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Detection and management of copper-tolerance in bacterial diseases of vegetables

Heidi Martin
QLD Department of Primary Industries

Project Number: VX99021

VX99021

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Level 1
50 Carrington Street
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Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

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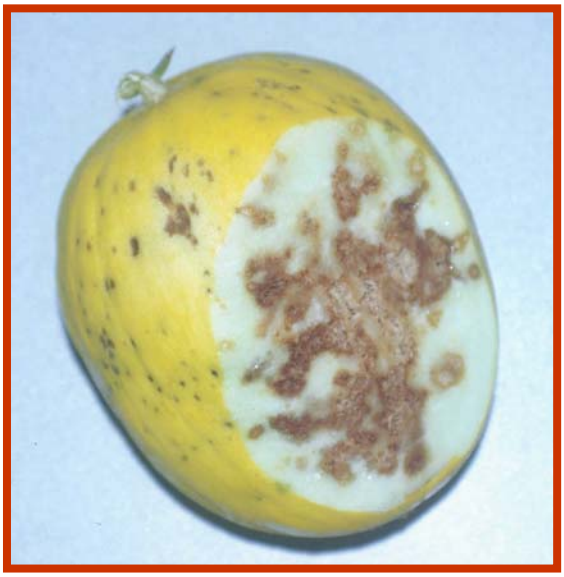
(Completion 31 March 2003)

Final Report



Heidi L. Martin *et al.*

Queensland Horticulture Institute

(HAL Project VX99021)

Project Leader: Heidi L. Martin
Plant Pathologist
Queensland Horticulture Institute
DPI Gatton Research Station
LMB 7, MS 437
Warrego Hwy
Gatton, QLD 4343

ph: 61 7 54662222
fax: 61 7 54623223
email: Heidi.Martin@dpi.qld.gov.au

Key Research Personnel:

Dr Peter M. Stephens (Senior Plant Pathologist, Applethorpe Research Station)
Mr Glenn A. Geitz (Spray Application Technologist, Gatton Research Station)
Mrs Vicki A. Hamilton (Experimentalist, Gatton Research Station)

This report summarises the results of a three-year study investigating the occurrence of copper-tolerant bacterial strains in Queensland populations of three important bacterial pathogens of vegetable crops. It provides information to industry about prevention and management of these diseases in Queensland**.**

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HAL Project VX99021

Detection and Management of Copper-Tolerance in Bacterial Diseases of Vegetables

(Completion 31 March 2003)

Final Report

Heidi L. Martin
Peter M. Stephens
Glenn A. Geitz
Vicki A. Hamilton
Rosemary A. Kopittke

Queensland Horticulture Institute

A final report prepared for Horticulture Australia:

Level 1
50 Carrington Street
SYDNEY NSW 2000

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1. Media Summary

Copper pesticides are the only products available to growers to combat outbreaks of bacterial diseases in vegetable crops in Australia. Copper products need to be applied to the plant prior to infection for them to be effective. Their efficacy is also compromised if coverage of the target plant is poor. In recent years there have been increasing reports by Queensland growers of poor control of bacterial spot of capsicum, bacterial fruit blotch of melons and black rot of brassicas despite frequent application of copper pesticides. At the same time, strains of bacterial plant pathogens tolerant of copper applied at recommended rates have caused disease control failures in a range of crops worldwide. This report details a three-year investigation into poor field control of bacterial spot, fruit blotch and black rot epidemics in Queensland. We sought to understand whether copper-tolerant bacterial strains of these pathogens were contributing to the poor field control. Also, we investigated ways to improve the efficacy of copper pesticides and improve disease control by optimising spray application techniques.

This work showed that copper-tolerance is present in Queensland populations of bacterial spot of capsicum and we demonstrated that copper-tolerant strains of bacterial spot increase in a population after 12 copper sprays and dominate a field population after 21 sprays. Copper-tolerant strains are contributing to poor control of this disease with copper sprays in the field, particularly in north Queensland production districts. Strains of the fruit blotch bacterium with tolerance to copper are also present in low frequency in north Queensland production districts. There was no evidence that copper tolerance exists in populations of black rot from the Granite Belt.

Copper hydroxide/mancozeb tank mix combinations should now replace existing industry standard treatments, because they gave better control of all three pathogens compared to copper hydroxide (Kocide DF) applied alone (the current industry standard). Air assisted boom sprayers fitted with twin jet nozzles were the best means of achieving overall spray coverage of capsicum, rockmelon and watermelon plants. Young capsicum plants should be sprayed with 300L/ha and this should be increased to 800L/ha when the plant canopy has fully developed. Volumes of 300L/ha for young watermelons, 500L/ha for young rockmelons and 1000L/ha for mature vines of both melon types are required for optimal spray coverage of melon crops.

2. Technical Summary

Regular applications of copper-based bactericides are currently used in pre-harvest preventative control programs against bacterial spot of capsicum (*Xathomonas campestris* pv. *vesicatoria*), bacterial fruit blotch of melons (*Acidovorax avenae* subsp. *citrulli*) and black rot of brassicas (*Xathomonas campestris* pv. *campestris*). In recent years, reports of poor control of these pathogens have been increasing in Queensland. Several factors may contribute to poor disease control efficacy including:

- incorrect timing of copper sprays
- poor spray coverage
- favourable environmental conditions and/or high disease pressure
- copper-tolerant bacterial strains

Reports of copper-tolerant strains in populations of a range of bacterial plant pathogens have been increasing worldwide. Outside Australia, copper tolerance in populations of bacterial spot of capsicum has caused severe crop losses in crops sprayed with copper at recommended rates. This project is the first investigation of copper-tolerance in Australian populations of bacterial spot of capsicum, bacterial fruit blotch of melons and black rot of brassicas. In addition, we sought to improve disease control by determining ways to increase the efficacy of copper bactericide formulations, as well as establishing optimal methods for delivery of copper sprays to the target host plants.

We screened over 220 bacterial isolates, against a range of concentrations of $CuSO_4$ in a laboratory assay. The maximum copper concentration that would support bacterial growth, was determined for each isolate. Isolates of bacterial spot of capsicum tolerant of copper concentrations $\geq$ 1.0mM were relatively common and constituted tolerant strains. Copper-tolerant isolates of bacterial fruit blotch were also identified in low frequency, however all the black rot isolates were sensitive to copper. In field studies, we demonstrated that copper-tolerant strains of bacterial spot of capsicum will dominate a pathogen population when copper sprays are routinely applied. We increased the proportion of copper-tolerant strains of *Xanthomonas campestris* pv. *vesicatoria* by applying weekly copper sprays to a field population. Identification of copper tolerance in Queensland populations of bacterial spot of capsicum, now means that growers will need to take all measures to maximise disease control, as well as implementing strict "non crop" periods, particularly in districts where secondary spread of the bacterial population into neighbouring crops is likely due to the close proximity of capsicum farms. These measures should help minimise dominance of the pathogen population by copper-tolerant strains.

Copper hydroxide/mancozeb mixtures provided better control of all three pathogens than copper hydroxide applied alone and this treatment should now constitute the industry standard treatment for control of these diseases. Field evaluations of spray rigs, application volumes, and nozzle types revealed that an air-assisted boom fitted with twin-jet nozzles provided superior spray coverage of capsicums, watermelons and rockmelons than other rig/nozzle combinations. Spray volumes of 300L/ha gave optimal coverage of young capsicum plants, the volume increasing to 800L/ha for coverage of mature capsicums. For melons, 300L/ha and 500L/ha should be used when spraying watermelon and rockmelon plants at an early flowering stage, increasing to 1000L/ha when vines are mature.

3. General Introduction

Bacterial spot of capsicum, fruit blotch of melons and black rot of brassicas are major bacterial disease problems currently facing the Australian capsicum, melon and brassica industries. These industries have an estimated combined value of more than A$250 million per year. Outbreaks of all three diseases have the potential to cause serious crop losses. Epidemics of the diseases are favoured by periods of warm weather and high humidity (rain, dew).

Bacterial spot of capsicum affects the leaves, stems and fruit of plants. When infection is severe, plants are defoliated causing fruit to become sunburnt. Fruit blotch of melons causes a severe internal rot of melon fruit that cannot easily be detected through external examination. Fruit is susceptible to blotch infection when young, and once infected, it develops brown internal cavities in the flesh, and secondary breakdown by soft rotting bacteria is common. Brassicas affected by the black rot bacterium develop brown patches on leaf margins and interveinal areas, and blackening of leaf veins. All three diseases result in unmarketable produce.

Chemical control options for these and other bacterial diseases are very limited, with copper sprays being the only products with bactericidal activity registered for use against these pathogens in Australia.

In Queensland, in recent years, reports from capsicum growers of poor field control of bacterial spot epidemics have been increasing, despite the routine use of copper bactericides. Similarly, on the Granite Belt, brassica growers have expressed concern about their apparent inability to control epidemics of black rot despite the use of copper.

Bacterial fruit blotch of melons, reported in Australia in 1967, was initially only known to affect watermelon crops in the south western Queensland melon production district. In the 1990's however the disease became prevalent in nurseries and then field crops in north Queensland, and a second strain of the pathogen appeared. The second strain was shown to be pathogenic to rockmelon, honeydew and watermelon, whereas the original strain was a pathogen of watermelon and honeydew only. This second strain, "the rockmelon strain" now dominates the bacterial population in north Queensland, whereas in the south "the watermelon strain" is still the most prevalent (probably due to its survival in populations of wild cucurbits in the southern Queensland production district). In recent years, melon producers in south Queensland have reported that copper sprays provide effective control of bacterial fruit blotch outbreaks. In north Queensland, however, growers report poor control of the disease in the field, even after frequent applications of copper.

Several factors may contribute to poor disease control, including:

- poor timing of copper sprays
- poor spray coverage
- favourable environmental conditions and/or high disease pressure
- copper-tolerant bacterial strains

Copper compounds are contact products with a protective mode of action. Consequently, the likelihood that pathogen populations will develop resistance to copper is considered to be relatively low. Despite this, the development of copper-

tolerant bacterial strains is recognised as an increasing problem world-wide for a range of bacterial pathogens including *Xanthomonas campestris* pv. *vesicatoria* (the causal agent of bacterial spot of capsicum), and it has been associated with poor levels of disease control in crops treated with copper at recommended label rates. There have been no records of copper-tolerance having developed in populations of bacterial fruit blotch or black rot worldwide. Copper bactericides do form the basis of preharvest preventative control programmes for both of these pathogens however, and in view of the development of copper-tolerant strains of bacterial pathogens worldwide, populations of fruit blotch and black rot are potentially at risk of developing copper-tolerant strains.

This project was initiated to investigate the failure by grower's to adequately control bacterial disease epidemics in the field. First, we investigated whether copper-tolerance is present in Queensland populations of these three pathogens, and whether it is contributing to control failures. Second, we examined methods of improving the efficacy of copper sprays and we sought alternative bactericides that may be used instead of or along with copper. Third, we investigated methods to optimise delivery of bactericide sprays to plant surfaces, so that maximum spray coverage is achieved.

4. Copper-tolerance: studies in detection and development in Queensland bacterial populations

4.1 Introduction

In Australia, and many other parts of the world, copper products are the only compounds that may be used to fight bacterial diseases in vegetable crops. Consequently, the prospect that copper may be ineffective due to the development of strains of bacteria resistant to copper, represents a major blow to the farmer's ability to control bacterial diseases. Reports of copper-tolerant bacterial strains in populations of plant pathogenic bacteria are increasing worldwide (Adaskaveg and Hine, 1985; Andersen *et al.*, 1991; Lee *et al.*, 1991; Ritchie and Dittapongpitch, 1991; Lee *et al.*, 1992; Spotts and Cervantes, 1995; Scheck *et al*, 1996; O'Garro, 1998; Scheck and Pscheidt, 1998).

Populations of *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot of capsicum, with resistance to copper have been reported from the USA (Marco and Stall, 1983; Cooksey *et al.*, 1990; Bender *et al*, 1990), Mexico (Adavaskeg and Hine, 1985) and the Caribbean Islands (O'Garro, 1998). In Australia, the copper-sensitivity status of populations of capsicum bacterial spot is unknown, however a low percentage of streptomycin resistance among Australian strains has been reported (Minsavage *et al.*, 1990). This may indicate the presence of copper-tolerance since in a US survey Ritchie and Dittapongpitch found that all streptomycin-resistant strains were also copper-resistant (1991).

Recently, copper-resistance was detected in Australian isolates of *Pseudomonas syringae* pv. *tomato*, the causal agent of bacterial speck of tomato (Tesoriero *et al.*, 1997). In this instance Tesoriero *et al.* suggested that introduction of resistant bacterial strains through contaminated seed was likely due to the rapid development of high level resistant types in southern Australian populations (1997). *Xanthomonas campestris* pv. *vesicatoria* is also known to be transmitted by seed. Therefore, aside from the possibility for gradual development of copper-tolerant strains of this pathogen through the regular use of copper-based bactericides in Australia, it seems likely, given the international traffic of seeds, that copper-tolerant strains may already have been introduced into Australia on contaminated seed.

No reports of copper-tolerance in populations of bacterial fruit blotch (*Acidovorax avenae* sub sp. *citrulli*) or black rot of brassicas (*Xanthomonas campestris* pv. *campestris*) have been reported in the literature. For both pathogens, copper bactericides form the basis of preharvest preventative control programmes. As such, and in view of the development of copper-tolerant strains of bacterial pathogens worldwide, populations of both pathogens are potentially at risk of developing copper-tolerant strains.

Recent control failures of all three pathogens in Queensland vegetable production districts has prompted this investigation into whether the presence of copper-tolerent strains in Queensland populations are contributing to control failures in the field.

4.2 Detection of copper-tolerance in Queensland bacterial pathogen populations

4.2.1 Bacterial spot of capsicum

4.2.1.1 Materials and Methods

Isolate Collection and Storage Isolates of *Xanthomonas campestris* pv. *vesicatoria* were collected from capsicum and chilli crops from Queensland production districts in 1999-2000. Isolations were made from infected leaves, stems and fruit by macerating small pieces of symptomatic tissue in drops of sterile de-ionised water and then streaking the macerate onto plates of nutrient agar (Difco) using a sterile inoculation loop. Following incubation of the plates for 48 hours at 28°C, pure cultures were obtained by re-streaking bacteria from single colonies onto fresh plates of nutrient agar. This procedure was repeated twice for each isolate to ensure the purity of the cultures. Each culture was tested for gram reaction and confirmed to be gram-negative. All isolates were identified as *Xanthomonas campestris* pv. *vesicatoria* by an agglutination test using a bacterial detection kit (Adgen Ltd.). To confirm pathogenicity, inoculum suspensions (10^8 cfu/mL) were prepared for a selection of the isolates. Inoculum was misted onto young capsicum plants cv. Heldor until runoff and the plants were incubated under high humidity for 48 hours. Re-isolation of the bacterium from symptomatic plant tissue was completed in order to fulfil Koch's postulates.

For storage, all isolates were inoculated into Cryobank™ (Mast Diagnostics) vials which were stored in a chest freezer at -20°C for the duration of the project. Lypholised cultures of each isolate were also prepared.

Copper-tolerance Screening Isolates were retrieved from storage by plating beads from Cryobank™ vials onto nutrient agar. Plates were incubated for 72 hours, after which a single bacterial colony was selected from each culture and inoculated into a flask containing 25mL of sterile peptone-yeast extract-glycerol broth. Each flask was shaken on a platform orbital shaker at 130 rpm. for 24 hours. Aliquots (1.0mL) were removed from each flask and inoculated into 8 flasks of PYE broth amended with different concentrations of $CuSO_4$ (0, 0.2, 0.3, 0.5, 0.7, 1.0, 1.1, 1.2mM $CuSO_4$). All flasks were shaken on a platform orbital shaker (130rpm) for 48 hours at 25°C. A dilution series (10^{-8}-10^{-1} fold dilutions) was prepared for each flask in sterile phosphate buffer and 1.0mL of each dilution was spread across a plate of nutrient agar with a sterile glass hockey stick. After inoculation, all plates were allowed to dry and then incubated at 28°C for 72 hours. Resulting bacterial colonies were counted and the highest concentration of $CuSO_4$ at which bacterial colony formation occurred was determined for each isolate.

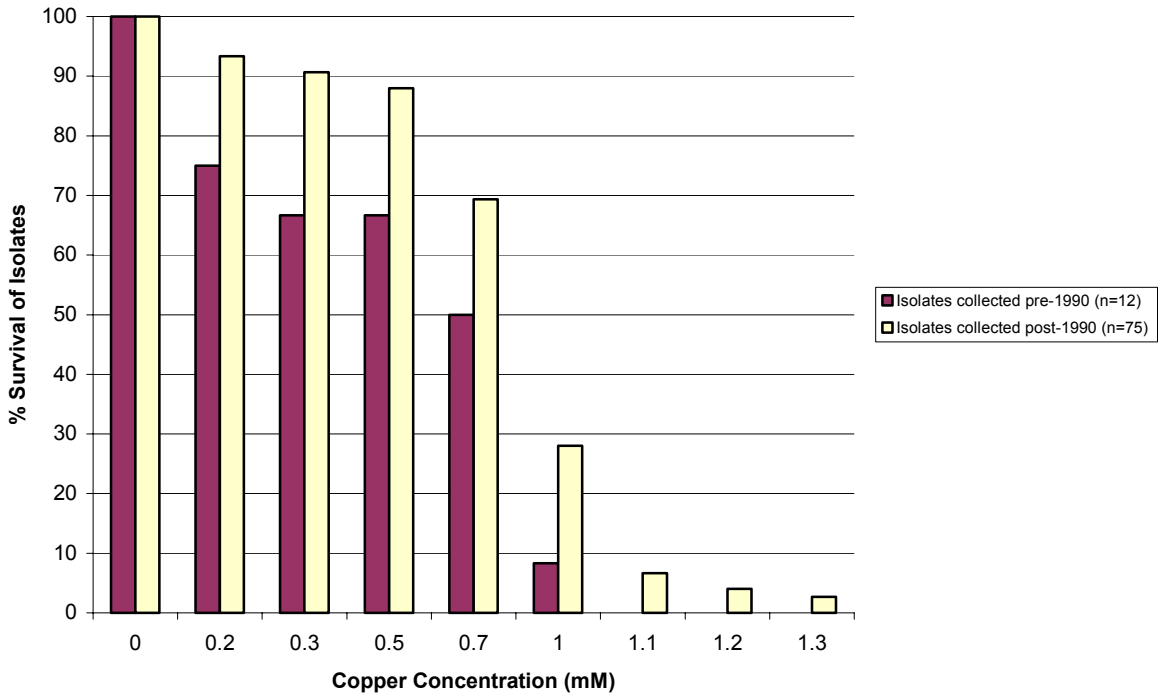
4.2.1.2 Results

Seventy-five *Xanthomonas campestris* pv. *vesicatoria* isolates were collected in this study. The majority of these (61) were from North Queensland production areas (Burdekin 21; Gumlu 22; Bowen 18), 4 were from Bundaberg, and 10 were collected from South Queensland (Lockyer Valley and Stanthorpe).

