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Key staff:	Dr S J Roberts, Plant Health Solutions
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The results and conclusions in this report are based on an investigation conducted over a one-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

Headline

- Two potential bacterial canker pathogens *(Pseudomonas syringae* pv. *morsprunorum (Psm)*; and *P. s.* pv. *syringae (Pss)*), behaved differently on plums and cherries and the spray treatments appeared to have different effects on their populations.
- Levels of both *Psm* and *Pss*, but especially *Psm* were greater on plum than on cherry.
- Levels of *Psm* were reduced by sprays with Cuproykylt and Serenade ASO, however, spray treatments appeared to have little effect on *Pss.*
- Disinfection of pruning knives or tools using a quick wipe or dip in disinfectant is unlikely to be effective.

Background and expected deliverables

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 plant 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 Research Centre in the 1960's and 70's), *Psm* was considered to be the primary cause of the disease in the UK. During a MAFF-funded survey of '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/carryover of (pathogen) inoculum. Such a

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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 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 HDC Factsheet with clear practical recommendations.

Summary of the project

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 the project's Industry Representatives, two rootstocks ('Saint Julien A' and 'Colt') and three scions (plum cv. 'Victoria'; cherries cvs. '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/bud populations of bacterial canker pathogens during this first year. These same plants will also be monitored for canker symptoms in subsequent years. The treatments are summarised in Table GS 1. They include: Coprokylt (copper oxychloride); the newly-approved biological control agent Serenade ASO (a strain of *Bacillus subtilis);* Bactime Cu L4F (*a* glucohumate product) which has shown promise against a bacterial disease on walnuts in Italy; Aliette 80 WG which showed promise in previous work (HNS 91: Roberts and Akram 2002); Dithane NT (mancozeb) in combination with Cuprokylt, which is also widely used in France and Australia for the control of bacterial pathogens of stone fruits and nuts.

Applications were made according to the following timings: 2 sprays at bud burst in spring, 2 sprays prior to budding and 2 autumn sprays. Sprays were applied 7-14 days apart depending on weather conditions, and planned for days when no rain was predicted in the

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following 24 h and applied as late in the day as possible. Approximately 12 individual stock hedge plants were allocated to each treatment, 2-3 mother plants and 100 rootstocks.

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

Code	Product	a.i.	Rate	Approval status
A	Cuprokylt plus adjuvant (Activator 90)	copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator	LTAEU for outdoor ornamental plant production
B	Serenade ASO	Bacillus subtilis	10 mL/L	SOLA for ornamental plant production
C	Bactime Cu L4F	copper + glucohumate	4 g/L	Not approved. Foliar fertiliser
D	Aliette 80WG	Fosetyl-aluminium	1 g/L	On-label approval for ornamental plant production
E	Dithane NT + Cuprokylt	Mancozeb + copper oxychloride	2 g/L Dithane + 3 g/L Cuprokylt	Dithane NT LTAEU for outdoor ornamental plant production Cuprokylt (see Code A)
U	Untreated control	-	-	-

Leaf/bud samples were collected from each treatment from each nursery on four occasions during the growing season and taken to the laboratory to determine the presence or absence and numbers of *Psm* and *Pss.* Visits were timed to occur shortly after sprays had been applied.

Both of the target pathogens were isolated from samples at both nurseries throughout the year. The main significant differences 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 Cuproykylt and Serenade ASO.
- Spray treatments had little effect on levels of *Pss.*
- Levels of *Pss* increased in the autumn.

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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 using 70% iso-propanol, Jet 5 (0.8%), bleach (1% chlorine, prepared using Presept(TM) tablets), or a hand sanitising gel (Deb, FloraFree). 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. Three rounds of testing were done.

