Project number	**TF 217**
Project title:	Improving the management of bacterial canker in stone fruits
Project leader:	Dr S J Roberts, Plant Health Solutions Ltd.
Report:	Final report, May 2015
Previous report:	None
Key staff:	Dr S J Roberts
Location of project:	PHS Laboratory, Ryton Gardens Poly-tunnel at Warwick Crop Centre
Industry Representative:	Mr Steve Castle, Mount Ephraim Farm, Kent
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AUTHENTICATION

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

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CONTENTS

GROWER SUMMARY

Headlines

- Copper oxychloride is still the most effective product to use against bacterial canker caused by *Pseudomonas syringae* pv. *morsprunorum*.
- Copper may not be so effective against bacterial canker caused by *Pseudomonas syringae* pv. *syringae,* as some strains were found to be copper resistant.
- There was no evidence for a benefit from any of the 'resistance inducers' or 'elicitors' or disinfectants applied as foliar sprays.

Background and expected deliverables

Bacterial canker of *Prunus* spp. has been an on-going problem for stone fruit growers for many years. It 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* potentially has a much wider host range, with the potential for cross infection between a number of different species and genera.

Bacterial canker can kill trees, but as well as cankers, these pathogens may also cause leaf spots/shot-holes, shoot die-back, flower blights, fruit spotting and rots, although the stem canker phase is probably the most economically important.

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. Therefore cankers are sometimes not seen until 18 months after the initial infection has taken place.

For many years (based on work done at East Malling Research 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 in 2001-02, led by the current project leader, it became clear that both pathogens were causing canker in England (Vicente *et al.* 2004).

An HDC-funded project on bacterial canker during nursery production (HNS 179) (Roberts 2013) has recently been completed.

As part of HNS 179 we reviewed (in 2012) (Roberts 2013) the global research literature on the control of bacterial canker and a factsheet is in production. We do not expect that the global situation has changed much since that time, therefore it was considered to be more cost-effective to re-target this information, changing the emphasis to fruit rather than nursery production.

Also as part of HNS 179 (Roberts 2013) we conducted three years of spray trials on trees during nursery production. The overall conclusion was that copper oxychloride was the most effective spray treatment. However, partly due to limitations in the in the scope of the project, and partly due to HDC policy, so-called 'grey-products' (i.e. products that are not marketed as plant protection products but may nevertheless provide some benefit) were not examined. HDC policy has now changed as a result of a change in guidance form CRD.

There have been recent reports from the USA that copper sprays have become ineffective due to the development of resistant pathogen strains (Scheck, Pscheidt and Moore 1996; Pscheidt 2013). There is no recent information (two strains were tested in HNS 91 in 2000) (Roberts and Akram 2002) on whether or not UK strains of the bacterial canker pathogens are resistant. As a result of the work in HNS 179 we have a collection of pathogen strains from trees which have been sprayed six times a year with copper for three years, plus strains from untreated trees. These strains therefore present an ideal opportunity to examine the potential for resistance to develop in the UK.

The main objectives of the project were to:

1. Perform preliminary evaluations of potential spray products.
2. Determine if there is any evidence of copper resistance in recent isolates of the pathogens.
3. Produce best-practice guidelines for the management of bacterial canker in plums and cherries during fruit production.

Summary of the project and main conclusions

Spray trials

Spray trials were carried out on plums (cv. Victoria) inoculated with *Psm.* Nine treatments plus an untreated control were examined. A number of potential 'resistance inducers' or 'elicitors' and other products (see Table 1) were included. The trees used were potted maidens growing in a polytunnel. Spraying and inoculation was done at two times of the year: in late spring to examine effects on leaf populations and leaf symptoms and in the autumn to examine the development of the canker phase resulting from leaf scar infections. In both cases all products (see Table 1) except the disinfectants were sprayed onto the trees one week before inoculation. The disinfectants were applied either the day before or day after inoculation.

Table 1. Spray treatments, rates, and timings

Code	Product (Active ingredient)	Rate*	Timing (relative to day of inoculation)	Basis for inclusion (approval status)
Un	Untreated control	-	-	Negative control
Cu	Cuprokylt (copper oxychloride) + Activator 90 wetter	3 kg/ha, 0.25 mL/L Activator	Spray 7 d before	Standard treatment (full approval)
Bi	Bion (acibenzolar-s-methyl)	60 g/ha	Spray 7 d before	Resistance inducer, positive reports vs. citrus canker (not approved)
Hx	Hexanoic acid	1 mM	Spray 7 d before	Resistance inducer, positive reports vs. citrus canker (not approved)
Ph	Phorce (phoshite)	2 L/ha	Spray 7 d before	Resistance inducer (foliar fertiliser, approval not required)
Fr	Frostect (Harpin protein)	200 g/ha	Spray 7 d before	Resistance inducer, indication of activity vs. fireblight (not a PPP, approval not required)
Se	Sentry R (plant extract from *Reynoutria* spp.) with Yuccah wetter	1% plus 0.04% wetter	Spray 7 d before	Resistance inducer (not a PPP, approval not required)
Fe	Fenomenal (fosetyl-aluminium and fenamidione)	2.25 kg/ha	Spray 7 d before	Contains fosetyl-aluminium, which had indications of benefit v. canker in HNS 179 (not approved)
J5	Jet 5 (peroxyacetic acid)	0.8%	Spray 1 d before/after	Disinfectant (not approved)
Xi	XzioX (chlorine dioxide)	50 ppm	Spray 1 d before/after	Disinfectant (not approved, but may be used to disinfect water)