Twelve isolates were also retrieved from live cultures held in Australian herbarium collections (DAR26930, DAR26931, DAR26932, DAR26933, DAR33341, DAR34895a, BRIP39063, BRIP38988, BRIP38998, BRIP38999, BRIP39009, GRS1910). These isolates were all collected prior to 1990 (1972-1986) and were used in this study to generate base-line information against which change in the copper-tolerance status of Australian populations of *Xanthomonas campestris* pv. *vesicatoria* could be monitored.

The percentage of *X. campestris* pv. *campestris* isolates collected pre-1990 that survived at the 9 concentrations of $CuSO_4$ are compared with the percentage survival of post-1990 isolates (Figure 1).

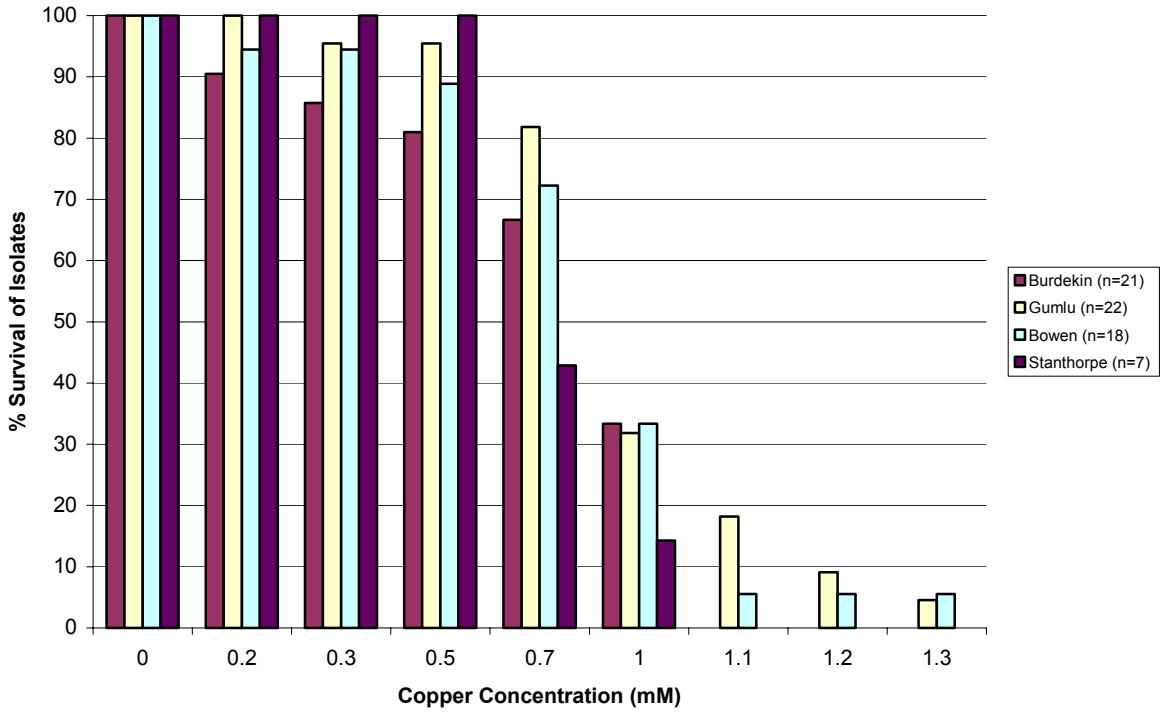
Figure 1: Survival of *Xanthomonas campestris* pv. *vesicatoria* isolates in Cu broths
Comparison of isolates collected pre and post-1990



Although the sample sizes for both groups differ, it is noteworthy that none of the pre-1990 isolates survived at copper concentrations in excess of 1.0mM, whereas nearly 7% of the post-1990 isolates did. For the pre-1990 isolates, 8.33% (1/12 isolates) survived at 1.0mM, compared to 28.0% (21/75) of the post-1990 sample. Similarly, the lower concentrations of $CuSO_4$ were lethal to a greater proportion of the pre-1990 isolates, with 0.2mM $CuSO_4$ preventing the growth of 25% of the pre-1990 isolates, whereas in excess of 90% of the post-1990 isolates were tolerant of the 0.2mM concentration.

A comparison of the copper-tolerance of the isolates between the districts studied (Figure 2), reveals that populations from the Gumlu and Bowen districts contained isolates that were tolerant of copper concentrations 6.5 times greater than the lowest lethal copper concentration studied (0.2mM). Isolates tolerant of copper concentrations in excess of 1.0mM were not recovered from any other district.

Figure 2: Survival of *Xanthomonas campestris* pv. *vesicatoria* isolates in Cu broths
Comparison of isolates collected from different production districts



4.2.2 Bacterial Fruit Blotch of Melons

4.2.2.1 Materials and Methods

Isolate Collection and Storage Isolates of *Acidovorax avenae* subsp. *citrulli* were collected from melon crops from Queensland production districts in 1999-2000. Isolations were made from infected leaves, stems and fruit by macerating small pieces of symptomatic tissue in drops of sterile de-ionised water and then streaking the macerate onto plates of King's medium B using a sterile inoculation loop. Following incubation of the plates for 48 hours at 28°C, pure cultures were obtained by re-streaking bacteria from single colonies onto fresh plates of King's medium B. This procedure was repeated twice for each isolate to ensure the purity of the cultures. Each culture was tested for gram reaction and confirmed to be gram-negative. All isolates were identified as *Acidovorax avenae* sub sp. *citrulli* by the BIOLOG system for identification of gram-negative bacteria. In addition, the strain of each isolate was determined through differential BIOLOG reactions. Ability to utilise the L-leucine carbon source (well G3, GN Microplate) was indicative of a "watermelon strain" isolate, whereas inability to utilise L-leucine but ability to utilise 2-amino ethanol (well H7, GN Microplate) was indicative of a "rockmelon strain" isolate (O'Brien and Martin, 1999). To confirm pathogenicity, inoculum suspensions (10^8 cfu/mL) were prepared for a selection of the isolates. Inoculum was misted onto young melon plants until runoff and the plants were incubated under high humidity for 48 hours. Re-isolation of the bacterium from symptomatic plant tissue was completed in order to fulfil Koch's postulates.

For storage, all isolates were inoculated into Cryobank™ (Mast Diagnostics) vials which were stored in a chest freezer at -20°C for the duration of the project. Lypholised cultures of each isolate were also prepared.

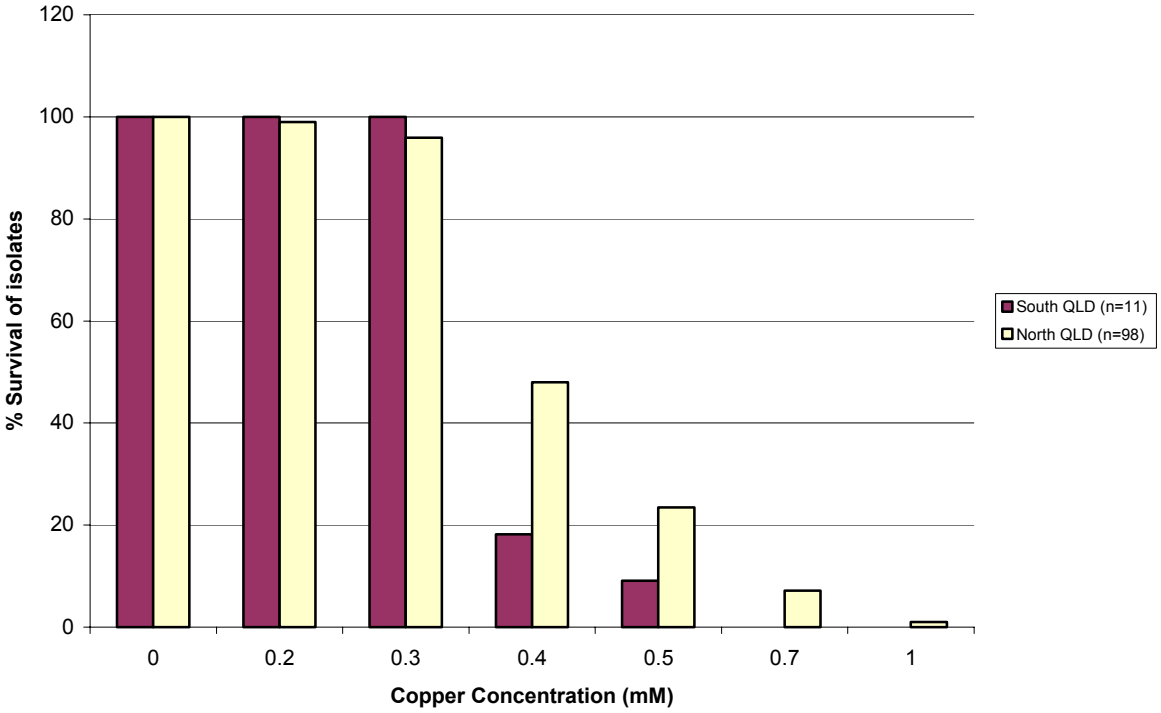
Copper-tolerance Screening Isolates were retrieved from storage by plating beads from Cryobank™ vials onto nutrient agar. Plates were incubated for 72 hours, after which a single bacterial colony was selected from each culture and inoculated into a flask containing 25mL of sterile peptone-yeast extract-glycerol broth. Each flask was shaken on a platform orbital shaker at 130 rpm. for 24 hours. Aliquots (1.0mL) were removed from each flask and inoculated into 6 flasks of broth amended with different concentrations of $CuSO_4$ (0, 0.2, 0.3, 0.5, 0.7, 1.0mM $CuSO_4$). All flasks were shaken on a platform orbital shaker (130 rpm) for 48 hours at 25°C. A dilution series (10^{-8}-10^{-1} fold dilutions) was prepared for each flask in sterile phosphate buffer and 1.0mL of each dilution was spread evenly across a plate of nutrient agar with a sterile glass hockey stick. After inoculation, all plates were allowed to dry and then incubated at 28°C for 72 hours. Resulting bacterial colonies were counted and the highest concentration of $CuSO_4$ at which bacterial colony formation occurred was determined for each isolate.

4.2.2.2 Results

One-hundred and nine *Acidovorax avenae* subsp. *citrulli* isolates were collected in this study. The majority of these (99) were from North Queensland production areas (Burdekin, Gumlu and Bowen) and 10 were collected from South Queensland (Chinchilla, Jandowae and Redlands).

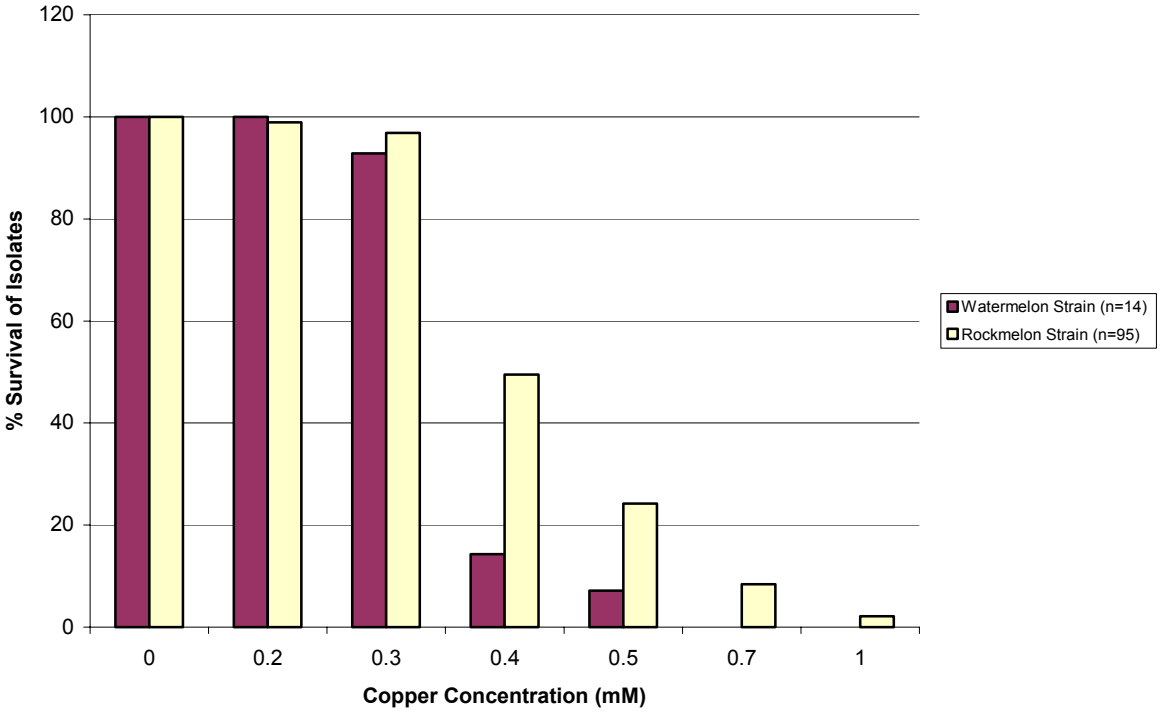
Since this disease has only recently become a major concern to the melon industry (since the mid 1990's), there were no isolates available from Australian herbarium collections from which to generate baseline information about the copper-tolerance in populations of the pathogen prior to the epidemics seen in the 1990's. The percentage of *A. avenae* subsp. *citrulli* isolates collected from north Queensland that survived at the 6 concentrations of $CuSO_4$ are compared with the percentage survival of south Queensland isolates (Figure 3).

Figure 3: Survival of *Acidovorax avenae* sub sp. *citrulli* isolates in Cu broths
Comparison of north and south QLD isolates



Queensland populations of *A. avenae* subsp. *citrulli* are currently more sensitive to copper than populations of *Xanthomonsa campestris* pv. *vesicatoria*. For both the north and south Queensland fruit blotch isolates, 0.4mM was sufficiently concentrated to prevent the growth of greater than 50% of the isolates tested. Although no south Queensland isolate was tolerant of copper concentrations in excess of 0.5mM, this result is not surprising, considering the low number of south Queensland isolates included in the study. Several isolates from North Queensland were tolerant of copper concentrations of 0.7-1.0mM, indicating that tolerant types are present in low frequency in the North Queensland pathogen population. It is unclear whether copper-tolerant types are present in the watermelon strain of the pathogen (Figure 4), because of the comparatively small number of South Queensland/watermelon strain isolates screened.

Figure 4: Survival of *Acidovorax avenae* sub sp. *citrulli* isolates in Cu broths Comparison between strains of the pathogen



4.2.3 Black Rot of Brassicas

4.2.3.1 Materials and Methods

Isolate Collection and Storage Isolates of *Xanthomonas campestris* pv. *campestris* were collected from brassica crops from the Granite Belt production district in 1999-2000. Isolations were made from infected leaves, by insertion of a sterile needle into infected leaf veins and plating of the bacteria onto nutrient agar from the tip of the needle. Following incubation of the plates for 48 hours at 28°C, pure cultures were obtained by re-streaking bacteria from single colonies onto fresh plates of nutrient agar. This procedure was repeated twice for each isolate to ensure the purity of the cultures. Each culture was tested for gram reaction and confirmed to be gram-negative. All isolates were identified as *Xanthomonas campestris* pv. *campestris* by an agglutination test using a bacterial detection kit (Adgen Ltd.). To confirm pathogenicity, inoculum suspensions (10^8 cfu/mL) were prepared for a selection of the isolates. Inoculum was misted onto young cauliflower plants until runoff and the plants were incubated under high humidity for 48 hours. Re-isolation of the bacterium from symptomatic plant tissue was completed in order to fulfil Koch's postulates.

For storage, all isolates were inoculated into Cryobank™ (Mast Diagnostics) vials which were stored in a chest freezer at -20°C for the duration of the project.

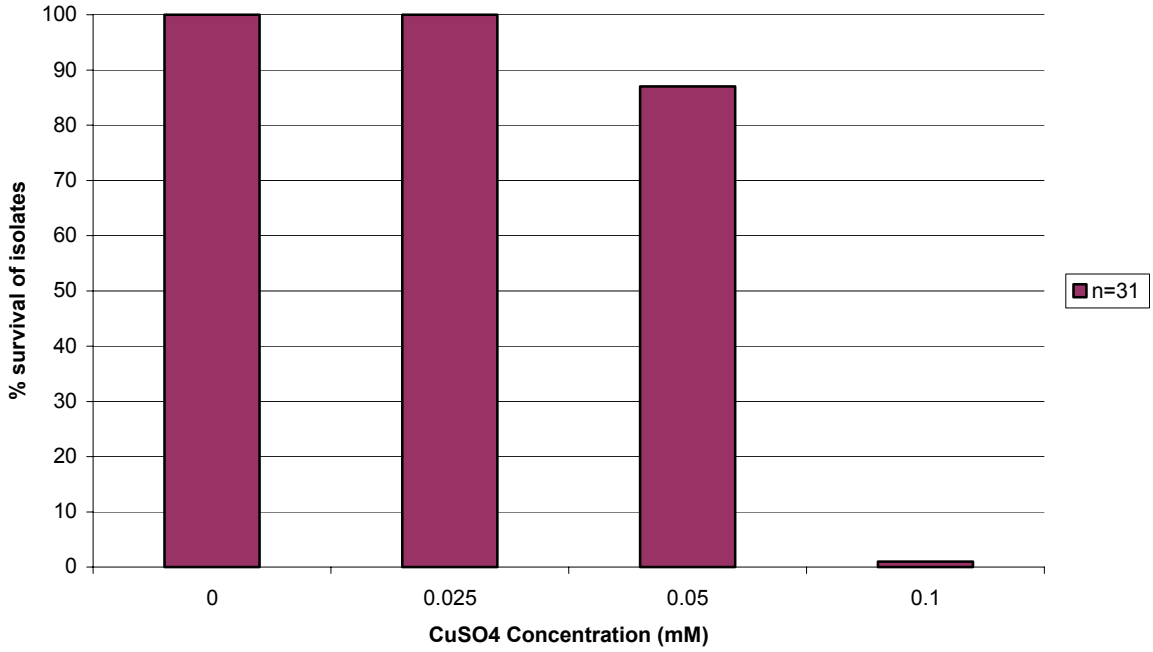
Copper-tolerance Screening Isolates were retrieved from storage by plating beads from Cryobank™ vials onto nutrient agar. Plates were incubated for 72 hours, after which a single bacterial colony was selected from each culture and inoculated into a flask containing 25mL of sterile casitone-yeast extract-glycerol broth. Each flask was shaken on a platform orbital shaker at 130 rpm. for 24 hours. Aliquots (1.0mL) were

removed from each flask and inoculated into 4 flasks of CYE broth amended with different concentrations of $CuSO_4$ (0, 0.01, 0.05 and 0.1mM $CuSO_4$). All flasks were shaken on a platform orbital shaker (130rpm) for 48 hours at 25°C. A dilution series (10^{-8}-10^{-1} fold dilutions) was prepared for each flask in sterile phosphate buffer and 1.0mL of each dilution was spread over a plate of nutrient agar with a sterile glass hockey stick. After inoculation, all plates were allowed to dry and then incubated at 28°C for 72 hours. Resulting bacterial colonies were counted and the highest concentration of $CuSO_4$ at which bacterial colony formation occurred was determined for each isolate.

