primary
Industries



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VG317

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**HORTICULTURAL
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CORPORATION**

**Partnership in
horticulture**

"INTEGRATED MANAGEMENT OF BACTERIAL HEAD ROT OF BROCCOLI"

HRDC REF. NO. QFVG 2240

**A FINAL REPORT PREPARED FOR THE HORTICULTURAL RESEARCH AND
DEVELOPMENT CORPORATION**

DECEMBER 1997

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1. INDUSTRY SUMMARY

During the life time of the project (1992-1997), bacterial head rot of broccoli was recorded predominantly in the Laidley and Gatton localities. In the Granite Belt, relatively dry warm conditions persisted throughout the survey period and losses from bacterial head rot were minimal.

Two bacterial isolates, identified as *Pseudomonas marginalis* and *Pseudomonas syringae*, reproduced the disease symptoms of bacterial head rot in glasshouse or field tests.

Three variety trials were conducted to assess the disease tolerance of broccoli cultivars. In the first trial, Shilo, Summer King, BR 130 and Coral suffered severe losses due to bacterial head rot. Another 14 varieties appeared relatively resistant. Disease severity was inversely correlated with the time taken from transplanting until harvest. Bacterial head rot was not recorded in the second trial. In the third trial, where broccoli heads were first damaged in order to obtain head rot, S & G BR327 and NW Hybrid NW702 exhibited the highest frequency of head rot, while SPS Maverick and SPS 938 exhibited the lowest percentage of heads with bacterial head rot.

In a field trial conducted in the Granite Belt in 1997, bacterial head rot only developed on those heads in which florets were damaged by cabbage white caterpillars and where *P.syringae* was applied to the head. Inoculum or damage caused by caterpillars alone, did not result in head rot.

Plant waxes, sodium hypochloride (100 µg / ml), codacide oil (5 ml / L) and agridex (1 ml / L) were each shown to cause discolouration to the broccoli head and were deemed unsuitable as protective agents.

Both copper oxychloride (50% WP available copper @ 5g / L) and copper hydroxide (50% WP available copper @ 2g / L), applied twice at early buttoning 7 days apart, reduced the incidence of bacterial head rot by >20% in one trial on cv. Generation. Calcium chloride (Stop-it), applied at 20 ml L⁻¹, once at early buttoning, also significantly reduced the severity of disease in cv. Greenbelt. Results suggested that hydrated lime applied as a dip on mature heads may be effective in reducing head rot in storage. However, post-harvest dips of copper hydroxide and phosphorous acid increased head rot.

Previous overseas studies and local observations have suggested that nitrogen influences the incidence and severity of bacterial head rot. A trial was established in which nitrogen was applied at 0,40, 80 and 160 kg / ha N, applied as ammonium nitrate, with 3 varieties of broccoli. Little bacterial head rot was recorded, but that which did occur was present in the 'high N' plots'. There was a significant positive response between marketable yield and nitrogen fertiliser. The estimated critical total nitrogen plant concentration in terms of marketable yield was 5.39%.

2. TECHNICAL SUMMARY

In order to reduce bacterial head rot in broccoli, the results from this study suggest that ;

- 1) The broccoli varieties Shilo, Summer King, BR130 and Coral possess little resistance to bacterial head rot. Dominator, Purple Mountain, Pheonix, NW182, Legacy and Dynamo exhibited little bacterial head rot and where possible, these varieties should be grown in areas highly susceptible to bacterial head rot.
- 2) Applications of copper oxychloride, copper hydroxide and calcium chloride can reduce the severity of bacterial head rot.
- 3) Any factor (eg. wind, hail, frost, machinery or insect damage) which causes injury to the broccoli head, is likely to increase the incidence and severity of bacterial head rot.
- 4) The two bacteria (*Pseudomonas marginalis* and *Pseudomonas syringae*), identified as causative agents of bacterial head rot, naturally occur in soil and water. They mainly spread in crops by rain splash and aerosols. Factors which reduce soil splash, would therefore be expected to reduce bacterial head rot.
- 4) The incidence and severity of bacterial head rot is influenced by nitrogen fertilisation. Nitrogen should only be applied according to soil / foliar / sap tests and not added to excess, especially after buttoning.
- 5) More information is required in order to determine the precise environmental conditions which lead to disease. This will help growers determine if and when the disease will occur and help in future studies to determine methods of control.

3. INTRODUCTION

3.1 Economic cost of bacterial head rot in broccoli

Bacterial head rot of broccoli (*Brassica oleracea* L. var. *italica* Plenck) is reported to be a problem in broccoli production areas in Australia, Canada, Ireland, the United Kingdom and United States. In Australia, a soft rotting of broccoli was reported for the first time by broccoli growers in Victoria in 1978. Since 1978, losses to head rot have been recorded from New South Wales, Queensland and Tasmania.

Disease losses can be severe and can average from 30 to 100% of the crop in some fields. In Victoria, head rot causes serious losses to growers, averaging 35% in the field and over 10% in transit and storage (Wimalajeewa *et al.*, 1987). Additional losses may occur in cold storage and in transit. In the Granite Belt, Darling Downs, and Lockyer Valley bacterial head rot is estimated to cost the industry 10% of the value of production.

3.2 Symptoms

The disease appears as water soaked florets that become necrotic, soft rotted and produce an unpleasant odour if wet conditions persist. The disease begins in the field causing major losses and also during postharvest, reducing storage life of the broccoli.

3.3 Epidemiology

The disease tends to occur mainly during periods of moderate temperatures and high relative humidity or rainfall. The disease also occurs under warm and wet conditions, but is less severe. The exact moisture conditions for the initiation of bacterial head rot have not yet been defined. However, these parameters have been elucidated for bacterial soft rot of potatoes. With potato tubers, decay occurs under conditions of oxygen depletion. A film of water can cause the tubers to become deficient in oxygen in a relatively short period of time, because of internal respiration requirements. This can reduce the host's ability to resist the pathogen. Ludy *et al* (1997) proposed that the same effect could be important in the development of soft rot of broccoli, when free moisture develops on the developing head. This can occur when fields are irrigated frequently by overhead sprinklers or subjected to prolonged dew periods. Under wet conditions, specific bacteria can also produce a natural wetting agent which acts on and disrupts the cuticle and surface wax of the broccoli. This may allow *P. marginalis* and other soft rotting bacteria to enter the broccoli head.

3.4 Disease Control

Alternative irrigation, disease tolerant cultivars, soil management, seedling disinfestation, biocidal agents and manipulation of plant nutrition and plant density have all been shown to have potential for improved control of bacterial head rot.

