

# Research Report

**HNS 179**

Management of bacterial canker of plums and  
cherries during nursery production

**Final Report 2010-13**

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

**Authentication**

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

Dr S J Roberts  
Director  
Plant Health Solutions

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

**Report authorised by:**

Dr S J Roberts  
Director  
Plant Health Solutions

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

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## Grower Summary

### Headlines

- Levels of the bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) and *P. s. pv. syringae* (*Pss*) were reduced by sprays of Cuprokylt (copper oxychloride) + wetter (Activator 90).
- There was no evidence for improved control by mixing Cuprokylt (copper oxychloride) with Dithane NT (mancozeb), or using a sticker (Nu-Film P) rather than a wetter.
- There was no evidence of a consistent benefit from the biological control agent Serenade ASO (*Bacillus subtilis* strain QST 713) either alone or alternating with Cuprokylt (copper oxychloride).
- The overall levels of the pathogens varied from year to year and with time of year, levels of both *Psm* and *Pss*, but especially *Psm* were greater on plum than on cherry.
- A practical approach to disinfection of pruning tools during field operations using isopropanol-impregnated disinfectant wipes such as 'Azo Wipes' has been identified.

### Background and objectives

Bacterial canker of *Prunus* species has been an on-going problem for HNS growers for many years and also causes losses to stone fruit growers. It was identified as a major concern during a survey of bacterial diseases of HNS in 1996-97 (HNS 71)

Bacterial canker may be caused by two distinct pathovars (pv.) of *Pseudomonas syringae*: pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). *Psm* is host specific to *Prunus* species, whereas *Pss* has a much wider host range, with the potential for cross infection between a number of different species and genera. Although the stem canker phase is the most economically important, these pathogens also cause leaf spots/shot-holes, bud death, shoot die-back and flower blights. It is important to note that stem cankers result from infections which have been initiated in the previous year, and may not always be obvious in the first year after infection. Thus cankers may not be observed until 18 months after the initial infection has taken place.

For many years (based on work done at East Malling in 1950's and 60's), *Psm* was considered to be the primary cause of the disease in the UK. During a MAFF-funded survey or 'Farm Woodland' cherries, led by the author, in 2001-02, it became clear that both pathogens were causing canker in England, it was also clear that trees were already contaminated with the pathogen on the nursery.

It is generally considered that the most effective way to control bacterial diseases is by an avoidance strategy, i.e. avoiding introduction or carry-over of inoculum. Such a strategy can usually be implemented effectively for seed-raised annual crops, but presents considerable challenges for vegetatively propagated perennials.

Growers are aware that good hygiene practices are important, and that secateurs/pruning knives, etc. should be disinfected, but the most practical and effective method(s) to achieve this are not clear.

The overall aim of the project was to identify management options which will be of benefit in the control of bacterial canker of *Prunus* species. To achieve this the project aimed to identify the main sources of primary inoculum on propagation nurseries; examine the potential of targeted treatments to reduce/eliminate inoculum; examine the relative merit of different practical approaches for cleaning/disinfection of pruning knives/secateurs; and critically review relevant scientific and advisory literature and draw together with the new experimental work to produce a fact-sheet with clear practical recommendations. This final report summarises the results for all three years of the project.

## Summary

### ***Spray trials and epidemiology***

Spray trials were located at two commercial tree production nurseries in the UK (England), one in the South and one in the Midlands. Following discussions with grower co-ordinators two rootstocks (Saint Julien A and Colt) and three scions (plum cultivar Victoria; cherry cultivars Stella and Kiku-shidare Sakura) were selected for the experimental work. The stock hedges used to produce cuttings for rootstocks and the mother plants used to produce bud-wood for grafting were located at one nursery. The rootstocks were planted, budded, and grown-on at both nurseries.

Six (five plus an untreated control) different treatments were examined for their effects on leaf and bud populations of the bacterial canker pathogens and also on development of canker and die-back symptoms (in the final year). The treatments are shown in Table 1. Three treatments were consistent throughout the three years: (A) Cuprokylt (copper oxychloride) + wetter (Activator 90); (B) the bio-pesticide Serenade ASO (*Bacillus subtilis* strain QST 713); (E) Cuprokylt + Dithane NT (mancozeb) tank mix (this mix is widely used in France and Australia for control of bacterial pathogens of stone fruits and nuts). Two treatments varied from year to year as a result of product withdrawals and review of the results of the previous years: (C1) Bactime Cu L4F (glucohumate + copper) in 2010 was replaced by (C2) Cuprokylt alternating with Serenade ASO in 2011 and 2012; (D1) Aliette 80WG (fosetyl-aluminium) in 2010 was replaced by (D2) Cuprokylt + Dithane NT mix plus wetter in 2011 and by (D3) Cuprokylt plus sticker (Nu-Film P) in 2012. Bactime Cu L4F (C1) was replaced as pathogen levels were worse than in the untreated samples. Aliette 80WG (D1) was replaced as it was being withdrawn from the market.

Applications were made according to the following timings and key growth stages: 2 x spring, as soon as possible after bud burst; 2 x summer, prior to budding; 2 x autumn sprays. Approximately 12 individual stock hedge plants, 2-3 mother plants and 100 rootstocks or maidens were allocated to each treatment.

<b>Table 1.</b> Treatment codes, products and rates used in spray trial.				
Code	Product	Active ingredient	Rate	Approval status
A	Cuprokylt + wetter (Activator 90)	Copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator 90	Label approval
B	Serenade ASO	Bacillus subtilis	10 mL/L	EAMU for ornamental plant production
C1	Bactime Cu L4F (Year 1)	Copper + glucohumate	4 g/L	N/A - foliar fertiliser
C2	Cuprokylt followed by Serenade (Years 2 and 3)			
D1	Aliette 80WG (Year 1)	Fosetyl-aluminium	1 g/L	No longer approved
D2	As E + Activator 90 (Year 2)			
D3	Cuprokylt + sticker (Nu-Film P) (Year 3)	Copper oxychloride	3 g/L Cuprokylt + 0.3 mL/L Nu-Film P	Label approval
E	Dithane NT + Cuprokylt	Mancozeb + copper oxychloride	2 g/L Dithane + 3 g/L Cuprokylt	Dithane NT – LTAEU Cuprokylt – Label approval
U	control, no treatment	N/A	N/A	N/A

Leaf and bud samples were collected from each treatment from each nursery during the growing season and taken to the laboratory for processing. Sampling visits were timed to occur shortly after sprays had been applied. Samples were extracted, diluted and plated onto semi-selective agar media to determine the presence/absence and numbers of *Psm* and *Pss*. The identities of the bacteria were confirmed by cultural, biochemical and (in the case of *Pss*) host tests.

Approximately 750 samples were collected over the three years. Both bacterial canker pathogens were isolated from samples at both nurseries throughout the year. The main conclusions can be summarised as follows:

- Levels of *Psm* and *Pss* were reduced by sprays containing Cuprokylt.
- The most consistent effects were obtained with Cuprokylt plus a wetter (Activator 90).
- There was no consistent benefit from mixing Cuprokylt with Dithane NT compared to Cuprokylt plus wetter.
- There was no benefit from using a sticker (Nu-Film P) rather than wetter (Activator 90).
- There was no benefit from Serenade ASO or alternating Serenade ASO and Cuprokylt compared to Cuprokylt alone.
- Levels of both *Psm* and *Pss*, but especially *Psm* were greater on plum than on cherry.

- The overall levels of pathogens varied from year to year and with the time of year: levels of *Psm* tended to be higher in spring and summer, levels of *Pss* were higher in spring and autumn

### **Disinfection of pruning tools**

The cutting edges of secateur blades or 'Stanley' knife blades were contaminated with a standard amount of a known strain of *Psm*. An attempt was then made to disinfect the blades by one of several methods (Table 2). Following 'disinfection' each blade was then used to make ten cuts in a plate of agar medium. Disinfection efficiency was then assessed on the basis of the number of cuts in the agar with bacterial growth. Results are summarised in Table 2.

<b>Table 2.</b> Summary of disinfection tests. Each replicate consisted of ten sequential cuts following disinfection of the contaminated blade. The percentage is the number of cuts giving bacterial growth: the lower the percentage the better the treatment.				
Code	Detail	Replicates	% cuts (5 x 10 <sup>7</sup> ) <sup>a</sup>	% cuts (1 x 10 <sup>6</sup> ) <sup>b</sup>
U	Untreated control.	20	99.9	99.3
SW	Spray with 70% iso-propanol, leave 30 s then wipe dry with paper towel.	20	16.9	0.8
SW2	Spray with 70% iso-propanol, wipe residue, repeat spray leave 30 s then wipe dry.	3	1.1	0.0
W	Wipe with Azo wipes (70% iso-propanol).	8	8.6	0.4
J5_0	Brief dip in Jet 5 (0.8%) then wipe dry.	19	48.2	3.4
J5_15	15 s dip in Jet 5 (0.8%) then wipe dry.	6	0.0	0.0
J5_30	30 s dip in Jet 5 (0.8%) then wipe dry.	7	0.3	0.0
Cl_0	Brief dip in 1% chlorine then wipe dry.	7	24.4	1.2
Cl_30	30 s dip in 1% chlorine.	1	0.0	0.0
GW	Rub edge of blade with alcohol hand gel between finger and thumb, wipe dry.	11	51.1	3.8
<sup>a</sup> Predicted % cuts with growth, adjusted to a standard inoculum concentration of 5 x 10 <sup>7</sup> CFU/mL				
<sup>b</sup> Predicted % cuts with growth, adjusted to a standard inoculum concentration of 1 x 10 <sup>6</sup> CFU/mL				

During the first rounds of testing done in 2010, we failed to identify a practical option for disinfection in the field. Given the wider potential importance of disinfection of pruning tools, further experiments were done in 2011 with lower inoculum concentrations and shorter drying times.

At lower inoculum doses and with shorter drying times, the efficacy of all treatments improved, and all gave significant reductions in potential pathogen transfer compared to the untreated control. Conversely, the level of disinfection achieved was reduced as inoculum increased and when drying was fan-assisted. Although long (30 second) dips in disinfectants (chlorine or Jet 5) were the most effective, these are not practical to implement in the field. Hence, whilst not the most effective when bacterial inoculum levels are high or when it is dried on, regular use of disinfectant wipes (impregnated with 70% iso-propanol as the active ingredient) are probably the most practical option for use in the field. The Azo Hard Surface Wipes used in the tests and similar products are readily obtained from a

number of suppliers, especially medical and clean-room suppliers. In addition, because such an approach is easily implemented and so more likely to be applied, it seems likely that the benefits of more frequent use may outweigh the lower efficiency compared to other methods.

### **Financial benefits**

Current industry estimates indicate potential losses from bacterial canker during nursery production and soon after final planting in the range £125,000 to £200,000 per annum. Based on current (April 2013) prices for Cuprokylt of £165 for 25 kg and Activator 90 of £29 for 5 L, the cost of six applications per annum would be less than £128 per ha, plus the labour cost of application.

### **Action points for growers**

- Disinfect pruning tools and knives as often as possible in the field using iso-propanol impregnated wipes such as 'Azo Wipes'.
- Copper sprays in the form of Cuprokylt + wetter (Activator 90) are still the most effective chemical control option available for bacterial canker. Other products containing the same active ingredient (copper oxychloride) would be expected to be equally effective, but were not tested in this project, and may be more limited in terms of the number of applications that can be applied.
- The highest levels of *Psm* were seen in the spring and summer, thus the current label recommendations for three sprays in late summer may be starting too late to have a significant impact, and spray applications should start in the spring

## Science Section

### Introduction

Bacterial canker of *Prunus* species has been an on-going problem for HNS growers for many years, and was identified as a major concern during a survey of bacterial diseases of HNS in 1996-97 (HNS 71) (Roberts 1997).

Bacterial canker may be caused by two distinct pathovars of *Pseudomonas syringae*: pathovar (pv.) *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). *Psm* is host specific to *Prunus* species, whereas *Pss* potentially has a much wider host range, with the potential for cross infection between a number of different species and genera. As well as stem cankers, these pathogens may also cause leaf spots or shot-holes, shoot die-back, bud death and flower blights, although the stem canker phase is probably the most economically important. They may also be present as epiphytes on leaf surfaces in the absence of disease symptoms.

It is important to note that stem cankers result from infections which have been initiated in the previous year, and may not always be obvious in the first year after infection. Thus cankers may not be observed until 18 months after the initial infection has taken place.

For many years (based on work done at East Malling in 1960's and 70's), *Psm* alone was considered to be the primary cause of the disease in the UK; whereas in Europe, South Africa and USA the disease has long been attributed to both pathovars of *P. syringae*.

The most extensive recent work on bacterial canker on *Prunus* species in the UK was done in the late 1990s, early 2000s. This Defra-funded work (WD0224 and WD0234) (Roberts & Vicente 2001) (Roberts & Vicente 2002) was on the biology, epidemiology and resistance of bacterial canker in cherry for farm woodlands, in collaboration with breeders at East Malling. The project sought to improve understanding of the pathogen, its taxonomy and variation, and develop improved methods for detection and discrimination, as a necessary pre-requisite both for studies on the epidemiology of this disease and for the development of improved methods for disease resistance screening. Although prior to this work, bacterial canker of sweet cherry was considered to be mainly caused by *Psm* in the UK; it was confirmed that both pathovars could be important in the UK (Vicente *et al.* 2004) and emphasised the need to select appropriate strains for resistance screening. As part of this work selective media were devised for isolation of the pathogens, and a rapid pathogenicity test using micro-propagated plantlets was developed (Vicente & Roberts 2003). It was also clear that trees were already contaminated with the pathogen on the nursery. It was suggested that control measures need to be targeted at producing, cleaning-up and maintaining disease-free stock plants, and minimising the likelihood of cross-infection between batches of cuttings and plants. In other studies on cherry laurel (*Prunus laurocerasus*) (Roberts 1998) symptomless contamination of stock plants was considered the most likely source of primary inoculum.

It is generally considered that the most effective way to control bacterial diseases is by an avoidance strategy, i.e. avoiding introduction and carry-over of inoculum. Such a strategy can usually be implemented effectively for seed-raised annual crops, but presents considerable challenges for vegetatively propagated perennials.

