Project Title:	Management of bacterial canker in *Prunus* spp.
Project number:	HNS 179
Project leader:	Dr Steven J Roberts
Report:	Annual Report, March 2012
Previous report	Annual Report, March 2011
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Location of project:	Plant Health Solutions, Warwick; HDRA, Ryton, Coventry; Grower nurseries
Project coordinators:	Mr Nick Dunn and Mr John Hedger
Date project commenced:	01 April 2010
Date completion due:	31 March 2013
Keywords:	diseases; bacteria; HNS; trees; plum; cherry; Pseudomonas

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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.

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Director
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Dr S J Roberts
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GROWER SUMMARY

Headline

- Levels of the potential bacterial canker pathogens *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) and *P. s.* pv. *syringae* (*Pss*) were reduced by sprays with Cuprokylt (copper oxychloride) and a Cuprokylt + Dithane NT (mancozeb) mix.
- Results with the biological control agent Serenade ASO (*Bacillus subtilis*) have not been consistent from year to year.
- The overall levels of bacteria were greater in 2011 than in 2010 and levels of both *Psm* and *Pss,* but especially *Psm,* continued to be greater on plum than on cherry.
- The pattern of variation in bacterial numbers with sampling period differed between years, and calls into question previous thinking on changes in populations through the growing season.
- A practical approach to disinfection of pruning tools during field operations using isopropanol-impreganated disinfectant wipes such as "Azowipes" has been identified.

Background and objectives

Bacterial canker of *Prunus* spp. 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 of *Pseudomonas syringae*: pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). *Psm* is host specific to *Prunus* spp., whereas pv. *syringae* 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 may also cause leaf spots/shot-holes, 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 1960's and 70'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/carry over of (pathogen) 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 is to identify management options which will be of benefit in the control of bacterial canker of *Prunus* spp. To achieve this the project will: aim 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 report summarises the results for the second year, and combined analysis of all data from both 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 cv. Victoria; cherries cv. 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. Following review of the first year results with grower representatives, two modifications to the year 1 (2010) treatments were agreed

(see Table 1). The 2010 Treatment C (C1; Bactime Cu L4F, glucohumate + copper) was replaced by Cuprokylt (copper oxychloride) alternating with Serenade (*Bacillus subtilis*) (C2) as pathogen levels were worse than in the untreated samples. The first year Aliette treatment (D1) was replaced by Cuprokylt plus Dithane mix plus wetter (D2) as Aliette will not be available in the future. The treatments are summarised in Table 1.

Applications were made according to the following timings: 2 x spring at bud burst, 2 x prior to budding, 2 x autumn sprays. Approx. 12 individual stock hedge plants were allocated to each treatment, 2-3 mother plants and 100 rootstocks or maidens.

Table 1. Treatment codes, products and rates used in spray trial.

Code	Product	Active ingredient	Rate	Approval status
A	Cuprokylt plus adjuvant (Activator 90)	copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator	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	Alternating Cuprokylt and Serenade ASO (Year 2)	copper oxychloride or *Bacillus subtilis*	3 g/L Cuprokylt or 10 mL/L Serenade ASO	Cuprokylt – Label approval Serenade ASO - EAMU for ornamental plant production
D1	Aliette 80WG (Year 1)	fosetyl-aluminium	1 g/L	Label approval
D2	As E plus Activator 90 (Year 2)		0.25 mL/L Activator	
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/bud samples were collected from each treatment from each nursery during the growing season and taken to the lab 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.*

Both of the target pathogens (i.e. either *Psm* or *Pss)* were isolated from samples at both nurseries throughout the year. The overall effects of treatments in 2011 are shown in Fig. 1 (percentage of leaves contaminated) and Fig. 2 (numbers of bacteria). The main statistically significant differences can be summarised as follows:

- Levels of *Psm* and *Pss* were reduced by sprays with Cuprokylt and Cuprokylt + Dithane NT.

- Results with Serenade ASO have not been consistent from year to year.
- There are indications that the new treatments introduced in 2011 (alternating Cuprokylt/Serenade ASO and Dithane NT + Cuprokylt + wetter) may reduce levels but with only a single year of results are not so significant.
- Levels of both *Psm* and *Pss*, but especially *Psm* continued to be greater on plum than on cherry.
- The overall levels of bacteria were greater in 2011 than in 2010.
- The pattern of variation with sampling period differed between years, and calls into question previous thinking on changes in populations through the growing season.

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.

Table 2. 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 (5×10^7)[a]	% cuts (1×10^6)[b]
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

[a] Predicted % cuts with growth, adjusted to a standard inoculum concentration of 5×10^7 CFU/mL
[b] Predicted % cuts with growth, adjusted to a standard inoculum concentration of 1×10^6 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, although further work was not originally scheduled in this project, 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 treatment 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 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 clear-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.

Action points for growers

- Disinfect pruning tools and knives as often as possible in the field using iso-propanol impregnated wipes such as Azowipes.
- Growers should be aware that canker symptoms may not become apparent until 18 months after infection has occurred, thus actions taken in one growing season may potentially have an impact on appearance of disease two seasons later.

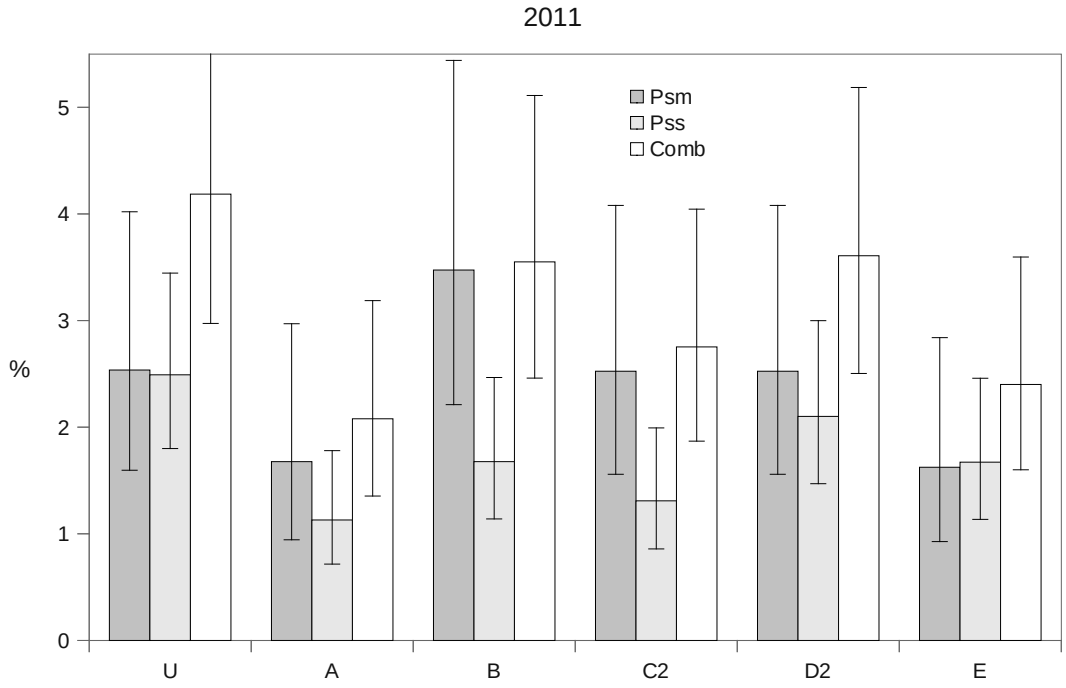

Figure 1: Effect of 2011 treatments on the estimated % of leaves contaminated with *Pseudomonas syringae* pv. *morsprunorum* (Psm), *P. s. pv. syringae* (Pss), and either or both (Comb). Bars represent the 95% confidence intervals.

