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The results and conclusions in this report are based on a series of laboratory based experiments conducted over a 3-4 month 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.

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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.

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Signature .... Date ....

Report authorised by:

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Signature .... Date ....

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Grower Summary

Headline

- A number of horticultural disinfectant products, a plant oil and a bio-control product were evaluated *in vitro* and found to be effective against *Xanthomonas campestris* pv. *campestris*, cause of black rot of crucifers, in laboratory studies. Chlorine dioxide ('Sanogene') and didecyl dimethyl ammonium chloride ('Sporekill') were found to be most effective in this initial *in vitro* test.
- Note – Not all products tested are registered for use in the UK – please see text below for details and ensure full compliance with all on or off-label recommendations. Consult www.pesticides.gov.uk for up-to-date label status.

Background and expected deliverables

The seed-borne pathogen *Xanthomonas campestris* pv. *Campestris* (*Xcc.*) causes black rot in Brassica seedlings during propagation. It has been estimated that even very low seed-borne infections e.g. <1 in 10,000 seed can lead to epidemic development of the disease in intensive monoculture systems such as Brassica propagation. There has been discussion in the industry that products such as Jet 5/Hyperox are becoming less effective in controlling the spread of infection from primary infector plants (R White : pers. comm.). This investigation focused on looking at the efficacy of possible alternative products for use in the future.

Information regarding one possible candidate product for this work was put forward by a prominent Brassica propagator. The product - 'Sporekill'™ is marketed as 'a horticultural disinfectant and plant sanitiser'. However, at present this product is not registered for use in the UK though is currently marketed in South Africa and Australia. This product, whose active ingredient is didecy dimethyl ammonium chloride, was tested alongside 'Jet 5' (hydrogen peroxide/per-acetic acid), 'Sanogene' (chlorine dioxide), Thyme Oil and the biological control product 'Serenade' (*Bacillus subtilis*) in an *in vitro* study to ascertain their relative efficacy in inhibiting the growth of *Xcc.*

It was hoped that this small-scale laboratory experiment would provide some initial comparative data to enable the propagation industry to make decisions regarding the potential for further study to ultimately gain improved control of black rot in Brassica seedlings and, ultimately, crops post-planting.

Summary of the project and main conclusions

- A number of horticultural disinfectant products along with Thyme oil and a bio-control product were tested in the laboratory to investigate their efficacy in inhibiting the growth of *Xanthomonas campestris* pv. *campestris* (*Xcc*) in an *in vitro* experiment.

- All products performed well, particularly at the higher concentrations and inhibited the growth of *Xcc* at a range of different time exposures.

- 'Sanogene' (chlorine dioxide) proved to be the most effective of all the products tested, with 'Sporekill' (didecyl dimethyl ammonium dioxide) proving only slightly less effective. Thyme oil and Jet 5 (per-acetic acid) were effective, though marginally less so than the two products named previously.

- The biological control product – 'Serenade' (*Bacillus subtilis*) could not be tested in the same way as the other products as its mode of action involves direct competition with *Xcc* in terms of nutrients, niches and the possible release of secondary (inhibitory) metabolites. The modified experiments showed that at the higher

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concentrations there was clear inhibition of *Xcc* in culture. However, whilst a qualitative effect was found, it was not possible to quantify it effectively in an *in vitro* test and hence form a clear idea of the potential efficacy of this product. It is therefore recommended that further work is undertaken with this product *in vivo*.

The majority of the products in this study (with the exception of 'Serenade') were tested by adding a known amount of a *Xcc* cell suspension to the product at a range of dilutions (1:0, 1:1, 1:10, 1:100, 1:1000 and 1:10,000). The bacteria/disinfectant mix was then sampled after 1, 5, 10, 30 and 60 minute exposure times. The samples were plated out onto artificial growth media (nutrient dextrose agar) and incubated for 72 hours. After this time the number of viable *Xcc* colonies (colony forming units or cfu's), arising from single cells of *Xcc* that had survived disinfection exposure, were counted.

The data from the experiments is shown in Figure 1. It is presented as the percentage inhibition of *Xcc* colonies compared to the sterile water treatment, included as a control for comparison purposes.

All the products under investigation were fully effective in inhibiting *Xcc* at concentrations of 1:100 and above. Similarly, most products were highly effective at the lower concentrations (1:1000 and 1:10,000) particularly at the longer time exposures. 'Sanogene' appeared particularly good in this regard. 'Sporekill' was marginally less effective at the 1: 10,000 dilution (1 minute exposure) but still provided a 99.8% inhibition of *Xcc*.

