Project title:	**Bacterial Diseases of HNS: Chemical Control**
Report:	**Final**
Project Number:	**HNS 91**
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Date Commenced:	**01 July 2000**
Date Completed:	**30 June 2002**
Keywords:	**HNS, bacterial diseases, disinfectants, ivy, *Philadelphus*, *Prunus***

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PRACTICAL SECTION FOR GROWERS

Disinfectants

 Alcohol (70% ethanol), bleach (sodium hypochlorite), Jet 5, Menno Florades and Panacide M were the most effective disinfectants in **laboratory tests**, with 99.999% kill under clean conditions and 99.99% kill in the presence of peat. Vitafect performed only marginally worse than the top five and would probably prove equally effective in routine use. Super Antibac required longer contact times.

 Wetcol 3 was consistently the most bactericidal of the copper-based pesticides in laboratory tests. The bactericidal properties of the other copper-based pesticides (Croptex Fungex, Cuprokylt, Cuprokylt FL) were more variable in the laboratory tests, and were affected by test conditions and isolate.

Spray Trials

 Aliette, Jet 5 and Wetcol 3 failed to give satisfactory control of bacterial diseases of ivy, *Philadelphus* and *Prunus*, despite up to 26 spray applications over 37 weeks. On the basis of these trials we cannot recommend any for protecting against, or eradication of, bacterial plant pathogens.

Objectives and background

Bacterial diseases cause major problems to growers of HNS. A previous HDC project (HNS 71: Bacterial diseases of HNS) identified the most important and widespread bacterial diseases of HNS. Currently the only bactericides registered as pesticides and available to growers in the UK are copper-based compounds. Bactericidal disinfectants offer potential for controlling spread of disease from infected leaf debris and other inoculum sources such as pots, surfaces and equipment. This project examined a range of disinfectants and pesticides for use as part of a disease management strategy for bacterial diseases of HNS.

During visits to nurseries, as part of HNS 71, it became apparent that growers were attempting a number of chemical control treatments with varying degrees of success. While some reported that copper-based compounds effectively controlled diseases on HNS species, others found the symptoms worsened or showed symptoms of phytotoxicity. Results with disinfectants were equally variable. It should be noted that the general disinfectants are not registered as pesticides and their application to crops for disease control is illegal. However, as disinfectants can play an important part in reducing potential sources of inoculum, it is still valuable to determine their efficacy for use in non-crop situations.

Clearly there was a need to investigate the differing claims of growers with respect to efficacy and phytotoxicity of these chemicals and, if they are effective, to

devise appropriate disease control programmes, based on epidemiological data, which minimise pesticide use and hazard.

The project was conducted in two stages. Phase one was limited to *in vitro* laboratory investigations of the bactericidal activity of a broad range of candidate chemicals, including pesticides and disinfectants, against a range of bacterial pathogens. The aim was to provide growers with objective information on the relative merits of different compounds for legal use as disinfectants. Phase two consisted of a spray trial to evaluate selected products from Phase one. Experiments at Phase two were designed to provide clear-cut answers on the potential of chemicals rather than to develop specific recommendations for spray programmes.

Summary of experiments and results

Phase one: plate and suspension tests

Table 1. Summary of laboratory tests on bactericidal (disinfectant) activity. See Science Section for details, rates and methods.

Fast[1] acting, complete[2] kill, clean and dirty[3] conditions	Alcohol (70% ethanol) Bleach (sodium hypochlorite) Jet 5 Menno Florades Panacide M
Slower acting, complete[2] kill, clean and dirty[3] conditions	Vitafect Super Antibac Wetcol 3
Slower acting, not always complete[2] kill, affected by conditions	Croptex Fungex Cuprokylt FL Cuprokylt
Failed to give complete[2] kill under any conditions	Aliette Copper sulphate Myacide

[1] Fast: 5 minutes contact time.
[2] Complete kill: below the detection threshold of the test.
[3] Dirty conditions: in the presence of 1% peat.

Thirty-five bacterial isolates from HNS plus five other bacterial plant pathogens (included as standards) were tested for inhibition by twelve bactericides/pesticides in plate tests. Growth of all forty isolates of bacterial pathogens was inhibited after 7 days incubation at 25ºC, by Aliette, Cuprokylt, Cuprokylt FL, Jet 5, Menno Florades, Myacide, Panacide M, Vitafect and Wetcol 3 at all three test concentrations (standard rate, half-rate, double rate). Growth of all except two isolates was inhibited by Super-

Antibac and copper (II) sulphate at all three test concentrations. There was no evidence of copper resistance in the 35 isolates from HNS.

Suspension tests for bactericidal activity were performed on fourteen compounds using strains of four bacterial pathogens from HNS in clean conditions and in the presence of peat and for three different contact times (5 min, 15 min, 30 min). All of the 14 compounds tested showed some bactericidal activity. Alcohol (70% ethanol), bleach (sodium hypochlorite), Jet 5, Menno Florades, and Panacide M reduced bacterial numbers to undetectable levels (< 250 cells/ml) at the shortest contact time (5 min) both in the presence and absence of peat. Other compounds required longer contact times to give complete kill (Super Antibac, Vitafect and Wetcol 3) or gave variable results depending on the test conditions and the bacterial strain (Croptex Fungex, Cuprokylt, Cuprokylt FL) or failed to give complete kill (Aliette, Myacide, copper (II) sulphate).

Phase two: spray trial with selected compounds

Three compounds were selected for spray trials on three plants species infected with different bacterial pathogens (*Xanthomonas hortorum* pv. *hederae* on ivy; *Pseudomonas syringae* pv. *philadelphi* on *Philadelphus*; *Pseudomonas syringae* on *Prunus avium*). Aliette was chosen as it is systemic and showed some bactericidal activity with longer contact times. Jet 5 was chosen as potentially the safest from the most effective biocides (note that experimental approval was required from PSD, as Jet 5 is not an approved pesticide). Wetcol 3 was chosen as the best performing, approved copper compound in the laboratory tests. Two spray regimes were followed: Routine (every two weeks) and Managed (according to weather conditions in the previous week and the presence of new un-protected growth).

Despite up to 26 spray applications over 37 weeks, none of the three compounds tested gave satisfactory control of any of the three bacterial diseases. There was some evidence of a slight reduction in disease with Wetcol 3 in ivy and *Philadelphus*, but not enough to be considered of commercial benefit. There was some evidence of a protectant effect of Aliette in Prunus, but again not enough to be considered of commercial benefit. Wetcol 3 showed some phytotoxicity to ivy and *Philadelphus*. Clearly the laboratory tests for bactericidal activity are poor indicators of efficacy as spays.

Action points for growers

- None of the compounds examined in the spray trial (Aliette, Jet 5, Wetcol 3) can be recommended for application to plants for the control of bacterial diseases of HNS.

- Growers must consider control of bacterial diseases through an overall disease management strategy: ensuring a clean start, combined with good hygiene and production practices/systems which reduce the risk of disease spread and infection.
- The disinfectants, alcohol (70% ethanol), bleach (sodium hypochlorite), Jet 5, Menno Florades and Panacide M, all proved to be equally effective bactericides within the limits of the tests performed and gave a reduction in bacterial numbers of equivalent to 99.999% kill under clean conditions and 99.99% kill in the presence of peat.
- Vitafect performed only marginally worse than the top five and would probably prove equally effective in routine use. Super Antibac required longer contact times than the other disinfectants.
- The bactericidal properties of the copper-based pesticides (Croptex Fungex, Cuprokylt, Cuprokylt FL, Wetcol 3) showed more variability, and were affected by test conditions and isolate. Wetcol 3 was consistently the most bactericidal of the copper-based pesticides, but this did not translate into efficacy in the spray trials.
- Aliette consistently had the lowest level of bactericidal activity.
- In terms of disinfectant activity there is little to choose between the compounds marketed as disinfectants, therefore selection of a disinfectant for use as part of a hygiene regime should depend on other considerations such as operator and environmental safety, plant toxicity and cost.

Practical and financial benefits

The hardy nursery stock industry is valued at over £300 million. Project HNS 71 indicated that a significant proportion of HNS subjects are affected by bacterial diseases. In susceptible crops direct losses from bacterial diseases are considerable. Additional losses can be attributed to the use of treatments that are only partly effective, or that are phytotoxic. By determining the efficacy of the chemicals currently available, ineffective or inappropriate treatments can be avoided, and future research can be targeted at those measures which are most likely to prove effective.

This project has clearly demonstrated that most disinfectants are effective against bacterial pathogens of HNS when used for general hygiene purposes, but Aliette, Jet 5 and Wetcol were not effective when applied as foliar sprays for control of bacterial diseases.

SCIENCE SECTION - INTRODUCTION

Bacterial diseases cause major problems to growers of HNS. HDC project HNS 71 (Bacterial diseases of HNS) (Roberts, 1997) identified the most important and widespread diseases of HNS. Currently the only bactericides registered as pesticides and available to growers in the UK are copper-based compounds. In addition to the need to control bacterial diseases on plants, spread from infected leaf debris and other sources such as pots, surfaces and equipment needs to be kept to a minimum if disease outbreaks are to be avoided. Disinfectants offer the potential for controlling these sources of infection.

During visits to nurseries, as part of HNS 71, it became apparent that growers were attempting a number of chemical control treatments with varying degrees of success. While some report that copper-based compounds effectively controlled diseases on HNS species, others found the symptoms worsened or showed symptoms of phytotoxicity. Results with disinfectants were equally variable. It should be noted that the general disinfectants are not registered as pesticides and their application to crops for disease control is illegal. However, as disinfectants can still play an important part in reducing potential sources of inoculum, it is still valuable to determine their efficacy for use in non-crop situations.

Clearly there was a need to investigate the differing claims of growers with respect to efficacy and phytotoxicity of these chemicals and, if shown to be effective, to devise appropriate disease control programmes, based on epidemiological data, which minimise pesticide use and hazard.

