Project title:	Apples and Pears: The use of Biological Control, Plant Health Promoters and copper to Effect Control of Fireblight (*Erwinia amylovora*)
Project number:	TF 183
Project leader:	Tim Biddlecombe FAST Ltd Crop Technology Centre Brogdale Farm Brogdale Road Faversham Kent ME13 8XZ
Report:	Final Report, December 2010
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Key words:	

CONTENTS

GROWER SUMMARY

Headline

In one year of this project when infection levels were high, Serenade ASO and Sentry P provided significant levels of control of fireblight.

Background and expected deliverables

Fireblight, caused by the bacterium *Erwinia amylovora*, is a widespread destructive bacterial disease of pome fruit trees and related plants. The prevalence of the disease tends to be cyclical depending on spring and summer weather conditions. There was a sharp increase in the incidence of the disease in 2006-2008. Typical symptoms include wilting and death of flower clusters and of young shoots causing a loss of fruiting wood but when infections become severe, it can lead to the death of the tree. Control in the U.K. is currently limited to cutting out diseased material and use of copper sprays which can lead to russeting of the fruit surface. In fact Defra states that "There are no effective chemical measures available in the U.K. to control Fireblight" (Defra, 2005). In the USA and parts of Europe, antibiotics are used but they are not permitted in the U.K.

A suitable method of control would benefit the industry by increasing cropping potential and reducing the need for cutting out infected shoots.

The introduction of certain biological control agents and plant health promoters offers the opportunity for potential Fireblight control. This project aimed to determine the effectiveness of PreTect (active ingredient Harpin), Regalis (active ingredient Prohexadione-calcium), copper, Sentry P (active ingredient *Bacillus pumilis*) and Sentry S (active ingredient *Bacillus subtilis*). In January 2009 Serenade ASO was granted a SOLA for use on tree fruit, and since the active ingredient (*Bacillus subtilis - strain QST 713*) is identical to that used in Sentry S, HDC agreed that Serenade ASO be used instead.

Summary of the project and main conclusions

Egremont Russet apple and Concorde pear are known to be particularly susceptible to the disease and were therefore chosen for the first year of this project. However, due to very low levels of infection within the Egremont Russet trial site, treatments were only applied to the Concorde site during the project's second (2009) and third (2010) year.

The only treatments that had a significant effect were Serenade ASO and Sentry P in the second year of the project (2009). Serenade ASO was the most effective treatment where it reduced infection levels by 89.5% compared to the control. In 2010, both Serenade ASO and Sentry P did reduce infection levels but to a reduced extent.

It should be noted that neither Sentry P, nor Pre-Tect are currently approved as crop protection products in the UK.

Figure 1 shows the effect of treatments on the percentage of shoots infected with Fireblight. Although PreTect and copper reduced infection levels of fireblight, the reductions were not statistically significant.

