Project Title:	Evaluation of disinfectants, biological and natural products for control of Brassica black rot (*Xanthomonas campestris* pv. *campestris*)
Project number:	FV 335
Project leader:	Dr Steven J Roberts
Report:	Final Report, February 2009
Previous report	None
Key staff:	Dr S J Roberts, Plant Health Solutions
Location of project:	Plant Health Solutions, Warwick; HDRA, Ryton, Coventry; Warwick-HRI, Kirton, Lincs.
Project coordinators:	Mr Ellis Luckhurst and Mr Roger White
Date project commenced:	01 March 2008
Date completion due:	28 February 2009
Keywords:	Brassicas, transplants, diseases, bacteria, seed, seed health, control, biological, organic

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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

Dr S J Roberts
Director
Plant Health Solutions

Signature ..... Date .....

Report authorised by:

Dr S J Roberts
Director
Plant Health Solutions

Signature ..... Date .....

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Grower Summary

Headline

- Weekly sprays of copper oxychloride reduced *Xanthomonas* to undetectable levels in Brassica transplants.

- Two copper oxychloride products have off-label approval for use on protected Brassica transplants: Cuprokylt FL (2001-0117) and Headland Inorganic Liquid Copper (2008-0156).

- Treatment of infested Brassica seed with the natural product Thyme oil or the biologicals 'Serenade' and 'Subtilex' reduced seed to seedling transmission of *Xanthomonas*.

Background and objectives

Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) was listed as a high priority disease in the HDC/BGA research strategy 2006/7 and continues to cause sporadic problems in Brassica production, especially in autumn and winter Brassicas. Although the disease has the potential to cause complete crop loss, more usually in the UK it leads to reduced quality and marketability (e.g. in cauliflowers) and increased trimming costs/losses (winter cabbage), thus the importance of the disease varies depending on market conditions.

The black rot pathogen is seed-borne and can spread rapidly during Brassica plant raising under favourable conditions, so that even relatively low levels of seed infestation can be epidemiologically significant. Although most seed companies test vegetable Brassica seed fro *Xcc,* there are no 'official' tolerance standards or requirements for seed to be free from the pathogen. Recent relaxation in the sensitivity of the seed test for *Xcc* contained in the rules of International Seed Testing Association (ISTA) may have increased the risk of seed with epidemiologically significant levels of infestation being introduced into production. Work by the author on modelling transmission, spread and likely seed test results also suggests, counter-intuitively, that the biggest risk is likely to occur with seed lots which have a high % infestation but low numbers of *Xcc* per infested seed. Such seed lots are unlikely to give a positive seed test result.

Previous HDC-funded projects have examined the frequency of seed infestation in commercial winter cauliflower seed lots in Cornwall (FV186) and the potential of copper sprays to control the spread of *Xanthomonas* during plant raising (FV 186a), weekly sprays of copper oxychloride significantly reduced the rate of spread, and HDC obtained SOLAs for use of copper oxychloride to control *Xanthomonas* both in transplants and in

the field. Unfortunately the SOLA for transplants refers to Cuprokylt FL, whereas the work done in FV 186a was done with a different formulation Cuprokylt (WP).

During a recent EC-funded project (STOVE, www.stove-project.net) a number of physical and biological seed treatments were evaluated for their efficacy against *Xcc.* The physical treatments (hot air, hot water, electron bombardment) all gave reductions in levels seed infestation, but their effectiveness in practice depends on the initial level of infestation in the seedlot. This also highlights a problem with seed treatments applied by the seed trade: seed lots which have been treated may give a negative test result but may still be infested at low (but epidemiologically significant) levels. A number of bio-control agents (BCAs) and essential oils were also screened for activity against *Xcc* in the EC project. Thyme oil and two commercial BCAs (MBI600 and Serenade) showed a high level of activity *in vitro*, but unfortunately it was not possible to evaluate their efficacy *in vivo* within the lifetime of the project.

An additional compound (Sporekill™) has been suggested as having potential activity against *Xcc*. This compound is in the group of Quaternary Ammonium Compounds which have biocidal/disinfectant activity against many bacteria, as a first step, this compound together with chlorine dioxide was tested for *in vitro* activity in HDC project FV 314, together with Thyme oil and Serenade under 'clean' conditions in comparison with Jet 5. The results indicated that all compounds are inhibitory (and possibly 'cidal) to *Xcc*, thereby re-confirming the work done in the EC STOVE project and providing additional information for chlorine dioxide and Sporekill.

This project aimed to examine the potential of these products for use in the control of *Xcc*.

Specific objectives

(1) Evaluate the efficacy of the biological compounds against black rot as seed-treatments

(2) Evaluate the ability of the disinfectants (Sporekill, Sanogene) to reduce populations of *Xcc in vivo.*

(3) Evaluate the efficacy of selected compounds against black rot during plant-raising

Summary of results and conclusions

Efficacy of biologicals/natural products as seed-treatments

Three seedlots were treated with biological control agents (BCAs: Subtilex, Serenade MAX) and the natural product Thyme oil. The seed lots were: a naturally infested lot; and two inoculated lots, one dip inoculated to simulate surface contamination only, one vacuum inoculated to simulate both internal and surface contamination.

The numbers of bacteria detected on the seed post-treatment, prior to sowing are shown in Fig. 1. All treatments reduced the apparent numbers of *Xcc* bacteria on the seeds

compared to the untreated control and these reductions were statistically significant across the three seedlots. The greatest reductions were achieved with the Thyme oil treatment where numbers were reduced to undetectable levels. The BCAs gave a smaller reduction with more variability between lots.

To assess transmission, seeds were sown in module trays, monitored for symptoms and sampled four weeks after sowing to estimate the proportion of contaminated but

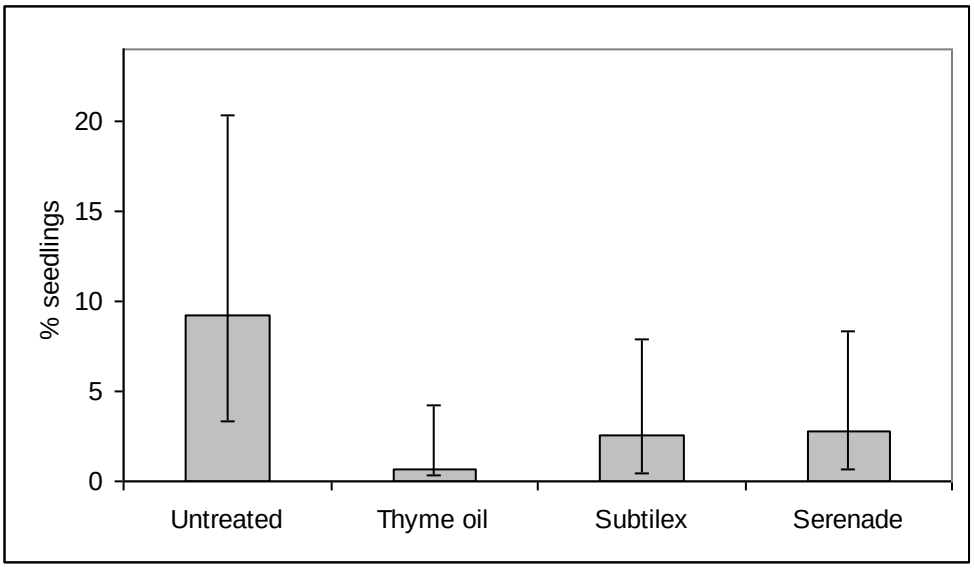

Figure 1. Transmission of *Xanthomonas campestris* pv. *campestris* in three Brassicas seedlots, following treatment of the seed with biologicals/natural products. Values are the mean % of seedlings infested (i.e. contaminated or infected). Bars represent the upper and lower 95% confidence limits.

symptomless plants. Values were then combined to obtain overall estimates of the proportions of contaminated plants (regardless of symptoms) (Figure 2).

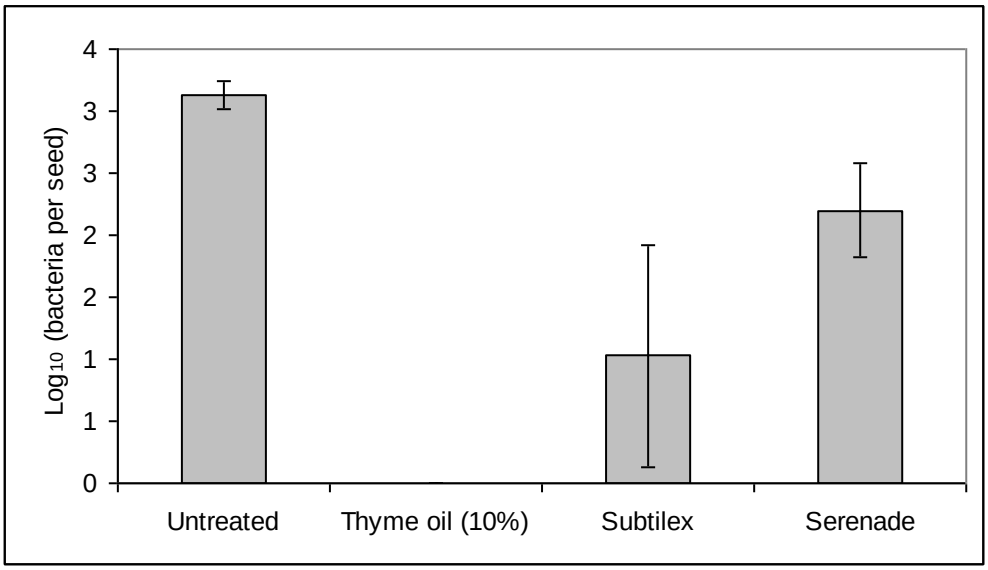

Figure 2. Combined results of pre-sowing, post-treatment seed tests for *Xanthomonas campestris* pv. *campestris* on seed used in transmission experiment. Values are the mean $\log_{10}$(no. of bacteria per seed) for the three seedlots. Bars represent the upper and lower 95% confidence limits.

All of the seed treatments reduced transmission of *Xcc* from seed to seedling, with no evidence of phytotoxicity or reductions in germination or emergence. Thyme oil gave the greatest reduction (from 9% to 0.6%, i.e. approx. 15-fold) and apparently eliminated transmission completely in the naturally infested seedlot). The two BCAs both gave similar reductions (from 9% to 2.5% and 2.8%, i.e. approx 3-fold).

Although experimental limitations and the nature of the data meant that these reductions were not statistically significant, given the previous results showing direct bactericidal/inhibitory effects on *Xcc* (STOVE, www.stove-project.net; and HDC project FV 314); it seems reasonable to consider that these results represent real effects.

It should be noted that the infestation levels in both the naturally infested and inoculated seed used in these experiments were much greater than a grower would expect to encounter in commercial practice, where the % seeds infested would be expected to be orders of magnitude lower. Thus, the reductions in transmission achieved with all of these treatments could prove to be beneficial in normal commercial practice where the starting level of seed infestation would be much lower.

Although the BCAs were less effective against *Xcc* than thyme oil overall, they should not be disregarded as they may have other benefits as seed treatments, e.g. impacts on other (e.g. soil-borne) pathogens.

Taken together with previous work, these results suggest that further research to confirm these results and develop practical treatments would be worthwhile.

Ability of the disinfectants to reduce populations *in vivo*

Two seedlots infested with *Xcc* (one naturally infested, and one inoculated) were sown in module trays and populations of the pathogen allowed to build up. Once populations were established, a single spray of each of the disinfectants (Sporekill and chlorine dioxide), thyme oil and copper oxychloride was applied and the population of *Xcc* determined the following day.