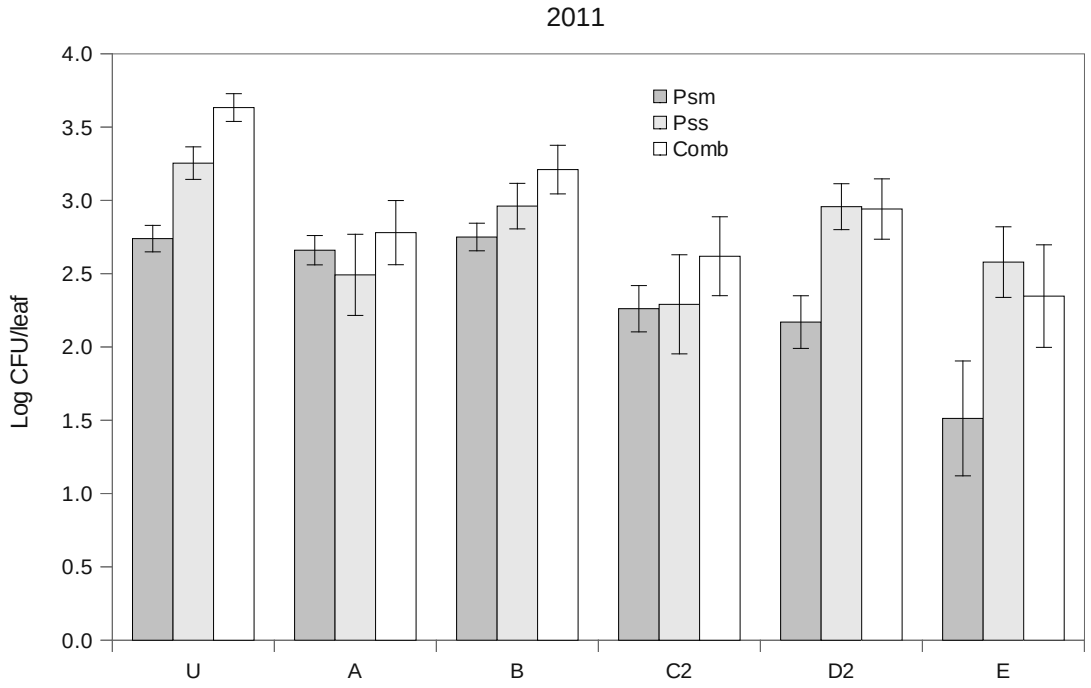

Figure 2: Effect of 2011 treatments on the mean Log_{10} of the number of *Pseudomonas syringae* pv. *morsprunorum* (Psm), *P. s.* pv. *syringae* (Pss), and either or both (Comb). Bars represent the approximate standard errors of the means.

SCIENCE SECTION

Introduction

Bacterial canker of *Prunus* spp. 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).

Bacterial canker may be caused by two distinct pathovars of *Pseudomonas syringae*: pv. *morsprunorum* (*Psm*) and pv. *syringae* (*Pss*). *Psm* is host specific to *Prunus* spp., 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/shot-holes, shoot die-back 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* spp. in the UK was done in the late 1990s, early 2000s. This Defra-funded work (WD0224 and WD0234) (Roberts and Vicente 2001; Roberts and 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 and 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/maintaining

disease-free stock plants, and minimising the likelihood of cross-infection between batches of cuttings/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/carry over of (pathogen) 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 and 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 and 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. *philadelphi*) 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 ≥99.999% kill under clean conditions and ≥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 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 < 1 year on young potted plants, only examined foliar symptoms.

A current top fruit project on fireblight (TF 183; Carew 2009) has not yet come to any conclusions due to the low incidence/absence of disease in the test orchards. Apart from copper and a growth regulator the other three products being trialled appear to come from one company (none are registered as pesticides). The authors claim that the product Sentry S is a 'Serenade equivalent' – this is unlikely to be the case – the species *Bacillus subtilis* is comprised of many different strains, the activities of these strains as bio-control agents are often quite strain specific. They are also examining a *harpin* based product – this has been 'around' for a number of years in the US (and is specifically derived from the *harpin* protein of the fireblight pathogen). It induces SAR (systemic acquired resistance) but it seems (from discussions with pathologists in other countries) that effects are rather marginal and it is apparently not widely used commercially in the US.

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* spp. 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 will be that bud-wood and/or rootstock material may be asymptomatically 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 year of the project have been reported previously (Roberts 2011). The main conclusions from the first year can be summarised as follows:

- Levels of both *Psm* and *Pss*, but especially *Psm* were greater on plum than on cherry.
- *Psm* was more frequent on buds and stock hedges
- *Pss* was less frequent on stock hedges
- Levels of *Psm* were reduced by sprays with Cuprokylt and Serenade ASO.
- Spray treatments had little effect on levels of *Pss.*
- Levels of *Pss* increased in the Autumn with the biggest increase at Site 2
- Effective disinfection of pruning tools requires disinfectant exposure times of up to 30 seconds.

This report contains the results of the work done in the second year of the project and combined analysis of the data from both years 1 and 2. In addition to the work originally scheduled for the second year of the project, additional disinfection experiments were done, to examine the effects of different experimental parameters on results.

Materials and Methods

Experimental design

The experimental design was essentially as described in the first year, but with the addition of 1st year rootstock material which was taken as cuttings in Autumn 2011.

Following discussion at a review meeting two modifications to the treatment plant were agreed (See Table 3). The first year glucohumate treatment C (C1) was replaced by Cuprokylt alternating with Serenade ASO (C2) as pathogen levels were worse than in the untreated samples. The first year Aliette treatment (D1) was replaced by Dithane NT + Cuprokylt mix plus wetter (D2) as the product will not be available in the future.

Table 3. Treatment codes, products and rates used in spray trial.

Code	Product	Active ingredient	Rate	Approval status
A	Cuprokylt plus adjuvant (Activator 90)	copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator	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 (Year 2)	copper oxychloride or *Bacillus subtilis*	3 g/L Cuprokylt or 10 mL/L Serenade ASO	Cuprokylt – Label approval Serenade ASO - EAMU for ornamental plant production
D1	Aliette 80WG (Year 1)	fosetyl-aluminium	1 g/L	Label approval
D2	As E plus Activator 90 (Year 2)		0.25 mL/L Activator	
E	Dithane NT + Cuprokylt	mancozeb + copper oxychloride	2 g/L Dithane NT + 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 as in 2010.

Sample collection and processing

Sample collection and processing was done in the same way as in 2010, and is summarised below:

Collect samples
↓
Stomach/extract
↓
Dilute and plate on selective media
↓
Incubate then count suspects
↓
Sub-culture suspects
↓
Test and confirm ID of suspects

Characterisation of suspect isolates

Suspect isolates characterised in the same way as in year 1: initial characterisation on the basis of appearance and production of fluorescent and other pigments on PAF medium,

levan production on SNA medium, and oxidase reaction; further characterisation of *P. syringae* isolates using the GATTa tests (gelatinase, aesculin hydrolysis, tyrosinase, utilisation of D-tartrate) (Vicente *et al.* 2004), colour of growth in NSB, and tobacco hypersensitivity reaction. Based on the results of these tests, isolates were assigned to either *Psm* or *Pss*.

Disinfection of pruning tools

The basic method was the same as in the first year, with the addition that different drying times and inoculum concentrations were also examined, it is summarised below:

Prepare suspension of bacteria in sugar/peptone (and conduct viable counts)

↓

Apply 100 µl drop to blade

↓

Leave in contact for 1-2 h (+ or – fan to enhance drying)

↓

Disinfect blade

↓

Make 10 sequential cuts in agar plate

↓

Incubate plates

↓

Record number of cuts with growth

Statistical analysis

The spray trial data were analysed in two ways. The effect of treatments on the proportion of leaves/buds contaminated/infected with the 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 treatment 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 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 304 leaf or bud samples were collected and processed in the second year, to give a total of 530 over the two years of the project, and considerably in excess of the budgeted numbers. A matrix of the material sampled, when and the potential pathogens detected is shown in Table 4. 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. 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 I and II) 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 tended to be 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 year 1 it was only rarely detected on cherries, and in year 2 it was never detected on cherries (Figs. 3 and 4). 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. 5) and location. These are also illustrated for each treatment on maidens in Appendix 4.