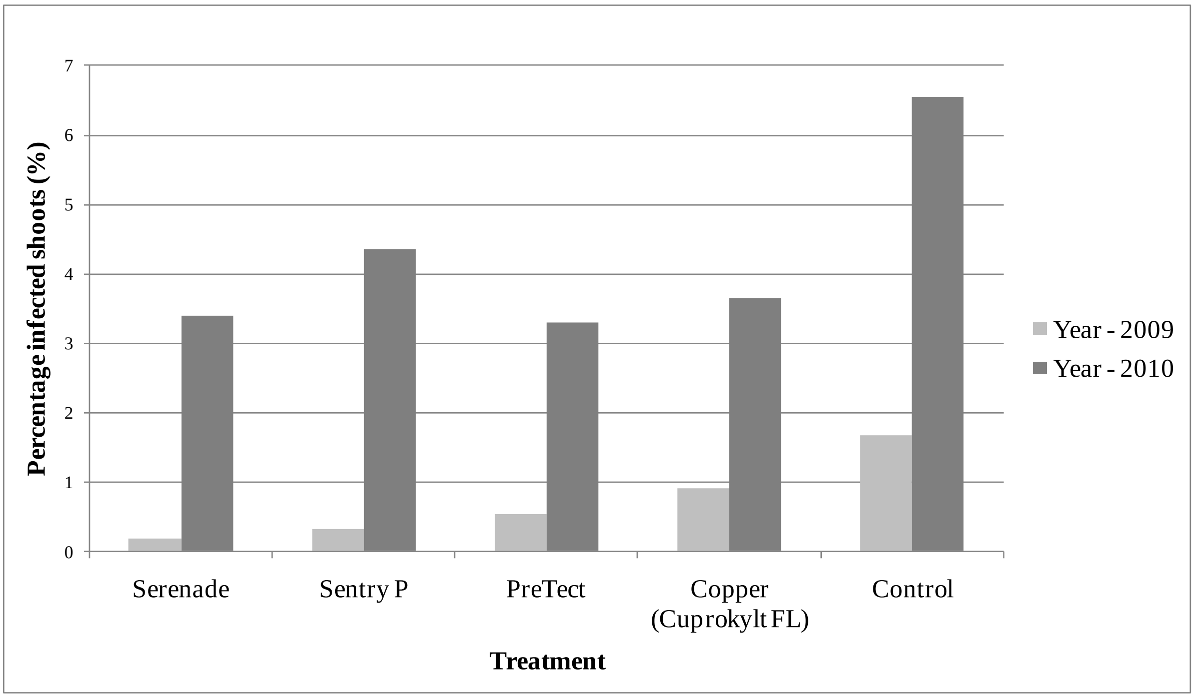

Figure 1. The effect of treatment on percentage of shoots infected with fireblight in 2009 (light grey bars) and 2010 (dark grey bars).

All treatments were less effective at controlling fireblight in 2010 than in 2009. This is probably explained by greater disease pressure caused by the higher rainfall during the blossom period in 2010. This disease pressure was calculated from the RIMpro *Erwinia* computer prediction model.

Because no treatment completely eliminated Fireblight, the use of any of these products should not be seen as the sole method of control, but rather should be used in conjunction with other methods. A single fireblight infection can, if left uncontrolled, ultimately lead to the death of the tree and provide a source of infection for further spread. The results show that even the most effective treatment did not achieve complete control with there being less than 1% infected shoots remaining in 2009, and therefore the use of other control methods such as cutting out of any infected shoots and burning, would still be required.

Further work is required to assess the impact of spray timings to specifically target infection periods, with the aim of reducing the number of applications and therefore improving the economic benefit of these treatments.

Financial Benefits

Yields were not recorded as part of the trial and it is difficult to make assumptions as to potential yield losses due to the nature of the disease. Cultural methods of control involve removing and burning infected shoots and branches, and disinfecting pruning tools. These are clearly expensive operations. The costs of some of the treatments are compared in Table 1.

Although Serenade ASO was the most effective treatment, it is expensive to purchase. Based on the increase in yield and typical savings made in labour costs achieved from its use, application could be difficult to justify except in seasons where infection risk is high and leads to significant numbers of tree deaths.

Table 1. Treatment Costs

Product	No. of Treatments	l/ha per treatment	£/ l or kg of Product	£/ha
Serenade ASO	15	10	£10.70	£1,605.00
PreTect	15	1	£29.00	£435.00
Copper (Cuprokylt FL)	1	2.5	£5.11	£12.78

Action points for growers

- Inspect vulnerable orchards routinely for fireblight symptoms during winter pruning, soon after bud break, during mid June, from late July to early August and soon after leaf fall. Inspect young trees more frequently.

- Make additional inspections after frost, following storms and when fireblight warnings are issued.

- Slice off bark to determine the extent of infection and then cut out diseased wood at least 30cm below the stained tissue on smaller wood (< 2cm diameter) and at least 50 cm below on larger wood (Figure 2).

F**igure 2.** An example of fireblight staining of apple wood (var. Egremont Russet).These samples come from the same shoot.

- Disinfect tools between cuts and burn diseased wood. Burning of diseased plant material is legal providing the grower has obtained the appropriate exemptions from the Environment Agency under 'The Environmental Permitting (England and Wales) Regulations.

- Apply a copper treatment at bud burst. This will give protective cover against fireblight and also scab. In orchards vulnerable to high levels of infection, consider applications of Serenade ASO timed to cover high risk infection periods e.g. blossom. Where possible, use disease forecasting models such as RIMpro Erwinia and ADEM Fireblight to identify these periods.

SCIENCE SECTION

Introduction

Fireblight is a serious disease of apples and pear and other members of the *Rosaceae* family caused by the bacterium *Erwinia amylovora.* The disease is native to North America, where recent outbreaks in Washington State caused growers to completely remove orchards, particularly of pears (Grove *et al*., 2003). The disease was later discovered in New Zealand in 1919. Its first noted occurrence in the U.K. was in 1957 in Kent and it is now widespread throughout the UK and most of the European Union. In 1997, after a couple of years of build up, there was a severe outbreak in the Emilia-Romagna region of Italy which resulted in the removal of 500,000 trees (Calzolari *et al*., 1999).Top of Form

Fireblight was classified as a notifiable disease, and it is now subject to quarantine regulations within the EU.

The pathogen's life cycle is shown in Figure 3 below. The fireblight bacteria over winter in cankers on the host plant. During the following spring the bacteria multiply at canker margins giving rise to primary inocula which then begin to ooze onto the canker's surface (Beer, 1991). Primary infection occurs at the onset of bloom, where rain splash and insects move the bacteria to apple and pear blossom and vegetative growing points.