Irrigation

Overhead irrigation favours disease development by increasing the relative humidity, decreasing temperatures and increasing diurnal dew periods. These changes favour bacterial growth and disease development. Ludy *et al* (1997) reported that in Oregon when the disease incidence was high, the incidence of bacterial head rot was reduced when irrigation was applied weekly compared to three times per week. In contrast, neither the amount of applied water nor timing (morning versus evening irrigation) had a consistent effect on bacterial head rot or yield of broccoli. As irrigation must be adequate during head initiation and enlargement for satisfactory yields of broccoli, more knowledge is required of irrigation effects on disease development and yield.

The other major factors which have been shown to influence bacterial head rot are investigated in this project and are discussed in the appropriate sections.

4. SUMMARY OF RESULTS

4.1 Incidence of bacterial head rot in the Granite Belt

In surveys of broccoli crops from 1992-97, Gatton, Laidley and Thornton were the localities most affected by bacterial head. High losses in the Lockyer Valley were associated with warm humid conditions after rainfall in excess of 50mm. In the Granite Belt and Brookstead, dry warm / hot conditions persisted through the survey period and losses from bacterial head rot were minimal.

4.2 Identification of the Causative Agent of Broccoli Head rot in Queensland

Introduction

Previous studies have suggested that the etiology of bacterial soft rot is apparently complex and is further complicated by the continuing uncertainty about the taxonomic status of some phytopathogenic fluorescent pseudomonads implicated in bacterial head rot. In a collation of crucifer diseases (Hansen, 1985), two bacteria, *Pseudomonas marginalis* pv. *marginalis* (Brown) Stevens and *Erwinia carotovora* (Jones) Bergey *et al*, were listed as the causal agents for bacterial head rot. In other studies, several bacteria have been shown to act as causal agents of bacterial head rot and it is likely that a complex of bacterial pathogens are responsible (Hildebrand, 1989).

Hildebrand (1989) isolated many *Pseudomonas* species and *Erwinia carotovora* subsp. *carotovora* from diseased heads. He considered fluorescent pseudomonads resembling *P.marginalis* to be primary pathogens. Virulent strains were identified as *P.fluorescens* Migula biovar II, *P.marginalis* and *P.fluorescens* biovar IV. Highly virulent strains produced pectolytic enzymes and surfactant like substances that apparently played a major role in successful pathogenesis. Avirulent strains produced a surfactant or pectolytic enzymes, but not both. When *E.carotovora* subsp. *carotovora* was coinoculated with these pectolytic strains of *Pseudomonas*, extensive rot occurred. In Oregon, *E.carotovora* subsp. *carotovora* was commonly isolated from symptomatic heads. When inoculated onto healthy heads under field conditions, disease frequently resulted. In Victoria, Australia, *P.marginalis* was shown to reproduce the symptoms of bacterial head rot in broccoli ((Wimalajeewa *et al.*, 1987). These results suggest that broccoli head rot appears to be most commonly associated with several species of *Erwinia* spp. and *Pseudomonas* spp., acting in combination. The apparent confusion in identifying the specific species of bacteria responsible for bacterial head rot in broccoli, required that identification of the bacteria causing bacterial head rot is confirmed in Queensland.

Method

Broccoli heads, in which head rot was in the early stages of development, were surface sterilised with a 1% solution of sodium hypochlorite, passed through 3 washes of sterile water, macerated in 2ml of sterile water with a mortar and pestle and stood for 30 min. The solution was subsequently plated onto Kings B medium. Single bacterial colonies were restreaked onto LB agar and single colonies subcultured onto LB slopes and stored at 4 C.

Bacterial isolates were subsequently streaked onto Kings B agar, incubated for 72h at 25 C, and suspended in sterile water to ca. 1×10^8 cells ml⁻¹. 1cm² of cotton lint was dipped into the bacterial

solutions (or water control) and placed on the heads of individual 4cm diameter florets of broccoli (cv. Summer King). Florets were either left undamaged or damaged by passing a sterile scalpel through the top 2 mm of the head prior to inoculation. There were 3 replicates of each treatment. Florets were placed into 250 ml plastic beakers, which contained 25 ml of sterile water and sealed with glad-wrap. Broccoli heads were kept upright by inserting the broccoli stalks into rubber holders, the same diameter as the base of the beaker. Beakers were incubated at 20 ± 3 C in the laboratory, with ca. 15 daylight hours.

A total of 25 bacterial isolates were evaluated. Broccoli heads were rated on a scale of 0 - 3. (0 = no rot present, 1 = water soaked lesions, 2 = rot over < 10% of the head, 3 = rot over > 10% of head).

Results

Two attempts were made to identify the causative agent(s) of broccoli head rot. In the first attempt, seven broccoli head rot isolates of fluorescent *Pseudomonad* bacteria were identified as causing head rot. These were identified by Dr Peter Fahy and Mr J Bradley at the Biological and Chemical Research Institute, NSW Department of Agriculture. The isolates belonged to *Pseudomonad* fluorescent groups I, II or IV, with some similarities to *P. marginalis*. Unfortunately these isolates lost pathogenicity during storage and could not be used in trials. In an attempt to confirm these original findings, this methodology was repeated a second time, using both damaged and undamaged heads. The results are detailed below ;

Bacterial Isolate	Disease Rating	
	Undamaged	Damaged
PS4bb	0	2.67
PS1bb	0.33	3.0
PS5bb	0.33	2.0
PS2bb	0	2
2	0	2.0
5	0	2.0
8	0	2.5
10	0	2.0
18	0.33	2.0
20	0	3.0
21	0	3.0
22	2.0	3.0
24	0	2.0
26	0	2.0
29	0	2.0
31	0	2.5
32	0	2.5
33	0	2.0
34	0	2.5
35	0	2.0
37	0.33	2.5
40	0	2.0
42	0	3.0
44	0	1.67
45	0	3.0

Bacterial isolate 22 was identified using Fatty Acid Methyl Ester Analysis by Bruce Hawke (CSIRO Division of Land and Water). The best correlation was with *Pseudomonas syringae glycinea* (0.83), *Pseudomonas putida* biotype A (0.742), *Pseudomonas fluorescens* biotype A (0.734) and *Pseudomonas syringae pisi* (0.725).