The test method above could not be used for the biological control product 'Serenade' (*B. subtilis*) as it resulted in blanket growth of the bio-control organism in the formulated product. We therefore devised an alternative, qualitative, technique to determine whether this product was inhibitory to *Xcc*. We used a droplet plate method whereby agar plates were initially 'seeded' with a cell suspension of *Xcc* spread across the plates using an 'L' shaped glass rod to create a bacterial 'lawn'. The plates were then incubated at 22°C for 24hrs. Droplets (2µl) of the bio-control product 'Serenade' were positioned in 5 locations on triplicate plates at the full range of concentrations. The plates were then incubated for a further 48hours. Following this period the plates were examined visually and we were able to detect zones of inhibition in the *Xcc* cultures (Figure 2). This demonstrated that the *B. subtilis* had out-competed *Xcc* either for a food source, through direct antagonism or via metabolite liberation into the agar.

Figure 2 : Example of zones of inhibition in 'Serenade' challenge inoculation plates.

The zones of inhibition were seen most clearly at the higher concentrations of 'Serenade' (1:0 – 1:100), with less marked zones observed in the two lower concentrations.

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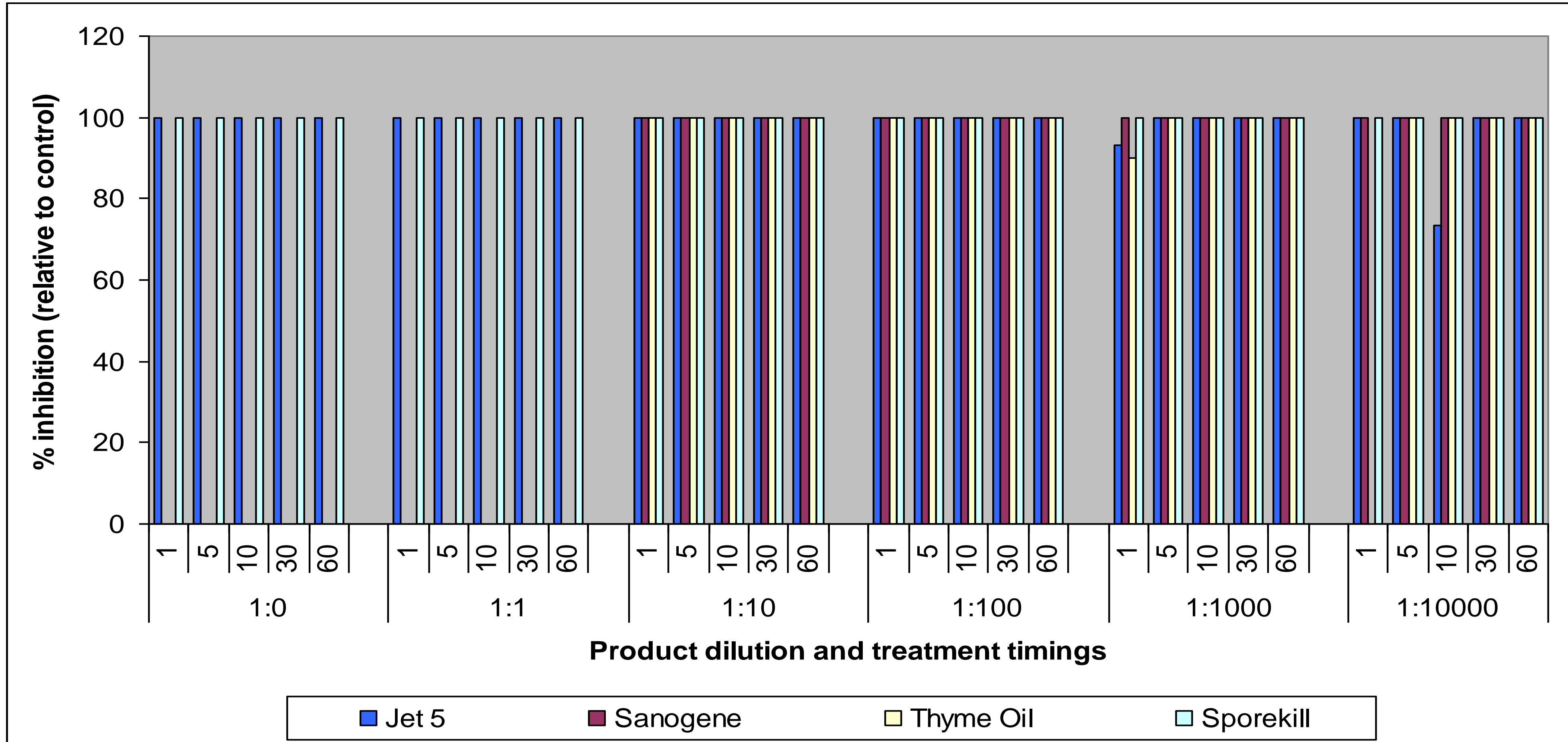
Figure 1. Summary chart of the inhibitory potential of the various disinfectants tested against *Xanthomonas campestris pv. campestris in vitro*.