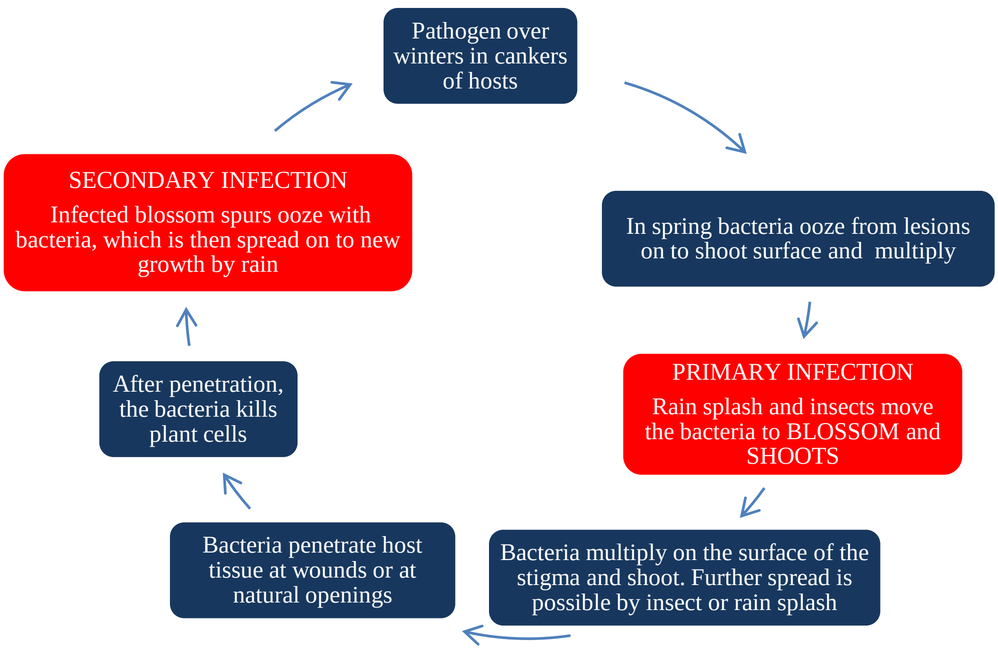

Figure 3. Fireblight life cycle.

In the epiphytic phase, the fireblight bacteria multiply on the surface of the stigma from where pollinating insects and rain then spread bacteria from an infected flower to other flowers throughout the orchard. Rain and subsequent moisture then enable the bacteria to penetrate the host tissue through natural openings in the flower or wounds, and go on to kill plant cells, moving within the branch to form a lesion. These lesions and infected blossom spurs ooze with bacteria, which are then spread onto new vegetative growth by rain splash, causing secondary infections (Beer, 1991).

Typical symptoms include wilting and death of flower clusters and withering and death of young shoots. Infections can potentially lead to the death of the tree. Figures 4 and 5 show shoots and branches infected by fireblight in the trial orchard.

Figure 4. Close up of a shoot showing typical symptoms of fireblight infection (Pear – Concorde).

Figure 5. Pear trees (Concorde) with infected shoots and branches.

The epiphytic phase is in practice, the only period where biological and chemical control measures can be effective in reducing infections (Trapman, 2008; Holtz *et al*., 2002). Once the pathogen has entered plant tissue, cutting out and burning of infected material is the only effective control method.

In the USA, antibiotics based upon streptomycin are used to protect the blossom from infection, but the development of antibiotic resistant strains of *Erwinia amylovora* has brought this approach into question (McManus *et al*., 2002). The use of antibiotics in the UK is not permitted. Control in the UK is limited to cutting out diseased material and copper sprays, which can cause russeting of fruit. In fact Defra states that "There are no effective chemical measures available in the UK to control fireblight" (Defra, 2005).

There are several products available in the U.K. that are existing or potential treatments for fireblight:

- **Copper** (e.g. in the form of copper oxychloride)
- **Serenade ASO** and Sentry S (containing *Bacillus subtilis*)
- **PreTect** (containing the protein harpin)
- **Sentry P** (containing *Bacillus pumilus*)
- **Regalis** (containing prohexadione-calcium)

The mode of action of copper is based upon the availability of copper ions that inactivate many essential biological processes, but treatment is only effective at the point of dispersal from an infected tree (or alternative host) to another (Steiner, 1998) and has proven to be satisfactory only when disease pressure is low to moderate. Treatments are generally limited to dormant and bud break periods because of the risk of fruit russeting (Holtz *et al*., 2002).