In the first round of testing it was clear that quick dips in Jet 5 or hypochlorite were ineffective, so in the second and third rounds, longer exposure durations were introduced. It appears so far the most reliable treatments are 15 or 30 second dips in Jet 5 or hypochlorite, with a repeated alcohol (iso-propanol) spray, the next best. However the relatively long dip treatments are not really practical for routine use during field operations. Wiping with disinfectant wipes or rubbing the blade with hand gel were almost completely ineffective. However, in this first round of testing, a high number of bacteria were applied to the blade and were allowed a long time (1 – 2 hours) to dry on to the blade before the cuts in the medium were made. A second round of testing will be conducted next year that will attempt to replicate commercial conditions more closely.

Financial benefits

Current industry estimates indicate potential losses from bacterial canker during nursery production and soon after final planting in the range of £125,000 to £200,000 per annum.

Action points for growers

- No clear action points have been identified at this early stage in the work. The spray trials and assessments will continue over the three years of the project to establish evidence-based action points.
- However, good hygiene practices are important and knives and secateurs should be disinfected as frequently as possible.
- 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.

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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

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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/carryover 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

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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:

1. Identify the main sources of primary inoculum
2. Examine the potential of targeted treatments to reduce/eliminate inoculum
3. Examine the relative merit of different practical approaches for cleaning/disinfection of pruning knives/secateurs.
4. 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.

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Materials and Methods

Experimental design

Given the perennial nature of the host and disease development, spray trials and assessments will continue 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 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, initially on leaf/bud populations of bacterial canker pathogens and also on development of canker symptoms (in subsequent years). The treatments are shown in Table 1, and include a copper-based bactericide and the newly-approved biological control agent Serenade ASO (a strain of *Bacillus subtilis), a* glucohumate product which has shown promise against a bacterial disease on walnuts in Italy. Mancozeb is also widely used in France and Australia in combination with copper for control of bacterial pathogens of stone fruits and nuts.

Approximately 12 individual stock hedge plants were allocated to each treatment, 2-3 mother plants and 100 rootstocks.

Table 2. Treatment codes, products and rates used in spray trial.

Code	Product	a.i.	Rate	Approval status
A	Cuprokylt plus adjuvant (Activator 90)	copper oxychloride	3 g/L Cuprokylt + 0.25 mL/L Activator	LTAEU for outdoor ornamental plant production
B	Serenade ASO	Bacillus subtilis	10 mL/L	SOLA for ornamental plant production
C	Bactime Cu L4F	copper + glucohumate	4 g/L	Not approved. Foliar fertiliser
D	Aliette 80WG	Fosetyl-aluminium	1 g/L	On-label approval for ornamental plant production
E	Dithane NT + Cuprokylt	Mancozeb + copper oxychloride	2 g/L Dithane + 3 g/L Cuprokylt	Dithane NT LTAEU for outdoor ornamental plant production Cuprokylt (see Code A)
U	Untreated control	n/a	n/a	n/a

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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, and 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/bud samples were collected from each nursery on four occasions during the growing season. Visits were timed to occur shortly after sprays had been applied. Two visits were required during summer 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. 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/twigs selected at random whilst walking around the tree. For bud-wood samples 5-10 branches/twigs were collected and prepared as bud-wood (i.e. leaves removed).

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 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 5 minutes and a tenfold 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 sectored plates of PAF and SNA media.

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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 SNA medium, and oxidase reaction. Based on these results isolates were considered to be potential *P. syringae* or not. Further characterisation of *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 NSB, and tobacco hypersensitivity reaction. Based on the results of these tests, isolates were assigned to either *Psm* or *Pss*. When available, some isolates were also inoculated into immature cherry fruit. A representative selection of isolates was frozen on glass beads in NB+15% glycerol for future reference and characterisation.

Statistical analysis

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 treatments means were obtained as predictions from the model. All analysis was performed using Genstat. In each case three separate analyses were done: combined data (i.e. either *Psm* or *Pss* detected), for *Psm* alone, for *Pss* alone.

Disinfection of pruning tools

A known strain of *Psm* 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 containing ca. 10^8 CFU/ml 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.