Thyme oil at 0.1% caused severe phytotoxicity (scorching) of the leaves - See figure 3

Figure 3. Phytotoxicity symptoms in brassica seedlings (S1064) following spray treatment with 0.1% Thyme oil

Bacterial numbers following treatment are shown in Fig. 4 for the plants grown from naturally infested seed. None of the differences between treatments can be considered statistically significant, although treatment with copper oxychloride appeared to reduce the number of *Xcc* on the plants compared to the control (from 10^4 to 10^2 CFU/plant);, this low level was mainly due to no *Xcc* detected in one of the three sub-samples. This was surprising given that the other compounds had apparently shown greater activity *in vitro* in the limited tests conducted as part of a an earlier project (HDC FV 314), but demonstrates the importance of testing *in vivo* as well as *in vitro.*

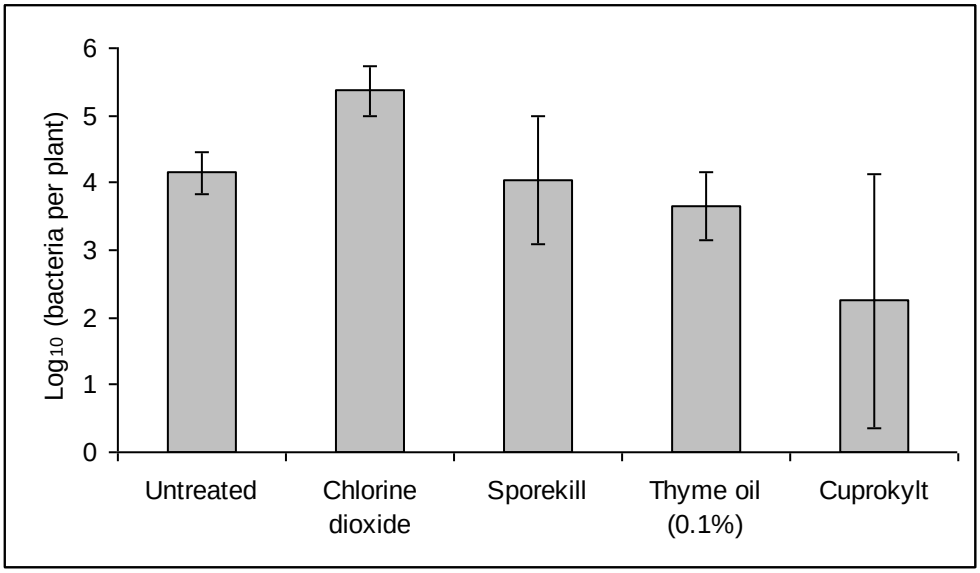

Figure 4. Numbers of *Xanthomonas campestris* pv. *campestris* detected on leaves of Brassica transplants grown from a naturally infested seedlot (S966) following a single treatment with 'disinfectants'. Bars represent the upper and lower 95% confidence limits.

Effect of treatments on spread of *Xcc* in transplants

Blocks of 15 '345' trays of Brassica transplants containing a single primary infector cell were treated with weekly sprays of chlorine dioxide, Sporekill, Cuprokylt, Cuprokylt FL, and Serenade ASO. Symptomatic and symptomless spread of the pathogen was then monitored.

A summary of the overall contamination levels is shown in Fig 5. Both of the copper oxychloride treatments (two different formulations of Cuprokylt) resulted in the absence of any detectable spread/increase in *Xcc* in the transplants, and reduced the number of plants infested 39 days after sowing by over 60%, from around 9% in the untreated control to less than 3% (the limit of detection). This reduction was statistically significant. Chlorine dioxide and Serenade ASO reduced the number of plants infested by around 40%, but these reductions were not statistically significant. Sporekill did not give any reduction.

The results for copper oxychloride support those of an earlier HDC project on the management of *Xcc* done by the author (HDC FV 186a). This work also showed a significant reduction in the spread of *Xcc* in transplants with weekly sprays of copper oxychloride and formed the basis for the original SOLA application for use in transplants. Despite the clear-cut results in project FV 186a and their further confirmation in this work, there appears to be little confidence in the value of copper sprays within the industry. It is possible that this is because growers have not been using/applying it in the same way and at the same rates as used in these and previous HDC trials. This suggests that further work may be appropriate to compare and define the minimum treatment parameters for success (i.e. minimum rates, application frequencies, irrigation

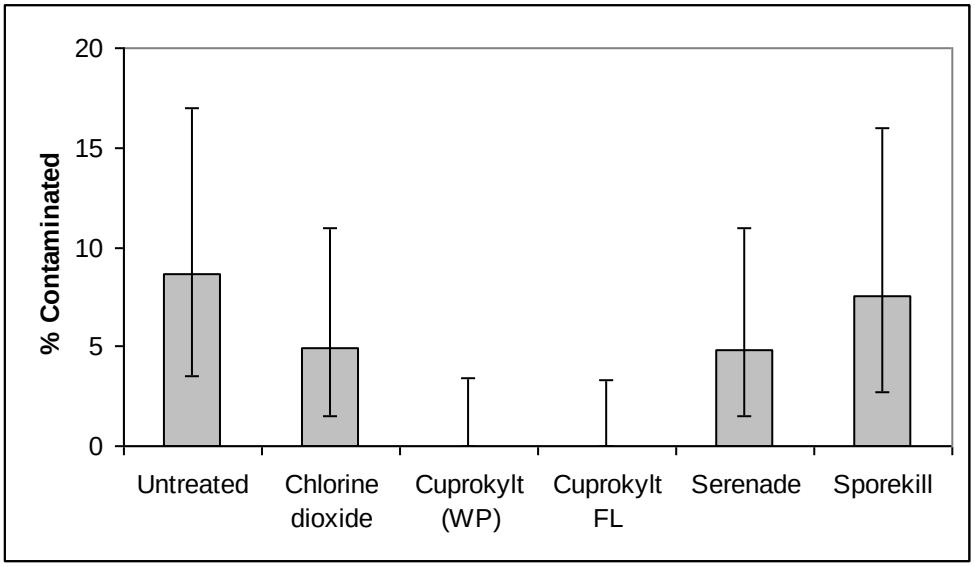

Figure 5. Overall % of Brassica transplants contaminated (both with and without symptoms) with *Xanthomonas campestris* pv. *campestris* 39 d after sowing and with weekly applications of treatments. Bars represent the upper and lower 95% confidence limits.

line dosing) together with taking transplants on into field plantings to demonstrate the value in practice.

The two different copper oxychloride formulations were included in the spread trial as the previous work in FV 186a had been done with the wettable powder formulation, whereas the current SOLA is for the 'FL' formulation, and it was considered possible that one of the reasons for lack of confidence may have been because the FL formulation was not as effective as the wettable powder formulation. These results suggest that there is little difference in the performance of the two formulations.

The chlorine dioxide treatment caused widespread and very noticeable scorching symptoms - see figure 6:

Figure 6. Leaf scorching in cauliflower transplants following chlorine dioxide treatment.

Equally, if not more importantly, levels of downy mildew were also much greater in the chlorine dioxide treatment, perhaps due to the scorching damage allowing greater opportunities for infection. Thus its use cannot be recommended on the basis of this experiment. However, the author is aware of some unpublished experimental work done recently in Germany where, in contrast to these results, dosing of the water supply with a lower dose of chlorine dioxide gave large reductions in *Xcc* (Krauthausen, pers. comm.). Further investigation of this approach to the application of chlorine dioxide may therefore be worthwhile.

Approval status of products

As at 28-Feb-2009.

Copper oxychloride has approval for off-label use on protected Brassica transplants as Cuprokylt FL (2001-0117) or Headland Inorganic Liquid Copper (2008-0156).

Serenade ASO (liquid formulation) has approval for off-label use on vegetable Brassicas (2009-0246) (Note that this does not include use on seeds).

Serenade MAX (powdered formulation) does not have approval as a plant protection product in the UK; it is registered as a fungicide in the USA.

Subtilex does not have approval as a plant protection product in the UK; it is registered as a biological fungicide in the USA.

Chlorine dioxide and Sporekill are biocides and do not have approval as plant protection products in the UK.

The status of thyme oil is unclear: it does not have approval as a plant protection product in the UK, but is widely used in mouthwashes and soaps, and for aroma-therapy.

Financial benefits

Based on Summer 2008 prices the cost of Cuprokylt FL treatment works out at ~8p per module tray for six treatments plus application costs.

Action points for growers

- Confirm that Brassica seed has been tested for *Xanthomonas* before purchase/use, and request information on the tolerance standards applied.
- Consider the routine use of weekly sprays with Cuprokylt FL or Headland Inorganic Liquid Copper during Brassica plant raising.
  IMPORTANT: at this stage there are no indications of how much flexibility is possible in such a spray programme without compromising efficacy (see suggestions for further work below). It is therefore important to use at the full rate specified in the relevant SOLA and apply at regular 7 day-intervals from 7 days after sowing.
- To ensure copper oxychloride treatments are applied effectively, growers may wish to consulting the report's author: Dr Steve Roberts, Plant Health Solutions.
- Consider funding further work on copper treatments to refine treatment parameters in transplants (i.e. determine flexibility) and treatment recommendations for field crops.
- Consider funding work to investigate the use of lower concentrations (of all products) in continuous-dosing systems.
- Consider funding further research to confirm the seed treatment results and develop practical treatments.
- There may be potential for significant impacts from subtle alterations in irrigation systems and management, and which may be counter-intuitive, consider the need for further work in this area.

Science Section

Introduction

Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) was listed as a high priority disease in the HDC/BGA research strategy 2006/7 and continues to cause sporadic problems in Brassica production, especially in autumn and winter Brassicas. Although the disease has the potential to cause complete crop loss, more usually in the UK it leads to reduced quality and marketability (e.g. in cauliflowers) and increased trimming costs/losses (winter cabbage), thus the importance of the disease varies depending on market conditions.

Previous work over a number of years by the author and funded by Defra at HRI focused on understanding the biology of the pathogen and the host/pathogen interactions, and obtaining quantitative data on the underlying epidemiology of the disease. The results of much of this work have been published in the scientific literature (Roberts *et al.* 1999;Roberts *et al.* 2007;Vicente *et al.* 2000;Vicente *et al.* 2001) and/or is available in reports at the DEFRA website (Roberts and Brough 2002;Roberts and Brough 2004). Spread during plant raising is most critical for the development of epidemics in the field and the conditions under which most Brassica transplants are produced are highly favourable for the spread of and/or infection by *Xcc*.

The black rot pathogen is seed-borne, but there are no 'official' tolerance standards or requirements for seed to be free from the pathogen. Recent relaxation in the sensitivity of the seed test for *Xcc* contained in the rules of ISTA may have increased the risk of seed with epidemiologically significant levels of infestation being introduced into production. In any case, statistically, 1 in 20 infested seedlots which exceed the tolerance standards and test sensitivity usually applied by seed companies may fail to be detected. Work on modelling transmission, spread and likely seed test results (Roberts 2006) suggests, counter-intuitively, that the biggest risk is likely to occur with seed lots which have a high % infestation but low numbers of *Xcc* per infested seed. Such seed lots are unlikely to give a positive seed test result.

Previous HDC-funded projects have examined the frequency of seed infestation in commercial winter cauliflower seed lots in Cornwall (FV186; (Roberts *et al.* 1998) and the potential of copper sprays to control the spread of *Xanthomonas* during plant raising (FV 186a; (Roberts and Brough 2000). In the latter project, weekly sprays of copper oxychloride significantly reduced the rate of spread, and HDC obtained SOLAs for use of copper oxychloride to control *Xanthomonas* both in transplants and in the field. Unfortunately the SOLA for transplants refers to Cuprokylt FL, whereas the work done in FV 186a was done with a different formulation Cuprokylt (WP).