The active ingredient of Serenade ASO (*Bacillus subtilis*) has multiple modes of action, where the beneficial bacteria:

- are able to out-compete the pathogen on the plant surface
- produce a range of lipopeptide metabolites that breakdown cell membranes, causing the pathogen to collapse and die (Edgecomb and Manker, 2010).
- prevent harmful disease spores from germinating by disrupting the germ tubes and mycelial growth (AgraQuest, 2002)
- are inducers of Systemic Acquired Resistance aiding the plant to resist infection (Fargro Ltd., 2008)

Trials in California were carried out to compare the efficacy of Serenade to control fireblight on the apple varieties Pink Lady, Granny Smith and Fuji. In 2000 Serenade gave the best levels of control on all varieties and results were statistically comparable with streptomycin treatments (Holtz *et al*., 2002). At the outset of this project, Sentry S (Plant Health Care Ltd.) was identified as a potential control agent. However, as another product Serenade ASO with the same active ingredient (*Bacillus subtilis – strain QST713*) had been granted a Specific Off Label Approval (SOLA 20090246) for use on tree fruit in January 2009, it was requested that it be used instead.
Sentry P (*Bacillus pumilus)* is known to have an effect on a range of diseases on a variety of crops and products with this active ingredient are widely available in the USA. However its effectiveness in the control of fireblight is unknown (Oregon State University Extension, 2010).

The active ingredient of PreTect is the protein harpin, which is produced naturally by plants infected by certain bacterial plant pathogens (e.g. fireblight). In many plants the presence of harpin induces Systemic Acquired Resistance (Plant Health Care Ltd., 2010) but it has no direct effect on the fireblight pathogen. Research carried out by Cornell University, U.S.A. in 1997 showed that the use of harpin reduced the incidence of fireblight in the apple variety Idared by 41% but proved to be significantly less effective than streptomycin treatments (Momol *et al*., 1999).

The principle use of Regalis (prohexadione-calcium) is as a growth regulator in apples. During product development it was noted that treated trees were less affected by fireblight as well as scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) (Rademacher, 2008). Although prohexadione-calcium is inactive as a bactericide, it is believed to reduce infections by reducing shoot growth leading to a reduction in the amount of soft tissue vulnerable to infection, and by changing the range of flavonoids metabolized in apple trees, induce physiological resistance to infection.

Trials in 2002 and 2003, on apple varieties 'Idared' and 'Freedom' showed that prohexadione-calcium achieved superior results to streptomycin treatments (Bubán *et al*., 2005).

The aim of this project was to assess the effectiveness of the range of products listed above in their control of fireblight.

Although not in the remit of the project, the output of two fireblight disease models was examined. These outputs informed the project as to the severity of infection periods but were not used to determine treatment timings. In 2009 RIMpro Erwinia, developed by Marc Trapman of Bio Fruit Advies, Holland, and ADEM Fireblight (developed by Warwick HRI based on earlier work at East Malling by Billing - Billing's Integrated System 1995) were used, with output based upon weather data generated by a locally based weather station. In 2010 only RIMpro Erwinia was used. For the models' output and a brief explanation on interpretation, see Appendix 1.

Materials and Methods

This trial was conducted at A. Hinge & Sons Ltd, Ham Green Farm, Upchurch, Sittingbourne, Kent by kind permission of Mr Robert Hinge. The farm has a known history of fireblight infection which was present in both apple and pear orchards in the season preceding the start of the trial. There are also other sources of infection in the vicinity of the trial plots from hedgerows and neighbouring orchards. Egremont Russet apple and Concorde (*Pyrus communis* Concorde) pear are known to be particularly susceptible to the disease and therefore one site of each variety was initially chosen for the first year of this project. However, due to very low levels of infection within the Egremont Russet site, treatments were only applied to the Concorde site during the project's second and third years (2009 and 2010). Because there was no current approval in the UK for the use of Regalis on pears at the time, assessing its effectiveness in controlling fireblight could not be continued.

The details of Concorde pear site are as follows:

Orchard name:	Shakespeare Pears
Soil Type:	Sandy loam
Planting detail:	Individually staked trees configured as single rows with tree spacings of 1.25m and row spacings of 3.6m giving a tree population of ~2200 per ha.

The trial was designed as a completely randomized block experiment with five blocks of five trees per treatment. All branches showing symptoms of fireblight prior to the beginning of the treatments were pruned out.

The individual products used, rates of use and timings are described in Table 2.

Table 2. Products and pesticides used in treatments.

Trt no.	Product - trade name	Active ingredient	Application rate	Application regime - timing	Approval status: *MAPP No* / *On-Label or SOLA* / *Expiry date*
1	Control				
2	PreTect	harpin	1 kg	15 weekly applications starting bud burst	NA
3	Copper (Cuprokylt FL)	Copper oxychloride	2.5 l/ha	Bud burst	8299 / On-Label / 31/12/2013
4	Sentry P	*Bacillus pumilis*	15 l/ha	15 weekly applications starting bud burst	NA
5	Serenade ASO	*Bacillus subtilis*	10 l/ha	15 weekly applications starting bud burst	14318 / SOLA - 20090246 - Top fruit / 25/11/2012

Table 3 lists the treatment application dates made during 2009 and Table 4 lists the application dates for 2010. In both cases the copper treatments were applied at bud-burst, and the *Bacillus* and harpin applications began soon after bud burst and consisted of fifteen weekly applications.