In some other countries (esp. USA) the antibiotic Streptomycin has been used for control of bacterial diseases, especially fireblight of apples and pears. It can be highly effective, but, as an antibiotic, its use is not permitted and is not likely to ever be permitted in the UK. Additionally in areas (such as the North Western USA) where its use has been widespread, resistance has inevitably developed, resulting in control failures and the deployment of the biological control agent *Pantoea agglomerans*. [Note that this has not been included in these trials as its mode of action is very specific in colonising flowers to prevent infection by competitive exclusion]

HDC projects FV 186a (Roberts & Brough 2000) and FV 335 (Roberts 2009) examined the efficacy of copper oxychloride and other products in reducing the rate of spread of a seed-borne bacterial pathogen (*Xanthomonas campestris* pv. *campestris*) during brassica transplant production [previous MAFF-funded work (Roberts *et al.* 1999; Roberts *et al.* 2007) had shown that this could be very rapid]. Weekly sprays with copper greatly reduced or even eliminated the spread of the pathogen (regardless of symptoms).

HNS 91 (Roberts & Akram 2002) evaluated the bactericidal properties of 14 disinfectants/pesticides in 'plate' tests against 20 bacterial strains representing a number of species and genera of plant pathogenic bacteria. A more limited set of bacteria was evaluated in suspension tests in both 'clean' and 'dirty' conditions. Spray trials were also conducted with a more limited number of products for control of bacterial leaf spots of ivy (*Xanthomonas*), Philadelphus (*Pseudomonas syringae* pv. *philadelphii*) and Prunus (*Pseudomonas syringae* pv. *syringae*). Most of the disinfectant products proved to be equally effective bactericides and gave a reduction in bacterial numbers of equivalent to  $\geq 99.999\%$  kill under clean conditions and  $\geq 99.99\%$  kill in the presence of peat. In the spray trials, there was some evidence of a slight reduction in disease with copper (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 80WG (fosetyl-aluminium) in Prunus plants, with a marked reduction in the mean disease levels compared to the other treatments; this difference (23% versus 42%) was visually perceptible, but again was considered commercially unacceptable. It should be noted that this trial, conducted over less than 1 year on young potted plants, only examined foliar symptoms.

Growers are aware that good hygiene practices are important, and that secateurs/pruning knives, etc. should be disinfected, but the most practical and effective method(s) to achieve this are not clear.

The overall aim of the project is to identify management options which will be of benefit in the control of bacterial canker of *Prunus* species. The specific objectives of the project are:

- Identify the main sources of primary inoculum
- Examine the potential of targeted treatments to reduce/eliminate inoculum
- Examine the relative merit of different practical approaches for cleaning/disinfection of pruning knives/secateurs.

- Critically review relevant scientific and advisory literature and draw together with the new experimental work to produce a fact-sheet with clear practical recommendations

The essential hypothesis behind the work was that bud-wood and/or rootstock material may be asymptotically contaminated with the pathogen(s) at propagation, and that targeting control measures at reducing or eliminating this contamination will result in lower levels of disease.

Results from the first and second years of the project have been reported previously (Roberts 2011, 2012). This report consolidates the methods, results and analysis of all three years of the project.

## **Materials and Methods**

### ***Experimental design***

Given the perennial nature of the host and disease development, spray trials and assessments were done over the three years of the project. The trials were located at two commercial tree production nurseries in the UK (England), one in the South and one in the Midlands. Following discussions with grower co-ordinators two rootstocks (Saint Julien A and Colt) and three scions (plum cultivar (cv.) Victoria; cherry cultivars Stella and Kiku-shidare Sakura) were selected for the experimental work/treatments. The stock hedges used to produce cuttings for rootstocks and the mother plants used to produce bud-wood for grafting were located at one nursery. The rootstocks were planted, budded, and grown-on at both nurseries.

Six (five plus untreated control) different treatments were examined for their effects on leaf/bud populations of bacterial canker pathogens and also on development of canker symptoms (in the final year). The treatments are shown in Table 3. Three treatments were consistent throughout the three years: (A) Cuprokylt (copper oxychloride) plus wetter (Activator 90); (B) Serenade ASO (*Bacillus subtilis* strain QST 713); (E) Cuprokylt + Dithane NT (mancozeb) tank mix (this mix is widely used in France and Australia for control of bacterial pathogens of stone fruits and nuts). Two treatments varied from year to year as a result of product withdrawals and review of the results of the previous years: (C1) Bactime Cu L4F (glucohumate + copper) in 2010 was replaced by (C2) Cuprokylt alternating with Serenade ASO (C2) in 2011 and 2012; (D1) Aliette 80WG (fosetyl-aluminium) in 2010 was replaced by (D2) Dithane NT + Cuprokylt tank mix plus wetter in 2011 as Aliette 80WG was due to be withdrawn, and by (D3) Cuprokylt + sticker (Nu-Film P) in 2011 to examine the effect of different adjuvants.

In the first year approximately 12 individual stock hedge plants (of each of the two species), 2-3 mother plants (of each of the three species/cultivars) and 100 rootstocks (of each of the two species and at each site) were allocated to each treatment. The rootstocks were budded in summer of the first year to become maidens in the second year, and further 1<sup>st</sup> year rootstock material which was taken as cuttings in Autumn 2011 was included in the second year.

Code	Product	Active ingredient	Rate	Approval status
A	Cuprokylt plus wetter (Activator 90)	Copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator 90	Label approval
B	Serenade ASO	Bacillus subtilis	10 mL/L	EAMU for ornamental plant production
C1	Bactime Cu L4F (Year 1)	Copper + glucohumate	4 g/L	N/A - foliar fertiliser
C2	Cuprokylt followed by Serenade ASO (Years 2 and 3)			
D1	Aliette 80WG (Year 1)	Fosetyl-aluminium	1 g/L	No longer approved
D2	As E plus wetter (Activator 90) (Year 2)			
D3	Cuprokylt + sticker (Nu-Film P) (Year 3)	Copper oxychloride	3 g/L Cuprokylt + 0.3 mL/L Nu-Film P	Label approval
E	Dithane NT + Cuprokylt	Mancozeb + copper oxychloride	2 g/L Dithane + 3 g/L Cuprokylt	Dithane NT – LTAEU Cuprokylt – Label approval
U	Control, no treatment	N/A	N/A	N/A

### ***Spray applications and timing***

Spray treatments were applied by the growers using a knapsack sprayer. The timing of spray applications was the same for all treatments: two sprays in spring at/soon after bud burst, two sprays in summer prior to budding, two sprays in autumn prior to leaf fall. Sprays were applied 7-14 days apart depending on weather conditions, planned for days when no rain was predicted in the following 24 h, and applied as late in the day as possible.

### ***Sample collection and processing***

Leaf and bud samples were collected from each nursery on three or four occasions during the growing season. Visits were timed to occur shortly after sprays had been applied. Two visits were required during summer in 2010 due to the different timing of budding for plum and cherry material (due to differences in maturity of the wood).

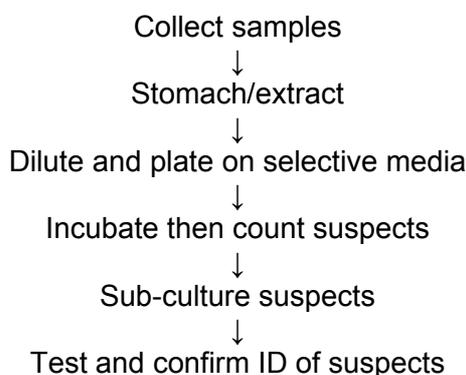
Individual leaves were collected by cutting the petiole with a pair of scissors, whilst holding a 'stomacher' bag underneath to catch it, to minimise handling and the potential for cross-contamination. For stock hedges and rootstocks growing in rows in the field, a single leaf was collected from individual plants at random intervals whilst walking along the row until sufficient leaves had been collected for the sample. For the mother-plants single leaves were collected from individual branches selected at random whilst walking around the tree. For bud-wood samples, 5-10 branches were collected and prepared as bud-wood (i.e. leaves removed). Equipment and hands were disinfected with 70% iso-propanol between samples.

Following collection, all samples were stored in polythene bags in the fridge overnight and until processing within two days of collection.

Leaf samples were processed in the same stomacher bag in which they were collected. Buds were excised from the collected twigs/branches and placed into a stomacher bag immediately before processing.

For processing, a minimal volume of sterile saline (0.85% NaCl) plus 0.02% Tween 20 was added to the plant material (leaves or buds) in a stomacher bag. The volume was adjusted according to the weight of plant material and number of leaves. The material was then stomached for five minutes and a dilution series prepared from the resulting extract. Aliquots (0.1 ml) of dilutions and the undiluted extract were then spread on plates of mP3 and MS3 selective media (Vicente *et al.* 2004). A positive control was also included for each batch of selective media. This consisted of a suspension of known strain of either *Pss* or *Psm* which was diluted and plated in the same way as the test samples. Plates were incubated at 25°C for 3-4 days and the number of suspect colonies of *P. syringae* on each plate recorded. If present, up to six suspect colonies were sub-cultured from each sample to sector plates of PAF and SNA media.

The procedure is summarised below:



### **Characterisation of suspect isolates**

Suspect isolates were initially characterised on the basis of appearance and production of fluorescent and other pigments on PAF medium, levan production on sucrose nutrient agar (SNA) medium, and oxidase reaction. Based on these results isolates were considered to be potential *P. syringae* or not. Further characterisation of potential *P. syringae* isolates was done using the GATTa tests (gelatinase, aesculin hydrolysis, tyrosinase, utilisation of D-tartrate) (Vicente *et al.* 2004). Colour of growth in nutrient sucrose broth (NSB), and tobacco hypersensitivity reaction were also tested. Based on the results of these tests, isolates were assigned to either *Psm* or *Pss*. In the first year, some isolates were also inoculated into immature cherry fruit, and in years 2 and 3, isolates identified as *Pss* based on GATTa tests were also tested for pathogenicity on lilac leaves (including some isolates from year 1). A representative selection of isolates was frozen on glass beads in nutrient broth (NB)+15% glycerol for future reference and characterisation.

### **Disease assessment**

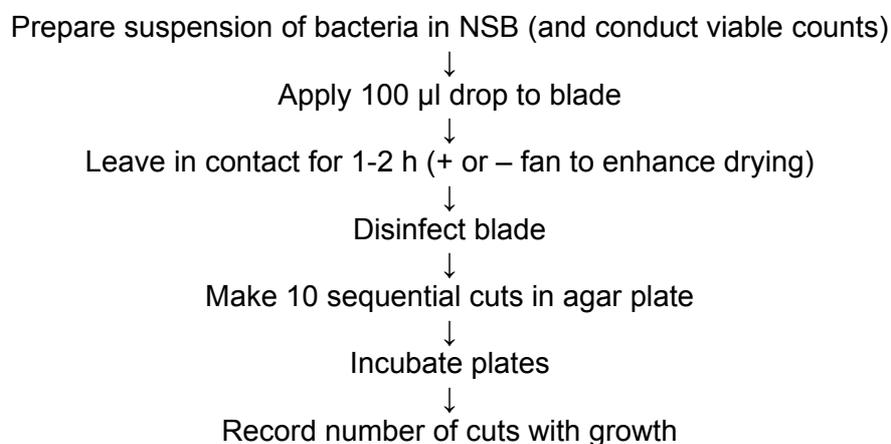
In spring 2012, the two year old production material common to both sites was visually assessed for disease levels. Each tree was inspected for the presence of canker lesions or the presence of die-back in current or previous years growth. Data were recorded as the number of trees with die-back out of the total number assessed.

### **Disinfection of pruning tools**

A known strain of *Psm* (5300) was grown for 24-48 h on PAF medium at 25°C. A small amount of growth from the plate was used to make a dense suspension in nutrient broth containing 5% sucrose (NSB). Aliquots (100 µl) of this suspension were then spread on one side of the cutting edge of secateur blades (dismantled from the handles) or 'Stanley' knife blades, and allowed to partially dry at room temperature (ca. 18-20°C) for 1 – 2 h. In year 2, the effects of different drying times and inoculum concentrations were also examined.

An attempt was then made to disinfect the blades by one of several methods using 70% iso-propanol, Jet 5 (0.8%), bleach (1% chlorine, prepared using Presept<sup>TM</sup> tablets), or a hand sanitising gel (Deb, FloraFree). Following 'disinfection' each blade was used to make ten cuts in a plate of PAF agar medium. Plates were then incubated for 2-4 days at 25°C and the number of cuts in the medium with bacterial growth recorded.

The procedure is summarised below:



### **Statistical analysis**

The spray trial data were analysed in two ways. The effect of treatments on the proportion of leaves/buds contaminated/infected with either *Pss* or *Psm* or both was analysed by fitting a series of generalised linear models with binomial error distribution and complementary log-log link function. The natural log of the number of leaves/buds in each sample was used as an offset. The effect of treatments on the numbers of bacteria per leaf/bud was analysed by fitting a series of generalised linear models with Poisson error distribution and a log link function. The number of leaves in each sample was used a weighting factor. In both cases treatments means were obtained as predictions from the model. In each case three separate analyses were done: combined data (i.e. either *Psm* or *Pss* detected), for *Psm* alone, for *Pss* alone.

The disease symptom data were analysed by fitting a series of generalised linear models with binomial error distributions and logit link function. Treatment means were obtained as predictions from the relevant model.

The disinfectant data were analysed by fitting a series of generalised linear models with binomial error distribution and logit link function. Treatment means were obtained as predictions from the relevant model.