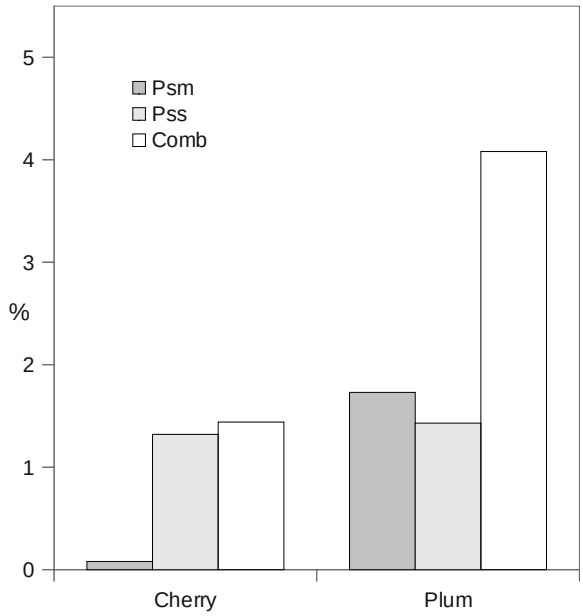

Figure 3: Mean percentage of leaves contaminated with potential pathogens (both years, all samples).

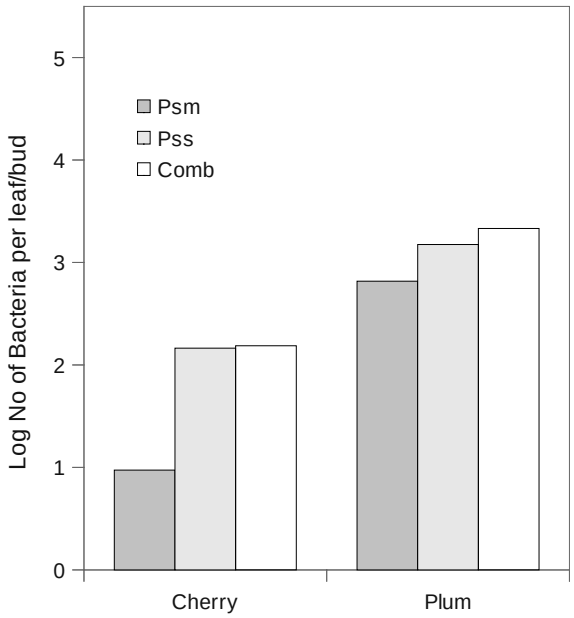

Figure 4: Mean numbers of bacterial canker pathogens detected on cherries and plums (both years, all samples)

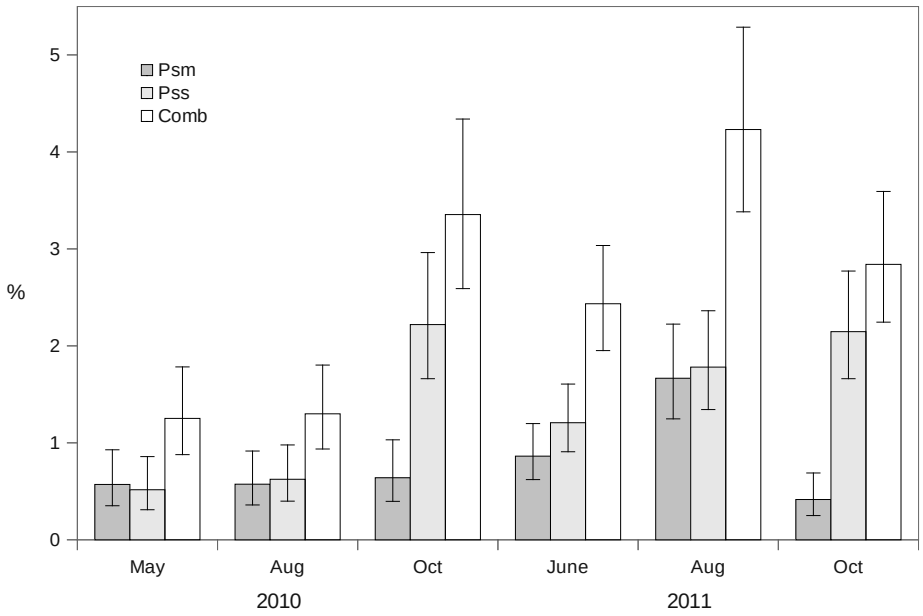

Figure 5: Mean % leaves contaminated with potential pathogens at each sampling period (all samples, both sites, both species). Bars represent the 95% confidence limits.

Proportion of leaves/buds infested

The overall percentages of leaves contaminated with potential bacterial canker pathogens for each treatment and year are shown in Fig. 6.

Psm (Plum only)

There was a significant effect of treatment, and significant interactions of treatment with year and production stage. Treatments A (Cuprokylt) and E (Cuprokylt + Dithane) both gave reductions in the proportion of contaminated leaves, in both years, but were less effective in 2011 than in 2010. The main cause of the significant interaction terms was that treatment B (Serenade ASO) appeared to be very effective in 2010, but failed to give any control on the newly planted rootstocks in 2011.

Pss

Unlike for *Psm* the effect of host was not significant. There was a significant effect of production stage and a host x stage interaction: stock hedges and 1st year cherry rootstocks appeared to have lower levels, and cherry mother-plants and 1st year plum rootstock had higher levels. Treatment had a significant effect: Treatments A (Cuprokylt), C2 (Cuprokylt/Serenade ASO) and E (Cuprokylt + Dithane NT) all gave significant reductions.

Table 4. Matrix showing the materials sampled and when and the micro-organisms detected.

					2010		2011			
Site	Treat	Species	Cultivar	Stage	May	Aug	Oct	June	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	
	B				nd	Pss		Psm + P	Psm + Pss	
	C				Psm	Psm		Pss	Psm	
	D				Pss	Pss		Pss?	Psm + Pss	
	E				Ps	Pss		Psm + P	Psm	
	U				Ps	Psm		Psm	Psm + Pss	
	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 – budded		nd	Ps	Psm	Pss	Psm + P
	B					nd	Pss	Psm	Psm	Pss
	C					Psm	Pss	Psm	Pss	Pss
	D					nd	Pss	Psm	Psm + P	Pss
	E					nd	Pss	nd	Pss	nd
	U					Psm	Psm + P	Pss	Psm + P	Psm + P
	A	avium	Colt	RS2 – budded		Pss	Pss	nd	nd	nd
	B					Psm	Pss	Pss	nd	nd
	C					nd	Pss	Pss	nd	nd
	D					nd	Pss	Pss	nd	nd
	E					nd	Pss	nd	nd	nd
	U					nd	Pss	Pss	nd	nd
	A	instita	SJ	RS1				Pss		Pss
	B							Psm		Psm + P
	C							Psm		nd
	D							Psm + Pss		Psm + P
	E							Pss		Psm
	U							Psm	Psm	Pss
	A	avium	Colt	RS1				Pss		nd
	B							nd		nd
	C							nd		nd
	D							nd		nd
	E							nd		nd
	U							nd	nd	nd
2	A	instita	SJ	RS2 – buddec	nd	nd	Ps	nd	Psm	Pss
	B				nd	nd	Pss	nd	Psm	Pss
	C				Psm	Psm	Pss	nd	Psm	Pss
	D					nd	Pss	Pss	Psm	Pss
	E				nd	nd	Pss	nd	Psm + P	Pss
	U				nd	Ps	Pss	nd	Psm + P	Pss
	A	avium	Colt	RS2 – buddec	nd	nd	Pss	nd	nd	Pss
	B				Pss	nd	Pss	nd	Pss	Pss
	C				nd	nd	Psm + P	nd	nd	Pss
	D				Pss	Pss	Ps	nd	nd	Pss
	E				nd	Pss	Pss	nd	nd	Pss
	U				Pss	Pss	Pss	nd	Pss	Pss

