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An investigation on head rot disease of broccoli crops grown for processing

Hoong Pung
Serve-Ag Research

Project Number: VG01082

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This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

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RESEARCH

FINAL REPORT

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Media Summary

In recent years, the production of broccoli produced for processing into frozen vegetable products has increased rapidly. Although head rot disease has been identified by the processors to be a major constraint to processing broccoli production in Tasmania, little is known of the primary causal organisms or pre-disposing factors. This project was therefore aimed at gaining a better understanding of the primary causal organisms, or factors that predispose broccoli to head rot.

This two-year project was funded by McCain Foods (Aust.) Pty Ltd, Simplot Australia Pty Ltd and Nufarm Australia Ltd, with matching funds from Horticulture Australia Limited. The major findings of the four areas of studies examined in this project are summarized as follows:

1. Etiology of head rot

- The potential of bacteria to cause rot on non-wounded heads is determined by two key characteristics, the presence of pectolytic activity and biosurfactant activity. On plants that have thick, waxy surfaces, like broccoli, the water soaking properties of biosurfactant-producing bacteria, help provide entry through natural openings. In addition, as the broccoli head increases in maturity, the partial opening of sepals increases, thereby making them vulnerable to bacterial and fungal invasion.

- In this study, fluorescent pseudomonads were the most common microbes isolated from water soaked and soft head rot lesions. The fluorescent pseudomonads were subsequently identified as *Psuedomonas fluorescens*, *P. marginalis*, and *P. tolassi*. The pectolytic positive and high biosurfactant producing *P. marginalis* was the most virulent bacteria, capable of causing rot on undamaged heads under humid conditions and without continuous wetting. Other bacteria types require damaged and/or continuous wetting of heads to cause rot.

- Fungi isolated from head rot include *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium compactum* and *Sclerotinia sclerotiorum*. The most common fungal rots were *Botrytis* and *Sclerotinia*. Fungi such as *Sclerotinia, Botrytis* and *Fusarium* did not appear to require damage for invasion. *Alternaria*, however, can only infect damaged and continuously wet florets, and therefore is likely to be a secondary invader.

2. Surfactant activity of agricultural chemicals

- All of the insecticides commonly used in broccoli crops showed none or very mild surfactant activity. Therefore, a wetting agent was often applied with the insecticide in order to improve wetting and uptake on waxy plant surfaces. Activator and Agral are commonly used in pesticide applications for insect or disease control in horticultural crops in Tasmania.

- At the beginning of this study in 2002, many growers were found to apply these adjuvants at the maximum rates recommended with insecticides on broccoli crops. However, as a result of findings in this study that high rates of these adjuvants may pre-dispose the wet florets to severe head rot, lower rates are recommended for use instead. The minimum rates recommended on labels were shown to be as effective in wetting the florets as higher rates of the adjuvants, with lower risks of bacterial invasion.

Media Summary (Cont.)

3. Bacterial control

- This study showed that all the copper-based products were less effective in the complete inhibition of very high populations of bacteria (in excess of $10^6 - 10^{10}$ cfu/mL). At the lower bacteria levels of 10^4 cfu/mL and below, complete bacteria inhibition was achieved with the copper treatments. Therefore, copper products are most effective when used as crop protectants to reduce initial bacterial population levels on plant surfaces, thus preventing diseases. They are likely to be less effective when used on a severely infected crop that already has a very high bacteria population.

- Unlike copper products, general disinfectants such as Sporekill and Path-X were shown to be highly effective in eradicating high populations of bacteria. The disinfectants, however, have no residual activities, and hence offer no prolonged protection from bacteria re-colonisation. The use of Sporekill at 0.1%, however, generally increases the incidence of black spots on matured heads, and requires further studies on its effects on plants before commercial use at the late crop stage.

- The frequent use of copper sprays can result in the prevalence of copper tolerant bacteria strains. However, the resistance development by the copper tolerant bacteria strains appears to be quantitative, whereby increasing the toxicity or the availability of free copper ions may still effectively control the copper resistant strains. Improved toxicity and efficacy could be achieved in part with new and better formulations of copper products. Alternatively, copper toxicity may be enhanced with the use of additives that enhance the solubility and uptake of copper ions or that interfere with the bacteria resistance mechanisms.

4. Black spots

- If severe, black spots affect the appearance of the broccoli heads, reducing their quality, as well as increasing the risk of secondary rot after harvest and storage. Black spots appeared to be associated with cell necrosis on the sepals of florets, and were not necessarily related to the bacterial head rot. This symptom increased with head maturity. In our field observations, nutrient deficiency, frost damage, and a prolonged period of wetness on florets tended to increase the incidence of black spots.

- In the presence of frequent frost conditions, copper treatments may have increased the incidence of black spots on the sepals of florets on maturing heads in a field trial. This study also indicated a possible link in concentrations of copper products in spray mixture, whereby higher copper product concentrations with low spray water volume of 210 L/ha tended to increase black spot incidence compared to a high volume spray of 400 L/ha. The pH of different copper products also appeared to be related to the incidence of black spots. No increase in black spot incidence was noted with the alkaline Champ DP, while the slightly acidic Cuprofix MZ tended to cause high incidence of black spots.

- In 2003, the use of the disinfectant Sporekill in mixtures with copper sprays also tended to increase black spot incidence, compared to copper sprays only. This may be related to increased wetting with Sporekill or other possible adverse effects on plant cells on sepals of florets. Therefore, although Sporekill and other disinfectants are highly effective in eradicating or reducing bacteria populations, further investigation is recommended to understand its impact on black spots.

Technical Summary

In recent years, the production of broccoli produced for processing into frozen vegetable products has increased rapidly. Although head rot disease has been identified by the processors to be a major constraint to processing broccoli production in Tasmania, little is known of the primary causal organisms or pre-disposing factors. This project was therefore aimed at gaining a better understanding of the primary causal organisms, or factors that predispose broccoli to head rot.

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1. Etiology of head rot:

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- In this study, fluorescent pseudomonads were the most common microbes isolated from water soaked and soft head rot lesions. The fluorescent pseudomonads were subsequently identified as *Psuedomonas fluorescens, P. marginalis*, and *P. tolassi.* The pectolytic positive and high biosurfactant producing *P. marginalis* was the most virulent bacteria, capable of causing rot on undamaged heads under humid conditions and without continuous wetting. Other bacteria types require damaged and/or continuous wetting of heads to cause rot.

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2. Surfactant activity of agricultural chemicals:

- All of the insecticides commonly used in broccoli crops showed none or very mild surfactant activity. Therefore, a wetting agent was often applied with the insecticide in order to improve wetting and uptake on waxy plant surfaces. Activator and Agral are commonly used in pesticide applications for insect or disease control in horticultural crops in Tasmania.

- At the beginning of this study in 2002, many growers were found to apply these adjuvants at the maximum rates recommended with insecticides on broccoli crops. However, as a result of findings in this study that high rates of these adjuvants may pre-dispose the wet florets to severe head rot, lower rates are recommended for use instead. The minimum rates recommended on labels were shown to be as effective in wetting the florets as higher rates of the adjuvants, with lower risks of bacterial invasion.

Technical Summary (Cont.)

- Most copper products have little or no surfactant activity, and did not wet the florets or predispose heads to head rot. Moderate surfactant activities recorded with Bravo and Tri-Base Blue are expected to be similar to the minimum rates of spray adjuvants, and are unlikely to have any adverse impact. Some dry copper hydroxide products were shown to increase surface tension between water droplets and the surface of the florets, making the surface even more water repellent.

3. Bacterial control

- An excess of copper ions can be damaging to plant tissues. Therefore, copper products formulated for agricultural use are almost insoluble in water at approximately pH 7.0. Copper sprays applied on to plants exist mainly as insoluble deposits of copper salts. Under moist conditions, carbon dioxide, plant and microbial exudates form weak organic acids in water on the plant surface, hence reducing plant surface pH. A small quantity of free copper ions (Cu^{2+}) is released from the insoluble copper deposits into this film of water. Cu^{2+} is toxic to fungal spores or bacterial cells.

- This study showed that all of the copper-based products were less effective in the complete inhibition of very high populations of bacteria (in excess of $10^6 - 10^{10}$ cfu/mL). Even though at 10^6 cfu/mL, the copper products Champ DP, Cuprofix MZ, and Tri-Base Blue did reduce the final population to approximately 1% of the initial population, the remaining 10^4 cfu/mL was still considered to be relatively high. At the lower bacteria levels of 10^4 cfu/mL and below, complete bacteria inhibition by Champ DP, Cuprofix MZ, and Tri-Base Blue was recorded.

- The reduced effectiveness of the copper products at very high bacteria levels may be associated to the proportion of free copper ions in solution and at equilibrium, bacterial cell population, and uptake by the bacteria. Therefore, copper products are most effective when used as crop protectants to reduce initial bacterial population levels on plant surfaces, thus preventing diseases. They are likely to be less effective when used on a severely infected crop that already has a very high bacteria population.

- General disinfectants, including those recommended for use to disinfect dam or washing water, such as Sporekill and Path-X, may be useful for drastic reduction of bacterial populations to low levels that can then be effectively controlled by copper based products. Unlike copper products, these disinfectants were shown to be highly effective in eradicating high populations of bacteria.

- The disinfectants, however, have no residual activities, and hence offer no prolonged protection from bacteria re-colonisation. Therefore, these disinfectants should be considered for use only as in additive to copper products, rather than as replacement products. The use of Sporekill at 0.1%, however, generally increased the incidence of black spots on matured heads, and requires further studies on its effects on plants before commercial use at the late crop stage.

- The frequent use of copper sprays can result in the prevalence of copper tolerant bacteria strains. One of the two strains of *P. marginalis* used in this study appeared to be more tolerant to copper than the other. The copper tolerant isolate came from a crop that had been sprayed with copper, while the more copper sensitive isolate was from an untreated crop.

Technical Summary (Cont.)

The resistance development by the copper tolerant bacteria strains appears to be quantitative, rather than a qualitative resistance to copper, whereby increasing the toxicity or the availability of free copper ions may still effectively control the copper resistant strains. Improved toxicity and efficacy could be achieved in part with new and better formulations of copper products. As an alternative, copper toxicity may be enhanced with the use of additives that enhance the solubility and uptake of copper ions, or that interfere with the bacteria resistance mechanisms.

4. Black spots

- If severe, black spots affect the appearance of the broccoli heads, reducing their quality, as well as increase the risk of secondary rot after harvest and storage. Black spots appeared to be associated with cell necrosis on the sepals of florets, and were not necessarily related to the bacterial head rot. This symptom increased with head maturity. In our field observations, nutrient deficiency, frost damage, and a prolonged period of wetness on florets tended to increase the incidence of black spots.

- In the presence of frequent frost conditions, copper treatments may increase the incidence of black spots on the sepals of florets on maturing heads in a field trial. The labels of many copper products have crop damage warnings that they should not be applied to cabbage crops if frost is likely. Similar warnings should also apply to their use on broccoli crops, particularly close to harvest, when the matured heads are most prone to damage.

- This study also indicated a possible link in concentrations of copper products in spray mixture, whereby higher copper product concentrations with low spray water volume of 210 L/ha tended to increase black spot incidence compared to a high spray water volume of 400 L/ha.

- The pH of different copper products also appeared to be related to the incidence of black spots. No increase in black spot incidence was noted with the alkaline Champ DP, while the slightly acidic Cuprofix MZ tended to cause high incidence of black spots.

- In both 2002 and 2003, whole crops have been observed to develop a purplish discoloration on the sepals of the florets. While some of these purplish spots might have developed into black spots most eventually disappeared. This general discolouration in the whole crop is believed to be due to a physiological response to poor translocation of nutrients under very cold conditions, or in a nutrient deficient crop.

- In 2003, the use of the disinfectant Sporekill in mixtures with copper sprays also tended to increase black spot incidence, compared to copper sprays only. This may be related to increased wetting with Sporekill or other possible adverse effects on plant cells on sepals of florets. Therefore, although Sporekill and other disinfectants are highly effective in eradicating or reducing bacteria populations, further investigation is recommended to understand its impact on black spots.

Technology Transfer

- Regular updates were given to R & D managers of the processing companies as findings became available during the project.
- A research meeting was held with staff from McCain Foods and Simplot, an Industry Development Officer and consultants, to present and discuss outcomes of the project studies, on 28th June 2002, at Serve-Ag Research, Devonport, Tasmania.
- Project outcomes were presented at Tasmanian vegetable extension days held at Devonport on 14th August 2002 and 5th September 2003. These were well attended by Tasmanian growers, industry representatives and researchers.
- A poster was presented at the International Plant Pathology Congress that was held at Christchurch, New Zealand on 3 - 7 February 2003. Copies of the poster were provided to Horticulture Australia and voluntary contributors.

Recommendations

- At the late crop stage when heads are maturing, use minimum label rates for spray adjuvants to minimize risk of damage to the florets by wetting agents.

- Copper products should be used primarily as crop protectants to reduce initial bacterial population levels on plant surfaces, thus preventing diseases.

- For head rot control in crops that are already infected, the addition of a disinfectant to copper sprays may assist in reducing bacterial populations to low levels that can then be effectively controlled by copper based products. The disinfectants, however, have no residual activities, and should be considered for use only as additives to copper products, rather than as replacement products. However, avoid the use of a disinfectant close to harvest, until safe lower rates that do not cause black spots can be recommended.

- Use of a high volume spray is preferable to a low volume spray for improved coverage and reducing the toxic effects of concentrated copper in a low volume spray.

- Crop damage warnings should be on the label, copper should not be applied on broccoli crops if frost is likely and close to harvest when the matured heads are most prone to blemishes or black spots.

Introduction

Background

Soft rot of broccoli heads is a common disease problem in broccoli production in Australia (Tasmania, New South Wales, Queensland, and Victoria), Canada, Europe and the United States of America. In Queensland and Victoria, broccoli is mainly produced for the fresh market, and *Pseudomonas marginalis*, a bacterial pathogen, has been identified as the cause of head rot, especially under hot and humid or wet conditions.

In contrast, broccoli is mainly produced for processing into frozen food products in Tasmania. Broccoli produced for processing is grown for a longer period than when produced for the fresh market, in order to obtain larger heads that are suitable for processing. With the longer growth period, processing broccoli tends to be more prone to head rot, especially in the cold, wet autumn period. As a result, head rot is a major limiting factor in the expansion of processing broccoli production.

