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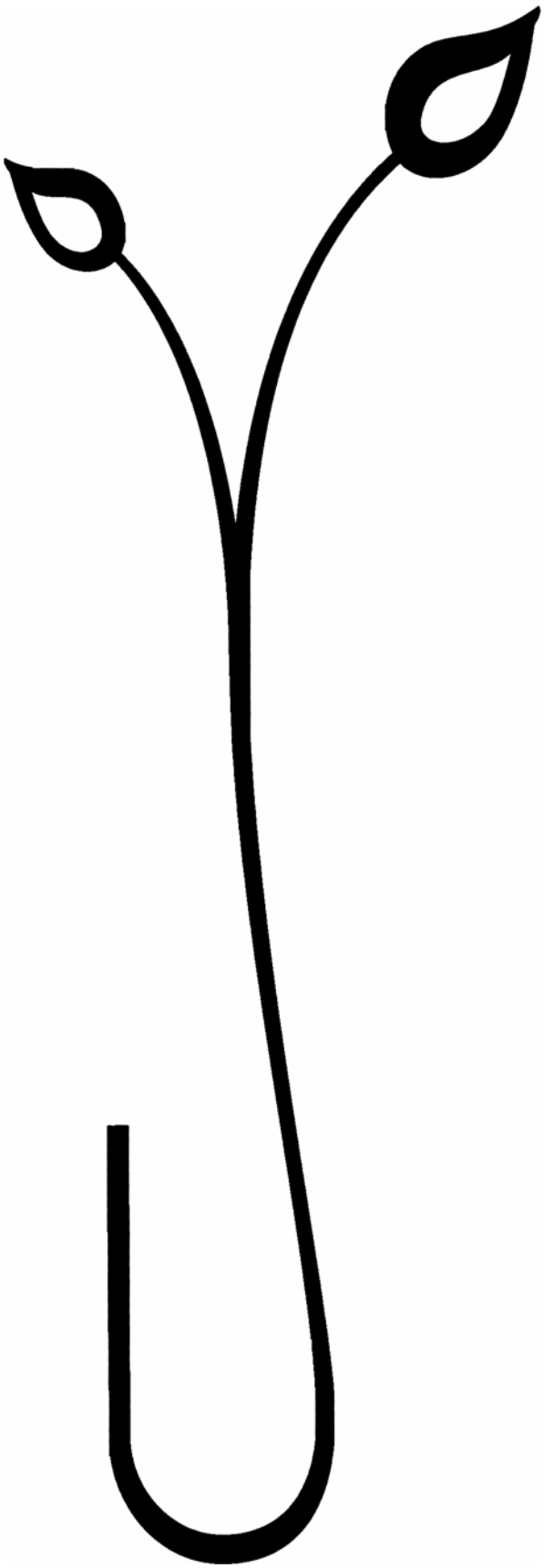

FINAL REPORT

FV 186a
Brassicas: use of copper sprays to control black rot during transplant production

Project title:	**Brassicas: use of copper sprays to control black rot during transplant production**
Report:	**Final**
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Objectives and background

Black rot of brassicas, caused by the bacterium *Xanthomonas campestris* pv *campestris* (*Xcc*), has been causing considerable and increasing concern to growers and seedsmen in the UK in recent years, particularly in winter cabbage and cauliflower crops. Although originally thought to cause serious problems in the South West, problems have now been reported in all the major *Brassica* production areas of England.

Symptoms are most frequently seen in the field as wedge-shaped yellow necrotic lesions developing from the edges of leaves. The pathogen colonises the vascular system giving rise to characteristic blackened veins. Infection often leads to premature defoliation, plants may be stunted and crop quality reduced. The disease also results in increased susceptibility to *Alternaria* and to secondary bacterial soft-rots which may result in complete crop loss. There are no approved chemicals available for control of black rot.

The disease is considered to be primarily seed-borne, and although there may be other sources of infection (e.g. soil, weeds, crop debris), their relative importance has not been established. Plant to plant spread in the field is by water splash and machinery. The majority of commercially available seed is tested for the presence of *Xanthomonas campestris* pv. *campestris*. One possible reason for the recent increases in the disease may be that the currently applied quality standard of 0.01% infection (1 in 10,000 seeds) is inadequate for the current intensive and centralised transplant production systems, where opportunities for pathogen dissemination are rife.

Thus, it is possible that many of the disease outbreaks seen in the field result from low levels of seed infection combined with very high rates of plant to plant and tray to tray spread of disease during transplant raising. Preliminary results from the current MAFF-funded project have confirmed that these rates of spread during plant-raising can be considerable. There is very little individual growers can do about the low levels of seed infection other than demand that seed is tested to the highest possible standards. Even then, there will always be the statistical possibility that some infected seed lots will get onto the market. Reducing the rate of disease spread and development during plant raising provides the next best target for control.

Currently the only registered pesticides with bactericidal activity are copper compounds. Although none are approved specifically for the control of black rot in brassicas, copper oxychloride has off-label approval for field application for control of bacterial spear rot in calabrese. The effectiveness of copper is limited to a protectant action and activity is likely to be highly dependant on being able to maintain sufficient concentration of copper on the leaves.