This project aimed to examine the potential of a range of disinfectants and pesticides for use as part of a disease management strategy for bacterial diseases of HNS and was conducted in two phases. In phase one, *in vitro* laboratory tests were done to examine the bactericidal activity of a broad range of candidate chemicals, both pesticides and disinfectants, against a range of bacterial pathogens. The aim was to provide growers with objective information on the relative merits of different compounds for legal use as disinfectants. In phase two, a spray trial was conducted to examined the efficacy of selected products from phase one. Experiments at phase two were designed to provide clear-cut answers on the potential of chemicals, but it was anticipated that further development work would be necessary to devise appropriate spray programmes, spray timings and application rates. The experimental details and results of the two phases are reported in separate sections.

PHASE ONE: *IN VITRO* STUDIES OF BACTERICIDAL ACITIVITY

Introduction

The aim of the *in vitro* studies was to provide objective information on the relative ability of compounds to inhibit the growth of and/or kill bacterial pathogens of HNS. Initially a large plate inhibition test was done. This examined inhibition of bacterial growth on agar plates and is an ideal method for screening compounds for activity against a large number of bacterial isolates. However, it only demonstrates the inhibitory action of compounds and not bactericidal activity (killing power). Both aspects may be important in determining the effectiveness of a compound for controlling disease. Subsequently, suspension tests were performed on a more limited set of isolates. Suspension tests are much more time-consuming, but provide more definitive information on the bactericidal properties of test compounds. They also allow testing in the presence/absence of interfering substances, as many biocides, which are highly effective in 'clean' conditions, may be rapidly inactivated in 'dirty' conditions i.e. in the presence of organic matter.

Materials and Methods

Bacterial Isolates

Thirty five isolates from a range of HNS species and locations were selected from the culture collection at HRI Wellesbourne (Table 1). These included a number of multiple strains of key species from different locations, many of which had been isolated as part of HNS 71 (Roberts, 1997). An additional five isolates representing key genera of bacterial plant pathogens were also included for comparative purposes (Table 1). Isolates were stored on glass beads at –76°C and recovered onto an appropriate growth medium prior to testing.

Test Products and Preparation of Working Solutions

Thirteen test products (pesticides/biocides) were selected with the advice of project co-ordinators (Table 2), and samples were obtained from the manufacturers/suppliers. Copper (II) sulphate ($CuSO_4$) was also included as a standard to indicate copper resistance. In the plate tests, products were tested at three concentrations: manufacturers' recommended rate , half-rate, and double-rate. In the suspension tests, products were tested at the recommended rate only. All stock solutions were made up in sterile RO (reverse osmosis) water. For liquid products, stock solutions were prepared volume for volume (v/v): an appropriate volume of product was aseptically pipetted into an appropriate volume of water. For solids/powders working solutions were prepared weight for volume (w/v): an appropriate amount of product was weighed and added to an appropriate volume of water.

Table 1. List of isolates used in inhibition plate test .

Number	Name	Host	Year
SC097	*Pseudomonas syringae* pv. *berberidis*	*Berberis gagnepainii*	1982
SC126	***Pseudomonas syringae* pv. *berberidis***	***Berberis julianae***	**1983**
5682	*Pseudomonas syringae* pv. *berberidis*	*Berberis thunbergii*	1996
5687A	*Pseudomonas syringae*	*Cornus* sp.	1996
5873A	*Pseudomonas syringae*	*Cornus* sp.	1997
5866A	*Pseudomonas syringae*	*Cotoneaster damneri*	1997
7764	*Erwinia amylovora*	*Cotoneaster* sp.	1999
5691B	***Xanthomonas hortorum* pv. *hederae***	***Hedera helix***	**1996**
5993	*Xanthomonas hortorum* pv. *hederae*	*Hedera helix*	1997
7053A	*Xanthomonas hortorum* pv. *hederae*	*Hedera hibernica*	1997
7183	*Xanthomonas hortorum* pv. *hederae*	*Hedera helix*	1997
7714	*Xanthomonas hortorum* pv. *hederae*	*Hedera colchica* cv. Dentata	1998
7731	*Xanthomonas hortorum* pv. *hederae*	*Hedera helix* cv. Buttercup	1998
7734	*Xanthomonas hortorum* pv. *hederae*	*Hedera algeriensis*	1998
7744^{T} (=NCPPB 939)	*Xanthomonas hortorum* pv. *hederae*	*Hedera helix*	1961
5994	*Pseudomonas syringae*	*Mahonia japonica*	1997
7038	*Pseudomonas syringae*	*Mahonia* sp.	1997
SC053^{T}	***Pseudomonas syringae* pv. *philadelphi***	***Philadelphus coronarius.***	**1982**
5875	*Pseudomonas syringae* pv. *philadelphi*	*Philadelphus virginalis*	1997
7017	*Pseudomonas syringae* pv. *philadelphi*	*Philadelphus coronarius*	1997
5357	*Pseudomonas syringae* pv. *syringae*	*Prunus avium*	1979
7016	*Pseudomonas syringae* pv. *morsprunorum*	*Prunus cerasifera*	1997
SC073B	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1982
5458B	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1995
5674A	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1996
5711	***Pseudomonas syringae* pv. *syringae***	***Prunus laurocerasus***	**1996**
5768	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1996
5769	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1996
5799	*Pseudomonas syringae* pv. *syringae*	*Prunus laurocerasus*	1996
5456A	*Pseudomonas syringae* pv. *syringae*	*Prunus lusitanica*	1995
5698	*Pseudomonas syringae*	*Spirea japonica*	1996
7055	*Pseudomonas syringae*	*Spirea* sp.	1997
7180	*Pseudomonas syringae*	*Spirea* sp.	1997
2070	*Pseudomonas syringae* pv. *syringae*	*Syringa vulgaris*	1988
7010	*Pseudomonas syringae* pv. *syringae*	*Syringa vulgaris*	1997
811^{T} (=NCCPPB 312)	*Erwinia carotovora* subsp. *carotovora*	*Solanum tuberosum*	1952
1159A	*Burkholderia gladioli* pv. *alliicola*	*Allium cepa*	1982
3811	*Xanthomonas campestris* pv. *campestris*	*Brassica oleracea*	?
5213	*Clavibacter michiganensis* subsp. *michiganensis*	*Lycopersicon esculentum*	1994
6237A	*Pseudomonas fluorescens*	*Brassica oleracea* (Calabrese)	1997

Isolates in bold were used in the Suspension Test.
^{T} Type strain

Plate Inhibition Test

Agar plates of a low complexity mineral salts medium, Casitone Yeast Extract Glycerol (CYEG) (Zevenhuizen et al. 1979), containing the test compounds (Table 2) at the manufacturers' recommended concentration, at half-rate and at double-rate were prepared. This medium was selected because it had been used previously in assays for copper tolerance (Zevenhuizen et al. 1979). Test compounds were added to the molten agar medium, after autoclaving and cooling to approx. 50°C, as stock solutions prepared to 10x final concentration. Sodium hypochlorite and ethanol were not examined in the plate test, as their inclusion in the agar medium was considered inappropriate.

Isolates were recovered from storage at –76˚C onto CYEG medium and incubated for 24-48 h at the normal growth temperature for each organism. Bacterial suspensions were prepared for each isolate in 3 ml of sterile RO water to give a final concentration of approximately 3 x 10^5 to 3 x 10^6 cells/ml. The bacterial suspensions were inoculated onto the amended CYEG medium (containing test product), under sterile conditions, using a multi-point inoculator (Denley Instruments). The multi-point inoculator simultaneously inoculates the surface of an agar plate with 1 µl of each of 20 bacterial suspensions. Control plates of CYEG medium containing no test product were also inoculated at the beginning and end of each batch of plates for comparison with the test plates.

The inoculated plates were incubated at 25ºC for 7 d and checked daily for the presence of growth at each inoculation point in comparison with the control plates containing no test product. Presence of growth was recorded as 1 and absence of growth was recorded as 0.

Suspension Tests

Four isolates (*Pseudomonas syringae* pv. *syringae* from cherry laurel, *P. syringae* pv. *philadelphi* from *Philadelphus, P. syringae* pv. *berberidis* from *Berberis*, and *Xanthomonas hortorum* pv. *hederae* from ivy; Table 1) were tested in the suspension test system. The method used followed the principles of the method contained in the British and European Standard (BSI, 1997), but modified to make the method more appropriate for the testing of plant rather than human pathogens. Peat was used as the interfering substance.

The test compounds were tested in batches of four to allow time for diluting and plating. A control was included in every batch, where the test product was replaced with 4 ml sterile water. Testing was carried out at room temperature (22ºC - 24ºC).

Table 2. Biocide and pesticide products tested for *in vitro* efficacy against a range of bacterial pathogens of hardy nursery stock.

Product Name	Active Ingredient (conc.)	Formulation	Recommended rate	Supplier
Alcohol	Ethanol	liquid	70%	BDH, Laboratory chemicals
Aliette[A]	Fosetyl aluminium (80% w/v)	WP	0.40%	Rhone-Poulenc, Essex
Bleach	Sodium hypochlorite	liquid	200 ppm Cl_2	Fisher Scientific
Copper sulphate	Copper (II) sulphate	Crystalline solid	0.5 mM	BDH Laboratory chemicals
Croptex Fungex[A]	Copper ammonium carbonate (80% w/w)	SC	0.63%	Hortichem Ltd, Wiltshire
Cuprokylt[A]	Copper oxychloride (84 % w/w)	WP	0.50%	Unicrop, Berkshire
Cuprokylt FL[A]	Copper oxychloride (473 g/L)	SC	0.50%	Unicrop, Berkshire
Jet 5	Peroxyacetic acid (5% w/w)	liquid	0.80%	Hortichem Ltd, Wiltshire
Menno Florades	Benzoic acid (90 g/l)	liquid	1%	Fargro Ltd, West Sussex
Myacide	Bronopol (99%)	Crystalline solid	0.10%	Boots Microcheck, Nottingham
Panacide M[A]	Dichlorophen (28%)	liquid	1%	Coalite Chemicals, Derbyshire
Super Antibac	Fruit acids	liquid	0.50%	Environmental Safe Solutions, Lincs.
Vitafect	Benzalkonium chloride (<5%)	liquid	1%	Vitax Ltd, Coalville
Wetcol 3[A]	Bordeaux mixture (30 g/l of copper)	SC	5%	Ford Smith & Co Ltd, London

[A] Approved pesticides

Isolates were recovered from -76˚C onto Nutrient Agar (NA, Difco). After incubation at 25°C for 24-48 h, stock suspensions were prepared for each isolate in 3 ml 0.1% peptone water to give a final concentration of approximately 3 x 10^8 cells/ml. For testing, 100 µl of bacterial stock suspension was pipetted into 1 ml sterile RO water or 1 ml RO water containing 5 % (w/v) peat and allowed to stand. After 10 min, 4 ml of a stock solution (1.25× final concentration) of the test product was added and allowed to stand for the appropriate contact time (5, 15 or 30 min). At the end of each contact time 0.5 ml was pipetted into 4.5 ml of Universal Quenching Agent (UQA) (Lambert et al. 1998) and left to stand for a minimum neutralisation time of 5 min. Three or four serial tenfold dilutions were prepared in 0.1% peptone and plated using the drop method of Miles and Misra (1933): 2 or 3 x 20 µl drops of each dilution and the un-diluted UQA suspension were pipetted onto sectors of NA plates. Plates were allowed to dry and then inverted for incubation at 25ºC for 1-3 d depending on the organism.