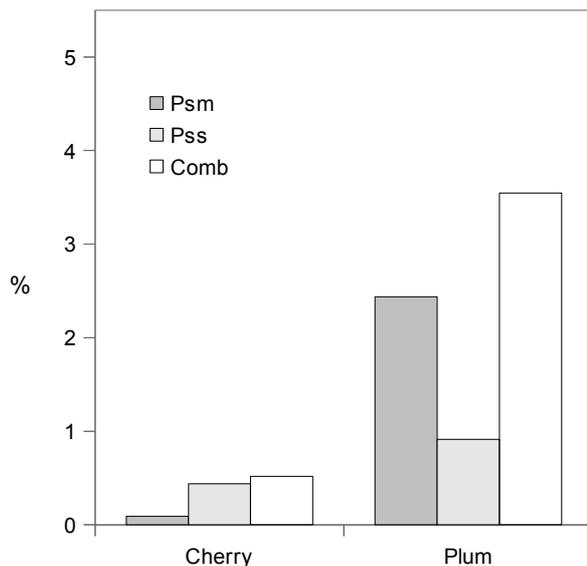
All analyses were performed using Genstat (Payne *et al.* 2005).

## Results

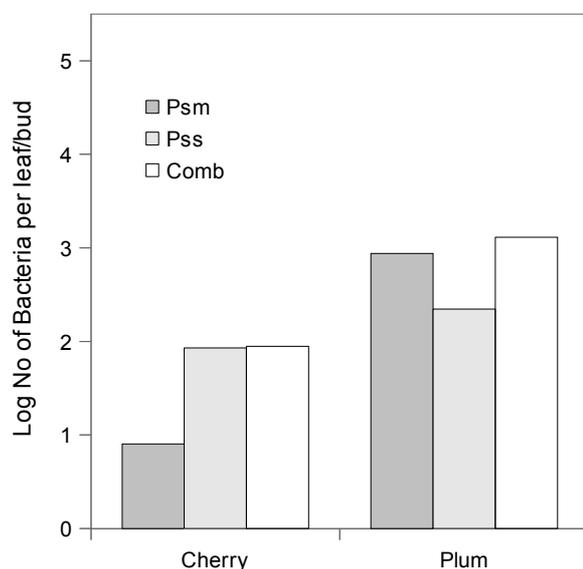
A total of 746 leaf or bud samples were collected and processed over the three years of the project. A matrix of the material sampled, when and the potential pathogens detected is shown in Appendix I. Both of the target pathogens (i.e. either *Psm* or *Pss*) were isolated from samples at both nurseries. In addition to strains identified as *Psm* or *Pss*, strains of *P. syringae* with characteristics which did not conform to either pathovar on the basis of the GATTA tests were also isolated, such strains were considered as non-pathogenic and so were excluded from the counts used for statistical analyses. Representative isolates initially characterised as *Pss* (from all years of the project) were also tested for pathogenicity on lilac; overall only about a third of these isolates were pathogenic on lilac, and so can be considered to be *Pss* (*sensu stricto*). Therefore, only these pathogenic *Pss* isolates were included in the counts used for the final statistical analyses. (Note that the data analyses in the annual reports for years 1 and 2 included all potential *Pss* isolates, irrespective of pathogenicity, as full pathogenicity data was not available at the time). For the purposes of analysis samples were grouped according to the broad host 'species' i.e. plum or cherry and stage of production (stock hedge, mother-plants, maidens, rootstocks).

Analyses of deviance (see Appendix II and III) were used to identify significant treatment factors. Means and standard errors for these factors were then obtained as predictions from models containing just the factors of interest. For brevity, only treatment factors considered to be the most important/significant are mentioned below. Some care is needed in interpreting the separate results for *Psm* and *Pss*, as the two organisms were often mutually exclusive in a given sub-sample, i.e. most suspects colonies sub-cultured tended to be one or the other.

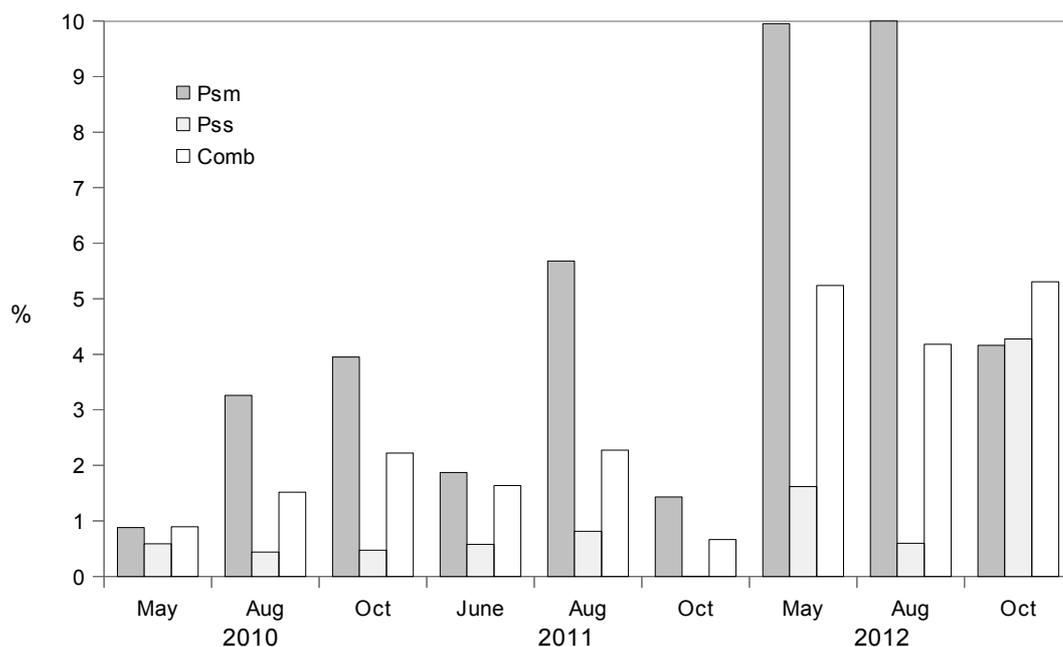
In initial analyses of the data, both in terms of the proportions contaminated and numbers of bacteria, it was clear that host was the most important factor determining the detection of *Psm*: in years 1 and 3 it was only rarely detected on cherries, and in year 2 it was never detected on cherries (Figs. 1 and 2). Therefore to simplify the statistical analyses, their interpretation, and presentation, analysis of *Psm* was restricted to Plums only. Inevitably, also, there were significant effects of sampling date (year, month) and interactions with location, i.e. the overall mean bacterial numbers varied with sampling date (Fig. 3) and location. These are also illustrated for each treatment on 'maidens' (rootstocks budded in 2010, headed back in 2011 to produce maidens, grown on in 2012) in Appendix VI.



**Figure 1.** Mean percentage of leaves contaminated with bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*)(all years, all samples).



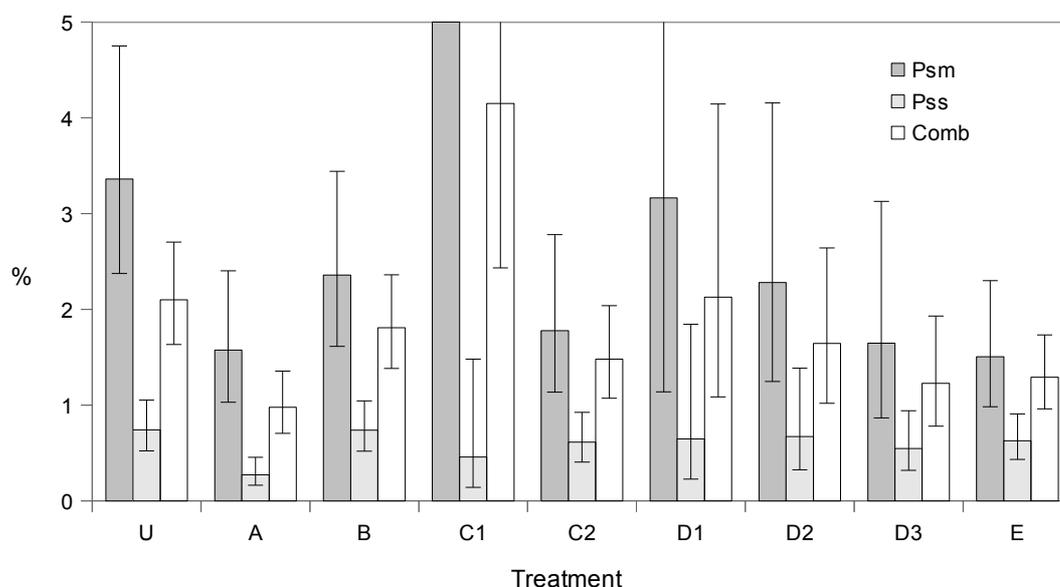
**Figure 2.** Mean numbers of bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*) detected on cherries and plums (all years, all samples).



**Figure 3.** Mean percentage of leaves contaminated with bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*, plum only), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*) at each sampling period (untreated samples only). *Psm* values for May and August 2012 were 9.95 and >40.8 respectively.

**Proportion of leaves/buds infested**

The overall percentages of leaves contaminated with potential bacterial canker pathogens for each treatment are shown in Fig. 4 and are summarised for each year in Fig 5.



**Figure 4.** Overall summary of the effects of treatments on the percentage of leaves contaminated with bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*, plum only), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*). The data have been adjusted for differences between years to allow presentation of treatments that were not applied in all years (model fitted: year + treatment). The value for *Psm* C1 is 12.3 (CI 6.1 to 23.8). Bars represent the 95% confidence limits.

*Psm (Plum only)*

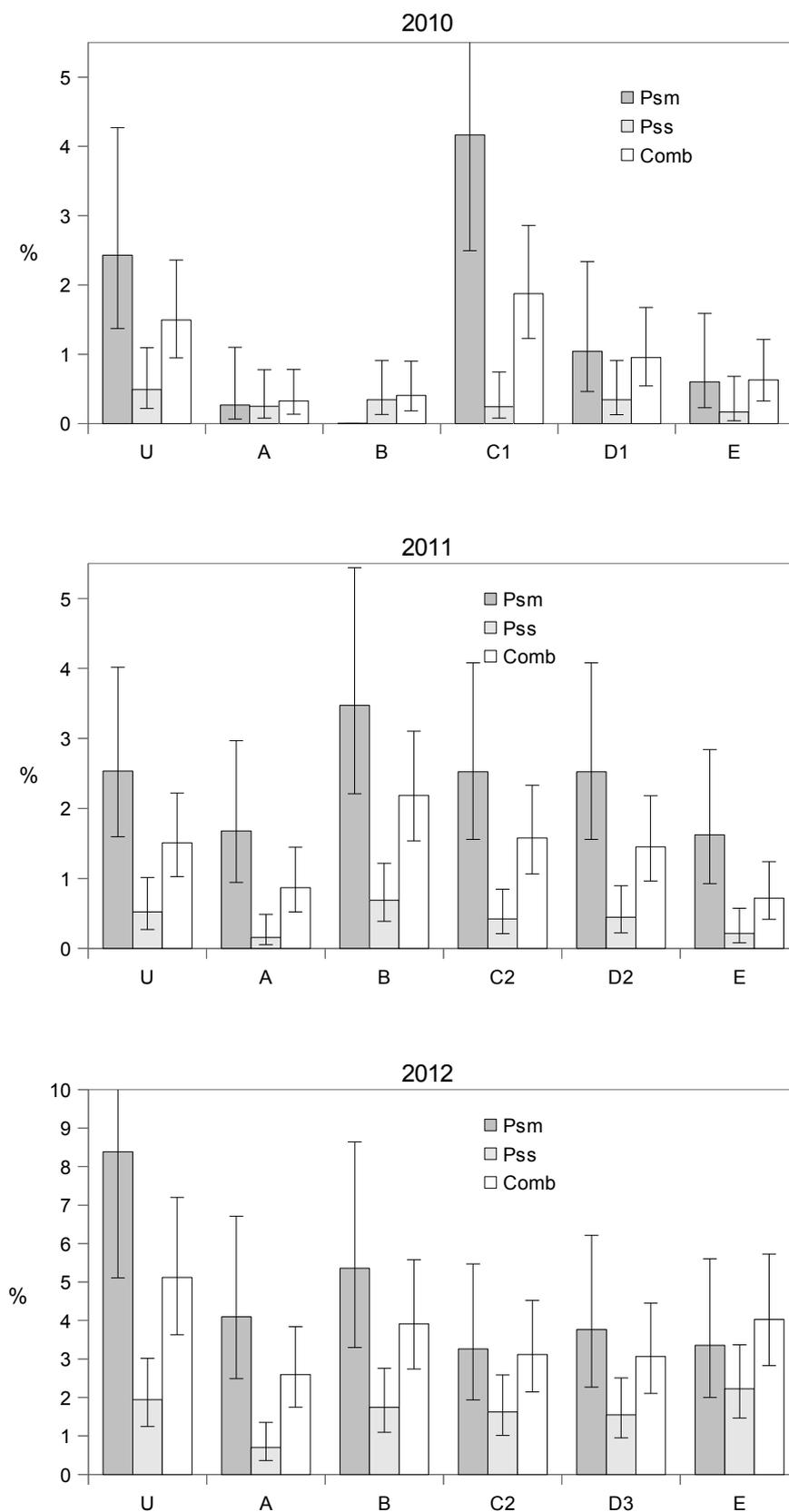
Year, month, production stage, treatment, and year x production stage and year x treatment interactions had significant effects on the proportion of plum leaves contaminated with *Psm* (Appendix II). Levels differed significantly from year to year, and were highest in 2012 (4.4%) and lowest in 2010 (1.1%). Overall levels differed with sampling time and tended to be higher in the summer (3.7%) and spring (2.6%) than the autumn (1.4%). The effect of production stage varied from year to year: stock hedges had the highest levels in 2010 and the lowest in 2011. Overall, treatments A (Cuprokyt + wetter) and E (Cuprokyt + Dithane NT) consistently gave significant reductions in the proportion of contaminated leaves compared to the untreated controls (Fig. 4). Treatment B (Serenade ASO) and C2 (alternating Cuprokyt and Serenade ASO) gave variable results from year to year, and treatments D1 (Aliette 80WG) and D3 (Cuprokyt + sticker) also gave significant reductions in the years they were included (Fig. 5).