The objective of this project was to investigate the use of copper oxychloride in a spray program during plant raising as a means of eliminating or reducing the rate of spread of *Xcc* during plant raising. MAFF-funded work had already established the appropriate experimental procedures for monitoring the rate of spread of *Xcc* from single infected seedlings, thereby mimicking the most likely commercial scenario. It should be noted that it is not sufficient to rely on visual observation of symptoms as spread of the pathogen greatly precedes symptoms. This project builds on experience gained during MAFF-funded work and will compare the rate of spread of *Xcc* in module trays sprayed with copper with the rate of spread in un-treated trays.

If spread during plant raising is the most important phase in the development of black rot epidemics, the approach suggested here is likely to have a much greater chance of success than using bactericidal sprays in field crops. In addition, because of the protected environment it may be easier to maintain adequate copper concentrations and/or combine with other measures to reduce the rate of spread/infection. By using the pesticide at the early stages of plant production in a relatively controlled environment the risks of residues remaining on the crop at harvest are minimised, as are the amount of pesticide required (hence costs) and any adverse environmental effects.

Summary of results

- A single cell in one tray at the end of each of four blocks of 15 '308' module trays was sown with seed inoculated with *Xcc*..
- Six sprays of copper oxychloride (Cuprokylt) were applied to half of the blocks of trays at the rate of 8.3 g of product/l with 0.25 ml/l of Agral wetter at weekly intervals.
- The development of disease symptoms was monitored visually and symptomless spread of the pathogen was monitored by carrying out leaf washings.
- Levels of black rot were significantly reduced in the copper-sprayed treatment.
- Reductions were seen in all of the measured disease parameters: apparent disease transmission, rate of symptom development, rate of disease and pathogen spread, pathogen numbers.

Action points for growers

- Copper compounds appear to have considerable potential as a tool for the management of black rot of Brassicas.
- It is not known whether the disease reductions achieved in this single experiment can be repeated or translated into disease reductions in the field.
- Further work is needed before recommendations can be made on the use of copper in the control of black rot in Brassica transplants.
- Growers should continue to implement other (non-chemical) disease management strategies for black rot.

Practical and financial benefits

The UK Brassica crop is worth over £200 million. Brassica growers are under constant pressure from the major retailers who are demanding ever greater standards of quality and uniformity. Foreign competition also contributes to the squeeze on grower returns. As a result, diseases which affect not only yield but also quality, growing and harvesting costs may have a major impact on the profitability of the crop. There is no doubt that *Xanthomonas* falls into this category and is perceived as one of the major disease problems by growers. Therefore information which may lead to elimination or improved control of the disease is of direct benefit to growers.

This experiment was intended as a first step to determine the *potential* of copper for use in the control of black rot of Brassicas. This potential has now been clearly demonstrated and further work is now needed to develop recommendations for turning this potential into a realistic and effective tool for the management of black rot.

SCIENCE SECTION

Introduction

Black rot of brassicas, caused by the bacterium *Xanthomonas campestris* pv *campestris* (*Xcc*), has been causing considerable and increasing concern to growers and seedsmen in the UK in recent years, particularly in winter cabbage and cauliflower crops. Although originally thought to cause serious problems in the South West, problems have now been reported in all the major *Brassica* production areas of England.

Symptoms are most frequently seen in the field as wedge-shaped yellow necrotic lesions developing from the edges of leaves. The pathogen colonises the vascular system giving rise to characteristic blackened veins. Infection often leads to premature defoliation, plants may be stunted and crop quality reduced. The disease also results in increased susceptibility to *Alternaria* and to secondary bacterial soft-rots which may result in complete crop loss. There are no approved chemicals available for control of black rot.

The disease is considered to be primarily seed-borne, and although there may be other sources of infection (e.g. soil, weeds, crop debris), their relative importance has not been established. Plant to plant spread in the field is by water splash and machinery. The majority of commercially available seed is tested for the presence of *Xanthomonas campestris* pv. *campestris*. One possible reason for the recent increases in the disease may be that the currently applied quality standard of 0.01% infection (1 in 10,000 seeds) is inadequate for the current intensive and centralised transplant production systems, where opportunities for pathogen dissemination are rife.

Thus, it is possible that many of the disease outbreaks seen in the field result from low levels of seed infection combined with very high rates of plant to plant and tray to tray spread of disease during transplant raising. Preliminary results from the current MAFF-funded project have confirmed that these rates of spread during plant-raising can be considerable. There is very little individual growers can do about the low levels of seed infection other than demand that seed is tested to the highest possible standards. Even then there will always be the statistical possibility that some infected seed lots will get onto the market. Reducing the rate of disease spread and development during plant raising provides the next best target for control.