4.2.3.2 Results

A total of 31 isolates of *Xanthomonas campestris* pv. *campestris* were collected in this study from brassica crops grown in the Granite Belt district of South Queensland. The percentage of isolates that survived at four concentrations of $CuSO_4$ in the laboratory are presented in Figure 5.

Figure 5: Survival of *Xanthomonas campestris* pv. *campestris* isolates in Cu broths



Of the 31 isolates screened, only 1 was tolerant of copper concentrations in excess of 0.05mM. There is therefore no evidence to suggest that copper-tolerance is contributing to control failures of black rot in the field in the Granite Belt district.

4.3 Field assessment of the rate of development of copper-tolerance in a population of *Xanthomonas campestris* pv. *vesicatoria* sprayed with different copper products

4.3.1 Introduction

We have demonstrated in the study described above, that copper-tolerant isolates of *Xanthomonas campestris* pv. *vesicatoria* are present in natural field populations of the pathogen in Queensland capsicum production districts. Presumably, these resistant types will begin to dominate the pathogen population if sufficient selection pressure is exerted to favour a change in population dynamics. In the case of bacterial spot of capsicum, the selection pressure is provided by foliar copper sprays. Currently, the number of copper sprays that need to be applied to a pathogen population to cause it to become dominated by copper-tolerant types is unknown and this will probably vary in response to a number of factors including, the quantity of inoculum, the environmental conditions and the concentration of copper sprays applied. We conducted this experiment in an attempt to determine whether such a population shift is possible if copper-sprays are routinely applied to crops in the field for management of the disease.

4.3.2. Materials and Methods

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 15cm spacings in single rows on Gatton Research Station on 13 December 2000. Seven rows were planted on beds (60m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. The experiment was a randomised complete block design with three blocks, each containing three treatments. Treatment plots were 3m long and were bordered at either end by 2m long guard plots. Whole rows were left as untreated guards between the treatment rows. Prior to transplant, all plants in the guard plots were inoculated with bacterial spot. Bacterial suspensions of six isolates (741(3), Gas(4), 761(2), 762(9), 763(8) and 1086) with tolerances to copper ranging from 0mM to 1.0mM were prepared and standardised to a concentration of 10^8cfu/mL with a turbidimeter. Equal volumes of each suspension were combined and the resulting mix was misted over young capsicum plants one week prior to transplant. The plants were incubated for 48 hours under high humidity after which the inoculated plants were transplanted into the guard plots on the day the field trial was planted. The following treatments were applied every 7 days to each block using a motorised backpack sprayer fitted with a 1m boom and 4 hollow-cone nozzles.

1. Untreated (check)
2. Kocide DF (copper hydroxide) (2.0kg/ha)
3. Kocide DF (copper hydroxide) (2.0kg/ha) + Mancozeb WG (Mancozeb) (2.0kg/ha)

Treatment applications commenced two weeks after transplant and continued for 21 weeks.

In each treatment plot, two plants were tagged. After 4 treatment applications, the two youngest leaves on each plant with symptoms of bacterial spot were removed.

Isolations from each individual lesion on each of the leaves were completed in the laboratory, by macerating a small section of tissue cut from each lesion in a drop of sterile de-ionsied water and streaking the macerate onto plates of nutrient agar. After obtaining pure cultures of each isolate and confirming its identity as *Xanthomonas campestris* pv. *vesicatoria* using a bacterial detection kit (Adgen Ltd), each was screened for tolerance to copper in the laboratory.

A single bacterial colony was selected from each culture and inoculated into a flask containing 25mL of sterile peptone-yeast extract-glycerol broth. Each flask was shaken on a platform orbital shaker at 130 rpm. for 24 hours. Aliquots (1.0mL) were removed from each flask and inoculated into 8 flasks of broth amended with different concentrations of $CuSO_4$ (0, 0.2, 0.3, 0.5, 0.7, 1.0, 1.1, 1.2mM $CuSO_4$). All flasks were shaken on a platform orbital shaker (130rpm) for 48 hours at 25°C. A dilution series (10^{-8}-10^{-1} fold dilutions) was prepared for each flask in sterile phosphate buffer and 1.0mL of each dilution was spread evenly across a plate of nutrient agar with a sterile glass hockey stick. After inoculation, all plates were allowed to dry and then incubated at 28°C for 72 hours. Resulting bacterial colonies were counted and the highest concentration of $CuSO_4$ at which bacterial colony formation occurred was determined for each isolate.

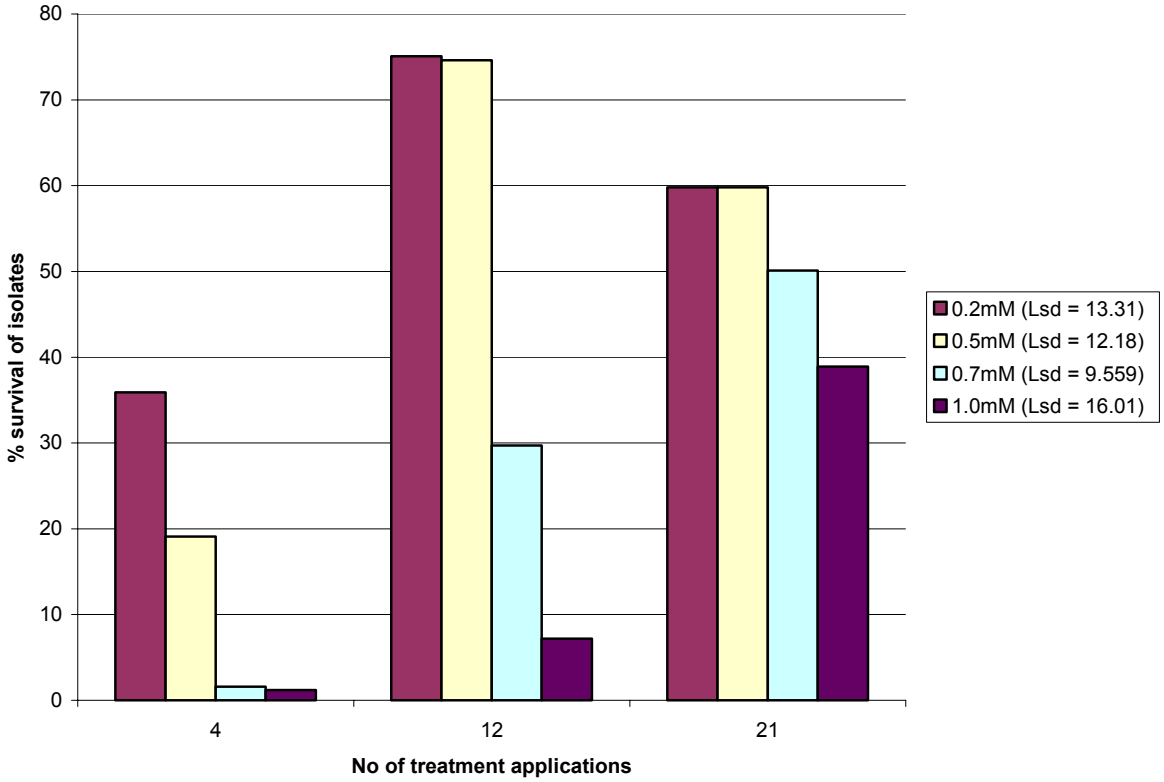
This procedure was repeated after the trial had been sprayed with 12 copper applications and again after 21 applications.

In each plot the percentage of the bacterial population tolerant to each copper concentration was determined for the three sampling times. The data were analysed as an unbalanced analysis of variance (ANOVA), with a split plot design.

4.3.3 Results

In total, 49 isolates were cultured from leaves collected after 4 treatments, 133 from leaves after 12 treatments and 129 from leaves after 21 treatments. The percentage of isolates from the total sample population that were tolerant of 4 of the 6 copper concentrations tested (0.2mM, 0.5mM, 0.7mM, 1.0mM) are presented for each sample time (Figure 6)

Figure 6: Survival of *Xanthomonas campestris* pv. *vesicatoria* isolates in Cu broths
Isolates collected from a field crop sprayed with copper bactericides



Data for the 0mM and 1.2mM concentrations were not included in the analysis and are not presented in Figure 6, because values for these concentrations were all 100% (0mM) or contained numerous 0% values (1.2mM). Inclusion of these results in the analysis would therefore have represented a violation of the assumptions of ANOVA.

Despite these exclusions, Figure 6 clearly indicates that after 4 copper sprays, the bacterial population was very sensitive to copper, with only 35.9% of the isolates surviving at the lowest copper concentration (0.2mM), compared with ≥ 60.0% of the isolates collected from the trial after 12 and 21 applications. Copper tolerant isolates of the pathogen become increasingly more frequent in the population after more treatment applications. Results for the two highest copper concentrations (0.7mM and 1.0mM) are particularly useful in illustrating this. After 4 exposures to copper sprays, only 1.6% of isolates were able to tolerate copper concentrations ≥ 0.7mM, whereas after 12 exposures this had increased to 29.7% of isolates. After 21 spray applications, copper-tolerant strains were dominating the population, with 50.1% of isolates tolerant of copper concentrations of 0.7mM or higher.

At a copper concentration of 0.7mM, there was a significant (P<0.05) interaction between sampling time and treatment (data not presented). After 21 sprays, plots sprayed with Kocide DF or Kocide DF + Mancozeb yielded significantly (P<0.05) higher proportions (60.5% and 67.9% respectively) of isolates that were tolerant of a 0.7mM copper concentration, compared to only 26.5% of isolates from untreated plots. A similar trend was also evident at the 1.0mM copper concentration, with 33.8% of Kocide treated isolates and 36.5% of Kocide + Manzate treated isolates being tolerant, compared to only 9.7% of isolates recovered from the untreated plots.

4.4 Discussion

In a recent study of copper-tolerance in *Xanthomonas campestris* pv. *vesicatoria* populations in Barbados, isolates of the pathogen were identified as being resistant to copper if they produced confluent growth on nutrient agar amended with 200ug/mM (0.80mM) of copper sulfate (Gore and O'Garro, 1999). Australian isolates of *Pseudomonas syringae* pv. *tomato* were characterised as copper-tolerant on the basis of growth at copper concentrations of 0.6-1.2mM, and resistant at concentrations of >1.6mM (Teseoriero, *et al*, 1997).

In our study, 28.0% of isolates (21/75) were tolerant of concentrations ≥1.0mM. If the copper tolerance assessments of these previous studies are considered, isolates in our study should be regarded as being copper-tolerant. Therefore, our screening work of Queensland populations of bacterial spot of capsicum indicates that copper-tolerant strains of the pathogen are contributing to the lack of efficacy of copper in controlling the disease that has been observed by many growers. It appears that tolerance has increased over time in Queensland populations, because prior to 1990 only one of 12 isolates was able to withstand a 1.0mM copper concentration, and no isolates collected before 1990 survived at concentrations in excess of 1.0mM.

In addition, our finding that copper-tolerant bacterial strains dominated a bacterial spot population after 21 exposures to copper, should be considered for management of this disease. This is particularly the case if sequential crops are to be planted or when capsicum farms are within close proximity to one another, where there is the possibility for carry-over of disease from older crops to new. In these instances the bacterial population will be exposed to many copper applications, increasing the likelihood that the population will become dominated by copper-tolerant strains.

This type of scenario may partially explain why the isolates tolerant of the highest copper concentrations in this study were from crops in the Gumlu district of north Queensland. Gumlu is a relatively small district of highly intensive capsicum production. Any bacterial spot population present in this district is likely to spread through sequential crop plantings or onto neighbouring farms, due to their close proximity to one another. With numerous growers all spraying copper for disease control, it is likely that the population will be exposed to many copper applications during the production season, potentially leading to an increase in the prevalence of copper-tolerant strains. It is important, therefore, that growers in this district (and in other districts where carry-over into neighbouring blocks is likely) employ a strict "no-plant" period so that over-summering of copper-tolerant bacterial strains does not occur. In addition, growers in these situations will need to pay particular attention to the timing of their copper sprays, the type of copper formulation used and the method of spray application, to ensure that disease control is maximised.
For bacterial fruit blotch of melons, several isolates from north Queensland were tolerant of copper concentrations in the 0.7mM-1.0mM range, indicating that copper-tolerant types are present in low frequency in the north Queensland pathogen population. It is unclear whether copper-tolerant types are also present in field populations of the watermelon strain, because of the comparatively small number of south Queensland/watermelon strain isolates collected.

For black rot, all isolates screened were sensitive to copper concentrations in excess of 0.1mM, indicating that copper-tolerance is not responsible for the control failures that have been observed by growers on the Granite Belt.

5. Optimising disease control: Assessment of putative bactericides

5.1 Introduction

Various possibilities for management of copper-tolerant bacterial strains have been proposed in the literature. Tank mixes of copper-based bactericides with EBDC fungicides (Conlin and Carter, 1983; Andersen *et al.*, 1991; Scheck and Pscheidt, 1998) or heavy metals such as iron (Lee *et al.,* 1993; Scheck and Pscheidt, 1998) or zinc (Adaskaveg and Hine, 1985; O'Garro, 1998) have been shown to improve disease control in the presence of copper-tolerant bacterial strains.

Enhanced toxicity of copper compounds and/or increases in the availability of free copper ions have been proposed as explanations for the synergistic responses when copper is mixed with fungicides or heavy metals (Adaskaveg and Hine, 1985; Andersen *et al.*, 1991; Lee *et al.*, 1993). In support of this second possibility, Scheck and Pscheidt reported that the amount of free cupric ions in solution was the only accurate predictor of formulation efficacy, but that this variable could not be estimated from the metallic copper content of the product (1998).

Several non-copper products have also been reported as valuable tools for control of several bacterial diseases. In particular, products that activate the natural defence mechanisms within plants (systemic acquired resistance) have proved useful (Romero *et al.*, 2001). One of these, acibenzolar-methyl gave superior control of bacterial spot of capsicum compared to registered copper bactericides, in a US study (Campbell *et al.*, 1997).

The timing of application is critical to the efficacy of copper products. This is particularly the case for copper products because they only have a protectant mode of action. Unlike systemic chemicals, ensuring plants are covered in adequate quantities of copper prior to bacterial infection underpins the efficacy of copper sprays.

Regardless of whether or not copper-tolerant strains are present in bacterial populations, methods to improve the efficacy of copper and alternatives to copper bactericides are required so that disease control can be maximised. We conducted a series of experiments to assess a range of bactericides for control of bacterial spot of capsicum, bacterial fruit blotch of melons and black rot of brassicas. In addition, we also examined means to improve the efficacy of copper sprays against these pathogens.

5.2 Bacterial spot of capsicum

5.2.1 Field assessment of copper formulations for control of bacterial spot

Introduction

Copper bactericides are the only products that are registered for control of bacterial diseases of vegetables in Australia. Currently, there is a range of products on the market, many of which are based on different copper compounds. There is uncertainty among capsicum growers about which copper products are most effective for control of bacterial spot. This trial was conducted to assess the efficacy of commercial copper products for bacterial spot control.

Materials and Methods

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 15cm spacings in single rows on Gatton Research Station on 13 December 2000. Seven rows were planted on beds (60m x 1.5m) that were irrigated by overhead irrigation applied through
solid set pipes. The experiment was a randomised complete block design with three blocks, each containing twelve treatments. Treatment plots were 3m long and were bordered at either end by 2m long guard plots. Whole rows were left as untreated guards between the treatment rows.

Prior to transplant, all plants in the guard plots were inoculated with bacterial spot. Bacterial suspensions of six isolates (741(3), Gas(4), 761(2), 762(9), 763(8) and 1086) with tolerances to copper ranging from 0mM to 1.0mM were prepared and standardised to concentrations of 10^8cfu/mL with a turbidimeter. Equal volumes of each suspension were combined and the resulting mix was misted over young capsicum plants one week prior to transplant. The plants were incubated for 48 hours under high humidity after which they were transplanted into the guard plots on the day the remainder of the trial was planted. The following treatments were applied every 7 days to each block using a motorised backpack sprayer fitted with a 1m boom and 4 hollow-cone nozzles.

1. Untreated (check)
2. Copper hydroxide (Kocide DF (2.0kg/ha))
3. Copper hydroxide + mancozeb (Kocide DF (2.0kg/ha) + Mancozeb WG(2.0kg/ha))
4. Copper hydroxide + mancozeb (Mankocide DF (2.0kg/ha))
5. Copper hydroxide + chlorothalonil (Kocide DF (2.0kg/ha) + Bravo 720 SC (2.0L/ha))
6. Copper hydroxide + acibenzolar methyl (Kocide DF (2.0kg/ha) + Bion (0.025gai/L))
7. Copper hydroxide + zinc sulfate (Kocide DF (2.0kg/ha) + $ZnSO_4$ (1800mg ai/L))
8. Copper hydroxide + iron chloride (Kocide DF (2.0kg/ha) + $FeCl_3.6H_2O$ (50ug/mL))
9. Copper oxychloride (Copperoxy DF (2.5kg/ha))
10. Copper ammonium carbonate (Liquicop (5L/ha))
11. Copper hydroxide liquid formulation (Kocide Liquid Blue (1.5L/ha))
12. Cuprous oxide (Norshield (2.0L/ha))

Treatment applications commenced two weeks after transplant and continued for 21 weeks. Due to phytotoxicity concerns, the acibenzolar methyl component of the copper hydroxide/acibenzolar methyl combination treatment was only included in the

spray program once in every three sprays. In weeks when acibenzolar methyl was not included, copper hydoxide alone was applied to the relevant plots.

Plants were assessed for disease development at three times throughout the experiment (22 March, 20 April, 16 May 2001). Single shoots on each of three plants were tagged in each plot and at the first and second assessment times the number of diseased leaves on the shoots were counted. The shoots were also rated for disease severity using a five point visual rating scale:

0 = nil disease
1 = slight disease
2 = moderate disease
3 = severe disease
4 = dead

At the third assessment time (16 May 2001), the three youngest leaves on each shoot showing disease symptoms were rated for disease severity using the following scale:

0 = nil disease
1 = < 1% leaf area affected
2 = 1-10% leaf area affected
3 = 11-25% leaf area affected
4 = 26-50% leaf area affected
5 = 51 – 75% leaf area affected
6 = >75% leaf area affected

Average % incidence of bacterial spot and average shoot ratings were determined for each plot at the first and second assessment times. Average leaf disease severity indices were calculated for the third assessment time.