N.B. The efficacy of 'Sanogene' and Thyme oil was only investigated at dilutions of 1:10 and above.

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Financial benefits

There are no immediate financial benefits from this laboratory based study. However, if the follow-on work on live plants can continue to demonstrate efficacy, and improve on the level of control of *Xanthomonas* that is currently achieved, then the economic benefit to the Brassica industry will be significant.

Action points for growers

- Growers should continue to source seed from reputable suppliers to minimize the risk of seed-borne *Xanthomonas*.
- Good hygiene measures should be deployed in propagation where they are practical and economic to minimize the risk of spread of the disease.
- Growers should walk crops regularly and rogue any suspect plants at the earliest opportunity though they should also note that *Xanthomonas* may be symptomless at this stage of infection in propagation
- Liberal use of disinfectants between crops is advisable to minimize the risk of carry-over of inoculum between crops.

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Science Section

Introduction

Black rot caused by seed-borne *Xanthomonas campestris* pv. *campestris* (*Xcc*) is a continuing problem for Brassica propagators. Although very low levels of seed infection may occur e.g. as low as 1:10,000 seed, this can still lead to exponential development of the disease in intensive monoculture systems such as that found in module production systems for Brassicas. Spread from a single infected leaf occurs when the organism is splashed from plant to plant during irrigation events (or during rainfall if the plants are stood outside to 'harden off'). Previously hydrogen peroxide/per-acetic acid products e.g. 'Jet 5', 'Hyperox' have been employed as water disinfection treatments prior to use for irrigation to help minimise spread of the pathogen. However, it is felt that 'Jet 5' is not effective enough (R White, *pers comm.*) and alternative disinfection products are required to provide effective control. One possible new product which is currently marketed in South Africa and Australia is 'Sporekill'™'. The product is based on didecyldimethyl ammonium chloride (DDAC) and is reported to be effective against a range of organisms (bacteria, fungi and viruses), including *Xanthomonas* spp.

This study described aimed to compare the efficacy of a number of products with known anti-bacterial properties, some of which are already used as proprietary horticultural disinfectants, in a small-scale *in vitro* study using different concentrations of the products and a range of exposure times against the *Xanthomonas* pathogen.

Materials and Methods

The products chosen for inclusion in this study were:

'Jet 5'	Hydrogen peroxide/per-acetic acid
'Sporekill'™	Didecyl dimethyl ammonium chloride (DDAC)
'Sanogene'	Chlorine Dioxide
Thyme Oil	-
'Serenade'	*Bacillus subtilis*

Where possible, the products were used at a range of dilutions, i.e. 1:0 (neat), 1:1, 1:10, 1:100, 1:1000 and 1:10000. However, in the case of 'Sanogene' and Thyme oil it was not practical, for health & safety reasons, to use them at the neat or 1:1 dilution and therefore they were only tested at 1:10, 1:100, 1:1000 and 1:10000 dilutions.

An isolate of *Xcc* was sourced (NCPPB 528) and grown on nutrient dextrose agar (NDA). A turbid suspension of the bacteria was produced in sterile de-ionised water (approximately 1×10^7 cells/ml) in sterile universal containers (UC's) and diluted to a working stock solution of approximately 1×10^4 cells/ml. Aliquots of 0.1ml were added to the prepared concentrations of the disinfectant products under investigation in UC's and vortexed well. Sterile de-ionised water was used as a control. The bacteria/disinfectant or bacteria/water (control) mixes were sampled after 1, 5, 10, 30, and 60 minute exposure times by plating 0.1ml aliquots of the mix onto triplicate plates of NDA. The sub-samples were spread using sterile spreaders and incubated at 22°C for 72 hours. After this time it was possible to count the number of *Xcc* colonies (colony forming units or cfu) present on each plate.