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The objective of this project was to investigate the use of copper oxychloride in a spray program during plant raising as a means of eliminating or reducing the rate of spread of *Xcc* during plant raising. MAFF-funded work has already established the appropriate experimental procedures for monitoring the rate of spread of *Xcc* from single infected seedlings, thereby mimicking the most likely commercial scenario. It should be noted that it is not sufficient to rely on visual observation of symptoms as spread of the pathogen greatly precedes symptoms. This project builds on the experience gained during MAFF-funded work and will compare the rate of spread of *Xcc* in module trays sprayed with copper with the rate of spread in un-treated trays.

Future MAFF-funded work will define the limits for acceptable infection levels in transplants.

If spread during plant raising is the most important phase in the development of black rot epidemics, the approach suggested here is likely to have a much greater chance of success than using bactericidal sprays in field crops. In addition, because of the protected environment it may be easier to maintain adequate copper concentrations and/or combine with other measures to reduce the rate of spread/infection. By using the pesticide at the early stages of plant production in a relatively controlled environment the risks of residues remaining on the crop at harvest are minimised, as are the amount of pesticide required (hence costs) and any adverse environmental effects.

Materials and Methods

Experimental design and layout

Healthy cauliflower seed was sown in '308' module trays (609 x 400 x 45 mm, 22 x 14 cells, 13.5 cm^3 cell volume, Linpac Material Handling, Dunstable, UK). Trays were set out in two blocks of 15 (5 x 3) on each of two benches in a glasshouse with a minimum set temperature of 18°C and venting at 20°C. A single cell in one tray at the end of each block was sown with artificially inoculated seed. One block on each bench was sprayed with copper oxychloride and one block was left untreated. Perspex screens were placed in-between the blocks on each bench to prevent spray drift and limit movement of inoculum. The two benches were separated by one empty bench (approx. 1.5 m). At 33 d after sowing the glasshouse vents were opened continuously to harden off the transplants. The experiment was run for six weeks, the duration of plant raising in normal commercial practice.

In addition, in order to estimate the effect of copper on apparent transmission of *Xcc*, two half-trays were sown entirely with inoculated seed. One was sprayed with copper and one was left untreated as above.

The glasshouse compartment and module trays were disinfected with Jet 5 following the manufacturers recommendations prior to the beginning of the experiment.

Seed and Inoculation

Cauliflower seed of a winter cultivar known to be susceptible to black rot (cv. Miracle) was obtained from Elsoms Seeds, Spalding, Lincolnshire, UK. An isolate of *Xcc* (3818A) was recovered from storage on glass beads at -76°C onto YDC agar (g l^{-1}: yeast extract, 10; $CaCO_3$, 20; Bacto agar, 15; glucose 20). This isolate had been obtained from a crop of cauliflower cv. Miracle which was part of a field trial in Cornwall, UK. After 24 h at 30°C, bacterial growth was scraped from the plate with a sterile spatula and suspended in 4.5 ml of sterile saline (0.85% NaCl) to give a concentration of approx. 10^8 cfu ml^{-1}. This was diluted tenfold with saline to provide the inoculum. Approx. 1,000 seeds were immersed in the bacterial suspension and a vacuum applied for approx. 7 min. The vacuum was released and the seed drained. It was then spread thinly in shallow trays on paper towels and left to dry overnight at room temperature in the air-flow of a fume hood. Bacterial numbers in the inocula were estimated by dilution and plating onto YDC using the drop method of Miles and Misra (Miles and Misra, 1933). After drying the seeds were stored in sealed polythene bags at approx. 4°C until sowing 5 days later.

Seed testing

The number of bacteria on the inoculated seed was determined after inoculation using a method based on International Seed Testing Association Working Sheet No 50. One sample of 30 seeds, three samples of ten seeds and seven samples of one seed were soaked for 2.5 h at room temperature in 3, 1 and 1 ml of sterile saline respectively. The resulting suspensions were diluted and 100 µl of each dilution spread on plates of FS agar medium (Schaad, 1989) with a bent glass rod. Plates were incubated at 30°C for 3 d before counting the number of typical *Xcc* colonies. The identity of a selection of typical colonies was confirmed using *Staphylococcus aureus* slide agglutination (Lyons and Taylor, 1990) with an antiserum specific for *Xcc*.

The health status of the healthy seed was confirmed by soaking aliquots of seeds in saline and diluting and plating as above. After testing the seeds were re-dried at room temperature by spreading out thinly in shallow trays on paper towels in the air-flow of a fume hood.

Tray filling and sowing

Module '308' trays were filled loosely with Levington F1 compost in a standard manner. The surface was levelled and then firmed by pressing it down with another similar tray so that the surface of the compost was 0.5-1 cm below the top of the tray. The seeds were sown with an automatic sower (Hamilton Seeder), one seed per module, and the trays covered with the same compost up to the top of the tray. Trays were numbered in order of sowing and transferred to the glasshouse, where they were arranged in 4 blocks of 15.

Prior to covering with compost the healthy seed in a single cell in the centre of four trays of healthy seed was replaced with two inoculated seeds. These trays were placed ate the end of each of the four blocks.

The two half-trays for estimation of transmission were sown entirely with inoculated seed by hand.