Table 3. The treatments and application dates in 2009.

Application Date 2009	PreTect	Copper (Cuprokylt FL)	Sentry P	Sentry S & Serenade ASO
April 14	✓	✓	✓	✓
April 22	✓		✓	✓
April 29	✓		✓	✓
May 6	✓		✓	✓
May 13	✓		✓	✓
May 20	✓		✓	✓
May 27	✓		✓	✓
June 3	✓		✓	✓
June 10	✓		✓	✓
June 17	✓		✓	✓
June 24	✓		✓	✓
July 1	✓		✓	✓
July 8	✓		✓	✓
July 15	✓		✓	✓
July 29	✓		✓	✓

Sentry S

Serenade

Table 4. The treatments and application dates in 2010.

Application number	Application date	Copper (Cuprokylt FL)	PreTect	Sentry P	Serenade ASO
1	Bud burst	✓			
2	16-Apr-10		✓	✓	✓
3	24-Apr-10		✓	✓	✓
4	01-May-10		✓	✓	✓
5	07-May-10		✓	✓	✓
6	14-May-10		✓	✓	✓
7	21-May-10		✓	✓	✓
8	28-May-10		✓	✓	✓
9	04-Jun-10		✓	✓	✓
10	12-Jun-10		✓	✓	✓
11	18-Jun-10		✓	✓	✓
12	25-Jun-10		✓	✓	✓
13	02-Jul-10		✓	✓	✓
14	08-Jul-10		✓	✓	✓
15	16-Jul-10		✓	✓	✓
16	23-Jul-10		✓	✓	✓

Assessments and analysis

Assessments were made post harvest. The 2009 assessment was carried out on the 24th September 2009 and the 2010 assessment on the 26th October 2010. The number of infected shoots per tree and the total number of shoots per tree were recorded and used to calculate the percentage of shoots infected with fireblight.

The effect of each treatment was determined by comparing the number of infected shoots to the total number of shoots per tree, expressed as a percentage. This was to avoid effects of the treatments being skewed by different sized trees. However as percentage data do not conform to a normal distribution especially as the percentages are calculated from low numbers of infected shoots, an arcsine transformation was used prior to analysis of variance (Hoshmand, 2006). Data was then subjected to Multiple Range Tests (95.0% LSD) to determine the significance of individual treatments.

Results

2008

Table 5. Total number of infected shoots per treatment 2008.

Treatment	Total no. of infected shoots
Copper (Cuprokylt FL)	3
PreTect	2
Sentry S (Serenade ASO)	2
Sentry P	0
Control	2

Table 5 shows the total number of infected shoots per treatment throughout the 5 treatment blocks for the year. Overall infection levels were low, and there were no significant differences between treatments and blocks.

2009

The trial results for both 2009 and 2010 are shown in Table 6 and Figure 6. The results are recorded as the average percentage of shoots per tree infected with fireblight per treatment. The chart shows that the treatments did have an effect in reducing the levels of infected shoots in comparison to the control.

The ANOVA indicated that overall, the treatments' effect on reducing the number of infected shoots was not significant (P=0.0626) and that the differences in the level of fireblight between blocks was also not significant (P=0.4386).

Whilst the treatments overall did not have a significant effect, when comparing the Control against individual treatments, the effect of Serenade ASO and Sentry P was significant as determined by the multiple range test.

Table 6. Average percentage (%) of shoots infected by fireblight.

Product	Year - 2009	Year - 2010
Serenade ASO	0.18%	3.40%
Sentry P	0.33%	4.36%
PreTect	0.54%	3.29%
Copper (Cuprokylt FL)	0.91%	3.65%
Control	1.68%	6.54%