Bacteria	Correlation Coefficient		
	Rep 1	Rep 2	Average
<i>Pseudomonas syringae</i>	0.80	0.86	0.83
<i>P.syringae glycinea</i>	0.80	0.86	0.83
<i>P.syringae tagetes</i>	0.78	-	*
<i>P.syringae phaseolicola</i>	-	0.69	*
<i>P.syringae pisi</i>	0.77	0.68	0.725
<i>Pseudomonas putida</i>	0.737	0.746	0.742
<i>P.putida biotype A</i>	0.737	0.746	0.742
<i>P.putida biotype B</i>	0.473	0.538	0.510
<i>Pseudomonas fluorescens</i>	0.735	0.733	0.734
<i>P.fluorescens biotype A</i>	0.735	0.733	0.734
<i>P.fluorescens biotype G</i>	0.608	0.707	0.658
<i>P.fluorescens biotype B</i>	0.631	0.697	0.664
<i>Chromobacterium</i>	0.636	0.656	0.646
<i>C. violaceum</i>	0.636	0.656	0.646

Discussion

P.marginalis was identified as causing broccoli head rot on undamaged heads. This result agrees with the only other previous report on the identification of the causative agent of broccoli head rot in Australia. This report showed *P.marginalis* to be a causative agent of bacterial head rot of broccoli in Victoria ((Wimalajeewa *et al.*, 1987).

In the second attempt, 5 bacterial isolates produced watersoaking in at least one out of three replicates on undamaged heads. Only one isolate (Number 22) produced rotting on undamaged heads. In contrast, 12 bacterial isolates produced rotting in at least one out of three replicates on damaged heads. Five bacterial isolates (PS 1bb, 22, 20, 42 and 45) produced rotting on all replicates. Only one isolate (number 22) produced rotting on both damaged and undamaged heads. This bacteria was identified as *Pseudomonas syringae*. The observation that bacterial head rot was recorded more commonly on damaged heads, suggests that in the field, damaged broccoli heads may be more susceptible to bacterial head rot.

Two bacteria were identified as capable of causing bacterial head rot on undamaged heads. These were *Pseudomonas syringae* and *P.marginalis*. As the broccoli heads were not surface sterilised it is possible that these bacteria were not acting alone, but were in combination with bacteria already present on the broccoli heads, to produce bacterial head rot.

4.3 Variety Trial to Identify Disease tolerant Broccoli cultivars

Introduction

Canaday et al., (1991) reported significant differences in susceptibility of broccoli to bacterial head rot caused by *Pseudomonas marginalis* (*P. fluorescens* biovar II) and other fluorescent *Pseudomonas* spp. Cultivars Green Defender and Shogun consistently appeared highly resistant to bacterial head rot. Cultivar Green Valiant was rated moderately resistant. Robertson et al. (1993) reported that the most susceptible calabrese (broccoli, *Brassica oleracea* var. *italica*) cultivar was Premium Crop ; cultivars with a high degree of resistance were Charade, Bacchus, Dixie, Exp 91, Hi Crown, Samurai and Shogun. Due to the constant development of new varieties and the fact that many of these varieties are not suitable for climatic conditions in Queensland, research was needed into the relative susceptibility of broccoli varieties commonly grown in Southeast Queensland.

Observations by DPI staff and broccoli growers in Queensland have noted that varieties with loose heads such as Pacific (Generation) are more susceptible to bacterial head rot. In contrast, varieties with tight heads such as Marathon (which do not retain irrigation / rain water on their heads) were observed to be less susceptible. These observations would agree with previous reports that horticultural characteristics (head tightness, doming, floret size and head weight / diameter ratio) are significantly correlated with genotype susceptibility to bacterial head rot.

Previous reports have also suggested that both disease incidence and severity are inversely correlated with the number of days from transplanting until harvest. As doming and floret size, are often associated with the number of days required for broccoli genotypes to reach harvest maturity, this factor might explain why disease severity can be correlated with these horticultural characteristics.

In the trials detailed below, heads of a similar size were harvested. This was necessary as both the incidence and severity of bacterial head rot has been shown to increase with head size (Canaday and Wyatt, 1992). Where only one harvest has been conducted, the variation in head size significantly affected the results (Canaday and Wyatt, 1992).

Results

Trial One

Twenty one summer varieties of broccoli from the seed companies S and G Sandoz Seeds, Henderson, South Pacific, New World and Arthur Yates were compared for tolerance to bacterial head rot. Macerated, previously frozen, head rot tissue was used as an inoculum in a trial planted at the Granite Belt Horticultural Research Station on the 18th October, 1995. The broccoli were grown under overhead irrigation according to standard horticultural practices and integrated pest management using *Bacillus thuringiensis*. Each variety was grown in a plot of 20 plants, 0.33m apart, replicated 6 times and arranged in randomised blocks.

Broccoli variety	Date Harvest Completed	Av. Head Wt.	% Marketability	% bacterial head rot
S & G Sandoz Seeds				
BR130 (S&G)	12.12.95	248	56	20 bc
Dominator	19.12.95	252	100	4 ef
Generation BR108	19.12.95	264	100	6 ef
Henderson				
Purple Mountain	19.12.95	252	100	4 ef
Marathon	24.12.95	298	98	6 ef
Greenbelt	24.12.95	332	84	22 bc
South Pacific Seeds				
Shilo	12.12.95	209	80	33 a
Coral	12.12.95	211	81	19 bcd
Maverick (1st plting)	20.12.95	266	88	9 def
Pheonix sps 566	25.12.95	315	100	4 ef
Lot R197FF	20.12.95	290	67	5 ef
Shilo 529	12.12.95	209	80	33 a
Maverick (2nd plting)	20.12.95	230	100	18 bcd
New World Seeds				
NW182	24.12.95	194	100	0 f
NW184	24.12.95	375	76	5 ef
Legacy 171	25.12.95	362	97	2 f
Hybrid Marathon	25.12.95	210	99	3 ef
Hybrid Greenbelt	20.12.95	256	83	6 def
Hybrid Legacy	25.12.95	393	95	7 def
Yates				
Summer King	12.12.95	260	80	33 b
YB-1290	20.12.95	366	77	13 cde
Sel 117	25.12.95	259	94	10 def
Dynamo	20.12.95	230	95	3 ef

Data converted to Arcsin prior to statistical analysis.