*All products were applied as a high volume spray, equivalent to 1000 L/ha

In the Spring treatment, inoculation was done by spraying the leaves with a suspension of Psm. Leaves were than sampled six days later and 'washed' to estimate pathogen populations, and leaf symptoms recorded two to three weeks later. The leaf inoculations successfully resulted in the development of typical disease symptoms. Cuprokylt (copper

oxychloride) was the only product that gave any reduction in pathogen populations compared to the untreated control, and although there was also a reduction in the percentage of infected leaves, this was not statistically significant. The autumn treatments were assessed the following spring. However, it appears that the inoculations failed as there was no disease development even in the untreated controls (we expected to at least see death/failure of some of the buds). Therefore no conclusions could be drawn about the effects of the treatments on leaf scar infections.

None of the treatments gave any indications of phytotoxicity.

Copper resistance

To check for copper resistance, twenty-two isolates of the bacterial canker pathogens, obtained from spray trials done as part of the previous HDC-funded project (HNS 179), were tested for copper resistance. Isolates came from both plums and cherries; some were from trees that had received up to 18 copper sprays over 3 years.

None of the eleven isolates of *Psm* showed any signs of copper resistance. However, most (seven out of eleven) of the *Pss* isolates showed some level of copper resistance. Thus, at least some of apparently inconsistent levels of control with copper sprays could be a result of the presence of copper resistant strains of *Pss,* particularly on cherry where *Pss* may be more prevalent. It should be noted that these 'resistant' strains are not completely resistant to copper and growth was still inhibited at higher copper concentrations, but it does highlight the need to understand which pathogen is responsible for causing disease in any particular orchard.

Table 2. Summary of copper sensitivity tests on 22 strains of *Pseudomonas syringae* isolated from copper-treated and un-treated plum and cherry trees.

Pathovar	Source	No. resistant	No. tested
Pseudomonas syringae pv. *morsprunorum*	Plum	0	7
	Cherry	0	4
Pseudomonas syringae pv. *syringae*	Plum	3	5
	Cherry	4	6

Financial benefits

There are no particular financial benefits arising directly from this project, but growers may be able to make savings by not applying sprays that may have little direct benefit.

The value of UK plum production is potentially around £12 million pa (Defra statistics 2011 value). The values for cherry production are no longer reported separately but are likely to be over £2 million (based on the most recent, 2007 figure). Bacterial canker has been a continuing problem for plum and cherry growers for many years. There are no definitive estimates for losses caused by bacterial canker and the impact of the disease on individual

growers is likely to vary considerably depending on factors such as orchard age, intensity of production, etc. However, even a conservative estimate of average losses of ca. 5% p.a. would mean reducing losses from bacterial canker could be worth in excess of £0.5 million p.a.

Action points for growers

- Copper sprays in the form of Cuprokylt + wetter (Activator 90) are still the most effective chemical control option available for bacterial canker caused by *Psm*.
- Copper sprays may be less effective against *Pss* due to the presence of resistance strains. It is therefore important to send samples for accurate diagnosis to understand the 'enemy'.
- In a previous project the highest levels of *Psm* were seen in the spring and summer, so the current label recommendations for three sprays in late summer may be starting too late to have a significant impact and growers may wish to consider earlier spray applications.
- New orchards should ideally be planted with pathogen-free trees.
- Growers should not rely on EU plant passports as an indication of health status and freedom from bacterial canker pathogens. Trees and their mother-plants should be inspected for disease symptoms when in leaf.
- Indexing of mother plants and trees for planting for pathogen should also be considered.

SCIENCE SECTION

Introduction

Bacterial canker of *Prunus* spp. has been an on-going problem for stone fruit growers for many years. It 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.

Bacterial canker can kill trees, but as well as cankers, these pathogens may also cause leaf spots/shot-holes, shoot die-back, flower blights, fruit spotting and rots, although the stem canker phase is probably the most economically important. It should be noted 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.