During a recent EC-funded project (STOVE, www.stove-project.net) a number of physical and biological seed treatments were evaluated for their efficacy against *Xcc.* The physical treatments (hot air, hot water, electron bombardment) all gave reductions in levels seed infestation; however they could not be relied upon to eradicate the pathogen. Hence their effectiveness in practice will depend on the initial level of infestation in the

seedlot to be treated. This also highlights a problem with seed treatments applied by the seed trade: seed lots which have been treated may give a negative test result but may still be infested at low (but epidemiologically significant) levels. A number of bio-control agents (BCAs) and essential oils were also screened for activity against *Xcc* in the EC project. Two commercial BCAs (MBI600 and Serenade) showed a high level of activity *in vitro*, but unfortunately it was not possible to evaluate their efficacy *in vivo* within the lifetime of the project. Several essential oils but especially thyme oil also showed a high level of activity against *Xcc in vitro*, but again was not fully evaluated *in vivo*.

Although in the past there have been difficulties with gaining approval for BCAs, etc., PSD now has in place a 'Biopesticides Scheme' with reduced fees to encourage more approvals for BCAs and natural products. In addition, two of the products are already registered outside of the UK and thyme oil is included in the EC list of substance permitted for organic agriculture.

An additional compound (Sporekill™) has been suggested as having potential activity against *Xcc*. This compound is in the group of Quaternary Ammonium Compounds which have biocidal/disinfectant activity against many bacteria, as a first step, this compound together with Sanogene (chlorine dioxide) was tested for *in vitro* activity in HDC project FV 314, together with Thyme oil and Serenade under 'clean' conditions in comparison with Jet 5. The results indicated that all compounds are inhibitory (and possibly 'cidal) to *Xcc*, thereby re-confirming the work done in the EC STOVE project and providing additional information for Sanogene and Sporekill.

This project aimed examine the potential of these products for use in the control of *Xcc*.

Specific objectives

(1) Evaluate the efficacy of the biological compounds against black rot as seed-treatments

(2) Evaluate the ability of the disinfectants (Sporekill, Sanogene) to reduce populations of *Xcc in vivo.*

(3) Evaluate the efficacy of selected compounds against black rot during plant-raising

Approaches

Experimental systems and approaches for work with the Brassica/*Xcc* pathosystem have been developed by the proposer over many years during previous Defra-, EC and HDC-funded work. The approaches used in this project made use of these systems and experience to maximise the likelihood of successful outcomes.

It is recognised that Brassica growers would ideally like to have options for disease control in the field as well as during propagation, however, by conducting initial evaluations in relatively controlled glasshouse conditions, un-necessary expenditure will be avoided on the basis that compounds which are ineffective under glasshouse conditions are unlikely to be effective in the field situation; if any compounds/products

give promising results in this study further studies of effectiveness in the field would be appropriate.

By examining the effects on transmission and spread separately, it was anticipated that much clearer and definitive results will be obtained, which will also give a clearer indication of how best to use the compounds in a commercial situation.

Materials and Methods

Sources of seed

Naturally infested and 'healthy' seed was sourced from various seed companies and tested to confirm their health status or the level of infestation.

The healthy seed lot used throughout the project was a widely grown autumn cauliflower variety. In order to be certain of its health status, a more stringent testing procedure than the ISTA (International Seed Testing Association) validated method was used: this included a centrifugation step and reduced sub-sample size both of which contribute to an increased analytical sensitivity of the test (see below). In addition the testing was done non-destructively and the tested seed was re-dried after testing, so that the actual seed which had been tested was used in the experiments.

Seed inoculation

Isolate 3818A of *Xcc* (Race 1, a standard isolate used in a number of previous Defra and HDC studies, and known to be pathogenic) was grown on YDC agar at 30°C for 48 h. Growth was scraped from the plate and suspended in sterile saline (0.85% NaCl). The resulting suspension was then diluted to provide sufficient volume for inoculation (approx. 60 ml) at a concentration of approx. 10^7 cfu ml^{-1}. Two aliquots of approx. 2,500 seeds of the healthy cauliflower seed lot were each immersed in approx. 30 ml of the bacterial suspension in conical flasks. One aliquot was left to stand on the bench. The other had a vacuum applied for approx. 5 min, which was then released. seed drained. In both cases after approx. 10 min the seeds were tipped out onto trays lined with absorbent paper towel. The towel was then removed and the seeds allowed to air-dry at room temperature in the lab for 2-3 d before packaging and storage in the fridge. Bacterial numbers in the inocula were estimated by dilution and plating onto YDC using the drop method of Miles and Misra (1933)

Seed testing

The health status of seedlots and numbers of bacteria on inoculated seeds were determined by a procedure based on the ISTA validated method (Roberts and Koenraadt 2005) with the addition of a centrifugation step. Briefly, multiple sub-samples of each lot of varying sizes (depending on expected infestation level) were suspended in sterile saline (plus 0.02% Tween 20) and shaken for 2.5 h at room temperature. An aliquot of the extract was also removed after 5 min shaking and centrifuged. The resulting suspensions were diluted and 100 µl of each dilution spread on plates of FS and mCS20ABN agar media with a bent glass rod. Plates were incubated at 30°C for 3-4 d before counting the number of typical *Xcc* colonies. The identity of a selection of typical

colonies was confirmed by sub-culture to sectored plates of YDC medium and pathogenicity testing on seedlings of Savoy cabbage cv. Wirosa.

Efficacy of biologicals/natural products as seed-treatments

Seed treatment

Three seedlots were used: a naturally infested lot (S0966), and two inoculated lots, one dip (S1065) and one vacuum inoculated (S1066). One aliquot of each lot was treated with Subtilex, one with Serenade MAX and one with Thyme oil.

In the case of the BCAs (Subtilex and Serenade MAX), which are formulated as dry powders, an amount of product equivalent to 20 mg per seed was weighed out into an appropriate container (either a conical flask or universal bottle depending on the amount of seed to be treated). The seeds were then added and the container shaken by hand to ensure the seeds received an even coating of product. In both cases there was a slight excess of powder remaining in the container.

In the case of thyme oil, a water emulsion of the oil was first prepared, using agar as an emulsifier. Agar (0.1% w/v) was dissolved in de-ionised water by heating, the oil was then added to the hot agar solution, which was shaken and sonicated to mix and then allowed to cool to room temperature. For treatment, seeds were immersed in the emulsion (in a glass container) for 15 min before tipping out onto absorbent paper and drying under an airflow.

Tray filling and sowing

Module '345' trays were filled loosely with Bulrush organic modular compost in a standard manner. The surface was levelled and then firmed so that the surface of the compost was 0.5-1 cm below the top of the tray. Single seeds were then placed in cells using vacuum 'tweezers' and covered with sieved compost up to the top of the tray.. Trays were set out on capillary matting on a glasshouse bench.

Watering

Trays were given an initial overhead watering by hand using a watering can fitted with a fine rose, subsequently all watering was via seep-hoses set out at intervals on the capillary matting. Watering was controlled via an irrigation timer connected to solenoid valves and set to irrigate daily at 0800 for 10 mins.

Records

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

Emergence. The number of emerged seedlings was counted at 8 d after sowing. The counts were made in two half trays from each treatment.

Disease symptoms. The number of plants showing black rot symptoms (chlorosis and/or necrosis with blackened veins) was recorded at four and five weeks after sowing.

Leaf washings. 'Leaf washings' to estimate apparent transmission were done at four weeks after sowing. One sample of 30 plants and two samples of two plants were collected from random positions in each tray.

Plants were cut off just below the cotyledons using sterile scissors and put into new stomacher bags (thick-walled polythene). A separate bag was used for each sample of plants, and, to avoid cross-contamination, scissors and hands were disinfected by wiping with 70% iso-propanol between samples. Plants with visible symptoms were separated and treated as separate samples. After collection, plants were stored in the fridge (*ca.* 4°C) until processing (< 24 h).

To process each sample, saline plus 0.02% Tween 20 was added to the stomacher bags (2 ml per plant), and the plants were then stomached for 5 min. The resulting extract was then diluted and 0.1 ml of each dilution and the original extract were spread on the surface of plates of FS and mCS20ABN selective media. Plates were then incubated for 3-4 days at 30°C and the numbers of suspect *Xcc* recorded. Suspect *Xcc*. colonies were then sub-cultured to sectored plates of YDC to confirm their identity by comparison with a positive control isolate.

Ability of the disinfectants to reduce populations *in vivo*

Design

The aim of this experiment was to mimic the natural development of *Xcc* populations on the surfaces of Brassica leaves and determine the effect of a single application of the test compounds on those populations. It would be tempting to create a population by simply spraying a suspension of bacteria onto the leaves of plants, however, because such a population has not developed over a period of time the physical locations of the bacteria on the leaves are likely to differ from those that have done so naturally, and results will not therefore be comparable to a 'natural' situation. For this reason, populations were allowed to develop from an initial low level following seed infestation, then once a significant leaf population had been established, the plants were treated and populations re-assessed.

Seeds and sowing

Two seed lots were used: a naturally infected seed lot (S966) and a healthy seedlot plus inoculated seed (S1064 + S1066). Both seed lots were sown in module '345' trays of Bulrush organic modular compost as described above. In the case of healthy + inoculated seed lot, all cells in the trays were sown with healthy seed, then in 21 cells (3 evenly-spaced rows of 7 evenly-spaced cells) the healthy seeds were replaced with 2 inoculated seeds.

Following sowing, trays were transferred to a glasshouse bench and watered daily at 08:00 via an overhead sprinkler system controlled by an electronic irrigation controller.. Glasshouse conditions were set to min 18/15°C and vent at 20/18°C (day/night).

Assessment of populations

At three weeks after sowing (2nd TL developing) a sample of 5 plants was collected from each tray to determine if a population of *Xcc* had developed. Samples were collected and processed in the same way as described previously.

The day following treatment, two samples of five and one sample of 30 plants were collected from each tray and processed as described previously.

Spray treatments

Spray treatments were applied 25 d after sowing at around 17:00. Each compound was applied in a total volume of 160 ml per tray using a handheld sprayer (Matabi 5 L) fitted with an 80° flat fan nozzle (yellow) and a constant pressure regulator set to 1.5 bar. To avoid the potential for cross-contamination of treatments, trays to be treated with each compound were removed from glasshouse bench and treated on the floor away from other trays.

Effect of treatments on spread of *Xcc* in transplants

Design

The experiment was done in the glasshouse facilities at Warwick-HRI, Kirton, and consisted of six treatments: two copper treatments (two different formulations of copper oxychloride), chlorine dioxide, Sporekill™, a biological, and an untreated control. The treatments were selected in collaboration with the grower coordinators following review of the earlier results. Each treatment was applied to a block of 15 '345' module trays set out in a 15 x 3 arrangement. To reduce the risk of cross-contamination between treatments, each block was separated by a barrier of clear polythene sheeting approx. 0.75 m high (see Appendix I and Photo 0862)

Seeds and sowing

Seeds of the healthy autumn cauliflower seed lot (S1064) were sown in module '345' trays of Bulrush organic transplant compost with 20% coir. Trays were filled loosely with compost and each cell compressed with a nippled roller. Seeds were sown with a St Moritz Auto Plate Seeder (3.00 mm plate) and then covered with the same compost.

In one tray in each block/treatment the seed in one cell in the centre of the tray was replaced by 5 vacuum inoculated seeds of the same lot (S1074) prepared as described previously (see page 12)

Following sowing, trays were transferred to the glasshouse and set out in blocks. Trays were raised off the floor by standing them on up-turned 5 L plant pots placed at the corners.

Watering

Trays were watered using an overhead moving gantry irrigation system. A horizontal spray boom with multiple nozzles providing a 'curtain' of water, was moved slowly up and down the transplants by means of a pulley connected to an electric motor under manual

control. Watering frequency and the number of passes was determined by the judgement of the glasshouse staff at Kirton.

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 approx. 3 weeks after sowing, an organic liquid feed (NuGro) was applied to all treatments at intervals according to perceived need by dosing the water supply line with a concentrated stock solution.