Trial 2

A second trial was established at the Granite Belt Horticultural Research Station, in which seedlings from 10 varieties of broccoli were sown, in a replicated, split plot design trial. There were 5 replicates of each treatment. Broccoli transplants were planted on the 5th February, 1997. Two rows of guards (broccoli cv. Maverick) were planted between replicates, with one guard between rows. Four rows of guards were planted around the whole trial. The soil was treated with dolomite (4 tons / ha) and terrafirma (5.55 tons / hect) prior to planting. In an effort to promote bacterial head rot, nitrogen (50 tons / hectare) was applied on the 12th February, 24th February, 12th March and 20th March. The last application was approximately at buttoning. Fertilisers were applied according to recommended practice.

The crop was watered 3 times a day from buttoning (for 1hr by overhead irrigation), and each head was individually inoculated 4 times, at ca. 2 day intervals (once the heads had reached 5cm in diameter. Inoculum consisted of 5 ml of sterile water or a comminuted suspension of broccoli heads containing bacterial head rot [Twenty-five grams of tissue (fresh weight) was taken from the margin of head rots, ground in a mortar and pestle and combined with 500 ml distilled water].

The weight and head of each head and the percentage head rot was measured at harvest ;

Broccoli variety	Av. Head Wt. (g)	Width (mm)	% bacterial head rot
S & G Sandoz			
Dominator	235 d	14.3 bc	0
Generation	257 bc	14.8 abc	0
Green Prince	167 e	13.4 ef	0
Marathon	246 cd	14.1 cde	0
Monaro	243 cd	14.8 bc	0
New World			
NW 702	255 bcd	15.6 a	0
South Pacific			
Maverick	247 cd	14.4 bcd	0
886	335 a	14.9 ab	0
Rainbow	170 e	13.1 fg	0
Henderson			
Amalia	187 e	13.6 def	0
Yates			
Sel 117	275 b	14.9 ab	0
Summer King	181 e	12.4 g	0

No bacterial head rot was recorded, despite the high amount of nitrogen fertilisers used and the heads being irrigated for approximately one hour, 3 times a day. Between the 27th March and 16th April, when the inoculum was applied, the weather was most often characterised by hot, sunny, dry conditions. It is likely that these conditions would have contributed to the death of the inoculated bacteria (through desiccation and UV radiation) and almost immediate evaporation of water present on the head after the morning and mid-day irrigations had been terminated. This, in turn, may also have limited the time for the florets to become deficient in oxygen and hence the development of bacterial head rot.

The broccoli heads were harvested when they appeared marketable. South Pacific 886 produced heads with a significantly greater fresh weight than any of the other varieties at the time of harvest. The p value for Smirnov's Chi squared approximation, between the head width and head weight was 0.8.

Trial 3

A third trial was established at the Granite Belt Horticultural Research Station. Broccoli transplants were planted on the 2nd April, 1997.

Each cultivar was replicated 3 times in plots measuring 3m x 6.6m. Each replicate was arranged into a randomised block. Urea was added @ 50 kg / ha on the 10th April, 24th April, 7th May, and 11th June in an attempt to make the broccoli plants more susceptible to infection by the causative agent(s) of bacterial head rot.

Once heads were ca. 5cm diameter, heads were damaged by using a scalpel to remove the top 2 mm of plant tissue over an area of 1 cm². These were immediately inoculated with sterile water or a comminuted suspension of broccoli heads containing bacterial head rot [Twenty-five grams of tissue (fresh weight) was taken from the margin of head rots, ground in a mortar and pestle and combined with 500 ml distilled water].

Plastic bags were placed over the heads in order to retain humidity. This procedure was conducted at 3pm. Preliminary experiments had shown that even with injury and using the comminuted suspension bacterial head rot suspension, infection was infrequent (presumably due to the warm sunny dry conditions present at the time of the trial). Due to the different rates of development of each broccoli head, it was necessary to inoculate heads on 6 occasions. Plastic bags were removed 96h after inoculation. Broccoli heads with a diameter of > 10 cm were harvested and rated visually for the amount of bacterial head rot. Heads were subsequently cut open and rated for the degree of rotting. Scales are detailed below ;

Seed Line	% of plants with head-rot	Visual rating*	Head Rot Rating [#]
1. S & G BR108	80	2.88	2.18
2. S & G BR 327	89	2.91	2.54
3. NW Hybrid Legacy	72	2.65	1.87
4. NW Hybrid NW 702	86	2.95	2.51
5. SPS Maverick (784)	57	2.63	2.03
6. SPS 938	49	2.52	1.76
7. Yates Endurance	71	2.66	2.00
8. Yates Hybrid 5722	60	2.39	2.12
9. Yates Hybrid 6251	61	3.01	2.62
P (LSD)	0.648	0.392	0.636

*Visual rating - 1 = no disease present, 2 = cell browning, 3 = cell disintegration, 4 = cell disintegration and odorous smell.

[#]Bacterial head rot rating - 0 = no rotting present, 1 = florets disintegrated, 2 = < 1cm length of stem rotted, 3 = > 1 cm stem length rotted.

Data converted to log values, prior to statistical analysis, in order to normalise the data.

Discussion

In trial one, bacterial head rot developed in early December after heavy rains and hail. Severe losses due to bacterial head rot were recorded in 4 varieties (Shilo, Summer King, BR130 and Coral), suggesting that these varieties possessed little resistance to bacterial head rot under the conditions of the trial. Each of these varieties matured on 12.12.95. Thereafter, dry conditions persisted until harvest was completed on 25.12.95. Moderate levels of head

rot (9-18%) were recorded in Maverick and YB-1290 and Sel 117, which matured between 20.12.95 and 25.12.95. Head rot was recorded at low levels (0-6%) in the 14 other varieties. Dominator, Purple Mountain, Pheonix, NW182, Legacy and Dynamo warrant further evaluation in southern Queensland as sources of resistance to bacterial head rot.

Previous reports have suggested that both disease incidence and severity are inversely correlated with the number of days from transplanting until harvest. The data from trial one, showing that cultivars which matured more quickly were more susceptible to head rot, would agree with this hypothesis. In order to avoid bacterial head rot, these results suggest that where bacterial head rot is likely to be a problem, cultivars should be chosen which have a relatively long time period between transplanting and harvest.