In Tasmania, the primary cause of broccoli head rot disease is not known. It is not possible to effectively manage the disease unless there is an improved understanding of the etiology of head rot, and factors that may pre-dispose the florets to decay. There have been field observations that fungal pathogens might be involved, as chlorothalonil fungicides (eg. Bravo) appear to reduce the incidence of head rot in some crops. There is also anecdotal evidence that certain pesticide sprays may accentuate the disease. The frequency of frost occurrences also appears to be related to increased head rot incidence. Moreover, lack of disease control by copper sprays has been reported, particularly in crops with relatively high disease incidence and favourable field conditions.

Aims

This two-year project, conducted in Tasmania, was aimed at gaining a better understanding of the primary causal organisms and factors that may predispose broccoli to head rot. In the second year, the effectiveness of copper products for bacterial inhibition and disease control was investigated. The studies conducted in this project could be divided into three areas as described below. A fourth area of study on black spot incidence and factors affecting its formation was carried out within all field studies.

1. Etiology of head rot

Broccoli with head rot in the field was examined in order to gain a better understanding of head rot development and disease symptoms. The organisms associated with head rot were also identified, and their ability to cause head rot was determined in laboratory studies.

2. Surfactant activity of chemicals

Laboratory evaluation on the surfactant activities of agricultural chemicals commonly used in horticultural crops, and their effects on head rot. Two field trials were also conducted to evaluate the effects of spray adjuvants, crop protectants and fertilisers, applied in foliar spray treatments, on head rot.

3. Bacterial control

In-vitro tests were conducted to determine the efficacies of different types of copper products and disinfectants for bacterial control. Four field trials were also conducted to evaluate the efficacy of copper products and disinfectants for controlling head rot, and their potential to cause blemishes or black spots under field conditions.

1. Etiology of head rot disease

Materials and Methods

Symptoms & microorganisms

Two processing broccoli crops affected by head rot disease were examined in the field at Wesley Vale in May - June 2001, and affected broccoli heads were collected from six infected crops for laboratory examinations to determine the types of microorganisms associated with the rots. Isolations were conducted for causal organisms on selective agar media for bacteria and fungi, and pure cultures were sub-cultured for species identification.

Biosurfactant test

A test for biosurfactant activity was conducted by gently mixing two loops (0.2 µmL lifted with a wire loop) of bacteria or fungal suspension, in 0.2 mL sterile distilled water on a sterile plastic petri dish. The presence of biosurfactant was detected by a reduction in the surface tension of the water droplet, therefore flattening and spreading the water droplet over a wider area. Bacteria or fungal suspensions were prepared from pure cultures multiplied on the appropriate agar media (King's B agar medium for *Pseudomonas* bacteria and potato dextrose agar for fungi). The diameter of the water droplet was measured at 30 minutes and 2 hours after mixing. The procedure was repeated once with the same results. Sterile distilled water was used as a control. Note that except for *Sclerotinia sclerotiorum*, fungal suspension consisted of spores. The suspension of *S. sclerotiorum* contained only fungal hyphae.

Soft rot on potato slices

Slices of potato tubers (8 mm thick) were disinfected by wiping the slices with household bleach (White King) that has 4% chlorine, and air-dried for 10 minutes before use. Six triangular depressions were then made using a sterile scalpel on each potato slice. Three of the depressions were inoculated with two loops of cloudy bacterial suspension, while the other three depressions were inoculated with sterile distilled water. The potato slice was then placed in a petri dish that was lined with moist filter paper, and incubated at room temperature (20-21°C). The depressions were checked for soft rot after two days incubation.

Pathogenicity test

Microbial suspensions of isolates of bacteria or fungus were prepared in sterile distilled water and diluted to approximately 1×10^8 colony forming units (cfu) per mL. Small pieces of cotton wool ($1.0cm^3$) were then soaked in the suspensions and placed on to wounded and non-wounded broccoli florets. The heads were covered with polythene bags to maintain high humidity, incubated at 15°C and examined after 7 days for soft rot. The test for each organism was repeated with three broccoli heads.

Etiology of head rot disease (Cont.)

Results

Symptoms

In the field, symptoms of the head rot process usually first became visible after periods of rain and cold conditions, when heads had remained wet for several days. Typically, the water soaked area of the head appeared bright green, and the soaked florets re-wetted readily after drying. In contrast, the florets that were not wet or soaked had a dull grey-green appearance and water rolled into beads. There was also intermediate wetting, where water film formed over the florets, partially wetting them.

Black lesions frequently developed on the sepals and pedicels of florets in the water soaked areas. The lesions were initially tiny, slightly raised and usually associated with the stomata. A dark discoloration was initially associated with the guard cells, but then spread to adjacent cells, and decay frequently developed on these affected florets. During long periods of continuous wetness, decay usually spread rapidly, resulting in a large dark green and soft rot area on the head. Bacteria were always found in association with the black lesions and the soft rot areas. The bacterial populations on the black lesions were generally lower than those of the soft rot areas, while little or no bacteria were found on florets that had no lesions.

Although bacteria were frequently found in association with head rot, some rots in the field were due to fungal invasion. Rots due to fungi tended to be relatively dry in appearance, and usually had light brown discoloration on the affected florets. Rots due to fungal infection also tended to spread to the stems beneath the florets, causing light brown discolouration of the affected stems. In contrast, bacterial rots tended to be confined to the florets, causing a dark green to black discolouration. Bacteria usually found in advanced rot due to fungal invasion are likely to be from secondary invaders.

Bacteria

Fluorescent pseudomonads were the most common microbes isolated from water soaked and soft head rot lesions. Fluorescent pseudomonads were also frequently isolated from tiny dark green to black lesions on sepals of florets.

Table 1.1: Bacteria strain identification based on physiological characteristics

LOPAT test	*P. fluorescens*	*P. fluorescens*	*P. marginalis*	*P. tolassi*
	Group IVa	Group Vb	Group Vb	Group Va
Levan formation	-	+	+	-
Oxidase reaction	+	+	+	+
Arginine dihydrolase reaction	+	+	+	+
Pectolytic activity on potato slice	+	-	+	-

Test showed positive (+) or negative (-) reaction.

Etiology of head rot disease (Cont.)

The fluorescent pseudomonads isolated were subsequently identified, according to their physiological characteristics, into four distinct groups, based on the LOPAT scheme tests (Table 1.1). Within each group, there appeared to be some variations between strains, based on their biosurfactant activities, and the virulence of the strains appeared to be closely linked to the pectolytic and biosurfactant activities (Table 1.2).

Most of the bacterial strains isolated from the head rot were *P. fluorescens*, which could be subdivided into two sub-groups, those that tested positive for pectolytic activity or macerating activity (Group IVa) and those that were negative (Group Vb). Bacterial strains in Group IVa could be further separated according to their ability to produce biosurfactant activity (Table 1.2). The strain that also possessed relatively mild biosurfactant activity, appeared to be more pathogenic than the one that didn't, where it was capable of causing lesions on undamaged florets under continuous wet condition (Table 1.2).

A third group of bacterial strains identified as *P. tolassi* did not possess pectolytic activity and is generally regarded as a non-pathogen. Some of the strains belonging to this group exhibited relatively mild biosurfactant activity (Table 1.2). All the *P. tolassi* strains were not capable of causing head rot on either undamaged or damaged heads under both humid and continuous wet conditions. This finding suggests that pectolytic activity is an important property that enables a bacterium to cause soft rot on florets.

Table 1.2: Pectolytic and biosurfactant activity and pathogenicity of flourescent *Pseudomonas* strains isolated from head rot disease of broccoli

	P. fluorescens			***P. marginalis***	***P. tolassi***	
	Group IVa		**Group Vb**	**Group Vb**	**Group Va**	
Culture	**5A**	**5B**	**5E**	**2B**	**2C**	**5D**
Key characteristics						
Pectolytic activity (Soft rot on potato slice)	+	+	-	+	-	-
Biosurfactant test						
0.5 hr	11	15	16	24	10	12
2.0 hr	11	15	17	26	10	15
Activity rating [1]	*	**	**	****	-	**
Lesions on undamaged broccoli heads						
Humid	-	-	-	+	-	-
Continuous wetting	-	+	+	+	-	-
Lesions on damaged broccoli heads						
Humid	-	-	-	+	-	-
Continuous wetting	+	+	+	+	-	-

Test showed positive (+) or negative (-) response.

[1] Surfactant activity rating with test on 0.4 mL droplet of distilled water: - = none, * = very mild, ** = moderate, **** = very high.

Etiology of head rot disease (Cont.)

Pectolytic and surfactant-positive strains identified as *P. marginalis* were isolated from soft rot, as well as from firm and tiny black lesions on sepals of florets. The frequency of *P. marginalis* isolation was the lowest among the three types of flourescent *Pseudomonas*. This low incidence may also be related to the difficulty in isolating *P. marginalis* on agar medium (Wimalajeewa *et al.* 1987). The bacterial strain produced a high level of biosurfactant activity, similar to that of some chemical surfactants (high rates of Agral and Activator), and was capable of causing rot on both undamaged and damaged heads under humid and continuous wet conditions. Studies conducted in Victoria and Queensland (Wimalajeewa *et al.* 1987, Stephens *et al.* 1997) and overseas (Hildebrand 1989, Canaday *et al.* 1991) showed that *P. marginalis* was responsible for causing extensive water soaking and spreading decay on non-wounded broccoli heads.

Fungi

Fungi isolated from head rot included *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium*, *Fusarium compactum* and *Sclerotinia sclerotiorum* (Table 1.4). The most common fungal rots were *B. cinerea* and *S. sclerotiorum*, which caused light brown discolouration of infected tissues. *B. cinerea* and *F. compactum* tended to cause localised dry rot, whereas rot due to *S. sclerotiorum* tended to spread rapidly to adjacent florets and stems beneath the florets, causing a large, extended rotting area. *A. alternata and Cladosporium* sp. were isolated from dark green, water soaked areas.

Table 1.3: Surfactant activity and pathogenicity of fungi isolated from broccoli head rot

No.	Fungus	Biosurfactant test			Broccoli head rot			
					Undamaged head		Damaged head	
		0.5h	2.0h	Activity rating[1]	Humid	Wet[3]	Humid	Wet
1	*Alternaria alternata*	10	10	-	-	-	-	+
2	*Botrytis cinerae*	12	12	*	+	-	-	+
3	*Cladosporium sp.*	10	10	-	-	-	-	-
4	*Fusarium* compactum	12	12	*	+	-	+	+
5	*Sclerotinia sclerotiorum*[2]	11	11	*	+	+	NT[4]	NT

[1] Surfactant activity rating: - = none, * = mild, ** = moderate, *** = high.
[2] Mycelium of *S. sclerotiorum* was used in the test.
[3] Continuous wetting of floret
[4] Not tested

Pathogenicity tests showed that *A. alternata* and *Cladosporium* sp. were likely to be non-pathogens, as they could not invade undamaged florets under humid or continuous wet conditions (Table 1.3). However, damaged and wet florets could be infected by *A. alternata*, suggesting that it could be a secondary invader. *B. cinerea, F. compactum* and *S. sclerotiorum* are likely to be pathogens, since they could invade undamaged florets under humid conditions. As with *P. marginalis*, these fungi required humid conditions but not a continuously wet period for invasion. It is interesting to note that all of these three fungi exhibited relatively low levels of biosurfactant activity, while none were caused by *A. alternata* and *Cladosporium* sp.. This indicated that biosurfactant activity might also play a key role in enabling fungal invasion on the waxy plant surface.

Etiology of head rot disease (Cont.)

Discussions

Cuticular hydrophobic wax layers cover broccoli leaves and florets, so that water from rainfall, irrigation or condensation remains mainly on the surface as discrete droplets. In the field, large discrete water droplets were often noted on the surface of broccoli heads. If cloudy, cool and wet conditions persist over a long period of time, water droplets may penetrate through the waxy layer, creating water soaked areas on the head, and even the entire head could become water soaked.

It has been shown in this study that a high proportion of the fluorescent pseudomonads commonly associated with black spot or head rot on broccoli heads produced biosurfactant activities. Biosurfactants produced by the bacteria enabled water to penetrate through the waxy surface. Therefore, apart from prolonged wet conditions, the wetting of heads may also be associated with the ubiquitous fluorescent pseudomonads bacteria on broccoli heads.

According to Hildebrand (1989), the potential of bacteria to cause rots on non-wounded heads is determined by two key characteristics: the presence of pectolytic activity and biosurfactant activity. The pectolytic fluorescent pseudomonads are important bacterial pathogens, causing soft rots on a diverse group of vegetables (Liao & Wells 1987).

On plants that have thick, waxy surfaces, like broccoli, the water soaking properties of biosurfactant-producing bacteria, help provide entry through natural openings (Hildebrand 1989). Stomata, and sepals of florets that do not overlap completely to close the reproductive parts of the flower, provide small openings for surfactant-mediated entry. The surfactant released by some fluorescent pseudomonads may also enable surfactant deficient strains to colonise the water soaked areas even though physical injury of tissues is not evident. Wimalajeewa *et al.* (1987) concluded that pectolytic strains of *P. viridiflava* and *Erwinia carotovora* were secondary invaders after *P. marginalis,* based on their inability to decay non-wounded tissue. Hildebrand (1989) suggested that pathogenic and saprophytic bacteria may compensate for their inherent deficiencies by interacting with each other. Surfactant-positive saprophytes, which colonise broccoli heads during wet weather, may play an important role in promoting decay by the surfactant-negative, pectolytic-positive strains. A relatively new area of microbiological study on biofilm showed that bacteria rarely occupies a space on its own, but will co-exist with other bacteria species or microorganisms in a thin film (Morris *et al.* 1997).

The etiology of fungal infection on the broccoli head appears also to be related to biosurfactant activity and entries through natural openings. The incomplete closing of sepals of some florets may provide entry for fungal spore infection by *S. sclerotiorum* as well as *Botrytis* and *Fusarium*. In this study, a higher incidence of hyphal growth was observed on florets with incomplete cover compared to few or none on those with complete cover. As the broccoli head increases in maturity, the partial opening of sepals increases, thereby making them vulnerable to fungal invasion.