Traditionally (based on work done at East Malling in 1960's and 1970's), *Psm* was considered to be the primary cause of the disease in the UK, but in the USA and elsewhere, *Pss* is often the most common cause of bacterial canker. However, during a MAFF-funded survey of 'Farm Woodland' cherries in 2001-02, it became clear that both pathogens were causing canker in England (Vicente *et al.* 2004). A recent HDC-funded project on bacterial canker during nursery production from 2010 to 2013 (HNS 179) (Roberts 2013) found that *Psm* was most prevalent on plum, whereas *Pss* was more common on cherry.

As part of HNS 179 we reviewed (in 2012) (Roberts 2013) the global research literature on the control of bacterial canker and a fact-sheet is in production. We do not expect that the global situation has changed much since last year, therefore it would be most cost-effective to re-target this information, changing the emphasis to fruit rather than nursery production.

Also as part of HNS 179 (Roberts 2013) we conducted three years of spray trials on trees during nursery production. The overall conclusion was that copper oxychloride was the most effective spray treatment. However, partly due to limitations in the scope of the project, and partly due to HDC policy, one area was not explored in the spray trials: so-called 'grey-products' i.e. products that are not marketed as plant protection products but may nevertheless provide some benefit. HDC policy has now changed as a result of a change in guidance form CRD.

There have been recent reports from the USA that copper sprays have become ineffective due to the development of resistant pathogen strains (Scheck, Pscheidt and Moore 1996; Pscheidt 2013). There is no recent information (two strains were tested in HNS 91 in 2000)

(Roberts and Akram 2002) on whether or not UK strains of the bacterial canker pathogens are resistant. As a result of the work in HNS 179 we have a collection of pathogen strains from trees which have been sprayed six times a year with copper for three years, plus strains from untreated trees. These strains therefore present an ideal opportunity to examine the potential for resistance to develop in the UK. The objectives of this project therefore were to:

(1) Perform preliminary glasshouse evaluations of potential spray products;

(2) Determine if there is any evidence of copper resistance in recent isolates of the pathogens;

(3) Produce best-practice guidelines for the management of bacterial canker in plums and cherries during fruit production.

Materials and methods

Plant material

Budded rootstocks (plum cv. Victoria budded on St. Julian A) were purchased as bare root dormant trees from F P Matthews Ltd. Trees had been budded in August 2013. Trees were lifted in late February and then cold-stored prior to collection and potting up on 17 March. Trees were potted into 8 L polythene pots of a peat-based potting mix with Forest Gold Plus, trace elements and a 12 month controlled release fertiliser.

The potted trees were grown in a poly-tunnel with open but screened ends at Warwick Crop Centre, and routinely hand-watered (by WCC staff) by application direct to the compost in the pot as required.

Trees were headed back to the grafted bud at the end of March when bud growth was beginning to initiate. Aluminium bud clips were applied to all trees to encourage vertical growth of the scion bud. Once significant growth of the scion bud had occurred (mid-May), suckers were removed, and lower buds rubbed out. Trees were then staked and tied in and set out in plots in three rows and three blocks. Each plot consisted of three or four trees in a row with a spacing of about 0.75 m between plots within the row, and spacing of about 2 m between rows. At the end of June, all trees were pruned back to a uniform height.

Following the first inoculation the trees became infested with rust mites. To control them a spray of Thiovit (sulphur) was applied and followed up with distribution of a predator mite (*Amblyseius andersonii*) in Gemini sachets (one per plot).

Spray treatments

Sprays were applied to the trees either one week before (resistance inducers, and copper) or the day before or day after inoculation (disinfectants), using a hand-held sprayer (Matabi 5L) fitted with a constant pressure regulator (adjusted to 1.5 bar) and an Orange Flat Fan Evenspray Nozzle (except for Cuprokylt which had a tendency to block the nozzle so a yellow nozzle was used for the Autumn sprays). Trees were sprayed from both sides to ensure even coverage. Products and rates are shown in Table 3. Trees were sprayed on two occasions: late spring, one week before leaf inoculations; autumn, one week before leaf scar inoculations.

Table 3. Spray treatments, rates, and timings

Code	Product (Active ingredient)	Rate*	Timing (relative to day of inoculation	Basis for inclusion (approval status)
Un	Untreated control	-	-	Negative control
Cu	Cuprokylt (copper oxychloride) + Activator 90 wetter	3 kg/ha, 0.25 mL/L Activator	Spray 7 d before	Standard treatment (full approval)
Bi	Bion (acibenzolar-s-methyl)	60 g/ha	Spray** 7 d before	Resistance inducer, positive reports vs. citrus canker (not approved)
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J5	Jet 5 (peroxyacetic acid)	0.8%	Spray 1 d before/after*	Disinfectant (not approved)
Xi	XzioX (chlorine dioxide)	50 ppm	Spray 1 d before/after*	Disinfectant (not approved)

*All products were applied as a high volume spray, equivalent to 1000 L/ha

Production and preparation of Inoculum

A recent isolate of *Psm* Race 1 that had been obtained from plum cv. Victoria in 2012 was used to prepare inoculum. The bacterium was sub-cultured to a plate of *Pseudomonas* Agar F (PAF, Difco) and incubated for two days at 25°C. Growth from the plate was scraped with a sterile loop and suspended in 20 mL of sterile deionised water (SDW). A 10 mL aliquot of this suspension was then further diluted in 1 L of SDW to produce a 'just visibly turbid' suspension, estimated to contain approx 1 x 10^6 CFU/mL, that was used as inoculum.