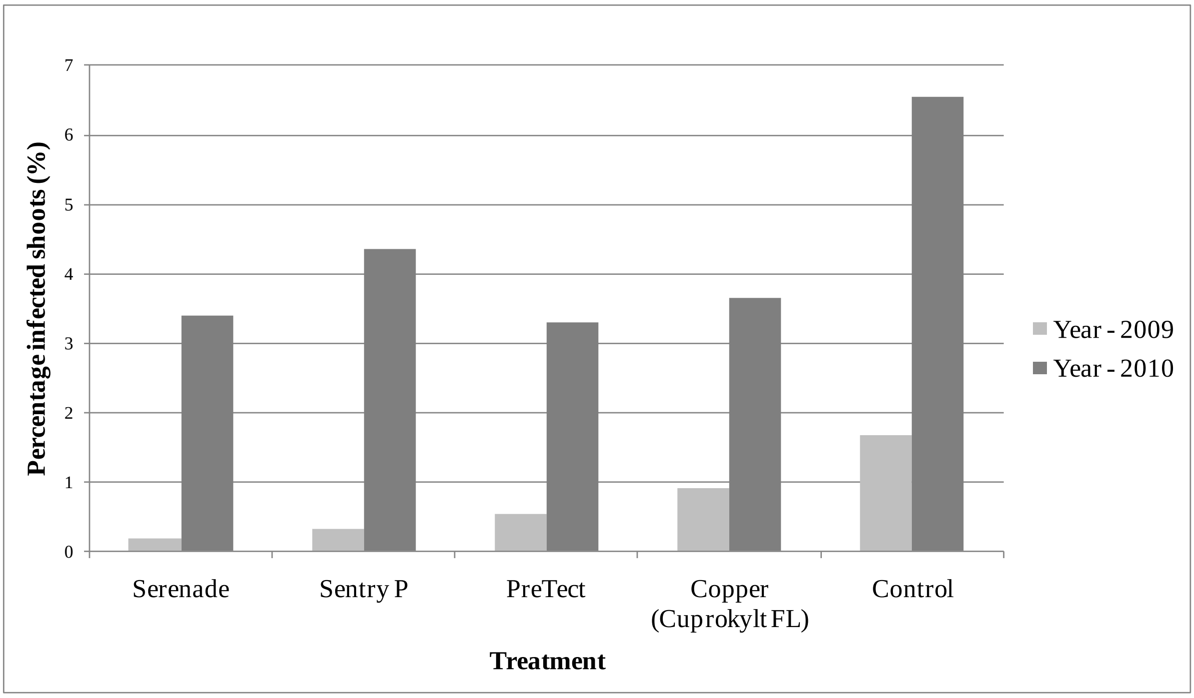

Figure 6. The effect of treatment on percentage of shoots infected with fireblight in 2009 (light grey bars) and 2010 (dark grey bars).

2010

Figure 6 shows that similarly to 2009, treatments did reduce levels of infection relative to the Control, but the ANOVA indicated that overall this effect was not significant (P=0.9432). There were also no significant differences in the level of fireblight between blocks (P=0.6423). Where in 2009, the effects of Sentry P and Serenade ASO were significant, in 2010 when comparing the Control against individual treatments, no individual treatment had a significant effect.

Discussion

Results from the statistical analysis, together with the data from Figure 6, indicate that the treatments were less effective during the 2010 season than during 2009. This difference in effectiveness could be partly explained by a potentially higher level of disease pressure during flowering in 2010 compared to 2009. Figure 7 shows rainfall during the blossom period for the 3 years of the trial. Rainfall during flowering was higher in 2010 than in 2008 and 2009 and would have improved conditions for the spread and colonization of the fireblight bacteria, at a period when the tree is most vulnerable to infection. This increase in disease pressure can be seen when comparing the prediction output from the RIMpro Erwinia for 2009 and 2010 (see Figures 8 and 9 in Appendix 1).

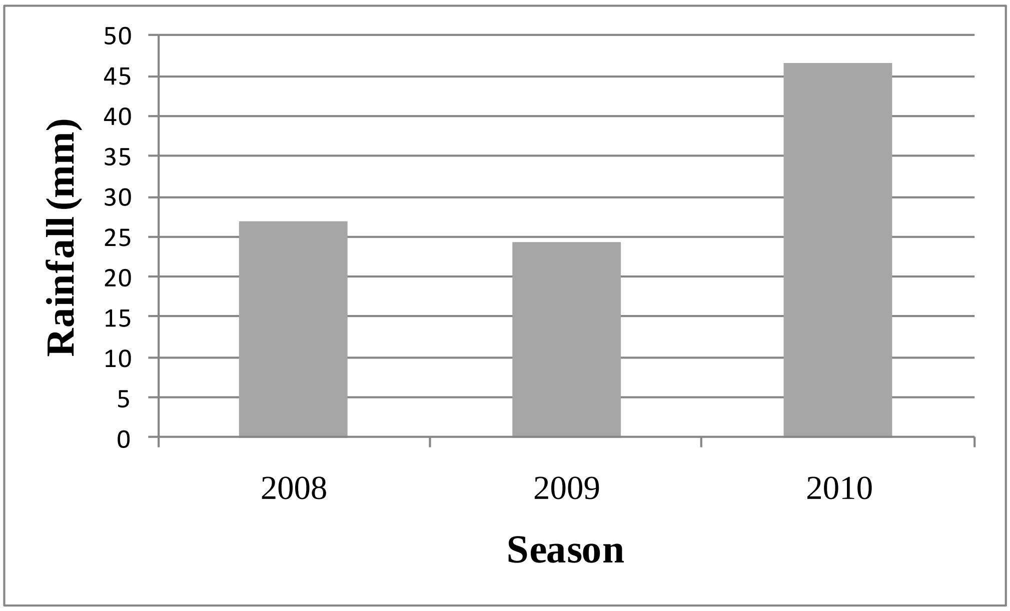

Figure 7. Total rainfall during bloom in the years shown. Data taken from the weather station at Brogdale Farm, Faversham, about 10 miles from the experimental site.