A different methodology had to be employed when testing the effect of 'Serenade' (*Bacillus subtilis*) on *Xcc* as initial attempts, using the method described above, resulted in blanket growth of *B. subtilis* across all plates with little or no growth of *Xcc* on any of the agar plates.

It was decided to use a qualitative *in vitro* inhibition test to examine the efficacy of 'Serenade' against *Xcc*. Triplicate plates were set up using the arrangement shown overleaf (Figure 3). A lightly turbid suspension of *Xcc* was produced (ca. 10^6 cells /ml) and 0.1ml aliquots were spread onto triplicate plates of NDA. The plates were then incubated for 24 hours to create

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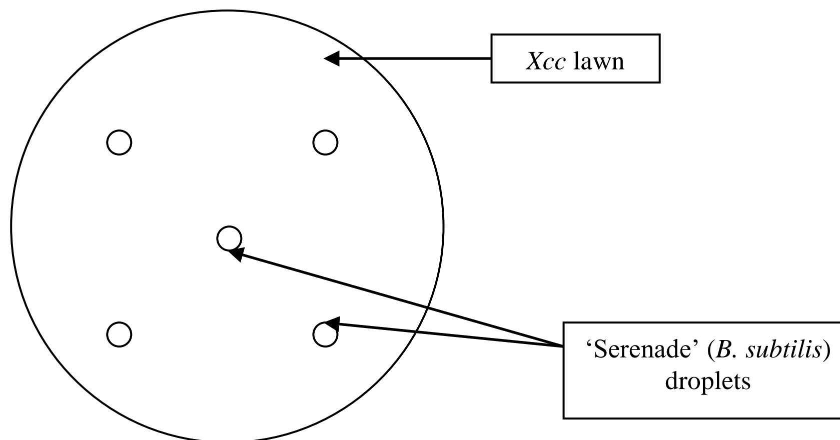
a bacterial ‘lawn’. Individual droplets (200µl) of ‘Serenade’ at the full range of concentrations were positioned on the plates as shown in the diagram. The plates were incubated for a further 48 hours. Although this test could not investigate any difference in time exposures, or be quantified accurately it did provide an indication of the relative activity of ‘Serenade’ against *Xcc*. A number of other small tests were also established on agar plates to investigate the inhibitory potential of ‘Serenade’ in a range of challenge inoculations with *Xcc*.

Figure 3 : Inhibition test plate for ‘Serenade’ (*Bacillus subtilis*) and *Xanthomonas campestris* pv. *campestris*