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$^{(TM)}$ tablets), or a hand sanitising gel (Deb, FloraFree). Following treatement 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 with bacterial growth recorded.

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Results

Overall a total of 204 leaf or bud samples were collected and processed. A matrix of the material sampled and when the potential canker pathogens were detected is shown in Table 2. Both of the target pathogens (i.e. either *Psm* or *Pss)* were isolated from samples at both nurseries throughout the year. 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, bud, rootstocks).

Analyses of deviance (see Appendix 1) 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. all suspects colonies sub-cultured tended to be the same. In the few cases where both *Pss* and *Psm* were detected this tended to be in separate sub-samples.

Proportion of leaves/buds infested

Psm

Host, production stage, and spray treatment were the most significant factors. *Psm* was detected more frequently on plums than on cherry (Table 3), particularly on buds and stock hedges. Of the spray treatments, A (Cuprokylt) and B (Serenade ASO) gave a significant reduction compared to the untreated control (Fig 1).

Pss

Month x site and production stage x species were the most significant factors. *Pss* was detected more frequently in plum buds than in other production stages, and at a lower frequency in the stock hedges of both plum and cherry. The frequency of *Pss* was higher in the autumn (Fig 2), with a bigger increase at Site 2 than at Site 1. Spray treatments did not have a significant effect, although the lowest values occurred for Treatments A (Cuprokylt) and E (Cuprokylt + Dithane NT).

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Table 3. Matrix showing the materials sampled and when and the micro-organisms detected.

Site	Treat	Species	Cultivar	Stage	May	Aug	Oct
1	A	instita	St. Julien	SH	nd		Pss
	B				nd		Pss
	C				Psm		Psm
	D				Psm		Psm
	E				Psm		Psm
	U				Psm		Psm
	A	avium	Colt	SH	Ps		nd
	B				Pss		Pss
	C				Ps		nd
	D				Psm		nd
	E				Psm		nd
	U				Psm		Pss
	A	domestica	Victoria	MP/Bud	nd	Psm	
	B				nd	Pss	
	C				Psm	Psm	
	D				Pss	Pss	
	E				Ps	Pss	
	U				Ps	Psm	
	A	avium	Stella	MP/Bud	nd	nd	
	B				Pss	nd	
	C				Pss	nd	
	D				Ps	nd	
	E				Ps	nd	
	U				Pss	nd	
	A	serrulata	Kiku-Shidare	MP/Bud		nd	
	B					nd	
	C					Pss	
	D					Pss	
	E					nd	
	U					Pss	
	A	instita	St. Julien	RS2		nd	Ps
	B					nd	Pss
	C					Psm	Pss
	D					nd	Pss
	E					nd	Pss
	U					Psm	Psm + Pss
	A	avium	Colt	RS2		Pss	Pss
	B					Psm	Pss
	C					nd	Pss
	D					nd	Pss
	E					nd	Pss
	U					nd	Pss
2	A	instita	St. Julien	RS2	nd	nd	Ps
	B				nd	nd	Pss
	C				Psm	Psm	Pss
	D				nt	nd	Pss
	E				nd	nd	Pss
	U				nd	Ps	Pss
	A	avium	Colt	RS2	nd	nd	Pss
	B				Pss	nd	Pss
	C				nd	nd	Pss + Psm
	D				Pss	Pss	Ps
	E				nd	Pss	Pss
	U				Pss	Pss	Pss

Key: SH = stock hedge; MP = mother-plant; Bud = budwoed; RS2 = 2nd yr. rootstock; nd = not detected; Pss = *Pseudomonas syringae* pv. *syringae;* Psm = *P. syringae* pv. *morsprunorum.*; Ps = *P. syringae*, not Pss or Psm.