2. Surfactant activity of chemicals

2.1. Laboratory study

Materials & Methods

Surfactant activity in water

The test methodology used to determine the surfactant activity by various agricultural chemical products was similar to that used for the biosurfactant test in Section 1. A list of spray adjuvants or wetting agents, copper based bacteriacides, biocontrol agent, fertiliser, fungicide and insecticides tested are included in Tables 2.1 and 2.2. The procedure was repeated once with the same results. Distilled water was used as a control. In 2003, additional tests were carried out with the three copper products Champ DP, Cuprofix MZ and Tri-Base Blue, which are based on ultrafine particles of copper.

The diameter of the water droplet was measured at 0.5 and 2.0 hours after treatment. The following surfactant rating was given, based on the diameter range of the water droplet: '-' = none at 10mm; '+' = mild activity with 11 - 12mm; '++' = moderate activity with 13 - 15mm; '+++' = high activity with 16 - 19mm; and '++++' = very high with 20mm or more.

Wetting of broccoli florets and head rot

The appropriate chemical rate was prepared (as described in Table 2.3) in 50mL water, and a small piece of cotton-wool (1.0cm^3) was soaked in the suspension and placed on top of a segment of broccoli floret. The wet cotton-wool was removed from the florets 1 hour after treatment. A second lot of florets were treated in the same manner, but kept in a moist chamber at room temperature overnight, and removed after 20 hours. Wetting of the florets at 1 hour and 20 hours after treatment was recorded. The re-wetting of the florets was also recorded in the 1 hour treatment, by allowing the florets to air-dry and then re-wet by spraying with water. All the treated florets were placed in a chamber after removing the wet cotton-wool, and high moisture on the florets was maintained by spraying with water that was inoculated with *P. tolassi* (isolate 18.8) twice a day.

The florets were assessed for necrotic lesions or early signs of head rot, at 2 and 3 days after the initial treatment. *P. tolassi* bacteria multiplied on King's B agar medium had no surfactant activity.

Chemical effects on bacteria

The effects of the chemical products, Activator, Agral, Kocide DF, Kocide SC and Flo Bordo, used in the surfactant test (Table 2.1), were also tested to determine if they had any inhibitory effects on bacteria. The non-pathogenic *P. tolassi* was used in the test. A small piece of agar (1cm^3) containing *P. tolassi* colonies that were multiplied on King's B agar medium for 4 days, was mixed vigorously with 50mL suspension of each chemical product at the appropriate concentration. The bacterial population in the suspension was approximately 10^6 cfu/mL. Approximately 60 minutes after mixing, the suspension (0.1mL) was spread over a King's B agar plate. Bacterial cells were also mixed with sterile distilled water for use as a control.

In the initial plate test, the three copper hydroxide based products, Kocide DF, Kocide SC and Flo Bordo, did not cause complete inhibition of high initial bacterial populations (cloudy bacterial suspension in excess of 10^{10} cfu/mL). Therefore, further tests were conducted with the Kocide DF suspension, by mixing it with high populations of *P. fluorescens and P. marginalis*, to determine if the lack of inhibition was related to the species of bacteria used. Sterile distilled water was used in place of the chemical suspension as a control.

Surfactant activity of chemicals (Cont.)

Results & Discussion

Table 2.1: Surfactant activity of agricultural chemical products

No.	Treatment	Product type [1]	Product Rate	Surfactant test (mm)		
				0.5 hour	2 hours	Surfactant rating [2]
1	Bravo [3]	Fu	1.8 L/200L	14	14	++
2	Kocide DF	Ba, Fu	2.2 kg/200L	11	11	+
3	Kocide Liquid Blue	Ba, Fu	1.0 L/200L	11	11	+
4	Champ	Ba, Fu	1.56 kg/200L	11	11	+
5	Tri-Base Blue	Ba, Fu	4.6 L/200L	14	14	++
6	Cuprofix MZ	Ba, Fu	3.5 kg/200L	11	11	+
7	Penncozeb DF750	Fu	200 g/100L	11	11	+
8	Penncozeb SC420	Fu	360 mL/100L	12	12	+
9	Companion	BCA	2.5 L/200L	12	12	+
10	Agri-Fos	Fu, Fe	2.5 L/200L	11	11	+
11	Grocal	Fe	5.0 L/1000L	11	11	+
12	Gypsum	Fe	2.5 kg/1000L	11	11	+
13	Chlorpyrifos	In	200 mL/100L	11	11	+
14	Chess	In	100 mL/100L	11	11	+
15	Success	In	400 g/250L	11	11	+
16	Pirimor	In	200 g/100L	11	12	+
17	Proclaim	In	300 g/400L	10	10	-
18	Activator	Ad	30 mL/100L	15	15	++
19	Activator	Ad	125 mL/100L	19	22	++++
20	Agral	Ad	18 mL/100L	12	12	+
21	Agral	Ad	100 mL/100L	21	22	++++
22	Agridex	Ad	50 mL/100L	11	11	+
23	Bond	Ad	100 mL/100L	11	11	+
24	Nu-Film	Ad	100 mL/100L	12	13	++
25	Synertrol	Ad	200 mL/100L	12	12	+
26	**Water only control**	-	N/a	**10**	**10**	-

1 Ad = adjuvant, Ba = bacteriacide, BCA = biocontrol agent, Fu = fungicide; Fe = fertilizer; In = insecticide
2 Surfactant rating: '-' = none; '+' = very mild activity; '++++' = very high activity
3 Used Bravo 720SC from Crop Care

Except for Proclaim, all of the insecticides showed very mild surfactant activity (Table 2.1). Proclaim showed no surfactant activity. As a result of the weak, or lack of, surfactant activity, a wetting agent was often applied with the insecticide in order to improve wetting and uptake on waxy plant surfaces. Among the bacteriacide and fungicide products tested, only Bravo and Tri-Base Blue showed moderate surfactant activity, and caused wetting of florets (Tables 2.1 & 2.3).

Surfactant activities of chemicals (Cont.)

Although there were differences in the surfactant activities between the different spray adjuvants or wetting agents (Table 2.1), they all caused the wetting of florets (Table 2.3). The highest surfactant activities were caused by the highest rates of Activator and Agral. These rates also appeared to pre-dispose the treated florets to severe head rot (Table 2.4). Activator and Agral are commonly used in pesticide applications for insect or disease control in horticultural crops. These adjuvants were shown to have no inhibitory effect on bacterial cells of *P. tolassi*.

Contrary to expectation, higher levels of head rot were observed in the 1 hour treatments compared to the 20 hour treatments (Table 2.4). This anomaly is likely to be due to the different lots of broccoli heads, used for the test, where the florets with their looser sepals in more matured heads in the 1 hour treatments, appeared to have greater susceptibility to head rot than the less matured heads used in the 20 hour treatments.

Table 2.2: The effects of spray adjuvants' rates on their surfactant activities

No.	Product	Product Rate (ml/100L)	Surfactant tests		
			0.5 hour	2 hours	Surfactant rating[1]
1a	Activator	120	16	23	++++
1b		60	16	18	+++
1c		30	15	15	++
1d		15	14	14	++
1e		7.5	12	12	+
1f		3.75	11	11	+
2a	Agral	100	21	22	++++
2b		18	13	14	++
2c		9	11	11	+
2d		3	11	11	+
2e		1.5	11	11	+
2f		0.75	11	11	+
2g		0.375	10	10	-
3	Water only	N/a	10	10	-

[1] Surfactant rating: '-' = none; '+' = mild activity; '++++' = very high activity

Reducing the rates of Activator and Agral reduced their surfactant activities (Table 2.2). The lower rates of Activator at 30 mL and Agral at 18 mL were shown to be as effective in wetting the florets as higher rates of the adjuvants (Table 2.3).

The other adjuvants, Agridex, Bond, Nu-Film and Synertrol, showed relatively low levels of surfactant activity at their recommended rates of application (Table 2.1). At the 20 hour treatment period, with the exception of Bond, these adjuvants did not cause head rot on the treated florets (Table 2.4).

Surfactant activities of chemicals (Cont.)

Table 2.3: The effects of agricultural chemical products on the wetting and re-wetting of florets at 1 and 20 hours after treatment

No.	Treatment	Product type [1]	Product rate	1 hour		20 hours
				Wetting of florets	Re-wet easily after drying	Wetting of florets
1	Water only	N/a	N/a	No	Partial	Partial
2	Activator	Ad	120 mL/100 L	Yes	Yes	Yes
3	Activator	Ad	60 mL/100 L	Yes	Yes	Yes
4	Activator	Ad	30 mL/100 L	Yes	Yes	Partial
5	Agral	Ad	18 mL/100L	Yes	Yes	Yes
6	Agral	Ad	9 mL/100 L	Yes	Yes	Yes
7	Agral	Ad	3 mL/100 L	Yes	Yes	Yes
8	Bond	Ad	100 mL/100 L	Yes	Yes	Partial
9	Nu-Film	Ad	100 mL/100 L	Yes	Yes	Yes
10	Agridex	Ad	50 mL/100 L	Yes	Yes	Yes
11	Synertrol	Ad	200 mL/100 L	Yes	Yes	Yes
12	Kocide DF	Ba, Fu	100 g/100 L	No	No	No
13	Kocide SC	Ba, Fu	100 g/100 L	No	Partial	Partial
14	Flo-Bordo	Ba, Fu	2.0 L/100 L	No	No	No
15	Bravo	Fu	1.8 L/300 L	Yes	Yes	NT
16	Chess	In	100 mL/100 L	Yes	Yes	Partial

1 Ad = adjuvant, Ba = bacteriacide, BCA = biocontrol agent, Fu = fungicide; Fe = fertilizer; In = insecticide

Kocide DF had no surfactant activity, and did not wet the florets or cause head rot (Tables 2.1, 2.3 & 2.4). In contrast, Kocide DF increased the surface tension between water droplets and the surface of the florets, causing water to roll up into raised droplets, hence reducing the contact surface area by the water droplets. Flo Bordo showed similar properties as Kocide DF, where the treated floret was not wet and did not have any rot (Tables 2.3 & 2.4). Kocide Liquid Blue, however, showed mild surfactant activity, and caused partial re-wetting of treated florets (Tables 2.1 & 2.3).

The water repelling effects of Kocide DF were lost if a Kocide DF + Activator mixture was used or if the Kocide DF treated florets were subsequently treated with Activator or Agral (Treatments 16-18, Table 2.4). The treated florets became soaked with the spray adjuvants, and were susceptible to head rot. This finding suggests that the benefits of Kocide DF applications, commonly used for head rot control in commercial crops, may be offset by the addition of Activator or Agral.

Surfactant activities of chemicals (Cont.)

Table 2.4: The effects of agricultural chemical products in pre-disposing treated florets to head rot at 2 and 3 days after treatment

No.	Treatment	Product type[1]	Product rate	Head rot severity after 2 days*		Head rot severity after 3 days*	
				1hr	20hr	1hr	20hr
1	Water only	N/a	N/a	0	++	0	++
2	Activator	Ad	120 mL/100 L	++	+++	+++	+++
3	Activator	Ad	60 mL/100 L	0	+++	+	+++
4	Activator	Ad	30 mL/100 L	0	0	+	+++
5	Agral	Ad	18 mL/100 L	0	+	+	++
6	Agral	Ad	9 mL/100 L	0	+	+	++
7	Agral	Ad	3 mL/100 L	0	++	0	++
8	Bond	Ad	100 mL/100 L	0	++	+	++
9	Nu-Film	Ad	100 mL/100 L	0	0	0	0
10	Agridex	Ad	50 mL/100 L	+	0	+	0
11	Synertrol	Ad	200 mL/100 L	++	0	++	0
12	Kocide DF	Ba, Fu	100 g/100 L	0	0	0	0
13	Kocide SC	Ba, Fu	100 g/100 L	+	0	++	0
14	Flo-Bordo	Ba, Fu	2 L/100 L	0	0	0	0
15	Chess	In	100 mL/100 L	+++	+	+++	+
16	Kocide DF + Activator at 1: 1 ratio	-	100 g/100 L; 60 mL/100L	+	NT	++	NT
17	Kocide DF, air-dry, then Activator	-	100 g/100 L; 30 mL/100 L	+++	NT	+++	NT
18	Kocide DF, air-dry, then Agral	-	100 g/100 L; 3 mL/100 L	0	NT	+	NT

* Head rot severity rating of florets that have been treated at 1hr and 20hr: '-' = none; '+' = mild; '++' = moderate; '+++' = severe

[1] Ad = adjuvant, Ba = bacteriacide, BCA = biocontrol agent, Fu = fungicide; Fe = fertilizer; In = insecticide

NT = not tested

When mixed with cloudy bacterial suspensions with very high population of bacteria (excess of 10^{10} cfu/mL), Kocide DF, Kocide SC and Flo Bordo did not cause a complete inhibition of *P. tolassi* cell growth and multiplication (*Photo 7).* The bacterial population and growth rate of the bacteria treated with the copper-based products were similar to those of the control plate (sterile water only plus bacteria). These products also did not cause complete inhibition with the other species of bacteria, *P. fluorescens and P. marginalis*, indicating that the lack of inhibition was not related to bacterial species. As a result of these findings, further studies on bacterial inhibition were conducted in 2003 in this project.

Surfactant activities of chemicals (Cont.)

2.2. Field Trials 2002

Materials & Methods

Two field trials were set up within commercial crops in Tasmania at Riana and Forest, to assess the effects of spray adjuvants, bacteriacides, fungicides and fertiliser on head rot under field conditions. The trial designs were complete randomised blocks, with five replicates, and plot size of 5 m long x 3.5 m wide (including a buffer plant row between replicate blocks). Two applications were made with a knapsack air-pressurised sprayer fitted with 3 m boom at 452 L/ha and 400 kPa. The first application was at approximately 10 weeks after planting, when the head sizes ranged from 20 mm to 50 mm, followed by a second spray, one week later.

Disease assessments were conducted on three plant rows within 4 m of each treatment plot. In Field Trial 2.1, the number of heads with no wet florets was recorded at 81 days after planting. The numbers of plants with head rot and black spot were recorded at 81 and 84 days after planting. In Field Trial 2.2, the number of heads with head rot or black spot was also recorded at 84, 91 and 103 days after planting. The number of affected heads was then converted to the percentage of total heads in the area assessed.

As the data values were not normally distributed and there were big differences between the data values of the five replicates within each treatment, due to the patchy distribution of the affected plants, the data was analysed using the non-parametric Kruskall-Wallis test in the Statgraphic Plus 4.1 statistical program. In the results, the median values were given instead of mean values.