Inoculation

Leaf inoculation was done on 03 June, one week after spray applications. Inoculation was done in two ways:

(a) by leaf infiltration, as this has been commonly used to demonstrate effects of resistance inducers as reported in the scientific literature, and is presumed to provide a direct indication of tissue resistance (as opposed to resistance resulting from physical barriers to ingress of bacteria into the tissues)

(b) by spraying onto the leaf surfaces, as this is considered to mimic the natural route for leaf infection.

For (a) a sterile syringe containing inoculum was pressed gently against the lower (abaxial) surface of a leaf and pressure applied to produce a water-soaked spot of a diameter equivalent to the diameter of the syringe outlet (i.e. 3-4 mm). This was done on six points on a single leaf on each tree.

For (b) the inoculum was sprayed onto the upper and mid-levels of the foliage with a hand-held mister to leave a surface coating of fine droplets with little or no run-off.

Leaf scar inoculation was done late afternoon on 2 October, one week after spray inoculations. Five leaves were removed from each of two or three branches on each tree and the freshly exposed leaf scars were immediately sprayed with inoculum using a hand-held 500 mL mister from both sides of the stem. Each of the inoculated branches was marked by applying a loop of green tying tape.

Following inoculation of both leaves and leaf scars, and the applications of post inoculation disinfectant sprays, trees were overhead irrigated for five minutes twice a day for five days to encourage infection.

Leaf sampling and estimation of leaf populations

Six days after inoculation, leaves were sampled to estimate populations of *Psm*. Sampling and processing was as described in HNS 179 (Roberts 2013). Essentially, five leaves were collected from each tree (10 or 15 per plot) into a stomacher bag. Leaves were then stomached in a minimal volume of sterile saline plus Tween 20, and the extract diluted and plated on mP3 and MS3 media. Plates were then incubated, and suspect *Psm* colonies counted and their identity confirmed.

Grower visits

Visits were made to plum and cherry growers in Kent and in Herefordshire. At each visit, general aspects of the disease were discussed, and attempts made to answer specific questions from the growers, explain the thinking behind the current project and present results obtained to date. Growers were questioned about their current approaches to bacterial canker control, growing systems, sourcing of plant material, and checks on health status.

Disease assessments

For the leaf inoculations, disease symptoms were recorded on two occasions: at approx. two and three weeks after inoculation. The number of infiltrated spots on the infiltrated leaves with necrosis were recorded, together with the number of leaves with symptoms (excluding the infiltrated leaves, and those removed for population counts) and maximum disease severity score (0-4 scale).

For the autumn leaf scar inoculations, disease symptoms were recorded in the following spring (late April and early May 2015) following bud burst. For each inoculated branch, on each tree, the number of buds (out of five inoculated) which had failed to grow or were showing other symptoms were recorded.

Copper sensitivity tests

Twenty-two isolates of *Psm* and *Pss* from cherry and plum were selected for testing. Isolates had been obtained from a three-year spray trial as part HNS 179 (Roberts 2013) from control trees which had not been sprayed with copper during the three-year trial or from trees that had received 18 sprays over the three years.

Isolates were recovered from the freezer and grown on PAF plates. Isolates were then sub-cultured to sectored plates of CYEG agar medium to provide inoculum for sensitivity testing. Two methods of testing were used: surface plating and liquid culture.

For surface plating, suspensions of each isolate were prepared in 2 mL of SDW using a (1 µl) loopful of growth from CYEG agar plates. A 5 µl drop of suspension of each isolate was then dropped onto duplicate plates of CYEG agar medium containing 0, 0.25, 0.5 and 1.0 mM copper (II) sulphate ($CuSO_4$). Plates were then incubated for up to seven days and the presence/absence of growth noted for each isolate on each plate.

For liquid culture, 200 µl aliquots of liquid CYEG medium containing 0, 0.25, 0.5 and 1.0 mM copper (II) sulphate ($CuSO_4$) were dispensed into a series of duplicate rows of a sterile 96-well plate with lid. A 5 µl drop of suspension of each isolate prepared as above was then added to each well of column. Plates were then incubated for up to seven days and the presence/absence of bacterial growth in each well was recorded.

Each isolate was tested at least twice.