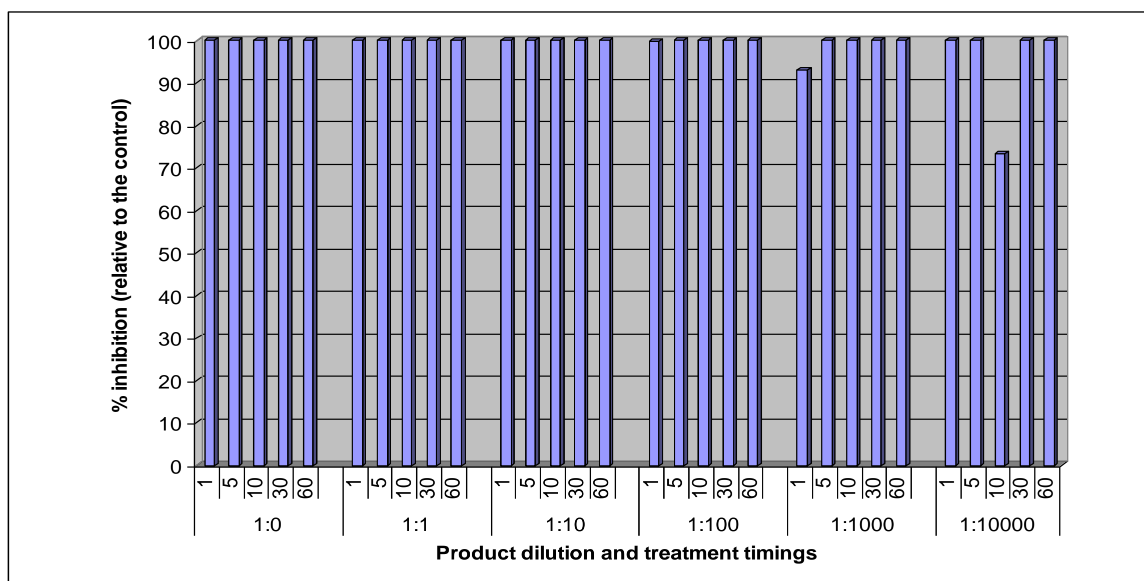
Results and Discussion

Following incubation of the agar plats as described, the number of colony forming units (cfu) of *Xcc* present on the test plates was recorded. The results for the different concentrations and exposure times for Jet 5, 'Sanogene', Thyme Oil and 'Sporekill'™ are shown in Figures 4-7. Results are presented as the % inhibition of the mean cfu of *Xcc* relative to the sterile water control at the same time exposures. Full data sets for each experiment are shown in Appendix 1. A reduction in the number of cfu in the control plates was observed (i.e. where *Xcc* was added to sterile de-ionised water) over the 60 minute sampling period, though this appeared slightly variable. The effect may have been due to lysing of the bacterial cells following exposure to a non-isotonic matrix, though this doesn't explain the overall variability of the results in this respect.

'Jet 5' (per-acetic acid/hydrogen peroxide)

A good level of inhibition of the bacteria was observed following disinfection with 'Jet 5' particularly at the higher concentrations of 1:100 and above (Figure 4). Low numbers of cfu, indicating viable *Xcc,* were detected following an exposure of 1 minute at the 1:1000 dilution rate; though a longer exposure time at this concentration appeared to be 100% effective. A slight dip in efficacy was also observed after a 10 minute exposure at the lowest concentration of 1:10000 though, given the positive results for reduced exposure times of 1 and 5 minutes, it suggests perhaps that this result is anomalous.

Figure 4 : Inhibitory potential of per-acetic acid/hydrogen peroxide ('Jet 5') against *Xanthomonas campestris pv. campestris in vitro*.

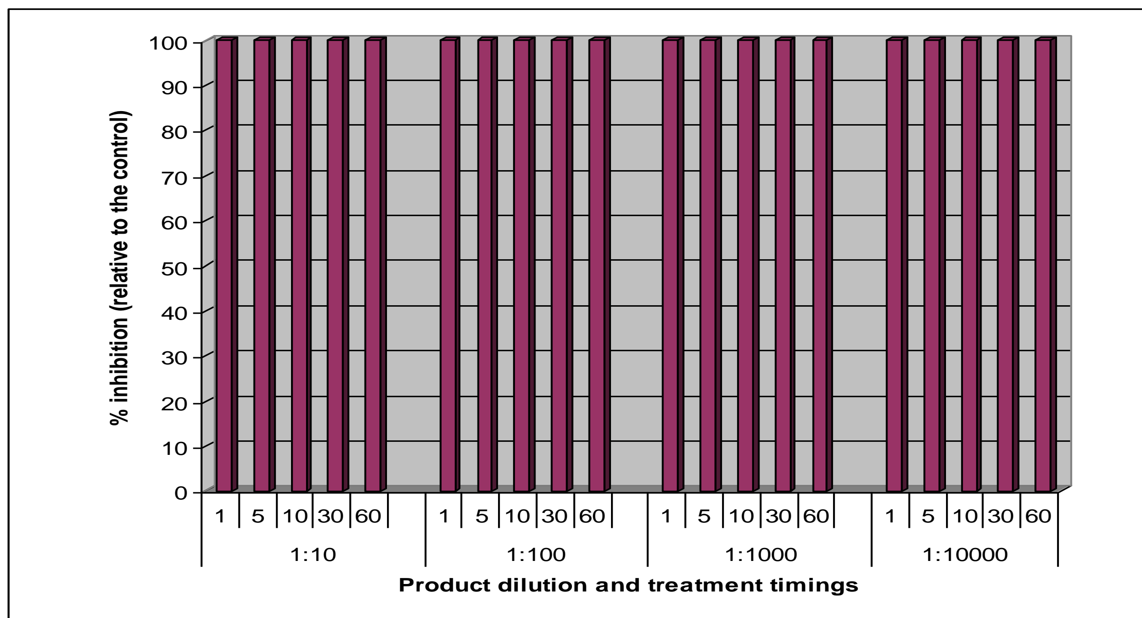


N.B. The result for this product in a 1:10,000 dilution with an exposure time of 10 minutes appears anomalous and cannot be accounted for.

'Sanogene' (chlorine dioxide)

Chlorine dioxide ('Sanogene') proved to be 100% effective against *Xcc* at the full range of dilutions and at the various exposure times evaluated in this *in vitro* test (Figure 5).