Treatment list for Trials 2.1 & 2.2

No.	Treatment	Product type [1]	Product Rate/ha	Application Schedule
1	Untreated control	N/a	N/a	N/a
2	Bravo 720SC	Fu	1.8 L/ha	2 spray applications, with 452 L/ha water. 1st spray at 10 weeks after planting, and 2nd spray at 11 weeks after planting.
3	Kocide DF	Ba, Fu	2.2 kg/ha	
4	Grocal MGB	Fe	2.5 kg/ha	
5	Agral	Ad	18 mL/100 L	
6	Agral	Ad	100 mL/100 L	
7	Activator	Ad	30 mL/100 L	
8	Activator	Ad	100 mL/100 L	
9	Agridex	Ad	50 mL/100 L	
10	Nu-Film	Ad	100 mL/100 L	
11	Agri-Fos	Fe, Fu	5 L/ha	
12	Path-X	Ba*	20 mL/1000 L	

* Path-X is an agricultural disinfectant, active ingredient is 120 g/L didecyldimethyl-ammonium chloride

[1] Ad = adjuvant, Ba = bacteriacide, BCA = biocontrol agent, Fu = fungicide; Fe = fertilizer; In = insecticide

Surfactant activities of chemicals (Cont.)

Results & Discussion

In Trial 2.1, there was no head rot at 81 days after planting, and relatively low head rot incidence at 84 days after planting (Table 3.1). At 84 days after planting, the Agral and Activator treatments (Treatments 5-8) tended to have higher head rot incidence compared to all other sprayed treatments. However, these Agral and Activator treatments did not appear to increase head rot incidence when compared to the untreated control.

Table 3.1: The effects of foliar spray treatments on head wetting, head rot and black spot incidence in Trial 2.1 at Riana

No.	Treatment	% Heads with no wetting	% Heads with rot *		% Black spots *
		81 DAP (4 DAS)	81 DAP (4 DAS)	84 DAP (7 DAS)	84 DAP (7 DAS)
1	Untreated control	20.7	0	5.7 b	0
2	Bravo 720SC	3.4	0	0 a	0
3	Kocide DF	3.4	0	0 a	6.5
4	Grocal MGB	6.3	0	0 a	0
5	Agral (18 mL/100 L)	9.4	0	3.1 b	0
6	Agral (100 mL/100 L)	3.0	0	3.0 ab	0
7	Activator (30 mL/100 L)	3.2	0	3.1 b	0
8	Activator (100 mL/100 L)	0.0	0	3.3 b	0
9	Agridex	16.1	0	0 a	0
10	Nu-Film	6.1	0	0 a	0
11	Agri-Fos	9.7	0	1.6 ab	0
12	Path-X	8.6	0	0 a	0

All results presented are median values.
DAP = days after planting, and DAS = days after the last spray application.
* Based on median value, the same letters within the same column are not significantly different according to the Kruskall-Wallis Test at 95% confidence level.

In Trial 2.2, the head rot incidence was very patchy, with no significant difference between treatments at 91 and 103 days after planting (Table 3.2). Unfortunately, as a result of the patchy distribution of the disease, it is not possible to draw any conclusions from this trial. The foliar spray applications were unlikely to have any residual effect at 103 days after planting, which was 3.5 weeks after the last spray applications.

It is noteworthy that the Kocide treatment in both trials increased the percentage of heads with black spots on the sepals of florets at 84 days after planting (Tables 3.1 & 3.3). When the black spots were kept moist at room temperature of about 20°C, bacterial decay was noted. This indicated that the necrotic tissues on the black spots could pre-dispose them to secondary rot by microorganisms, thereby affecting the quality of affected heads after harvest and storage.

Surfactant activities of chemicals (Cont.)

Results & Discussion (Cont.)

The small black spots appeared to be unrelated to the head rot. Although the Kocide treatments increased the incidence of black spot, there was no increase in the incidence of head rot (Tables 3.1 & 3.2). This also indicates that the primary causes of the two diseases are different. Almost all of the head rots were due to bacterial invasion. There was a very low incidence of fungal rot, which was caused by *Botrytis* or *Sclerotinia,* in both trials.

Table 3.2: The effects of foliar spray treatments on head rot incidence in Trial 2.2 at Forest

No.	Treatment	% Heads with rot *		
		84 DAP (6 DAS)	91 DAP (13 DAS)	103 DAP (25 DAS)
1	Untreated control	0	0	3.1
2	Bravo 720SC	0	2.9	6.1
3	Kocide DF	0	3.1	4.0
4	Grocal MGB	0	0	7.4
5	Agral (18 mL/100 L)	0	3.0	6.1
6	Agral (100 mL/100 L)	0	3.1	8.8
7	Activator (30 mL/100 L)	0	0	10.0
8	Activator (100 mL/100 L)	0	0	0
9	Agridex	0	0	3.0
10	Nu-Film	0	0	2.9
11	Agri-Fos	0	3.4	6.5
12	Path-X	0	3.4	3.4

DAP = days after planting, and DAS = days after the last spray application.

* Based on median value, there was no significant difference between different treatments according to the Kruskall-Wallis Test at 95% confidence level.

In Trial 2.2, at 91 days after planting, many heads developed a purplish discoloration on the sepals of the florets, which was difficult to distinguish from black spot when the florets were wet. Therefore, the black spot incidence assessed at 91 days after planting was not accurate, and is, therefore, not presented in the results. It is believed that the purplish coloration, which disappeared at 103 days, was due to a physiological response to poor translocation of nutrients under very cold conditions.

The relatively high incidence of black spot at 103 days after planting in all treatments could be related to increased head maturity and the onset of cold weather conditions, with increased exposure to frost (Table 3.3).

Surfactant activities of chemicals (Cont.)

Results & Discussion (Cont.)

Table 3.3: The effects of foliar spray treatments on black spot incidence in Trial 2.2 at Forest.

No.	Treatment	% Heads with black spot (median value) *	
		84 DAP	103 DAP
1	Untreated control	4.0	15.6
2	Bravo 720SC	0	16.7
3	Kocide DF	8.0	18.8
4	Grocal MGB	0	15.6
5	Agral (low)	0	9.1
6	Agral (high)	0	21.9
7	Activator (low)	0	15.6
8	Activator (high)	0	15.6
9	Agridex	0	13.3
10	Nu-Film	0	18.5
11	Agri-Fos	0	29.6
12	Path-X	0	24.1

DAP = days after planting, and DAS = days after the last spray application.

* Based on median value, there was no significant difference between different treatments according to the Kruskall-Wallis Test at 95% confidence level.

3. Bacterial control

3.1. Laboratory study

Materials & Methods

Three tests were conducted in *in-vitro* studies on bacterial control. Test 1 was conducted to determine the effects of various copper products (Photo 15) and disinfectants on very high population (~1 x 10^9 cfu/mL) of *P. marginalis 1.22 & 18.6*, *P. fluorescens 18.7*, and *P. tolaasi 18.8*. Test 2 examined the efficacies of different copper products and a disinfectant (Sporekill) on different population of *P. marginalis 18.6.* Test 3 was carried out to determine the product's volatility, especially with disinfectants, using a treated filter-paper disk test on *P. marginalis 18.6.*

All laboratory tests were conducted on King's B medium on agar plates. The bacterial colonies of all the Pseudomonas species used in the study fluoresced under UV light. A bacterial suspension was prepared by diluting a small wire-loop of a 5-day-old colony with sterile distilled water. The resulting bacterial suspension was clear. Different bacteria populations were prepared with serial dilutions. In the spread plate test, a bacterial suspension was mixed with an equal volume of a chemical suspension, and 0.1 mL of the resulting mixture was then spread over the agar surface, within 30 minutes after mixing, and incubated at 23°C for 48 hours.

Product volatility was examined in Test 3 by soaking two filter paper disks in chemical suspension at the desired concentrations, and placing the disks on to a King's B medium plate, which had bacteria (*P. marginalis* 1.22), spread over the surface. A third disk soaked with distilled water only was also placed in the same plate as a control. After 48 hours of incubation, the zone of bacterial inhibition from the disk was noted.

Copper products tested were Kocide Liquid Blue, Champ DP, Cuprofix MZ and Tri-Base Blue. Disinfectants tested were: Path-X (1.2%), White King (1.0%), Sporekill (0.1% and 1.0%), and Oxine (0.01%, 0.1% and 1%). The concentrations of copper products used in the tests were based on the maximum product rates on their label for brassica crops.

Results & Discussion

In Test 1, when mixed with very high bacterial population in suspension, no copper products appeared to show any inhibition on the spread plate test on *P. marginalis, P. fluorescent,* and *P. tolassi* (Table 3.1). This lack of inhibition by the copper products however, appeared to be related to a quantitative response to bacterial population, as demonstrated in Test 2.

The pH values of the different copper products in suspension varied. Some, like Champ DP and Kocide Liquid Blue were alkaline, while Copper oxychloride and Cuprofix MZ were acidic. The solubility and release of free copper ions from the relatively insoluble copper salts are affected by pH of solution, with more copper ions expected to be released under acidic conditions.

In Test 2, at the highest *P. marginalis* population of 10^6 cfu/mL, the different copper products reduced the initial bacterial population to approximately 10^4 cfu/mL (Figure 3.1). Even though the final bacterial population was approximately 1% of the initial population, at 10^4 cfu/mL, this resulting bacterial population was still considered too high for effective bacterial control. At the lower populations of 10^4, 10^3 and 10^2 cfu/mL, complete bacterial inhibition by the different copper products was noted (Figure 3.1). This showed that the sensitivity of the bacteria to copper treatment was related to its population. This finding also demonstrates that copper products are most effective when used before a build-up of very high bacterial population.

Typically, copper is most effective when applied as a preventative crop protectant before disease develops. Among the copper products tested, Tri-Base Blue appeared to be more effective than Champ DP (Photo 16).

Table 3.1: The effects of different copper products on very high initial population of *P. marginalis* in *in-vitro* spread plate Test 1

Treatment	pH value of test solution	Product rate/100L water	Active ingredient in final test solution	Bacterial Inhibition			
				***P. marginalis* 1.22**	***18.6***	***P. fluorescent 18.7***	***P. tolassi 18.8***
Tri-Base Blue	7.2	420 mL	0.08%	N	N	N	N
Cuprofix MZ	6.1	300 g	0.036% Cu + 0.09% Mz	N	N	N	N
Champ DP	10.2	140 g	0.052%	N	N	N	N
Copper oxychloride	6.3	250 g	0.10%	N	N	N	N
Kocide Liquid Blue	9.8	1000 mL	0.36%	N	N	N	N
Oxine	-	-	1%	Y	Y	Y	-
Path-X	-	-	1.2%	Y	Y	Y	-
Sporekill	6.0	-	1.2%	Y	Y	Y	-
White King	-	-	1%	Y	Y	Y	-
Distilled water for Control	6.2	-	-	N	N	N	N

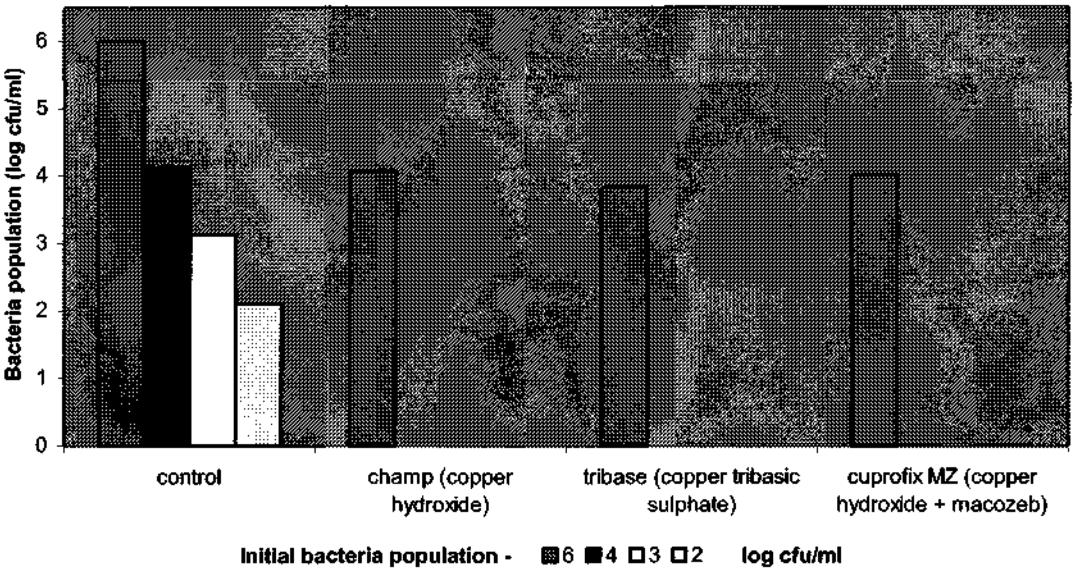
a.i. = active ingredient; see Appendix i for details on a.i. and product formulation.
Based on minimum label product rate to be applied in 100L water volume.
N = plate completely covered with bacterial colonies; Y = complete bacterial inhibition.

In Test 1, the various disinfectants used in this study, sodium hypochlorite, quaternary ammoniums, and carbon chlorine dioxide were found to be highly effective in eradicating bacteria, causing complete bacteria inhibition even at very high bacterial population (Table 3.1, Photo 14). These findings suggest that disinfectants may be useful to provide a rapid knock-down effect of high bacteria population in infected crops. As these disinfectants generally dissipate rapidly after application, they have no residual effects. Therefore, for disease control in the field, a disinfectant could potentially be used in a mixture with a copper product to provide an immediate reduction in bacteria population, while the copper provides bacterial control over a longer period.

In Test 3, when treated filter paper disks were placed on plate inoculated with bacteria, all copper products and the disinfectants (White King, Path-X, and Sporekill) were shown to be non-volatile and immobile (Table 3.2). Bacterial inhibition only occurred when the bacteria were in contact with the chemical treated filter-paper disks. Oxine (based on chlorine dioxide) is highly volatile, causing a wide zone of inhibition in the surrounding areas close to the Oxine treated filter paper disks. Increasing the rates of Oxine from 0.01%, 0.1% and 1%, increased the zone of inhibition (Photo 17). These findings demonstrate that except for Oxine, most disinfectants are non-volatile contact bacteriacides and hence, require good coverage for bacterial eradication.