Statistical analysis

The effect of treatments on the numbers of bacteria per leaf 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. Means and standard errors were obtained as predictions from the model, after fitting the appropriate model terms.

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

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

Results

Spring leaf inoculations

In the 'leaf wash' assessment relatively high numbers of *Psm* were detected on leaves from all treatments and ranged from about 10^6 to 10^7 CFU per leaf. The effect of the spray treatments on leaf populations of *Psm* is shown in Figure 1. The overall analysis of deviance indicated a marginally significant effect of spray treatment on leaf populations, with Cuprokylt the only treatment to give a significant reduction compared to the control, and some treatments appearing to result in an increase compared to the control.

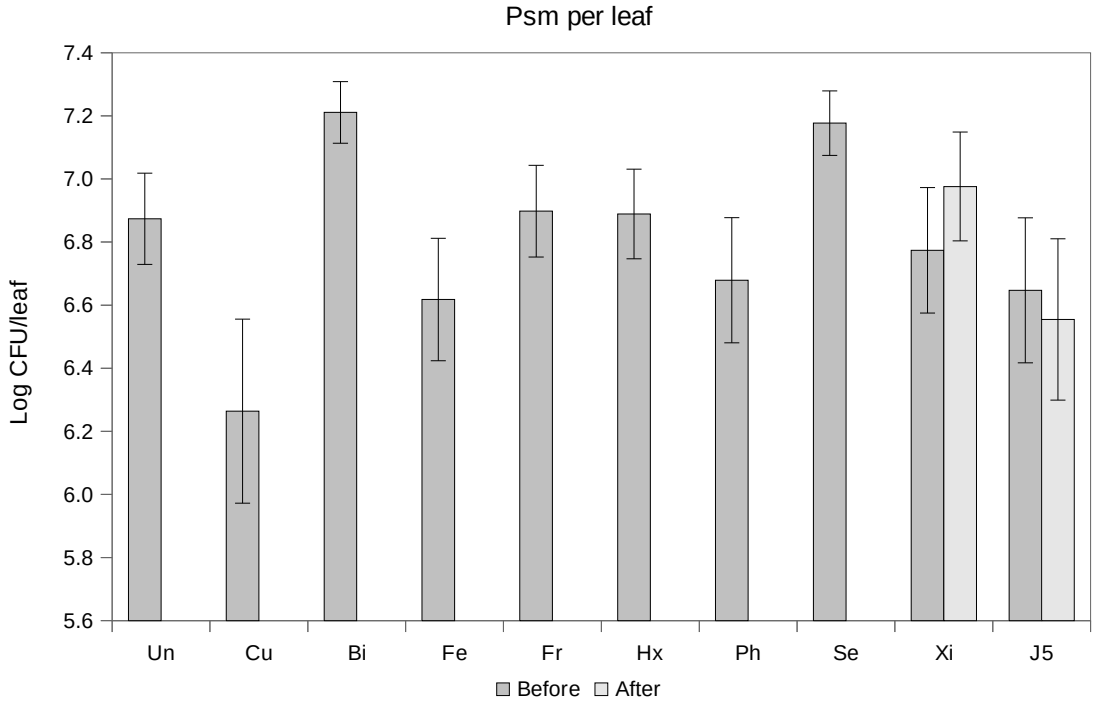

Figure 1. Effect of spray treatments on the leaf populations of *Pseudomonas syringae* pv. *morsprunorum* (*Psm*), six days after inoculation. For treatment codes see Table 3.

A similar pattern was seen in the proportion of leaves with symptoms (excluding the infiltrated leaves and leaves removed for population counts) (see Figure 2). The analysis of deviance indicated that overall there were no significant effects of spray treatments on the proportion of leaves with symptoms. Thus, although Cuprokylt and Xziox applied the day before inoculation had the lowest proportions of leaves with symptoms, these reductions were not significant.

In the infiltrated leaves, all six infiltrated spots on all leaves on all treatments resulted in typical symptoms of a dark initially water-soaked area, the area then became necrotic with water soaked margins and then dropped out to leave a hole. There were no differences between treatments and so a formal analysis was not done.

There were no signs of phytotoxicity with any of the treatments.