Plates were checked daily and recorded when individual colonies could be distinguished easily on the control plate(s). The number of colonies in each drop at each dilution was recorded as a number from 0 to 30, m (>30), or, c (confluent). Plates were incubated for a further 1-2 d after the initial recording to check for

additional slower growing colonies (which could occur from damaged cells) and were counted if necessary.

Data analysis

All results were entered in Excel spreadsheets. Detailed statistical analysis of the suspension test results was performed using the generalised linear modelling facilities of Genstat (Payne et al. 1993); a model with Poisson error and a log link function was fitted to the mean count at the countable dilution with the log of the dilution factor treated as an offset. Compounds which reduced bacterial numbers below the detection threshold of the test for all isolates/conditions/contact times (i.e. results all zeroes) were excluded from the statistical analysis. For the remaining compounds, zero results were replaced by a number ten times (i.e. 1 $\log_{10}$ unit) lower than the detection threshold of the test, in order to obtain mean values.

Results

Plate Inhibition Test

All of the 12 compounds tested showed some inhibitory activity. All bacterial isolates grew on the positive control plates of CYEG medium containing no test product and no growth occurred on negative control plates inoculated with sterile RO water.

Growth of all isolates was inhibited, after 7 d of incubation at 25ºC, by Aliette, Cuprokylt, Cuprokylt FL, Jet 5, Menno Florades, Myacide, Panacide M, Vitafect and Wetcol 3 at all three test concentrations. Only two isolates showed growth on any test compound: one (*Burkholderia gladioli,* 1159A), included as a standard, was not inhibited by half-rate Super Antibac after 2 d incubation, by 0.25 mM copper (II) sulphate after 1 d and by 0.5 mM Copper (II) sulphate after 2 d; the other (*P. syringae* pv. *syringae* from cherry laurel, 5711) was not inhibited by 0.25 mM copper (II) sulphate after 5 d of incubation.

Suspension Test

All of the 14 compounds tested showed some bactericidal activity, i.e. a reduction in bacterial numbers recovered after contact with the test products, compared to controls containing no test product, both in the presence and absence of interfering substance (peat). The results are summarised in Tables 3 and 4, as the mean $\log_{10}$ reduction in the number of bacterial cells in the original suspension. These values were obtained as the predictions from a generalised linear model containing the significant terms (see Analysis of Deviance in Appendix II).

Table 3. 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 (see Appendix II).

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 4. Mean $\log_{10}$ reduction in bacterial numbers after 5 min contact time for each isolate/compound in absence (clean) and presence of peat. Predictions obtained from a generalised linear model containing significant parameters (see Appendix II).

Compound	Rank[1]	Isolate							
		5691B		5711		SC126		SCO53	
		Red[n]	s.e	Red[n]	s.e	Red[n]	s.e	Red[n]	s.e
Clean									
Alcohol	1	5.0		5.7		4.7		4.6	
Aliette	14	1.0	0.3	0.0	0.2	-0.8	0.4	0.1	0.3
Bleach	1	5.0		5.7		4.7		4.6	
Copper sulphate	8	3.8	0.4	3.9	0.4	2.0	0.4	2.5	0.3
Croptex Fungex	11	4.4	0.7	2.3	0.3	1.6	0.5	0.9	0.4
Cuprokylt	12	3.9	0.4	2.2	0.3	0.3	0.4	0.2	0.3
Cuprokylt FL	10	3.4	0.4	3.4	0.2	1.7	0.5	1.7	0.4
Jet 5	1	5.0		5.7		4.7		4.6	
Menno Florades	1	5.0		5.7		4.7		4.6	
Myacide	13	0.3	0.3	1.3	0.3	3.6	1.1	1.1	0.4
Panacide M	1	5.0		5.7		4.7		4.6	
Super Antibac	9	3.1	0.4	3.5	0.3	2.4	0.7	1.0	0.3
Vitafect	6	7.2	3.4	6.7	2.4	4.6	2.3	5.1	2.3
Wetcol 3	7	4.3	0.9	6.0	1.1	4.1	2.0	4.6	2.2
Max. detectable reduction		5.0		5.7		4.7		4.6	
Peat									
Alcohol	1	4.6		5.4		3.0		2.4	
Aliette	12	2.2	0.4	0.8	0.4	1.4	0.5	1.9	0.5
Bleach	1	4.6		5.4		3.0		2.4	
Copper sulphate	14	0.6	0.3	0.4	0.3	-0.2	0.3	0.0	0.3
Croptex Fungex	6	5.8	1.0	3.4	0.6	4.0	0.8	3.0	0.7
Cuprokylt	11	3.6	0.5	1.5	0.3	0.9	0.5	0.5	0.4
Cuprokylt FL	13	1.3	0.4	0.9	0.3	0.6	0.5	0.3	0.4
Jet 5	1	4.6		5.4		3.0		2.4	
Menno Florades	1	4.6		5.4		3.0		2.4	
Myacide	10	0.2	0.3	0.8	0.3	4.6	1.2	1.7	0.5
Panacide M	1	4.6		5.4		3.0		2.4	
Super Antibac	9	2.7	0.4	2.8	0.4	3.1	0.8	1.3	0.5
Vitafect	7	4.3	2.5	3.5	0.5	2.7	2.3	2.8	2.6
Wetcol 3	8	1.9	0.4	3.2	0.6	2.7	2.1	2.9	2.2
Max. detectable reduction[3]		4.6		5.4		3.0		2.4	

[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.

Five compounds (Ethanol, Jet 5, Menno Florades, sodium hypochlorite and Panacide M) reduced bacterial numbers to undetectable levels at all contact times both in the presence and absence of peat (Table 5). The results for the other compounds were more complex, with varying degrees of bactericidal activity, generally increasing with increasing contact time; some performed better either in clean conditions or in the presence of peat. Some caution should be applied to apparent improvements to bactericidal activity in the presence of peat, as recovery of isolates in control tubes was poorer, effectively reducing the sensitivity of tests.

Vitafect, reduced bacterial numbers to undetectable levels at all contact times in clean conditions, but just failed to do so in the presence of peat.

The copper-based pesticides (i.e. Wetcol, Cuprokylt, Cuprokylt FL, Croptex fungex) were generally slower acting, reducing numbers to undetectable levels only at the longer contact times. They also varied in the response to the presence of peat, some apparently being more effective in the presence of peat. However recovery of control isolates was relatively poorer in the peat controls, effectively reducing the sensitivity of these tests. Wetcol 3 was consistently the most bactericidal copper compound both in clean conditions and in the presence of peat.

Three compounds (Aliette, Copper (II) sulphate, Myacide) did not reduce numbers of all bacterial isolates to undetectable levels under any conditions/contact time.

In general the four bacterial isolates tested responded to contact with all test

Table 5. Contact times which reduced bacterial numbers of all isolates below the detection threshold of the test (250 cfu/ml) in the absence (clean) and presence of peat (1 % w/v)

Compound	Rank[1]	Test organism/contact time (min)	
		Clean	Peat
Alcohol	1	5, 15, 30	5, 15, 30
Bleach	1	5, 15, 30	5, 15, 30
Jet 5	1	5, 15, 30	5, 15, 30
Menno Florades	1	5, 15, 30	5, 15, 30
Panacide M	1	5, 15, 30	5, 15, 30
Vitafect	6	5, 15, 30	-
Super Antibac	7	30	15, 30
Wetcol 3	7	15, 30	30
Croptex Fungex	9	-	15, 30
Cuprokylt FL	9	30	30
Cuprokylt	11	-	30
Aliette	12	-	-
Copper sulphate	12	-	-
Myacide	12	-	-

[1]Rank – in order of efficacy, 1 = most effective, 12 = least effective

compounds in a similar way, although there were differences in susceptibility to the different copper compounds.

Myacide was not neutralised by the Universal Quenching Agent, so that bacteriostatic effects were apparent in the lower dilutions, where the test bacteria were not recovered. At the higher dilutions the effect was removed so that bacterial colonies were recovered. This was consistent for all four test isolates.

Discussion

All of the compounds examined in the plate tests inhibited growth of almost all of the forty isolates of bacterial plant pathogens and could not be distinguished on the basis of these tests. It was anticipated that detailed examination of bactericidal activity in the suspension tests would be performed on only a subset of the most effective compounds in the plate tests and at several concentrations. However, as it was not possible to discriminate between them, all compounds were tested in the suspension tests, but at only a single concentration.