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Combined

For the combined results of both pathogens, month, host and spray treatment were the most significant factors. The proportion of leaves infested was greater in October than in August and May. Plums had a higher frequency than cherry, particularly buds and stock hedges. Treatment A (Cuprokylt) gave the biggest reduction in the proportion of contaminated leaves compared to the control (Fig 1). Treatment E (Cuprokylt + Dithane NT) also gave a significant reduction. Values for treatments B and D were also lower than the control but not significantly so.

Table 4. Estimated % leaves/buds contaminated with potential canker pathogens, and mean $\log_{10}$ of the number of bacteria per leaf/bud

Host	Psm		Pss		Comb	
	%	*Log_{10} Count*	*%*	*Log_{10} Count*	*%*	*Log_{10} Count*
Cherry	0.18	1.17	1.02	2.23	1.27	2.26
Plum	1.12	2.68	1.04	3.04	2.62	3.2

Numbers of bacteria

Psm

Host and spray treatment were the most important factors affecting the mean number of *Psm* per leaf. Numbers of *Psm* were greater on plum than on cherry. All treatments except treatment C (Bactime Cu L4F) reduced numbers compared to control, with the biggest reductions from A (Cuprokylt) and B (Serenade ASO).

Pss

Host and month were the most important factors affecting the mean number of *Pss* per leaf. Numbers were greater on plum than on cherry and increased in the autumn.

Combined

For the combined numbers, host had the biggest overall effect with numbers much greater on plum than on cherry. There was also a significant site x month interaction: at Site 1 numbers were similar at each assessment, but at Site 2 there was a sharp increase in the autumn. Numbers were lower for Treatment A (Cuprokylt), but this effect was only marginally significant.

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Disinfection of tools

The results are summarised in Table 4. Three rounds of testing were done on separate occasions. With some of the treatments modified according to the results of the previous round.

Table 5. Summary of disinfection tests. Each replicate consisted of ten 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	Reps	%
U	Untreated control	5	100
SW	Spray with 70% iso-propanol, leave 30 s then wipe dry with paper towel.	5	60
SW2	Spray with 70% iso-propanol, wipe residue, repeat spray leave 30 s then wipe dry with paper towel.	3	10
W	Wipe with Azo wipes (70% iso-propanol), more care to ensure visible residue removed.	2	100
J5_0	Brief dip in Jet 5 (0.8%) then wipe dry	4	92.5
J5_15	15 s dip in Jet 5 (0.8%) then wipe dry	3	0
J5_30	30 s dip in Jet 5 (0.8%) then wipe dry	4	2.5
Cl_0	Brief dip in 1% hypochlorite then wipe dry	1	100
Cl_30	30 s dip in 1% hypochlorite then wipe dry	1	0
GW	Rub edge of blade with alcohol hand gel between finger and thumb, wipe dry	2	95

Thus, in the first round of testing it was clear that quick dips in Jet 5 or hypochlorite were ineffective, so in the second and third rounds, longer exposure durations were introduced. It appears that so far the most reliable treatments 15 or 30 second dips in jet 5 or hypochlorite, with a repeated alcohol (iso-propanol) spray, the next best.

Discussion

Epidemiology and spray treatments

It appears that the two potentially pathogenic pathovars, *Psm* and *Pss*, behaved differently on the two host 'species' and the spray treatments appeared to have different effects on their populations.

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Both the proportion of tissues affected and numbers were greater on plum than on cherry with biggest difference occurring for *Psm.* Also, whereas numbers of *Psm* were similar throughout the season there was a big increase in *Pss* in the autumn. Given previous work on bacterial canker, the autumn increase for *Pss* was not surprising, but *Psm* was expected to behave in a similar way. However it is possible that this may be an artefact of the experimental details/methodology, as in an individual sub-sample the two organisms tended to be mutually exclusive: when detected, dilution plates were dominated by either one or the other. It is possible that these are real effects, but only a much higher sampling frequency would be able to resolve this.