Percentage incidence values were arcsin transformed, however in no instance did the transformation improve the residual or normal plots. Consequently, untransformed data was used in the analyses. All data was analysed as a one-way ANOVA (randomised blocks) design using Genstat 5.0 for Windows.

Results

Data for average shoot ratings are presented in Table 1. At the first assessment time (22 March), no significant ($P>0.05$) visual differences in disease severity were observed (data not presented). By the second assessment, however, differences were apparent, although most of the treatments did not cause significant ($P<0.05$) reductions in disease symptoms compared to the unsprayed checks.

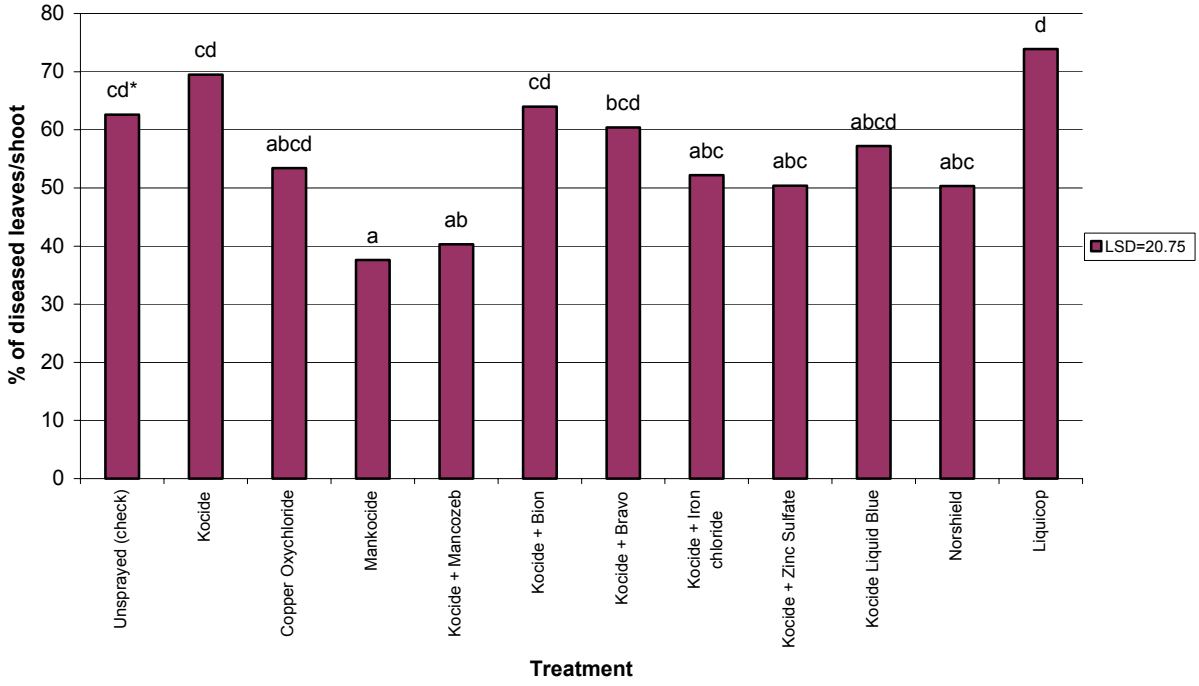
Table 1: Average shoot ratings for capsicum plants treated with different copper sprays

Treatment	Assessment 2 (20/4/01)
Mankocide DF	1.553**a**
Kocide DF+ Mancozeb WG	1.557**a**
Copperoxy DF	1.890**ab**
Norshield	2.000**abc**
Kocide DF + Bion	2.110**abcd**
Kocide DF + Bravo SC	2.110**abcd**
Kocide DF + $ZnSO_4$	2.167**abcde**
Kocide Liquid Blue	2.443**bcde**
Kocide DF + $FeCl_3$	2.447**bcde**
Kocide DF	2.670**de**
Liquicop	2.780**e**
Unsprayed (check)	2.557**cde**
Lsd	**0.6489**

The industry standard treatment Kocide DF performed poorly, giving no bactericidal benefit compared to the unsprayed plots. Mankocide DF was the best performer in the trial, closely followed by the Kocide DF + Mancozeb WG treatment combination. Both of these treatments significantly reduced symptoms on the foliage ($P<0.05$), as did Copperoxy DF, another standard industry product.

This same result is apparent if the data for the % of diseased leaves/shoot are considered. Trends were similar at both the first and second assessment times, although for simplicity only data from the second assessment time (20/4/01) is presented (Figure 7).

Figure 7: Incidence of diseased leaves on capsicum plants sprayed with different Cu products. Assessment (20 April 2001).



* Treatments with the same letters are not significantly different at the 5% level

Mankocide DF and the Kocide DF+ Mancozeb WG combination were the only treatments to give significant reductions in disease incidence compared to the untreated plots ($P<0.05$).

5.2.2 Field assessment of optimal application rates and frequencies for copper products for control of bacterial spot in capsicums

Introduction

The efficacy of copper products for control of bacterial diseases is substantially influenced by the frequency and rate of spray applications. This is particularly the case for copper products because they only have a protectant mode of action. Unlike systemic chemicals, ensuring plants are covered in adequate quantities of copper prior to bacterial infection, underpins the efficacy of copper sprays. This experiment was designed to test the most effective spray pattern for copper . We wished to test if lower concentrations of copper, applied more frequently offer superior disease control than higher concentrations applied less often.

Materials and Methods

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 30cm spacings in single rows on Gatton Research Station on 18 December 2001. Seven rows were planted on beds (90m x 1.5m) that were irrigated by overhead irrigation applied through
solid set pipes. The experiment was a completely randomised design with a factorial structure. There were 2 reps of each of treatment combination. Treatments were 3

copper products applied at two application rates and three application frequencies, as well as an untreated check as follows:

Product		Rate		Application Frequency
copper hydroxide (Kocide DF)		full label		5 days
copper oxychloride (Copperoxy DF)	x	half label	x	7 days
copper hydroxide + mancozeb (Mankocide DF)				10 days

Plots were 5m long and were bordered at either end by 1m long guard plots. Whole rows were left as untreated guards between the treatment rows.

Prior to transplant, all plants in the guard plots were inoculated with bacterial spot. Bacterial suspensions of six isolates (741(3), Gas(4), 761(2), 762(9), 763(8) and 1086) with tolerances to copper ranging from 0mM to 1.0mM were prepared and standardised to concentrations of 10^8cfu/mL with a turbidimeter. Equal volumes of each suspension were combined and the resulting mix was misted over young capsicum plants one week prior to transplant. The plants were incubated for 48 hours at high humidity after which they were transplanted into the guard plots on the day the remainder of the trial was planted. Treatments were applied to each plot using a motorised backpack sprayer fitted with a 1m boom and 4 hollow-cone nozzles.

Plants were assessed for disease development twice throughout the experiment (14 February 2002, 11 March 2002). Single shoots on each of three plants were tagged in each plot and at each assessment time the number of diseased leaves on the shoots were counted. The tagged shoots and the whole plants were also rated for disease severity using a five point visual rating scale:

0 = nil disease
1 = slight disease
2 = moderate disease
3 = severe disease
4 = dead

Average % incidence of bacterial spot and average shoot ratings were determined for each plot at the assessment times. Percentage incidence values were arcsin transformed for the analyses. Data for the untreated plots were ignored in the analysis because it could not be analysed in the factorial design. All data was analysed as a four-way general ANOVA with a factorial structure using Genstat 5.0 for Windows.

Results

The overriding factor governing the disease control efficacy in this experiment was the formulation of fungicide. At both assessment times, the frequency of application and the application rate of products used, had no significant impact (P>0.05) on the level of disease control (data not presented). Data for the average shoot assessment on 14th February 2002 are presented in Table 2.

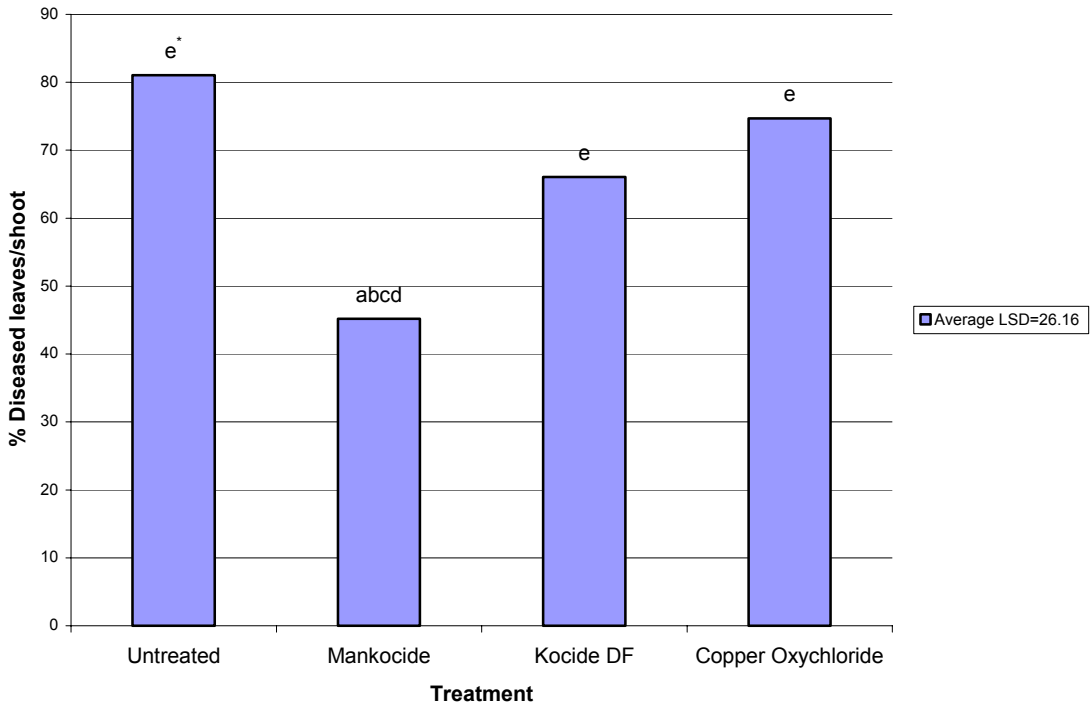
Table 2: Average capsicum shoot assessments for plots treated with different copper products. First assessment (14 February 2002)

Treatment	Average Shoot Assessments
Mankocide DF	0.527**abcd**
Kocide DF	0.888**abcde**
Copperoxy DF	1.264**ef**
Untreated (check)	2.000**f**
Lsd (P<0.05)	**0.7613**

Tagged shoots in plots treated with Mankocide DF displayed fewer disease symptoms than untreated shoots or those treated with Copperoxy DF. The level of disease on shoots in Mankocide plots was the lowest of any treatment, however the disease severity on shoots in Kocide DF treated plots was statistically comparable to Mankocide DF treated shoots (P<0.05). Although there were differences in disease severity between shoots treated with different products, the % of infected leaves on shoots treated with the different products did not differ significantly (P<0.05) at the first assessment time.

By the second assessment, 11 March 2002, the types of copper sprays applied were producing significant differences in the % of infected leaves on treated shoots (Figure 8).

Figure 8: Percentage of leaves on tagged capsicum shoots with bacterial spot symptoms.
Second assessment (11 March 2002)



*Treatments with the same letters are not significantly different at the 5% level.

Mankocide DF was the only product applied that significantly reduced disease incidence below that of the untreated shoots.

5.2.3. Glasshouse examination of the ability of spray-tank adjuvants to influence the pre and post-infectional activity of copper hydroxide

Introduction

Copper compounds have a protectant mode of action against bacterial diseases. Because of this, achieving optimal spray coverage of plants with copper is imperative if maximum disease control is to be achieved. A range of spray-tank adjuvants were examined here, in an attempt to improve spray coverage, and hence, disease control on capsicum plants in the glasshouse.

Materials and Methods

Capsicum seedlings cv. Heldor were transplanted, one per pot, into 208 5" pots of UC mix at the Indooroopilly Plant Protection Unit on 13 December 2000. After 5 days, half of the plants (104 plots) were sprayed until the point of runoff with Kocide DF (2.0g/L)/adjuvant mixtures. Four pots were sprayed with each treatment as follows:

Kocide DF + DcTron Plus (2mL/L)
Kocide DF + Agridex (2mL/L)
Kocide DF + Synertrol (2mL/L)
Kocide DF + Codacide (1.5mL/L)
Kocide DF + Hasten (1mL/L)
Kocide DF + Agral (2mL/L)
Kocide DF + X-77 (0.2mL/L)
Kocide DF + Pulse Penetrant (1mL/L)
Kocide DF + Spraymate LI-700 (1mL//L)
Kocide DF + Uptake (2mL/L)
Kocide DF + Bond (1mL/L)
Kocide DF
Untreated

All plants (208 pots) were then inoculated with either a copper-sensitive isolate of *Xanthomonas campestris* pv. *vesicatoria* (762(3)) or a copper tolerant isolate (Gas 7). Bacterial suspensions of both isolates were prepared and standardised to a concentration of 10^8cfu. Equal volumes of the suspensions were combined and the resulting mix was misted onto the plants. Half of the pre- and half of the post-inoculation treated plants were inoculated with the sensitive strain, and the remaining plants were inoculated with the resistant strain. After inoculation, the plants were enclosed in plastic bags and incubated at 25°C for 48 hours. After removal of the plants from the bags, the plants that had not been treated with Kocide /adjuvant mixtures prior to inoculation, were sprayed with the treatments until the point of runoff. The pots were randomly arranged on 4 benches in a glasshouse, such that each bench contained one pot of each treatment combination.

Two weeks after inoculation, the plants were again enclosed in plastic bags and incubated for 48 hours, after which time they were removed and rated for disease development. The two most severely affected leaves were visually assessed for disease severity using a 7 point rating scale as follows:

0 = nil disease
1 = <1% leaf area affected
2 = 1-10% leaf area affected
3 = >10-25% leaf area affected
4.= >25-50% leaf area affected
5 = >50-75% leaf area affected
6 = >75% leaf area affected

Each plant was also given a rating for phytotoxicity, based on the severity of foliar symptoms. A 7 point rating scale was used for this assessment as follows:

0 = nil phytotoxicity
1 = mild symptoms on <1 leaf/plant
2 = mild symptoms on <25% leaves/plant
3 = > 25% of leaves showing moderate symptoms
4 = severe symptoms on >25%-50% of leaves
5 = severe symptoms on 50-75% of leaves
6 = severe symptoms on >75% of leaves

Average ratings were obtained for each plant, and the data were analysed as a randomised complete block design with factorial structure using the general analysis of variance procedure (ANOVA) in Genstat 5.0 for Windows.

Results

Disease severity was very low in this trial, even on control plants that were not treated with Kocide DF. In terms of disease severity, regardless of whether a copper-sensitive or tolerant isolate was used, plants inoculated before treatment showed significantly fewer disease symptoms than those inoculated after (P<0.05). Disregarding the effects of inoculum type and application time, none of the treatment combinations proved any better than Kocide DF alone in reducing disease severity (P<0.05). The low levels of disease recorded in the control plants tended to indicate, however that there was a deficiency in the inoculation protocol. Both Pulse and Uptake caused significant (P<0.05) phytotoxicity when applied in combination with Kocide DF.

5.2.4 Discussion

For control of bacterial spot of capsicum, Mankocide DF should now replace Kocide DF as the standard industry treatment. The combination treatment of Kocide DF and Mancozeb WG also performed strongly and was significantly better than Kocide DF applied alone. In the event that Mankocide DF is unavailable, the Kocide DF/Mancozeb WG combination should be used.

5.3 Bacterial Fruit Blotch of Melons

5.3.1. Field assessment of optimal application rates and frequencies for copper products for control of bacterial fruit blotch of melons

Introduction

The efficacy of copper products for control of bacterial diseases is substantially influenced by the frequency and rate of spray applications. This is particularly the case for copper products because they only have a protectant mode of action. Unlike systemic chemicals, ensuring plants are covered in adequate quantities of copper prior to bacterial infection, underpins the efficacy of copper sprays. This experiment was designed to test the most effective spray pattern for copper. We wished to test if lower concentrations of copper, applied more frequently offer superior disease control than higher concentrations applied less often.

Materials and Methods

Seedlings of rockmelon cv. Eastern Star were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Redlands Research Station on 19 December 2001. Seven rows were planted on beds (70m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. The experiment was a completely randomised design with a factorial structure. There were two replicates of each of treatment combination. Treatments were three copper products applied at two application rates and three application frequencies, as well as an untreated check as follows:

Product		Rate		Application Frequency
copper hydroxide (Kocide DF)		full label		5 days
copper oxychloride (Copperoxy DF)	x	half label	x	7 days
copper hydroxide + mancozeb (Mankocide DF)				10 days

Plots were 4m long and were bordered at either end by 1m long guard plots. Whole rows were left as untreated guards between the treatment rows.

Prior to transplant, all plants in the guard plots were inoculated with bacterial fruit blotch. Bacterial suspensions of four isolates (1739, 367(2), 363(3) and 353(1)) were prepared and standardised to concentrations of 10^8cfu/mL with a turbidimeter. Equal volumes of each suspension were combined and the resulting mix was misted over young melon plants two weeks prior to transplant. The plants were incubated for 48 hours at high humidity after which they were transplanted into the guard plots on the day the remainder of the trial was planted. Treatments were applied to each plot using a motorised backpack sprayer fitted with a 1m boom and 4 hollow-cone nozzles.

Plants were assessed for disease development at harvest on 26 February 2002. Individual runners were selected from three plants in each plot. The number of infected leaves per runner was determined and each leaf the selected runners was rated for disease severity using a six point visual rating scale:

0 = nil disease
1 = 1-<10% leaf area affected
2 = 10-<25% leaf area affected
3 = 25-<50% leaf area affected
4 = 50-<75% leaf area affected
5 = >75% leaf area affected

All fruit were collected from each plot and each was rated for disease both externally and internally:

External rating

0 = nil disease
1 = mild (slight watersoaking)
2 = moderate (watersoaking and/or break in netting)
3 = severe (breaks in netting with cracking)

Internal rating

0 = nil disease
1 = slight (localised infection)
2 = moderate (infection extending into flesh)
3 = severe (infection of flesh and seed cavity)
4 = rotten (severe infection with secondary breakdown)

Data for the untreated plots were ignored in the analysis because it could not be analysed in the factorial design. All data was analysed as a four-way general ANOVA with a factorial structure using Genstat 5.0 for Windows.