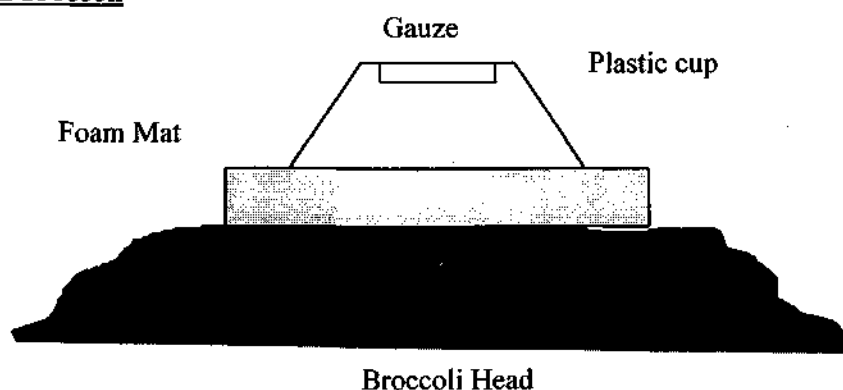
In trials two and three, the weather was warm and dry ; conditions not conducive for the establishment and development of bacterial head rot. In trial two, no bacterial head rot was recorded despite the heads being inoculated with a suspension of diseased heads, high nitrogen being applied and the plants being irrigated 3 times a day for ca. 2h. In order to try to ensure that bacterial head rot occurred in trial 3, heads were first damaged before being inoculated and the heads covered in plastic bags to retain humidity. Although large differences were found in cultivar susceptibility, these differences were not significant, due to the large variation between replicates. Part of this variation was probably caused by rabbits and wild pigs damaging broccoli heads around the circumference of the trial site. Although the rabbits were controlled, efforts to trap the pigs were unsuccessful. Damage of broccoli heads and plants may have lead to an increased severity and incidence of bacterial head rot.

Although trials (1) and (3) were conducted under different conditions and using different techniques, both trials indicated that NWS Hybrid Legacy and Maverick were relatively resistant to bacterial head rot, compared to the other cultivars grown.

4.4 Influence of broccoli head injury on the incidence of bacterial head rot

Previous *in vitro* results suggested that bacterial head rot may be promoted by injury to the broccoli heads. In order to evaluate this hypothesis, a field trial was conducted on broccoli (cv. Summer King). Heads were inoculated with either one, two or three mature caterpillars (Cabbage White), each ca. 15mm in length, enclosed within a trap. There were 5 replicates of each treatment.

Trap used to retain caterpillars on broccoli heads



After 48h the traps and caterpillars were removed and the percentage damage of the florets in the area covered by the trap approximated. The bacteria (*Pseudomonas syringae* ; culture 22), which had previously been cultured for 48h on Kings B medium and resuspended in sterile rain water (ca. 10^8 cells / ml), was then inoculated onto the tops of each broccoli head (1.8 ml). Controls were inoculated with 1.8 ml sterile rain water. Irrigation water was applied 3 times a day for one hour duration until the time of harvest.

Five days after inoculation, broccoli heads were harvested and rated for bacterial head rot on the following scale ;

0 = no disease, 1 = browning, but no rot, 2 = rot + smell (< 50% of test area), 3 = rot + smell (> 50% of test area)

Results

Treatment	Percentage Caterpillar Damage	Disease Rating
<u>No Caterpillars</u>		
- Inoculum	0.0 b	0.0 b
+ Inoculum	0.0 b	0.0 b
<u>One caterpillar</u>		
- Inoculum	40.0 a	0.2 b
+ Inoculum	44.0 a	1.6 a
<u>Three Caterpillars</u>		
- Inoculum	43.0 a	0.2 b
+ Inoculum	60.8 a	1.5 a

Numbers in the same column followed by the same letter were significantly different from each other.

These results demonstrate that under the trial conditions, bacterial head rot only occurred when both the heads were damaged and inoculum was present. Inoculum or damage alone did not cause bacterial head rot. The Kolmogorov-Smirnov test was conducted to test the association between the amount of damage and the amount of disease in those treatments in which inoculum was added. The two-tailed Kolmogorov-Smirnov statistic = 0.31, with a p value (Smirnov's Chi-Squared approximation) = 0.07.

The observation that bacterial head rot did not occur without inoculum would suggest that the causal bacteria is not ubiquitous in the environment. In contrast, Canaday et al (1991) suggested that as bacterial head rot could be consistently induced when heads of maturing broccoli were kept wet in Tennessee, USA, the causal bacterium was ubiquitous in the environment at this location. They further suggested that as this disease is widely scattered in fields not previously cropped to broccoli, that the causal bacterium was of a soilborne origin. In the trials described above, overhead irrigation was used and with the weakly structured soils present in the Granite Belt, soil splash would have been expected to be severe. The fact that broccoli head rot did not appear in this trial, suggests that at least at this site, the causal agent(s) were probably not present in the soil or the conditions were not sufficiently conducive.

These results are specific for the bacterial isolate used in these trials. However, they do suggest that bacterial head rot is more likely to occur when the broccoli head is injured. Although the above field trial was conducted with caterpillars, it is likely that damage caused by human damage, wind, hail stones, frost or other insect damage will cause a similar increase in bacterial head rot.

4.5 Chemical Control of Bacterial Head Rot of Broccoli

Control of this disease with commonly used fungicides and bactericides has proved largely unsuccessful. A variety of treatments were evaluated in this project ;

Plant Waxes, Sodium Hypochloride, Codacide Oil and Agridex

The ability of these compounds to reduce bacterial head rot were evaluated in trials established at Laidley. All treatments were applied at late buttoning and 7 and 14 days later.

- Plant waxes (apple wash and peach lustre wax) were evaluated for their ability to reduce bacterial head rot on Marathon broccoli. Although both waxes reduced bacterial head rot from 6.3 to 3.2%, they also turned the heads yellow and rendered the broccoli unmarketable.
- Sodium hypochloride (100 ug ml⁻¹) reduced bacterial head rot on cv. Marathon from 2.3% to 0.83% (P>0.05), but also caused the heads to turn yellow.
- In a trial evaluating the use of codacide oil (5ml / L) and Agridex (1ml /L) on cv. Marathon, no bacterial head rot was recorded in the control. However, both codacide oil and agridex caused darkening of the broccoli heads.

Further work on these compounds was terminated.

Chemical Control

In Scotland, a large variety of pesticides have been trialed without success. Only Cuprokyt (copper oxychloride) was shown to control bacterial head rot (Harling, 1994). Robertson et al. (1993) reported that application of copper oxychloride reduced disease by 80% in 1988 ; copper oxychloride reduced disease by 47% in 1987. Copper oxychloride was also successful in an earlier trial (Brokenshire and Robertson, 1986). Two sprays of copper oxychloride at "button" stage (heads 2.5cm in diam.) and 7 days later were shown to be most effective treatment (Robertson and Brokenshire, 1992).