Watering

Trays were water using an overhead gantry irrigation system. Spray heads, with five nozzles providing a 'curtain' of water, were moved slowly up and down the benches by pulleys connected to electric motors. Watering was controlled by an electronic irrigation controller connected to solenoid water valves and the motors driving the spray-heads. Each watering cycle consisted of two passes (once up and once down) along each bench.

The amount of water delivered in a pass was estimated by placing shallow circular trays of known diameter in the path of the spray heads and measuring the volume of water collected.

From 15 d after sowing, a liquid feed (0.33 g l^{-1} KNO_3; 40 ppm N) was applied to all treatments, with every watering, by dosing the water supply line with a concentrated stock solution.

Copper sprays

Copper oxychloride was obtained as a wettable powder formulation (Cuprokylt) containing 50% w/w copper from Universal Crop Protection Ltd., Berkshire, UK. Six sprays of copper were applied at approximately weekly intervals from one week after sowing with a Knapsack sprayer. It was applied at the rate of 8.3 g of product/l with 0.25 ml/l of Agral wetter. This rate was derived from the current SOLA rate for use to control Spear rot on Calabrese. Plants were sprayed until leaves were evenly wetted and the pesticide was just beginning to run off.

Sprays were applied to dry foliage out of direct sunlight and plants were not watered again until the day after spraying.

Records

Temperature. Air temperature was recorded at 30 min intervals using a Tinytag temperature logger.

Emergence. The number of emerged seedlings was counted at 9 d after sowing. The counts were made in two trays chosen at random from each of the four blocks of 15 trays (total of eight trays counted).

Disease symptoms. The location and number of all plants showing black rot symptoms (chlorosis and/or necrosis with blackened veins) was recorded at 2, 4 and 6 weeks after sowing.

Isolations. In order to confirm that the observed black rot symptoms were caused by Xcc, isolations were attempted from at least one plant from each (inoculated) treatment with symptoms considered to be typical. Small (1-2 mm^2) pieces of diseased tissue were comminuted in a drop sterile saline on a sterile microscope slide and observed by phase contrast light microscopy before streaking out the suspensions on plates of YDC. The identity of isolates was confirmed by slide agglutination.

Leaf washings. Leaf washings to estimate apparent transmission in the trays sown with 100% inoculated seeds were done at two weeks after sowing; ten randomly selected plants were sampled from each tray. Leaf washings to estimate the proportion of infested (=contaminated/infected symptomless) plants were done at 6 weeks after sowing; two samples of plants of varying sizes were taken at seven distances (1, 5, 10, 20, 45, 70, 98 cells) from the cells sown with the inoculated seed.

Plants were cut off just below the cotyledons using sterile scissors and put into clean polythene bags. A separate polythene bag was used for each sample of plants and scissors were disinfected by wiping with 70% ethanol between samples. Plants with visible symptoms were separated and treated as separate samples. After collection, plants were stored in a coldroom (*ca.* 4°C) until processing (< 24 h).

Plant samples were put into conical flasks with sterile saline (0.85 % NaCl) and 0.02% tween and shaken for 30 min on a wrist-action shaker. The volume of saline was adjusted according to the size and number of plants in the sample (approx. 2 ml/plant). After shaking, the extracts were diluted (10^{-1} and 10^{-2}) and 100 µl of the undiluted extract and of each dilution was spread onto plates of FS agar with a bent glass rod. Plates were incubated at 30°C for 3 d and the number of typical *Xcc* colonies counted. Identities of the colonies were confirmed by sub-culture to YDC for comparison with the inoculated isolate and by slide agglutination.

Statistical analysis

The mean number of bacteria per seed was estimated as a weighted mean, using the number of seeds in the sample as the weighting factor. The effect of the copper sprays on the proportion of plants with visible symptoms, on the proportion of symptomless contaminated plants (as estimated by the proportion of positive samples) and on the mean numbers of bacteria per plant was studied using the generalised linear modelling facilities of Genstat V (Payne et al. 1993). Analysis was done with treatments specified as qualitative factors to obtain an analysis of deviance equivalent to an analysis of variance.

Table 1. Approximate transmission rate of *Xcc* in the final trays grown with inoculated seed for each copper treatment.

Treatment	% of plants with symptoms	% of plants infested	Mean number of *Xcc* per plant
Un-sprayed	100	100	1.1 (±0.6)E+06
Sprayed	0	60	6.4 (±5.6)E+03

Table 3. Most probable number estimates of the final percentage of infested plants together with upper and lower 95% confidence limits.

Treatment	Bench	Incl. inoculated cells			Ex-inoculated cells		
		% infested	Lower limit	Upper limit	% infested	Lower limit	Upper limit
Un-sprayed	2	24.0	13.0	39.4	22.0	[illegible]	37.3
	4	14.0	7.7	23.3	13.0	6.8	22.0
	Combined	18.0	11.4	25.3	16.0	10.2	23.7
Sprayed	2	0.0	0.0	2.6	0.0	0.0	2.7
	4	4.0	1.3	9.3	3.1	0.8	8.0
	Combined	1.9	0.6	4.3	1.4	0.4	3.7

Table 2. Number (and percentage) of plants with symptoms for each copper treatment.