*Pss*

There were significant effects of host, site, year and production stage (Appendix II). There was a significant host x treatment interaction, and a marginal main effect of treatment. Overall on plums levels of pathogenic *Pss* were much lower than *Psm*, on cherries levels of *Psm* were lower than *Pss* (Fig 1). Levels were higher on site 2 (1.3%) than on site 1 (0.5%), and higher in 2012 than in 2010 and 2011. Levels were also highest on mother-plants (1%) and lowest on stock hedges (0.08%) and rootstocks (0.8%). The treatment x host interaction indicated that whereas Treatment A (Cuprokyt + wetter) gave consistently lower levels than the untreated on both plum and cherry, other treatments gave inconsistent effects on the two hosts.

*Combined*

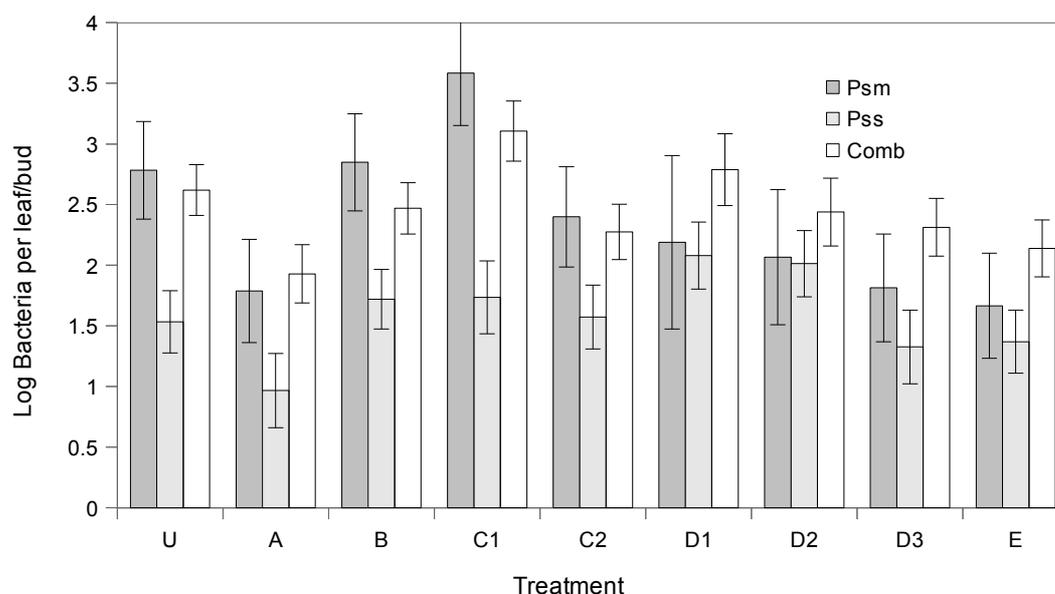
There were significant effects of host, year, production stage and stage x host and stage x year, treatment and treatment x year interaction (Appendix II). The host inevitably had a major effect (Fig. 1), due to the presence of both *Psm* and *Pss* on plums, but mainly only *Pss* on cherries. Levels on Plum were higher than cherry for all stages but particularly on the mother-plants (5.8% vs 0.8%) and the 1<sup>st</sup> year rootstocks (2.7% vs 0.2%). Levels were highest in 2012 (3.6%) and lowest in 2010 (0.9%). Overall, treatment A (Cuprokyt + wetter) gave the biggest and most consistent reductions (Fig 4). Other treatments were more variable giving significant reductions in some years but not in others: B (Serenade ASO, 1/3 years), D3 (Cuprokyt + sticker, 1/1 years) and E (Cuprokyt + Dithane NT, 2/3 years) (Fig. 5).



**Figure 5.** Effect of treatments on the percentage of leaves contaminated with bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (Psm, plum only), *P. s.* pv. *syringae* (Pss), and either or both (Comb) for each year. Note the different scale in 2012. Bars represent the 95% confidence limits.

### Numbers of bacteria

The overall mean numbers of potential bacterial canker pathogens for each spray treatment and year are shown in Fig. 6. Summaries of the data for each year of the project are shown in Fig. 7.



**Figure 6.** Overall effects of treatments on the  $\log_{10}$  of the mean number of *Pseudomonas syringae* pv. *morsprunorum* (*Psm*, plum only), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*). Note that the scale is a  $\log_{10}$  scale, so that a unit represents a tenfold difference in bacterial numbers. The data have been adjusted for differences between years to allow presentation of treatments that were not applied in all years (model fitted: date + treatment). Bars represent the standard errors of the means.

#### *Psm* (plum only)

Year, sampling time, site x year, treatment and several treatment interaction terms had significant effects on the mean numbers of *Psm* per leaf (Appendix III). Overall numbers were higher in 2012 (3.6  $\log_{10}$  CFU/leaf) than in 2010 (2.5  $\log_{10}$  CFU/leaf) and 2011 (2.7  $\log_{10}$  CFU/leaf) and were higher in the spring (3.3  $\log_{10}$  CFU/leaf) than in summer (2.5  $\log_{10}$  CFU/leaf) and autumn (2.2  $\log_{10}$  CFU/leaf). Interpreting the effect of treatments was complicated due to significant interaction terms (in order of importance): treatment x stage, treatment x site, treatment x year x stage. Overall, treatments A (Cuprokylt), C2 (alternating Cuprokylt and Serenade ASO), D3 (Cuprokylt + sticker) and E (Cuprokylt + Dithane NT) gave significant reductions in numbers of *Psm* compared to the untreated (Fig 6). The greatest reductions were by treatments A and E. The most important interaction term, treatment x stage, resulted from variable performance of treatments B, C1 and D3 on particular production stages. The treatment x site interaction indicated that treatments were relatively less effective at site 2 than at site 1. The treatment x year interaction indicated varying efficacy from year to year: in 2010 all treatments except treatment C1 (Bactime Cu L4F) reduced numbers compared to the untreated control; in 2011 only treatment E

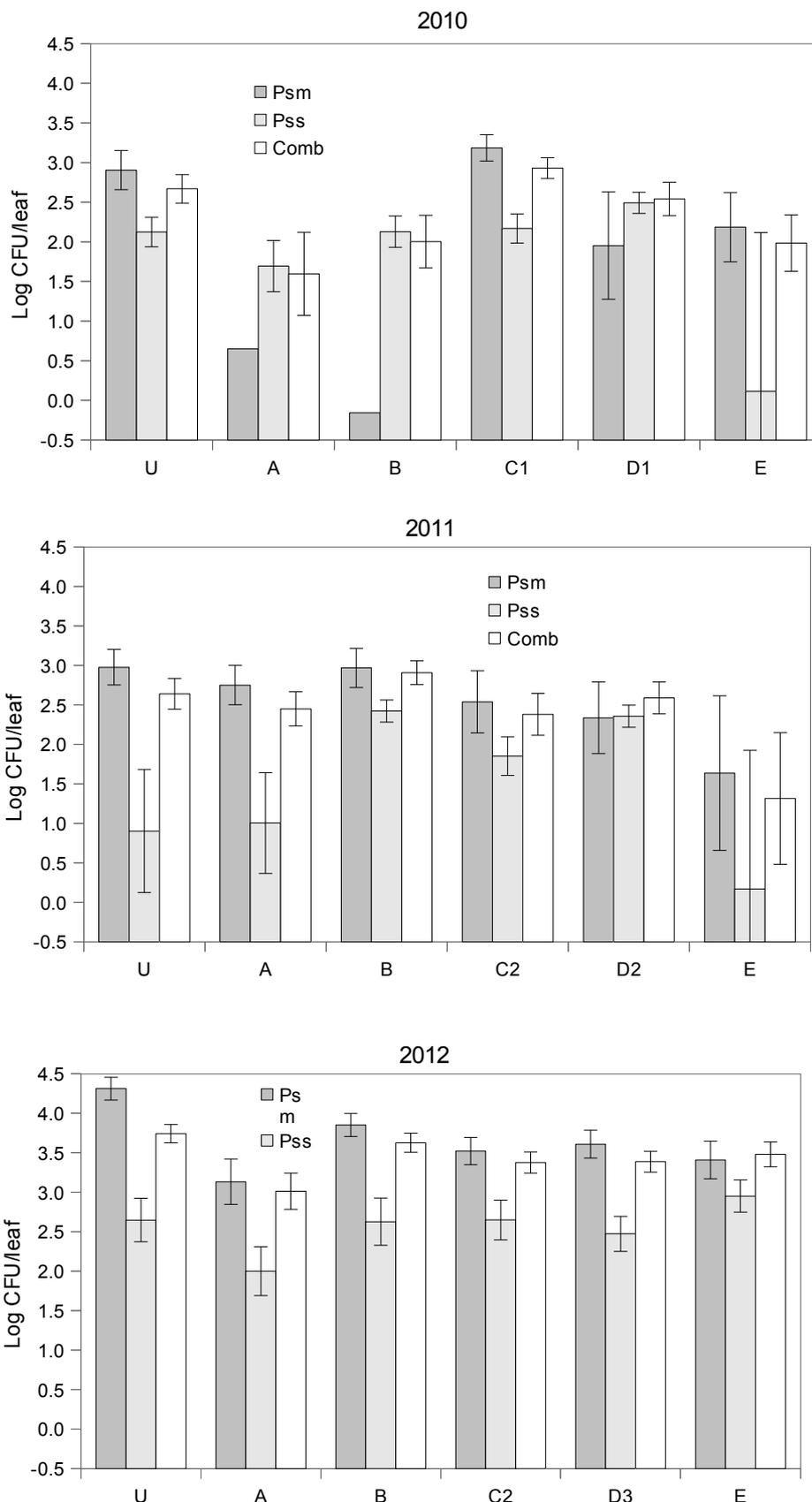
(Cuprokyt + Dithane NT) gave a significant reduction; in 2012 treatments A, D3 and E gave significant reductions (Fig. 7).

### *Pss*

Site, host x site, month, year x month, production stage, treatment, and several treatment interaction terms had significant effects on mean number of *Pss* per leaf (Appendix III). The host x site interaction term indicated that numbers were greater on plum at site 2 (3.0 Log<sub>10</sub> CFU/leaf) than on plum at site 1 and cherries at both sites (1.9 to 2 Log<sub>10</sub> CFU/leaf). Factors associated with sampling date (month, year x month) indicated a tendency for populations to dip in the summer compared to spring and autumn (1.9 vs. 2.4 and 3.2 Log<sub>10</sub> CFU/leaf). Production stage also had significant effect with greater numbers on mother-plants (2.3 Log<sub>10</sub> CFU/leaf) and maidens (2.4 Log<sub>10</sub> CFU/leaf) than on root-stocks (1.4 Log<sub>10</sub> CFU/leaf) and and stock hedges (0.5 Log<sub>10</sub> CFU/leaf).

### *Combined*

Host, year, month, production stage, treatment and several treatment interaction terms had significant effects on the mean number of *Psm* and *Pss* combined (Appendix III). Numbers were 15X greater on plum (3.1 Log<sub>10</sub> CFU/leaf) than on cherry (1.9 Log<sub>10</sub> CFU/leaf). Numbers were 10X greater in 2012 (3.5 Log<sub>10</sub> CFU/leaf) than in 2010 (2.5 Log<sub>10</sub> CFU/leaf) and 2011 (2.5 Log<sub>10</sub> CFU/leaf). Numbers were greatest in spring (3.1 Log<sub>10</sub> CFU/leaf) and lowest in the summer (2.2 Log<sub>10</sub> CFU/leaf). Numbers were greater on motherplants (3.2 Log<sub>10</sub> CFU/leaf) than on stock hedges (2.1 Log<sub>10</sub> CFU/leaf), root-stocks (1.7 Log<sub>10</sub> CFU/leaf) and maidens (2.2 Log<sub>10</sub> CFU/leaf). The treatment interactions terms made interpretation of effects difficult. Overall treatments A, C2, D3 and E gave significant reductions compared to the untreated control, with the greatest reduction with treatment A (Fig 6). The treatment x site interaction suggested that there were no significant reductions at site 2. The treatment x production stage interaction indicated variation in relative efficacy on the different production stages: e.g. treatment E was better than A on mother plants and root-stocks, A was better than E on maidens and stock hedges. The year x site x treatment interaction was mainly a result of the lack of control by any treatments at site 2, particularly in 2012.

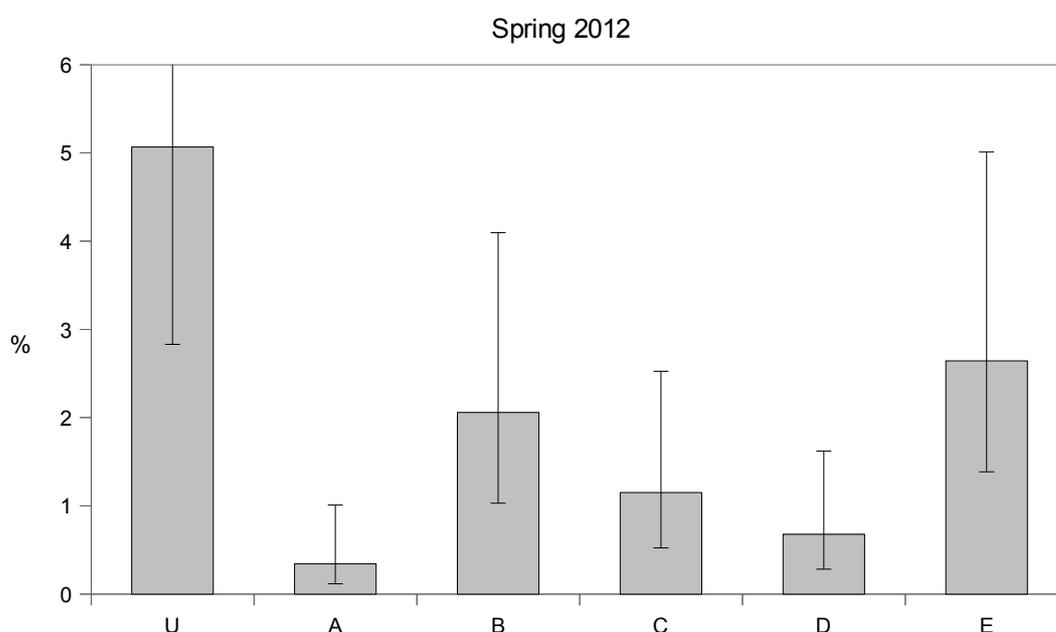


**Figure 7.** Effects of treatments on the log<sub>10</sub> of the mean number of *Pseudomonas syringae* pv. *morsprunorum* (*Psm*, plum only), *P. s.* pv. *syringae* (*Pss*), and either or both (*Comb*) for each year. Note that the scale is a log<sub>10</sub> scale, so that a unit represents a tenfold difference in bacterial numbers. Bars represent the standard errors of the means.