In order to evaluate the use of copper formulations and phosphorous acid for the control of bacterial head rot in the Granite Belt, a field trial was established at the Granite Belt Horticultural Research Station using broccoli cv. Generation. Broccoli were grown for 7 weeks to head expansion stage in single plant plots, each replicated 15 times. The chemicals were applied twice at early buttoning, using a pressurised hand sprayer with adjustable nozzle, 7 days apart. The heads were then inoculated with a macerated culture of diseased heads, containing approx. 10^8 cells ml^{-1} at early buttoning (heads 2.5cm diam.).

Treatment	% Bacterial Head Rot (Oct 93)
Copper oxychloride (50% WP available copper @ 5g / L)	33
Copper hydroxide (50% WP available copper @ 2g / L)	26
Potassium phosphite (200g / L at 5ml / L)	50
Control	55

A further trial was established at Landley using broccoli cv. Marathon

Treatment	% Bacterial Head Rot (Oct 93)
Sodium Hypochlorite (100 ug / ml)	0.83
Benzalkonium chloride (100 ug /ml)	45.0
Control	2.3

These results suggest that copper hydroxide, copper oxychloride and hydrated lime can give limited control. The copper fungicides caused no phytotoxicity and spray residues were at acceptable levels. However, hydrated lime produced white residues on the broccoli. Sodium hypochlorite at 100 μg / ml discoloured the broccoli heads. Even at 250 μg /ml a.i., sodium hypochlorite delayed but did not fail to prevent culture 22 from reaching turbidity, when 10^6 cells ml^{-1} , was inoculated into 100ml of Kings B medium and incubated on a rotary shaker at 22 C at ca. 100 rpm. These results, together with the experimental evidence from Scotland, suggest that copper fungicides may be an effective method of controlling this disease.

Field trial (2)

Calcium content of the host cell wall has been shown to be associated with increased resistance to pathogens (Conway, 1982). Biggs et al., (1994) reported that after evaluating calcium acetate, calcium sulphate, calcium silicate, calcium heptagluconate, calcium oxide, calcium succinate, calcium chloride and calcium hydroxide, that calcium propionate was the most effective form of calcium to stop lesion formation by the peach canker fungus *Leucostoma personii*. Bateman and Lumsden (1965) reported that the resistance of beans to *Rhizoctonia solani* was related to the plant calcium concentration.

The trial detailed below, assessed whether bacterial head rot could also be controlled by the application of calcium. A field trial was established at the Granite Belt Horticultural Research Station, using the following treatments ;

Treatments	Rate	Application
Copper oxychloride	5g / litre	13h after inoculation
Copper hydroxide	2g / litre	13h after inoculation
CaCl ₂ (Stop-it)	20 ml / litre	At buttoning
Calcium propionate	1.86g / litre (pH 6.0)	At buttoning

Broccoli (Greenbelt) was sown on the 26th March and grown according to the normal commercial practice. At buttoning, calcium compounds were added, till runoff, to the appropriate plants. Due to the different times in broccoli development, calcium was applied either on the 17th and 23rd May or the 6th June. Once heads were ca. 5cm diameter, they were either left undamaged or damaged by using a scalpel to remove the top 1mm of plant tissue over an area of 1 cm². Where appropriate, these were immediately inoculated with sterile water or a comminuted suspension of broccoli heads containing bacterial head rot [Twenty-five grams of tissue (fresh weight) was taken from the margin of head rots, ground in a mortar and pestle and combined with 500 ml distilled water].

Plastic bags were placed over the heads in order to retain humidity. This procedure was conducted at 3pm. Preliminary experiments had shown that even with injury and using the comminuted suspension bacterial head rot suspension, infection was infrequent (presumably due to the warm sunny dry conditions present at the time). The following morning (10 am), chemical solutions were applied over the heads till run-off and the plastic bags replaced. Due to the different rates of development of each broccoli head, it was necessary to have 6 inoculation times. Plastic bags were removed 72h after inoculation. There was a total of 10 treatments, with 4 reps per treatment, each rep being laid out in a randomised square. Each plot contained 40 (8 x 5) plants, with the outer line of 22 plants in each plot being treated as a guard row.

Broccoli heads were harvested when they were > 10cm in diameter. Heads were cut through to the stem and rated on the following scale ;

0 = no disease, 2 = water soaking, 3 = cells disintegrated, 4 = cells disintegrated plus smell.

Treatment	Damaged / Undamaged ± Inoculum	Disease rating
Control	Damaged + Water	2.18 c
Control	Damaged + Inoculum	2.87 a
CaCl ₂	“ “	2.21 bc
Ca Propionate	“ “	2.84 a
Calcium oxychloride	“ “	2.61 ab
Calcium hydroxide	“ “	2.97 a
Control	Undamaged + Water	0.0 d
Control	Undamaged + Inoculum	0.05 d
CaCl ₂	“ “	0.0 d
Ca Propionate	“ “	0.0 d
Calcium oxychloride	“ “	0.0 d
Calcium hydroxide	“ “	0.0 d

Numbers in the same column followed by the same letter were not significantly different from each other.

Wrapper leaves were removed from the calcium treated broccoli plants 4 days after the calcium solution was applied. The percentage calcium in these leaves was analysed.

Treatment	% calcium in wrapper leaves
Control (Damaged + Water)	0.75 a
Control (Damaged + Inoculum)	0.76 a
CaCl ₂ (Damaged)	0.82 a

Numbers in the same column followed by the same letter were not significantly different from each other.

Discussion

The results agree with previous findings that in the absence of broccoli head damage, broccoli head rot is unlikely to occur. The conditions of this experiment were again relatively warm and sunny, with no hail damage.

The only treatment that resulted in a significant reduction in head rot was the application of calcium chloride. However, the calcium content in the wrapper leaves surrounding the head after calcium chloride application was not significantly higher than the water control. Calcium propionate was not effective in reducing bacterial head rot. This is despite calcium propionate having a long history as a food additive and a well known inhibitor of certain moulds and bacteria in stored grain (Raeker *et al.*, 1992).