Spray applications

Spray treatments were applied at 7 d intervals from 7 d after sowing at around 15:00. Except for the biological treatment, each compound was applied in a total volume of 160 ml per tray using a handheld sprayer (Matabi 5 L) fitted with an 80° flat fan nozzle (yellow) and a constant pressure regulator set to 1.5 bar. To avoid cross-contamination a separate sprayer was used for the pesticide, disinfectant, and biological treatments. The product rates used are shown in Table 1. Efforts were made to ensure that plants were well-watered prior to treatment. Following treatment, watering was delayed for as long as possible (at least until the following morning, and longer if possible).

Assessments

Symptoms. Plants were observed weekly and the appearance and location of black rot disease symptoms recorded.

Confirmation of transmission. A single module tray was sown with the inoculated seed (S1074) on the same day as the main experiment and maintained in a separate glasshouse at a remote site, for observation purposes.

At 21 d after sowing a single plant sample was removed from the inoculated cell in each of the treatment blocks to confirm the presence of *Xcc.* Each plant was macerated in 1 ml saline plus Tween 20, then diluted and plated on FS and mCS20ABN media.

Leaf washing. Leaf washings to estimate the proportion of infested (=contaminated/infected symptomless) plants were done at 39 days after sowing; two samples of plants of varying sizes were taken at six distances (1, 8, 17, 37, 70, 93 cells) from the primary infectors (i.e. the cells sown with the inoculated seed).

Samples were processed as described previously (see page 13).

Statistical analyses

The proportions of seeds infested were calculated using the STPro™ seed test analysis program (Ridout and Roberts 1995)

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 overall mean numbers of *Xcc* per plant were calculated by fitting a generalised linear model with a Poisson error distribution, log link functions and using the number of plants in a sample as a weighting factor.

The effect of the spray treatments 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.

Results

Efficacy of biologicals/natural products as seed-treatments

Seedlings began to emerge at 3-4 d after sowing. Emergence was similar in both seedlots and in all treatments with no significant differences and an overall mean emergence of around 92%. There was also no evidence of phytotoxicity in any of the treatments.

The proportion of seeds infested and the numbers of bacteria detected on the seed prior to sowing are shown in Table 2 and Figures 1 and 2 . All treatments reduced the apparent numbers of *Xcc* bacteria on the seeds compared to the control and these reductions were statistically significant across the three seedlots. The greatest reductions were achieved with the Thyme oil treatments where numbers were reduced to undetectable levels. The BCAs gave a smaller reduction with more variability between lots.

Visible disease symptoms were observed on plants at the time of sampling (4 weeks after sowing) and the number recorded in each tray. The leaf washings on the symptomless plants were used to obtain a separate estimate of the proportion of contaminated but symptomless plants and these values were then combined with the proportion of plants with symptoms to obtain an overall estimate of the overall proportions of contaminated plants (regardless of symptoms) (**Table 3**, Figure 3).

All treatments appeared to reduce the rate of transmission compared to the untreated control, but due to the nature of the data these differences cannot be considered as statistically significant. The greatest reduction (from 9 to 0.6%) occurred with 10% Thyme oil treatment, the two BCAs gave similar reductions (to 2.5 and 2.8%)

The numbers of Xcc on symptomless plants showed a similar pattern to the proportion of plants contaminated. The numbers on plants with symptoms did not differ between treatments and ranged from 3 to 8 x 10^6 CFU per plant.

Ability of the disinfectants to reduce populations *in vivo*

The bacterial numbers on plants at the pre-treatment sampling (4 d before treatment) were up to 8 x 10^5 CFU per plant on the plants grown from a naturally infested seedlot, with transmission confirmed in all trays. Numbers were up to 5 x 10^4 CFU per plant on those grown from the spiked healthy seedlot (S1064), but in this case results were more

variable and transmission/spread was not confirmed in all trays. No symptoms were apparent in plants grown from either seedlot.

[A decision was made to go ahead with treatment, on the assumption that failure to confirm transmission/spread in all trays may have been due to the relatively small number of plants sampled for this purpose.]

Thyme oil at 0.1% caused severe phytotoxicity (scorching) of the leaves (see Photo 0609)

Bacterial numbers following treatment are shown in Table 4. *Xcc* was not detected in the untreated control for the spiked seed lot (S1064), this makes comparisons of treatment effects in the plants grown from this seedlot meaningless, hence only the results for the naturally infested lot (S966) are shown in Fig. 4. In the plants grown from naturally infested seed, the treatment with copper appeared to reduce the number of *Xcc* on the plants compared to the control (from 10^4 to 10^2 CFU/plant), this low level was mainly due to no *Xcc* detected in one of the three samples. None of the differences between treatments can be considered statistically significant.

Effect of treatments on spread of *Xcc* in transplants

The mean average of the half-hourly temperature records from the time of sowing until the time at which samples were collected was 19.9°C (Range 8.4 to 39.8). Each watering cycle (i.e. 2 passes of the gantry) was estimated to deliver the equivalent of 5 mm of water, and the plants received an average of 0.7 cycles per day from sowing to sampling.

Phytotoxicity

Plants treated with chlorine dioxide showed very obvious phytotoxicity symptoms. These were manifest initially as pale brown marginal necrosis on the cotyledons following the first treatment and later as pale brown necrotic spots and margins on the leaves of most plants (see Photo 0931). Plants treated with copper also showed some slight phytotoxicity symptoms. These were more cumulative and only noticeable by comparison with other treatments, and were manifest as slightly smaller, harder, more blue-coloured leaves.

General appearance

During a visit to the trial 35 d after sowing, one of the grower co-ordinators was asked to comment on the appearance of plants in each treatment:

Untreated. Generally okay. Some DM, but less than chlorine dioxide treated. No cotyledons retained.

Copper oxychloride. Both formulations very similar. ‘Harder’ looking plants compared to other treatments, but not obvious when viewed in isolation. Some DM, but less than untreated. No cotyledons retained.

Chlorine dioxide. Worst looking plants. Lots of leaf scorching. High level and very obvious DM. No cotyledons retained.

Serenade ASO. Generally okay, very similar to untreated. Some DM, but less than chlorine dioxide treated.

Sporekill. Best looking plants. Majority of cotyledons still retained. Some DM but appears to be less than other treatments.

A typical tray from each treatment is shown in Photo 0992.

Symptoms

Black rot symptoms were first seen on the cotyledons of seedlings growing from inoculated seeds at 14-18 d after sowing. Subsequently symptoms were not observed in any other plants until shortly before sampling (35 d after sowing) and even then only in one or two plants neighbouring the inoculated cells (Table 5).

***Symptomless spread of* Xcc (Leaf washing)**

Proportion of plants. The overall maximum likelihood estimates (obtained using STPro) of the final percentages of infested (=infected or contaminated) plants in each treatment 39 d after sowing together with 95% confidence limits and the maximum distance at which infested plants were detected are shown in Table 5 and Fig. 5. The highest proportion was in the untreated block where the proportion of plants infested was 8.6%. The lowest levels were in the copper treated transplants where *Xcc* was not detected at all at the final sampling (this included plants in the inoculated cells), these reductions were statistically significant compared to the untreated control. The chlorine dioxide and Serenade ASO treatments were about half the levels of the untreated transplants (4.8 and 4.9%), but these reductions were not statistically significant. Only a marginal reduction was obtained with Sporekill and this was not significant.

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

$$\ln - \ln(1 - p) = \ln(N) + a + b\ln(dist)$$

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 (Payne *et al.* 1993). Unfortunately fitting these models was problematical:

(a) because there had been no *Xcc* detected in the copper treatments these data could not be included (i.e. all values zero);

(b) because of the limited amount of spread in the experiment as a whole (i.e. many ‘zeroes), the models often failed to converge or even diverged.

An attempt was also made to obtain to obtain parameters for the previously developed spread model (Roberts *et al.* 2007) for each treatment separately. Again no values for spread could be obtained for the copper treatments as there was no spread, and the

model failed to converge for the Serenade ASO and chlorine dioxide treatments. Values were obtained for the untreated plants and Sporekill treated plants, but the differences in parameter values were not significant.

Numbers of bacteria. The overall mean numbers of *Xcc* per plant are shown in Fig 6. These values were calculated by fitting a generalised linear model with a Poisson error distribution, log link functions and using the number of plants in a sample as a weighting factor. Numbers showed a similar pattern to those for the proportion of plants contaminated, with the highest levels in the untreated control, and all treatments showing a reduction compared to the control, but only the copper treatments were significantly lower. The upper confidence limits for the copper treatments shown in Fig 6 represent the detection limit (30 CFU per plant, P=0.95)

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. Model parameters are shown in Table 6, and again values could not be obtained for the copper treatments because there was no spread. The parameters values for *b* indicate the steepness in the decline in bacterial numbers with distance from the primary infectors and suggest that all treatments increased the rate of decline (i.e. reduced the spread) compared to the control.

Discussion

Efficacy of biologicals/natural products as seed-treatments

All of the seed treatments appeared to reduce the transmission of *Xcc* from seed to seedling, with no evidence of phytotoxicity or reductions in germination or emergence.. The natural product thyme oil gave the biggest reductions (on average approx. 15-fold, and apparently eliminated transmission in the naturally infested seedlot), with smaller reductions (on average approx. 3-fold) for the BCAs. However, experimental limitations and the nature of the data meant that these reductions were not statistically significant.

In the case of thyme oil, the pre-sowing seed tests indicated a significant reduction in both the % of seeds infested and the numbers of *Xcc* per seed, and it has previously been shown to have direct bactericidal/inhibitory effects on *Xcc* (STOVE, www.stove-project.net; and HDC project FV 314); thus it seems reasonable to consider that this is a real effect.

In the case of the BCAs the pre-sowing seed tests did not show such a clear cut reduction in either the % of seeds infested or the numbers of *Xcc* per seed (although Serenade MAX gave a reduction in the %, and both gave reduction in numbers); this was entirely to be anticipated as, being BCAs, they were not expected to have an impact until the pathogen became active (i.e. during germination and emergence). Given that both have previously been shown to have direct inhibitory effects on Xcc (STOVE,

www.stove-project.net; and HDC project FV 314), it again seems reasonable that the observed reductions in transmission were real effects.

It should be noted that, for experimental convenience (i.e. to keep the size of the experiments within practical bounds and minimise costs) the infestation levels in both the naturally infested and inoculated seed used in these experiments was much greater than a grower would expect to encounter in commercial practice, where the % seeds infested would be expected to be orders of magnitude lower. Thus the reductions in transmission achieved with all of these treatments could prove to be beneficial in normal commercial practice where the starting level of seed infestation would be expected to be much lower.

Although the biological treatments were less effective against *Xcc* than thyme oil overall, they should not be disregarded as they may have other benefits as seed treatments, e.g. direct impacts on other (e.g. soil-borne) pathogens.

Taken together with previous work, these results suggest that further research to confirm these results and develop practical treatments would be worthwhile.

Effect of disinfectants/natural product on *Xcc* populations *in vivo*

Cuprokylt was the only treatment that gave an observable reduction in the numbers of *Xcc* compared to the untreated control, with a reduction from approx. 10^4 to 10^2 following a single spray application. This was surprising given that the other compounds had apparently shown greater activity *in vitro* in the limited tests conducted as part of a an earlier project (HDC FV 314).

Two possible reasons for the apparent failure of the disinfectants to reduce the numbers of *Xcc* are:

1. Populations were allowed to develop naturally as a result of seed transmission and subsequent spread, it is possible that the bacteria were physically located in (micro-) niches on the leaf surfaces which the disinfectants could not penetrate.
2. If leaves were already infected rather than surface-contaminated, the bacteria inside the leaf tissues would be protected from the action of the disinfectants. Although there were no disease symptoms at the time of spray applications, and the numbers detected in the pre-test did not indicate infection, symptoms did develop later on some plants.

In this experiment all compounds were applied 'as is', i.e. no additional wetters/adjuvants, etc. were added to the spray solution/suspension. It is possible that addition of a wetter/adjuvant may have improved the activity of some compounds in the case of (1). However, it should be noted that this is unlikely for Sporekill which already has considerable surfactant activity and visibly gave the best/most even coverage.