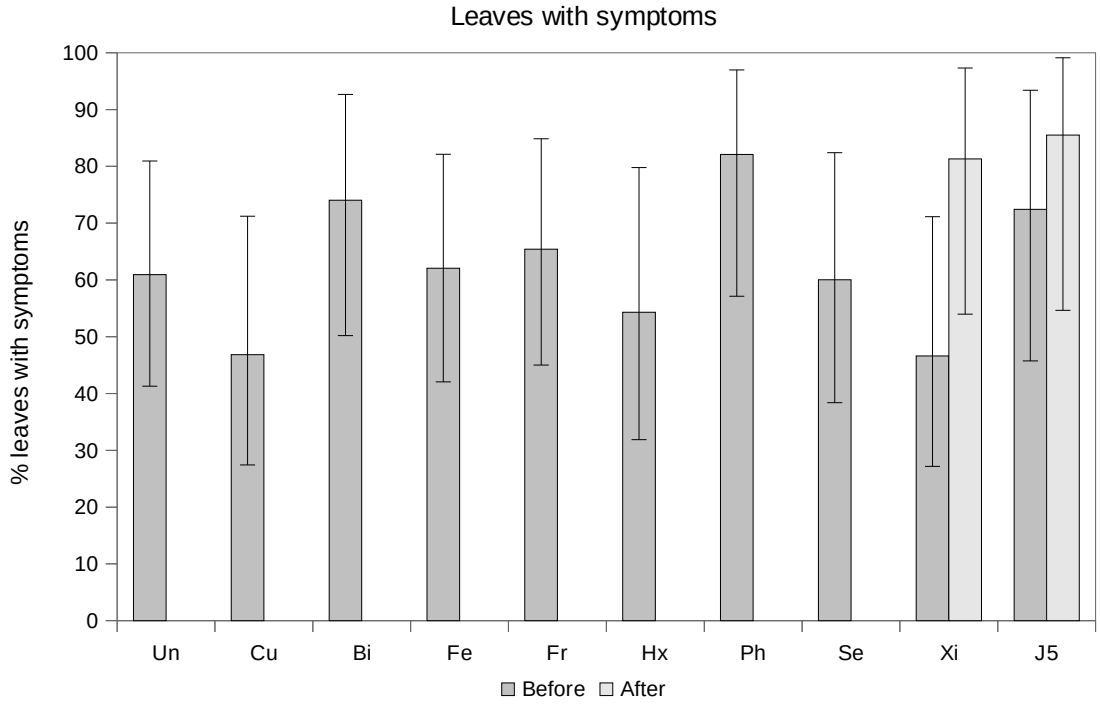

Figure 2. Effect of spray treatments on the % of leaves with disease symptoms about 2 weeks after inoculation with *Pseudomonas syringae* pv. *morsprunorum*. For treatment codes see Table 3.

Autumn leaf scar inoculations

There was little or no evidence of any disease development in the spring following autumn leaf scar inoculations, with nearly all re-growth on all trees looking extremely healthy. Symptoms of bud death were seen in only two plots out of 30 (three out of 96 trees, four branches). In particular, there was no visible disease on any of the untreated control trees, making detailed statistical analysis pointless.

Copper sensitivity tests

There was a marked difference in copper sensitivity between isolates of *Psm* and *Pss* (Table 2). Growth of all 11 of the *Psm* isolates was inhibited at all concentrations of copper, and so can be considered fully sensitive. Whereas seven out of the 11 *Pss* isolates grew in the presence of 0.25 ppm copper or more and can be considered resistant. Of the resistant *Pss* isolates three out of five were from plum and four out of six were from cherry, with no apparent relationship to the treatments (untreated vs. Cuprokylt treated) or number of Cuprokylt treatments that the trees had received.

Table 4. Summary of copper sensitivity tests on 22 strains of *Pseudomonas syringae* isolated from copper-treated and un-treated plum and cherry trees.

Pathovar	Source	No. resistant	No. tested
Pseudomonas syringae pv. *morsprunorum*	Plum	0	7
	Cherry	0	4
Pseudomonas syringae pv. *syringae*	Plum	3	5
	Cherry	4	6

Grower visits

Some common themes and observations emerged during grower visits:

- Plant material for most recent plantings, especially cherry on Gisela rootstocks, had invariably been sourced from continental Europe;
- Little or no specific attention had been given to the health status of the imported plant material, with respect to bacterial canker, relying on implied health as a result of the EU plant passport system;
- Orchards may have an expected lifetime of up 25 years, but it is not clear to what extent bacterial canker contributes to a reduction;
- There is little or no formal diagnosis of 'canker' symptoms;
- 'Bacterial canker' often appears to be worse in recent plantings of cherry on Gisela rootstocks;
- Severe outbreaks in recent plantings has sometimes resulted in death of trees and grubbing of orchards within six years of planting;
- Growers attempt control through the use of the standard three autumn sprays with copper-based products, with additional spray treatments attempted including phosphites, Pretect (harpin protein), Serenade;
- Growers are generally aware of the risks of transfer of the pathogen on pruning equipment and infection through pruning cuts/wounds, but may struggle to achieve adequate disinfection due to time constraints and lack of information about the best options;
- Many cherry crops are covered for part of the season using various types of polythene tunnels, this is targeted at ensuring fruit quality, but may have a benefit in the management of bacterial canker.

Material for a factsheet is in preparation.