Overall, treatment A (Cuprokyt + wetter) was the only treatment that gave a significant reduction compared to the untreated control (Fig. 6). The host x treatment interactions suggested that other treatments (C2, D2, D3,E) gave significant reductions on cherry but not on plum. The year x treatment interaction indicated a reduction by A in 2010, no reductions in 2011 and A in 2012 (Fig. 7). The treatment x site interaction suggested that there were no significant reductions at site 2, and only A at site 1

### **Disease assessment**

No canker symptoms were seen at either site, and most of the die-back observed was in plums at site 1. Statistical analysis indicated a significant effect of treatment (See Appendix VI). Results are summarised in Figure 8. Treatments A (Cuprokyt + wetter) and D (Aliette 80WG in 2010, Cuprokyt + Dithane NT + wetter in 2011) gave the largest reductions compared to the untreated control, treatment C (Bactime Cu L4F in 2010, alternating Cuprokyt and Serenade ASO in 2011) also gave a significant reduction.



**Figure 8.** Effect of treatments on the percentage of trees with die-back in spring 2012. Error bars represent 95% confidence intervals.

### **Disinfection of tools**

The results are summarised in Table 4. A total of nine rounds of testing were done on separate occasions (three in 2010 and six in 2011). All the testing done in 2011 was additional to the originally planned work in the project. Some of the treatments were modified according to the results of the previous round.

Analysis of deviance (Appendix V) indicated that inoculum dose (i.e. numbers of bacteria) and the disinfection method were the most significant factors affecting disinfection efficiency, with forced drying (fan) also significant. All of the disinfection treatments reduced the number of cuts with growth compared to the control, although the brief dips in chlorine or Jet 5 disinfectants, and rubbing the blade with an alcohol gel were less effective than the others.

**Table 4.** Summary of disinfection tests. Each replicate consisted of ten sequential cuts following disinfection of the contaminated blade. The percentage is the number of cuts giving bacterial growth: the lower the % the better the treatment.

Code	Detail	Replicates	% cuts ( $5 \times 10^7$ ) <sup>a</sup>	% cuts ( $1 \times 10^6$ ) <sup>b</sup>
U	Untreated control	20	99.9	99.3
SW	Spray with 70% iso-propanol, leave 30 s then wipe dry with paper towel.	20	16.9	0.8
SW2	Spray with 70% iso-propanol, wipe residue, repeat spray leave 30 s then wipe dry.	3	1.1	0.0
W	Wipe with Azo wipes (70% iso-propanol).	8	8.6	0.4
J5_0	Brief dip in Jet 5 (0.8%) then wipe dry	19	48.2	3.4
J5_15	15 s dip in Jet 5 (0.8%) then wipe dry	6	0.0	0.0
J5_30	30 s dip in Jet 5 (0.8%) then wipe dry	7	0.3	0.0
Cl_0	Brief dip in 1% chlorine then wipe dry	7	24.4	1.2
Cl_30	30 s dip in 1% chlorine	1	0.0	0.0
GW	Rub edge of blade with alcohol hand gel between finger and thumb, wipe dry	11	51.1	3.8

<sup>a</sup> Predicted % cuts with growth, adjusted to a standard inoculum concentration of  $5 \times 10^7$  CFU/mL  
<sup>b</sup> Predicted % cuts with growth, adjusted to a standard inoculum concentration of  $1 \times 10^6$  CFU/mL

## Review of the literature on control of bacterial canker

A bibliographic search was made of the scientific literature using Google Scholar and the following search terms: "bacterial canker" Prunus and control and *Pseudomonas*. This generated around 603 results. Many of the resulting papers only *referred* to control rather than being *about* control. A search was also made with same terms using Google in order to identify more popular articles that are not part of the 'scholarly literature'; this did not provide any new insights and mostly found articles repeating the same general information. A selected summary of key articles are shown in the following sections and tables (5-8). A general conclusion from all the literature is that control of bacterial canker is difficult.

The strategies to control bacterial canker (or any plant disease) can be summarised under four main headings: avoidance, cultural controls, resistance, chemical/biological control.

### Avoidance

In practical terms, a disease avoidance strategy for bacterial canker would mean the production of pathogen-free planting material on pathogen-free sites. No information was found on this aspect.

### Cultural control

There were a number of papers describing attempts at control through cultural practices, examining three main approaches: plant nutrition, soil pH, grafting systems (Table 5). Most of the work has been done in the USA, and with trees infected with *Pss*. The work on nutrition suggests poor nutrition may lead to increased susceptibility to *Pss*. For pH, trees were more susceptible to disease, or the symptoms caused by *Pss* were more severe, when pH was relatively low. Several studies demonstrated that grafting high (>1 m) reduced

losses, presumably because the rootstocks were less susceptible to disease than the scions.

**Table 5.** Selected literature examining the effects of cultural practices on bacterial canker.

Authors	Factors examined	Crop	Pathogen <sup>1</sup>	Location	Main conclusion(s)
(Spotts <i>et al.</i> 1990)	N	Cherry	<i>Pss</i>	Oregon, USA	No effect
(Southwick <i>et al.</i> 1997)	N	Plum	<i>Pss</i>	California, USA	Low N increased canker
(Sayler & Kirkpatrick 2003)	Biannual N-P-K + micronutrients	Plum	<i>Pss</i> ?	California, USA	Reduced disease severity
(Cao <i>et al.</i> 2011)	N,P,K,Ca,Mg,Fe	Peach	<i>Pss</i>	California, USA	Mineral nutrients play only a minor role
(Weaver 1975)	Soil pH	Peach	<i>Pss</i>	Georgia, USA	Susceptibility reduced at pH ≥ 6.4
(Melakeberhan <i>et al.</i> 2000)	Soil pH	Cherry	<i>Pss</i>	Michigan, USA	Low soil pH pre-disposed seedlings to <i>Pss</i>
(Grubb 1944)	Graft height	Cherry	<i>Psm</i>	East Malling	Less disease in high -worked trees
(Schofield & Clift 1959)	Graft height	Plum	<i>Psm</i> ?	Midlands	Losses reduced on high-worked trees
(Prunier <i>et al.</i> 1997)	Graft height	Apricot	<i>Pss</i>	France	High grafted were more resistant
(Sayler <i>et al.</i> 2002)	Graft height	Plum	<i>Pss</i>	California, USA	Incidence and mortality reduced with high-budding

<sup>1</sup> A '?' is used to indicated that the pathogen was not clearly identified in the paper.

### **Resistance and rootstocks**

Summaries of selected literature examining resistance to bacterial canker can be found in Table 6 and the influence of rootstocks in Table 7. A number of studies have reported differences in susceptibility, and there have been a number of attempts at selecting both resistant scions and resistant rootstocks. In the USA only *Pss* has been considered. Methods have varied from study to study, sometimes relying on natural infection, sometimes inoculation via wounds or via leaf scars. Results from some studies have been conflicting: e.g. (Krzyszewska 1990) found F12/1 to be resistant to *Pss* whereas (Vicente & Roberts 2003) found it to be just as susceptible as other lines, some of this discrepancy may be due to differences in test methods. There is also a report of opposite results for *Pss* and *Psm* within a single study (Allen & Dirks 1978). A major problem in resistance breeding has been how to define and assess resistance and which pathogen strains to use, and this

was one of the main foci of the MAFF-funded work led by the author (Roberts & Vicente 2002).

One study (Theiler-Hedtrich 1994) indicated that bacterial canker susceptibility in sweet cherry was evenly distributed in seedlings from crosses with both a highly resistant and susceptible parents, suggesting a lack of heritability.

<b>Table 6.</b> Selected literature examining resistance to bacterial canker.					
<b>Authors</b>	<b>Crop</b>	<b>Pathogen(s)<sup>1</sup></b>	<b>Less susceptible</b>	<b>More susceptible</b>	<b>Location</b>
(Wilson 1933)	Cherry	<i>Pss</i> ?	Black Tartarian	Lambert, Napoleon	California, USA
(Grubb 1944)	Cherry	<i>Psm</i> ?	Many, Roundel most resistant	Napoleon and many others.	East Malling
(Vries 1965)	Cherry	<i>Pss</i> ?	<i>P. avium</i> x <i>P. cerasus</i>	<i>P. avium</i>	Netherlands
(Allen & Dirks 1978)	Cherry	<i>Psm</i>	Windsor, Victor	Vic, Hedelfingen, Bing	Canada
(Allen & Dirks 1978)	Cherry	<i>Pss</i>	Hedelfingen, Bing	Viva, Venus, Vega, Victor	Canada
(Theiler-Hedtrich 1985)	Cherry		12/1200 seedlings. 4/29 cultivars.		Switzerland
(Garrett 1986)	Cherry	<i>Psm, Pss</i>	Jl 14039	Napoleon	EMRS
(Young 1987)	Cherry	<i>Pss</i>	Dawson	Bing	NZ
(Krzyszewska 1990)	Cherry	<i>Pss</i>	F12/1	Napoleon, Corum	Oregon
(Scortichini <i>et al.</i> 1995)	Wild cherry	<i>Psm</i>	Alpe 2	Montemignaio, Piantata Catenaia, Puzzolo, Raggiolo, Pozzacce 2, Pozzacce I, Paradision	Italy
(Vicente & Roberts 2003)	Cherry (mostly wild)	<i>Psm</i>	FD1-57-4/166 (race 1), Cobtree (race 2)		UK (lab)
(Vicente & Roberts 2003)	Cherry (mostly wild)	<i>Pss</i>	None	All 19 (including F12/1)	UK (lab)
(Santi <i>et al.</i> 2004)	Wild cherry	<i>Pss, Psm, Psp, Psa</i>	Inconsistent results		France
(Thomidis & Exadaktylou 2008)	Cherry	<i>Pss</i>	None	All 30	Greece (lab)
(Spotts <i>et al.</i> 2010)	Cherry	<i>Pss</i>	Regina, Rainier	Bing, Sweetheart	Oregon, USA

<sup>1</sup> A '?' is used to indicate that the pathogen was not clearly identified in the paper.

**Table 7.** Selected literature examining the influence of rootstocks on bacterial canker.

Authors	Crop(s)	Pathogen(s) <sup>1</sup>	Results/conclusions	Location
(Wormald 1934)	Plum	<i>Psm</i>	Victoria very susceptible on Brussels, Brompton, Common Plum, or Myrobalan B	East Malling
(Wilson 1953)	Cherry	<i>Pss</i> ?	F1-1, F5-5, F12-1 induce resistance in scion	California, USA
(Montgomery <i>et al.</i> 1943)	Plum	<i>Psm</i>	Victoria on Myrobalan B, Warwickshire Drooper, Utility prevented stem canker	East Malling
(Garrett 1986)	Cherry	<i>Psm, Pss</i>	No effect	East Malling
(Sayler <i>et al.</i> 2002)	Plum	<i>Pss</i>	Lovell peach reduced incidence and mortality	California, USA
(Spotts <i>et al.</i> 2010)	Cherry	<i>Pss</i>	Gisella 6 90% dead at 3 yrs, Colt 0%	Oregon, USA

<sup>1</sup> A '?' is used to indicated that the pathogen was not clearly identified in the paper.

### **Chemical control**

Selected papers on chemical control are summarised in Table 8. Copper compounds have been the most widely used and there are a number of reports of successful control with several applications during the growing season and variable timings. In two recent reports where control was not successful (Sayler & Kirkpatrick 2003; Renick *et al.* 2008), sprays were applied mainly during the dormant season. Copper resistance has been reported in *Pss* from cherry (Sundin *et al.* 1989), but was not detected in *Psm* in the same study. Also the resistance, carried on a plasmid, could be transferred from copper-resistant to copper-sensitive strains of *Pss*, but not from *Pss* to *Psm* (Sundin *et al.* 1989). The antibiotic Streptomycin has also been tested successfully, but it is unlikely that its use would ever be permitted in the UK, and where it has been used extensively resistance is likely to develop (Scheck *et al.* 1996)

**Table 8.** Selected literature on chemical control of bacterial canker.

Authors	Crop	Pathogen(s) <sup>1</sup>	Compounds	No. and timing of sprays	Results	Location
(Montgomery <i>et al.</i> 1943)	Plum	<i>Psm</i>	Bordeaux mixture	3 weeks after petal fall	appreciable control of shot-hole and branch cankers, but not stem cankers	East Malling
(Moore 1946)	Cherry	<i>Psm</i>	Bordeaux mixture	autumn and spring	promising control	East Malling
(Crosse & Bennett 1957)	Cherry	<i>Psm</i>	Streptomycin	2 blossom, 2 autumn	~90% reduction in leaf infections, 50 to 67% reduction in branch infections	East Malling
(Boyd & Paton 1958)	Plum	<i>Psm</i> ?	Streptomycin paint	Brushed onto bark at 3 mo. intervals	Marked reduction in incidence of stem cankers	Edinburgh
(Crosse & Bennett 1959)	Cherry	<i>Psm</i>	Bordeaux mixture	Aug, Sept, Oct	>90% reduction	East Malling
(Olson & Jones 1983)	Sour cherry	<i>Psm</i>	Tri-basic copper sulphate (TBS), copper salts of fatty acids (Citcop 4E)	7-9 sprays at 7-10 day intervals from April to July	Tri-basic copper gave significant reduction in populations.	Michigan, USA
(Wimalajeewa <i>et al.</i> 1991)	Apricot and cherry	<i>Pss</i>	copper hydroxide (apricot), Bordeaux mixture (cherry)	2 Autumn, 1 Winter, 2 pre-bloom	>67% reduction	Australia
(Sayler & Kirkpatrick 2003 p. 200)	Plum	<i>Pss</i>	Copper	throughout dormant season	no reduction	California, USA
(Renick <i>et al.</i> 2008)	Cherry	mainly <i>Pss</i>	copper sulphate, copper hydroxide	2 dormant, 1 reduced rate post-bloom	Inconsistent in reducing Ps populations	Michigan, USA

<sup>1</sup> A '?' is used to indicated that the pathogen was not clearly identified in the paper.