More detailed studies on the *in vitro* activity of a range of disinfectants and including various copper compounds have been done by the author as part of a previous HDC project (Roberts and Akram 2002) (HDC HNS 91) and for convenience a table from this report, summarising and ranking the efficacy of the compounds examined, is reproduced in Table 7. The work used ISO standards as the basis for the test protocols. In general

the copper compounds ranked poorly compared to the disinfectants when compared under 'clean' conditions and with short contact times. However, under 'dirty' conditions and with longer contact times, they were as effective as the disinfectants. The work done in FV 314 only examined efficacy under 'clean' conditions, using de-ionised water as the diluent and without a quenching agent, so is most likely to have over-estimated the bactericidal power of the compounds examined.

Thyme oil when sprayed directly onto leaves at a concentration of 0.1% caused significant scorching/phytotoxicity (see Photo x), therefore, regardless of any disinfectant activity it is not suitable for foliar application.

Effect of treatments on spread of *Xcc* in transplants

Both of the copper oxychloride treatments (two different formulations of Cuprokylt) resulted in the absence of any detectable spread/increase in *Xcc* in the transplants, and reduced the number of plants infested 39 d after sowing by over 60% from around 9% in the untreated control to less than 3% (the limit of detection). This reduction was statistically significant. Chlorine dioxide and Serenade ASO reduced the number of plants infested by around 40%, but these reductions were not statistically significant. Sporekill did not give any reduction.

The results for copper oxychloride support those of an earlier HDC project on the management of *Xcc* done by the author (Roberts and Brough 2000) (HDC FV 186a). This work also showed a significant reduction in the spread of *Xcc* in transplants with weekly sprays of copper oxychloride and formed the basis for the original SOLA application for use in transplants. Despite the clear-cut results in project FV 186a and their further confirmation in this work, there appears to be little confidence in the value of copper sprays within the industry. It is possible that this is because growers have not been using/applying it in the same way and at the same rates as used in these and previous HDC trials. This suggests that further work may be appropriate to compare and define the minimum treatment parameters for success (i.e. minimum rates, application frequencies, irrigation line dosing) together with taking transplants on into field plantings to demonstrate the value in practice.

The two different copper oxychloride formulations were included in the spread trial as the previous work in FV 186a had been done with the wettable powder formulation, whereas the current SOLA is for the 'FL' formulation, and it was considered possible that one of the reasons for lack of confidence may have been because the FL formulation was not as effective as the wettable powder formulation. These results suggest that there is little difference in the performance of the two formulations.

The chlorine dioxide treatment caused widespread and very noticeable scorching symptoms. This was not observed in the previous (single treatment) experiment, and may have been due to an interaction with the higher temperatures, and greater direct sunlight during the spread trial compared to the previous experiment. Equally, if not more importantly, levels of downy mildew were also much greater in the chlorine dioxide treatment, perhaps due to the scorching damage allowing greater opportunities for

infection. Thus regardless of any benefits in the management of *Xcc*, its use cannot be recommended on the basis of this experiment. However, the author is aware of some unpublished experimental work done recently in Germany where, in contrast to these results, dosing of the water supply with a lower dose of chlorine dioxide gave large reductions in *Xcc* (Krauthausen, pers. comm.). Further investigation of this approach to application of chlorine dioxide may therefore be worthwhile.

It should be noted that during the development of this proposal, consideration was given to the possibility/option of dosing the irrigation supply lines during the planning/project proposal stage, however, this would have greatly added to the project costs, due to the need for separate irrigation systems for each treatment. Therefore it was decided to maintain a simple approach initially and consider dosing systems once the best treatment had been identified.

Sporekill gave no apparent reduction in Xcc. This was surprising given the promising results obtained *in vitro* in project FV 314 and anecdotal reports from other parts of the world. Nevertheless, Sporekill-treated transplants had the best visual appearance and retained their cotyledons much longer than the other treatments, and also had noticeably lower levels of downy mildew (see Table 8). It seems quite likely that the retention of the cotyledons was due to the prevention of infection with downy mildew. As the first infections with *Xcc* in Brassica transplants occur in the cotyledons, it is possible that by promoting cotyledon retention, Sporekill may have given *Xcc* populations a greater opportunity to become established than in the other treatments.

An important feature of these spread experiments was that spread of *Xcc* and development of symptoms even in the untreated control was much more limited than expected based on the previous experiments. A prime reason for this may be that the watering system in this experiment was different (although in both cases an overhead moving gantry was used). The original intention had been to use the same system and glasshouse as used previously (at WHRI-Wellesbourne), however, planned decommissioning of the facility meant that the experiment was moved to WHRI-Kirton at a late stage.

Although both systems supply water via an overhead moving gantry, as has been suggested by the author in previous proposals to HDC, small differences in the details of watering systems such as nozzle size/type and water delivery rate may have relatively large impacts on disease spread. In these experiments the system delivered a larger volume of water per pass, meaning that the overall number of passes/watering cycles was reduced.

Another factor may have been that because the watering was under manual control, the operator would sometimes only water with one pass: as the primary infectors were at one end of each block of transplants, and spread has been shown to be directional, the effect of the watering pass would depend on the direction in relation to the location of the primary infectors.

Conclusions

- The probability of transmission of *Xcc* from seed to seedling can be reduced using seed treatments: physical or biological
- Weekly sprays with copper oxychloride are still the best option for reducing the rate of spread of *Xcc* in Brassica transplants
- The BCAs may be some benefit in reducing spread in organic systems or where there may be benefits against other diseases.
- Subtle differences in irrigation systems and watering management may have a big impact on the spread of *Xcc*.

Approval status of products

As at 28-Feb-2009.

Copper oxychloride has approval for off-label use on protected Brassica transplants as Cuprokylt FL (2001-0117) or Headland Inorganic Liquid Copper (2008-0156).

Serenade ASO (liquid formulation) has approval for off-label use on vegetable Brassicas (2009-0246).

Serenade MAX (powdered formulation) does not have approval as a plant protection product in the UK; it is registered as a fungicide in the USA.

Subtilex does not have approval as a plant protection product in the UK; it is registered as a biological fungicide in the USA.

Chlorine dioxide and Sporekill are biocides and do not have approval as plant protection products in the UK.

The status of thyme oil is unclear: it does not have approval as a plant protection product in the UK, but is widely used in mouthwashes and soaps.

Future work

- Based on these results and previous work, further research to confirm the seed treatment results and develop practical treatments would be worthwhile.
- Further work to refine and demonstrate the use of copper sprays in transplants would be worthwhile: i.e. compare and define the minimum treatment parameters for success (i.e. minimum rates, application frequencies) together with taking transplants on into field plantings to demonstrate benefits in the field.
- The apparently contradictory results for chlorine dioxide obtained during recent work in Germany, suggest that it would be worthwhile to investigate the use of lower concentrations (of all products) in continuous-dosing systems.
- The benefit of Sporekill as part of a downy mildew management program is worthy of further investigation.
- The results indicated that there may be potential for significant impacts from subtle alterations in irrigation systems and management, and which may be counter-intuitive, emphasising the need for further work in this area.

Technology Transfer

Roberts, S.J. *Black rot of Brassicas*. Presentation to Plant Propagators Ltd technical meeting, Stamford, 02 October 2008.

Acknowledgements

I am grateful to the various seed companies who provided the seed lots for experimental work. This work was greatly helped by the efforts of the staff at the propagation unit at W-HRI Kirton: Martin Gray and Andrew Flatters.

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Table 1. Treatments and application rates/volumes used in trial to assess the spread of *Xanthomonas campestris* pv. *campestris* in Brassica transplants

Block	Product Name (form)	ml or g/L	Rate[1] per tray (mL or g)	Vol. per tray (mL)	Notes
1	Serenade ASO (liquid suspension)	7-5	0.3	48-72	Biological control agent, reduced volume compared to others. SOLA 2009-0246.
2	Cuprokylt FL (liquid suspension)	16	2.5	160	SOLA 2001-0117.
3	Untreated			0	
4	Tristel Fusion (liquid)	20	3.2	160	Chlorine dioxide (100 ppm final conc.). Not approved
5	Cuprokylt (WP, powder)	8.2	1.3	160	plus Activator 90 0.25 mL/L. Not approved.
6	Sporekill (liquid)	0.5	0.08	160	QAC disinfectant. Not approved.

[1] Rate of product.

Table 2. Post-treatment, pre-sowing *Xanthomonas campestris* pv. *campestris* seed test results for three seedlots used in transmission studies, values are the mean, upper and lower 95% confidence limits.

		% infested			Log10(Xcc per seed)		
Seedlot	Treatment	Mean	Lower	Upper	Mean	Lower	Upper
Nat. inf. (S966)	Untreated	25	12.0	43	3.9	3.8	4.0
	Thyme oil (10%)	1.7	0.1	7	0.0	0.0	–
	Subtilex	21	9.3	39	-0.8	-3.1	1.5
	Serenade MAX	6.9	2.2	15	3.7	3.6	3.9
Dip (S1065)	Untreated	75	57.0	88	2.3	2.1	2.5
	Thyme oil (10%)	1.7	0.1	7	0.0	0.0	–
	Subtilex	86	70.0	95	2.5	2.2	2.7
	Serenade MAX	21	12.0	32	-0.2	-1.3	1.0
Vacuum (S1066)	Untreated	75	57.0	88	3.0	2.7	3.3
	Thyme oil (10%)	<5	0.0	5	0.0	0.0	–
	Subtilex	>90	90.0	100	1.8	1.5	2.2
	Serenade MAX	>90	90.0	100	2.7	2.2	3.1

Table 3. Percentage of seedlings contaminated and showing symptoms with Xanthomonas campestris pv. campestris following seed treatment

		Contaminated			Symptoms			Combined		
Seedlot	Treatment	Mean	Lower	Upper	Mean	Lower	Upper	Mean	Lower	Upper
Nat. inf. (S966).	Untreated	6.9	0.35	44.0	0.0	0.00	0.87	6.9	0.4	44.5
	Thyme oil (10%)	0.0	0.00	8.4	0.0	0.00	0.87	0.0	0.0	9.2
	Subtilex	6.9	0.35	44.0	0.0	0.00	0.87	6.9	0.4	44.5
	Serenade MAX	3.0	0.17	13.0	0.0	0.00	0.87	3.0	0.2	13.8
Dip (S1065)	Untreated	3.0	0.17	13.0	1.0	0.26	2.81	3.9	0.4	15.4
	Thyme oil (10%)	0.0	0.00	8.4	1.6	0.69	3.72	1.6	0.7	11.8
	Subtilex	0.0	0.00	8.4	0.6	0.12	2.32	0.6	0.1	10.5
	Serenade MAX	0.0	0.00	8.4	0.6	0.12	2.32	0.6	0.1	10.5
Vacuum (S1066)	Untreated	12.0	12.00	100.0	1.6	0.69	3.72	13.4	12.6	100.0
	Thyme oil (10%)	6.9	0.35	44.0	0.3	0.02	1.80	7.2	0.4	45.0
	Subtilex	3.0	0.17	13.0	0.0	0.00	0.87	3.0	0.2	13.8
	Serenade MAX	6.9	0.35	44.0	1.0	0.26	2.81	7.8	0.6	45.6

Table 4. Effect of various spray treatments on the mean numbers of *Xcc* (log_{10}(bacteria) per plant) on Brassica transplants grown from two different seedlots. Values are the mean, lower and upper 95% confidence limits obtained as predictions from a GLM model in Genstat.

Treatment	Lot 966 (Naturally inf.)			Lot 1064 (spiked)		
	Mean	Lower	Upper	Mean	Lower	Upper
Untreated	4.15	3.85	4.46	nd		
Chlorine dioxide	5.36	4.99	5.73	2.67	1.20	4.15
Sporekill	4.04	3.09	5.00	nd		
Thyme oil (0.1%)	3.66	3.15	4.17	2.84	1.51	4.16
Cuprokylt	2.25	0.37	4.14	3.65	2.81	4.49

nd – not detected.