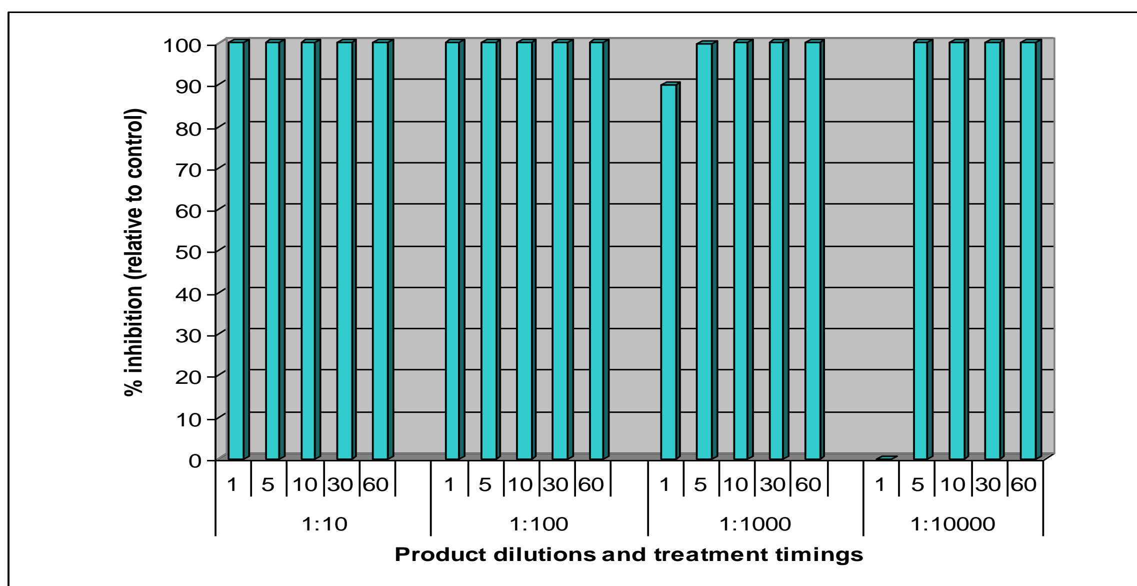
Figure 5 :Inhibitory potential of chlorine dioxide ('Sanogene') against *Xanthomonas campestris pv. campestris in vitro*.



Thyme Oil

Thyme oil was not used neat or at the 1:1 rate as for some of the other products tested. It proved to be 100% effective in this *in vitro* study at the two higher concentrations (1:10 and 1:100) though a slight reduction in efficacy was observed following a 1 minute exposure time at the 1:1000 dilution (Figure 6). At the lowest concentration of the product (1:10000) no inhibition of the bacteria was observed following a 1 minute exposure. However, a longer exposure time of 5 minutes and above proved to be effective at this lowest concentration tested.

Figure 6 : Inhibitory potential of Thyme Oil against *Xanthomonas campestris pv. campestris in vitro*.



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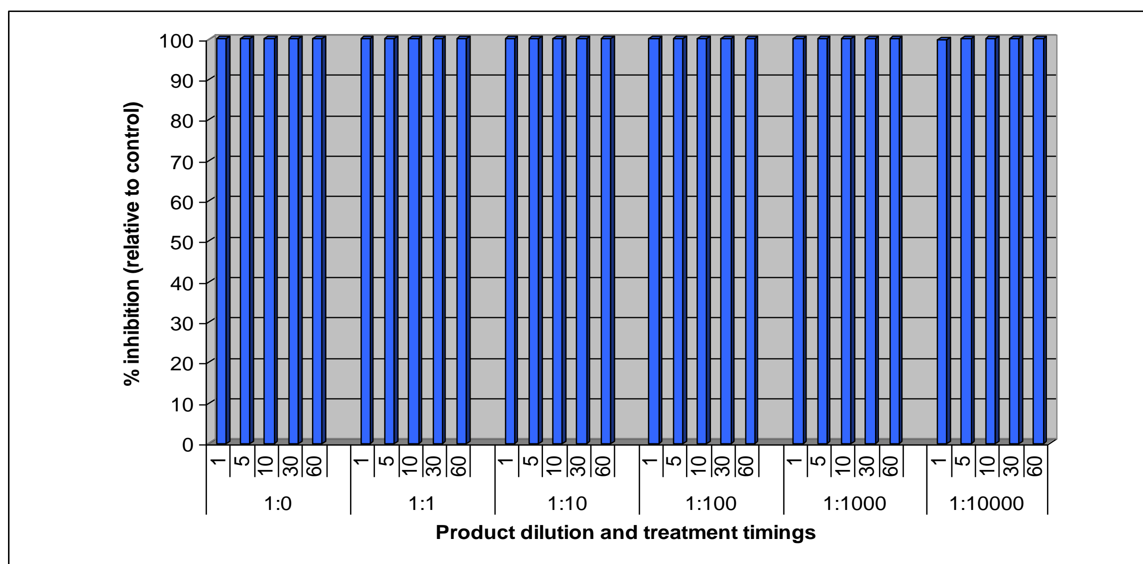
‘Sporekill’ (didecyl dimethyl ammonium chloride (DDAC))