Treatment	Bench	Week 2	Week 4	Week 6
Un-sprayed	2	2 (0.05)	4 (0.09)	86 (2.04)
	4	1 (0.02)	5 (0.12)	32 (0.72)
	Mean	1.5 (0.04)	4.5 (0.11)	59 (1.38)
Sprayed	2	0 (0.00)	0 (0.00))	0 (0.00)
	4	0 (0.00)	0 (0.00)	2 (0.05)
	Mean	0 (0.00)	0 (0.00)	1 (0.02)

Maximum likelihood estimates of the proportions of contaminated plants together with their 95% likelihood-based confidence intervals were obtained using the STPro program (Ridout and Roberts, 1995).

Results

The suspension used to inoculate the seed contained 4.2 x 10^7 cfu/ml. The weighted mean number of *Xcc* per inoculated seed was 1.2 cfu.

Temperature in the glasshouse during the six weeks of the experiment ranged from 13.5 to 36.1°C with a mean of 21.4°C. On average trays received one watering cycle (2 passes) per day equivalent to 1.5 mm.

The mean percentage emergence was 93.34%

Transmission

At two weeks after sowing all ten of the plants sampled in the un-sprayed tray were infested with *Xcc* and showed symptoms whereas only six out of the ten plants sampled from the sprayed tray were infested and none showed any symptoms (Table 1). All of these differences were statistically significant (χ^2 test).

Symptoms

The numbers of plants with symptoms at each assessment are shown in Table 1. Figure 1 shows the locations of the symptomatic plants in each block of trays at 6 weeks and Figure 2 shows the change in numbers of plants with symptoms over time. In the unsprayed treatment symptoms were first apparent on plants in the inoculated cells 2 weeks after sowing and increased to a final level of 1.38% of plants with symptoms at 6 weeks.. In the copper-sprayed treatment symptoms did not appear until 6 weeks even in the inoculated cells giving a final level of 0.02% of plants with symptoms. Overall the proportion of plants with symptoms in the sprayed treatment was 69 times smaller than in the un-sprayed at 6 weeks. This difference was highly significant ($P < 0.001$) based on a χ^2 test.

Leaf washing

Proportion of plants. The overall most probable number estimates (from STPro) of the final percentages of infested (=infected or contaminated) plants in each treatment 6 weeks after sowing together with 95% confidence limits are shown in Table 2. In the unsprayed treatment *Xcc* was detected in 23 out of 31 samples (21/29 exc. inoculated cells) giving an overall estimated proportion of plants infested of 18%. In the sprayed treatments *Xcc* was detected in only 4 out of 29 samples (3/27 exc. inoculated cells) giving an overall estimated proportion of plants infected of 1.9%. The proportion of infested plants decreased with increasing distance from the inoculated cell.

More detailed analysis of the proportion of plants infested in relation to distance from the inoculated cell was done by fitting the complementary log-log model:

$$\ln\left[-\ln(1-p)\right] = \ln(N) + a + b\ln(dist)$$

where p is the proportion contaminated/infected, N is the number of plants in the sample (defined as an offset in Genstat), *dist* is the distance from the source (inoculated cell), a is a constant representing fixed treatment effects and b is a coefficient for the effect of distance from the source.

A series of models was fitted to obtain an analysis of deviance similar to analysis of variance (Table 4) (Payne et al. 1993). From the table it can be clearly seen that spraying with copper had a significant effect on the proportion of plants contaminated, irrespective of distance from the source. Fitting separate coefficients for each copper treatment for ln(*dist*) was significant. The model parameters are shown in Table 4 and the resulting estimated values for the proportion of infested plants in relation to distance are shown in Fig 3.

Numbers of bacteria. The overall mean numbers of *Xcc* per plant are shown in Table 5. More detailed analysis of the numbers of bacteria in relation to the distance from the source was done by fitting the model:

$$\ln(m) = \ln(dil) + a + b\ln(dist)$$

where m number of colonies counted, *dil* is the dilution factor, *dist* is the distance from the source (inoculated cell), a is a constant representing fixed treatment effects and b is a coefficient for the effect of distance from source.

A series of models was fitted to obtain an analysis of deviance similar to analysis of variance (Table 6). From the table it can be seen that spraying with copper gave a significant reduction in the mean number of *Xcc* per plant. In addition plants with symptoms in the unsprayed treatments also carried significantly more *Xcc* per plant than symptomless plants. The mean number of *Xcc* per plant decreased with increasing distance from the inoculated cell. Fitting separate coefficients for ln(*dist*) was significant for each copper treatment but not for symptoms. The model parameters are shown in Table 7, and the resulting estimated values for the numbers of *Xcc* per plant in relation to distance from source are shown in Fig 4.