Table 5. Overall percentages of Brassica transplants infested 39 day after sowing following spread from a single primary infector cell in a block of 15 '345' trays, together with the maximum distance (in cells) from the primary infector at which Xcc was detected, the number with symptoms and the maximum distance at which they were detected.. Estimate, lower and upper 95% confidence limits obtained using STPro (Ridout and Roberts 1995).

Treatment	Estimate (%)	Lower	Upper	Max dist.	No. with symps.	Max. dist.
Untreated	8.6	3.5	17	37	3	2
Chlorine dioxide	4.9	1.5	11	5	0	
Cuprokylt (WP)	0	0	3.4	0	0	
Cuprokylt FL	0	0	3.3	0	0	
Serenade ASO	4.8	1.5	11	17	1	2
Sporekill	7.5	2.7	16	70	3	1

Table 6. Parameter estimates (and their standard errors) for the model describing the relationship between the number of Xcc bacteria and distance from source: $\ln(m) = \ln(dil) + a + b\ln(dist)$

Treatment	*a*		*b*	
	estimate	s.e.	estimate	s.e.
Untreated	15.14	0.44	-3.10	0.22
Chlorine dioxide	10.55	1.35	-6.78	2.15
Cuprokylt (WP)	na		na	
Cuprokylt FL	na		na	
Serenade ASO	14.52	0.50	-4.32	0.40
Sporekill	14.50	0.52	-4.05	0.38

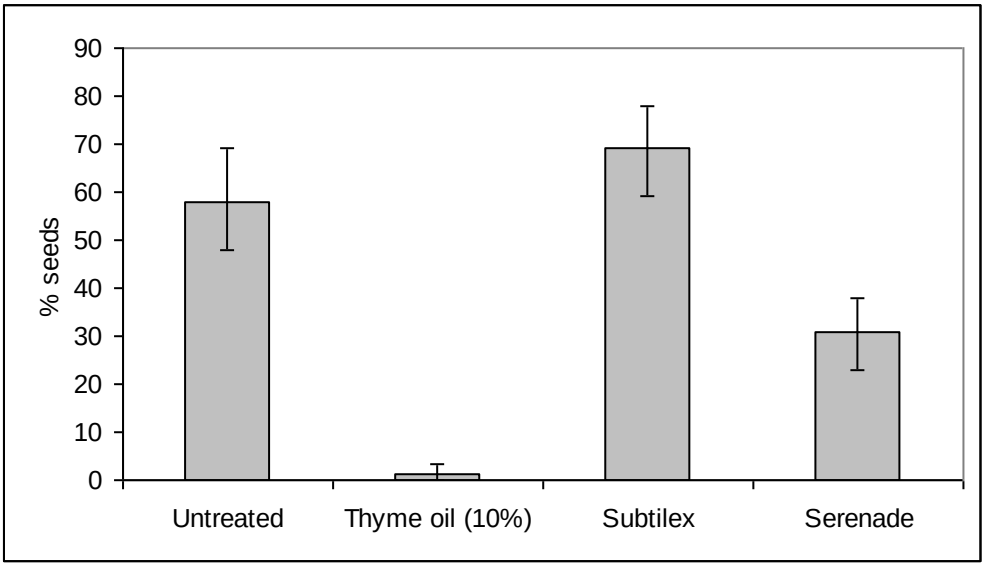


Figure 1. Combined results of pre-sowing, post-treatment seed tests for *Xanthomonas campestris* pv. *campestris* on seed used in transmission experiment. Values are the mean percentage of seeds infested for the three seedlots. Bars represent the upper and lower 95% confidence limits.

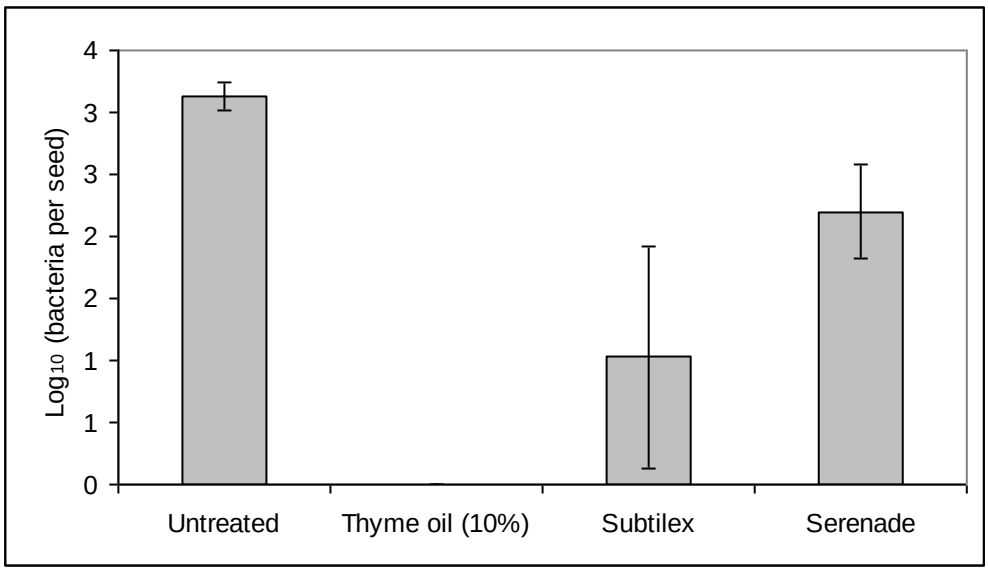


Figure 2. Combined results of pre-sowing, post-treatment seed tests for *Xanthomonas campestris* pv. *campestris* on seed used in transmission experiment. Values are the mean $\log_{10}$(no. of bacteria per seed) for the three seedlots. Bars represent the upper and lower 95% confidence limits.

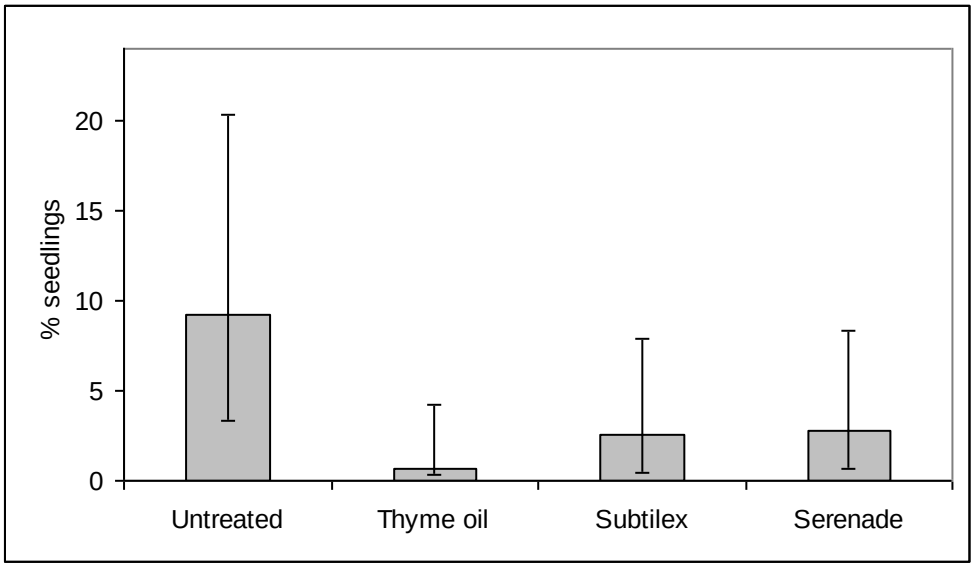


Figure 4. Transmission of *Xanthomonas campestris* pv. *campestris* in three Brassicas seedlots, following treatment of the seed with biologicals/natural products. Values are the mean % of seedlings infested (i.e. contaminated or infected). Bars represent the upper and lower 95% confidence limits.

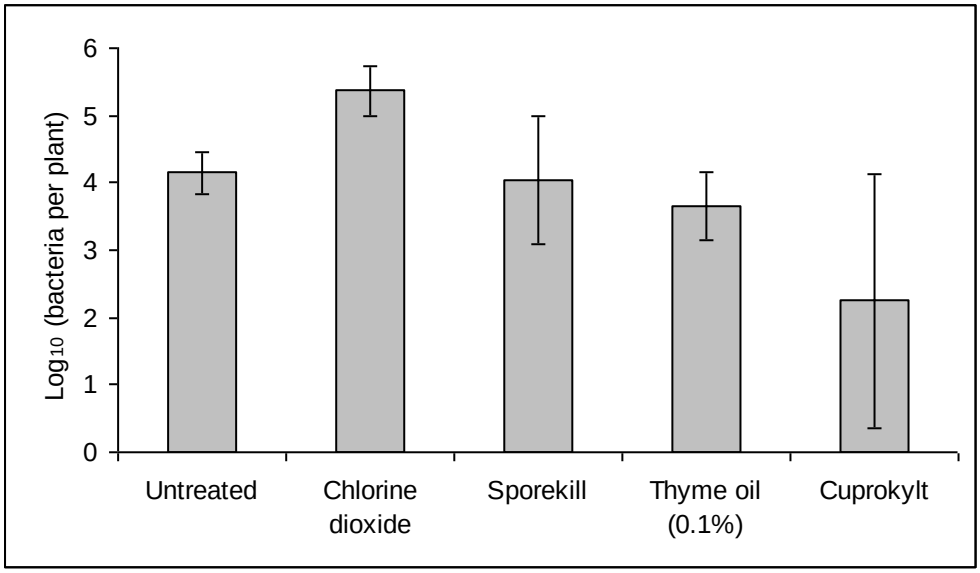


Figure 3. Numbers of *Xanthomonas campestris* pv. *campestris* detected on leaves of Brassica transplants grown from a naturally infested seedlot (S966) following a single treatment with 'disinfectants'. Bars represent the upper and lower 95% confidence limits.

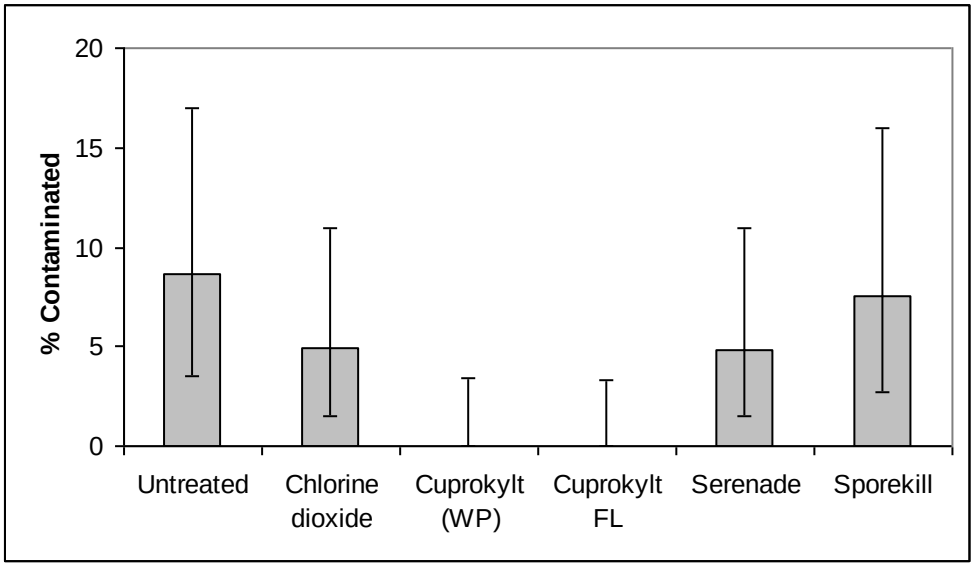


Figure 5. Overall % of Brassica transplants contaminated (both with and without symptoms) with *Xanthomonas campestris* pv. *campestris* 39 d after sowing and with weekly applications of treatments. Bars represent the upper and lower 95% confidence limits.