It appears that spray treatments had little or no effect on *Pss*, but significant effects on *Psm.* Cuprokylt and Serenade ASO gave significant reductions in both the proportion of contaminated tissues and the numbers of *Psm.* The Bactime Cu L4F had no effect or even gave a slight increase. Aliette and the combined Cuprokylt + Dithane NT treatments gave smaller but significant reductions in populations, but not proportions of leaves. One surprising aspect of these results is that the Cuprokylt + Dithane NT combined did not appear to be as effective as Cuprokylt alone; given that the copper concentration was the same in both cases, it would be expected that even if there was no benefit from the Dithane NT, this treatment would give similar results to the Cuprokylt alone. It is possible that the inclusion of the adjuvant Activator 90 (a non-ionic surfactant/wetter) in the Cuprokylt-alone treatment was the reason for this difference. It remains to be seen if these reductions in levels of *Psm* have any impact on the levels of canker symptoms in subsequent years.

Disinfection of tools

These results suggest that effective disinfection of pruning knives/secateurs in the field may be more difficult than generally perceived. A quick dip in disinfectant and wipe dry with a paper towel would appear to be unlikely to prevent transmission of the pathogen from one cut to the next. Only with longer exposures to the disinfectant of 15 or 30 secs was disinfection generally effective and even then was not always 100%. However, to achieve such exposure times during labour-intensive field operations, may prove to be impractical. The two approaches to disinfection that were considered to be easiest to implement (wiping with disinfectant wipes, and using rubbing with a disinfectant hand gel) proved to be more or less ineffective.

It should be noted that the test system as implemented could be considered as a very stringent test (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. In results reported elsewhere, disinfection can be more easily achieved if attempted

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immediately after application of inoculum to the blade, but this probably also represents an unrealistic situation.

Given the wider potential importance of disinfection of pruning tools, although further work was not scheduled in this project, further investigation of practical approaches to disinfection in the field will be considered next year.

Conclusions

It is important to keep in mind that these conclusions are based on relatively limited sampling in only a single season; therefore these interpretations should be treated with a great deal of caution.

- 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 Cuproykylt 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.
- A practical approach to disinfection of pruning tools during field operations has not identified.

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Knowledge and Technology Transfer

No formal knowledge transfer at present.

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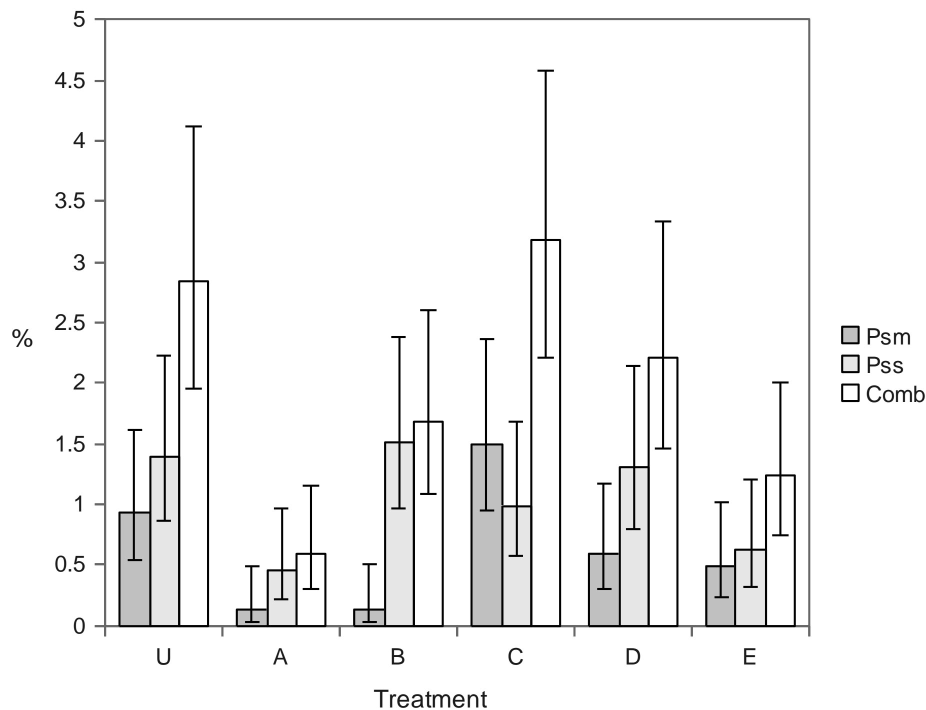

Figure 1. Effect of treatments on the estimated % of leaves contaminated with *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (Comb). Bars represent the 95% confidence limits.