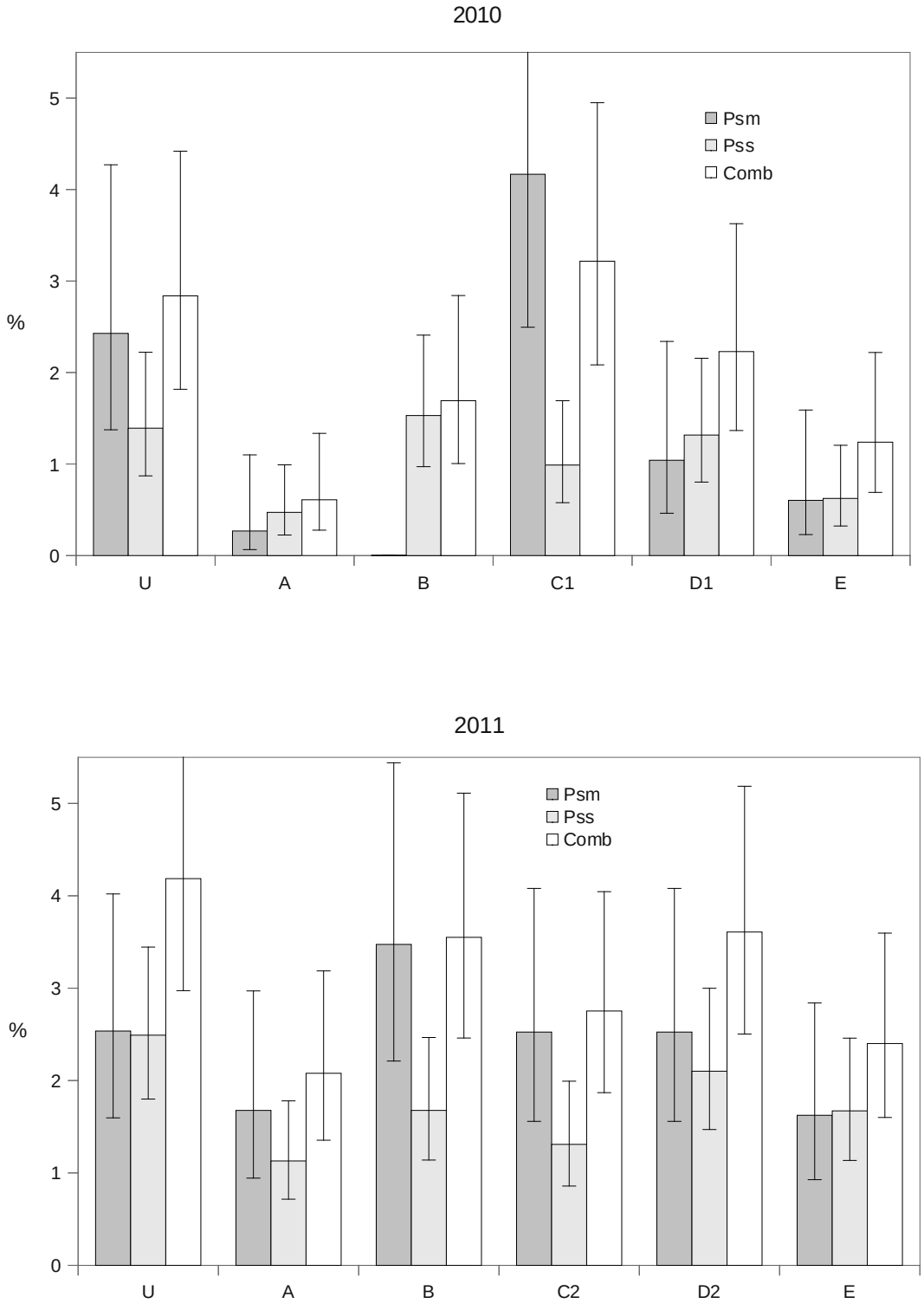

Figure 6: Effect of treatments on the estimated % of leaves contaminated with *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s*. pv. *syringae* (*Pss*), and either or both (Comb). Bars represent the 95% confidence limits.

Combined

For the combined data for both potential canker pathogens, the host inevitably had a major effect, due to the presence of both *Psm* and *Pss* on plums, but mainly only *Pss* on cherries. The significance of terms in the model followed a similar pattern to *Pss* alone. There was a significant effect of production stage and a host x stage interaction: plums had a higher levels at all stages but particularly on the mother-plants and the 1st year rootstocks. Treatment also had a significant effect: Treatment A (Cuprokylt) and Treatment E (Cuprokylt + Dithane NT) both gave significant reductions in the proportion of contaminated leaves compared to the untreated control.

Numbers of bacteria

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

Psm

Site had a major effect on the overall mean numbers of Psm per leaf: numbers were greater at site 1 (2.6 Log_{10} CFU) than at Site 2 (1.7 Log_{10} CFU). Interpreting the effect of treatments was complicated due to significant interaction terms: treat.year, treat.stage, and treat.year.stage. In year 1 all treatments except treatment C1 (Bactime Cu L4F) reduced numbers compared to the untreated control, but in year 2 only treatment E (Cuprokylt + Dithane NT) gave a significant reduction. This change in year 2 was driven mainly by higher numbers detected on all treatments on mother-plants and maidens compared to year 1, particularly at the August sampling.

Pss

Host was one of the most important factors affecting the mean number of *Pss* per leaf: numbers of *Pss* were greater on plum (2.8 Log_{10} CFU/leaf) than on cherry (2.0 Log_{10} CFU/leaf). Factors associated with sampling date and location and their interactions (site, month, site.month, host.year.month) were also highly significant indicating fluctuations in the overall numbers and particularly increases in the Autumn. Production stage also had significant effect with greater numbers on mother-plants and 1st year rootstocks than on maidens and stock hedges. Spray treatment also had a significant effect on the numbers of *Pss,* although there were significant interactions with site and year, these were relatively less important than for *Psm* and overall all treatments except C1 (Bactime Cu L4F in year 1) reduced numbers compared to the untreated control. The treat.site interaction suggested that treatments were generally less effective at Site 2 than at Site 1. The treat.year

interaction suggested that treatments B and E (Serenade ASO and Cuprokylt + Dithane NT) were more effective in 2011 than 2010.

Combined

For the combined numbers, host had the biggest overall effect with numbers much greater on plum (3.3 Log_{10} CFU/leaf) than on cherry (2.2 Log_{10} CFU/leaf). There was also a significant effect of production stage with maidens and rootstocks having greater numbers than stock hedges and mother-plants. Interpreting the effect of the spray treatments was complicated by a several significant interaction terms, particularly treat.year and treat.site. In year 1 Treatment A (Cuprokylt) gave a significant reduction in the total numbers of potential pathogens; in year 2 all except treatment B (Serenade ASO alone) gave a significant reduction. Treatments also performed differently at the two sites: at site 1 treatments A, B, C2, D2, E all gave reductions, but at site 2 only treatment D1 gave a significant reduction.