‘Sporekill’™ was highly effective at the 1:0, 1:1, 1:10, 1:100 and 1:1000 concentrations and the product resulted in 100% inhibition of the *Xcc* at all time exposures. A slight drop in efficacy of the product was observed at the lowest concentration (1:10000) following a 1 minute exposure time, though the product still proved to be 100% effective at the longer exposure times at this lowest concentration (Figure 7).

Figure 7 : Inhibitory potential of ‘Sporekill’™ (didecyl dimethyl ammonium chloride (DDAC)) against *Xanthomonas campestris pv. campestris in vitro*.



‘Serenade’ (*Bacillus subtilis*)

Tests to investigate the efficacy of ‘Serenade’ employed a different methodology as described in the Materials & Methods section. Investigations, which compared the growth of *Xcc* and ‘Serenade’ in challenge inoculations on agar plates, indicated that ‘Serenade’ was effective, to a greater or lesser extent, in inhibiting the growth of *Xcc* (Figure 8). The two upper plates show that the growth of *Xcc* was completely inhibited by the growth of the bio-control product ‘Serenade’ containing Bacillus *subtilis*. Little or no growth of *Xcc* was seen on the two lower plates suggesting perhaps that the ‘Serenade’ inhibits the growth of *Xcc* either through direct competition for nutrients or by releasing inhibitory metabolites into the agar.

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Figure 8 : Challenge inoculations between the black rot pathogen *Xanthomonas campestris* pv. *campestris* and the bio-control agent *Bacillus subtilis* ('Serenade')

N.B. The *Bacillus subtilis* ('Serenade') is the off-white cultures on the plates above. The yellow bacterium inoculated along the marked green lines is *Xanthomonas campestris* pv. *campestris.* Note the strong inhibition of *Xcc* where it is adjacent to *B. subtilis*.

Subsequent tests to investigate the effect of different concentrations of the product 'Serenade' on *Xcc* resulted in clear zones of inhibition, particularly at the higher concentrations of 'Serenade' (Figures 9-15). However, this test merely provides a qualitative indication of the level of inhibition. It was not possible, using this technique to quantify the results.

The zones of inhibition at the 1:1000 and 1:10,000 dilution rates of 'Serenade' were less clear, though it should be noted that the concentration of *Xcc* to produce the bacterial 'lawn' on the plates was exceptionally high. It is possible, that where the pathogen is present at a lower population level *in vivo* the overall efficacy of this product at the lower concentrations may be improved. It will be important therefore to further validate these results *in planta* to accurately determine the efficacy of this product against black rot in Brassicas grown in intensive module production systems.

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Figures 9-15 : Relative efficacy of the bio-control product 'Serenade' (*Bacillus subtilis*) in a qualitative inhibition test with *Xanthomonas campestris* pv. *campestris* at different concentrations on agar in the laboratory .

Figure 9 : *Xcc* and sterile water (control)

Figure 10 : *Xcc* and 'Serenade' (neat)

Figure 11 : *Xcc* and 'Serenade' at 1:1 dilution

Figure 12 : *Xcc* and 'Serenade' at 1 : 10 dilution

Figure 13 : *Xcc* and 'Serenade' at 1: 100 dilution

Figure 14 : *Xcc* and 'Serenade' at 1:1000 dilution

Figure 15 : *Xcc* and 'Serenade' at 1:10000 dilution

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Conclusions

All of the products under investigation resulted in moderate to excellent inhibition of *Xcc* in this *in vitro* study. 'Sanogene' (chlorine dioxide) resulted in 100% inhibition at all concentrations over all time exposures in our tests. 'Sporekill' (didecyl dimethyl ammonium chloride) performed well with a very slight drop in inhibition at the 1:10,000 dilution at the shortest time exposure. However, it remained 100% effective at a time exposure>1 minute. Thyme oil was slightly less effective and viable *Xcc* was recovered at 1:1000 dilutions exposed for 1 minute. At this concentration, an exposure time of >5 minutes was required for 100% efficacy. The standard product Jet 5 (hydrogen peroxide/per-acetic acid), included here for comparison, was only moderately effective and viable cells of *Xcc* were detected even in the 1:100 dilution exposed for 1 minute. Increasing the exposure time improved efficacy as with the other products.