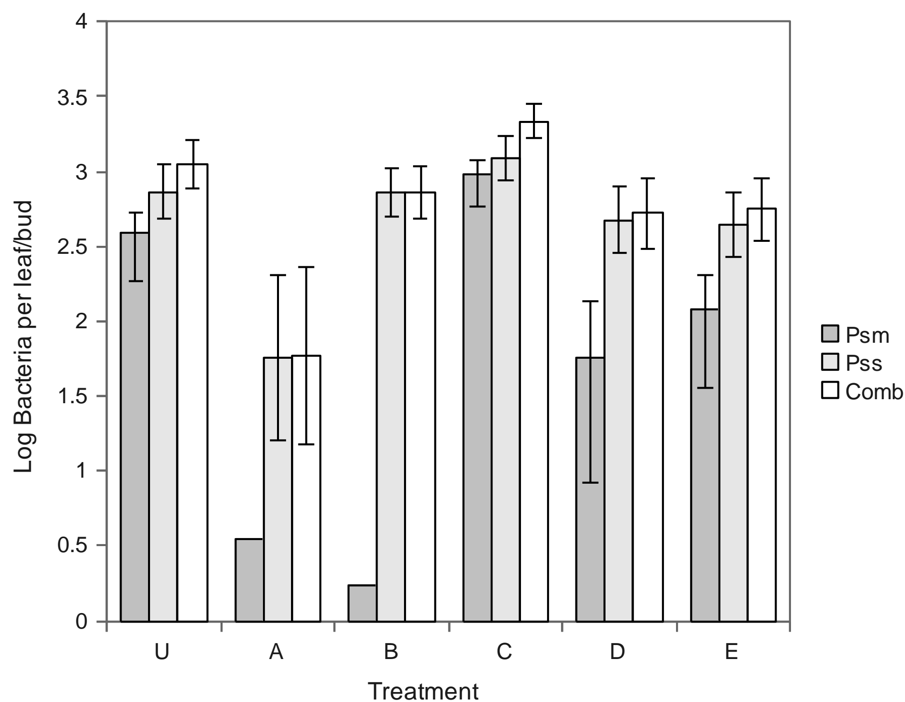

Figure 2. Effect of treatments on the log_{10} of the mean number of *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (Comb). Bars represent the standard errors of the means.