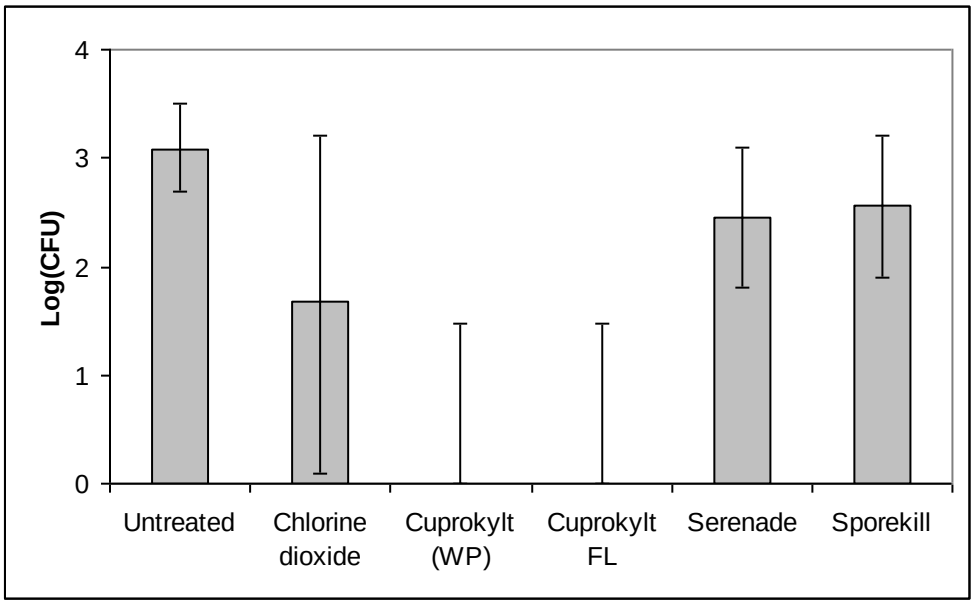


Figure 6. Overall mean numbers of *Xanthomonas campestris* pv. *campestris* detected in Brassica transplants (without symptoms) 39 d after sowing and with weekly applications of treatments. Bars represent the upper and lower 95% confidence limits.

Table 7. Predicted mean $\log_{10}$ reduction in bacterial numbers for each contact time in the absence (clean) and presence of peat. Predictions obtained by fitting a generalised linear model containing significant parameters (extracted from HDC HNS 91 Final Report (Roberts and Akram 2002)).

Compound	Rank[1]	Contact time (min)					
		5		15		30	
		Red[n]	s.e.[2]	Red[n]	s.e	Red[n]	s.e
Clean							
Alcohol	1	5.0		5.0		5.0	
Aliette	14	0.1	0.1	0.6	0.1	1.5	0.2
Bleach	1	5.0		5.0		5.0	
Copper sulphate	8	3.1	0.2	3.7	0.2	4.6	0.4
Croptex Fungex	11	2.3	0.2	3.4	0.3	5.2	0.4
Cuprokylt	12	1.7	0.2	3.9	0.4	7.1	0.9
Cuprokylt FL	10	2.5	0.2	4.3	0.4	7.0	1.1
Jet 5	1	5.0		5.0		5.0	
Menno Florades	1	5.0		5.0		5.0	
Myacide	13	1.6	0.3	2.6	0.4	4.2	0.6
Panacide M	1	5.0		5.0		5.0	
Super Antibac	9	2.5	0.2	5.1	0.8	9.0	2.0
Vitafect	6	5.9	1.9	6.1	1.8	6.3	1.9
Wetcol 3	7	4.7	1.0	5.2	1.0	5.8	1.9
Max. detectable reduction		5.0		5.0		5.0	
Peat							
Alcohol	1	3.9		3.9		3.9	
Aliette	12	1.6	0.3	2.7	0.3	4.5	0.5
Bleach	1	3.9		3.9		3.9	
Copper sulphate	14	0.2	0.2	1.4	0.3	3.3	0.6
Croptex Fungex	6	4.1	0.6	5.9	0.7	8.6	0.9
Cuprokylt	11	1.6	0.2	4.4	0.5	8.7	1.1
Cuprokylt FL	13	0.8	0.2	3.2	0.5	6.9	1.1
Jet 5	1	3.9		3.9		3.9	
Menno Florades	1	3.9		3.9		3.9	
Myacide	10	1.8	0.4	3.5	0.5	6.1	0.9
Panacide M	1	3.9		3.9		3.9	
Super Antibac	9	2.5	0.4	5.7	0.9	10.5	2.1
Vitafect	7	3.3	1.2	4.1	1.3	5.3	1.9
Wetcol 3	8	2.7	0.8	3.7	1.0	5.3	2.1
Max. detectable reduction[3]		3.9		3.9		3.9	

[1]Rank – in order of efficacy, 1 = most effective, 14 = least effective
[2]s.e. – standard error, not estimable for some treatments.
[3]Values greater than the maximum detectable reduction should be considered to be equivalent, but are included for ranking purposes.

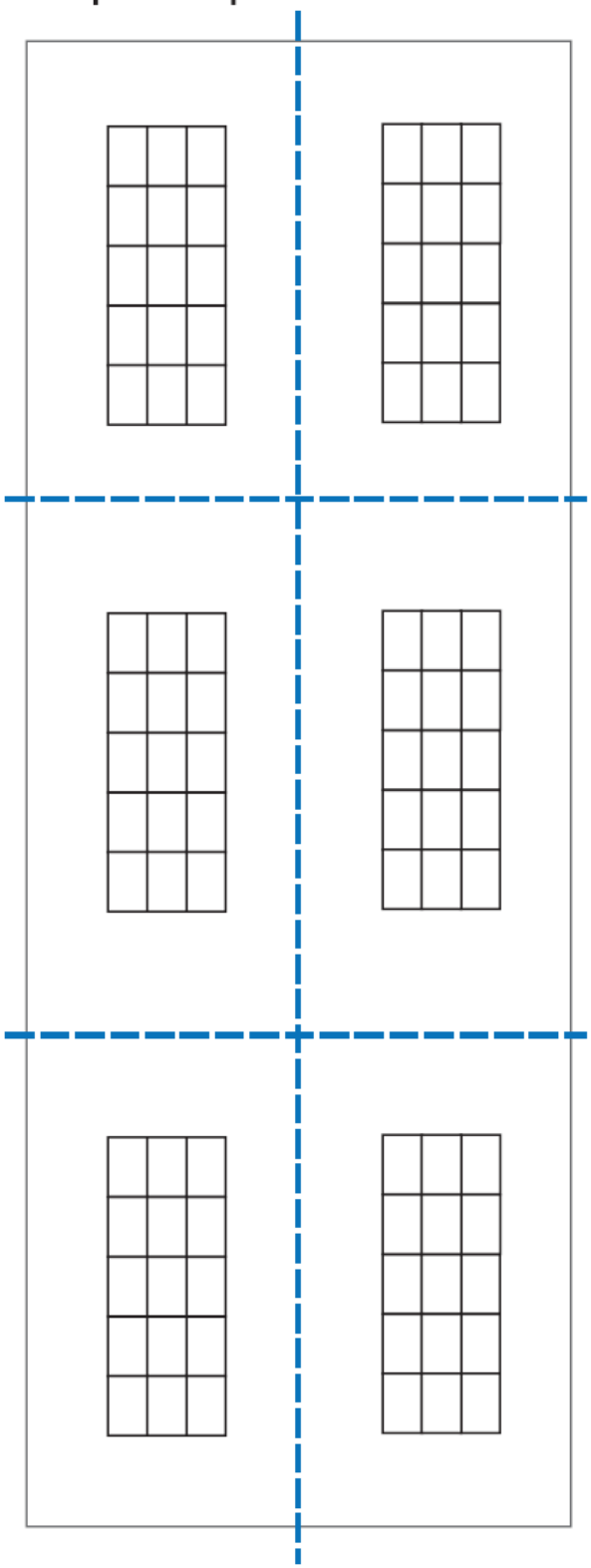
Table 8.Downy mildew levels in Brassica transplants in the edge row of a central module tray.

Treatment	%
Untreated	77
Chlorine dioxide	100
Cuprokylt (WP)	52
Cuprokylt FL	37
Serenade	78
Sporekill	35

n=22

Appendix I

FV 335 Layout of *Xcc* Spread Experiment at Kirton



Direction of irrigation gantry

Approx. 0.75 m high polythene barrier to minimise splash/spread between treatments

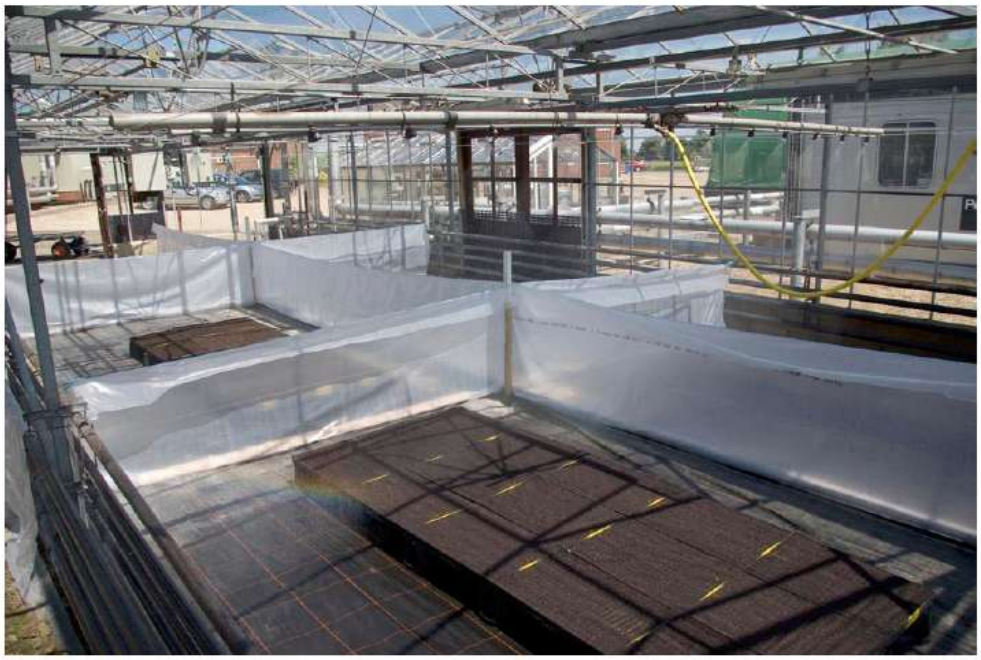
6 Blocks of 15 trays

Prepared by Steve Roberts
Plant Health Solutions
23 May 2008

Appendix II

IMGP0609
ıytotoxicity symptoms in brassica seedlings (S1064) following spray treatment with 0.1% Thyme oil

IMGP0627
Black rot (Xanthomonas campestris pv. campestris) symptoms in brassica seedlings



IMGP0862
Overview of experiment to examine the effect of different treatments on the spread of Xanthomans in brassica transplants

IMGP0931
Leaf scorching in cauliflower transplants following chlorine dioxide treatment. HDC trial to examine treatments to reduce the rate of spread of Xanthomonas (E890)

Appendix III

NOTICE OF APPROVAL Number: 0117/2001

FOOD AND ENVIRONMENT PROTECTION ACT 1985
CONTROL OF PESTICIDES REGULATIONS 1986 (S.I. 1986 NO. 1510):
APPROVAL FOR OFF-LABEL USE OF AN APPROVED PESTICIDE PRODUCT

This approval provides for the use of the product named below in respect of crops and situations, other than those included on the product label. Such "off-label use", as it is known, is at all times done at the user's choosing, and the commercial risk is entirely his or hers.