## Discussion

### ***Epidemiology and spray treatments***

These results represent a considerable amount of effort with around 750 sub-samples collected and processed over the three years. Analysis and interpretation of the data was difficult due to significant interaction effects (particularly for the spray treatments). These interaction effects indicate variable/inconsistent effects from year to year, between sites and at different production stages. Unlike the analysis in the first two years, the analysis of *Pss* in this report is based on isolates that had been confirmed as pathogenic on lilac; only about a third of isolates identified as potential *Pss* on the basis of the cultural and biochemical tests (i.e. GATTa) were pathogenic on lilac, therefore some of the data summaries and conclusions presented in this report will differ from those in the previous years' reports. Previous studies have shown that not all isolates identified as potential *Pss* on the basis of cultural and biochemical tests are necessarily pathogenic on lilac (by definition *Pss* must be pathogenic on lilac). At the time of preparing the earlier reports, data on the pathogenicity of potential *Pss* isolates was not available, therefore analysis was based on the numbers of potential *Pss* detected rather than confirmed *Pss* as in this report. The results indicated a major difference in pathogen populations on the two hosts: both the proportion of leaves contaminated and the numbers of both *Psm* and *Pss* were greater on plums than on cherries. This was particularly the case for *Psm*, which was detected on cherries relatively rarely: only occasionally in 2010 and 2012, and not at all in 2011. Therefore, to avoid artefacts caused by excessive numbers of zeroes, analysis of *Psm* was restricted to plums.

In most of the analyses there were significant effects of sampling date and location (i.e. year, month, site and their interaction), suggesting that populations may change over relatively short-term periods, possibly driven by local weather factors (e.g. rainfall, temperatures) in the days or weeks prior to sampling. Overall, the proportions of contaminated/infected leaves and numbers of bacteria were highest in 2012 and lowest in 2010, possibly a result of the much higher rainfall experienced at both sites in 2012 (Table 9). Populations of *Psm* tended to be highest in the spring, but proportions of leaves/buds contaminated were highest in the summer. Numbers of *Pss* followed a different pattern and apparently dipped in the summer. The relatively high levels of *Psm* detected in the summer contradicts conventional wisdom that numbers decline in the summer. However, it is important to consider that these apparent peaks may represent short term fluctuations in populations as a result of the environmental conditions immediately before each sampling date, i.e. there may have been as much fluctuation in populations in the periods between sampling dates as between individual sampling dates.

<b>Table 9.</b> Total rainfall and average temperature April to October for each year and each site*.				
Year	Site 1		Site 2	
	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)
2010	345	12.8	331	13.5
2011	233	13.7	401	14.2
2012	646	12.1	729	13.2

\*Data obtained from nearest official Meteorological Office station to each site.

Production stage consistently had significant effects on pathogen levels, with a general tendency for higher proportions and numbers on motherplants than at other stages, particularly in plums. The blocks of motherplants selected for these trials were intentionally relatively old, with the expectation that they might have high inoculum levels and therefore demonstrate clearer differences between treatments.

Interpreting the effects of the spray treatments was complicated by the presence of relatively significant interaction terms in some analyses: year, site and production stage for *Psm* and combined; year and site for *Pss* counts. These interaction terms are indicative of a variable level of control by the different treatments. The most notable interaction being a general lack of efficacy from any treatments at site 2. There is no obvious explanation for this difference between sites, and we might speculate that it could be due to one or more of: differences in timing of spray applications in relation to local weather; differences in application efficiency; differences in disease levels in surrounding crops; differences in the underlying infection vs. contamination levels (inoculum within the leaf will be less sensitive to sprays than inoculum on the leaf surfaces); differences between pathogen populations at the two sites and their resistance to copper. Nevertheless treatment A (Cuprokyt + wetter) gave the most consistent reductions in pathogen levels throughout the three years. Treatment A also resulted in the lowest levels of die-back on production trees assessed in spring 2012. Other treatments that contained Cuprokyt also gave significant reductions, particularly treatment E (Cuprokyt + Dithane NT), but overall none were significantly better than A. Thus, the main conclusion is that Cuprokyt + wetter was usually effective in reducing levels of bacterial canker pathogens, and there was no advantage of mixing it with Dithane, or using a sticker (Nu-Film P) rather than a wetter (Activator 90), or alternating with Serenade ASO.

We included several novel treatments for the control of bacterial canker, either as alternatives to copper or to improve its efficacy. The biological control agent Serenade has claimed benefits against bacterial canker (US label), and although it looked very promising in 2010, failed to live up to that promise in subsequent years. Possibly control with Serenade ASO could be improved with more frequent applications throughout the growing season, as applications are permitted every seven days until harvest, although given the relatively high cost of Serenade, this would be difficult to justify based on these results. Bactime Cu LF4 was selected based on promising preliminary results against *Xanthomonas* on walnuts in Italy and was considered to have resistance inducing effects (i.e. systemic

acquired resistance); pathogen levels were worse than the untreated control and combined with grower distaste for using it (very strong smell) was abandoned. Aliette 80WG showed some promise in 2010, but was abandoned due to pending withdrawal of the product from the market due to changes in legislation.

The current general recommendation for control of bacterial canker in the UK (as in the label recommendation for Cuprokyt) is to apply three sprays from late summer to autumn. It is likely that this is based on work at East Malling in the 1950s (Crosse & Bennett 1959) with the idea of reducing the populations on the leaves and so reduce the likelihood of infection via leaf scars which would lead to canker. Presumably, also on the basis that leaf infection in spring and summer was of little consequence. In other work (see Table 8) spray applications have been used at other times of the year, including during the dormant period and in early spring. All of the previous work on chemical control of bacterial canker has been targeted at orchard trees for fruit production. This project was targeted at nursery production with the underlying approach being to try and minimise inoculum levels at all stages of production, and attempt to keep newly budded trees and maidens as free from inoculum as possible. Thus, the approach to spray timing in this project was based on the aim of trying to (a) minimise the build-up of inoculum in the spring and thus prevent leaf and shoot infections, (b) minimise transfer of inoculum from mother-plants during budding, (c) minimise the potential for infection during the budding process, (d) minimise inoculum levels in the autumn to prevent leaf-scar infections. During the three years of this project high levels of one or other of the pathogens could be found at any time during the season, and *Psm* levels in particular seemed to peak in spring or summer rather than autumn. Thus, the approach to spray timing in this project would seem to be justified. However, as there were no direct comparisons of the impact of different timings in this project, definitive conclusions cannot be made and further work to investigate different spray timings may be warranted. It is also possible that more effective control could be achieved with the development of a 'forecasting' system based on a model for the development and spread of pathogen populations in relation to weather parameters.

It is also important to consider that continuing dependence on only one product for control of bacterial canker increases the likelihood of resistance developing (if not already at site 2). Therefore it is essential that other approaches to control are developed. The literature review suggests that whilst there may be some potential to select for resistance to *Psm*, this seems less likely for *Pss*. Disease avoidance through the use of tested/indexed high health starting material, possibly produced through micro-propagation, and grown on under protection is one approach that has not been tested, and its success would depend on the rate of re-contamination.

The trees used for the spray trials in this project were generally surrounded by much larger blocks of trees which were untreated, there was also little separation between 'plots'. This means that there is a high likelihood of interference between 'plots' and a constant influx of inoculum to the trees in the trial. Therefore it is likely that these trials will have under-estimated the effect of any treatments which were successful in reducing the levels of bacterial canker pathogens, and we would expect to see more effective control if sprays are

applied consistently to all susceptible species at the whole nursery level than seen in these trials.

### **Disinfection of tools**

During the first rounds of testing done in 2010, we failed to identify a practical option for disinfection in the field. It was considered that the test system as implemented was a stringent test and perhaps more stringent than would occur in practice due to the relatively high numbers of the pathogen and the partial drying of the inoculum onto the blade. Given the wider potential importance of disinfection of pruning tools, further experiments were done in 2011 with lower inoculum concentrations and shorter drying times.

At lower inoculum doses and with shorter drying times, the efficacy of all treatments improved, and all gave significant reductions in pathogen transfer compared to the untreated control. Conversely, the level of disinfection achieved was reduced as inoculum increased and when drying was fan-assisted. Although long (30 s) dips in disinfectants (chlorine or Jet 5) were the most effective, these are not practical to implement in the field. Hence, whilst not the most effective when bacterial inoculum levels are high or when it is dried on, regular use of disinfectant wipes (impregnated with 70% iso-propanol as the active ingredient) are probably the most practical option for use in the field. The Azo Hard Surface Wipes used in the tests and similar products are readily obtained from a number of suppliers, especially medical and clean-room suppliers. In addition because such an approach is easily implemented and so more likely to be applied, it seems likely that the benefits of more frequent use may outweigh the lower efficiency compared to other methods.

Notably during the testing we also made up to 50 cuts with the untreated control blades without any apparent reduction in bacterial growth on the test plates.

### **Conclusions**

- Levels of *Psm* and *Pss* were reduced by sprays containing Cuprokylt.
- The most consistent effects were obtained with Cuprokylt plus a wetter (Activator 90).
- There was no consistent benefit from mixing Cuprokylt with Dithane NT compared to Cuprokylt plus wetter.
- There was no benefit from using a sticker (Nu-Film P) rather than wetter (Activator 90),
- There was no consistent benefit from using Serenade ASO alone or alternating Serenade ASO and Cuprokylt compared to Cuprokylt alone.
- We would expect to see better control at the whole nursery level than in these trials due to high likelihood of interference between 'plots' and constant influx of inoculum.
- Levels of both *Psm* and *Pss*, but especially *Psm* were greater on plum than on cherry.

- The overall levels of pathogens varied from year to year, and levels of *Psm* tended to be higher in spring and summer.
- A practical approach to disinfection of pruning tools during field operations using disinfectant wipes such as 'Azo Wipes' has identified.

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## Knowledge and Technology Transfer

The disinfection results have been reported to herbaceous growers at the HPTDG in Feb 2012.

Article in HDC News February 2013 on disinfection of pruning tools.

An HDC Fact-sheet will be published in 2013.

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# Appendix I

Site	Treat	Species	Cultivar	Stage	2010			2011			2012		
					May	Aug	Oct	June	Aug	Oct	May	Aug	Oct
1	A	instita	SJ	SH	nd		Pss	nd		nd			
	B				nd		Pss	nd		Pss			
	C				Psm		Psm	Pss		Psm			
	D				Psm		Psm	nd		Psm			
	E				Psm		Psm	Psm + Pss		Pss			
	U				Psm		Psm	Psm		Psm			
	A	avium	Colt	SH	Ps		nd	nd		nd			
	B				Pss		Pss	Pss		nd			
	C				Ps		nd	Pss		nd			
	D				Psm		nd	Pss		nd			
	E				Psm		nd	Pss		nd			
	U				Psm		Pss	Pss		Pss			
	A	domestica	Vic	MP/Bud	nd	Psm		Psm + P;	Psm + Pss		Psm	Psm	Psm
	B				nd	Pss		Psm + P;	Psm + Pss		Psm	Psm + P;	Psm
	C				Psm	Psm		Pss	Psm		Psm	Psm + P;	Pss
	D				Pss	Pss		Pss?	Psm + Pss		Psm	Psm + P;	Psm + P;
	E				Ps	Pss		Psm + P;	Psm		Pss	Psm + P;	Psm + P;
	U				Ps	Psm		Psm	Psm + Pss		Psm + P;	Psm	Psm + P;
	A	avium	Stella	MP/Bud	nd	nd		nd	Pss?				
	B				Pss	nd		nd	Pss				
	C				Pss	nd		Pss	Pss				
	D				Ps	nd		Pss	Pss				
	E				Ps	nd		Pss	Pss				
	U				Pss	nd		Pss	Pss				
A	serrulata	Kiku	MP/Bud		nd			Pss					
B					nd			Pss					
C					Pss			nd					
D					Pss			Pss					
E					nd			Pss					
U					Pss			Pss					
A	instita	SJ	RS2 (2010)	nd	Ps		Psm	Pss	Psm + P;	(Pss)		Psm + P;	Psm + P;
B				nd	Pss		Psm	Psm	Pss	Psm + P;	Psm + P;	Pss	
C				Psm	Pss		Psm	Pss	Pss	Psm + P;	Psm	Pss	
D				nd	Pss		Psm	Psm + P;	Pss	Psm + P;	Psm + P;	Pss	
E				nd	Pss		Pss	nd		Psm + P;	Psm + P;	Psm + P;	
U				Psm	Psm + P;	Pss	Psm + P;	Psm + P;	Psm + P;	Psm + P;	Psm	Psm + P;	
A	avium	Colt	RS2 (2010)	Pss	Pss		nd	nd	nd	nd	nd	nd	
B				Psm	Pss		nd	nd	Pss	nd	Pss		
C				nd	Pss		Pss	nd	nd	Pss	Pss	Pss	
D				nd	Pss		Pss	nd	nd	nd	Pss	nd	
E				nd	Pss		nd	nd	nd	Pss	Pss	Psm	
U				nd	Pss		Pss	nd	nd	(Pss)	nd	Psm	
A	instita	SJ	RS1 (2011)				Pss		Pss				
B							Psm		Psm + Pss				
C							Psm		nd				
D							Psm + Pss		Psm + Pss				
E							Pss		Psm				
U							Psm	Psm	Pss				
A	avium	Colt	RS1 (2011)				Pss		nd	Pss	nd	nd	
B							nd		nd	nd	Pss	Pss	
C							nd		nd	(Pss)	nd	nd	
D							nd		nd	(Pss)	nd	Pss	
E							nd		nd	nd	nd	nd	
U							nd		nd	nd	Pss	nd	Pss
2	A	instita	SJ	RS2 (2010)	nd	nd	Ps	nd	Psm	Pss	Psm + P;	Psm + P;	Pss
	B				nd	nd	Pss	nd	Psm	Pss	Psm + P;	Psm	Psm + P;
	C				Psm	Psm	Pss	nd	Psm	Pss	Psm + P;	Psm + P;	Pss
	D					nd	Pss	Pss	Psm	Pss	Psm + P;	Pss	Psm + P;
	E				nd	nd	Pss	nd	Psm + P;	Pss	Psm + P;	Psm + P;	Pss
	U				nd	Ps	Pss	nd	Psm + P;	Pss	Psm	Psm	Psm + P;
	A	avium	Colt	RS2 (2010)	nd	nd	Pss	nd	nd	Pss	Pss	Pss	Psm + P;
	B				Pss	nd	Pss	nd	Pss	Pss	nd	Pss	Psm + P;
	C				nd	nd	Psm + P;	nd	nd	Pss	nd	Pss	nd
	D				Pss	Pss	Ps	nd	nd	Pss	nd	(Pss)	nd
	E				nd	Pss	Pss	nd	nd	Pss	Pss	nd	nd
	U				Pss	Pss	Pss	nd	Pss	Pss	nd	Pss	Pss

## Appendix II

Abbreviations used in analyses of deviance tables:

Stage2 – stage in production, i.e. mother-plants, stock-hedges, rootstocks, maidens.