In the suspension tests, Alcohol, bleach (sodium hypochlorite), Jet 5, Menno Florades and Panacide M were clearly the most effective bactericides under all conditions, and could not be differentiated within the limits of the test performed here. Statistical analysis of the data for the other compounds was problematical as interpretation was highly dependant on the precise model fitted to the data; effectively a result of the significant high-order interaction effects. As a result, producing overall rankings for the other compounds was difficult: the rankings changed according to conditions and contact time. Nevertheless, Vitafect, Super Antibac and Wetcol 3 could be grouped together as the next most consistently effective compounds. The copper based compounds Croptex Fungex, Cuprokylt and Cuprokylt FL formed a third group with more variable performance and a slower kill rate. Aliette consistently had the lowest bactericidal activity overall, giving only a small reduction at the longest contact time under clean conditions. However, in common with some of the copper compounds, its activity seemed to be enhanced in the presence of peat. As the suspension medium for the test was not buffered, it is possible that some of this apparent enhancement is a result of a reduction in pH due to the addition of peat, although this was not checked.

Sodium hypochlorite is generally considered to perform relatively poorly in the presence of organic matter, the amount of peat used in these tests (1% w/v in final test suspension) did not appear to reduce its efficacy. It may be appropriate in future tests to challenge with larger amounts of interfering substances.

Myacide is used as a preservative in the cosmetics industry. It is bacteriostatic (inhibitory) at very low concentrations (as demonstrated by its continuing effects even after 100-fold dilution of the test concentration), but has only limited bactericidal activity

The relatively poor performance of copper (II) sulphate compared to the formulated copper pesticides highlights the importance of formulation in obtaining maximum bactericidal activity.

Copper resistance in bacteria is generally considered to be manifest as growth in the presence of 1 mM copper (II) sulphate. Although one HNS isolate (5711) grew in the presence of 0.25 mM copper (II) sulphate, this should not therefore be considered as copper resistance. *Burkholderia gladioli* has previously been reported (Goto et al. 1994) as being less sensitive to copper than other bacteria, which is consistent with its growth in the presence of 0.5 mM copper (II) sulphate in these experiments.

PHASE TWO: SPRAY TRIAL

Introduction

The aim of the spray trial was to evaluate the potential of the most promising chemicals identified in Phase one to control bacterial diseases of HNS. The selection of chemicals and the experimental design was agreed at a review meeting with one of the grower co-ordinators. The trial was designed to allow investigation of both eradicant effects (on inoculated spreader plants) and protectant effects (on neighbouring un-inoculated plants). Chemicals were applied with relatively high frequency in order to maximise the chances of obtaining clear-cut effects, rather than with the intention of developing a practical spray programme.

Materials and Methods

Experimental design

The results of the *in vitro* screening (Phase one) were reviewed with HDC staff and the project co-ordinators. Three compounds (Aliette, Jet 3, Wetcol) were selected for efficacy studies. Aliette was chosen, as although *in vitro* it was one of the poorest performing compounds, it did show some bactericidal activity with longer contact times and, as it is systemic, it was considered that it may be more effective *in planta*. Jet 5, although it is a disinfectant and cannot legally be applied to plants, was chosen as potentially the safest from the most effective biocides; although not an approved pesticide, it was considered that if clear efficacy could be demonstrated it could provide the manufacturers with greater impetus to seek approval. Wetcol was chosen as the best performing, approved copper compound in the *in vitro* tests.

Three pathogen/host combinations were selected for study: *Pseudomonas syringae* on *Prunus avium* (potential broad host range pathogen on high value deciduous host); *Xanthomonas hortorum* pv. *hederae* on ivy (different pathogen genus on evergreen host with different canopy structure); *Pseudomonas syringae* pv. *philadelphi* on *Philadelphus* (well characterised specific pathogen on widely-grown deciduous host).

In the original proposal the trials were to be conducted at two sites; it was agreed that trials would be done at only one site (HRI-Wellesbourne), but with two spraying frequencies/regimes. Thus in the final design four chemical treatments (three compounds plus untreated control) were applied to three plant species according to two different spraying regimes. The trial was laid out in blocks comprising each of the three plant species, with each treatment combination (i.e. chemical × regime) applied to a single block.

Within each block, each of the three species were set out in sub-blocks of 5 × 7 plants (Fig. 1). Five plants in the central column of each sub-block of *Philadelphus* and *Prunus* were inoculated with the appropriate bacterial pathogen.

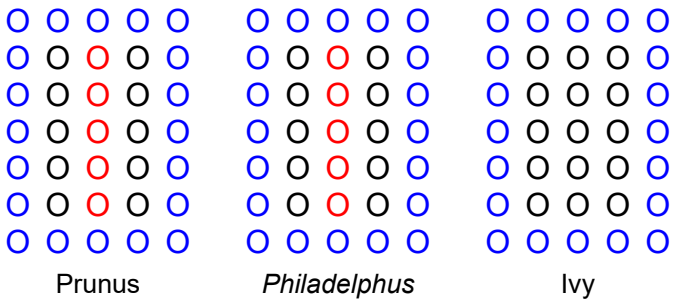

Fig. 1. Typical layout of a single block of plants showing the inoculated plants (O), assessed un-inoculated plants (O), and uninoculated guard plants (O).

Plant Material

All plant material was purchased from commercial growers, delivered to HRI-Wellesbourne and potted up as necessary during August 2001.

Ivy plants (*Hedera helix* cv. Green Ripple) were obtained as liners and potted up into 3 litre pots of coarse peat containing: Osmacote Plus 12-14 months (Scotts: 15+8+11+2 Mg + micronutrients, Autumn) 4 kg/m^3; Ground magnesium limestone 1.5 kg/m^3; SuScon Green 750 kg/m^3.

Philadelphus plants (*Philadelphus coronarius* cv. Aureus) were obtained as rooted plugs and potted up into 2 litre pots of coarse peat containing: Osmacote Plus 12-14 months (Scotts: 15+8+11+2 Mg + micronutrients, Autumn) 4kg/m^3; Ground magnesium limestone 1.5 kg/ m^3; SuScon Green 750 kg/ m^3.

Prunus avium plants were obtained as 60-90 cm plants growing in 2 litre pots which had been potted up in February 2001. The potting medium was coarse peat containing: Dolmitic limestone 0.5 kg/l; Osmacote Plus 12-14 (15+9+11+2 Mg, trace elements B, Cu, Fe, Mn, Mo, Zn) 0.3kg/l; Suscon Green pellets 0.6 g/l; horticultural grit for pot stabilisation. Osmacote Plus tablets (Scotts: 15+10+12+2Mg + trace elements, 5-6 months) were applied to all *Prunus* plants in the trial in April 2002 at a rate of 2 pellets per 2 litre pot.

Bacterial Isolates and inoculation

Prunus plants were inoculated with *Pseudomonas syringae* pv. *syringae* isolate HRI 5725 which had been obtained from a canker on wild cherry in the UK in 1990. *Philadelphus* plants were inoculated with the type strain of *Pseudomonas syringae* pv. *philadelphi,* isolate HRI SC053. Ivy plants were already showing symptoms of natural infection with *Xanthomonas hortorum* pv. *hederae* when received and therefore inoculation was not necessary.

Bacterial isolates were recovered from storage at –76°C onto King's medium B (King et al. 1954) and incubated at 25°C for 24 h. A turbid suspension was prepared

Table 6. Products and rates used in spray trial to examine efficacy against a range of bacterial pathogens of hardy nursery stock.

Product	Active Ingredient (conc.)	Formulation[2]	Rate	Supplier
Aliette[1]	Fosetyl aluminium (80% w/v)	WP	1 g/l	Rhone-Poulenc, Essex
Jet 5	Peroxyacetic acid (5% w/w)	liquid	6.6 ml/l	Hortichem Ltd, Wiltshire
Wetcol 3[1]	Bordeaux mixture (30 g/l of copper)	SC	50 ml/l + 7.5 ml/l vegetable oil	Ford Smith & Co Ltd, London

[1] Approved pesticides
[2] WP – wettable powder, SC – suspension concentrate

for each isolate in 500 ml of sterile tap water using bacterial growth from four plates of medium.

At the beginning of the trial (August 2001), leaves of the *Philadelphus* plants were damaged with a steel- pinned 'dog-brush' and the bacterial suspension (isolate HRI SC053) was sprayed onto the foliage. Leaves of the *Prunus* plants were inoculated in a similar way with isolate HRI 5275, but in addition several leaves were pulled off the stem to expose fresh scars and a drop of the bacterial suspension (isolate HRI 5275) was placed on scars. Plants to be inoculated were removed from the blocks prior to inoculation and kept separate. They were replaced in the blocks three weeks after inoculation, when symptoms were clearly visible.

Philadelphus and *Prunus* plants were inoculated again the following spring, in April 2002. Bacterial suspensions were prepared as described previously and plants from the central inoculated column were removed, sprayed with the bacterial suspension and replaced.

Chemical rates and application

As Jet 5 is not an approved pesticide, experimental approval was obtained from PSD (Pesticide Safety Directorate) before the trial was started. Chemicals were diluted according to the manufacturers' suggested rates (Table 6) and applied by a member of the Horticultural Services staff with appropriate qualifications using a Knapsack sprayer to give complete cover of foliage without drenching. Each block of plants received approximately 2 litres of diluted product.

Spraying programmes

Table 7 gives a detailed breakdown of the spray application dates for each plant species under each spray regime.

In the *Routine* spray programme, plants were sprayed every two weeks throughout the trial (ivy) or until leaf fall in the autumn and from bud burst in the spring (*Philadelphus* and *Prunus*).

Table 7. Spray application dates during spray trial to examine the efficacy of selected chemicals in controlling bacterial diseases of HNS. All compounds (Aliette, Jet 5, Wetcol) were applied on each occasion.