3. Bacterial control (Cont.)

Figure 3.1: Effects of copper products against different population of *P. marginalis*



Further tests with different rates of two disinfectants showed that lower rates of Sporekill at 0.1% and Oxine at 0.1 and 0.01% were also effective in causing complete bacterial inhibition in a suspension of high initial bacterial population of *P. marginalis*.

It should also be noted that while the high volatility of Oxine is beneficial, this property also makes it vulnerable to dissipation and hence loss of its activities if not kept stored under cool conditions in airtight containers. The same applies for the chlorine-based product White King. Quaternary ammonium based products are less volatile, and are commonly used for disinfecting dam water, irrigation and spray water, and washing water in vegetable processing.

Table 3.2: The effects of different products on treated filter paper disks on very high initial population of *P. marginalis* in Test 3

Treatment	Product rate	Complete inhibition of bacteria on contact	Zone of inhibition away from treated disks
Tri-Base	420 mL/100 L	N	N
Cuprofix MZ	300 g/100 L	N	N
Champ	140 g/100 L	N	N
Copper oxychloride	250 g/100 L	N	N
Kocide Liquid Blue	1.0 L/100 L	N	N
Oxine	1.0%	Y	Y
Path-X	1.2%	Y	N
Sporekill	1.0%	Y	N
White King	1.0% chlorine	Y	N
Untreated control	-	N	N

3. Bacterial control (Cont.)

Among the different bacteria used in the test, *P. marginalis* 18.6 appeared to be more tolerant of copper than *P. marginalis* 1.22, and *P. fluorescent* 18.7 was more sensitive than the two *P. marginalis* isolates (Photo 16). This difference in copper tolerance between the two *P. marginalis* isolates may be attributed to the origin of the isolates, where isolate 18.6 came from a crop that had been sprayed with copper, while isolate 1.22 was from an untreated crop.

There is evidence that sole reliance on the use of copper sprays for bacterial disease control has resulted in an increase in the prevalence of copper-tolerant strains (Martin & Hamilton 2003, Scheck *et al.* 1998, Cooksey 2002). In Queensland, Martin & Hamilton (2003) found that isolates of *Xanthomonas campestris pv. vesicatoria* (bacterial spot of pepper) collected between 1999 and 2000 tended to have higher tolerance to copper than those collected before 1987. Furthermore, in a field study, the proportion of bacterial isolates that tolerated copper concentrations > 0.7 mM increased from only 1.6% after four sprays of copper hydroxide at seven day intervals to 29.7% after 12 sprays and 50.1% after 21 sprays (Martin & Hamilton 2003).

3. Bacterial control (Cont.)

3.2. Field Trials 2003

Materials & Methods

In 2003, four field trials were set up within commercial broccoli crops to assess the effects of different copper products, with and without a disinfectant (Sporekill) under field conditions. Trials 3.1 - 3.3 were conducted on processing broccoli crops during autumn, while Trial 3.4 was conducted on a fresh market broccoli crop in winter.

All trial designs were complete randomised block with plot size of 5 m long x 1.6 m wide beds. , There were four replicates in Trials 3.1 & 3.2, three replicates in Trial 3.3 and two replicates in Trial 3.4. All trials had two spray applications, using a knapsack air-pressurised sprayer with 400 L water/ha. Spray application details are in Appendix iii. In Trials 3.1 & 3.2, bacterial suspension of *Pseudomonas marginalis* was applied to plants in the middle of the trial area in replicate blocks 2 and 3 at approximately one hour before chemical spray applications.

Trials 3.1 & 3.2 were conducted to evaluate the efficacies of various products with and without a disinfectant (Sporekill) for head rot control under field conditions (Table 3.3). A combination of iron chelate and copper hydroxide (Champ DP) was also examined.

Table 3.3: Treatment list for Field Trials 3.1 & 3.2

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated control	N/a	N/a
2	Mancozeb 750 g/kg DF	2.2 kg	Two spray applications : 1st spray when broccoli heads reached 50mm in diameter, and 2nd spray 10-14 days after.
3	Champ DP	1.55 kg	
4	Champ DP + Sporekill	1.55 kg + 100 mL/100 L	
5	Champ DP + chelated iron	1.55 kg + 85 g/100 L	
6	Cuprofix MZ	3.5 kg	
7	Cuprofix MZ + Sporekill	3.5 kg+ 100 mL/100 L	
8	Tri-Base Blue	3.0 L	
9	Tri-Base Blue + Sporekill	3.0 L+ 100 mL/100 L	
10	Sporekill	100 mL/100 L	

3. Bacterial control (Cont.)

Trials 3.3 & 3.4 were conducted to evaluate the phytotoxic effects of copper based products, with and without Sporekill, on broccoli heads under field conditions (Table 3.4). Two rates of water volumes, at 250 L and 400 L per hectare, were used as described on the treatment list. Each product was diluted at two water rates, in order to obtain two extremes in product concentrations for different copper concentrations. These water rates are within the range that is commonly used by growers in field spray applications.

Table 3.4: Treatment list for Trials 3.3 & 3.4

No.	Treatment	Product Rate	Water rate (L)	Product concentrat-ion (per 100L water)	Copper concentrati on (per 100L water)
1	Untreated control	N/a	N/a	N/a	N/a
2	Kocide Liquid Blue	1.65 L	210	660 g	238 g
3	Champ DP	1.55 kg	210	620 g	233 g
4	Cuprofix MZ	3.5 kg	210	1400 g	168 g
5	Tri-Base Blue	3.0 L	210	1.2 L	228 g
6	Kocide Liquid Blue	1.65 L	400	413 g	149g
7	Kocide Liquid Blue + Sporekill	1.65 L + 100 mL/100 L	400	413 g	149g
8	Champ DP	1.55 kg	400	387 g	145 g
9	Champ DP + Sporekill	1.55 kg + 100 mL/100 L	400	387 g	145 g
10	Cuprofix MZ	3.5 kg	400	875 g	105 g
11	Cuprofix MZ + Sporekill	3.5 kg + 100 mL/100 L	400	875 g	105 g
12	Tri-Base Blue	3.0 L	400	750 mL	142.5 g
13	Tri-Base Blue + Sporekill	3.0 L + 100 mL/100 L	400	750 mL	142.5 g
14	Sporekill	100 mL/100 L	400	-	-

In the disease assessments, three plant rows within 4 m of each treatment plot were assessed for head rot and black spots. Head rot was due to bacterial rot, whereas the black spots indicated blemishes or cell necrosis on sepals of florets that may have been due to phytotoxic effects. The total number of heads assessed was also recorded, and the percentage of bacterial head rot or black spot was tabulated.

As the data values were not normally distributed and there were big differences between the data values of the replicates within each treatment due to the patchy distribution of the affected plants, the data was analysed using the non-parametric Kruskall-Wallis test on treatment median values in the Statgraphic Plus 4.1 statistical program. Therefore, in the results, the median values are given instead of mean values.

3. Bacterial control (Cont.)

Results & Discussion

In Trial 3.1, there was a negligible level of head rot in the trial area and there were no differences between treatments in the incidence of head rot (p = 0.60) (Table 3.5). In Trial 3.2, there were also no differences between treatments in the incidence of head rot at three days (p = 0.12) and at one day (p = 0.28) before commercial harvest at 92 and 96 days after planting (DAP) (Table 3.6).

Table 3.5: Treatment effects on the incidence of heads with obvious black spot and head rot in Trial 3.1 at Forrest, Tasmania

No.	Treatment	Product rate/ha	% Black spot * (85 DAP, 9 DAS)	% Head rot * (85 DAP, 9 DAS)
1	Untreated control	N/a	0.0	0.02
2	Mancozeb DF	2.2 kg	0.0	0.00
3	Champ DP	1.55 kg	0.0	0.00
4	Champ DP + Sporekill	1.55 kg + 100 mL/100 L	8.0	0.00
5	Champ DP + chelated iron	1.55 kg + 85 g/100 L	2.1	0.00
6	Cuprofix MZ	3.5 kg	0.0	0.00
7	Cuprofix MZ + Sporekill	3.5 kg+ 100 mL/100 L	2.0	0.00
8	Tri-Base Blue	3.0 L	0.0	0.00
9	Tri-Base Blue + Sporekill	3.0 L+ 100 mL/100 L	4.2	0.00
10	Sporekill	100 mL/100 L	0.0	0.00

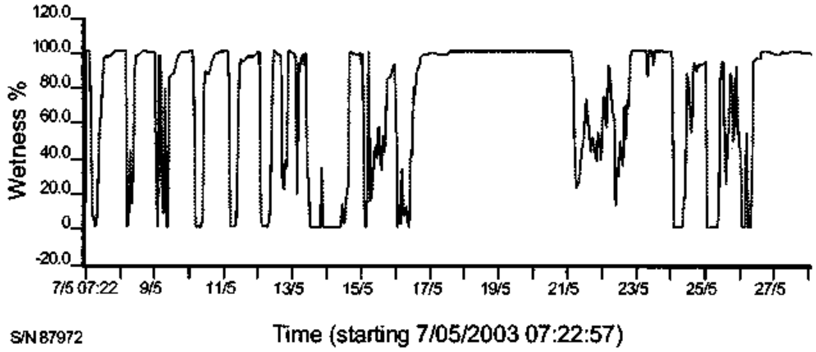
All data sets are based on median values.
DAP = days after planting, and DAS = days after the first spray application.
No assessment was conducted after 2^{nd} spray, as crop was harvested one day after the last spray
* No statistical differences between treatments according to the Kruskall-Wallis Test at 95% confidence level.

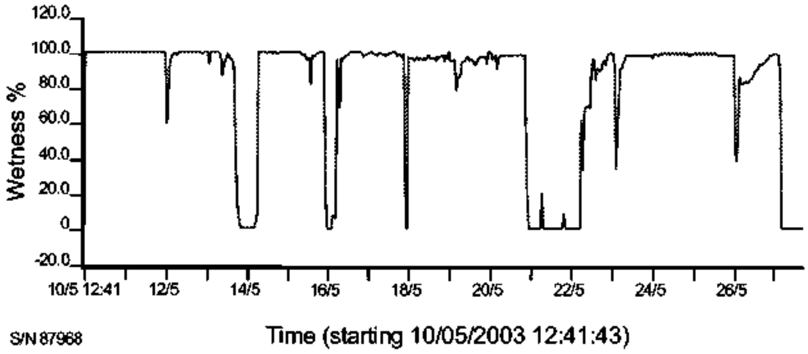
Table 3.6: Treatment effects on the incidence of heads with obvious black spot and head rot in Trial 3.2 at Boat Harbour, Tasmania

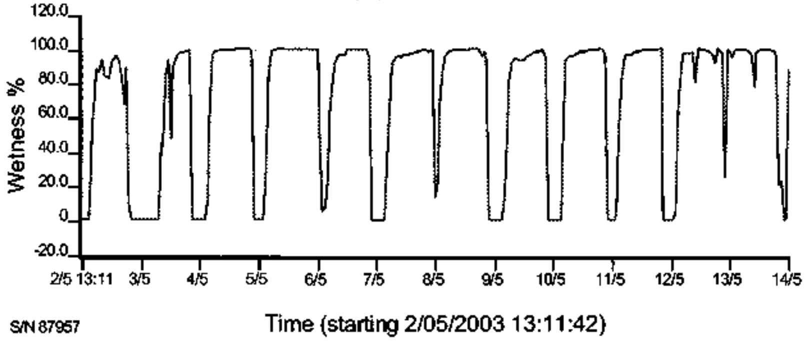
No.	Treatment	Product rate/ha	% Black spot (92 DAP, 3 DAS) *	% Head rot	
				(92 DAP, 3 DAS) *	(96 DAP, 7 DAS) *
1	Untreated control	N/a	15.5	0.0	8.4
2	Mancozeb DF	2.2 kg	0.0	0.0	8.8
3	Champ DP	1.55 kg	4.5	0.0	4.5
4	Champ DP + Sporekill	1.55 kg + 100 mL/100 L	9.2	0.0	0.0
5	Champ DP + chelated iron	1.55 kg + 85 g/100 L	17.8	0.0	0.0
6	Cuprofix MZ	3.5 kg	25.8	5.6	9.1
7	Cuprofix MZ + Sporekill	3.5 kg+ 100 mL/100 L	39.4	4.2	4.2
8	Tri-Base Blue	3.0 L	17.4	4.2	9.5
9	Tri-Base Blue + Sporekill	3.0 L+ 100 mL/100 L	23.6	0.0	5.0
10	Sporekill	100 mL/100 L	11.9	0.0	3.8

All data sets are based on median values.
DAP = days after planting, and DAS = days after the last spray application.
* No statistical differences between treatments according to the Kruskall-Wallis Test at 95% confidence level.