Discussion

The first spray trial to examine the effect of spray treatments on foliar disease and pathogen populations, indicated that the most effective treatment against bacterial canker was Cuprokylt and that none of the resistance inducers (RIs) or disinfectants were likely to be of any benefit. Cuprokylt was included as the standard control treatment. It should be noted that the copper spray was applied at the same time as the resistance inducers (i.e. seven days before inoculation) and it was anticipated that it would not be very effective when applied this far in advance of the inoculation. As there was no overhead irrigation between spray applications and inoculation, it would seem likely that the copper residue persisted on the leaves in sufficient concentration to have an effect on pathogen populations.

Results for the resistance inducers were disappointing, with none of them giving any indication of a positive benefit. For several of them, there have been reports of significant effects against bacterial pathogens, when a single spray is applied prior to inoculation, and this was the basis for our approach. We also considered applying some of the products as a drench rather than a foliar spray as there is evidence that this may give a higher and more persistent level of induced resistance (Francis, M.I. *et al.* 2009). It is also possible that repeated pre-treatments might prove effective. It would have been useful to have examined these different aspects as part of this project, but given the limited resources, we considered that it was better to test a wider range of products at a single time-point, with a single application method, rather than a reduced number of products at multiple time-points and/or with different application methods. It might also be argued that the inoculum concentration we used for the leaf inoculations was too high (thereby providing an unrealistic challenge for the products tested). However we consider that the bacterial concentration applied (ca. 10^6 CFU/mL) was not excessive.

The two disinfectants did not have any apparent effect on pathogen populations when applied either the day before or the day after inoculation. Both products are bactericidal to *Psm* at the concentrations used, however, it is clear from this study that any effects are likely to be short-lived. Thus we would expect that if they had been applied at the same time as inoculation we may have seen an effect.

The failure to obtain any disease following leaf scar inoculations in the autumn was disappointing. This highlights one of the challenges in working with this disease of a perennial crop: a repeat inoculation would require a further year of work, but resources preclude this being done. The inoculation method used and the timing was similar to that used by in previous studies (Crosse and Garrett 1966; 1970) where they obtained 80% infection. It is possible that this failure was due to too low an inoculum concentration, but the

concentration was similar to that used earlier for leaf inoculation where the majority of inoculated leaves became infected. We also observed and ensured that the freshly exposed leaf scars were all fully wetted by the inoculum. We also aimed to ensure success by twice daily overhead irrigation for a few days after inoculation. It is also possible that the failure for disease to develop was due to the prevailing environmental conditions over the winter, rather than inoculation failure per se. The inoculated trees in the previous work at East Malling were grown outdoors and therefore may have been exposed to colder temperatures than the trees in the more protected environment of this experiment (although the lowest temperature recorded in the poly-tunnel. was -5.4°C), which may have an influence on the susceptibility of plant tissues to invasion by the bacteria.

When assessing the trees for disease in the spring following the inoculations, the overall impression was of the apparent overall high health status of the trees. Apart from the failure of buds to grow on one or two individual trees, there appeared to be no evidence of disease, and all leaves and buds appeared to be very healthy. Following the leaf inoculation infected leaves tended to senesce prematurely, and except for the weeks following the inoculations, the trees had received no overhead water. Thus, it appears that simply growing trees under protection with sub-irrigation may be a way of producing high-health trees for planting. The question remains whether this high-health status would continue once trees are more exposed to the elements and also the rate of re-infection from surrounding inoculum. It would have been interesting to have followed this up by testing and observing the health status of the trees, but the limited resources allocated to the project precluded any further investigation.

The copper sensitivity tests provided very clear results, with a marked contrast between the two canker pathogens. The results indicated that there is no evidence for copper resistance in *Psm* even from trees that had received 18 copper sprays, whereas for *Pss* copper resistance was found in strains, irrespective of their known exposure to copper sprays. These results are in line with reports from North America, where resistance has also been found in *Pss*, but not in *Psm* (Sundin, Jones and Fulbright 1989; Scheck, Pscheidt and Moore 1996). Results from previous studies also suggest that the copper resistance in *Pss* is not transferable to *Psm* (Sundin *et al.* 1989). The lack of copper sensitivity in some *Pss* strains may at least partly explain the reports of variable levels of control achieved with copper-based bactericides, and this may be particularly the case in cherry, where the results from the previous HDC project (Roberts 2013) indicated that *Pss* may be more prevalent in cherry (at least in nurseries). Thus, where *Pss* is the primary cause of the disease, successful control with copper-based bactericides would seem to be less likely. On the other hand, we might speculate that the use of copper sprays has reduced the

prevalence of *Psm*, and selected for *Pss*. Alternatively, given that a large proportion of recent plantings of modern cherry cultivars/rootstocks have used imported material (where *Pss* has traditionally been considered more important), we wonder if the industry has imported both *Pss* and copper-resistant *Pss* with this plant material. However, it is important to note that most of the isolates tested came from only two locations and we do not have any data on the relative prevalence of the two pathogens in fruit production, nor whether copper resistance is present. This highlights the importance of sending samples for diagnosis and identification of the pathogen: the control strategy may need to be different depending on whether the cause is *Psm* or *Pss*.