Week	Date	Routine sprays			Managed sprays			Notes
		Ivy	*Phil.*	*Prunus*	Ivy	*Phil.*	*Prunus*	
-1	19-Sep-01	□	□	□				
1	03-Oct-01	□	□	□	□	□	□	Routine on 05 Oct.
2	10-Oct-01				□	□	□	
3	17-Oct-01	□	□	□	□	□	□	
4	24-Oct-01				□	□	□	
5	31-Oct-01	□	□	□	□	□	□	
2	07-Oct-01				□	□	□	
7	14-Nov-01	□	□	□	□	□	□	Last Autumn on *Phil.*
9	28-Nov-01	□		□	□		□	Last Autumn on *Prunus*
10	06-Dec-01				□			
11	13-Dec-01	□						
13	28-Dec-01	□			□			
15	09-Jan-02	□						
16	16-Jan-02				□			
17	23-Jan-02	□						
18	30-Jan-02				□			
19	06-Feb-02	□			□			
20	13-Feb-02				□			
21	20-Feb-02	□	□			□		1st Spring on *Phil.*
22	27-Feb-02				□	□		
23	06-Mar-02	□	□		□	□		
24	13-Mar-02				□	□	□	1st Spring on *Prunus*
25	20-Mar-02	□	□	□	□	□	□	
26	27-Mar-02				□	□	□	
27	03-Apr-02	□	□	□	□	□	□	
28	10-Apr-02				□	□	□	Aphox on *Prunus*
29	17-Apr-02	□	□	□				Aphox on *Prunus*
30	24-Apr-02							Aphox on *Prunus*
31	01-May-02	□	□	□	□	□	□	
33	15-May-02	□	□	□				
34	22-May-02				□	□	□	
35	29-May-02	□	□	□	□	□	□	
36	07-Jun-02				□	□	□	
37	12-Jun-02	□	□	□	□	□	□	Last spray
	Total sprays	20	14	13	26	20	18	

Table 8. Major events during spray trial to examine the efficacy of selected chemicals in controlling bacterial diseases of HNS.

Date	Days	Action
24-Aug-01	-33	Plants received
30-Aug-01	-27	Ivy and *Philadelphus* potted up
05-Sep-01	-21	*Prunus* and *Philadelphus* inoculated
19-Sep-01	-7	1st Routine spray
26-Sep-01	0	Inoculated plants introduced
27-Sep-01	1	Disease assessment 1 – Ivy and inoculated plants
03-Oct-01	7	1st Managed spray
14-Oct-01	18	Last autumn spray for *Philadelphus*
28-Oct-01	32	Last autumn spray for *Prunus*
22-Nov-01	57	Disease assessment 2 – Ivy only
14-Jan-02	110	Disease assessment 3 – Ivy only
18-Feb-02	145	Disease assessment 4 – Ivy only
20-Feb-02	147	1st Spring spray for *Philadelphus* (routine)
13-Mar-02	168	1st Spring spray for *Prunus* (managed)
07-Apr-02	193	Disease assessment 5 – all
09-Apr-02	195	*Prunus* and *Philadelphus* re-inoculated
06-May-02	222	Disease assessment 6 – all
05-Jun-02	252	Disease assessment 7 – all
12-Jun-02	259	Final spray application
25-Jun-02	272	Disease assessment 8 – all
28-Jun-02	275	Experiment completed

In the *Managed* spray programme, a decision whether to spray or not was taken each week, based on the following criteria: the presence of significant un-protected new growth, or the occurrence of one or more 'significant' spread/infection events in the previous week. As for the routine spray programme, ivy plants were sprayed throughout and *Philadelphus* and *Prunus* were sprayed only when leaves were present.

A significant spread/infection event was considered to have occurred if the mean rainfall rate was greater than 1 mm per hour and with a rainfall duration of greater than 1 h. Rainfall was recorded at 10 min intervals using a tipping bucket rain gauge connected to an electronic data logger. Data from the logger was downloaded and summarised weekly.

Disease assessment

Disease assessments were done at approximately monthly intervals when leaves were present on the plants. At each assessment the total number of leaves and the number of leaves with visible disease symptoms was recorded for each inoculated plant and for five un-inoculated plants on each side of the inoculated plants (see Fig 1). For

Philadelphus, in addition to leaves with typical leaf spot disease symptoms, the number of leaves with brown necrotic edges/areas was also recorded.

Data Analysis

Data for each disease assessment was recorded in a spreadsheet. Data for each plant species was analysed separately using the generalised linear modelling (GLM) facilities of Genstat (Payne et al. 1993). A model with binomial error distribution and a logit link function was fitted to the number of leaves with disease symptoms.

Results

The trial was run for a total of 37 weeks from introduction of the inoculated plants into the sub-blocks of *Philadelphus* and *Prunus* plants (September 2001 to June 2002), a diary of the trial is given in Table 8, and the dates of all spray applications are given in Table 7. Plants in the *Managed* spray regime received more sprays than those in the *Routine* spray regime due to a higher frequency of spray applications during wet weather and rapid plant growth. In addition to the planned sprays, *Prunus* plants also received aphicide sprays in the Spring to control a severe aphid infestation.

Disease

The disease assessment data for each plant species are summarised graphically in Figs. 2 to 6. The data (and their approximate standard errors) were obtained as predictions from the model containing all terms in Genstat. The analysis of deviance for each plant species is shown in Appendix III. The relative importance of treatment effects (model terms) was assessed by examination of the relative size of the mean deviance values which can be considered approximately equivalent to an F-test in a conventional analysis of variance. However, as these are GLM models, exact significance tests are not possible.

Ivy. Data for the first disease assessment were excluded from the final overall analysis, as they represented the initial starting level of disease. The analysis of deviance indicated a significant effect of chemical treatment on disease, but no effect of spray programme. Examination of overall means (Table 9) showed slight reduction in disease with the Wetcol treatment. This is also seen in the graphs where the Wetcol treatment gave the lowest disease levels at most assessments. Both the Aliette and the Jet 5 treatment failed to give any overall disease reduction compared to the control.

It should be noted that the apparent decline in disease levels at the seventh assessment was due to dropping of older infected leaves combined with a flush of new growth.

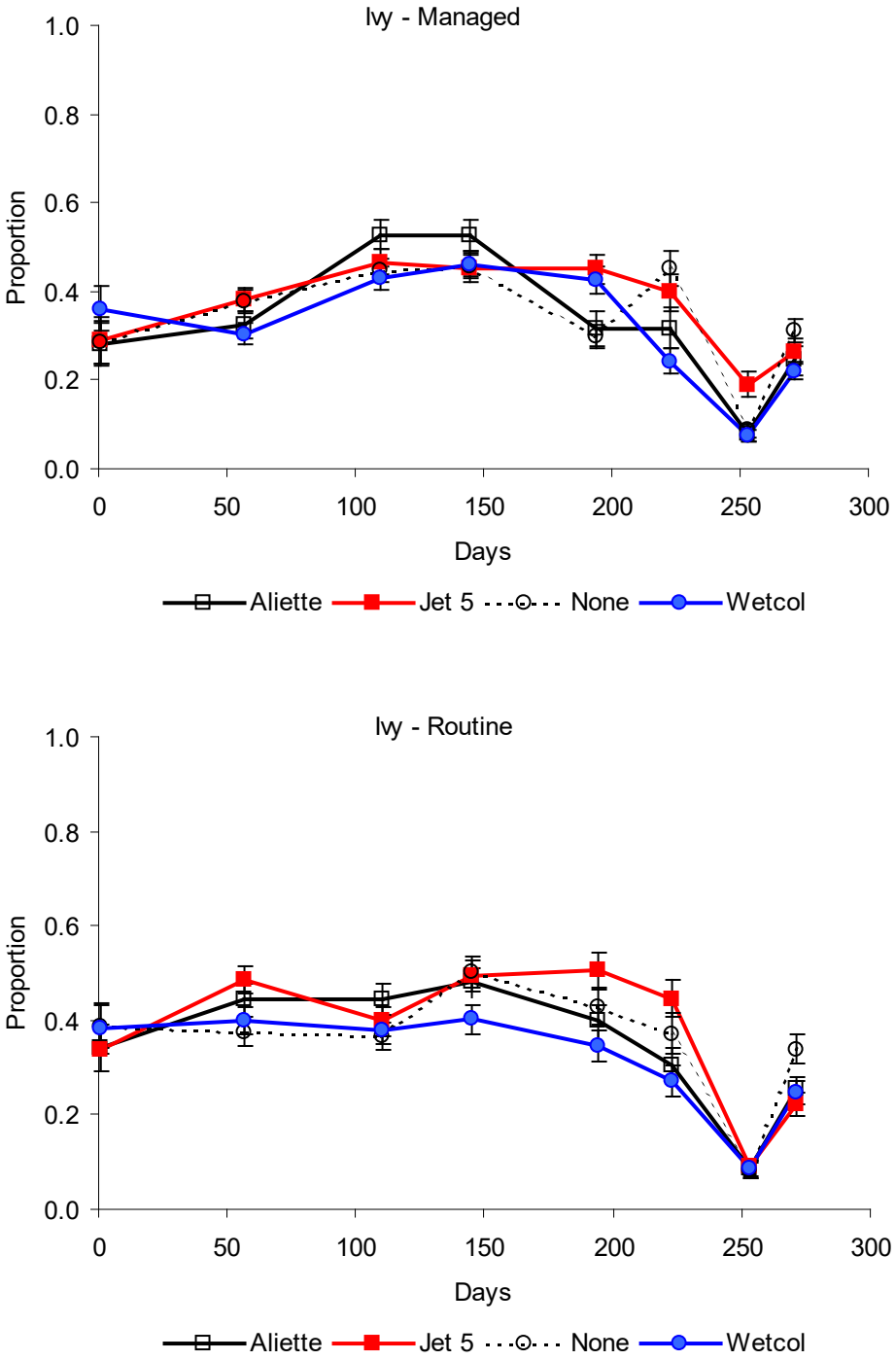

Fig. 2. Proportion of ivy leaves with bacterial leaf spot (*Xanthomonas hortorum* pv. *hederae*) at each assessment during spray trial with managed and routine spray programmes. Bars represent standard errors.