Previous studies have demonstrated that calcium can inhibit the development of plant fungal pathogens without causing an associated increase in calcium in the surrounding plant tissue. Biggs and Peterson (1990) in a study on the application of exogenous materials to peach bark wounds, reported calcium chloride to be the most promising treatment for reducing disease caused by *Leucostoma persoonii* (peach canker fungus). In a further study, Biggs *et al.* (1994) reported that lesion length due to *L. persoonii* was reduced by more than 70% when excised peach twigs were dipped in calcium silicate or calcium propionate for 15 mins prior to inoculation. However, the calcium content in the bark was not greater than in the distilled deionised control. Calcium chloride did not stimulate the production of lignin or suberin. These authors suggested that the Ca²⁺ ions may have formed cross bridges between pectic acids in the plant cell walls, thus making the cell walls more resistant to digestion. The possibility that Ca²⁺ acts in a similar manner in reducing bacterial head rot of broccoli is supported by the observation that breakdown of pectins in the broccoli head appears to be part of the disease process (Hildebrand, 1989). Kohle *et al.* (1985) suggested that the Ca²⁺ ion can also stimulate the production of phytoalexins and / or phenols. Alternatively the calcium ion or undissociated compounds may have acted directly on the bacterial pathogens or modified membrane permeability, electron transport and / or enzyme activity, resulting in reduced bacterial growth or virulence.

Although neither of the copper compounds in this study reduced bacterial head rot, these compounds were only applied once ; in contrast to earlier studies where copper compounds were applied twice and showed to successfully reduce the severity of bacterial head rot.

Biological Control

The commercially available biological control agent Victus, a fluorescent *Pseudomonad* registered for the control of bacterial blotch, was trialed at the Granite Belt Horticultural Research Station. Drought conditions prevented the development of the disease. The efficacy of this product could therefore not be evaluated.

4.6 Influence of nitrogen on bacterial head rot

Introduction

Broccoli yields generally respond to high rates of nitrogen fertilisation (Dufault and Luther, 1985). One management practice of concern is the use of these high rates of nitrogen to maximise yields. Canaday and Wyatt (1992) reported that as side-dressings of ammonium nitrate increased from 38 to 152 kg / ha, the incidence and severity of bacterial soft rot increased in a susceptible broccoli cultivar (Premium), but had no effect in a resistant one (Shogun). The length of time necessary for heads to reach maturity decreased with increased N and this in turn was correlated with increased disease severity. In addition to bacterial head rot, head quality and the incidence of hollow stem have been reported to increase with the amount of N applied (Dufault and Luther, 1985).

The relationship between nitrogen application, total yield, marketable yield, % plant recovery per plot and bacterial head rot was investigated in a trial at Gatton in the Lockyer Valley. Four nitrogen rates were applied (0, 40, 80, 160 kg / ha N applied as ammonium sulphate), with 3 varieties of broccoli (Greenking, Greenbelt and Shilo) varying in susceptibility to bacterial head rot, and 3 plant spacings (165mm, 330 mm and 495 mm). There was 3 replicates per treatment with a plot size of 1.5 x 4.95 m². The trial area had 2 plantings of sorghum prior to the establishment of this trial in an effort to reduce the soil N.

Results

Due to the early planting time and the mild growing conditions, the variety Greenbelt performed poorly and data from this variety was not included in the statistical analysis of results.

Relationship between nitrogen fertiliser and bacterial head rot

Unseasonably warm autumn conditions were present during the course of this trial. These conditions were likely to have contributed to the low frequency of bacterial head rot (2 -3%) that were observed. This head rot was observed predominantly in plots receiving high levels (200 kg / ha) of nitrogen.

Total Yield

Positive responses to nitrogen fertiliser were recorded with the highest nitrogen treatment yielding higher than the other three treatments (P=0.05). Shilo produced higher yields than Greenking and yields increased as spacing decreased (P=0.05).

Marketable Yield

There was a significant response to nitrogen fertiliser. However, there was no significant difference between 165 and 330 mm spacing or between Greenking and Shilo. Shilo produced more marketable yield than Greenking.

Individually, the application of 80 kg / ha significantly increased both total and marketable yields of Shilo. Only at the highest rate of N (160 kg N / ha) was there a response to N for Greenking.

Weight of Heads

Weight per head significantly increased with applied N, but decreased as plant spacing decreased. There was no significant difference in weight of heads (both total and marketable) between Greenking and Shilo.

% Recovery

Recovery significantly increased as spacing increased. There was a significant N x variety interaction. Greenking had the highest recovery at 160 kg N / ha. For Shilo, recovery decreased with increasing N fertiliser.

Indicators of Nitrogen Status

N % of Heads

For both Greenking and Shilo, the application of N had little effect on total N%. Concentrations of N averaged across spacings ranged from 5.65 - 5.78% for Greenking and 5.19 - 5.41% for Shilo. Total N was higher in Greenking (5.72%N cf. 5.35%N).

Sap Nitrate

At 24 days after transplanting, differences in sap NO₃ concentrations were very small (6960 - 7460 mg NO₃ / L) over the various nitrogen treatments. Thirty-nine days after transplanting, differences between plants receiving different amounts of N were only significant for Shilo. The range of concentrations were : Greenking 2660 - 3395 mg NO₃ / L : Shilo 2730 - 3985 mg NO₃ / L.

Nitrate-N in dried midribs

For both varieties there were significant differences in NO₃-N concentrations with N applied. Concentrations ranged from 7190 - 10080 mg NO₃-N / kg for Greenking and from 7930 - 11650 mg NO₃-N / kg for Shilo.

These results indicate that nitrate accumulated in the midribs of wrapper leaves as the N rate increased. Total N% was high in all treatments, even in plants receiving nil N. This indicates that soil N was high in the control soil, so as to negate sufficient response to applied N or that the test was insensitive as an indicator of N status.

Relationships between broccoli yields and N concentrations using the commercial plant spacing 330mm.

For both Greenking and Shilo, although yields increased with applied N, the application of N did not significantly improve total and marketable yields.

	Nitrogen fertiliser rate (N kg /ha)			
	0	40	80	160
Greenking				
Total Yield	6.43	6.64	6.39	9.01
Marketable Yield	4.67	5.10	4.70	6.73
Shilo				
Total Yield	6.81	7.54	8.27	8.33
Marketable Yield	4.00	5.18	6.55	4.49

For Shilo, coefficients of determination (R^2) for the quadratic, square root quadratic and linear regressions of Y (yield) vs X (%N or sap NO_3 or $\text{NO}_3\text{-N}$ in tissue) were all < 0.05 , indicating that relationships were not significant. This can be attributed, in part, to the narrow range of yields recorded. The most significant relationship was for marketable yield vs. total yield in heads for Shilo (R^2 of 0.48).