Table 6. Mean number of *Xcc* per plant (estimated from fully parameterised model)

Distance	Treatment			
	Un-sprayed		Sprayed	
	No symptoms	Symptoms	No symptoms	Symptoms
0	6.8 (±8.5) E+06	6.8 (±12) E+05	-	
1	1.4 (±1.1) E+07	0.0 (±3.6) E+00	-	
5	1.4 (±5.1) E+06	1.7 (±1.0) E+07	5.1 (±10) E+03	-
10	2.2 (±6.8) E+03	4.6 (±9.2) E+06	7.7 (±14) E+02	-
20	7.5 (±12) E+03	1.9 (±2.7) E+07	0.0 (±1.4) E+00	-
45	5.8 (±3.9) E+04	-	0.0 (±4.1) E+00	-
70	1.2 (±11) E+02	-	1.7 (±1.1) E+04	-
98	2.4 (±4.5) E+00	-	0.0 (±1.0) E+00	-
Mean	8.2 (±23) E+04	1.2 (±0.8) E+07	1.2 (±1.4) E+04	-

Table 4. Analysis of deviance for proportion of plants infested

Source of deviance	d.f.	Mean deviance
Ln(*dist*)	1	33.9
Treat	1	38.6
Ln(*dist*).Treat	1	5.5
Bench + Treat.Bench	2	3.1
Residual	51	0.9
Total	56	2.3

Table 5. Parameter estimates for the proportion of plants infested

Parameter	Estimate	s.e.
Un-sprayed	4.82	1.38
Sprayed	-2.06	1.19
Ln(*dist*) (Un-sprayed)	-1.80	0.40
Ln(*dist*) (Sprayed)	-0.66	0.38

Discussion

These results clearly indicate that weekly spray-applications of copper oxychloride (as Cuprokylt) to Brassica transplants give significant reductions in black rot caused by *Xcc*. Reductions were seen in all of the measured disease parameters: apparent disease transmission, rate of symptom development, rate of disease and pathogen spread, pathogen numbers. However, whether these reductions can be translated into an effective level of disease control in the field is still open to question. The transplants grown in this experiment were not planted out into the field, therefore there is no evidence that the reduction in disease levels achieved in these transplants can be translated into disease reductions in the field. Further carefully designed and controlled experiments are needed to determine the benefits of copper applications to transplants in terms of field disease.

Some caution should be exercised in interpreting these results as they represent a single experiment done in a single year, with a single rate of copper to a single cultivar, with a single isolate of *Xcc,* in a single environment. Further work is clearly needed to determine the repeatability of these results in other seasons and production systems.

The application rate, formulation and adjuvant used in this experiment were based on those used in the SOLA for field application of Cuprokylt to control spear rot of calabrese. More effective control may be achieved with different application rates and/or frequencies

Before starting this experiment there was concern that the high frequency of application would lead to phytotoxicity. In the system used in this experiment there was no visual evidence of toxicity. It is possible that the daily overhead-watering between copper applications limited the build-up of copper residues on the plants and/or in the compost. Under different conditions (watering system, frequency, temperature) toxicity may occur even with the same application rates.

For many growers the preferred method of application of pesticides to transplants might be by dosing the irrigation water. It would be premature to extrapolate to this method of

application for the reasons stated above and because the formulation (WP) may not be appropriate for in-line dosing systems.

It is not known whether the disease reductions observed here resulted from continuous maintenance of bacteristatic or bactericidal copper residue levels on the leaf surfaces or a from short-term bactericidal levels immediately following each application. It will therefore be important to determine copper residue levels at regular intervals during future experiments, and to determine the minimum inhibitory and 'cidal concentrations for *Xcc*. Failure to understand the basis for copper's effectiveness as a bactericide may be one reason why it has not been demonstrated as an effective bactericide by previous workers.

Copper is available as a pesticide in a number of different copper compounds and formulations, it is quite feasible that compounds/formulations other than the one used in this experiment may prove more effective in control black rot. Further work to investigate these other compound/formulations is essential.

Table 7. Parameter estimates for numbers of *Xcc* per plant

Parameter	Estimate	s.e.
Un-sprayed	15.86	0.95
Sprayed	8.13	3.65
Ln(*dist*) (Un-sprayed)	-1.60	0.43
Ln(*dist*) (Sprayed)	0.20	0.89
No symptoms	-2.37	0.98
Symptoms	0	-

It is important to recognise that it is unlikely that copper-based pesticides will prove to be a panacea for black rot, but rather they should be considered as one of a number of weapons in the armoury for the management of the disease.

Conclusions

- Copper compounds appear to have considerable potential as a tool for the management of black rot of Brassicas.
- Further work is needed before any recommendations can be made on the use of copper in the control of black rot in Brassica transplants.