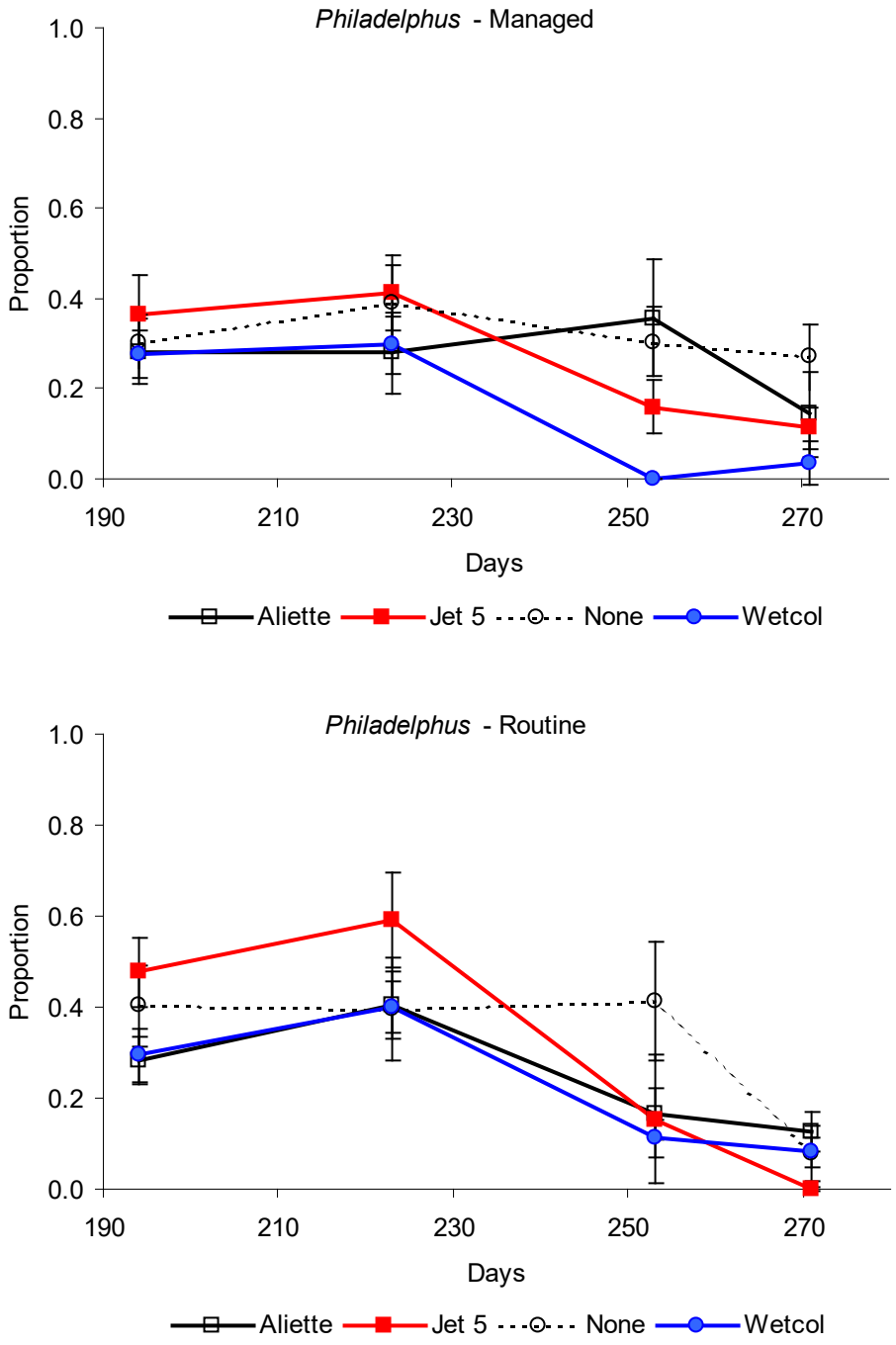

Fig. 3. Proportion of inoculated *Philadelphus* leaves with bacterial disease symptoms (brown lesions) at each assessment during spray trial with managed and routine spray programmes. Bars represent standard errors.

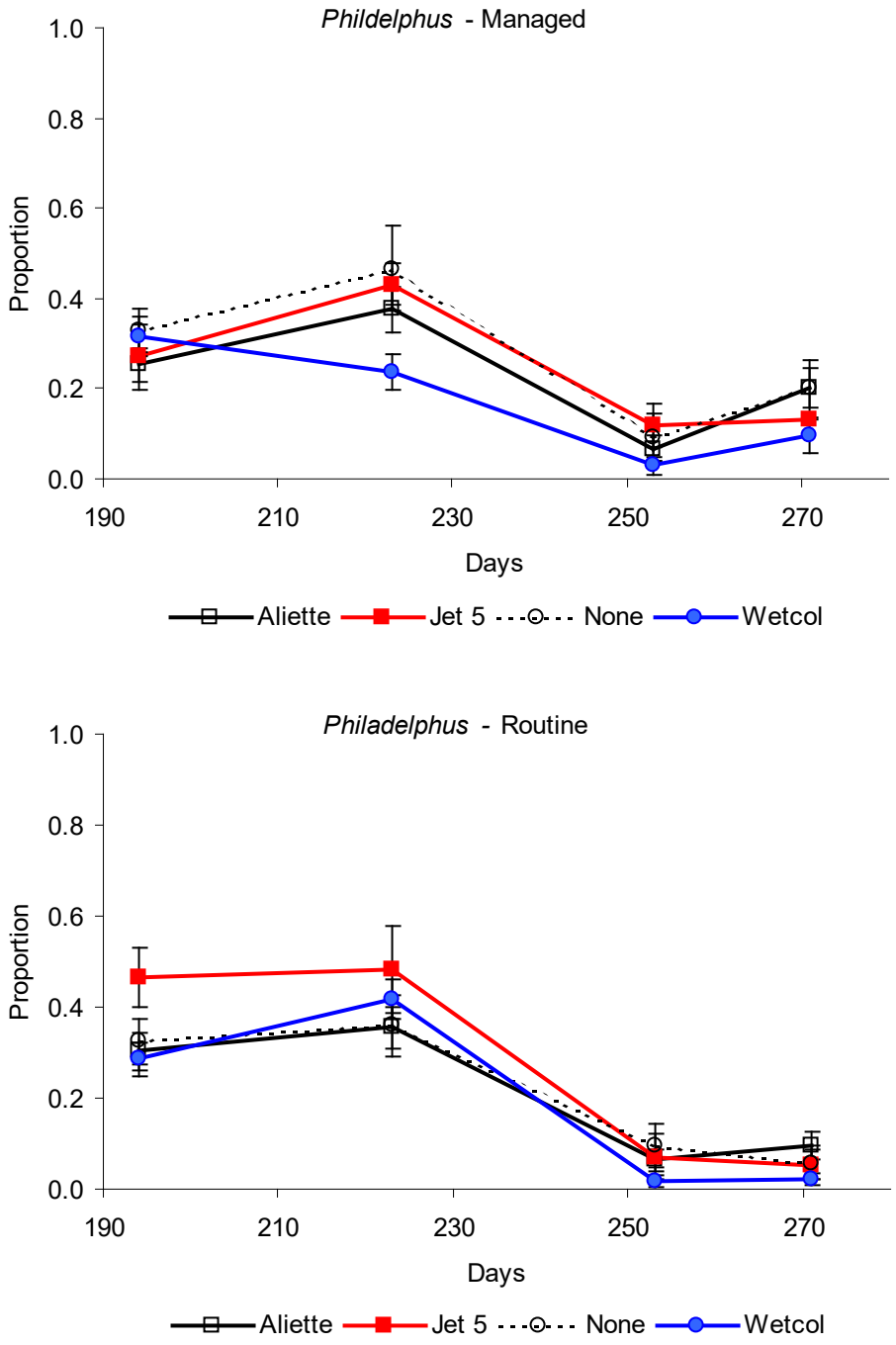

Fig. 4. Proportion of un-inoculated *Philadelphus* leaves with bacterial disease symptoms (brown lesions) at each assessment during spray trial with managed and routine spray programmes. Bars represent standard errors.