Figure 3.2: Leaf wetness of crops in Trials 3.1 – 3.4


Trial 3.1 at Forrest


Trial 3.2 at Boat Harbour


Trial 3.3 at North Motton

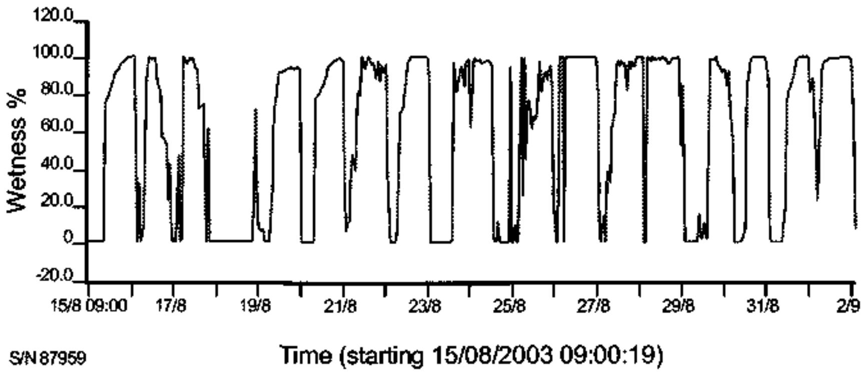
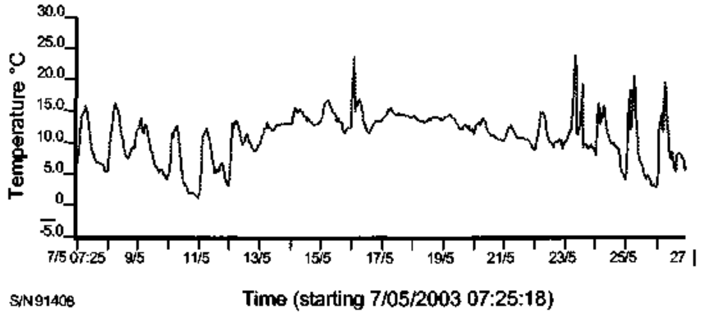
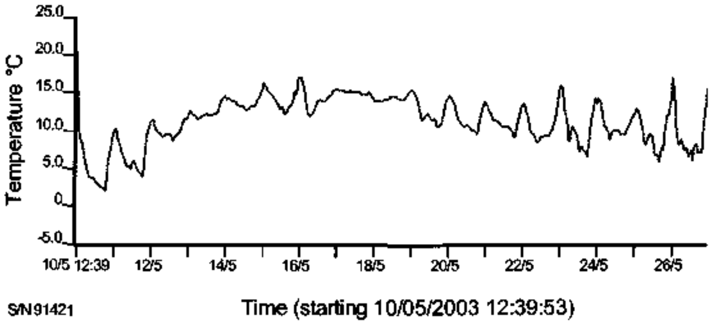

Trial 3.4 at Forth

Figure 3.3: Field temperatures for Trials 3.1 – 3.4

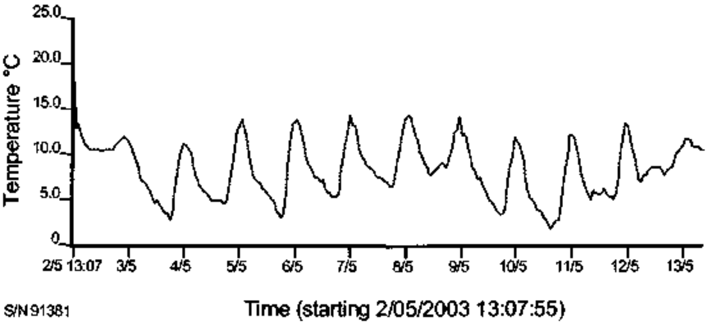
Trial 3.1 at Forrest



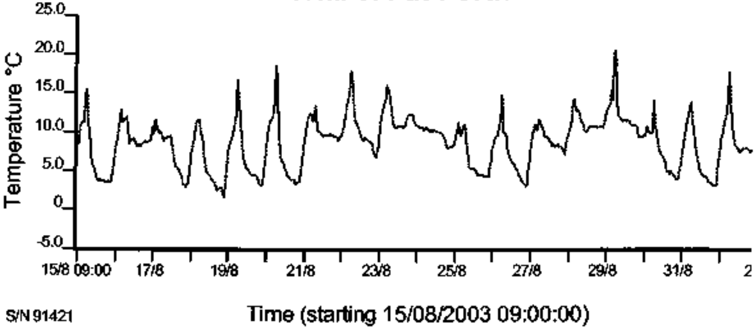
Trial 3.2 at Boat Harbour



Trial 3.3 at North Motton



Trial 3.4 at Forth



3. Bacterial control (Cont.)

In Trial 3.1, the relatively low incidence of head rot may have been due to the relatively dry conditions during the crop period, and only one prolonged wet period between 17/05/03 to 21/05/03 (Figure 3.2). Plant wetness in the crop, as measured by the leaf wetness sensors, occurred mainly as a result of periodic cycle of overnight water condensation and dew formation on plants.

The crop in Trial 3.2 was subjected to wetter conditions than that in Trial 3.1 (Figure 3.2). The multiple prolonged periods of wetness during the late crop stage between 10/05/03 to 26/05/03 were likely to predispose heads to a higher incidence of head rot in Trial 3.2. The atmospheric temperature in the crops in Trials 3.1 and 3.2 were similar (Figure 3.3). Temperatures in the crops were mainly above 5°C and dropped to below 3°C, which is conducive to frost, on only one occasion in Trial 3.1 at crop maturity. This suggests that wet conditions were more important as a predisposing condition, rather than temperature, in these trials.

Broccoli heads are also known to produce glucosinolates, which can convert to the active isothiocynates that are active against bacteria and fungal invasion. However, as the heads mature, the bioactive compounds become less concentrated and hence, less effective against pathogens (J. Kirkegaard, CSIRO, per. comm.). Therefore, in addition to prolonged wet conditions, head maturity increases susceptibility to bacteria and fungal invasion. Fungal infection of heads by *S. sclerotiorum* was also mainly observed only on matured heads. In Trial 3.2, head rot incidence recorded at one day prior to commercial harvest was generally higher than at three days before harvest (Table 3.6).

Table 3.7: Treatment effects on the incidence of heads with obvious black spot and head rot in Trials 3.3 at North Motton and 3.4 at Forth, Tasmania

No.	Treatment	Water Volume (L/ha)	Metallic copper concentration (g/100 L water)	Trial 3.3		Trial 3.4	
				% Black spot * (14/5/03)	% Head rot * (14/5/03)	% Black spot * (02/9/03)	% Head rot * (02/9/03)
1	Untreated control	400	N/a	3.8	0.02	0.0	0.0
2	Kocide Liquid Blue	250	238	13.3	0.07	0.0	0.0
3	Champ DP	250	233	0.0	0.00	0.0	0.0
4	Cuprofix MZ	250	168	7.7	0.00	0.0	0.0
5	Tri-Base Blue	250	228	7.1	0.07	0.0	0.0
6	Kocide Liquid Blue	400	149	0.0	0.00	0.0	0.0
7	Kocide Liquid Blue + Sporekill	400	149	0.0	0.00	0.0	0.0
8	Champ DP	400	145	0.0	0.00	0.0	0.0
9	Champ DP + Sporekill	400	145	7.7	0.00	0.0	0.0
10	Cuprofix MZ	400	105	0.0	0.00	0.0	0.0
11	Cuprofix MZ + Sporekill	400	105	6.7	0.07	0.0	0.0
12	Tri-Base Blue	400	142	0.0	0.00	0.0	0.0
13	Tri-Base Blue + Sporekill	400	142	0.0	0.08	0.0	0.0
14	Sporekill	400	N/a	7.7	0.08	0.0	0.0

All data sets are based on median values.

DAP = days after planting, and DAS = days after the last spray application.

* No statistical differences between treatments according to the Kruskall-Wallis Test at 95% confidence level.

There were also no significant differences in black spot incidence between treatments in Trial 3.1 ($p = 0.09$) and Trial 3.2 ($p = 0.10$) (Tables 3.5 & 3.6). In Trial 3.1, black spot incidence was also relatively low with mild symptoms, and was not considered to be a quality problem. No heads were rejected in the trial due to black spot in the crop at harvest. The black spot incidence in Trial 3.2 was much higher than in Trial 3.1. This may be related to wetter conditions in Trial 3.2. However, it should also be noted that most of the heads with black spot in Trial 3.2 were also harvested for commercial use. Black spots observed in the two trials appeared to be unrelated to frost damage.

It is interesting to note that there appeared to be higher black spot incidence on heads treated with Sporekill-copper mixtures in both trials, when compared to copper spray only (Tables 3.5 - 3.7). It is possible that the high surfactant activity by the Sporekill may have a superficial effect on the waxy surface of the floret. Therefore, although the disinfectant is highly effective in eradicating bacteria, further studies are recommended to determine rates that will have no adverse effects in field applications. If a disinfectant is used in an infected crop, the lowest possible rate should be used.

Among the four trials conducted in 2003, Trial 3.2 had the highest incidence of black spots. This could be due to a prolonged period of wetness during the trial period. Although in Trial 3.2, there was no significant difference in the black spot incidence between treatments ($p = 0.101$) at the 95% confidence level (Table 3.6), it is interesting that heads treated with Champ DP appeared to have the lowest incidence of black spot. Different copper products had different pH values (Table 3.1). Champ had a high pH value (pH 10.2 in solution), while Cuprofix MZ that had a relatively low pH (pH 6.1) tended to result in a high incidence of black spots. Sporekill is also acidic (pH 6.0), and its low pH value may also have contributed to higher black spot incidence. As the solubility of copper is expected to increase with lower pH, these findings suggest that the increase in black spot incidence might be associated with increased free copper ions. Copper sprays are known to predispose treated plants to frost damage and many product labels caution against their use on cabbage crops if frost is likely to occur. Similar caution should also apply to broccoli crops, especially on plants with maturing heads close to harvest.

In Trials 3.3 and 3.4, there were negligible levels of head rot (Table 3.7). There were no differences in the black spot incidence between treatments in Trial 3.3 ($p = 0.98$). Apart from black spot, no other adverse effects were observed. It is worth noting, however, that at low water volume, there appeared to be more black spots than in similar treatments at high water volume. This may be related to copper product rate. The highest black spot incidence appeared to be recorded with the highest copper concentration in spray mix with Kocide Liquid Blue in 210 L water per hectare. In general, plants treated with copper products alone also tended to have fewer numbers of head rot with high volume sprays of 400 L/ha than the low volume sprays at 210 L/ha. Field observations by field staff also generally noted improved head rot control with high volume spray applications by growers on commercial crops. Matured plants in Trial 3.4 were relatively small and sparse due to late planting and poor growth. No head rot or black spot were noted in Trial 3.4 due to rapid drying and lack of prolonged wet period (Figure 3.2).

General Discussions

Head rot epidemiology

The potential of bacteria to cause rots on non-wounded heads is determined by two key characteristics: the presence of pectolytic activity and biosurfactant activity (Hildebrand 1989). The pectolytic fluorescent pseudomonads are important bacterial pathogens, causing soft rots on a diverse group of vegetables (Liao & Wells 1987). On plants that have thick, waxy surfaces, like broccoli, the water soaking properties of biosurfactant-producing bacteria, help provide entry through natural openings (Hildebrand 1989). Biosurfactants released by some fluorescent pseudomonads may also enable surfactant deficient strains to colonise the water soaked areas even though physical injury of tissues is not evident (Wimalajeewa *et al.* 1987, Hildebrand 1989).

Stomata, and sepals of florets that do not overlap completely to close the reproductive parts of the flower, help provide small openings for surfactant-mediated entry by bacteria and fungal pathogens. As the broccoli head increases in maturity, the partial opening of sepals increases, thereby making them vulnerable to bacterial and fungal invasion. In this study, a higher incidence of bacterial head rot, as well as fungal rot by *Sclerotinia*, *Botrytis* and *Fusarium*, was noted on matured heads. Less mature heads harvested for the fresh market rarely get infected by bacteria or fungal pathogens. Low susceptibility of young heads may partly be associated with the presence of high levels of glucosinates in plant tissues (J. Kirkegaard, CSIRO, per. comm.). These observations suggest that bacterial and fungal head rot may be managed with head maturity and early harvest timing, particularly in the presence of prolonged wet field conditions.

Surfactant activities of agricultural chemicals

All of the insecticides commonly used in broccoli crops showed none or very mild surfactant activity. Therefore, a wetting agent is often applied with the insecticide in order to improve wetting and uptake on waxy plant surfaces. Activator and Agral are commonly used in pesticide applications for insect or disease control in horticultural crops in Tasmania. At the beginning of this study in 2002, many growers were known to apply these adjuvants at the maximum rates recommended with insecticides on broccoli crops. However, as a result of findings in this study, high rates of these adjuvants may pre-dispose the wet florets to severe head rot and lower rates are recommended for use instead. The minimum rates recommended on labels were shown to be as effective in wetting the florets as higher rates of the adjuvants, with lower risks to bacterial invasion.

Most copper products have little or no surfactant activity, and do not wet the florets or pre-dispose heads to head rot. Moderate surfactant activities recorded with Bravo and Tri-Base Blue are expected to be similar to the minimum rates of spray adjuvants, and unlikely to have any adverse impact. Excessive metallic copper ions, however, may have phytotoxic effects on plant tissues and cause burning or blemishes similar to black spots on sepals of florets.

Bacterial control

An excess of copper ions is damaging to plant tissues. As a result of the phytotoxic effects, copper products formulated for agricultural use are almost insoluble in water at approximately pH 7.0. Copper sprays applied to plants exist mainly as insoluble deposits of copper salts (Menkissoglu & Lindow 1991). These products are designed to release a small, but constant, supply of free copper ions whenever the plant surface is wet.

Under moist conditions, carbon dioxide, and plant and microbial exudates, form weak organic acids in water on the plant surface, hence reducing plant surface pH. A small quantity of free copper ions (Cu^{2+}) is released from the insoluble copper deposits into this film of water (Arman & Wain 1958, Lee *et al.* 1993). Any fungal spores or bacterial cells that come in contact with this moisture will absorb the free copper ions. Once absorbed, the free copper ions will disrupt cellular enzyme systems.

If too many copper ions are released at one time, phytotoxicity and damage to the florets and foliage can occur. The phytotoxicity symptoms include burning of leaves, and black spots or blemishes on florets. Factors that can result in excessive release of free copper ions are; the spray mixture is too acidic (less than pH 6.0 to 6.5), copper formulations are contaminated with soluble copper compounds, or formulations that release too many copper ions. This study also indicated a possible link in concentrations of copper products in spray mixture volumes. Higher copper product concentrations with low spray water volumes of 210 L/ha tended to increase black spot incidence compared to high spray water volumes of 400 L/ha. Furthermore, as mentioned in the previous sub-heading, low pH of resulting copper spray mixture may also contribute to phytotoxicity.

Particle sizes of a copper product have been shown to affect their efficacy (Torgeson *et al.* 1967). Formulations with smaller particles produce improvements in disease control through better coverage, rain-fastness, longevity of the product, and release of copper ions on plant surfaces. The smaller the particle size, the greater the number of particles per gram, and therefore the greater the release of copper ions for antibacterial activity. Smaller particles give superior coverage and improved efficacy in disease control. Tri-Base Blue and Champ DP are examples of copper products that are made of ultra-fine particles of less than 1 micron. New copper product formulations such as these have enabled the use of lower rates of metallic copper for disease control, thereby reducing the potential environmental risk. In this study, although Champ DP appeared to be less effective than Tri-Base Blue in inhibiting *P. marginalis* in an *in-vitro* test, it appeared to be more effective in reducing black spots and bacterial head rot in a field trial. These differences in efficacies suggest the complex interacting factors between copper type, particle size, pH, toxicity and other formulation properties. According to Scheck & Pscheidt (1998), the amount of free copper ions in solution is the only predictor of formulation efficacy, which could not be estimated from the metallic copper content of a product.