Results

Disease infection levels in this trial were low and the trial was severely affected by leaf miner
immediately prior to harvest, which made assessment extremely difficult. No significant differences were found between the treatments in terms of the quantity or quality of harvestable melons ($P>0.05$). In terms of foliar assessments however, treatment with Mankocide resulted in significantly reduced foliar disease severity compared to treatment with either Kocide DF or Copperoxy DF, regardless of the rate or frequency of application. In contrast, disease incidence was influenced by the frequency of fungicide application. Significantly fewer infected leaves were recovered from plots treated at 5 and 7 day intervals compared to those treated every 10 days ($P<0.05$). For the lowest disease incidence and severity, therefore, it would seem that Mankocide DF applied every 5 or 7 days would be most likely to give superior disease control.

5.3.2 Assessment of copper/mancozeb ratios for field control of bacterial fruit blotch

Introduction

Mankocide DF is a commercial formulation of copper hydroxide (300g/kg) + mancozeb (150g/kg). Copper is an environmental pollutant that accumulates in agricultural water and soil as a result of spray runoff. Minimising the quantity of copper that is applied in a copper/mancozeb mix is therefore desirable from an environmental as well as a copper-resistance management standpoint. This trial was conducted to determine if the ratio of copper:mancozeb could be reduced without compromising disease control.

Materials and Methods

Seedlings of rockmelon cv. Eastern Star were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Redlands Research Station on 19 December 2001. Seven rows were planted on beds (70m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. The experiment was a randomised complete block design with 3 replicate plots of each treatment. Nine treatments were applied using a motorised backpack sprayer fitted with a 1m boom and 4 hollow-cone nozzles:

Kocide DF (copper hydroxide 2.0g/L)
Mancozeb WG (mancozeb 2.0g/L)
Mankocide DF (2.0g/L)(1.333g $Cu(OH)_2$ + 0.667g mancozeb)
Copper hydroxide + Mancozeb (2.0g/L) (1.0g $Cu(OH)_2$ + 1.0g mancozeb) **(1:1)**
Copper hydroxide + Mancozeb (2.0g/L) (1.333g $Cu(OH)_2$ + 0.667g mancozeb) **(2:1)**
Copper hydroxide + Mancozeb (2.0g/L) (0.667g $Cu(OH)_2$ + 1.333g mancozeb) **(1:2)**
Copper hydroxide + Mancozeb (2.0g/L) (0.5g $Cu(OH)_2$ + 1.5g mancozeb) **(1:3)**
Copper hydroxide + Mancozeb (2.0g/L) (0.4g $Cu(OH)_2$ + 1.6g mancozeb) **(1:4)**

Plots were 5m long and were bordered at either end by 1m long guard plots. Whole rows were left as untreated guards between the treatment rows.

Prior to transplant, all plants in the guard plots were inoculated with bacterial fruit blotch. Bacterial suspensions of four isolates (1739, 367(2), 363(3) and 353(1)) were prepared and standardised to concentrations of 10^8cfu/mL with a turbidimeter. Equal volumes of each suspension were combined and the resulting mix was misted over young melon plants two weeks prior to transplant. The plants were incubated for 48 hours at high humidity after which they were transplanted into the guard plots on the day the remainder of the trial was planted.

Plants were assessed for disease development at harvest on 26 February 2002. Individual runners were selected from three plants in each plot. The number of infected leaves per runner was determined and each leaf was rated for disease severity using a six point visual rating scale:

All fruit were collected from each plot and each fruit was rated for disease both externally and internally:

External rating

0 = nil disease
1 = mild (slight watersoaking)
2 = moderate (watersoaking and/or break in netting)
3 = severe (breaks in netting with cracking)

Internal rating

0 = nil disease
1 = slight (localised infection)
2 = moderate (infection extending into flesh)
3 = severe (infection of flesh and seed cavity)
4 = rotten (severe infection with secondary breakdown)

All data was analysed as a one way ANOVA in randomised complete blocks using Genstat 5.0 for Windows.

Results

Disease infection levels in this trial were extremely low, which made assessment very difficult. Consequently, no significant differences were found between the treatments in terms of the quantity or quality of harvestable melons ($P>0.05$) or the incidence or severity of foliar symptoms ($P>0.05$).

5.3.3 Field assessment of spray tank adjuvants for improving the efficacy of Mankocide sprays for bacterial fruit blotch in rockmelon

Introduction

Copper compounds have a protectant mode of action against bacterial diseases. Because of this, achieving optimal spray coverage of plants with copper is imperative if maximum disease control is to be achieved. A range of spray-tank adjuvants were examined here, in an attempt to improve spray coverage, and hence, disease control on rockmelon plants.

Materials and Methods

Experiment 1

Seedlings of rockmelon cv. Eastern Star were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Gatton Research Station on 18 December 2001. Seven rows of rockmelon planted on beds (90m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Assessments were conducted once, 11 weeks after transplant, on 7th March 2002. Nine treatments were compared as follows:

Untreated (check)
Mankocide DF (2.0g/L)
Mankocide DF (2.0g/L) + Synertrol Oil (2.0mL/L)
Mankocide DF (2.0g/L) + DCTron Plus (2.0mL/L)
Mankocide DF (2.0g/L) + Pulse Penetrant (1.0mL/L)
Mankocide DF (2.0g/L) + Hasten (1.0mL/L)
Mankocide DF (2.0g/L) + Bond (1.0mL/L)
Mankocide DF (2.0g/L) + Product A (0.375mL/L)
Mankocide DF (2.0g/L) + Product B (2.5mL/L)

All sprays were applied with a modified motorised backpack hydraulic sprayer (Solo model 442)(with air assistance) fitted with hollow cone nozzles (Albuz ATR red nozzles). The trial was completed as a completely randomised design, with 3 replicate plots per treatment. Plots comprised two rows, 4 metres long, with one metre guard row plots at either end.

Helios 500SC (Uvitex), a fluorescent tracer, was added to water in the spray tank at rate of 20g/ha. The tracer was sprayed at 300L/ha and 4 bar (56psi). All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry and leaves were collected from the tops and bottoms of the plant canopy. Ten leaves were sampled per plot. After collection, the leaf area of each leaf that was sampled was determined using a leaf area meter (model LIA6100).

To remove the tracer from the leaves, each leaf was shaken 50 times in a glass jar containing 30mL of ethyldigol. The ethyldigol was filtered to remove any debris and the concentration of tracer recovered from each sample was measured using a digital fluorometer. The amount of spray deposit per area of leaf was determined. This was expressed as the quantity of tracer recovered (ng tracer/cm^2 of leaf) per quantity applied per hectare (g/ha).

Data were analysed as a one-way ANOVA (without blocking) using Genstat 5.0 for Windows.

Experiment 2

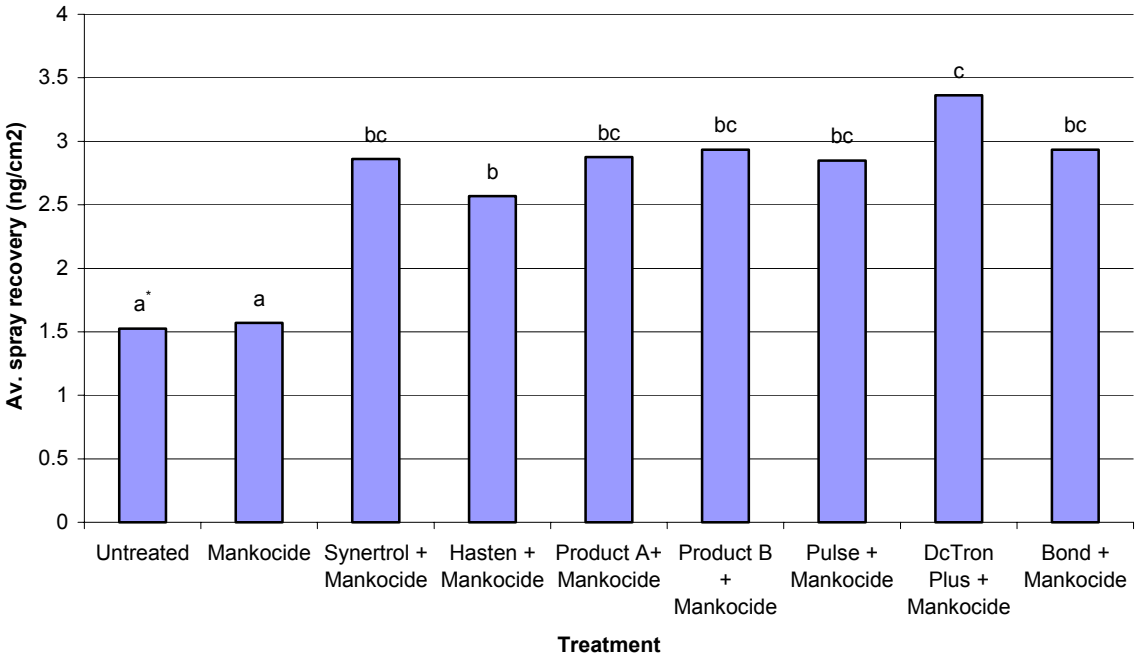
This experiment was repeated at Gatton Research Station with rockmelon seedlings transplanted on 22 October 2002. In this case, the trial was inoculated with bacterial fruit blotch, to determine whether the improved spray coverage provided by the addition of adjuvants to the spray tank, resulted in superior disease control. Unfortunately, however, because of the prevailing drought conditions (extremely low humidity and rainfall) at the time the trial was conducted, a disease epidemic was not iniated in the trial and hence no data on the efficacy of adjuvants for fruit blotch control was obtained. Consequently, only the results of Experiment 1 are presented here.

Results

All the spray-tank adjuvants assessed in this trial significantly ($P<0.05$) improved spray coverage of rockmelon leaves, compared to water or Mankocide DF alone (Figure 10). The DCTron Plus/Mankocide combination produced the highest coverage of any treatment, although the level of coverage provided by this treatment

was not significantly greater than that of 5 of the other adjuvant/Mankocide combinations (Pulse, Synertrol, Product A, Product B or Bond).

Figure 10: Average spray deposit recovered from rockmelon leaves sprayed with different spray-tank adjuvants



*Treatments with the same letters are not significantly different at the 5% level.

This trial does not provide any information about how the improved coverage affects disease control efficacy. Future work is needed to establish this important link.

5.3.4 Discussion

Mankocide DF appears to be a superior product to either copper oxychloride or Kocide DF for reducing severity of bacterial fruit blotch on melon crops. This product should be applied every 5 –7 days with a spray-tank adjuvant to improve spray coverage of the melon plant.

5.4 Black rot of brassicas

5.4.1 Field assessments of putative bactericides for control of black rot of brassicas

Materials and Methods

Experiment 1

Seedlings of cauliflower cv. Freemont were raised in plastic speedling trays and transplanted at 0.5m spacings into 14 double rows (0.75m x 36m) and 4 double rows (0.75m x 24.5m) on Applethorpe Research Station (24 October 2000). The block was irrigated overhead through sprinklers. The experiment was a randomised complete block design with five blocks, each containing six treatments. Treatment plots were 4m long

Treatment applications commenced on 8 November 2000 with a 15L knapsack sprayer fitted with a wand attachment and single hollow cone nozzle. Treatments were applied every 7 days as follows:

1. Untreated (check)
2. Agri-R-Phos (phosphorous acid)(5.0mL/L)
3. Vigor-Cal (10.0mL/L)
4. Kocide DF + Mancozeb WG (copper hydroxide (2.0g/L) + mancozeb (2.0g/L))
5. Tsunami (1.0mL/L)
6. Molasses (10.0mL/L)

Prior to transplant, selected trays of plants were inoculated with the black rot bacterium. A bacterial suspension of *Xanthomonas campestris* pv. *campestris* was prepared and it was misted over young capsicum plants two weeks prior to transplant. The plants were incubated for under high humidity and they were transplanted into the trial on 16 November 2000, one week after commencement of the first treatment application

Treatment applications continued until the week prior to harvest and assessment on 8 January 2001. Thirty-eight plants in each plot were assessed for disease incidence and severity. The total number of leaves and number of infected leaves was counted on each plant. Disease incidence was expressed and the % infected leaves per plant. Each plant was also given a visual rating for disease severity based on a 4 point rating scale:

0 = nil disease
1 = slight disease (marginal leaf necrosis on >75% of leaves. Total leaf area affected: 5-15%
2 = moderate disease (extensive marginal leaf necrosis, as well as necrotic areas on >90% leaves infected. Total leaf area affected: 15-30%
3 = severe disease (necrotic areas on 100% of leaves. Total leaf area affected: >30%

Percentage incidence values were arcsin transformed, however in no instance did the transformation improve the residual or normal plots. Consequently, untransformed data was used in the analyses. All data was analysed as a one-way ANOVA (randomised blocks) design using Genstat 5.0 for Windows.

Experiment 2

Seedlings of cauliflower cv. Cauldron were raised in plastic speedling trays and transplanted at 0.5m spacings into 15 double rows (0.75m x 36m) on Applethorpe Research Station (21 February 2001). The block was irrigated overhead through sprinklers. The experiment was a randomised complete block design with five blocks, each containing five treatments. Treatment plots were 4m long

Treatment applications commenced on 28 February 2001 with a motorised hydraulic knapsack (Silvyn) fitted with a wand attachment and a single hollow cone nozzle Treatments were applied every 7 days as follows:

1. Untreated (check)
2. Kocide DF(copper hydroxide)(2.0g/L)
3. Kocide DF + Mancozeb WG (copper hydroxide (2.0g/L) + mancozeb (2.0g/L))
4. Kocide DF + Mancozeb WG + Bond (copper hydroxide (2.0g/L) + mancozeb (2.0g/L) + Bond (1.4mL/L))
5. Fertiliser (GF303, 303g/plot applied every 2 weeks)

Prior to transplant, selected trays of plants were inoculated with the black rot bacterium. A bacterial suspension of *Xanthomonas campestris* pv. *campestris* was prepared and it was misted over young capsicum plants two weeks prior to transplant. The plants were incubated under high humidity and they were transplanted into the trial on 16 November 2000, one week after commencement of the first treatment application

Cauliflower leaves affected with black rot were obtained from a commercial grower on 28 March 2001. The leaves were blended, and the macerate was diluted in water to a total volume of 30L (3.2×10^8 cfu/mL *X. campestris* pv. *vesicatoria*). The inoculum suspension was then sprayed over the trial plots in the late afternoon. This process was repeated on the 20 April 2001, with an inoculum suspension of 5.8×10^7cfu/mL of the bacterium. Treatments were applied until 2 weeks prior to harvest and assessment on 18 May 2001.

Twenty-five plants in each plot were assessed for disease incidence and severity. The total number of leaves and number of infected leaves was counted on each plant. Disease incidence was expressed as the % infected leaves per plant. The diameter of each cauliflower was also measured and each plant was also given a visual rating for disease severity based on a 4 point rating scale:

0 = nil disease
1 = slight disease (marginal leaf necrosis on >75% of leaves. Total leaf area affected: 5-15%)
2 = moderate disease (extensive marginal leaf necrosis, as well as necrotic areas on >90% leaves infected. Total leaf area affected: 15-30%)
3 = severe disease (necrotic areas on 100% of leaves. Total leaf area affected: >30%)

Percentage incidence values were arcsin transformed, however in no instance did the transformation improve the residual or normal plots. Consequently, untransformed data was used in the analyses. All data was analysed as a one-way ANOVA (randomised blocks) design using Genstat 5.0 for Windows.

Results

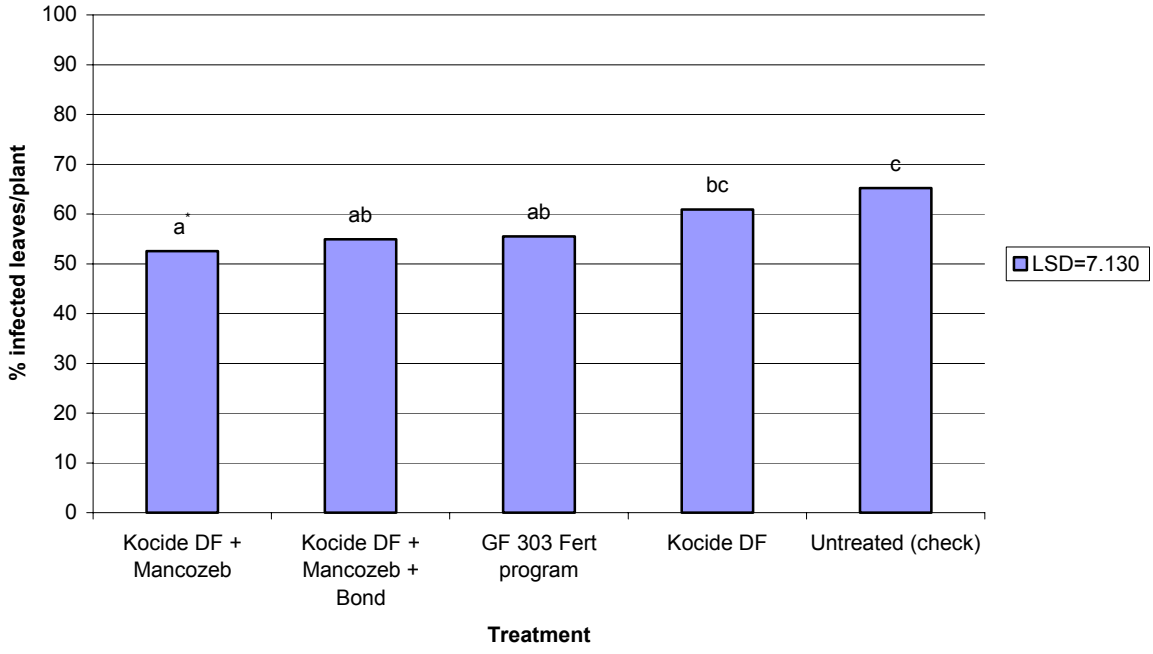
Experiment 1

The disease pressure in this trial was very low. An epidemic failed to develop in the trial due to unfavourable weather conditions at the time the trial was conducted. Rainfall and humidity were both extremely low for the duration of the trial. Consequently, no significant differences ($P<0.05$) were detected between the treatments in this trial. Disease incidence and severity and the diameter of the cauliflowers were comparable in all replicates, regardless of the treatment applied.

Experiment 2

Data for disease incidence is presented in Figure 11. Plots treated with the Kocide DF/Mancozeb combination had the lowest incidence of black rot of any of the treated plots. The level of control provided by the Kocide DF/Mancozeb treatment was statistically comparable to that provided by the Kocide/ Manocozeb/Bond treatment and the fertiliser treatment program (GF303 applied fortnightly). All three of these treatments outperformed the industry standard treatment Kocide DF ($P<0.05$). Kocide DF did not reduce disease incidence compared to the untreated plots ($P<0.05$) (Figure 11).

Figure 11: Incidence of black rot on plants treated with putative bactericides



*Treatments with the same letters are not significantly different at the 5% level.