From the assessments carried out in 2009 and 2010, the only treatments that had a significant effect were Serenade ASO (and Sentry S – see note in Materials and Methods) and Sentry P applied in 2009, with Serenade ASO being the most effective treatment reducing infection levels by 89.5%. Although the effect was less in 2010 (47.9%), these could prove to be valuable reductions in vulnerable orchards carrying a high value crop. Figure 6 shows that PreTect and copper also reduced infection levels in each of these years but these were not statistically significant decreases.

Copper is typically applied in orchards for the control of scab (*Venturia inaequalis*). Although the copper treatment did not have a significant effect, the fact that infection was reduced by a single application does indicate that this treatment could have a place in any control system developed for fireblight. The time that elapsed between the application of copper and the date of the first infection period predicted by ADEM Fireblight in 2009 was more than two months. Copper was applied on 14th April, while the first predicted infection period occurred on 22nd June. Whilst the copper would not have still been present to be effective during the periods of infection in June and July, it is conceivable that the copper may have killed the fireblight bacteria as it began to ooze from cankers earlier in April, reducing the potential for further infection.

The principle aim of this project was to assess the effectiveness of the four products, and with the exception of the copper treatment, this was achieved by applying the products every week from mid-April until the end of July (15 applications), thereby maintaining a protective cover whether fireblight infection periods occurred or not. Maintaining this cover will be costly in terms of product and time, raising the question of economic viability. Further work is required to assess the effectiveness of targeting these products at specific infection periods using computer based disease forecasting models with the aim of reducing the number of applications required and improving economic viability.

The use of any of these products should not be seen as the sole measure of control, but should be used in conjunction with other methods. A single fireblight infection can, if left uncontrolled, ultimately lead to the death of the tree and provide a source for further infection. This project showed that, although levels of disease were reduced, complete control was not achieved with any treatment, and any infection that did occur would oblige the grower to incorporate the use other control methods, such as cutting out of infected shoots and burning, causing further expense to the business.

Conclusions

1. In both years application of the four products did result in reduced percentages of infected shoots. However, only Serenade ASO and Sentry P in 2009 caused statistically significant reductions in 2009. Timing applications of these products based on prediction models needs to be tested to maximise their effectiveness especially for Serenade ASO, where the cost is a significant barrier to its regular use.

2. No treatment resulted in complete eradication of the disease and so any infection that did occur would oblige the grower to incorporate other control methods such as monitoring orchards carefully and cutting out infected shoots as soon as they appear. Further details of these are given in the HDC's Apple Best Practice Guide.

3. The use of the treatments described here do show potential however to form part of an overall control strategy for fireblight control, especially in high risk seasons where infections could result in significant tree deaths.

Appendix 1 - Fireblight Infection Prediction Models

The protocol was designed to compare the efficacy of four products against fireblight by making regular applications throughout the growing season. Models have been developed for fireblight which use weather data to predict infection periods. Whilst outside the scope of the project, the results of the models are shown here to demonstrate the difference in infection risk between 2009 and 2010.