This study showed that all the copper-based products were less effective in the complete inhibition of very high populations of bacteria (in excess of 10^6 – 10^{10} cfu/mL). Even though at 10^6 cfu/mL, Champ DP, Cuprofix MZ, and Tri-Base Blue did reduce the final population to approximately 1% of the initial population, the remaining 10^4 cfu/mL was still considered to be relatively high. At the lower bacteria levels of 10^4 cfu/mL and below, complete bacterial inhibition by Champ DP, Cuprofix MZ, and Tri-Base Blue were recorded.

The reduced effectiveness of the copper products at very high bacterial levels may be associated with the proportion of free copper ions in solution and at equilibrium, bacterial cell population, and uptake by the bacteria. Therefore, copper products are believed to be most effective when used as crop protectants to reduce initial bacterial population levels on plant surfaces, thus preventing diseases. It is likely to be less effective when used on a severely infected crop that already has a very high bacterial population.

General disinfectants, including those recommended for use to disinfect dam or washing water, such as Sporekill and Path-X, may be useful for drastic reduction of bacterial populations to low levels that can then be effectively controlled by copper based products. Unlike copper products, these disinfectants were shown to be highly effective in eradicating high populations of bacteria. It should be noted however, that the disinfectants have no residual activities, and hence offer no prolonged protection from bacteria re-colonisation. Therefore, these disinfectants should be considered for use only as an additive to copper products, rather than as replacement products.

In recent studies conducted in Queensland, Martin & Hamilton (2003) showed that the reliance on copper sprays by pepper producers for bacterial spot of pepper has resulted in the prevalence of copper tolerant bacteria strains. In this study with *in-vitro* tests, one of the two strains of *P. marginalis* used appeared to be more tolerant to copper than the other. This difference in copper tolerance between the two *P. marginalis* isolates could be attributed to the origin of the isolates, where the copper tolerant isolate came from a crop that had been sprayed with copper, while the more copper sensitive isolate was from an untreated crop.

According to Cooksey (2002), the higher tolerance to copper ions may be partly related to the loss of toxicity when proteins produced by the copper resistant bacteria can bind with multiple copper ions. However, the resistant development by the copper tolerant bacteria strains appears to be quantitative, rather than a qualitative resistance to copper (Lee *et al.* 1993), whereby increasing the toxicity or the availability of free copper ions may still effectively control the copper resistant strains.

The use of increased rates of copper products is not an option due to the increased risk of phytotoxicity, and the general trend of reducing rather than increasing metallic copper rates. Improved toxicity and efficacy could be achieved in part with new and better formulations of copper products. As an alternative, copper toxicity may be enhanced with the use of additives that enhance the solubility and uptake of copper ions or that interfere with the resistance mechanisms. Additives that have shown enhancement of copper sensitivity include fungicides, ferric chloride, zinc chloride and aluminium nitrate (Cooksey 2002). Scheck *et al.* (1998) found that while all copper-based bactericides reduced the population size of copper-sensitive strains of *Pseudomonas syringae pv. syringae* by 50%, only cupric hydroxide mixed with mancozeb or ferric chloride reduced the population size of the copper-resistant strains by an equivalent amount.

The increased toxicity of mancozeb mixtures with copper based bacteriacides appears to be associated with the ability of the dithiocarbamate anion to chelate copper and transport cupric ions to copper susceptible sites within bacterial cells (Medhekar & Borapai 1981). Lee *et al.* (1993) showed that adding iron in the form of ferric chloride to cupric hydroxide resulted in increased toxicity to copper resistant strains of *Xanthomonas campestris pv juglandis*, on walnut. The ferric ion apparently has a direct physiological effect on *X. campestris pv juglandis*, resulting in an increased susceptibility to the toxic effect of cupric ion.

General Discussions (Cont.)

The addition of ferric chloride to cupric hydroxide also increases the availability of cupric ions on leaf surfaces by lowering the pH and through cation exchange between cupric ion and ferric ion. Unfortunately, in this study, we were not able to compare the treatment mixture of iron chelate + Champ DP against Champ DP alone, due to little or no head rot in the treatments.

Black spots

The small black spot was not necessarily related to the bacterial head rot. If severe, black spots may affect the appearance of the heads, thereby reducing their quality or make them unmarketable. In this study, when the black spots were kept moist under room temperature, about 20°C, bacterial decay was noted on the spots that may spread and cause head rot. Therefore, black spots may increase the risk of secondary rot by bacteria, thereby affecting the quality of affected heads after harvest and storage.

Black spots appeared to be associated with cell necrosis on the sepals of florets. This symptom increased with head maturity. In our field observations, nutrient deficiency, frost damage, and prolonged period of wetness on florets tended to increase black spot incidence. In the presence of frequent frost conditions in 2002, Kocide DF treatments consistently increased the incidence of black spots on the sepals of florets on maturing heads in a field trial. This increase in black spots appeared to be related to increased susceptibility to frost damage. The labels of many copper products have crop damage warnings that they should not be applied onto cabbage crops if frost is likely. Similar warnings should also apply on their use on broccoli crops, particularly close to harvest, when the matured heads are most prone to damage.

The pH of different copper products also appeared to be related to the incidence of black spots. The copper product Champ, which has a relatively high pH value, tended to cause lower black spot incidence, while copper products like Cuprofix MZ resulted in a high incidence of black spot.

In both 2002 and 2003, whole crops have been observed to develop a purplish discoloration on the sepals of the florets. While some of these purplish spots might have developed into black spots, most eventually disappeared. This general discolouration in the whole crop is believed to be due to a physiological response to poor translocation of nutrients under very cold conditions, or in a nutrient deficient crop.

In 2003, the use of the disinfectant Sporekill in mixtures with copper sprays also tended to increase black spot incidence, compared to copper sprays only. This may be related to increased wetting with Sporekill or other possible adverse effects on plant cells on sepals of florets. Therefore, although Sporekill and other disinfectants are highly effective in eradicating or reducing bacteria populations, further investigation is recommended to understand their impact on black spots.

Acknowledgments

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Appendix i - Product Formulations

Treatment	Product type [1]	Active Ingredient (a.i.)	Concentration of a.i.	Formulation
Activator	Ad	Isopropanol, polyalkoxylated alkyl alcohol and oleic acid	900 g/L	Emulsifiable concentrate
Agral	Ad	Nonyl phenol ethylene oxide condensate	600 g/L	Liquid concentrate
Agridex	Ad	Paraffin base petroleum oil. Polyol fatty acid esters and polyethoxylated polyol fatty acid ester emulsifier	730 g/L 149 g/L	Emulsifiable concentrate
Bond	Ad	Butadiene copolymer nonyl phenol ethoxylate type	400 g/L 100 g/L	Liquid concentrate
Nu-Film-17	Ad	Di-1-p-Menthene	904 g/L	Emulsifiable concentrate
Synertrol	Ad	Emulsifiable vegetable oil	832 g/L	Emulsifiable concentrate
Companion	BCA	*Bacillus subtilis*	6.8 x 10^8 cfu/cc	Liquid concentrate
Grocal	Fe	Calcium Nitrogen Magnesium Boron	17% 10% 4% 1%	Liquid concentrate
MicroGyp (Gypsum)	Fe	Calcium sulphur	21.6% calcium 16.3% sulphur	Wettable powder
Librel Fe	Fe	chelated iron (Fe)	12%	Water soluble powder
Agri-Fos	Fe, Fu	Phosphorous acid	400 g/L	Suspension concentrate
Bravo	Fu	Chlorothalonil	720 g/L	Suspension concentrate
Penncozeb DF750	Fu	Mancozeb	750 g/kg	Dry flowable
Penncozeb SC420	Fu	Mancozeb	420 g/L	Suspension concentrate
Chess	In	Pymetrozine	250 g/kg	Wettable powder
Chlorpyrifos	In	Chlorpyrifos	500 g/L	Emulsifiable concentrate
Pirimor	In	Pirimicarb	500 g/kg	Wettable granules
Proclaim	In	Emamectin benzoate	44 g/kg	Water soluble granules
Success	In	Spinosad	120 g/L	Emulsifiable concentrate

1 Ad = spray adjuvant, BCA = biocontrol agent, Fu = fungicide; Fe = fertilizer; In = insecticide

Appendix i - Product formulation (Cont.)

Treatment	Product type [1]	Active Ingredient (a.i.)	Concentration of a.i.	Formulation
Champ DP WG	Ba, Fu	Cupric hydroxide	375 g/kg	Flowable dry prill
Flo Bordo	Ba, Fu	Cupric hydroxide	50 g/L	Liquid concentrate
Kocide DF	Ba, Fu	Cupric hydroxide	400 g/kg	Dry flowable
Kocide Liquid Blue	Ba, Fu	Cupric hydroxide	360 g/L	Suspension concentrate
Cuprofix MZ	Ba, Fu	Tribasic copper sulphate + mancozeb	120 g/kg + 300 g/kg	Liquid concentrate
Tri-Base Blue	Ba, Fu	Tribasic copper sulphate	190 g/L	Emulsifiable concentrate
Copper oxychloride WP	Ba, Fu	Copper oxychloride	500 g/kg	Wettable powder
Path-X	Dis	Didecyldimethyl-ammonium chloride (Quartenary ammonium)	120 g/L	Liquid concentrate
Sporekill	Dis	Didecyldimethyl-ammonium chloride (Quartenary ammonium)	120 g/L	Liquid concentrate
White King	Dis	Sodium hyphochloride, active is available chlorine	42 g/L (4% available chlorine)	Liquid concentrate

[1] Ad = adjuvant, Ba = bacteriacide, Fu = fungicide

Appendix ii - Agar Medium

Recipe for King's B agar medium

Proteose peptone No. 3	20.0 g
Agar	15.0 g
Glycerol	10 mL
K2HPO4	1.5 g
MgSO4.7H2O	1.5 g
De-ionised water	1 L

Appendix iii - Field Trial Details

Trials conducted in 2002

Spray application details for Trials 2.1 and 2.2

Application Equipment				
Equipment	Knapsack precision sprayer fitted with 3 m boom			
Jets	Fan jets - Spray Systems 110-SF-05			
Volume (L/ha)	452			
Pressure (kPa)	400			
Treatment Applications				
	Trial 2.1		**Trial 2.2**	
Location	Riana, Tasmania		Forrest, TAsmania	
Dates	29/04/02	06/05/02	04/05/02	11/05/02
Treatments Applied	2-12	2-12	2-12	2-12
Temperature (^{o}C)	18	13	7	16
Relative Humidity (%)	41	63	87	87
Cloud Cover (%)	99	30	0	0
Wind Direction & Speed (m/sec)	S/E 0-1	Nil	Nil	Nil
Soil Moisture	Wet	Wet	Surface moist	Wet
Crop Stage	Early head, button stage Head diameter range of 20-40 mm	Late head Head diameter range of 90-115 mm	Early head Head diameter range of 5-40 mm	Early head to late head Head diameter range of 55-120 mm

Chronology of Events

Date	DAP*	Trial 2.1 (Riana)
18/02/02	0	Transplants (cv. Marathon) planted in the field. Crop planted for processing broccoli.
24/04/02	65	Trial pegged.
29/04/02	70	1st foliar fungicide application of Treatments 2-12.
06/05/02	77	2nd foliar fungicide application of Treatments 2-12. Some heads were fully exposed, while others were still covered by either their own leaves or leaves from adjacent plants.
10/05/02	81	1st disease assessment.
13/05/02	84	2nd disease assessment.
16/05/02	87	Disease assessment of incubated heads.
Date	**DAP***	**Trial 2.2 (Forrest)**
22/02/02	0	Transplants (cv. Marathon) planted in the field. Crop planted for processing broccoli.
02/05/02	69	Trial pegged.
04/05/02	71	1st foliar fungicide application of Treatments 2-12. Leaves and heads were soaked following Activator application with Treatments 7 and 8.
11/05/02	78	2nd foliar fungicide application of Treatments 2-12.
17/05/02	84	1st disease assessment.
24/05/02	91	2nd disease assessment.
05/06/02	103	3rd disease assessment.

* DAP = *Days after planting*

Appendix iii - Field Trials (Cont.)

Trials conducted in 2003

Spray application details for Trials 3.1 and 3.2

Application Equipment				
Equipment	Knapsack precision sprayer fitted with 1.5m boom			
Jets	Cone jets - Spray Systems TX18			
Volume (L/ha)	410			
Pressure (kPa)	400			
Treatment Applications				
	Trial 3.1		Trial 3.2	
Location	Forrest, Tasmania		Boat Harbour, Tasmania	
Dates	07/05/03	16/05/03	10/05/03	20/05/03
Treatments Applied	2-10	2-10	2-10	2-10
Temperature (^{o}C)	5.1	13	12	15.9
Relative Humidity (%)	90	73	45	76
Cloud Cover (%)	90	100	1	5
Wind Direction & Speed (m/sec)	Nil	Nil	Nil	N/W 0-1
Soil Moisture	Wet	Wet	Wet	Wet
Crop Stage	Early head Head diameter range of 20-65 mm	Late head Head diameter range of 50-140 mm	Early head Head diameter range of 30-60 mm	Early head to late head Head diameter range of 60-100 mm

Spray application details for Trials 3.3 & 3.4

Application Equipment				
Equipment	Knapsack precision sprayer fitted with 1.5m boom			
	Trial 3.3		**Trial 3.4**	
Jets	Cone jets - Spray Systems TX10	Cone jets - Spray Systems TX18	Cone jets - Spray Systems TX10	Cone jets - Spray Systems TX18
Volume (L/ha)	250	410	210	391
Pressure (kPa)	400	400	400	400
	Treatment Applications			
	Trial 3.3		**Trial 3.4**	
Locations	North Motton, Tasmania		Forth, Tasmania	
Dates	01/05/03	08/05/03	15/08/03	26/08/03
Treatments Applied	2-14	2-14	2-16	2-16
Temperature (^{o}C)	13	15	7	10
Relative Humidity (%)	52	43	60	60
Cloud Cover (%)	90	80	99	0
Wind Direction & Speed (m/sec)	N/a	Nil	Nil	Nil
Soil Moisture	Wet	Wet	Wet	Wet
Crop Stage	Early head Head diameter range of 20-60 mm	Late head Head diameter range of 70-115 mm	Early head Head diameter range of 4-6 mm	Early head Head diameter range of 6-9 mm

Appendix iii - Field Trials (Cont.)