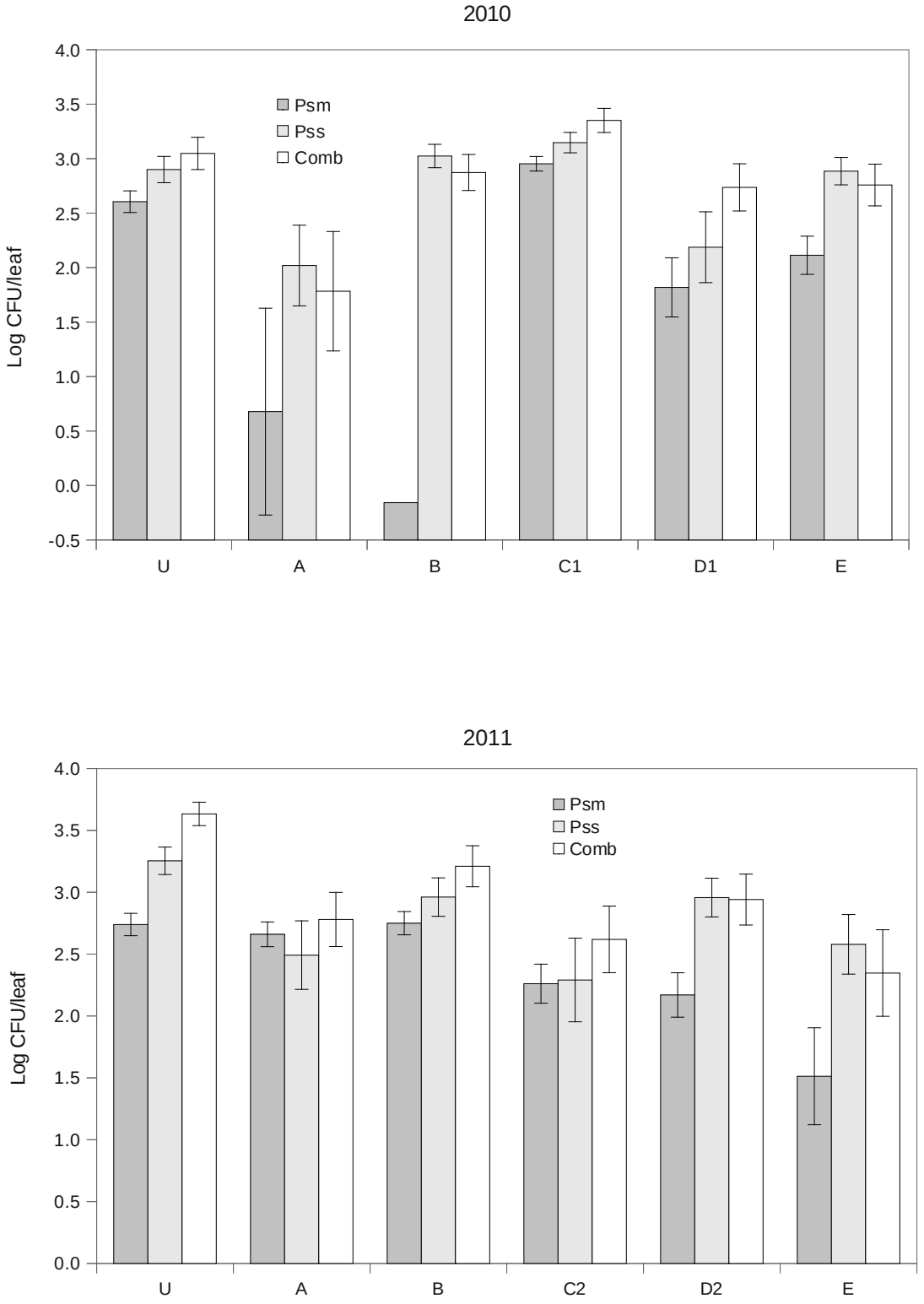

Figure 7: Effect of treatments on the $\log_{10}$ of the mean number of *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (Comb). Bars represent the standard errors of the means.

Disinfection of tools

The results are summarised in Table 5. 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.

Table 5. 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 (5×10^7)[a]	% cuts (1×10^6)[b]
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 Azowipes (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

[a] Predicted % cuts with growth, adjusted to a standard inoculum concentration of 5×10^7 CFU/mL
[b] Predicted % cuts with growth, adjusted to a standard inoculum concentration of 1×10^6 CFU/mL

Analysis of deviance (Appendix III) 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.

Discussion

Epidemiology and spray treatments

In the second year the two potential bacterial pathogens, *Psm* and *Pss*, continued to behave differently on the two host 'species' and the spray treatments appeared to have different effects on their populations. Although occasionally detected on cherry in 2010,

Psm was not detected at all on cherry in 2011, therefore to avoid artefacts caused by excessive numbers of zeroes, analysis of *Psm* was restricted to plums.

Overall the proportions of contaminated/infected leaves and numbers of bacteria were higher in 2011 than in 2010, and particularly at the summer (August) sampling. This peak, at site 2, corresponded to very obvious and severe shot-hole symptoms on the plum leaves of all treatments. This peak in numbers in the summer contradicts conventional wisdom that numbers decline in the summer. In all 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, driven by local weather factors (e.g. rainfall, temperatures) in the days or weeks prior to sampling. We will attempt to acquire weather data from the Meteorological Office from nearby weather stations to investigate this in the final year of the project.

Production stage also appeared to have significant effects, but these differed for *Psm* (highest on mother-plants) and *Pss* (highest on mother-plants, lowest on stock hedges), and combined (highest on 1st year rootstocks). However, it is important to note that these may to some extent be artefacts of the methods and that *Pss* and *Psm* tended to mutually exclusive on the isolation (dilution) plates.

Interpreting the effects of the spray treatments was complicated by the presence of relatively significant interaction terms in some analyses: year and production stage for *Psm*; year and site for *Pss* counts and combined counts. Overall it appears that treatments A and E (Cuprokylt and Cuprokylt + Dithane NT) are giving significant reductions in both *Psm* and *Pss,* whereas treatment B (Serenade ASO) is more variable, giving reductions in in 2010 but not in 2011. It appears that treatment A (Cuprokylt) was relatively less effective in 2011 than in 2010, but treatment E (Cuprokylt + Dithane NT) was relatively more effective in 2011 than in 2010. The treatments generally do not appear to be as effective at Site 2 compared to Site 1, although overall pathogen numbers were lower at Site 2 than at Site 1.

The new treatments in 2011 (C2 and D2, Cuprokylt/Serenade ASO and Cuprokylt + Dithane NT + adjuvant) are also giving indications of reduction, but as they have only been tested over one year, the reductions are not so significant.

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, although further work was not originally scheduled in this project, 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 treatment 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 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 clear-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 with Cuprokylt and Cuprokylt + Dithane NT.
- Results with Serenade ASO have not been consistent from year to year.
- There are indications that the new treatments introduced in 2011 (alternating Cuprokylt/Serenade ASO and Cuprokylt + Dithane NT + wetter) may reduce levels but with only a single year of results are not so significant.
- Levels of both *Psm* and *Pss*, but especially *Psm* continued to be greater on plum than on cherry.
- The overall levels of bacteria were greater in 2011 than in 2010.
- The pattern of variation with sampling period differed between years, and calls into question previous thinking on changes in populations through the growing season.
- A practical approach to disinfection of pruning tools during field operations using disinfectant wipes such as "Azo-wipes" has identified.

Acknowledgements

The author would like to thank the nurseries and staff involved in and supporting the trials.

Knowledge and Technology Transfer

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

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Appendix I

Abbreviations used in analyses of deviance tables:

Stage2 – stage in productions, 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. The nature of the models means that there are no absolute signficance tests, so ratios and significance values should be taken as a guide only.

Summaries of analyses of deviance for proportions of leaves

Psm proportions, all data:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	192.8355	192.8355	192.84	<.001*
+ Site	1	3.2607	3.2607	3.26	0.071
+ Year	1	9.6480	9.6480	9.65	0.002
+ Year.Month	4	46.8847	11.7212	11.72	<.001*
+ Site.Year	1	6.0281	6.0281	6.03	0.014
+ Site.Month	2	37.0975	18.5487	18.55	<.001*
+ Site.Year.Month	2	11.5775	5.7887	5.79	0.003
+ Cultivar	3	32.4284	10.8095	10.81	<.001*
+ Host.Site	1	3.8408	3.8408	3.84	0.050
+ Host.Site.Month	4	13.9108	3.4777	3.48	0.008
+ Stage2	3	11.5433	3.8478	3.85	0.009
+ Host.Stage2	3	8.7949	2.9316	2.93	0.032
+ Treat2	7	41.7645	5.9664	5.97	<.001*
+ Year.Treat2	3	20.6109	6.8703	6.87	<.001*
+ Year.Month.Treat2	20	38.9984	1.9499	1.95	0.007
+ Host.Treat2	7	21.0808	3.0115	3.01	0.004
+ Site.Treat2	7	41.6537	5.9505	5.95	<.001*
+ Stage2.Treat2	19	115.7917	6.0943	6.09	<.001*
+ Samp.Med	440	100.6679	0.2288	0.23	1.000
Residual	476	152.8167	0.3210		
Total	1005	911.2347	0.9067		

* MESSAGE: ratios are based on dispersion parameter with value 1

Psm proportions, Plum only

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Site	1	3.9710	3.9710	3.97	0.046
+ Year	1	16.6463	16.6463	16.65	<.001*
+ Year.Month	4	50.2861	12.5715	12.57	<.001*
+ Site.Year	1	8.1198	8.1198	8.12	0.004
+ Site.Month	2	54.2207	27.1103	27.11	<.001*
+ Site.Year.Month	2	10.7602	5.3801	5.38	0.005
+ Stage2	3	10.6286	3.5429	3.54	0.014
+ Treat2	7	40.4624	5.7803	5.78	<.001*
+ Year.Treat2	3	27.8685	9.2895	9.29	<.001*
+ Year.Month.Treat2	20	42.4176	2.1209	2.12	0.002
+ Site.Treat2	7	22.3919	3.1988	3.20	0.002
+ Stage2.Treat2	19	120.1703	6.3248	6.32	<.001*
+ Samp.Med	181	86.5884	0.4784	0.48	1.000
Residual	225	133.0097	0.5912		
Total	476	627.5414	1.3184		