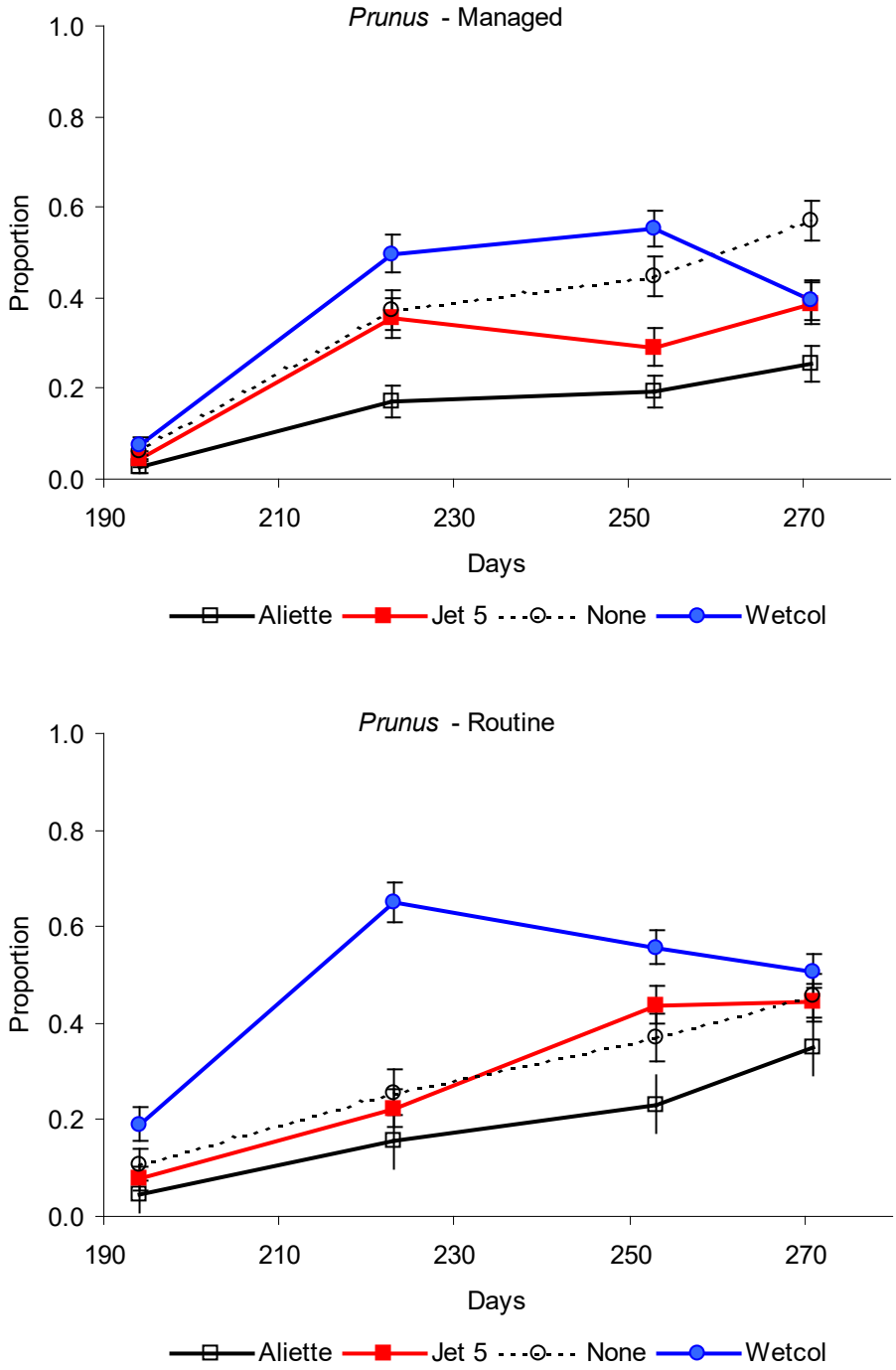

Fig. 5. Proportion of un-inoculated *Prunus* leaves with bacterial disease symptoms at each assessment during spray trial with managed and routine spray programmes. Bars represent standard errors.

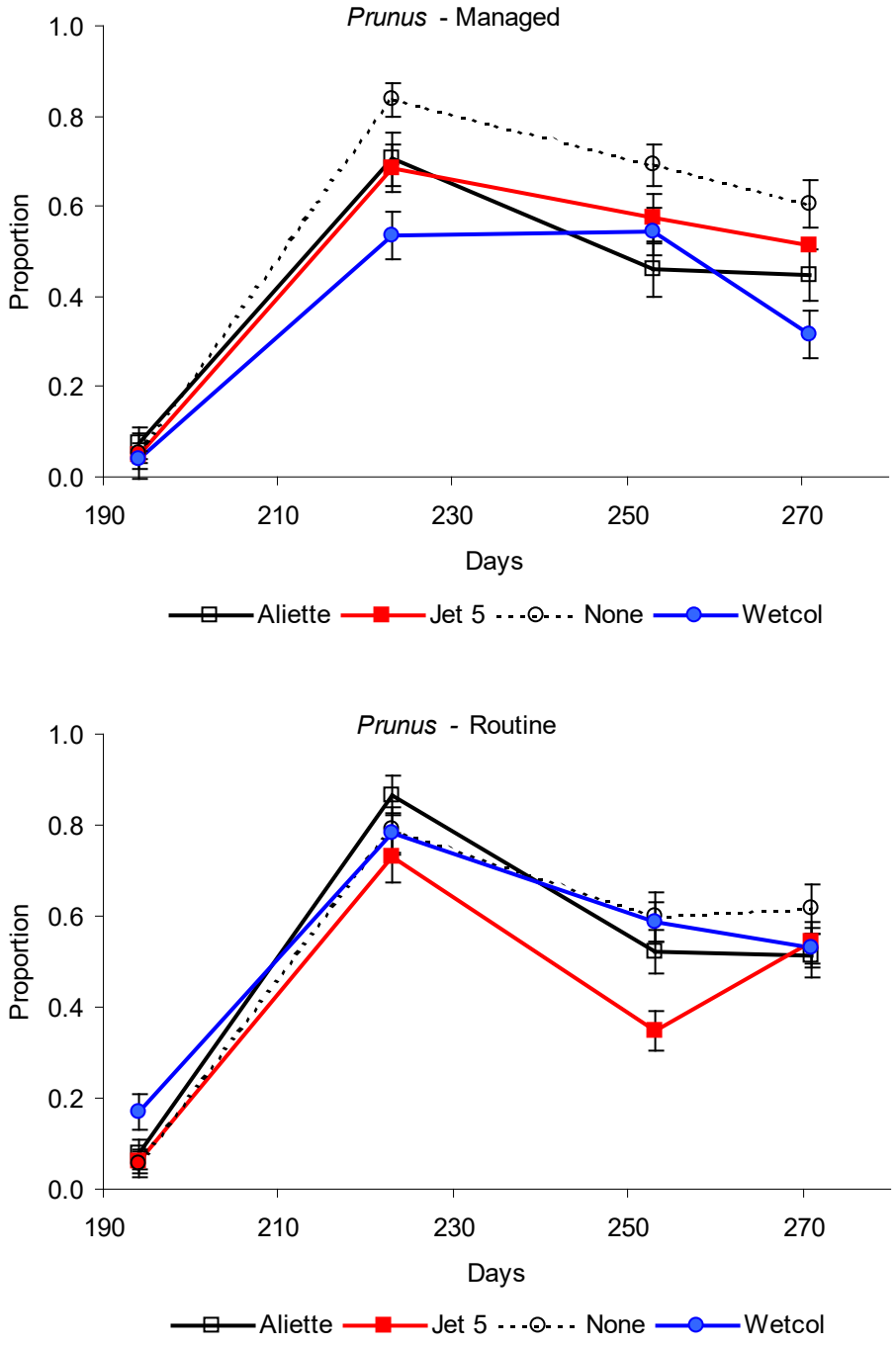

Fig. 6. Proportion of inoculated *Prunus* leaves with bacterial disease symptoms at each assessment during spray trial with managed and routine spray programmes. Bars represent standard errors.

Table 9. Overall mean proportions (and approximate standard errors) of ivy leaves with disease symptoms for each chemical treatment. Data were obtained as predictions from a generalised linear model; assessments 2 to 8.

Chemical	Mean	s.e.
Aliette	0.34	0.015
Jet 5	0.37	0.014
None	0.35	0.014
Wetcol	0.31	0.013
	Dispersion[1] = 7.6	

[1] Dispersion factor from analysis of deviance used for estimation of standard errors

Philadelphus. In order to ensure adequate disease pressure, inoculated plants were re-inoculated in the Spring. Results for inoculated and un-inoculated plants were analysed separately. Results for the initial disease assessment in the Autumn were excluded from the analysis. The analyses of deviance indicated a marginal effect of chemical treatment on disease symptoms (brown lesions) in inoculated plants, but no effect of spray programme. Wetcol gave a slight reduction in the overall mean disease levels (brown lesions) compared to the other treatments (Table 10) in inoculated plants.

Table 10. Overall mean proportions (and approximate standard errors) of *Philadelphus* leaves with brown necrotic lesions for each chemical treatment and for inoculated and un-inoculated plants. Data were obtained as predictions from a generalised linear model; assessments 5 to 8.

Chemical	Un-inoculated		Inoculated	
	Mean	s.e.	Mean	s.e.
Aliette	0.23	0.021	0.26	0.031
Jet 5	0.27	0.033	0.31	0.035
None	0.25	0.031	0.33	0.036
Wetcol	0.20	0.019	0.21	0.022
	Dispersion[1] = 4.73		Dispersion[1] = 2.69	

[1] Dispersion factor from analysis of deviance used for estimation of standard errors

Prunus. As disease levels were very low at the first assessment in the Spring (Assessment 5, 193 days), plants were re-inoculated 2 days later, and only data from subsequent assessments were included in the final analysis. Results for inoculated and un-inoculated plants were analysed separately. In the case of un-inoculated plants, the analysis of deviance indicated a major effect of chemical treatment on disease levels, but no effect of spray programme (i.e. application frequency). Thus for un-inoculated

Table 11. Overall mean proportions (and approximate standard errors) of *Prunus* leaves with brown necrotic lesions for each chemical treatment and for inoculated and un-inoculated plants. Data were obtained as predictions from a generalised linear model; assessments 6 to 8.

Chemical	Un-inoculated		Inoculated	
	Mean	s.e.	Mean	s.e.
Aliette	0.23	0.030	0.57	0.032
Jet 5	0.36	0.034	0.55	0.031
None	0.42	0.038	0.68	0.031
Wetcol	0.53	0.032	0.55	0.030
	Dispersion[1] = 17.2		Dispersion[1] = 7.97	

[1] Dispersion factor from analysis of deviance used for estimation of standard errors.

plants Aliette gave a marked reduction in the mean disease levels compared to the other treatments; this difference (23% versus 42%) was visually perceptible, but was nevertheless still commercially unacceptable. In the case of the inoculated plants, the analysis of deviance indicated that there were no consistent effects of either chemical treatment or spray programme (significant interaction terms). However, it would appear from the graphs (Fig 6) and overall means that all three chemicals gave a reduction in disease in the managed spray programme compared to the untreated control. This effect should be interpreted with considerable caution, as the spray programme treatment was confounded with a block effect.

Phytotoxicity

There was no evidence of phytotoxicity towards any of the three plant species by Aliette or Jet 5. In the case of Wetcol, however, there was evidence of some phytotoxicity with both ivy and *Philadelphus.* For both species, leaves of Wetcol treated plants had a 'harder' appearance and tended to be slightly smaller, with plants overall having a poorer appearance than the other treatments.

Discussion

Despite up to 26 spray applications over 37 weeks, none of the three compounds tested gave satisfactory control of any of the three bacterial diseases.

Although Wetcol gave a statistically significant reduction in disease in both Ivy and *Philadelphus,* the magnitude of the reduction was relatively small and it is thought that this would be unlikely to translate into an economic benefit in commercial practice, especially where disease pressure is high. In addition, the Wetcol treated Ivy and *Philadelphus* plants had a poor appearance due to phytotoxicity and the presence of visible spray residues. However, given that there was some reduction in disease, it is possible that the compound may have some benefit in situations where disease pressure is lower, e.g. under protection and/or with capillary or drip watering systems.