There were no significant differences between any of the treatments in terms of disease severity ($P<0.05$).

5.4.2 Discussion

Combination treatments Kocide DF + Mancozeb (with or without adjuvant) provided the best control of black rot. Tank mixing copper hydoxide with mancozeb should now represent standard industry practice, rather than application of copper hydroxide alone. Kocide DF alone was ineffective. Plants treated solely with Kocide DF displayed symptoms as severe as those in the untreated plants. It is noteworthy also, that plants treated with the GF 303 fertiliser program had disease levels similar to those in the Kocide DF + Manozeb treated plots. This fertiliser program was utilised by a local grower in the Granite Belt district and its efficacy demonstrates that plants in sound nutritional health are less susceptible to the black rot pathogen.

6. Optimising disease control: Spray application assessments

6.1 Introduction

Copper is the only chemical option for control of bacterial spot, fruit blotch and black rot. Because it only has a protective mode of action, optimisation of the delivery and distribution of the copper over plant surfaces is critically important, since poor coverage will result in poor disease control.

A range of ground based spray equipment is used throughout Queensland vegetable production districts. The standard hydraulic nozzle boom sprayer (either linkage mounted or trailed) is the most widely used rig type. Depending on the target crop, some growers use attachments such as short droppers (200-400mm long) to improve spray coverage. All spray equipment needs to be regularly calibrated (measuring individiual nozzle outputs, replacing worn nozzles and testing the sprayer output), so that the correct quantity of bactericide can be added to the spray tank.

The canopy architecture of the target crop markedly influences spray droplet penetration and distribution over the plant. Spray droplet distribution is difficult to manipulate when spraying is done with a boom from above the plant canopy. When spraying from over the top, the deposit is highest in the top part of the canopy and declines rapidly lower down. Specifically, achieving coverage of the underside of the leaves is particularly difficult especially when the plant canopy is dense. Despite this, for effective bacterial disease control, coverage of the bottom sections of plant canopies and undersides of leaves is critical. It is in these locations, the most protected locations in the canopy, that bacterial diseases are most likely to become established, since they remain wet longest and are the regions of highest humidity in the canopy.

We conducted a series of experiments to determine the optimum spray application methods for capsicum and melon plants at different stages of development.

6.2 Bacterial spot of capsicum

6.2.1 Assessment of Spray Rigs

6.2.1.1 Materials and Methods

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 15cm spacings in single rows on Gatton Research Station. Seven rows were planted on beds (40m x 1.5m) that were irrigated by overhead irrigation applied through
solid set pipes. Spray rig assessments were conducted twice throughout the crop lifecycle, at early flowering (15 January 2002), and during harvest (12 March 2002). Four types of spray rigs were compared in the trial:

Hardi twin-force air assisted sprayer (with air-assist)
Hardi twin-force air assisted sprayer (without air-assist)
Modified Hardi linkage sprayer fitted with 300mm droppers
Solo model 442 motorised hydraulic knapsack sprayer.

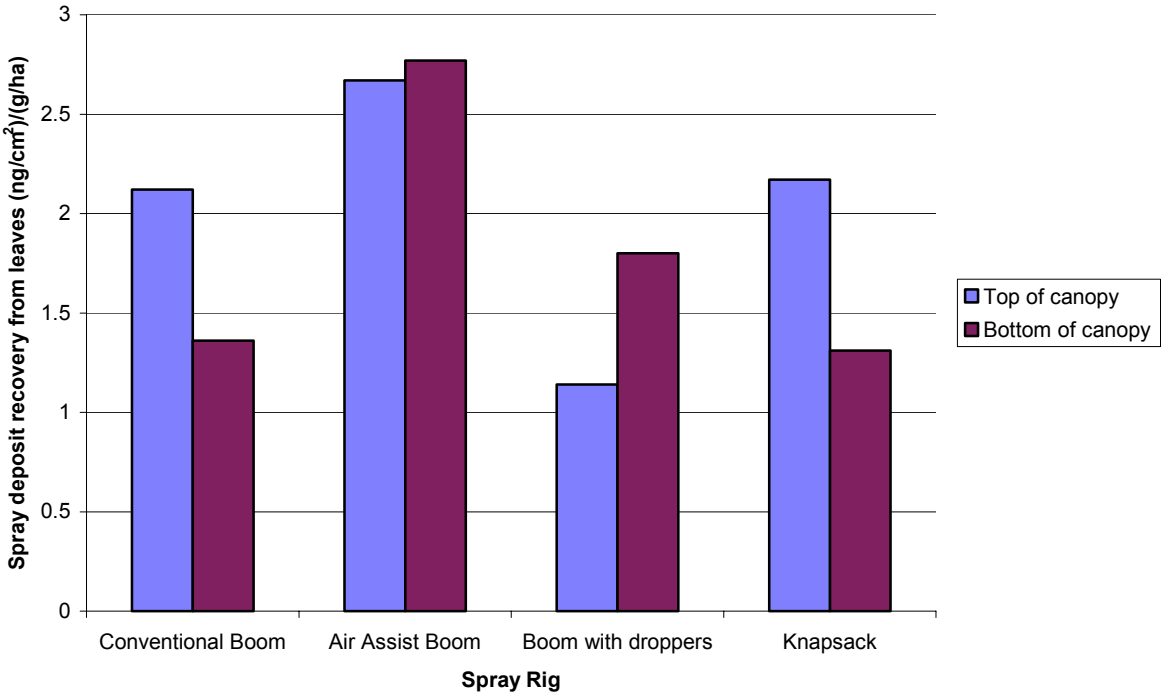
A uniformity trial layout was used comprising four plots. Each plot was 15m long x 2 rows wide. Helios 500SC (Uvitex), a fluorescent tracer, was added to water in the spray tank at rate of 20g/ha. The tracer was sprayed from each rig type at 300L/ha and 4 bar (56psi) using hollow cone (Albuz ATR red) nozzles. Each of the four plots was sprayed using a different rig type. All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry and leaves were collected from both the tops and bottoms of the canopies of 15 plants spaced evenly along each of the sprayed row lengths, such that a total of 30 plants were sampled for each rig type. At the first sampling time, leaves were collected from the tops and bottoms of the plant canopies. At the second sampling time, leaves were collected from 4 positions on each plant, the top outer canopy, the top inner canopy, the bottom outer canopy and the bottom inner canopy. After collection, the leaf area of each leaf that was sampled was determined using a leaf area meter (model LIA6100).

To remove the tracer from the leaves, each leaf was shaken 50 times in a glass jar containing 30mL of ethyldigol. The ethyldigol was filtered to remove any debris and the concentration of tracer recovered from each sample was measured using a digital fluorometer. The amount of spray deposit per area of leaf was determined. This was expressed as the quantity of tracer recovered (ng tracer/cm^2 of leaf) per quantity applied per hectare (g/ha).

6.2.1.2 Results

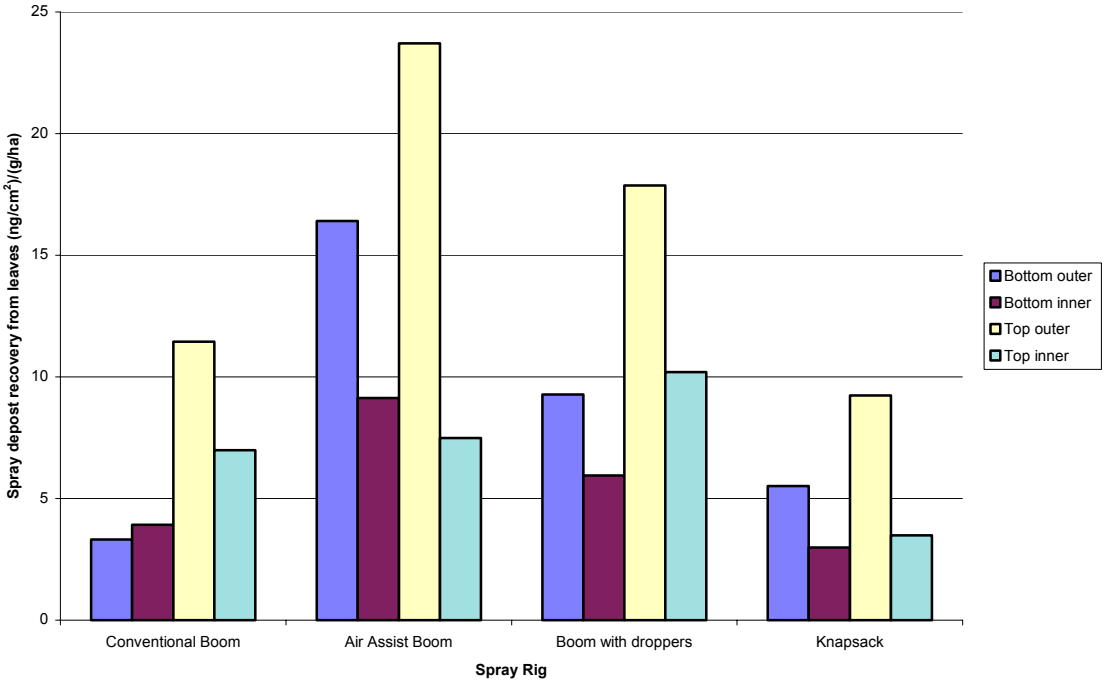
At an early stage of plant development, the air assisted spray rig produced the greatest coverage of any of the rigs tested, and the spray deposit was evenly distributed over the whole plant canopy (Figure 12). Both the conventional boom and the knapsack performed similarly, with greater coverage of the top of the canopy and relatively poor coverage at the bottom of the canopy. Droppers on a conventional boom assisted substantially with coverage of the lower canopy, however with this rig configuration, coverage of the upper portions of the canopy was compromised.

Figure 12: Droplet recovery from young capsicum plants sprayed using different rigs (early flowering stage)



Similar trends were evident for the mature plants, with the air-assisted boom being the rig of choice for achieving the best overall canopy coverage. Droppers on the conventional boom were not as effective at covering the bottom sections of the canopy when the canopy was denser (Figure 13). Despite this, adding droppers to the conventional boom did provide substantially better coverage of entire plants, compared to when a conventional boom was used without droppers. On the basis of this result we suggest to growers who do not have access to an air-assisted spray rig, that dropper added to a conventional boom are a worthwhile addition to help improve spray coverage of capsicum plants.

Figure 13: Droplet recovery from capsicum plants sprayed using different rigs (mid-harvest stage)



6.2.2 Assessment of Application Volumes

6.2.2.1 Materials and Methods

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 15cm spacings in single rows on Gatton Research Station. Seven rows were planted on beds (40m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Spray volume assessments were conducted twice throughout the crop lifecycle, at early flowering (15 January 2002), and at first pick (12 March 2002). All sprays were applied using a motorised hydraulic knapsack sprayer (Solo model 442) fitted with hollow cone nozzles (Albuz red 2.0 ceramic) At the assessment on 15 Januray 2002, four spray volumes were compared (100, 200, 300 and 400L/ha). For the March 12 assessment the spray volumes tested were 300, 400, 800 and 1000L/ha.

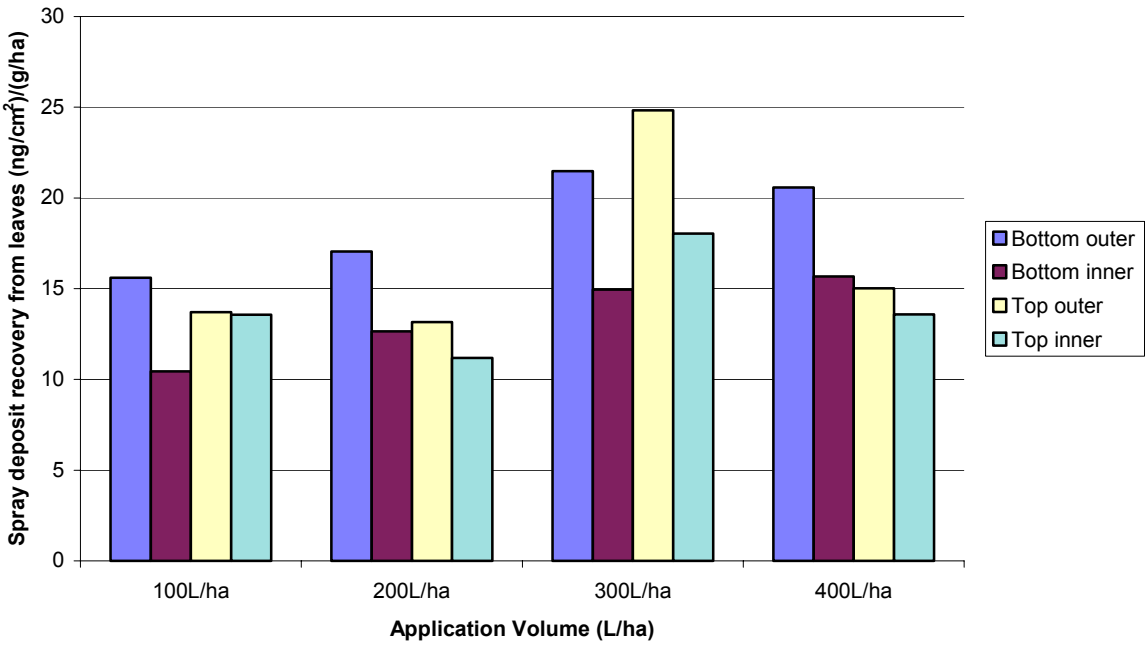
The experiment was completed as a randomised complete block design with 5 replications. Plots were 5.0m long, with 2.0m long guard rows at the end of each plot. Helios 500SC (Uvitex), a fluorescent tracer, was added to water in the spray tank at rate of 20g/ha. The tracer was sprayed at 4 bar (56psi) and 5km/hr. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry and leaves were collected from both the tops and bottoms of the canopies of 2 plants from each plot, such that a total of 10 plants were sampled for each application volume. At each sampling time, leaves were collected from 4 positions on each plant, the top outer canopy, the top inner canopy, the bottom outer canopy and the bottom inner canopy. After collection, the leaf area of each leaf that was sampled was determined using a leaf area meter (model LIA6100).

To remove the tracer from the leaves, each leaf was shaken 50 times in a glass jar containing 30mL of ethyldigol. The ethyldigol was filtered to remove any debris and the concentration of tracer recovered from each sample was measured using a digital fluorometer. The amount of spray deposit per area of leaf was determined. This was expressed as the quantity of tracer recovered (ng tracer/cm^2 of leaf) per quantity applied per hectare (g/ha). Data were analysed as a one-way ANOVA (completely randomised design) using Genstat 5.0 for Windows.

6.2.2.2 Results

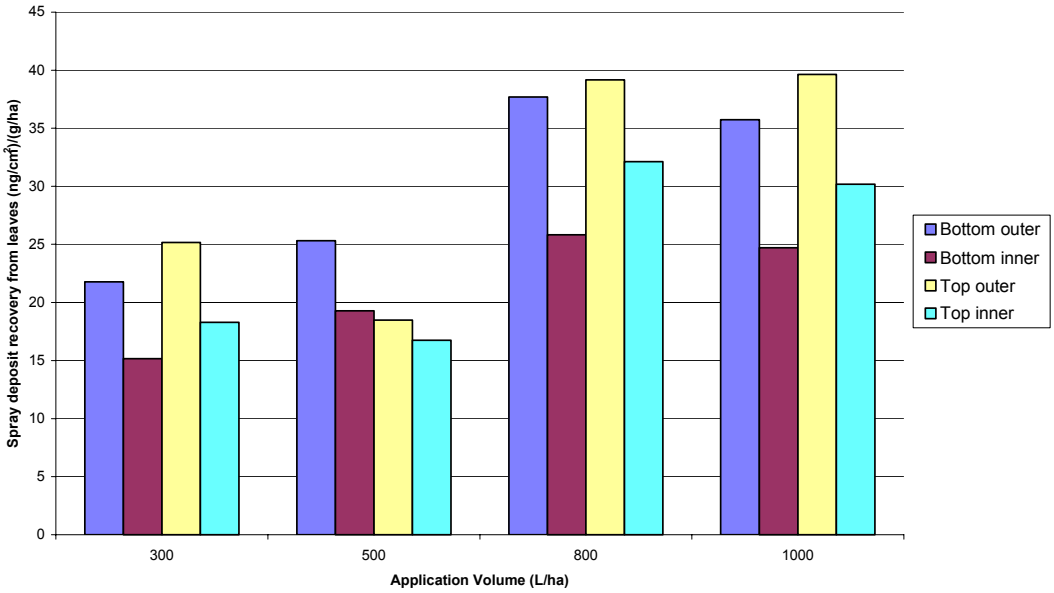
When the plants were young, before they had developed a full canopy, 300L/ha appeared to result in the best overall plant coverage. Volumes greater than 300L/ha (in this case 400L/ha) resulted in loss of chemical through spray runoff (Figure 14)

Figure 14: Spray recovery from capsicum plants sprayed with different volumes (early flowering stage)



Once a full plant canopy had developed (mid harvest stage), a spray volume of 800L/ha produced significantly ($P<0.05$) greater coverage of all portions of the plant canopy than lower volumes (300, 500L/ha). No additional benefit was gained by spraying plants at volumes greater than 800L/ha at this stage. At 1000L/ha, no additional coverage ($P>0.05$) was achieved and it can be assumed from these results that the additional volume was lost to the surrounding environment as runoff (Figure 15).

Figure 15: Spray recovery from capsicum leaves sprayed with different volumes (mid harvest stage)



6.2.3 Assessment of Nozzle Types

6.2.3.1 Materials and Methods

Experiment 1

Seedlings of capsicum cv. Heldor were raised in plastic speedling trays and transplanted at 15cm spacings in single rows on Gatton Research Station on 13 December 2000. Seven rows were planted on beds (60m x 1.5m) that were irrigated by overhead irrigation applied through
solid set pipes.

On 12 March 2001, a uniformity trial layout was used comprising four plots. Each plot was 25m long x 3 rows wide and a buffer of 10m was left between the plots. . Treatments were applied using a Hardi twin-force air assisted sprayer (with no air-assistance) Helios 500SC (Uvitex), a fluorescent tracer, was added to water in the spray tank at rate of 20g/ha. The tracer was sprayed at 300L/ha and 4 bar (56psi). Four nozzle types were compared in the trial:

Albuz ceramic hollow cone red (2.0)
Teejet TJ60 1103 twin jet nozzles
Teejet TP 11003 flat fan nozzles
Teejet AI 11003 air induction nozzles

Each of the four plots was sprayed using a different nozzle type. All sprays were applied with a single pass of the spray rig in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry and leaves were collected from 4 positions on each plant, the top outer canopy, the top inner canopy, the bottom outer canopy

and the bottom inner canopy. Ten plants were sampled per treatment. After collection, the leaf area of each leaf that was sampled was determined using a leaf area meter (model LIA6100).