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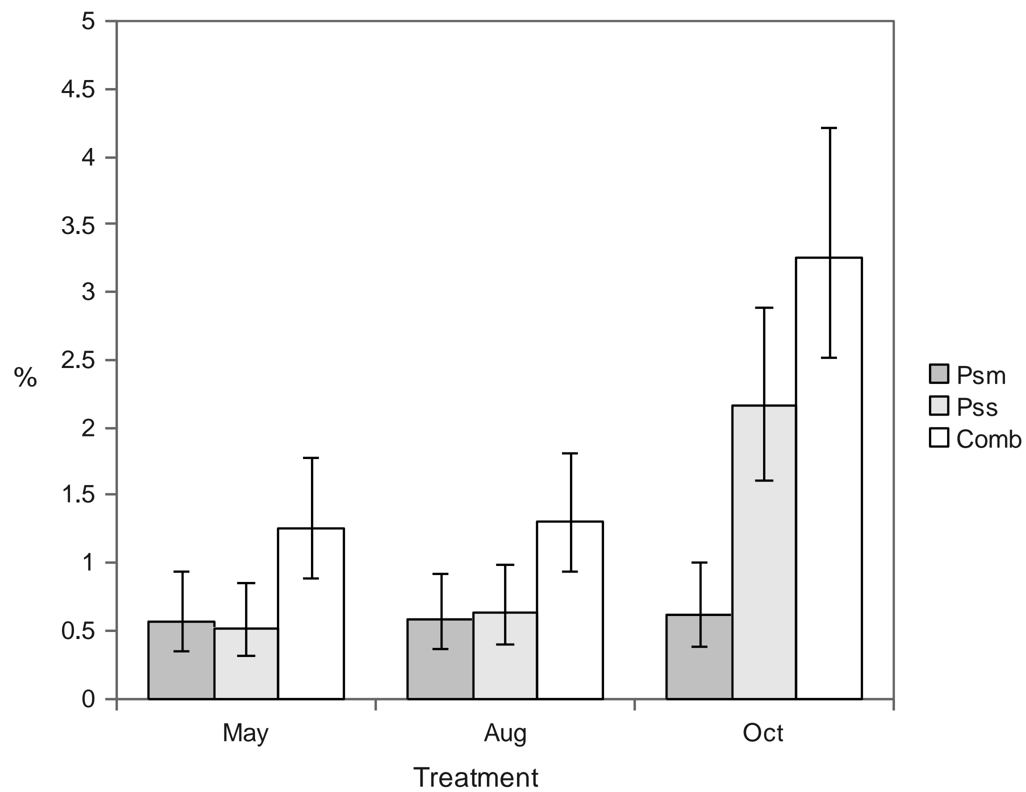

Figure 3. Effect of time of sampling month on the estimated % of leaves contaminated with *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), *P. s.* pv. *syringae* (*Pss*), and either or both (Comb). Bars represent the 95% confidence limits.

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APPENDIX 1

Summaries of analyses

For Psm proportions:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Site	1	4.7283	4.7283	4.73	0.030
+ Month	2	0.1241	0.0621	0.06	0.940
+ Site.Month	2	2.4458	1.2229	1.22	0.294
+ Host	1	34.4994	34.4994	34.50	<.001***
+ Site.Host	1	2.4739	2.4739	2.47	0.116
+ Site.Month.Host	4	14.8020	3.7005	3.70	0.005
+ Stage	3	24.9156	8.3052	8.31	<.001***
+ Host.Stage	3	5.9546	1.9849	1.98	0.114
+ Treat	5	54.8168	10.9634	10.96	<.001***
+ Host.Treat	5	28.3656	5.6731	5.67	<.001
+ Site.Treat	5	30.7020	6.1404	6.14	<.001
+ Stage.Treat	15	43.5380	2.9025	2.90	<.001
+ Samp.Med	178	13.4145	0.0754	0.08	1.000
Residual	178	44.3050	0.2489		
Total	403	305.0855	0.7570		

MESSAGE: ratios are based on dispersion parameter with value 1

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For Pss
proportions:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Site	1	4.3152	4.3152	4.32	0.038
+ Month	2	38.3211	19.1606	19.16	<.001***
+ Site.Month	2	19.9843	9.9921	9.99	<.001**
+ Host	1	0.1202	0.1202	0.12	0.729
+ Site.Host	1	0.0383	0.0383	0.04	0.845
+ Stage	3	31.6154	10.5385	10.54	<.001**
+ Host.Stage	3	18.8583	6.2861	6.29	<.001
+ Treat	5	17.0067	3.4013	3.40	0.004
+ Host.Treat	5	9.1992	1.8398	1.84	0.101
+ Site.Treat	5	15.8377	3.1675	3.17	0.007
+ Stage.Treat	15	31.4628	2.0975	2.10	0.008
+ Samp.Med	182	131.0064	0.7198	0.72	0.998
Residual	178	150.0971	0.8432		
Total	403	467.8626	1.1609		