It is difficult to be sure of the potential efficacy of 'Serenade' due to the nature of the testing carried out in this laboratory based study. There was a clear suggestion of inhibition of growth of *Xcc*, on the agar plates, particularly at the higher concentrations of the product. However, it was not possible to quantify the effect to allow comparison with the other products under investigation.

The results from this single experiment suggest that 'Sporekill' (didecyl dimethyl ammonium chloride) may well prove to be a useful disinfectant to aid the control of *Xcc* in horticultural Brassicas, subject to the appropriate regulatory requirements within the Biocides Directive. The on-label rate for this type of use is stated as 50-100ml/100l water and this equates to a 1:1000–1:2000 dilution rate. This product performed well at this concentration in the study reported here. However, it should be considered that many other factors may influence the efficacy of this product in an *in vivo* situation e.g. temperature, UV radiation, product stability in the presence of organic material, microbial population, pH and hardness of water etc. It should also be noted that the product label states that ''Sporekill' has wetting characteristics' and this may be of concern in terms of stripping leaf wax on Brassicas which may leave the foliage open to other foliar infections e.g. downy mildew and also potentially increase the risk of phytotoxicity of other products applied for pest and disease control.

The remaining products also performed well and may be worthy of consideration as alternative control products for *Xcc* on Brassica seedlings, especially as there appears to be concern regarding the efficacy of Jet 5 (Mr R White, *pers comm*).

It is therefore recommended that the products evaluated here are further tested in an *in vivo* study to ensure efficacy and crop safety in a commercial cropping situation. Also, progress with the Biocides Directive gathers pace and it will be important to fully explore the potential for commercial development with each of these experimental products in the near future.

Technology transfer

It is too early to make any recommendations for technology transfer from this small piece of laboratory based work. However, the initial results are promising and will be of interest to the Brassica propagators and growers who face a constant challenge of black rot arising as a result of seed- and debris-borne infection by *Xanthomonas campestris* pv. *campestris*. It will, however, be equally important to ensure the industry is aware of the potential limitations of securing some of the products for use and also appreciating that these early *in vitro* studies may not necessarily be 'mirrored' in *in vivo* studies under commercial conditions.

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Appendix 1 : Replicated data sets for the various disinfectant products evaluated against *Xanthomonas campestris* pv. *campestris.*

Table 1 : Replicated data set for hydrogen peroxide/per-acetic acid ('Jet 5')

Disinfectant concentration	Exposure time (minutes)	No of cfu/plate			Mean	% inhibition*
1:0	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:100	1	1	0	0	*0.3*	99.7
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1000	1	8	10	6	*8.0*	93.0
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10000	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	12	0	0	*4.0*	73.3
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100

* % inhibition relative to growth on control plates (sterile de-ionised water)

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Table 2 : Replicated data set for chlorine dioxide ('Sanogene')

Disinfectant concentration	Exposure time (minutes)	No of cfu/plate			Mean	% inhibition*
1:10	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:100	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1000	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10000	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100

* % inhibition relative to growth on control plates (sterile de-ionised water)

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Table 3 : Replicated data set for didecyl dimethyl ammonium chloride (DDAC or 'Sporekill')

Disinfectant concentration	Exposure time (minutes)	No of cfu/plate			Mean	% inhibition*
1:0	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:100	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1000	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10000	1	1	C	0	*0.5*	99.8
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100

* % inhibition relative to growth on control plates (sterile de-ionised water)
C=contaminated plate

Table 4 : Replicated data set for Thyme Oil

Disinfectant concentration	Exposure time (minutes)	No of cfu/plate			Mean	% inhibition*
1:10	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:100	1	0	0	0	*0.0*	100
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:1000	1	42	9	17	*22.7*	90.0
	5	2	0	0	*0.7*	99.7
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100
1:10000	1	172	432	164	*256.0*	0.0
	5	0	0	0	*0.0*	100
	10	0	0	0	*0.0*	100
	30	0	0	0	*0.0*	100
	60	0	0	0	*0.0*	100

*** % inhibition relative to growth on control plates (sterile de-ionised water)**

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