Treat2 – spray treatment.

Med – medium, i.e. the agar medium used for dilution plating (MS3 or mP3).

Samp – the individual sample.

The tables show the raw output from Genstat, with asterisks marking those terms considered most important/significant based on the relative values for mean deviance. The nature of the models means that there are no absolute significance tests, so ratios and significance values should be taken as a guide only.

### Summaries of analyses of deviance for proportions of leaves

*Psm* proportions, Plum only

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Site	1	1.2212	1.2212	0.07	0.794
+ Year	2	66.5437	33.2718	1.86	0.156*
+ Month	2	47.0010	23.5005	1.31	0.269*
+ Year.Month	4	38.2315	9.5579	0.53	0.711
+ Site.Year	2	9.1404	4.5702	0.26	0.775
+ Site.Month	2	12.5734	6.2867	0.35	0.704
+ Site.Year.Month	4	71.6667	17.9167	1.00	0.406
+ Stage2	3	20.5318	6.8439	6.84	<.001**
+ Treat2	8	49.5424	6.1928	6.19	<.001**
+ Year.Stage2	3	26.5454	8.8485	8.85	<.001**
+ Year.Treat2	7	41.9223	5.9889	5.99	<.001**
+ Year.Month.Treat2	30	93.3149	3.1105	3.11	<.001
+ Site.Treat2	8	34.4633	4.3079	4.31	<.001
+ Stage2.Treat2	20	66.3125	3.3156	3.32	<.001
+ Year.Stage2.Treat2	10	28.8249	2.8825	2.88	0.001
+ Samp.Med	253	163.9595	0.6481	0.65	1.000
Residual	333	256.1697	0.7693		
Total	692	1027.9646	1.4855		

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Pss proportions:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	29.1604	29.1604	4.08	0.043*
+ Site	1	36.9529	36.9529	5.17	0.023*
+ Year	2	86.9771	43.4885	6.08	0.002**
+ Month	2	9.2914	4.6457	0.65	0.522
+ Year.Month	4	18.5921	4.6480	0.65	0.627
+ Site.Year	2	1.2655	0.6328	0.09	0.915
+ Site.Month	2	13.0885	6.5443	0.92	0.400
+ Host.Site	1	13.9843	13.9843	1.96	0.162
+ Site.Year.Month	3	21.4460	7.1487	1.00	0.392
+ Stage2	3	35.6341	11.8780	11.88	<.001**
+ Host.Stage2	3	8.1161	2.7054	2.71	0.044
+ Year.Stage2	4	1.3879	0.3470	0.35	0.846
+ Treat2	8	21.5632	2.6954	2.70	0.006 *
+ Year.Treat2	7	13.6755	1.9536	1.95	0.057
+ Host.Treat2	8	33.1709	4.1464	4.15	<.001**
+ Site.Treat2	8	23.1720	2.8965	2.90	0.003 *
+ Stage2.Treat2	21	37.8596	1.8028	1.80	0.013
+ Samp.Med	622	504.9328	0.8118	0.81	1.000
Residual	609	217.4910	0.3571		
Total	1311	1127.7612	0.8602		

Combined proportions:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	301.2945	301.2945	37.38	<.001**
+ Site	1	0.6878	0.6878	0.09	0.770
+ Year	2	197.1048	98.5524	12.23	<.001**
+ Month	2	13.8580	6.9290	0.86	0.423
+ Year.Month	4	25.5727	6.3932	0.79	0.529
+ Site.Year	2	13.4308	6.7154	0.83	0.435
+ Site.Month	2	14.3833	7.1917	0.89	0.410
+ Host.Site	1	1.7788	1.7788	0.22	0.639
+ Site.Year.Month	4	32.2569	8.0642	1.00	0.406
+ Stage2	3	42.0668	14.0223	14.02	<.001**
+ Host.Stage2	3	20.5848	6.8616	6.86	<.001**
+ Year.Stage2	4	26.0028	6.5007	6.50	<.001**
+ Treat2	8	63.7712	7.9714	7.97	<.001**
+ Year.Treat2	7	34.2694	4.8956	4.90	<.001*
+ Host.Treat2	8	23.4039	2.9255	2.93	0.003
+ Site.Treat2	8	16.7859	2.0982	2.10	0.032
+ Stage2.Treat2	21	58.2677	2.7747	2.77	<.001
+ Samp.Med	664	702.0057	1.0572	1.06	0.149
Residual	692	305.7350	0.4418		
Total	1437	1893.2607	1.3175		

## Appendix III

### Summaries of analyses of deviance for counts of bacteria

To facilitate plotting of data and calculation of meaningful standard errors, a value of 0.1 was substituted for zero actual counts.

*Psm* counts, plum only:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Site	1	20250.	20250.	0.76	0.383
+ Year	2	1097188.	548594.	20.59	<.001**
+ Month	2	1399728.	699864.	26.27	<.001**
+ Site.Year	2	409018.	204509.	7.68	<.001*
+ Year.Month	4	337727.	84432.	3.17	0.013
+ Site.Month	2	10046.	5023.	0.19	0.828
+ Site.Year.Month	4	106564.	26641.	1.00	0.406
+ Stage2	3	55738.	18579.	2.76	0.042
+ Treat2	8	1169772.	146222.	21.73	<.001**
+ Year.Treat2	7	205340.	29334.	4.36	<.001
+ Site.Treat2	8	580149.	72519.	10.78	<.001*
+ Stage2.Treat2	20	1683456.	84173.	12.51	<.001*
+ Year.Stage2.Treat2	13	872584.	67122.	9.98	<.001*
+ Site.Year.Treat2	7	38410.	5487.	0.82	0.575
+ Samp.Med	275	1088172.	3957.	0.59	1.000
Residual	321	2159522.	6727.		
Total	679	11233664.	16544.		

*Pss* counts:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	66831.	66831.	5.17	0.023
+ Year	2	120341.	60170.	4.66	0.009
+ Site	1	146269.	146269.	11.32	<.001**
+ Month	2	163514.	81757.	6.33	0.002*
+ Year.Site	2	174615.	87308.	6.76	0.001*
+ Year.Month	4	214297.	53574.	4.15	0.002*
+ Site.Month	2	44666.	22333.	1.73	0.177
+ Host.Site	1	178159.	178159.	13.79	<.001**
+ Host.Site.Month	4	48727.	12182.	0.94	0.438
+ Host.Year.Month	6	109562.	18260.	1.41	0.205
+ Year.Site.Month	3	38754.	12918.	1.00	0.392
+ Stage2	3	226105.4	75368.5	32.71	<.001**
+ Host.Stage2	3	34502.3	11500.8	4.99	0.002
+ Treat2	8	334176.1	41772.0	18.13	<.001**
+ Year.Treat2	7	189538.8	27077.0	11.75	<.001*
+ Host.Treat2	8	801225.7	100153.2	43.46	<.001**
+ Site.Treat2	8	238180.8	29772.6	12.92	<.001*
+ Stage2.Treat2	21	62080.6	2956.2	1.28	0.173
+ Year.Site.Treat2	7	62783.2	8969.0	3.89	<.001
+ Year.Stage2.Treat2	18	25946.7	1441.5	0.63	0.883
+ Samp.Med	588	1355001.0	2304.4	1.00	0.492
Residual	587	508963.8	867.1		
Total	1286	5144239.9	4000.2		

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Combined counts:

Accumulated analysis of deviance

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Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	1277621.	1277621.	11.20	<.001**
+ Year	2	1969876.	984938.	8.63	<.001**
+ Site	1	61462.	61462.	0.54	0.463
+ Month	2	1150530.	575265.	5.04	0.006*
+ Year.Site	2	542244.	271122.	2.38	0.093
+ Year.Month	4	388576.	97144.	0.85	0.492
+ Site.Month	2	125949.	62974.	0.55	0.576
+ Host.Site	1	329529.	329529.	2.89	0.089
+ Host.Year	2	339497.	169749.	1.49	0.226
+ Host.Year.Month	6	102763.	17127.	0.15	0.989
+ Year.Site.Month	4	456320.	114080.	1.00	0.406
+ Stage2	3	432713.	144238.	43.59	<.001**
+ Host.Stage2	3	21443.	7148.	2.16	0.090
+ Treat2	8	1596694.	199587.	60.32	<.001**
+ Year.Treat2	7	288627.	41232.	12.46	<.001*
+ Host.Treat2	8	392223.	49028.	14.82	<.001*
+ Site.Treat2	8	541747.	67718.	20.46	<.001**
+ Stage2.Treat2	21	1679398.	79971.	24.17	<.001**
+ Year.Site.Treat2	7	484509.	69216.	20.92	<.001**
+ Year.Stage2.Treat2	18	863424.	47968.	14.50	<.001*
+ Samp.Med	631	2088287.	3309.	1.00	0.491
Residual	656	1611543.	2457.		
Total	1397	16744976.	11986.		

## Appendix IV

### Analysis of deviance for die-back

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Host	1	171.861	171.861	108.51	<.001*
+ Site	1	177.320	177.320	111.95	<.001*
+ Host.Site	1	60.408	60.408	38.14	<.001*
+ Treat	5	73.690	14.738	9.30	<.001*
Residual	33	52.268	1.584		
Total	41	535.548	13.062		

## Appendix V

Abbreviations used in the analysis:

logd – the natural log of the dose applied to the blade

Fan – whether or not a fan was used to increase the drying speed

Drying – the duration of drying

Method – the disinfection method

The tables show the raw output from Genstat, with asterisks marking those terms considered most important/significant based on the relative values for mean deviance. The nature of the models means that there are no absolute significance tests, so ratios and significance values should be taken as a guide only.

### Analysis of deviance for disinfection tests

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ logd	1	67.363	67.363	22.80	<.001**
+ Drying	2	1.074	0.537	0.18	0.834
+ Fan	1	21.828	21.828	7.39	0.008*
+ Method	9	1202.354	133.595	45.22	<.001**
+ Drying.Method	11	16.700	1.518	0.51	0.888
+ Fan.Method	5	34.749	6.950	2.35	0.050
+ Drying.Fan.Method	3	35.643	11.881	4.02	0.011
Residual	68	200.899	2.954		
Total	100	1580.609	15.806		

Fit logd + Method, Predictions

logd = 17.82

Method	p	LCL	UCL
C1_0	24.37	7.63	55.69
C1_30	0.00	0.00	100.00
GW	51.11	25.68	75.98
J5_0	48.19	30.06	66.80
J5_15	0.00	0.00	100.00
J5_30	0.25	0.00	11.49
SW	16.92	7.77	32.99
SW2	1.12	0.10	11.28
U	99.97	94.30	100.00
W	8.64	2.42	26.51

logd = 13.82

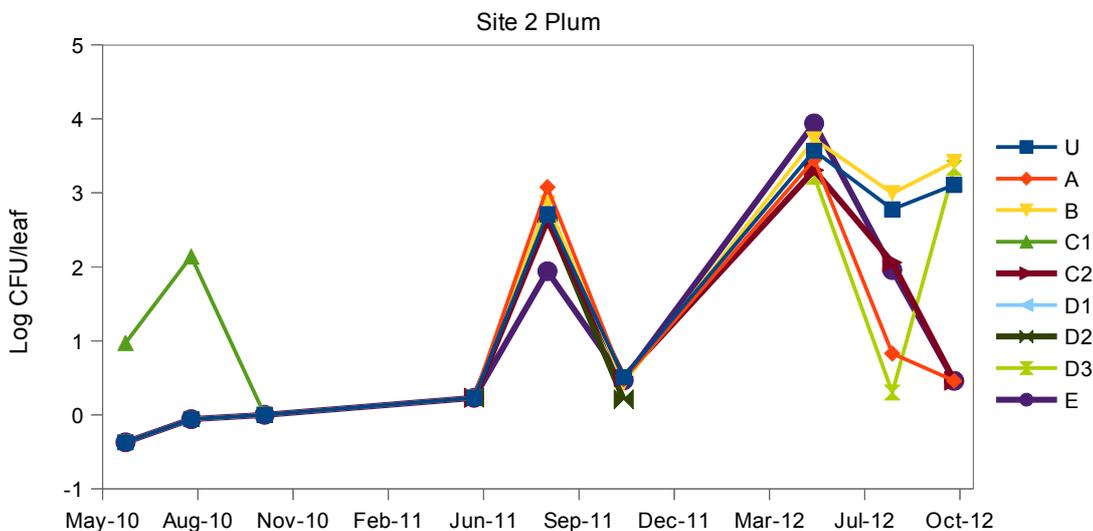
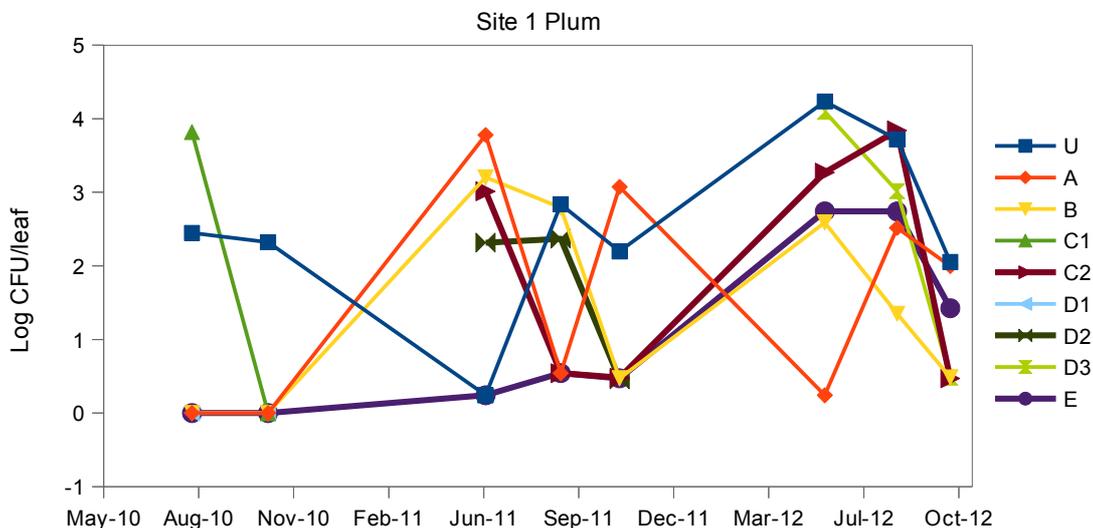
Method	p	LCL	UCL
C1_0	1.22	0.208	6.76
C1_30	0.00	0.000	100.00
GW	3.84	0.991	13.73
J5_0	3.43	1.102	10.17
J5_15	0.00	0.000	100.00
J5_30	0.01	0.000	0.64
SW	0.77	0.170	3.44
SW2	0.04	0.003	0.72
U	99.28	39.362	100.00
W	0.36	0.051	2.50