* MESSAGE: ratios are based on dispersion parameter with value 1

Pss proportions:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	0.9837	0.9837	0.98	0.321
+ Site	1	6.6480	6.6480	6.65	0.010
+ Year	1	16.9372	16.9372	16.94	<.001*
+ Year.Month	4	51.7665	12.9416	12.94	<.001*
+ Site.Year	1	0.1226	0.1226	0.12	0.726
+ Site.Month	2	79.0584	39.5292	39.53	<.001*
+ Site.Year.Month	2	12.8707	6.4353	6.44	0.002
+ Cultivar	3	15.5265	5.1755	5.18	0.001*
+ Host.Site	1	0.2108	0.2108	0.21	0.646
+ Host.Site.Month	4	23.4362	5.8590	5.86	<.001*
+ Stage2	3	32.2409	10.7470	10.75	<.001*
+ Host.Stage2	3	32.2051	10.7350	10.74	<.001*
+ Treat2	7	37.5414	5.3631	5.36	<.001*
+ Year.Treat2	3	6.3405	2.1135	2.11	0.096
+ Year.Month.Treat2	20	58.3547	2.9177	2.92	<.001
+ Host.Treat2	7	25.9679	3.7097	3.71	<.001
+ Site.Treat2	7	18.9112	2.7016	2.70	0.008
+ Stage2.Treat2	19	54.5932	2.8733	2.87	<.001
+ Samp.Med	440	416.9490	0.9476	0.95	0.779
Residual	476	358.3372	0.7528		
Total	1005	1249.0016	1.2428		

* MESSAGE: ratios are based on dispersion parameter with value 1

Combined proportions:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Host	1	101.6166	101.6166	101.62	<.001*
+ Site	1	0.0134	0.0134	0.01	0.908
+ Year	1	28.5711	28.5711	28.57	<.001*
+ Year.Month	4	54.1387	13.5347	13.53	<.001*
+ Site.Year	1	1.1658	1.1658	1.17	0.280
+ Site.Month	2	66.2071	33.1036	33.10	<.001*
+ Site.Year.Month	2	15.1514	7.5757	7.58	<.001*
+ Cultivar	3	12.6115	4.2038	4.20	0.006
+ Host.Site	1	2.4511	2.4511	2.45	0.117
+ Host.Site.Month	4	46.5677	11.6419	11.64	<.001*
+ Stage2	3	29.4846	9.8282	9.83	<.001*
+ Host.Stage2	3	50.3158	16.7719	16.77	<.001*
+ Treat2	7	68.1250	9.7321	9.73	<.001*
+ Year.Treat2	3	4.9081	1.6360	1.64	0.179
+ Year.Month.Treat2	20	68.8179	3.4409	3.44	<.001
+ Host.Treat2	7	25.4788	3.6398	3.64	<.001
+ Site.Treat2	7	14.0115	2.0016	2.00	0.051
+ Stage2.Treat2	19	48.5846	2.5571	2.56	<.001
+ Samp.Med	440	500.9774	1.1386	1.14	0.023
Residual	476	330.3007	0.6939		
Total	1005	1469.4990	1.4622		

* MESSAGE: ratios are based on dispersion parameter with value 1

Appendix II

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	92821.8	92821.8	355.37	<.001	*
+ Year	1	37.5	37.5	0.14	0.705	
+ Month	2	110246.3	55123.2	211.04	<.001	*
+ Site.Year	1	19378.5	19378.5	74.19	<.001	*
+ Year.Month	2	18453.1	9226.5	35.32	<.001	
+ Site.Month	2	47869.8	23934.9	91.63	<.001	*
+ Site.Year.Month	2	1513.4	756.7	2.90	0.057	
+ Stage2	3	82406.1	27468.7	105.16	<.001	*
+ Treat2	7	246680.6	35240.1	134.92	<.001	*
+ Year.Treat2	3	90700.2	30233.4	115.75	<.001	*
+ Site.Treat2	7	2794.4	399.2	1.53	0.159	
+ Stage2.Treat2	19	312948.9	16471.0	63.06	<.001	*
+ Year.Stage2.Treat2	8	141515.8	17689.5	67.72	<.001	*
+ Samp.Med	193	148812.2	771.0	2.95	<.001	
Residual	221	57724.8	261.2			
Total	472	1373903.3	2910.8			

Pss counts:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.	
+ Host	1	450573.6	450573.6	747.51	<.001	*
+ Year	1	1777.0	1777.0	2.95	0.087	
+ Site	1	527427.7	527427.7	875.02	<.001	*
+ Month	2	1229930.2	614965.1	1020.24	<.001	*
+ Year.Site	1	515.2	515.2	0.85	0.356	
+ Year.Month	2	22969.1	11484.6	19.05	<.001	
+ Host.Year.Month	5	262649.8	52530.0	87.15	<.001	*
+ Site.Month	2	330615.2	165307.6	274.25	<.001	*
+ Year.Site.Month	2	8747.0	4373.5	7.26	<.001	
+ Host.Site	1	869.7	869.7	1.44	0.230	
+ Host.Site.Month	2	1464.3	732.2	1.21	0.298	
+ Stage2	3	417034.9	139011.6	230.62	<.001	*
+ Host.Stage2	3	39100.6	13033.5	21.62	<.001	
+ Treat2	7	484285.3	69183.6	114.78	<.001	*
+ Year.Treat2	3	119441.8	39813.9	66.05	<.001	*
+ Host.Treat2	7	66736.9	9533.8	15.82	<.001	
+ Site.Treat2	7	227244.0	32463.4	53.86	<.001	*
+ Stage2.Treat2	19	215468.1	11340.4	18.81	<.001	
+ Year.Site.Treat2	3	13468.4	4489.5	7.45	<.001	
+ Year.Stage2.Treat2	8	24173.8	3021.7	5.01	<.001	
+ Samp.Med	446	352585.8	790.6	1.31	0.002	
Residual	463	279079.6	602.8			
Total	989	5076158.0	5132.6			

Combined counts:

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Host	1	1321222.	1321222.	623.42	<.001*
+ Year	1	112117.	112117.	52.90	<.001*
+ Site	1	91302.	91302.	43.08	<.001*
+ Month	2	1796429.	898215.	423.82	<.001*
+ Year.Site	1	10827.	10827.	5.11	0.024
+ Year.Month	2	10787.	5394.	2.54	0.080
+ Host.Year.Month	5	260708.	52142.	24.60	<.001
+ Site.Month	2	3822214.	1911107.	901.75	<.001*
+ Year.Site.Month	2	45530.	22765.	10.74	<.001
+ Host.Site	1	5382.	5382.	2.54	0.112
+ Host.Site.Month	2	3116.	1558.	0.74	0.480
+ Stage2	3	716699.	238900.	112.72	<.001*
+ Host.Stage2	3	59870.	19957.	9.42	<.001
+ Treat2	7	1609129.	229876.	108.47	<.001*
+ Year.Treat2	3	450438.	150146.	70.85	<.001*
+ Host.Treat2	7	196662.	28095.	13.26	<.001
+ Site.Treat2	7	626740.	89534.	42.25	<.001*
+ Stage2.Treat2	19	1172526.	61712.	29.12	<.001
+ Samp.Med	457	2062732.	4514.	2.13	<.001
Residual	462	979127.	2119.		
Total	988	15353559.	15540.		