To remove the tracer from the leaves, each leaf was shaken 50 times in a glass jar containing 30mL of ethyldigol. The ethyldigol was filtered to remove any debris and the concentration of tracer recovered from each sample was measured using a digital fluorometer. The amount of spray deposit per area of leaf was determined. This was expressed as the quantity of tracer recovered (ng tracer/cm^2 of leaf) per quantity applied per hectare (g/ha).

Experiment 2

On 11 April 2002, an experiment was conducted on a commercial capsicum farm to evaluate different spray rig and nozzle type combinations. The trial was established as a completely randomised design with three replicate plots of each treatment. Plots comprised 10m row lengths of capsicum planted in double-rows. Each plot was separated by two metre guard plots at either end, and an untreated row was positioned between each treated row as a buffer to minimise spray drift contamination. Eight treatments were applied as follows:

Albuz ceramic hollow cone red (2.0) (motorised hydraulic knapsack, no air assist)
Teejet TJ60 1103 twin jet nozzles (motorised hydraulic knapsack, no air assist)
Teejet TP 11003 flat fan nozzles (motorised hydraulic knapsack, no air assist)
Teejet AI 11003 air induction nozzles (motorised hydraulic knapsack, no air assist)
Albuz ceramic hollow cone red (2.0) (modified knapsack, air assist)
Teejet TJ60 1103 twin jet nozzles (modified knapsack, air assist)
Teejet TP 11003 flat fan nozzles (modified knapsack, air assist)
Teejet AI 11003 air induction nozzles (modified knapsack, air assist)

Helios 500SC (Uvitex), a fluorescent tracer, was added to water in the spray tank at rate of 20g/ha. The tracer was sprayed at 300L/ha and 4 bar (56psi). All sprays were applied with a single pass of the spray rig (5km/hr) in an east-west direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry and leaves were collected from 4 positions on each plant, the top outer canopy, the top inner canopy, the bottom outer canopy and the bottom inner canopy. Ten plants were sampled per plot. After collection, the leaf area of each leaf that was sampled was determined using a leaf area meter (model LIA6100).

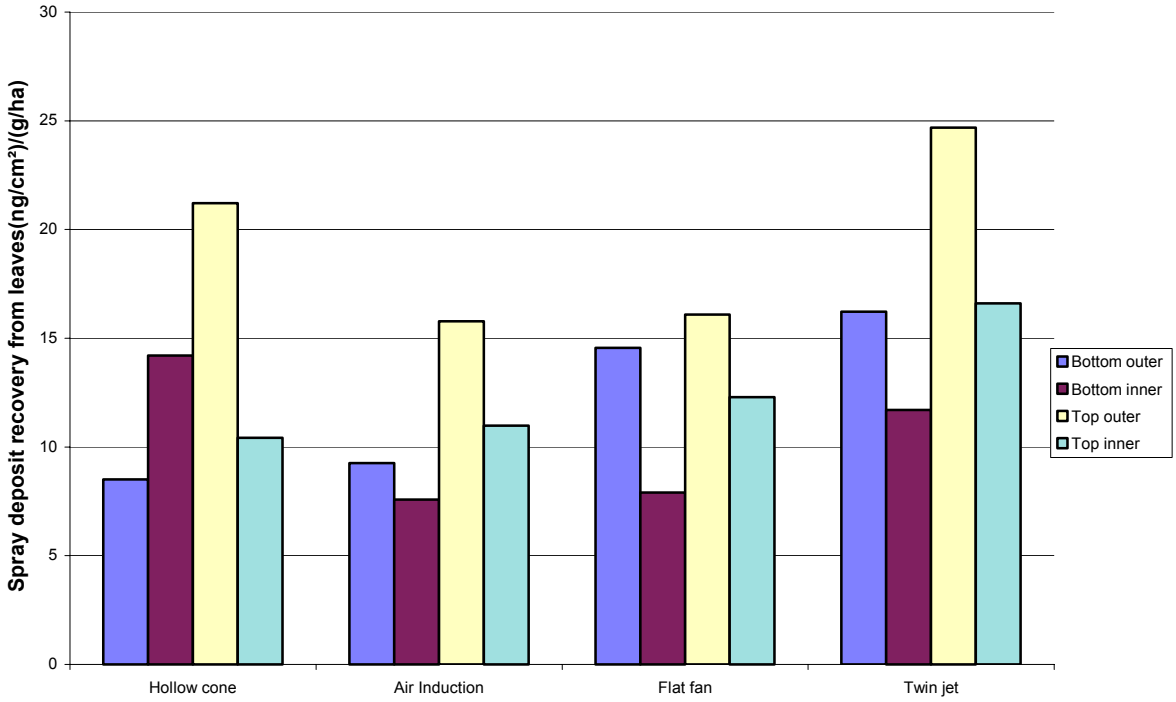
To remove the tracer from the leaves, each leaf was shaken 50 times in a glass jar containing 30mL of ethyldigol. The ethyldigol was filtered to remove any debris and the concentration of tracer recovered from each sample was measured using a digital fluorometer. The amount of spray deposit per area of leaf was determined. This was expressed as the quantity of tracer recovered (ng tracer/cm^2 of leaf) per quantity applied per hectare (g/ha).

6.2.3.2 Results

Experiment 1

Twin jet nozzles appeared to provide the best overall spray coverage at all parts of the plant canopy when they were applied with a conventional hydraulic boom (Figure 16).

Figure 16: Spray recovery from capsicum leaves collected from different portions of the plant canopy. Comparison of nozzle types



Experiment 2

The observation that twin –jet nozzles appeared to provide superior overall spray coverage of plants than other nozzle types with a conventional boom (Experiment 1) was tested here in a replicated field trial. Regardless of whether sprays were applied with a conventional boom or with an air assisted rig, the twin-jet nozzles out performed the other nozzle types in providing coverage of the lower plant canopy ($P<0.05$)(Table 3) and were equivalent to the best nozzle types for coverage of the upper plant canopy ($P<0.05$)(Table 3).

Table 3: Spray deposit recovery from capsicum leaves collected from different portions of the plant canopy. Comparison of spray nozzles.

Nozzle Type	Bottom outer leaves	Bottom inner leaves	Top outer leaves	Top inner leaves
Flat Fan	2.716**a**	2.521**a**	3.117**a**	3.139**a**
Hollow Cone	3.082**ab**	2.852**b**	3.511**b**	3.039**a**
Air Induction	3.224**b**	2.951**b**	3.062**a**	3.582**b**
Twin Jet	3.794**c**	3.544**c**	3.627**b**	3.540**b**
LSD (*P*<0.05)	**0.4812**	**0.2508**	**0.2592**	**0.3719**

The air-assisted spray rig provided significantly ($P<0.05$) better coverage of the bottom portion of the capsicum plant canopy than the conventional rig. For the top portions of the plant canopy, both rig types provided equivalent spray coverage (Table 4).

Table 4: Comparison of a conventional boom and an air-assisted boom for providing coverage of different portions of the capsicum plant canopy

Spray rig type	Bottom outer leaves	Bottom inner leaves	Top outer leaves	Top inner leaves
Air assisted	3.435**b**	3.190**b**	**NSD**	**NSD**
Conventional	2.973**a**	2.743**a**	**NSD**	**NSD**
LSD (*P*<0.05)	**0.3403**	**0.1773**	**-**	**-**

6.2.4 Discussion

An air-assisted boom fitted with twin jet nozzles was the best spray rig combination for optimal spray coverage of the capsicum plant. When the plants were young (at the early-flowering stage) a spray volume of 300L/ha gave optimal coverage of all portions of the plant canopy. At this stage of growth, the point of runoff was exceeded by greater volumes, with the excess chemical being lost from the plant as runoff. For plants with a fully developed canopy (during harvest) the spray volume should be increased to 800L/ha. In the event that an air-assisted sprayer is not available, substantial improvement in spray coverage of capsicums may be achieved by attaching short droppers to a conventional rig. Although, not as comprehensive as the coverage from an air-assisted rig, the conventional boom/dropper combination provided a more even coverage of the entire plant canopy compared that provided by a conventional boom without droppers. The conventional boom alone predominately distributed spray in the top portions of the plant canopy.

6.3 Bacterial Fruit Blotch of Melons

6.3.1 Assessment of Spray Rigs

6.3.1.1 Materials and Methods

Seedlings of rockmelon cv. Eastern Star and watermelon cv. Phantom were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Gatton Research Station. Three rows of rockmelon and three of watermelon were planted on beds (90m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Spray rig assessments were conducted once, 8 weeks after transplant (14 February 2001), when the plant canopy was at its most dense. Two types of spray rigs were compared in the trial and each was utilised on both the rockmelons and the watermelons:

Hardi twin-force air assisted sprayer (with air-assist)
Hardi twin-force air assisted sprayer (without air-assist)

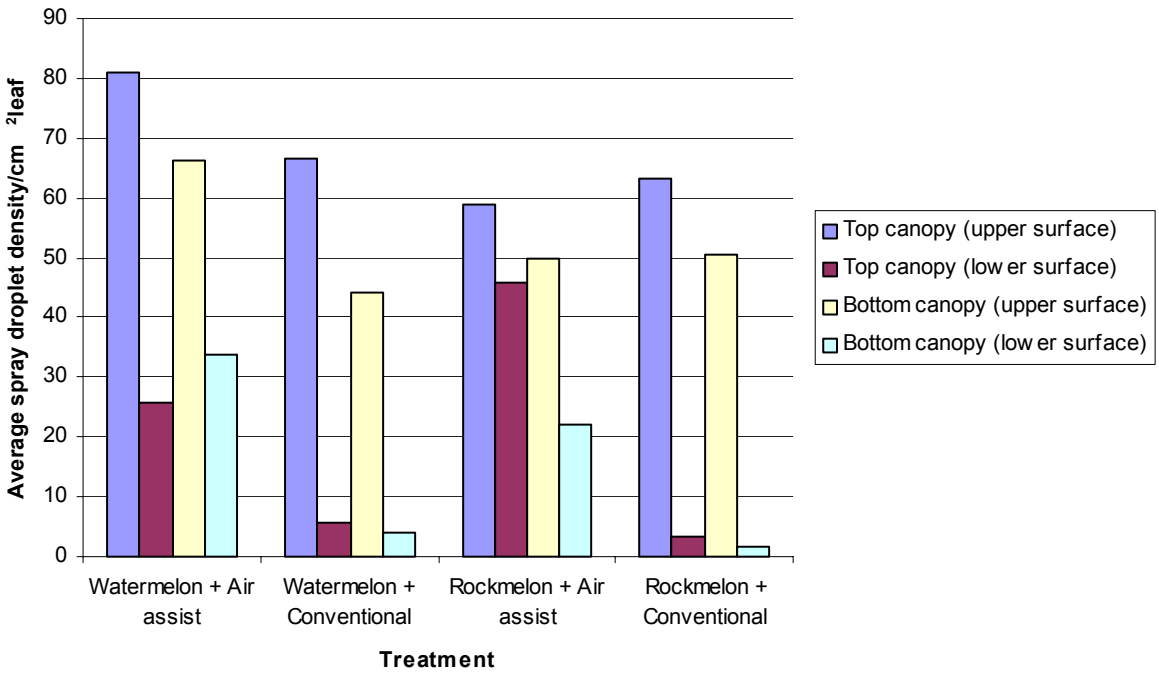
A uniformity trial was established comprising four plots. Each plot was 40m long x 3 rows wide. A UV fluorescent pink dye was added to water in the spray tank at rate of 2%. The dye was sprayed from each rig type at 300L/ha and 4 bar (56psi) using hollow cone (Albuz ATR red) nozzles. One plot of rockmelon and one of watermelon was sprayed with each rig type. All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry for 1-2 hours and 5 leaves were selected at random from the top of the plant canopy and 5 from the bottom of the plant canopy from each plot.

For each leaf sampled, the density of spray droplets was determined for 5 locations on the top and 5 locations on the bottom of each leaf under an ultra violet light in a dark room. The average droplet density for the upper and lower leaf surfaces was determined. For each leaf the percentage spray coverage was also calculated

6.3.1.2 Results

The air-assisted spray rig outperformed the conventional rig in providing greater overall canopy spray coverage for both watermelon and rockmelon. In particular, the air assistance provided improved spray coverage of the underside of the leaves, regardless of their position in the plant canopy (Figure 17).

Figure 17: Spray droplet density recovered from upper and lower leaf surfaces of leaves collected from the top and bottom of the plant canopy in rockmelon and watermelon crops



6.3.2 Assessment of Application Volumes

6.3.2.1 Materials and Methods

Seedlings of rockmelon cv. Eastern Star and watermelon cv. Phantom were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Gatton Research Station on 8 January 2001. Six rows of rockmelon and six of watermelon were planted on beds (180m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Spray volume assessments were conducted twice, 3 weeks after transplant at early flowering (30 January 2002) and at harvest (6 March 2002). Three spray volumes were compared, 300L/ha, 500L/ha and 1000L/ha. All sprays were applied with a Hardi twin-force air assisted boom (with air assist) fitted with hollow cone nozzles (Albuz ATR red nozzles).

Two separate trials were conducted, one for watermelons and the other for rockmelons. The trials were conducted as completely randomised designs, with 6 replicate plots per treatment. Each row was divided into 12 x 15m plots, so that there were a total of 36 plots for watermelons and 36 for rockmelons. At the first assessment time, half the plots were sprayed with the treatments and at the second assessment, the remaining half were used (to avoid potential cross contamination of leaves with fluorescent dye sprayed in an earlier assessment) Treatments were randomly assigned to plots. A UV fluorescent pink dye was added to water in the spray tank at rate of 2%. The dye was sprayed from each rig type at 300L/ha and 4 bar (56psi) using hollow cone (Albuz ATR red) nozzles. All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry for 1-2 hours and 5 leaves were selected at random from each plot.

For each leaf sampled, the density of spray droplets was determined for 5 locations on the top and 5 locations on the bottom of each leaf under an ultra violet light in a dark room. The average droplet density for the upper and lower leaf surfaces was determined. For each leaf the percentage spray coverage was also calculated.

6.3.2.2 Results

The three application volumes trialed (300L/ha, 500L/ha and 1000L/ha) all produced a similar coverage of upper and lower leaf surfaces of watermelon plants (Figure 18). When the canopy of the watermelon plants was dense during harvest, 300L/ha and 500L/ha gave inferior coverage of leaf surfaces, particularly those at the bottom of the plant canopy, than was provided by 1000L/ha (Figure 19). For early stages of plant growth, therefore, 300L/ha is sufficient volume to achieve optimal spray coverage of watermelons. Any additional volume at this time is lost as runoff. However, as the plants mature and the canopy density increases, spray volumes need to be increased to 1000L/ha to ensure that optimal coverage is attained.

Figure 18: Spray droplet recovery on watermelon leaves (upper and lower leaf surfaces) collected from different parts of the plant canopy. Early flowering stage.

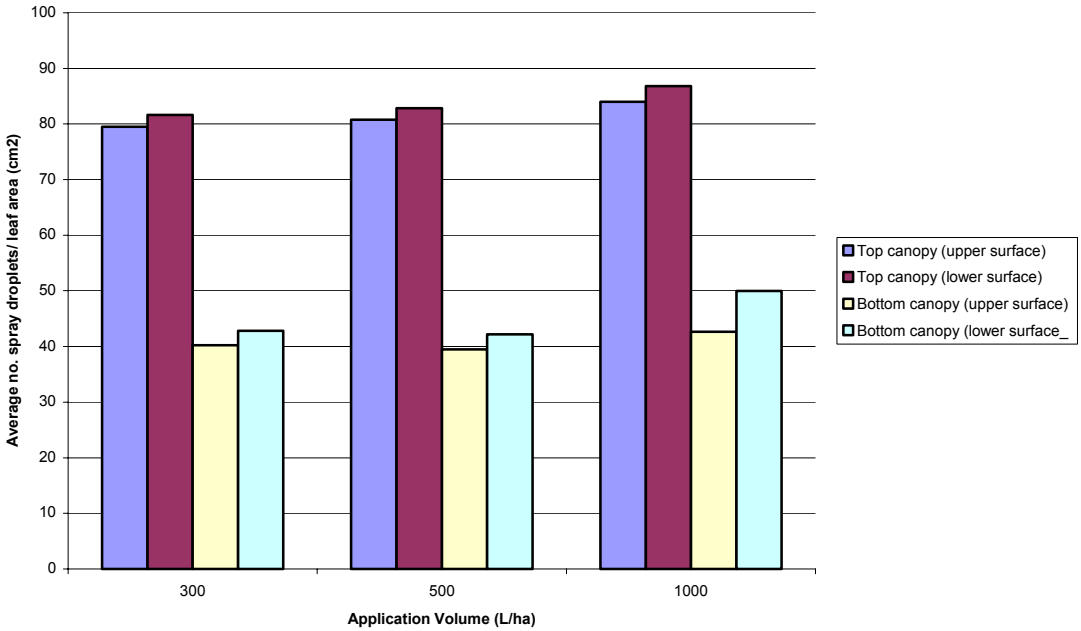
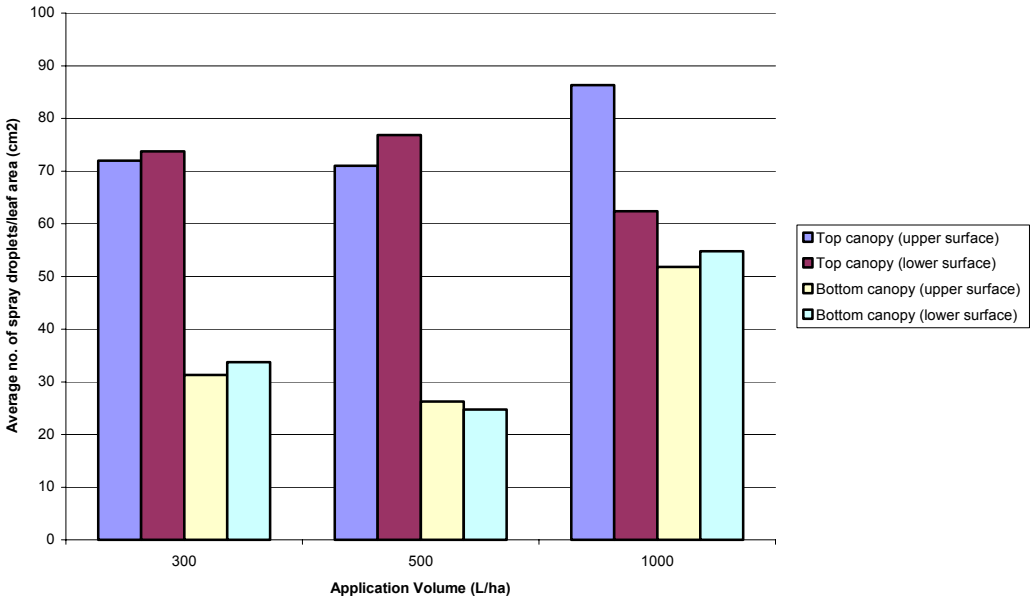
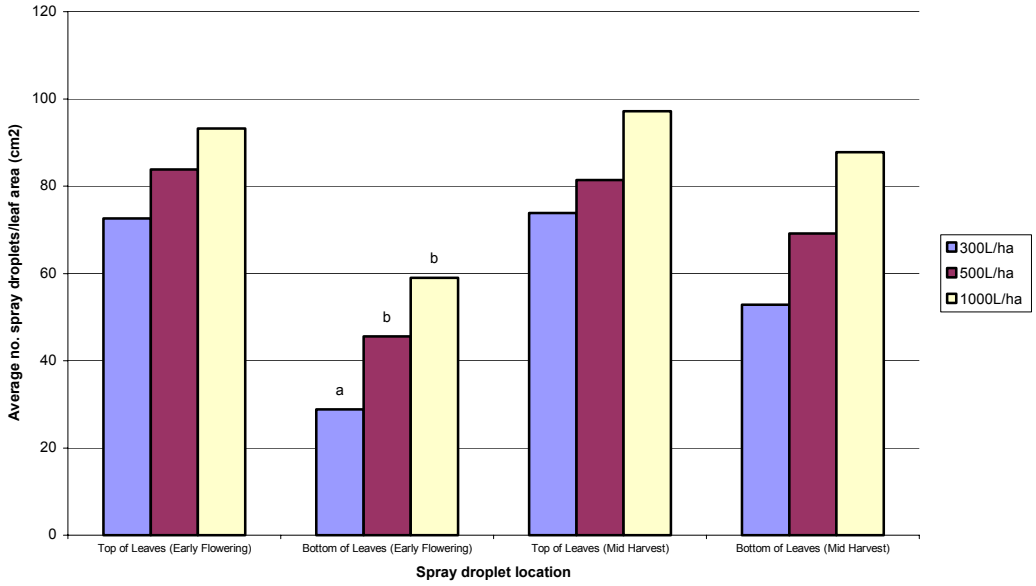


Figure 19: Spray droplet recovery on watermelon leaves (upper and lower leaf surfaces) collected from different parts of the plant canopy. Early harvest stage.