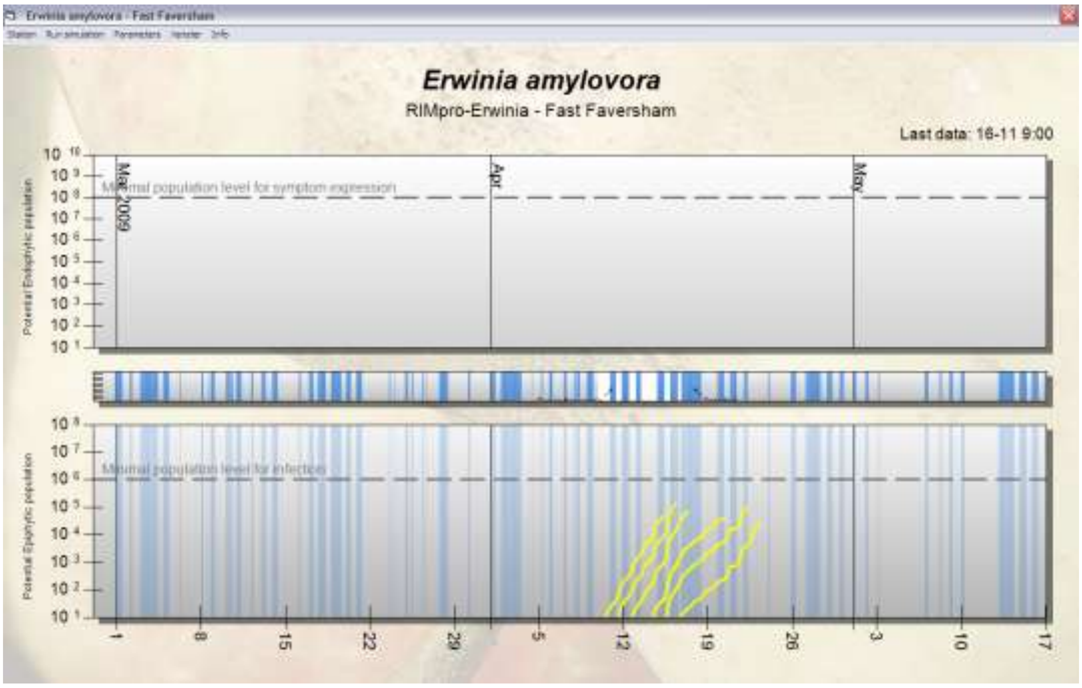

Figure 8. Output from RIMpro Erwinia - March to May 2009

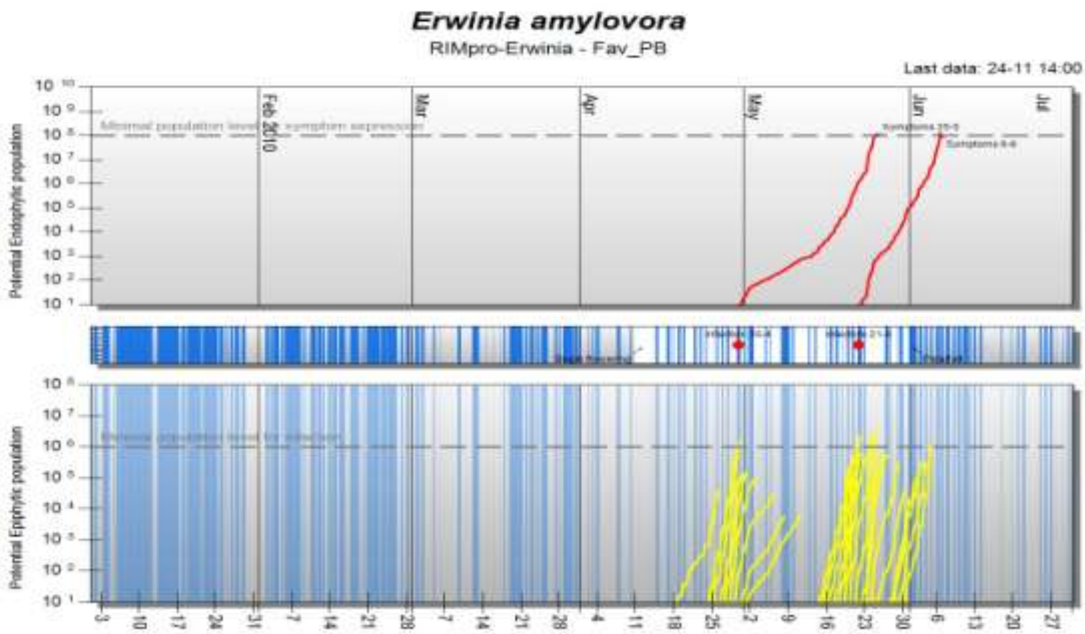

Figure 9. Output from RIMpro Erwinia - February to June 2010

There are several models that have been devised to forecast the infection of fruit trees by fireblight. Good examples of these are:

- BIS95 - Billing
- RIMpro Erwina
- Maryblyte
- Cougar Blight

Billing's Integrated System 1995 (BIS95) was, until recently, the most commonly used model, and has been incorporated into HRI forecasting software such as ADEM (Morph4). Another model, RIMpro Erwinia, is currently under development by Marc Trapman and has been incorporated into the RIMpro suite of prediction models.

The software behind all computer based prediction models use 3 types of data input:

- **Weather data** is used to predict the pathogen survival, rate of population growth, and spread. Weather data should ideally be generated from a local station.
- **Plant phenology** i.e. date of full blossom and petal fall, is used by the model to determine the periods at which the plant is vulnerable to infection.
- **Model configuration** parameters that describe, for example, the maximum and minimum temperatures between which the pathogen develop.

This data is then used by the model to generate an output that describes potential periods of population growth and infection. Figures 8 and 9 show the output generated by RIMpro Erwinia for 2009 and 2010 respectively.

Interpretation

RIMpro Erwinia's output is divided into three charts, **Potential Epiphytic Population** (lower chart), **Growth Stage and Infection Dates** (middle) and **Potential Endophytic Population** (top).

The **Potential Epiphytic Population** chart contains the most important information. It traces the potential growth of bacterial populations up to the 'minimum population required for infection' while the fireblight pathogen is on the tree's tissue surface. The output on this chart acts as an aid in timing applications during the only period where, in practice, control products can have an effect on reducing infection levels.

The Infection Dates and **Potential Endophytic Population** charts depict the point at which the pathogen gains entry in to the plant's tissue via wounds and natural openings in the blossom and the possibility of the disease developing.

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