Appendix III

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
Cl_0	24.37	7.63	55.69
Cl_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

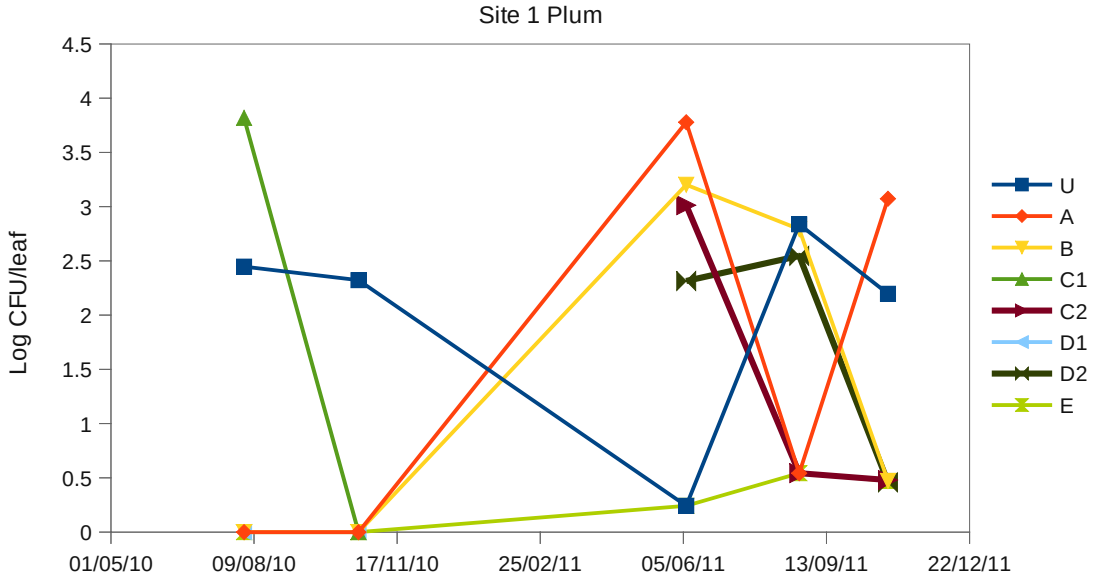
Method	p	LCL	UCL
Cl_0	1.22	0.208	6.76
Cl_30	0.00	0.000	100.00
GW	3.84	0.991	13.73
J5_0	3.43	1.102	10.17
J5_15	0.00	0.000	100.00
J5_30	0.01	0.000	0.64
SW	0.77	0.170	3.44
SW2	0.04	0.003	0.72
U	99.28	39.362	100.00
W	0.36	0.051	2.50

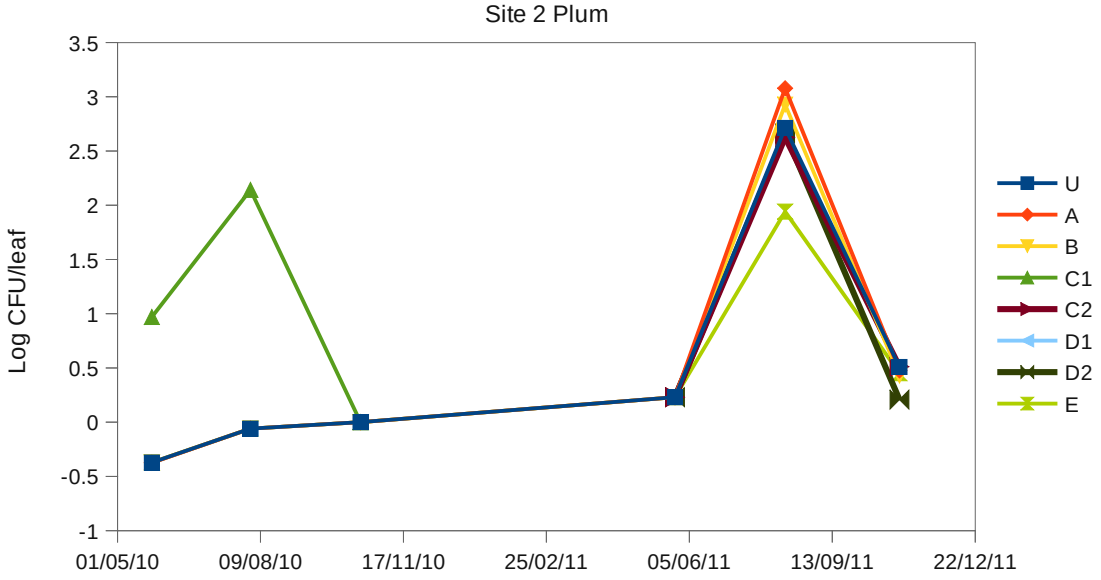
Appendix IV

Bacterial numbers on maidens at each site

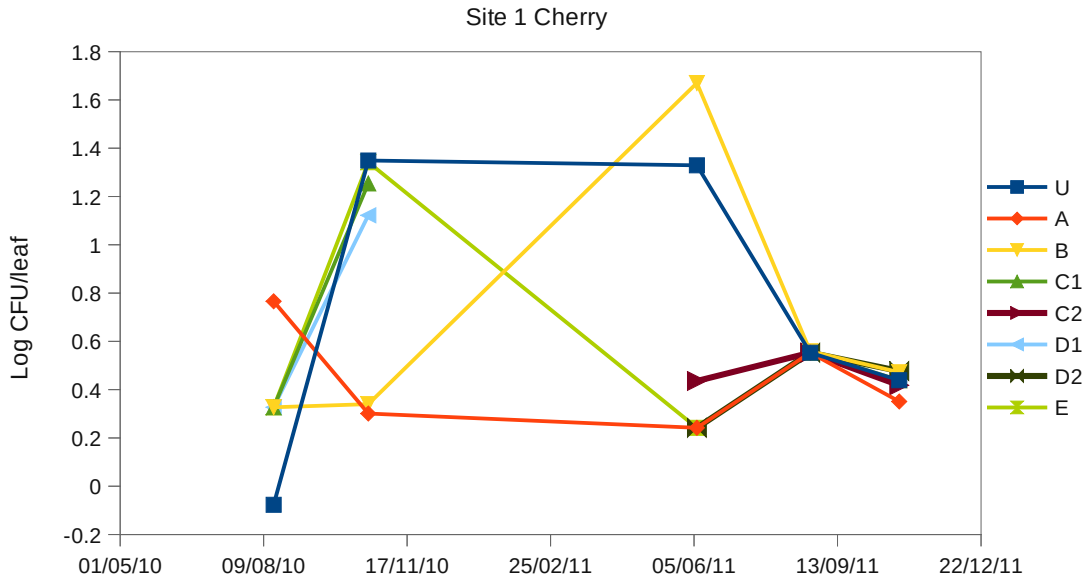
These plants were 2nd year rootstocks in 2010, budded in August 2010, and headed back in spring 2011.

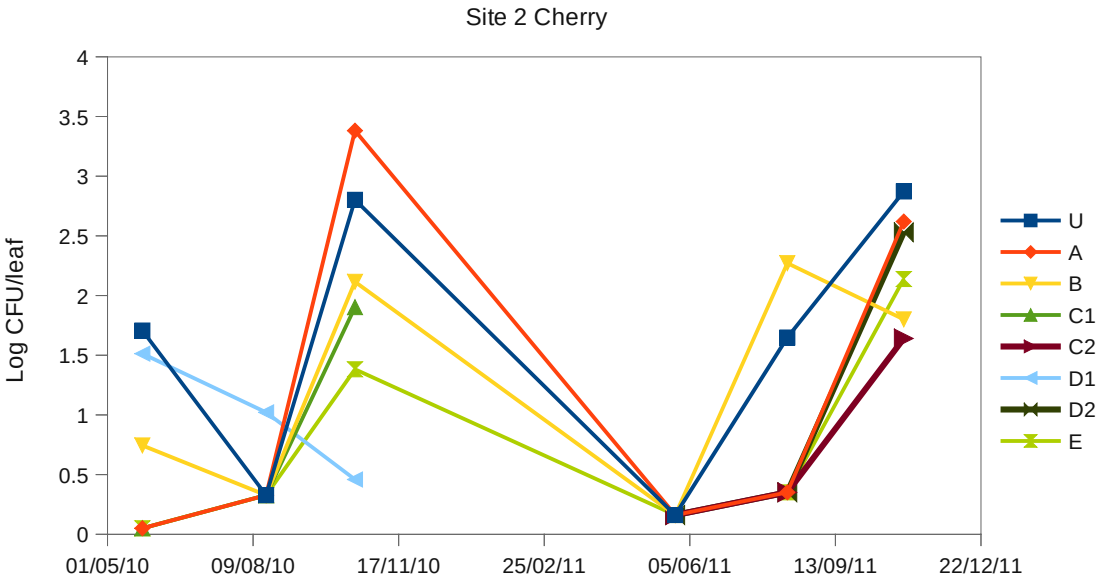
Psm (plums only)