The effects seen with the *Prunus* were quite different from the other two species. Aliette gave a very marked and visibly perceptible reduction in disease in the un-inoculated *Prunus* plants. Despite these reductions all plants still had significant numbers of diseased leaves, therefore control could not be considered adequate.

Of the three compounds examined in the spray trial, Jet 5 was the most bactericidal in the *in vitro* tests, whereas it was the least effective in the spray trial. This clearly demonstrates that relative *in vitro* activity is a poor predictor of relative performance in disease control *in planta*. Thus, although Wetcol was the most bactericidal of the copper compounds *in vitro*, it is possible that other copper formulations may be more effective for disease control *in planta*. The continuous presence of visible spray residues on leaves of Wetcol treated plants indicates the presence of insoluble forms of copper, whereas the toxicity of copper to bacteria is related to the concentration of copper ions (Cu^{2+}) in solution. Other copper formulations could be more effective if they result in higher concentrations of soluble copper ions on leaf surfaces.

There was no effect of spray programme on disease levels. Clearly it is unlikely that there will be an effect of spray programme, without a major effect of chemical treatment. At the beginning of the experiment, it was anticipated that the 'managed' spray programme would lead to fewer spray applications overall than the 'routine' spray programme. In fact, the reverse was true as a result of prolonged periods of wet weather in both the Autumn of 2001 and the Spring of 2002.

CONCLUSIONS

In the *in vitro* tests, all of the compounds inhibited the growth of bacterial pathogens of HNS and all of the compounds tested had some bactericidal activity.

Jet 5, Menno Florades, Panacide M, bleach (sodium hypochlorite) and alcohol (ethanol) all proved to be equally effective bactericides within the limits of the tests performed, giving a reduction in bacterial numbers of greater than or equal to 5 $\log_{10}$ units (99.999% kill) under clean conditions and greater than or equal to 4 $\log_{10}$ units (99.99% kill) in the presence of peat.

Vitafect performed only marginally worse than the top five and would probably prove equally effective in routine use. Super Antibac required longer contact times than the other disinfectants.

The bactericidal properties of the copper-based compounds showed more variability, and were affected by test conditions and isolate. Wetcol 3 was consistently the most bactericidal of the copper-based pesticides.

Aliette consistently had the lowest level of bactericidal activity.

In terms of disinfectant activity, based on these results, there is little to choose between the compounds marketed as disinfectants, therefore selection of a disinfectant for use as part of a hygiene regime should depend on other considerations such as operator and environmental safety, plant toxicity and cost.

In the spray trial, none of the compounds tested gave a satisfactory level of disease control. However, there was some evidence of a slight reduction in disease in ivy and *Philadelphus* plants sprayed with Wetcol 3 and a more significant reduction in disease in un-inoculated *Prunus* plants sprayed with Aliette. Given that these small reductions were achieved with a relatively high frequency of spray application, it would seem unlikely that any economic benefit could be achieved with these chemicals.

RECOMMENDATIONS FOR FURTHER WORK

An important criteria for comparing the effectiveness of disinfectants, in use, is the effect of dilution on their activity; within the scope of this project it was not possible to compare disinfectants in the suspension test at a range of concentrations. Further work comparing activity of the best disinfectants in the suspension test at a range of dilutions and with increasing amounts of interfering substance might allow differentiation between them. It would also be valuable to conduct tests on their effectiveness in a surface disinfection test.

There was some indication of a reduction in disease with Wetcol-sprayed ivy and *Philadelphus* plants, but not sufficient for this compound to give effective control. This compound was chosen for the trials as it was the most effective of the copper compounds *in vitro.* As it would appear that *in vitro* activity is not a reliable indicator

of *in planta* activity, it may be worthwhile to conduct further trials with the other copper compounds.

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APPENDIX I - PLATE INHIBITION TEST RESULTS

Table I-1. Growth of individual bacterial isolates on plates of agar medium containing disinfectant and pesticide compounds at half-, recommended- and double- rate. A value of '0' indicates no growth, a value of '1' indicates growth.

Compound	Concen-tration	Isolate Number																		
		5698	5711	5768	5769	5799	SC073B	5994	7038	7055	7180	5687A	5873A	5866A	6237A	2070	5357	7010	5456A	5458B	5674A
Aliette	0.80%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.40%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.20%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Croptex fungex	1.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.63%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.31%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cuprokylt	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cuprokylt FL	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jet 5	1.60%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.80%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.40%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menno Florades	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myacide	0.20%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.10%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Panacide M	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Super Antibac	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitafect	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wetcol 3	10.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copper (II) sulphate	1mM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.5mM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25mM	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
None	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table I-1 continued. Growth of individual bacterial isolates on plates of agar medium containing disinfectant and pesticide compounds at half-, recommended- and double- rate. A value of '0' indicates no growth, a value of '1' indicates growth.

Compound	Concen-tration	Isolate number																		
		1159A	5682	SC126	SC097	7016	5875	7017	SC053	7053A	7183	7714	7731	7734	5993	7744	5691B	3811	5213	7764	811
Aliette	0.80%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.40%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.20%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Croptex fungex	1.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.63%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.31%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cuprokylt	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cuprokylt FL	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jet 5	1.60%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.80%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.40%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menno Florades	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myacide	0.20%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.10%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Panacide M	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Super Antibac	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25%	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitafect	2.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wetcol 3	10.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5.00%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.50%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copper (II) sulphate	1mM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.5mM	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.25mM	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
None	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX II – ANALYSIS OF DEVIANCE FOR SUSPENSION TEST

Table II-1. Analysis of deviance for the effect of biocides on the numbers of bacterial pathogen recovered in *in vitro* suspension test. Contact time as a variable. Zero count values replaced by: 0.05 (10× less than detection threshold)

Source	d.f.[1]	mean deviance	deviance ratio	
Isolate	3	451.9	62.2	*
Test batch	20	107.0	14.7	*
Control	1	16793.9	2310.0	*
Isolate.Control	3	428.1	58.9	*
Control.Conditions	2	462.6	63.6	*
Control.Compound	8	357.0	49.1	*
Time.Control	2	761.0	104.7	*
Isolate.Control.Comp	24	80.2	11.0	*
Isolate.Control.Cond	6	120.5	16.6	*
Control.Cond.Comp	8	254.0	34.9	*
Time.Isolate.Control	6	78.6	10.8	*
Time.Control.Comp	8	67.4	9.3	*
Time.Control.Cond	1	106.7	14.7	*
Isolate.Control.Cond.Comp	24	13.7	1.9	
Time.Isolate.Control.Comp	24	10.8	1.5	
Time.Isolate.Control.Cond	3	14.1	1.9	
Time.Control.Cond.Comp	8	8.5	1.2	
Residual	212	7.3		
Total	363	96.2		

[1] d.f. – degrees of freedom

* Significant terms included in model to form tables of predictions

APPENDIX III – ANALYSES OF DEVIANCE FOR SPRAY TRIAL

Table III-1. Analysis of deviance for the effect of chemical and spray programme on the proportion of ivy leaves with bacterial leaf spot, excluding assessment 1.

Source	d.f.[1]	mean deviance	deviance ratio	
Assessment	6	416.6	54.53	*
Chemical	3	26.3	3.44	*
Programme	1	0.98	0.13	
Chem.Prog	3	0.14	0.02	
Assess.Chem.Prog[2]	42	7.64		
Assess.Chem	18	8.61	3.40	
Assess.Prog	6	13.29	5.25	*
Assess.Chem.Prog	18	4.78	1.89	
Residual	773	1956.9	2.53	
Total	828	4857.3	5.866	

[1] d.f. – degrees of freedom
[2] Residual term for assessment of significance of treatment effects
* Terms considered to be significant

Table III-2. Analyses of deviance for the effect of chemical and spray programme on the proportion of leaves with bacterial disease on un-inoculated and inoculated *Philadelphus* plants, excluding assessment 1.

	Un-inoculated				Inoculated			
Source	d.f.[1]	mean deviance	deviance ratio		d.f.	mean deviance	deviance ratio	
Assessment	3	188.0	39.75	*	3	47.1	17.53	*
Chemical	3	8.6	1.82		3	9.3	3.48	*
Programme	1	0.1	0.01		1	5.6	2.10	
Chem.Prog	3	2.1	0.45		3	1.6	0.58	
Assess.Chem.Prog[2]	21	4.7			21	2.7		
Assess.Chem	9	4.3	2.00		9	3.2	1.59	
Assess.Prog	3	12.2	5.72	*	3	1.6	0.81	
Assess.Chem.Prog	9	2.7	1.26		9	2.5	1.22	
Residual	163	2.1			63	2.0		
Total	194	5.38			94	3.87		

[1] d.f. – degrees of freedom
[2] Residual term for assessment of significance of treatment effects
* Terms considered to be significant

Table III-3. Analyses of deviance for the effect of chemical and spray programme on the proportion of leaves with bacterial disease on un-inoculated and inoculated *Prunus* plants, excluding assessments 1 and 5.

	Un-inoculated				Inoculated			
Source	d.f.[1]	mean deviance	deviance ratio		d.f.	mean deviance	deviance ratio	
Assessment	2	25.5	1.48		2	144.8	18.17	*
Chemical	3	236.8	13.73	*	3	32.0	4.02	*
Programme	1	7.4	0.43		1	10.9	1.37	
Chem.Prog	3	18.7	1.09		3	23.6	2.96	
Assess.Chem.Prog[2]	14	17.2			14	8.0		
Assess.Chem	6	27.9	6.07	*	6	8.3	2.33	
Assess.Prog	2	4.9	1.06		2	21.6	6.02	*
Assess.Chem.Prog	6	10.7	2.33		6	3.2	0.91	
Residual	215	4.6			96	3.6		
Total	238	8.6			119	7.7		

[1] d.f. – degrees of freedom

[2] Residual term for assessment of significance of treatment effects

* Terms considered to be significant

