Project Title	Brassicas: Evaluating the use of novel biocides for the control of *Xanthomonas campestris* pv. *campestris* in modules during propagation.
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Project leader:	Dr G M McPherson, Stockbridge Technology Centre.
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Project coordinator:	Mr Neil Hutchinson, Plant Propagators Association
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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.

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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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

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*.

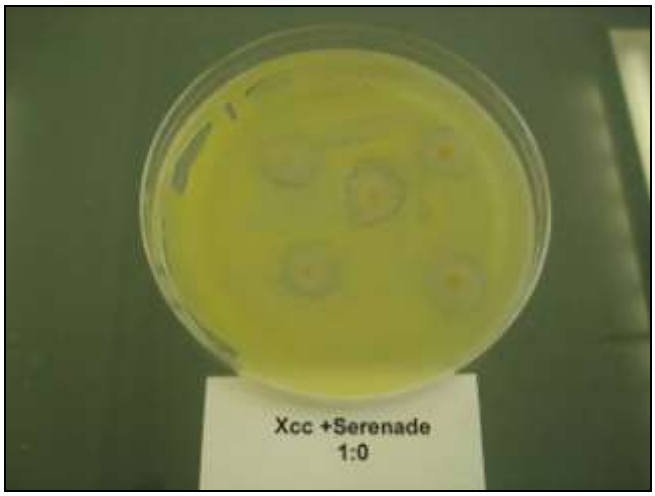
The majority of the products in this study (with the exception of 'Serenade') were tested by adding a known amount of a *Xcc* cell suspension to the product at a range of dilutions (1:0, 1:1, 1:10, 1:100, 1:1000 and 1:10,000). The bacteria/disinfectant mix was then sampled after 1, 5, 10, 30 and 60 minute 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*.

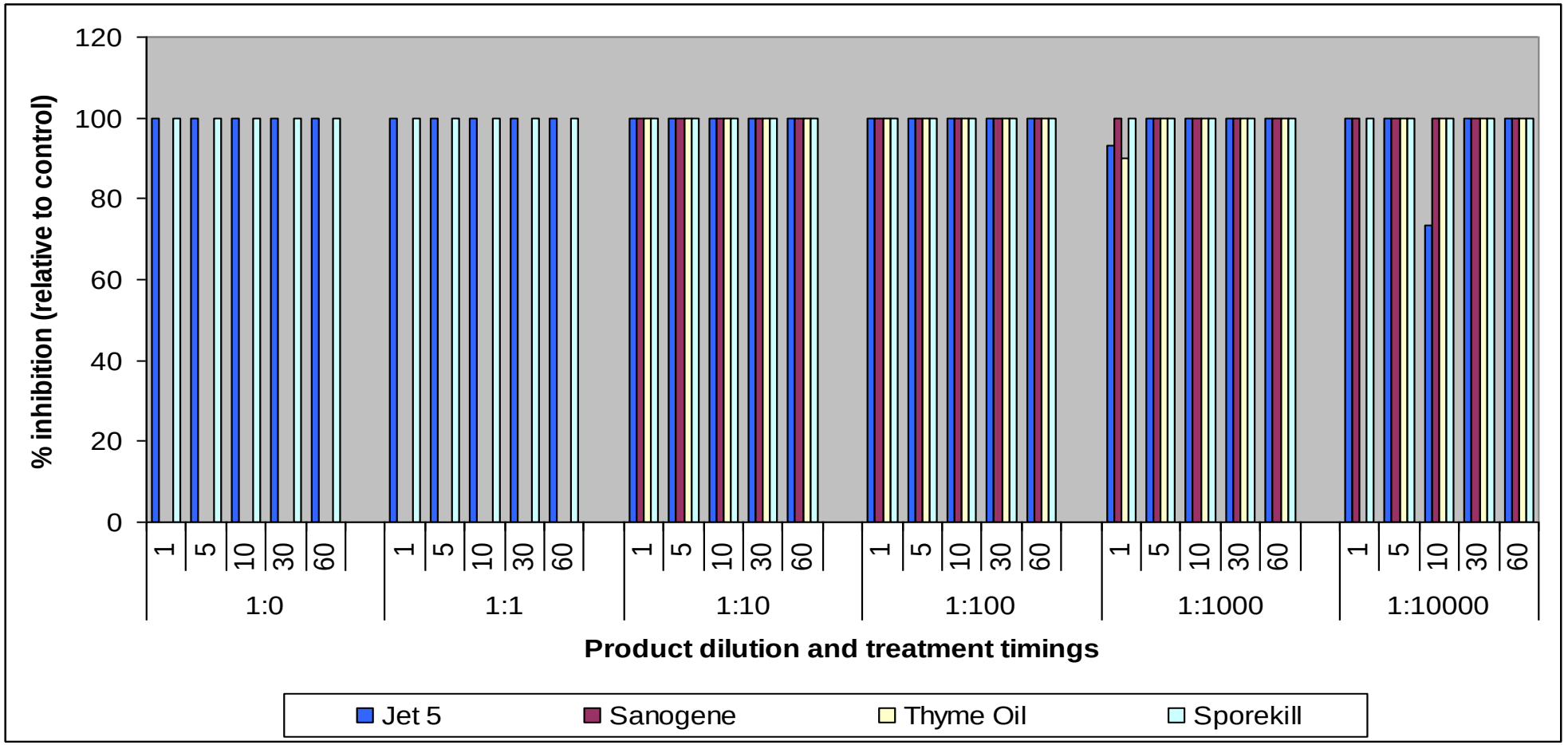
The test method above could not be used for the biological control product 'Serenade' (*B. subtilis*) as it resulted in blanket growth of the bio-control organism in the formulated product. We therefore devised an alternative, qualitative, technique to determine whether this product was inhibitory to *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.

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.

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.