## Appendix VI

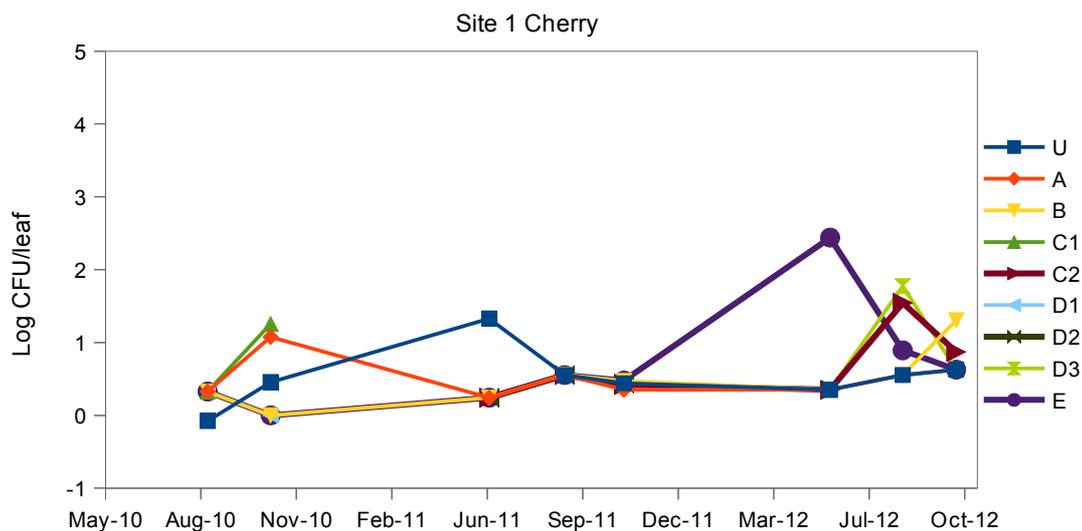
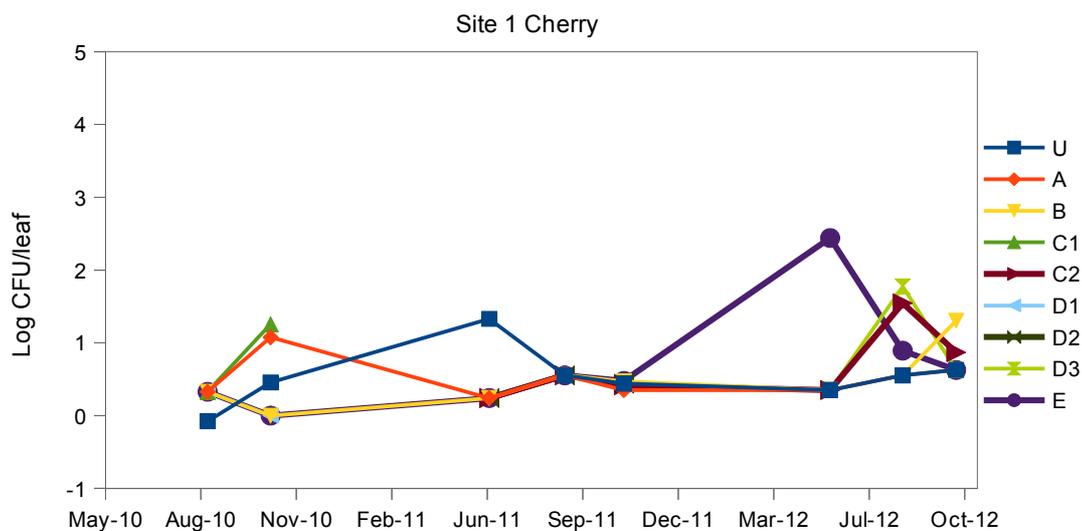
### Bacterial numbers on 'maidens' at each site

The following graphs show the estimated mean numbers of bacterial canker pathogens on plants that were sampled throughout all three years of the project at both sites. These plants were 2<sup>nd</sup> year rootstocks in 2010, budded in August 2010, and headed back in spring 2011. The values at each data point represent a weighted mean of the log<sub>10</sub> of the number of colony forming units (CFU) per leaf, based on just two sub-samples (one of 5 leaves and one of 35 leaves) and therefore should be interpreted with some caution. In addition, the lines joining points are provided to guide the eye and should not be taken to imply the numbers that were present between sampling dates.

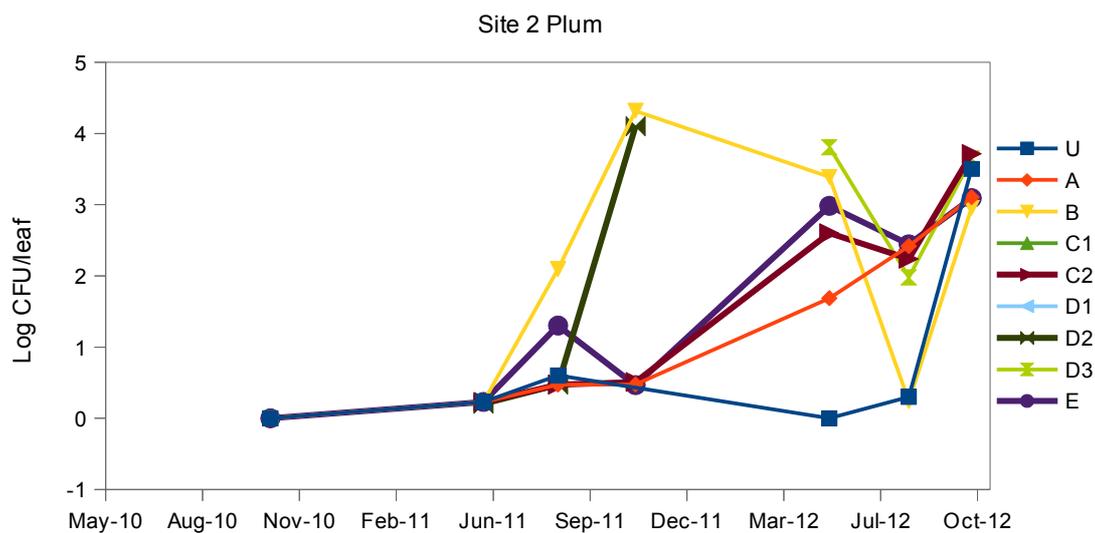
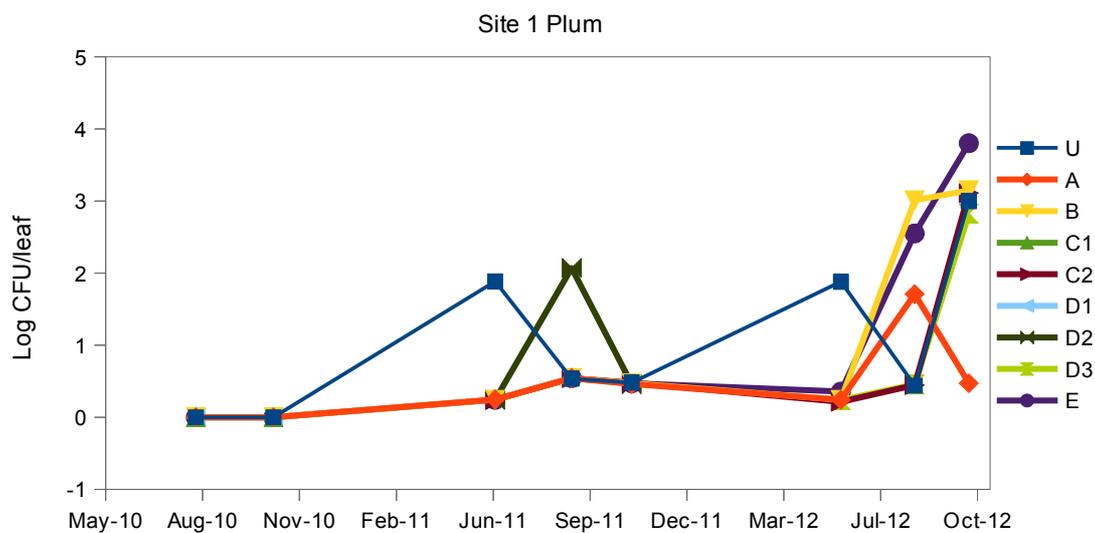
#### *Psm (plums only)*



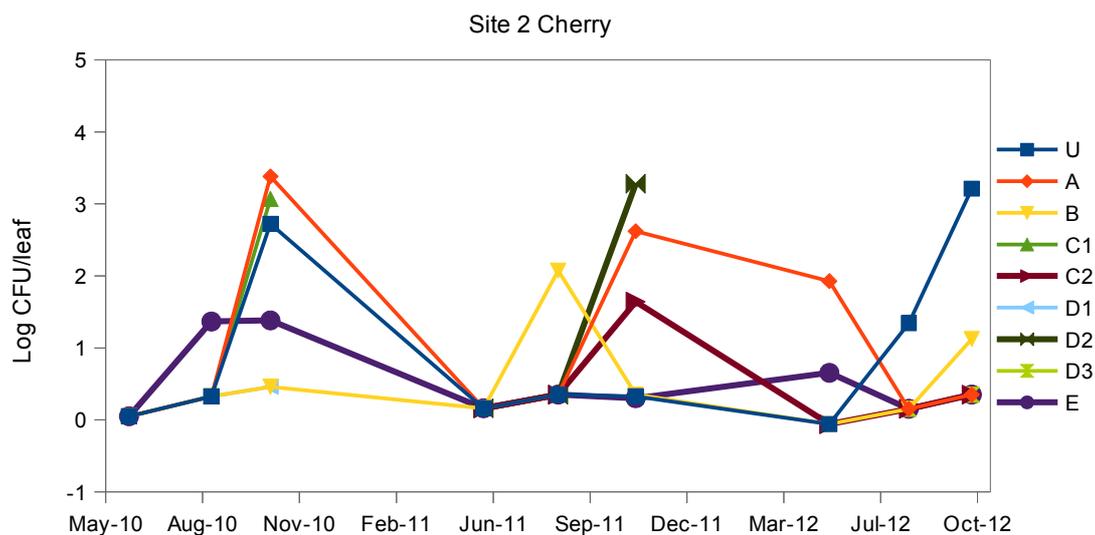
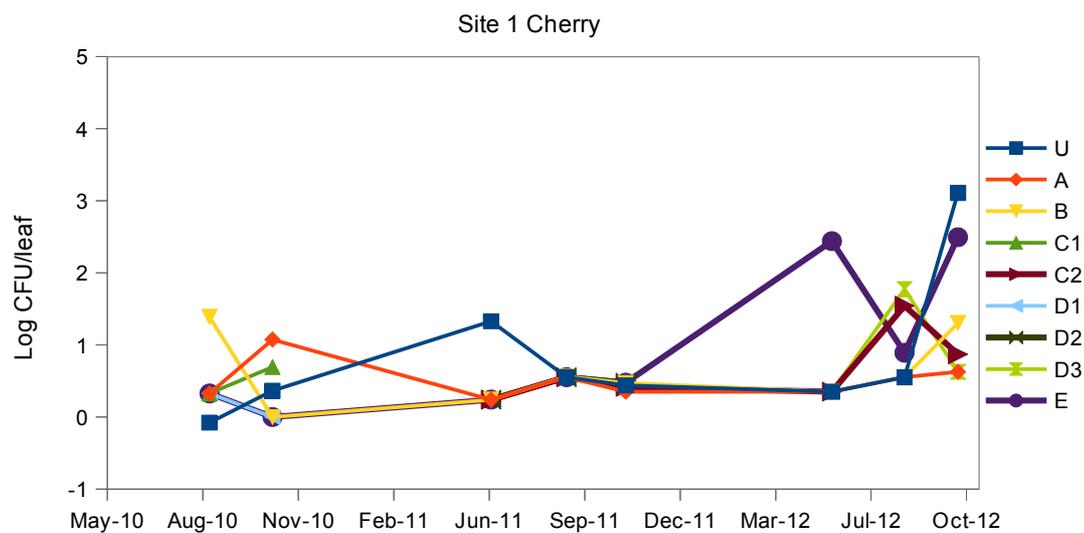
**Pss (cherries)**



**Pss (plums)**



**Combined (cherry)**



**Combined (plum)**