The grower visits highlighted that growers are not very pro-active about the health status of trees they are buying to plant new orchards. Given the level of investment involved and the long term nature of the investment, this is an important aspect that needs attention.

Taken together, and particularly the failure to obtain disease following the leaf scar inoculation, these results highlight that there is still much detail about the biology and epidemiology of this disease that remain to be resolved, e.g. environmental requirements for infection and particularly canker development, timing of leaf scar infection in relation to plant physiology, perennation, and differences between the two pathogens, etc.

Conclusions

- Cuprokylt was the most effective spray treatment against *Pseudomonas syringae* pv. *morsprunorum*, giving a significant reduction in pathogen populations.
- None of the resistance inducers gave any indication of a potential benefit.
- Neither of the disinfectants showed any significant effect on pathogen populations.
- The project has highlighted a number of aspects of the biology and epidemiology of this disease that remain to be resolved.

Knowledge and Technology Transfer

Visits to growers in Kent (Sept 2014) and Herefordshire (Jan 2015)

Project Profile article in Tree Fruit Review 2015

Presentation at HDC Top Fruit Meeting, 26 March 2015

Factsheet in preparation (expected publication in autumn 2015)

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References

Crosse, J.E. and Garrett, C.M.E. (1966) Bacterial canker of stone-fruits: Infection experiments with Pseudomonas mors-prunorum and P. syringae. *Annals of Applied Biology*, **58**, 31–41.

Crosse, J.E. and Garrett, C.M.E. (1970) Pathogenicity of Pseudomonas morsprunorum in Relation to Host Specificity. *Journal of General Microbiology*, **62**, 315–327.

Francis, M.I., Redondo, A., Burns, J.K. and Graham, H.H. (2009) Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of bacterial canker. *European Journal of Plant Pathology*, **124**, 283–292.

Payne, R., Harding, S., Murray, D., Soutar, D., Baird, D., Welham, S., Kane, A., Gilmour, A., Thompson, R., Webster, G. and Tunnicliffe Wilson, G. (2005) *The Guide to Genstat Release 8. Part 2: Statistics*. VSN International, Oxford, UK.

Pscheidt, J.W. (2013) Timely Blueberry Disease Control. URL http://www.growingproduce.com/fruits-nuts/berries/timely-blueberry-disease-control/ [accessed 13 January 2014]

Roberts, S.J. (2013) *Management of Bacterial Canker in Prunus Spp. HDC Project HNS 179. Final Report 2010-2013*. AHDB, Stoneleigh, UK.

Roberts, S.J. and Akram, S. (2002) *HDC HNS 91. Bacterial Diseases of HNS: Chemical Control. Final Report 2000-2002*. HDC, Kent, UK.

Scheck, H.J., Pscheidt, J.W. and Moore, L.W. (1996) Copper and streptomycin resistance in strains of Pseudomonas syringae from Pacific Northwest nurseries. *Plant disease*, **80**, 1034–1039.

Sundin, G., Jones, A. and Fulbright, D. (1989) Copper resistance in Pseudomonas syringae pv. syringae from cherry orchards and its associated transfer in vitro and in planta with a plasmid. *Phytopathology*, **79**, 861–865.

Vicente, J.G., Alves, J.P., Russell, K. and Roberts, S.J. (2004) Identification and discrimination of Pseudomonas syringae isolates from wild cherry in England. *European Journal of Plant Pathology*, **110**, 337–351.

Appendices

Analysis of deviance for leaf populations

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Accumulated analysis of deviance
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                                          mean  deviance approx
Change                d.f.     deviance deviance     ratio  F pr.

+ Block                  2       53297.    26649.      4.38  0.020
+ Treat                  9      263905.    29323.      4.82  <.001
+ Treat.Timing           2        7431.     3715.      0.61  0.548
+ Block.Treat.Timing    18       75329.     4185.      0.69  0.800
+ Med                    1      291629.   291629.     47.96  <.001
Residual                37      224979.     6081.

Total                   69      916570.    13284.
```

Analysis of deviance for proportion of leaves with symptoms

```
Accumulated analysis of deviance
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                                          mean  deviance approx
Change                d.f.     deviance deviance     ratio  F pr.
+ Block                  2       14.194     7.097      4.36  0.018
+ Treat                  9       15.964     1.774      1.09  0.388
+ Treat.Timing           2        3.110     1.555      0.95  0.392
Residual                50       81.481     1.630

Total                   63      114.749     1.821
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