For the variety Greenking, sap nitrate at both sampling times was poorly related to final yields (max. R^2 of 0.36). Large variation in the sap test results may have contributed to this poor relationship. Details of the regressions yield vs N in dried tissue are described below ;

(a) Total Yield (Y) vs. Total N in heads (X)

$$Y = -216.8 \text{ (t/ha)} + 79.05 X - 6.95X^2 \quad R^2 = 0.67$$

$$Y_{\text{max}} = 8.0 \text{ (t/ha)} \text{ (dy/dx = 0, X = 5.69\%N)}$$

$$90\% Y_{\text{max}} = 7.2 \text{ (t/ha)}$$

Estimated critical concentration = 5.35%N

(b) Marketable Yield (Y) vs. Total N (X)

$$Y = -151.0 \text{ (t/ha)} + 54.6 X - 4.75X^2 \quad R^2 = 0.63$$

$$Y_{\text{max}} = 5.75 \text{ (t/ha)} \text{ (dy/dx = 0, X = 5.76\%N)}$$

$$90\% Y_{\text{max}} = 5.18 \text{ (t/ha)}$$

Estimated critical concentration = 5.39%N

(c) Total Yield (Y) vs. $\text{NO}_3\text{-N}$ in dried mid-rib (X)

$$Y = -14.14 \text{ (t/ha)} + 0.0047 X - 0.00000027X^2 \quad R^2 = 0.65$$

$$Y_{\text{max}} = 6.31 \text{ (t/ha)} \text{ (dy/dx = 0, X = 8,703 mgNO}_3\text{-N/kg)}$$

$$90\% Y_{\text{max}} = 5.68 \text{ (t/ha)}$$

Estimated critical concentration = 7280 mg NO_3 N/kg

(d) Marketable Yield (Y) vs. $\text{NO}_3\text{-N}$ in dried mid-rib (X)

$$Y = -12.12 \text{ (t/ha)} + 0.00388 X - 0.00000022X^2 \quad R^2 = 0.70$$

$$Y_{\text{max}} = 4.98 \text{ (t/ha)} \text{ (dy/dx = 0, X = 8,818 mgNO}_3\text{-N/kg)}$$

$$90\% Y_{\text{max}} = 4.48 \text{ (t/ha)}$$

Estimated critical concentration = 7380 mg NO_3 N/kg

The estimated critical nitrogen concentration of 5.35 - 5.39% contrasts with the critical value of 5.00% reported by Reuter and Robinson (1986), In 'Plant Analysis - An Interpretation Manual'. Reuter and Robinson (1986) reported that an adequate range was 5.6 - 6.1%. An adequate $\text{NO}_3\text{-N}$ concentration of 9000mg $\text{NO}_3\text{-N}$ is defined in the manual, but no critical value is shown.

Discussion

Previous studies have shown that nitrogen fertilisation can have a significant effect on the incidence of bacterial head rot and to the marketable yield of the broccoli crop. In this trial, bacterial head rot was recorded only rarely. It is likely that this was due to the uncondusive environmental conditions. Canaday and Wyatt (1992) irrigated broccoli crops up to 5 times a day in order to make the conditions conducive to bacterial head rot. Should this methodology have been adopted in this trial, it is likely to have lead to increased leaching and denitrification of the nitrogen. This would have reduced the influence of nitrogen on bacterial head rot.

This trial was therefore unable to confirm the findings of Canaday and Wyatt (1992). This report demonstrated that the rate of nitrogen fertilisation had a negligible effect on soft rot incidence and severity in a broccoli cultivar with some resistance, but increased the incidence and severity in a susceptible variety. If this trend holds true for all susceptible and resistant cultivars, it has important implications for the management and control of bacterial head rot.

4.7 Post harvest chemical control

Hydrated lime (70g / L) applied as a dip on mature heads reduced head rot during storage from 14% to 7% ($P>0.05$). However, copper hydroxide, H_3PO_3 + Agral increased head rot.

5. DISCUSSION

Control of bacterial head rot in broccoli

This project has confirmed previous findings that *Pseudomonas marginalis* to be causal agent of bacterial head rot on undamaged broccoli heads. *Pseudomonas syringae* was also identified as a possible causative agent of bacterial head rot. As these trials were not conducted on 'sterile' heads, it is possible that these bacteria alone, did not cause bacterial head rot, but that they worked in conjunction with other bacteria already present on the head. Both types of bacteria naturally occur in soil and water and are mainly spread in crops by rain splash and aerosols. Factors which reduce soil splash may therefore, in addition to the factors previously listed in this project, also help reduce bacterial head rot.

Cultivars exhibiting little resistance to bacterial head rot were identified. This information will help growers identify which cultivars require preventative action to prevent the development of bacterial head rot. This project confirmed studies in Scotland showing that copper oxychloride was effective against this disease. In Scotland, copper oxychloride (Cuprokyt) has obtained an off-label approval to control bacterial head rot (Harling, 1994). Despite tests on a wide range alternative products copper oxychloride was the only chemical shown to be effective. This project demonstrated that copper hydroxide and calcium chloride may also help reduce the disease severity of bacterial head rot. These chemicals need to be applied at early buttoning. Application at late buttoning when wet weather prevails, is likely to be too late to be effective, because bacterial numbers will already be too high.

Most of the field trials in this project were conducted in the Granite Belt. Only in one trial was substantial bacterial head rot recorded without the heads being mechanically damaged. This trial (unlike other trials) was subjected to a hailstorm after buttoning. This observation would therefore support the hypothesis that broccoli is more susceptible to head rot after the heads have been damaged. Broccoli should therefore, where possible, be grown in sheltered spots, away from frost hollows and under netting. Caterpillars numbers should be rigorously controlled, especially after buttoning. Mechanical damage as a prerequisite for bacterial head rot development may only be important when the environmental conditions are not totally conducive for head rot development (such as often occur in the Granite Belt). In other states, where environmental conditions are more conducive, the development of bacterial head rot may be less dependant upon mechanical damage of the heads.

This project has highlighted the need to determine the precise conditions of temperature, rainfall, relative humidity and leaf wetness which lead to disease. This information is needed to help growers forecast if and when the disease will occur and when preventative measures should be applied. This research would also help overcome a major difficulty experienced in this project (of bacterial head rot occurring inconsistently in field trials), by ensuring that conditions conducive to head rot are present in trials evaluating the efficacy of possible control treatments.

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7. PUBLICATION SCHEDULE

Two papers detailing the results of this work will be submitted for publication in the Australian Journal of Experimental Agriculture by the end of July, 1998. An information sheet will also be made available to broccoli growers by the end of July, 1998.

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