Table 8. Analysis of deviance table for numbers of *Xcc* per plant

Source of deviance	d.f.	mean deviance
Ln(*dist*)	1	31828
Treat	1	57952
Symptoms	1	35219
Ln(*dist*).Treat	1	18959
Ln(*dist*).Symptoms	1	8510
Bench + Treat.Bench	2	5656
Residual	49	3500
Total	56	5987

Recommendations for further work

- Repeat the experiment reported here to ensure repeatability of results.
- Determine minimum application rates and frequency to achieve control.
- Investigate benefits/problems of higher application rates/frequencies.
- Investigate alternative copper compounds, formulations and adjuvants.
- Investigate feasibility of application by dosing irrigation lines.
- Determine minimum inhibitory and 'cidal concentrations of copper for *Xcc*.
- Determine copper residue levels present on the leaves and relate to efficacy.
- Plant treated/untreated transplants out in the field and take through to final crop maturity. to establish effects on crop growth and field levels of disease.
- Investigate potential of field applications of copper for control of disease in field crops.
- Investigate alternative transplant production systems which minimise application of overhead water.

The above proposals could be most effectively and efficiently carried out as part of a limited number of major experiments extending the initial work reported here.

Acknowledgements

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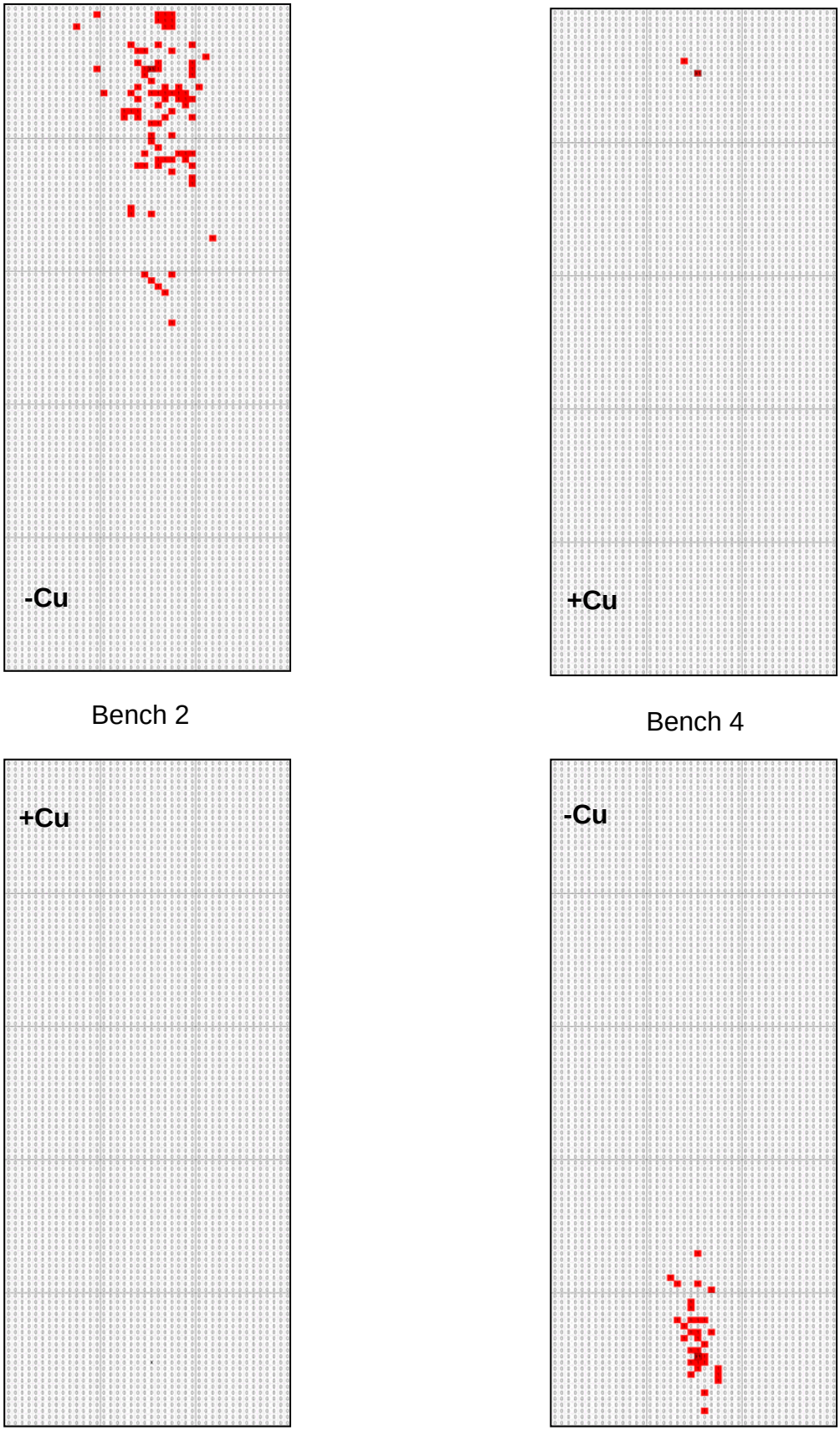

Figure 1. Distribution of plants with black rot symptoms (shaded cells) in blocks of cauliflower transplants, sprayed/un-sprayed with copper oxychloride, six weeks after sowing