The conditions below are statutory. They must be complied with when the off-label use occurs. Failure to abide by the conditions of approval may constitute a breach of that approval, and a contravention of the Control of Pesticides Regulations 1986 (as amended). The conditions shown below supersede any on the label which would otherwise apply.

Level and scope:	Notice is hereby given that in exercise of the powers conferred by Regulation 5 of the Control of Pesticides Regulations 1986 (SI 1986/1510) (as amended) and of all other powers enabling them in that behalf, the Secretary of State and the Scottish Ministers (as regards Scotland) and the National Assembly for Wales and the Secretary of State (acting jointly as regards Wales) have given full approval for the use of
Product name:	Cuprokylt FL containing
Active ingredient:	270 g / l copper oxychloride
Marketed by:	Universal Crop Protection Limited under MAFF Number 08299 subject to the conditions relating to off-label use set out below:
Date of expiry:	31 December 2013 (subject to the continuing approval of MAFF 08299).

Use:

Field of use:	**ONLY AS A HORTICULTURAL FUNGICIDE**

Crops/situations:	*Maximum individual dose*:	*Maximum total dose*:	*Maximum number of treatments*: (per crop)	*Latest time of application*:
Chinese cabbage during propagation	See 'Other specific restrictions'	-	6	Prior to planting out
Protected crops of broccoli, Brussels sprout, cabbage, calabrese, cauliflower, choi sum, collards (including spring greens), kale	See 'Other specific restrictions'	-	6	Prior to planting out

Other specific restrictions:

(1) This product must only be applied in accordance with the terms of this approval, the product label and/or leaflet and any additional guidance on off-label approvals.

(2) Since this product is harmful to livestock, all livestock must be kept out of treated areas for at least 3 weeks after treatment.

(3) The maximum in-use concentration must not exceed 16 ml product/litre of water.

Signed by: susan d porting
Signing time: Monday, September 25 2006, 11:30:51 GMT
Location: York
Reason to sign: For the Pesticides Safety Directorate

PSD Digital Signature

Date of issue 25 September 2006

EXPLANATORY NOTES

1. This Notice of Approval is number 0117 of 2001.
2. This notice will be published on the PSD website.
3. Application Reference Number: COP 2005/01229

ADVISORY INFORMATION

This approval relates to the use of 'Cuprokylt FL' (M08299) as a horticultural fungicide for the control of black rot (*Xanthomonas campestris*) and spear rot (*Pseudomonas flourescens*) on Chinese cabbage during propagation, protected crops of broccoli, Brussels sprout, cabbage, cauliflower, calabrese, collard (spring greens), kale and choi sum. Applications to be made via conventional hydraulic sprayer, air-assisted hydraulic sprayer, knapsack sprayer or overhead irrigation system at a rate of 16 mls product in 1 litre of water per 1.5 square metres.

This approval has been re-issued with the addition of approval for use on protected choi sum. This use was formerly approved under the Long Term Arrangements for Extension of Use. This notice sets out the full conditions of the agreed extrapolations.

Earlier versions of Notice of Approval Number 0117 of 2001 remain valid.

NOTICE OF APPROVAL Number: 0156/2008

FOOD AND ENVIRONMENT PROTECTION ACT 1985
CONTROL OF PESTICIDES REGULATIONS 1986 (S.I. 1986 NO. 1510):
APPROVAL FOR OFF-LABEL USE OF AN APPROVED PESTICIDE PRODUCT

This approval provides for the use of the product named below in respect of crops and situations, other than those included on the product label. Such "off-label use", as it is known, is at all times done at the user's choosing, and the commercial risk is entirely his or hers.

The conditions below are statutory. They must be complied with when the off-label use occurs. Failure to abide by the conditions of approval may constitute a breach of that approval, and a contravention of the Control of Pesticides Regulations 1986 (as amended). The conditions shown below supersede any on the label which would otherwise apply.

	Notice is hereby given that in exercise of the powers conferred by Regulation 5 of the Control of Pesticides Regulations 1986 (SI 1986/1510) (as amended) and of all other powers enabling them in that behalf, the Secretary of State and the Scottish Ministers (as regards Scotland) and the Welsh Ministers and the Secretary of State (acting jointly as regards Wales) have given
Level and scope:	full approval for the use of
Product name:	Headland Inorganic Liquid Copper containing
Active ingredient:	435 g / l copper oxychloride
Marketed by:	Headland Agrochemicals Ltd under MAPP Number 13009 subject to the conditions relating to off-label use set out below:
Date of expiry:	31 December 2013 (subject to the continuing approval of MAPP 13009).

Use:

Field of use:	**ONLY AS AN AGRICULTURAL/HORTICULTURAL FUNGICIDE**

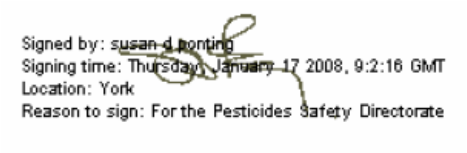
Crops/situations:	*Maximum individual dose*:	*Maximum total dose*: (litres product / ha / crop)	*Maximum number of treatments*: (per crop)	*Latest time of application*:
Protected crops of broccoli, Brussels sprout, cabbage, calabrese, cauliflower, Chinese cabbage, choi sum, collard, kale during propogation, Pak Choi, tat soi	See 'Other Specific Restriction' (3)	-	6	Prior to planting out.
Outdoor crops of broccoli, Brussels sprout, cabbage, calabrese, cauliflower, Chinese cabbage, choi sum, collard, kale, Pak Choi, tat soi	10 litres product / ha	40	-	3 days before harvest.
Outdoor crops of bulb onion, garlic, leek, salad onion, shallot	4 litres product / ha	20	-	14 days before harvest.
Watercress	1 ml product / 1 m^2 of seedling bed	-	2	Prior to planting out.
Cobnut, hazelnut, walnut	3 litres product / ha	-	-	3 months before harvest.

Other specific restrictions:

(1) This product must only be applied in accordance with the terms of this approval, the product label and/or leaflet and any additional guidance on off-label approvals.

(2) Since this product is harmful to livestock, all livestock must be kept out of treated areas for at least 3 weeks after treatment.

(3) The maximum in-use concentration for protected crops of broccoli, Brussels sprout, cabbage, cauliflower, calabrese, collard, kale, Chinese cabbage, Pak Choi, choi sum and tat soi during propagation must not exceed 16 ml product per 1 litre of water.



PSD Digital Signature

Date of issue 16 January 2008

EXPLANATORY NOTES

1. This Notice of Approval is number 0156 of 2008.

2. This notice will be published on the PSD website.

3. Application Reference Number: COP 2007/01726

ADVISORY INFORMATION

This approval relates to the use of 'Headland Inorganic Liquid Copper' (M13009) as an agricultural horticultural fungicide:

For the control of black rot and spear rot on protected crops of broccoli, Brussels sprout, cabbage, cauliflower, calabrese, collard, kale, Chinese cabbage, Pak Choi, choi sum and tat soi during propagation. Applications to be made at a rate of 16 ml product in 1 litre of water per 1.5 square metres via conventional hydraulic, air-assisted hydraulic and knapsack sprayers or via an overhead irrigation system.

For the control of black rot and spear rot on outdoor crops of broccoli, Brussels sprout, cabbage, cauliflower, calabrese, collard, kale, Chinese cabbage, Pak Choi, choi sum and tat soi. Applications to be made at a rate of 10 litres of product in 200 litres of water per hectare via conventional hydraulic, air-assisted hydraulic and knapsack sprayers

For the control of bacterial rot, in particular *Pseudomonas spp*, on outdoor crops of bulb onion, salad onion, shallot, garlic and leek. Applications to be made at a rate of 4 litres of product in 200 litres of water per hectare via a conventional hydraulic sprayer.

For the control of *Phythium spp* and *Rhizoctonia spp* on watercress during propagation applied via conventional hydraulic and hand held sprayers. The maximum concentration should not exceed 5 ml of product per 1 litre of water

For the control of bacterial cankers and blight on hazelnut, cobnut and walnut. Applications to be made at a rate of 3 litres of product in 500 litres of water per hectare applied via conventional hydraulic, hydraulic air assisted and hand held sprayers.

FOOD AND ENVIRONMENT PROTECTION ACT 1985: PART III
PLANT PROTECTION PRODUCTS REGULATIONS 2005
PLANT PROTECTION PRODUCTS (BASIC CONDITIONS) REGULATIONS 1997

(All references to this legislation in this Notice of extension of approved use and in any annexes to it are to the same as amended to the date of issue of this notice).

This extension of the approved use provides for the use of the product named below in respect of crops and situations, other than those included on the product label. Such an 'an extension of use', is at all times done at the user's choosing, and the commercial risk is entirely theirs.

The conditions below are obligatory. They must be complied with when the extension of use occurs. Failure to abide by the conditions of approval may constitute a breach of that approval, and a contravention of the Plant Protection Products Regulations 2005. For the purposes of this extension of use only, the conditions and/or requirements shown below supersede any corresponding conditions and/or requirements set out on the label or otherwise provided for under the main approval which would otherwise apply.

NOTICE OF Extension of use
Number: 0246 of 2009

Level and scope:	Notice is hereby given that the Secretary of State, in exercise of the powers conferred by regulation 10 of the Plant Protection Products Regulations 2005 (S.I. 2005/1435) ("PPPR") has given an extension of the approved use of
Product name:	Serenade ASO containing
Active ingredient:	1.34 % w/w bacillus subtilis (strain QST 713)
Product approval holder:	AgraQuest Inc (Company registration no. 69592)
Marketed by:	Fargro Limited under MAPP Number 14318

AS FOLLOWS:

USE:

Field of use:	**ONLY AS A HORTICULTURAL FUNGICIDE**

Crops/situations:	*Maximum individual dose*: (litres product / ha)	*Maximum total dose*:	*Maximum number of treatments*:	*Latest time of application*:
Bulb vegetables, fruiting vegetables, herbs, leafy vegetables, legumes, ornamental plant production, root and tuber crops, stem vegetables, top fruit, vegetable brassicas	10	-	Every 7 days to harvest	-
Cane fruit	10	-	Every 7 days to harvest	-
Bilberry, blackcurrant, blueberry, cranberry, gooseberry, redcurrant, ribes hybrids, outdoor strawberry, table grapes, wine grapes	10	-	Every 7 days to harvest	-
Canary grass, figs, hops	10	-	Every 7 days to harvest	-

Operator Protection:

(1) Engineering control of operator exposure must be used where reasonably practicable in addition to the following personal protective equipment:

Operators must wear suitable protective clothing (coveralls) and suitable protective gloves when handling the concentrate.

(2) However, engineering controls may replace personal protective equipment if a COSHH assessment shows that they provide an equal or higher standard of protection.

Other specific restrictions:

This product must only be applied in accordance with the terms of this approval, the product label and/or leaflet and any additional guidance on extensions of use.

Signed by: sue d ponting
Signing time:Thursday, January 29 2009, 9:50:54 GMT
Location: York
Reason to sign: For the Pesticides Safety Directorate

PSD Digital Signature

Date of issue: 29 January 2009

Date of expiry: 25 November 2012 [Subject to the continuing approval of MAPP 14318]

EXPLANATORY NOTES

1. This Notice of Extension of use is number 0246 of 2009.
2. This notice will be published on the PSD website.
3. Application Reference Number: COP 2008/00993 PP

ADVISORY INFORMATION

This approval relates to the use of 'Serenade ASO' (M14318) as a horticultural fungicide for the control of Botrytis spp. in protected and outdoor crops as listed above.

Crop situations have been defined according to their primary crop groups (please see crop hierarchy for further details) with the exception of the 'soft fruit' group. The 'soft fruit' primary group has been divided into the parent group 'cane fruit' and the basic crop or situations of the 'bush/small fruit crops'.

Applications should be made via conventional hydraulic sprayers including air assisted sprayers and hand held sprayers in a minimum of 100 litres water per hectare and a maximum of 1000 l water/ha (to just before run-off).