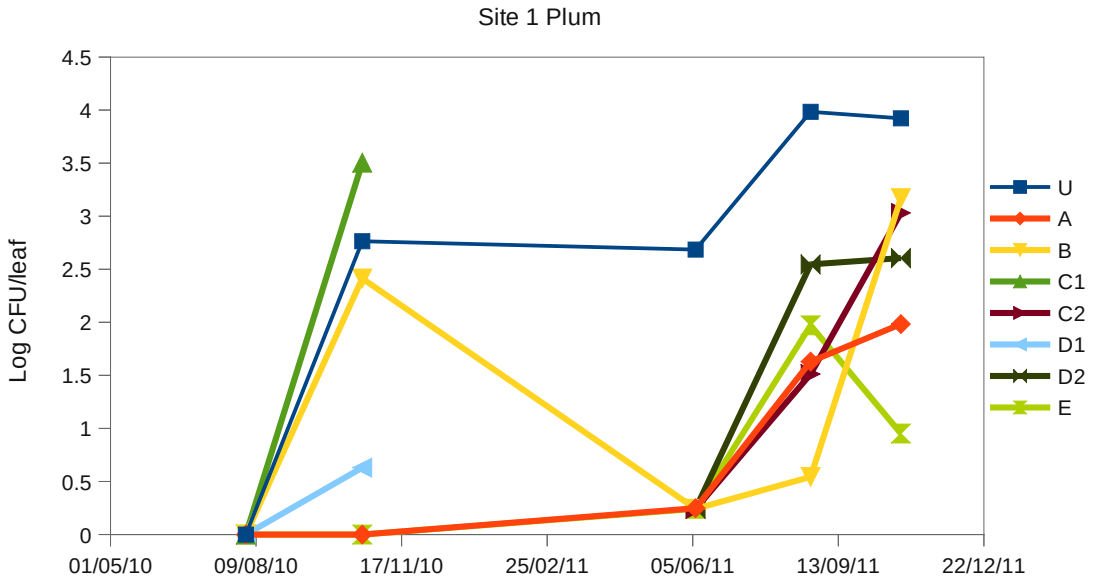


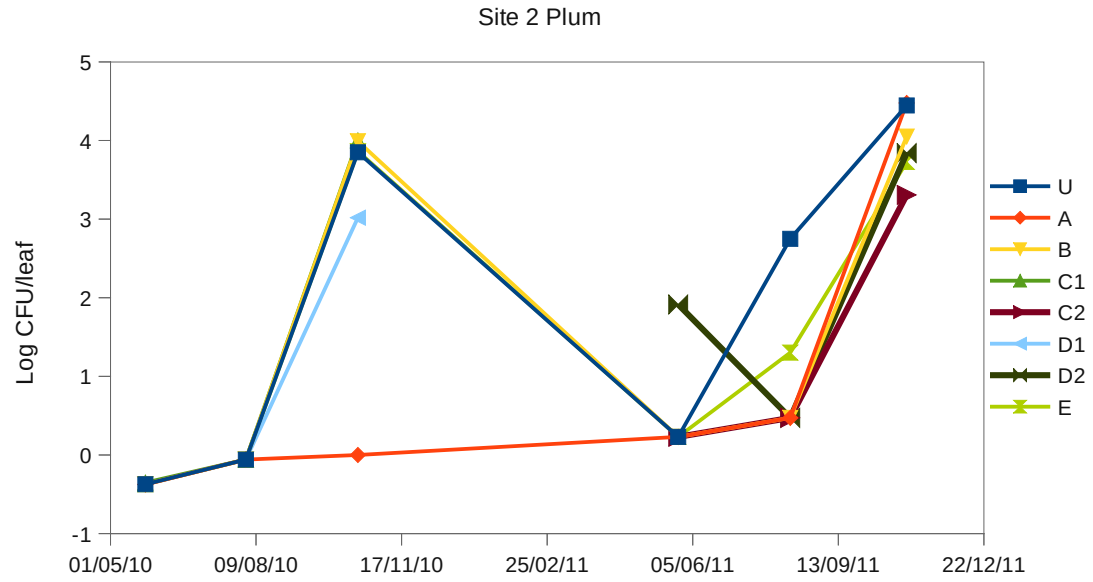
Pss (cherries)



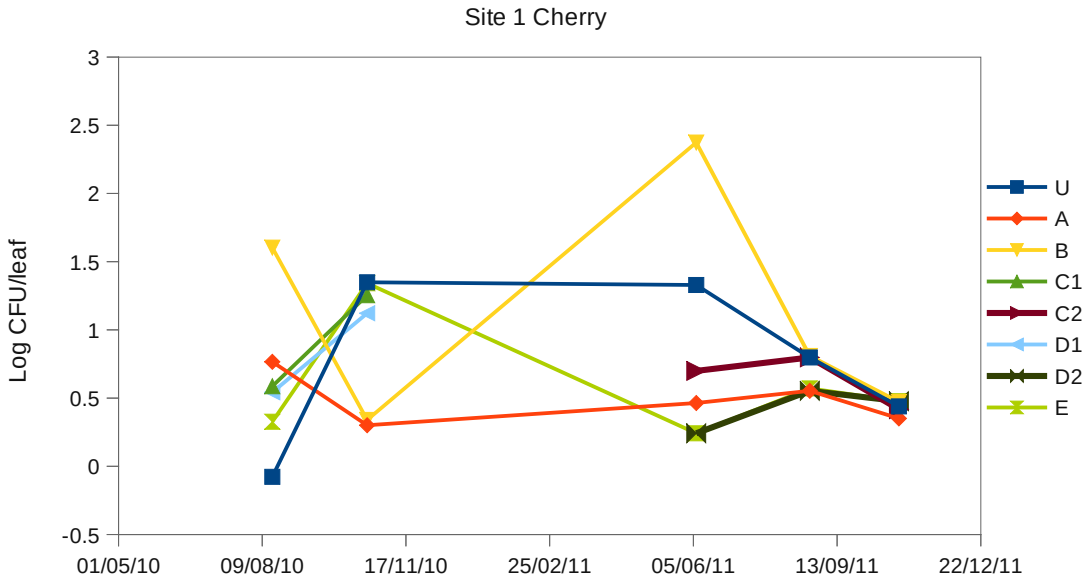


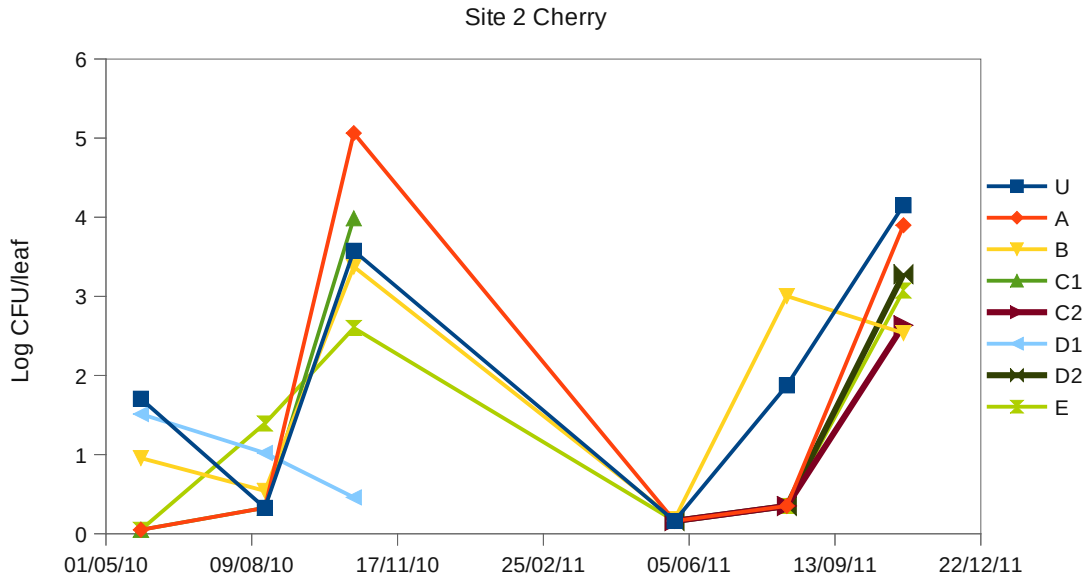
Pss (plums)





Combined (cherry)





Combined (plum)