At the early flowering stage of plant development, all three volumes trialed produced comparable coverage on the upper surfaces of rockmelon leaves, however 300L/ha was significantly inferior to either 500 or 1000L/ha in providing coverage of the lower leaf surfaces (P<0.05). At the mid harvest stage, 1000L/ha gave greater coverage of both upper and lower leaf surfaces, however the magnitude of the coverage was not significantly (P<0.05) greater than that provided by either 300L/ha or 500L/ha (P>0.05) (Figure 20).

Figure 20: Droplet coverage on rockmelon leaves sprayed with varying application volumes. Leaves collected at early flowering stage and at harvest



6.3.3 Assessment of Nozzle Types

6.3.3.1 Materials and Methods

Experiment 1

Seedlings of watermelon cv. Phantom were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Gatton Research Station. Three rows were planted on beds (90m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Nozzle assessments were conducted once, 10 weeks after transplant (26 February 2001), when the plant canopy was at an advanced stage of growth. Four nozzle types were compared in the trial:

Albuz ceramic hollow cone red (2.0)
Teejet TJ60 1103 twin jet nozzles
Teejet TP 11003 flat fan nozzles
Teejet AI 11003 air induction nozzles

A uniformity trial layout was used comprising four plots. Each plot was 15m long x 3 rows wide, and a 10m buffer of untreated plants was positioned between the plots. A UV fluorescent pink dye was added to water in the spray tank at rate of 2%. The dye was sprayed from each nozzle type at 300L/ha and 4 bar (56psi) using a conventional boom (Hardi twin-force air assisted sprayer (without air-assist)). One plot was sprayed with each nozzle type. All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry for 1-2 hours and 5 leaves were selected at random from the top of the plant canopy and 5 from the bottom of the plant canopy from each plot.

For each leaf sampled, the density of spray droplets was determined for 5 locations on the top and 5 locations on the bottom of each leaf under an ultra violet light in a dark room. The average droplet density for the upper and lower leaf surfaces was determined. For each leaf the percentage spray coverage was also calculated

Experiment 2

Seedlings of watermelon cv. Phantom were raised in plastic speedling trays and transplanted at 0.5m spacings in single rows on Gatton Research Station on 18 December 2001. Six rows were planted on beds (90m x 1.5m) that were irrigated by overhead irrigation applied through solid set pipes. Nozzle assessments were conducted once, 12 weeks after transplant (13 March 2002), when the plant canopy was at its most dense. Four nozzle types were compared in the trial:

Albuz ceramic hollow cone red (2.0)
Teejet TJ60 1103 twin jet nozzles
Teejet TP 11003 flat fan nozzles
Teejet AI 11003 air induction nozzles

Treatments were applied with two spray rig types, a motorised hydraulic knapsack (model Solo 442)(Conventional boom) and a modified motorised hydraulic knapsack with air assistance (model Solo 442)(Air assisted boom). The trial was conducted as a completely randomised design with 3 replicate plots per treatment. Plots comprised single row lengths 5m long, with 2m long unsprayed guard plots at either end. Single rows were left as unsprayed guard rows between the treated rows, to help minimise cross contamination of plots with spray drift.

A UV fluorescent pink dye was added to water in the spray tank at rate of 2%. The dye was sprayed at 300L/ha and 4 bar (56psi). All sprays were applied with a single pass of the spray rig (5km/hr) in a south-north direction. During spraying the wind was blowing from the SE at between 0 and 5 $msec^{-1}$. After the sprays had been applied, plants were allowed to dry for 1-2 hours and 10 leaves were selected at random from each plot.

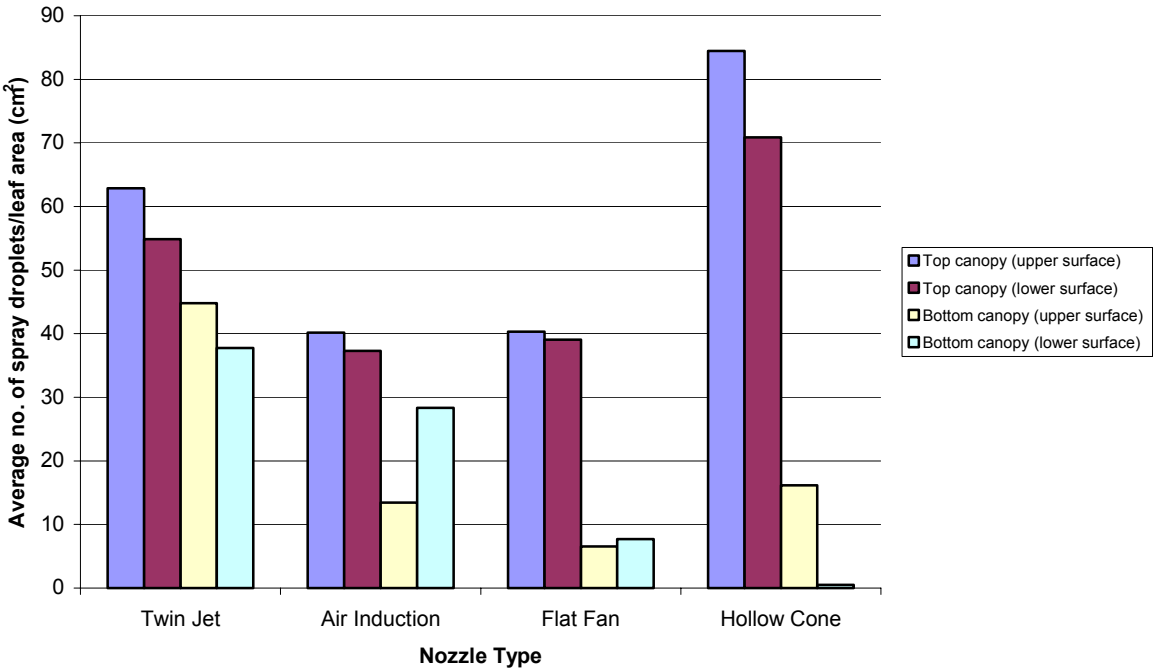
For each leaf sampled, the density of spray droplets was determined for 5 locations on the top and 5 locations on the bottom of each leaf under an ultra violet light in a dark room. The average droplet density for the upper and lower leaf surfaces was determined. For each leaf the percentage spray coverage was also calculated

6.3.3.2 Results

Experiment 1

Even spray coverage throughout the entire plant canopy is the objective when spraying melon plants. With this in mind, twin jet nozzles were the most effective of the nozzles in the trial. Twin jet nozzles provided the most even canopy coverage. The hollow cone nozzles performed exceptionally well in providing maximum spray coverage to the top section of the canopy, however, the superior upper canopy coverage was at the expense of coverage of leaves at the bottom of the canopy. The flat fan and air induction nozzles both failed to provide high levels of coverage on either side of leaves collected from anywhere in the canopy (Figure 21).

Figure 21: Spray coverage on watermelon leaves sprayed with different nozzle types using a conventional boom. Leaves collected from the upper and lower canopy



Experiment 2

In this trial, optimal spray coverage of the undersides of rockmelon leaves was achieved with twin jet or hollow cone nozzles fitted to an air-assisted spray boom. These two treatments achieved significantly greater droplet densities on the bottom of the leaves ($P<0.05$) than any other nozzle/rig combination trialed (Table 5).

Table 5: Average number of spray droplets recovered per cm^2 from the bottom surfaces of rockmelon leaves sprayed with different spray rig/nozzle combinations

Nozzle	Spray Rig	Average no. spray droplets/cm^2
flat fan	air	73.1**a**
flat fan	conventional	83.5**a**
air induction	conventional	85.6**a**
air induction	air	108.8**ab**
hollow cone	conventional	113.1**ab**
twin jet	conventional	156.5**b**
twin jet	air	253.6**c**
hollow cone	air	272.5**c**
LSD (*P*<0.05)		**55.90**

Coverage of the topsides of the leaves was significantly greater with twin jet nozzles, than when other nozzles were used ($P<0.05$)(Table 6). However, air assisted spray application produced significantly poorer coverage of leaf tops compared to the conventional rig, regardless of which nozzle was used (Table 7).

Table 6: Average number of spray droplets recovered per cm^2 from the top surfaces of rockmelon leaves sprayed with different nozzles

Nozzle	Average no. spray droplets/cm^2
air induction	449.9**a**
flat fan	547.9**b**
hollow cone	654.0**c**
twin jet	759.6**d**
LSD (*P*<0.05)	45.15

Table 7: Average number of spray droplets recovered per cm^2 from the top surfaces of rockmelon leaves sprayed with different spray rigs

Spray Rig	Average no. of spray droplets/cm^2
air assisted	582.1**a**
conventional	623.5**b**
LSD(*P*<0.05)	**31.93**

Twin jet nozzles appear, on the basis of this experiment to be the nozzle of choice for achieving optimal spray coverage of entire leaf surfaces of the rockmelon plant. Since coverage of the underside of leaves is more likely to be a limiting factor to spray application efficacy than coverage of the topsides, it seems reasonable to conclude from the results that air assisted spray application should be utilised for rockmelons, since the air assistance in combination with twin jets or hollow cones, provide significantly greater droplet coverage on leaf undersides than other nozzle/rig combinations.

6.3.4 Discussion

An air-assisted spray rig fitted with twin jet nozzles was the most effective combination for optimal spray coverage of both watermelon and rockmelon. It proved more difficult to achieve coverage of all surfaces of rockmelon plants than it did to adequately cover the watermelon canopy. At an early flowering stage, 300L/ha provided the maximum coverage of the upper and lower leaf surfaces at all positions in the watermelon plant canopy, whereas 500L/ha was required to achieve maximum coverage of the rockmelon plant at the same stage of development. At early harvest, when the canopies of both melon types were at their most dense, 1000L/ha was needed to achieve coverage of the entire plant canopy for both watermelons and rockmelons.

7. General Discussion

The detection of copper-tolerance in Queensland populations of bacterial spot of capsicum and the dominance of a field population of the pathogen by tolerant strains after regular applications of copper, are the most important findings in this study. This information provides strong evidence that failure of copper bactericides to control field epidemics of bacterial spot in Queensland can be attributed, at least in part, to the presence of copper-tolerant strains in the pathogen populations. For capsicum growers, managing copper tolerance in bacterial spot populations will involve:

- use of copper hydroxide/mancozeb spray combinations
- optimising spray coverage of the capsicum plant
  - air assisted spray rig (or conventional boom with droppers if an air assisted rig is not available)
  - twin-jet nozzles
  - spray volumes of 300L/ha (young plants – early flowering) and 800L/ha for mature plants (during harvest)
- employment of a "capsicum-free" plant period in order to minimise carry-over of tolerant strains into subsequent crops

These measures will become particularly important in growing districts where farms are in close proximity to one another or if sequential capsicum crops are to be planted, where there is the possibility for carry-over of disease from older crops to new. In these instances the bacterial population will be exposed to many copper applications, increasing the likelihood that the population will become dominated by copper-tolerant strains.

Copper-tolerant strains of bacterial fruit blotch were also found in low frequency in north Queensland. This study has provided important base-line information on the copper-sensitivity status of bacterial fruit blotch populations in Queensland. The populations will need to be monitored over the coming years to determine if these copper-tolerant strains increase in frequency. All isolates of black rot were sensitive to copper.

For all three bacterial diseases examined in this project, mancozeb + copper hydroxide combination treatments (either Mankocide or mancozeb + Kocide DF) provided the most effective disease control. These results are in accordance with those of similar studies conducted outside Australia (Conlin and McCarter, 1983; Andersen *et al.*, 1991; Ritchie and Bennett, 1991 and Scheck and Pscheidt, 1998). Our work also suggested that adjuvants may assist in improving spray coverage of the plant and consequently, improving disease control. This avenue should be investigated further in additional studies to demonstrate the relationship between disease control and improved spray coverage.

Finally, our studies into spray application optimisation highlighted the benefits of both air assisted spray rigs and twin-jet nozzles. Both melons and capsicums are difficult crops to spray effectively; melons because of their prostrate growth habit and capsicums because of their deep, dense canopy. In both instances air assistance helped to force fine droplets into the canopy, the added air movement pushing air

currents carrying fine droplets into the bottom of the canopy and up under the leaves. Twin jet nozzles have two small orifices, one angled forwards and one angled backwards. The two small orifices allow large quantities of very fine droplets to be produced, which, when applied with an air-assisted boom, are transported on air currents into the plant canopy. In contrast, flat fans and air induction nozzles produce much larger droplets, which are not transported as easily by air currents from an air-assisted boom. Consequently, the inferior performance of the flat fan and air induction nozzles compared to the twin jets was not surprising. In the field assessment of application volumes on capsicum plants, we established 300L/ha to be the optimal volume for capsicums at an early flowering stage and 800L/ha for capsicums at a mature stage. These results support previous work conducted in Spain, in which 300L/ha was identified as the point of runoff for 40cm high capsicum plants (early flowering stage) and 800L/ha was identified a the point of runoff for 95cm high capsicum plants (harvest stage).

Copper hydroxide/mancozeb spray combinations, spray volumes approaching the point of runoff applied with air assisted booms fitted with twin jet nozzles and "capsicum free" plant periods should now be employed collectively to manage copper-tolerance in Queensland populations of bacterial spot of capsicum.

8. Technology Transfer

A series of grower workshops was completed from 20th-29th May 2002 to communicate the research results from this project to the Queensland melon and vegetable industries. Workshops were held at four locations across the state: Chinchilla, Bundaberg, Gumlu and Ingham. The workshops were also scheduled to coincide with a visit by Dr Meg McGrath, Cornell University, New York. Dr McGrath, a world authority on cucurbit powdery mildew, capsicum bacterial spot and other vegetable diseases, presented her research results on powdery mildew and capsicum bacterial spot to the Queensland growers, and also provided information to local industry on several severe melon and capsicum diseases which are not yet present in Australia. Heidi Martin and Glenn Geitz both presented results from the current project at these meetings. As a component of the workshops, on farm spray application demonstrations were held so that growers could assess for themselves the nozzle type that gave the best spray deposit on capsicums and melons in a growers field (Plates 1 and 2). Workshop attendees were each provided with a folio of information, including: a booklet entitled " Efficient Pesticide Application in Vegetable Crops", summaries of research results by Heidi Martin, Glenn Geitz and Meg McGrath and information on current chemical registrations for capsicum and melons and fungicide mode of action groups. The workshop was repeated in the Lockyer Valley on 15 August 2002. More than 60 people attended the workshop series and information from the meeting was subsequently mailed to a further 10 growers after a pest and disease information meeting was held in Bowen in March 2003.

Plate 1: Spray application demonstration, capsicum (Bundaberg workshop, 22 May 2002)

Plate 2: Growers assessing spray droplet coverage using black light (Bundaberg workshop, 22 May 2002)

Several newspaper releases on the project have also been published: "Queensland Country Life", July 11, 2002, pg 27 and "Rural Weekly", July 5th 2002. Articles have also been published in "Melon News" (the magazine of the Australian Melon Association), Vol 12, June 2002, pg 2 and in the Bundaberg district growers magazine.

Heidi Martin and Glenn Geitz also attended the National Melon Conference, 25th-27th July 2002 in Melbourne. Posters were prepared and displayed at the conference on project outcomes. An abstract about the project work was also included in the conference proceedings. All newspaper articles, and conference materials are included in Appendix 1.

9. Recommendations

- Copper-tolerance in Queensland populations of bacterial spot of capsicum (*Xanthomonas campestris* pv. *vesicatoria*) is present and is responsible, at least in some instances, for poor field control despite the regular use of copper sprays.
- Copper-tolerant strains of bacterial spot will dominate the pathogen population if regular copper sprays are applied.
- Copper-tolerant isolates of bacterial fruit blotch of melons (*Acidovorax avenae* subsp. *citrulli*) were detected in low frequency in north Queensland pathogen populations.
- Black rot isolates collected from the Granite Belt were all sensitive to copper.
- Copper hydroxide/mancozeb sprays should be utilised as the standard industry treatment for control of all three pathogens in Queensland
- Twin jet nozzles and air assisted spray rigs should be utilised in spraying capsicum and melon crops.
- For capsicums, if an air assisted rig is not available, droppers should be attached to a conventional boom.
- Young capsicum plants (early flowering stage) should be sprayed at a rate of 300L/ha, and this should be increased to 800L/ha when the plants are mature.
- For watermelons, 300L/ha is optimal when plants are at an early flowering stage, and this should be increased to 1000L/ha when the vines are mature
- For rockmelons, 500L/ha is optimal when plants are at an early flowering stage, and this should be increased to 1000L/ha when the vines are mature

10. Acknowledgements

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12. Appendix