MESSAGE: ratios are based on dispersion parameter with value 1

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Combined proportions:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
+ Site	1	0.0139	0.0139	0.01	0.906
+ Month	2	28.5512	14.2756	14.28	<.001**
+ Site.Month	2	13.6894	6.8447	6.84	0.001
+ Host	1	18.2421	18.2421	18.24	<.001**
+ Site.Host	1	6.1459	6.1459	6.15	0.013
+ Site.Month.Host	4	13.9565	3.4891	3.49	0.007
+ Stage	3	19.6060	6.5353	6.54	<.001
+ Host.Stage	3	20.3001	6.7667	6.77	<.001
+ Treat	5	47.0220	9.4044	9.40	<.001*
+ Host.Treat	5	10.1347	2.0269	2.03	0.072
+ Site.Treat	5	22.4097	4.4819	4.48	<.001
+ Stage.Treat	15	36.1504	2.4100	2.41	0.002
+ Samp.Med	178	169.1695	0.9504	0.95	0.670
Residual	178	153.0691	0.8599		
Total	403	558.4603	1.3858		

MESSAGE: ratios are based on dispersion parameter with value 1

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Psm counts:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Site	1	68987.7	68987.7	172.36	<.001*
+ Month	2	149544.3	74772.2	186.81	<.001*
+ Site.Month	2	40593.0	20296.5	50.71	<.001
+ Host	1	220099.8	220099.8	549.90	<.001****
+ Site.Host	1	12290.3	12290.3	30.71	<.001
+ Site.Month.Host	4	6044.8	1511.2	3.78	0.006_
+ Stage	3	123742.5	41247.5	103.05	<.001
+ Host.Stage	3	3556.2	1185.4	2.96	0.034
+ Treat	5	609425.1	121885.0	304.52	<.001****
+ Host.Treat	5	24556.4	4911.3	12.27	<.001
+ Stage.Treat	15	670614.9	44707.7	111.70	<.001
+ Samp.Med	182	53654.0	294.8	0.74	0.979
Residual	174	69643.8	400.3		
Total	398	2052752.7	5157.7		

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Pss counts:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Site	1	313359.	313359.	208.98	<.001**
+ Month	2	1479651.	739826.	493.38	<.001***
+ Site.Month	2	894865.	447432.	298.39	<.001**
+ Host	1	863010.	863010.	575.53	<.001***
+ Site.Host	1	309602.	309602.	206.47	<.001**
+ Site.Month.Host	4	79190.	19797.	13.20	<.001
+ Stage	3	282439.	94146.	62.79	<.001
+ Host.Stage	3	2798.	933.	0.62	0.602
+ Treat	5	349193.	69839.	46.57	<.001
+ Host.Treat	5	179820.	35964.	23.98	<.001
+ Stage.Treat	15	453693.	30246.	20.17	<.001
+ Samp.Med	181	337105.	1862.	1.24	0.075
Residual	175	262413.	1500.		
Total	398	5807138.	14591.		

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Combined counts:
Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Site	1	142315.	142315.	77.33	<.001*
+ Month	2	856033.	428017.	232.58	<.001**
+ Site.Month	2	1652807.	826403.	449.06	<.001***
+ Host	1	1082286.	1082286.	588.10	<.001***
+ Site.Host	1	192803.	192803.	104.77	<.001*
+ Site.Month.Host	4	81029.	20257.	11.01	<.001
+ Stage	3	267956.	89319.	48.53	<.001
+ Host.Stage	3	30670.	10223.	5.56	0.001
+ Treat	5	675131.	135026.	73.37	<.001*
+ Host.Treat	5	190151.	38030.	20.67	<.001
+ Stage.Treat	15	1048317.	69888.	37.98	<.001
+ Samp.Med	181	614502.	3395.	1.84	<.001
Residual	175	322054.	1840.		
Total	398	7156054.	17980.		

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