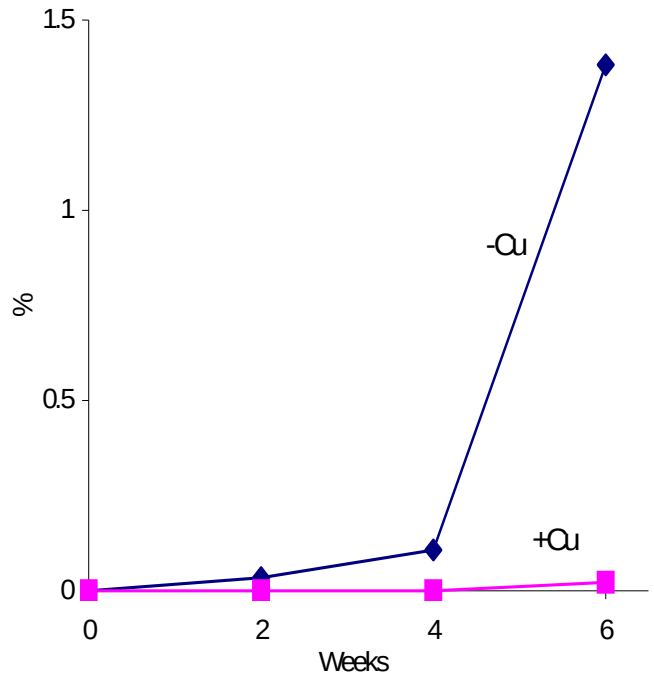

Figure 2. Development of black rot symptoms in cauliflower transplants sprayed/un-sprayed with copper oxychloride.

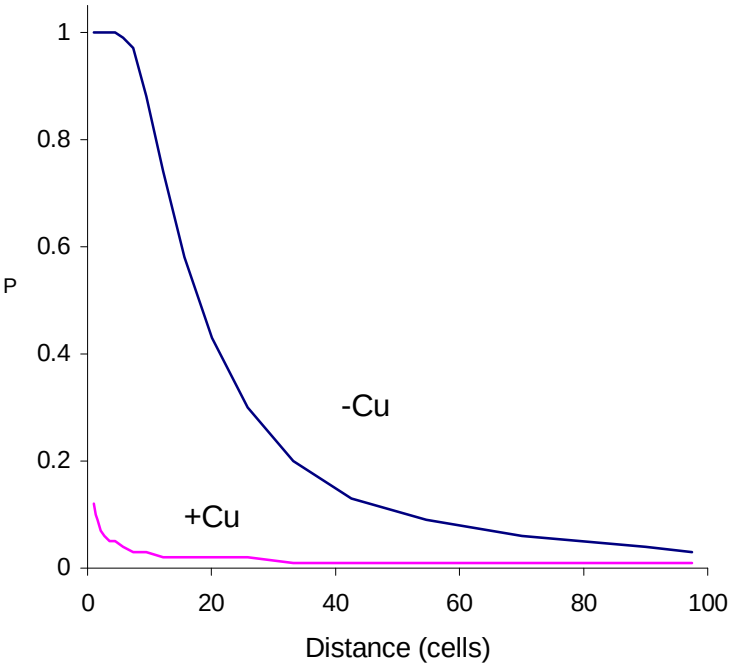

Figure 3. Change in estimated proportion of black rot infested transplants with distance from primary inoculum source in copper sprayed/un-sprayed blocks.

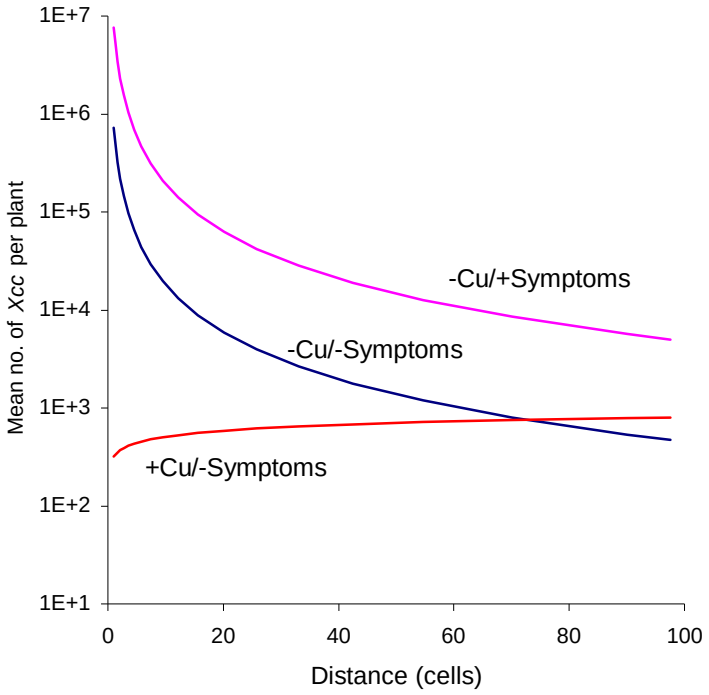

Figure 4. Mean no. of *Xcc* per plant in relation to distance from primary inoculum source for copper sprayed/un-sprayed cauliflower transplants, six weeks after sowing.