Chronology of Events

Date	DAP*	Trial 3.1 (Forrest)
20/02/03	0	Transplants (cv. Marathon) planted in the field. Crop planted for processing broccoli.
06/05/03	75	Trial pegged.
07/05/03	76	*P. marginalis* applied onto broccoli in Replicates 2 and 3 in the middle of the trial area, approximately 30 minutes before treatment applications.
07/05/03	76	1st foliar fungicide application of Treatments 2-10.
16/05/03	85	2nd foliar fungicide application of Treatments 2-10. Whole crop showed nutrient deficient symptoms, with purple discolouration on heads.
16/05/03	85	Disease assessment.
17/05/03	86	Commercial harvest.
Date	**DAP***	**Trial 3.2 (Boat Harbour)**
20/02/03	0	Transplants (cv. Marathon) planted in the field. Crop planted for processing broccoli.
06/05/03	75	Trial pegged.
10/05/03	79	*P. marginalis* applied onto broccoli in Replicates 2 and 3 in the middle of the trial area, approximately 30 minutes before treatment applications.
10/05/03	79	1st foliar fungicide application of Treatments 2-10.
20/05/03	89	2nd foliar fungicide application of Treatments 2-10.
23/05/03	92	1st disease assessment.
27/05/03	96	2nd and final disease assessment.
28/05/03	97	Commercial harvest.

* DAP = Days after planting

Chronology of Events

Date	DAP*	Trial 3.3 (North Motton)
13/02/03	0	Transplants (cv. Marathon) planted in the field. Crop planted for processing broccoli.
29/04/03	75	Trial pegged.
01/05/03	77	Pseudomonas marginalis, was applied onto broccoli in Replicates 1 and 2 only, before spray applications (30 minutes before copper applications) at 410 L/ha.
01/05/03	77	1st foliar fungicide application of Treatments 2-14.
05/05/03	81	No head rot evident in the trial area.
08/05/03	84	2nd foliar fungicide application of Treatments 2-14.
14/05/03	90	1st disease assessment.
15/05/03	91	Commercial harvest.
22/05/03	98	Checked trial area for heads rejected in commercial harvest.
Date	**DAP***	**Trial 3.4 (Forth)**
10/05/03	0	Transplants (cv. Marathon) planted in the field. Crop planted for fresh market broccoli.
11/08/03	93	Trial pegged.
15/08/03	97	1st foliar fungicide application of Treatments 2-16.
26/08/03	108	2nd foliar fungicide application of Treatments 2-16.
26/08/03	108	1st disease assessment.
02/09/03	115	2nd disease assessment, with no change since 1st disease assessment. White blister observed on leaves and heads in the crop, but not in the trial area.
08/09/03	121	Commercial harvest commenced.

DAP = Days after planting

Appendix iv - Photographs

Broccoli heads with bacterial rots (Photos 1-2) and fungal rots due to Botrytis *(Photo 3) and* Sclerotinia *(Photo 4).*

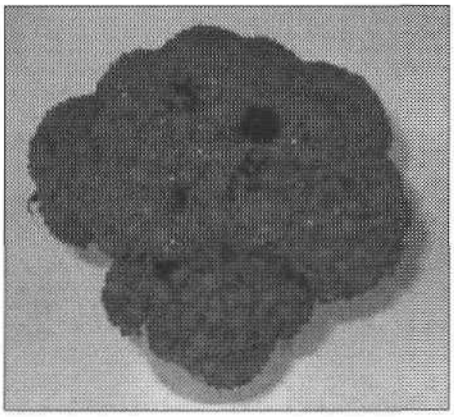


Photo 1

Photo 2

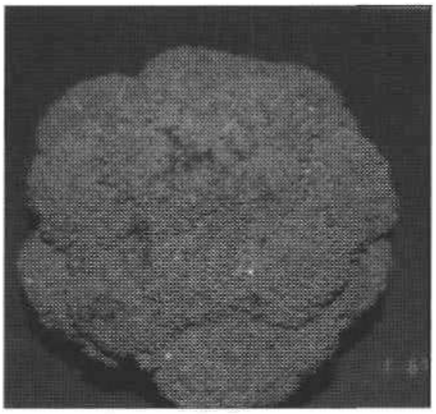


Photo 3

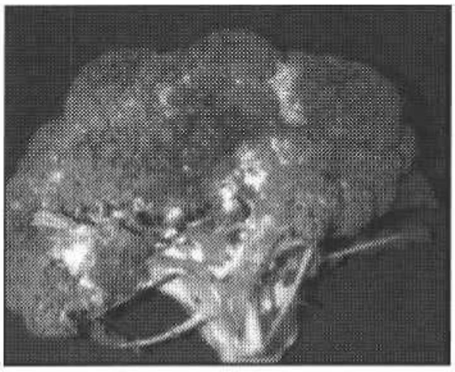


Photo 4

Fluorescent Pseudomonas marginalis *(Photo 5) on King's B agar medium and soft rot on potato slice by pectolytic positive* Pseudomonas *bacteria (Photo 6).*

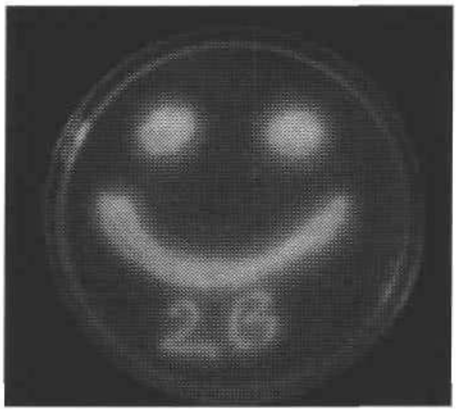


Photo 5

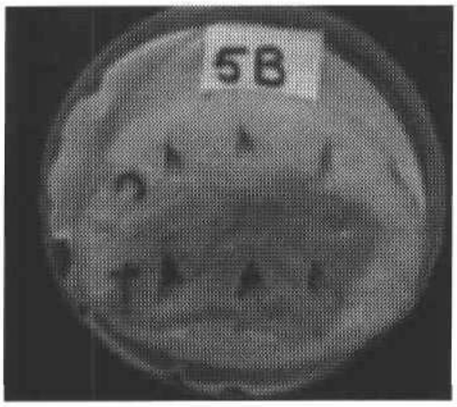


Photo 6

Appendix iv - Photographs (Cont.)

Black spots induced by frost damage on Kocide treated head at the Riana field trial in 2002 (Photo 7). Close-up of the black spot on florets (Photo 8).

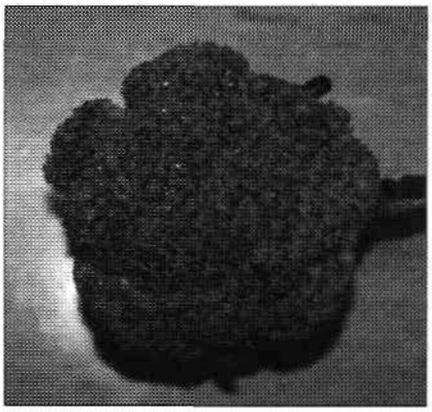


Photo 7

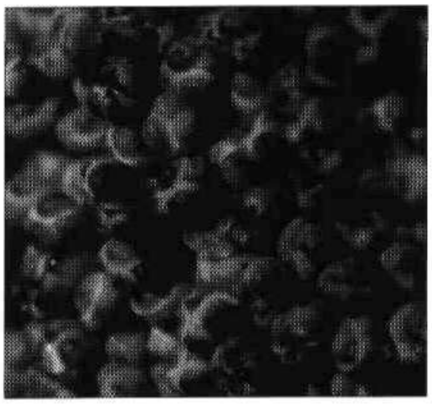
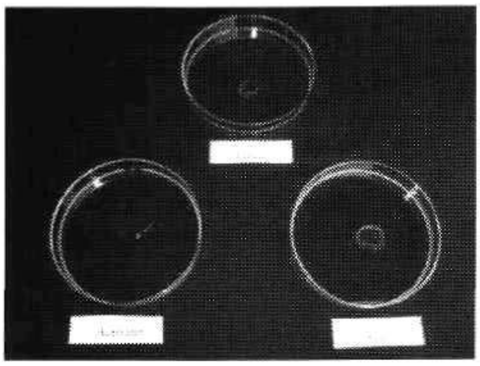


Photo 8

Photo 9: Test for surfactant activity of the spray adjuvants 120 ml/100 L Activator (bottom left) and 12 ml/100 L Agral (bottom right) by measuring the spread of the water droplet in comparison to water only (top plate).



Appendix iv - Photographs (Cont.)

Close-up photos of water beads on copper hydroxide treated florets (Photo 10) and water soaked florets following the application of Activator (Photo 11).

Photo 10

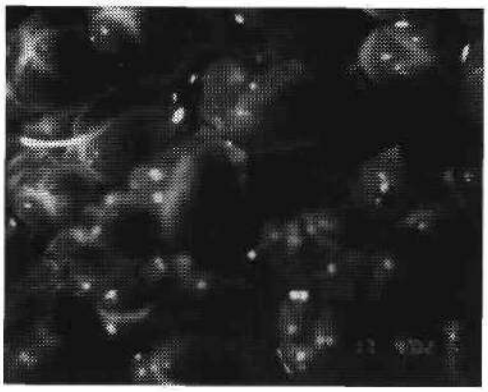


Photo 11

The wetting of florets by the different spray adjuvants (Photo 12), and bacterial rot after inoculation with P. tolassi *and incubation under moist condition (Photo 13).*

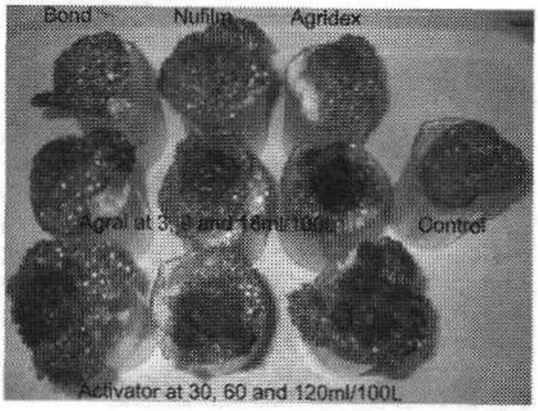


Photo 12

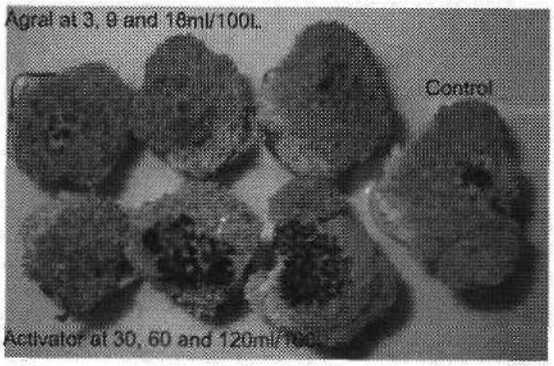


Photo 13

Photo 14: No bacterial inhibition by the copper products Tri-Base Blue, Kocide Liquid Blue, and water only on very high population level of fluorescent P. marginalis *agar plates (top plates left to right), and complete bacteria inhibition by the disinfectants White King (1% chlorine), 1% Oxine, and 1.2% Path-X (bottom plates: left to right)*

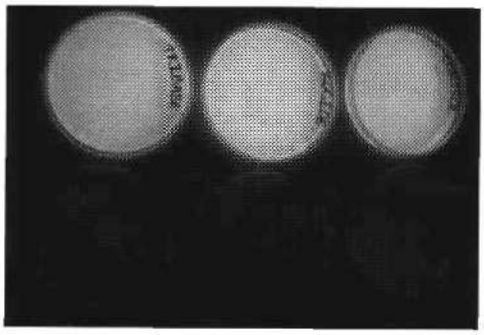


Photo 15: The copper products used in the laboratory study in 2003, left to right - Tri-Base blue, Cuprofix MZ, Champ DP, Kocide Liquid Blue, and Copper oxychloride (Photo 15)

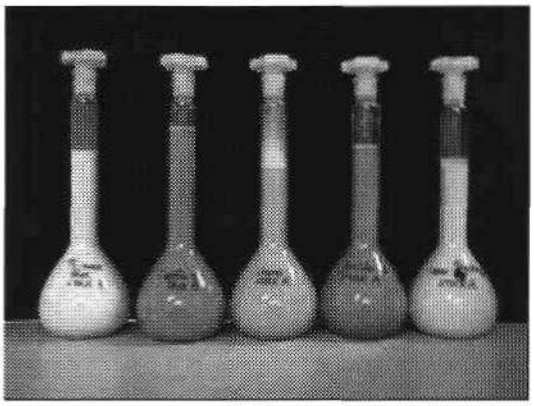


Photo 16: The efficacy of Champ DP and Tri-Base Blue on different bacteria isolates (Photo 16)

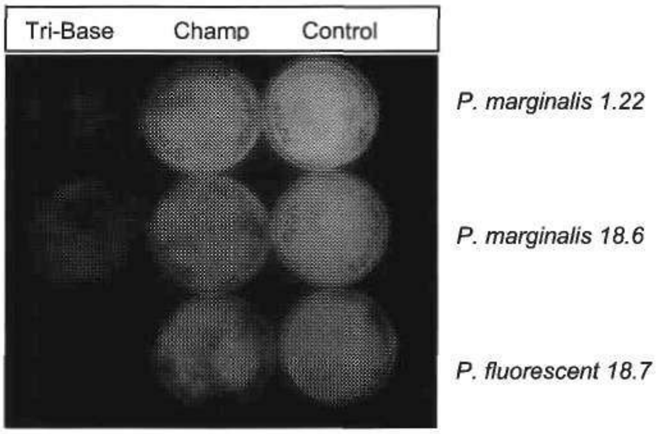


Photo 17: The effects of filter paper disks soaked with various disinfectants on P. marginalis *1.22 at* 10^6 *cfu/ml; top 3 plates - White King (1% chlorine), 1% Sporekill & 1% Oxine; and bottom 3 plates - 1.2% Path-X, 0.1% Sporekill & 0.1% Oxine*