Project title:	Raspberry: Efficacy of novel products for the control of *Phytophthora rubi* root rot
Project number:	SF 123
Project leader:	Erika F. Wedgwood, ADAS
Report:	Final report, March 2014
Previous reports:	Annual report, March 2012 Annual report, March 2013
Key staff:	Erika Wedgwood Tracey Evans Sarah Mayne Chloe Whiteside Sean Allison Abby Wood Robert Drummond Steve Richardson
Location of project:	ADAS Boxworth, Boxworth, Cambridge, CB23 4NN
Industry representative:	Tim Place, Church Farm, Tunstead, Norwich, NR12 8RQ
Date project commenced:	1 April 2011
Date project completed (or expected completion date):	31 March 2014

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

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

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.

Dr Erika F. Wedgwood
Research Scientist
ADAS

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

Report authorised by:

Dr Barry Mulholland
Head of Horticulture
ADAS

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

CONTENTS

GROWER SUMMARY

Headline

- A novel plant Protection product has been identified which offers equivalent control of raspberry root rot to Paraat.

Background and expected deliverables

Soil-borne *Phytophthora rubi* (previously known as *Phytophthora fragariae* var. *rubi*) can infect raspberry roots causing root rot, leading to the visible wilting of stem, petiole and leaf tissues and over time, the death of whole plants. Other species of Phytophthora can also cause root rot, but *P. rubi* is the most common and serious form (Kennedy and Duncan, 1991). Sections of the crop row and their resulting fruit yields are lost for the remainder of the crop's life. Once the soil is contaminated by the pathogen, any replacement plants are also likely to succumb to infection.

Another Phytophthora species (*P. idaei*) has also been found causing root rotting in Rubus species, but unlike *P. rubi*, it does not cause the crop to wilt. It is likely that resting spores of other Phytophthora species survive in land re-used for raspberries, even after a gap of five years or more. The resting spores will be stimulated to germinate when roots grow out into the soil. The motile zoospores produced are mobile in irrigation water. Once a plant becomes infected, the pathogen multiplies and neighbouring plants become infected as zoospores spread. Although some crops are grown in soilless substrates, contamination of the substrate can still occur resulting in root infection. Contamination can occur by irrigating with spore-contaminated water which has been drawn from open reservoirs. It can also occur where growing containers are stood directly on woven ground-cover and roots are allowed to develop through the woven material into contaminated soils below. The introduction of symptomless plants which are initially thought to be healthy is another common way of contaminating soilless substrates.

In soil grown crops, growers can fumigate the soil before planting using products such as Basamid (97% w/w dazomet) or chloropicrin, although the future availability of chloropicrin is uncertain. Experience suggests that soil fumigation does not totally eliminate the presence of *P. rubi* spores however and there is still a risk that infection will occur. At present, there is no commercial soil test available for detecting the presence of *P. rubi* in

soils or soilless substrates, although research in HDC Project SF 130 aims to develop one. Until a commercial test is widely available, growers are unable to determine the risk of infection occurring in soils or fields destined for cane fruit production.

As a control measure against *P. rubi*, growers usually apply fungicide drenches both in autumn and spring, applied to the root zone. The EAMU for SL567A (44.7% w/w metalaxyl-M) has been relied upon for some years, but resistance to metalaxyl has been reported in other crops such as potato (when used to control *Phytophthora infestans*). Other products used by raspberry growers include Shirlan (fluazinam) and Paraat (500 g/kg dimethomorph), but there is always a greater chance of resistance developing in pathogens where products have only a single mode of action. In commercial practice, none of the currently approved products provide complete control, so alternative products would be beneficial to the industry.

The aim of this project was to identify new drench treatments that protect raspberries from root infection by *P. rubi.*
Specific objectives were:

- To identify suitable products for the control or suppression of Phytophthora root rot in raspberry.
- To test products using inoculated growing media to determine their efficacy in the prevention of *P. rubi* infection in raspberry.
- To provide information to growers and the relevant chemical companies on any products that have efficacy and to collaborate with the industry to secure new EAMU approvals to control *P. rubi* in raspberry.

Summary of the project and main conclusions

Objective 1 – Identification of candidate products for root rot control

Five products with potential to control Phytophthora root rot in raspberry were identified in Year 1 of the project. The newly identified products were HDC F181, HDC F182, HDC F183 and Prestop (*Gliocladium catenulatum*). HDC F182 has recently been registered in the UK for use on outdoor strawberries against red core and crown rot, with its efficacy against *Phytophthora cactorum* demonstrated in HDC project SF 99. HDC F183 is approved for use against the closely related potato blight pathogen, *Phytophthora infestans*. Prestop is a biopesticide with full label recommendation for use against root pathogens on cane fruit and an EAMU for outdoor crops. Ranman Twinpack (cyazofamid) was initially

selected for assessment, but was removed from the final selection as it was considered highly unlikely to gain an extension of use from potato. After consultation with the HDC Soft Fruit Panel and the product's suppliers (then BASF, now Bayer), a second biological product, Serenade ASO (*Bacillus subtilis* strain QST 713) was chosen as an alternative candidate. This was chosen as it is known to have activity in soil against Phytophthora species. It already has an EAMU approval for use on raspberries and also for trees in amenity situations and forest nurseries, against Phytophthora root rot. The fifth candidate, HDC F181 was a chemical product shown in HDC Project SF 99 to give control of the Phytophthora species causing crown rot in strawberries.

Objective 2 – Evaluation of products for control of P. rubi in raspberry

The evaluation of plant protection products in an inoculated trial commenced in 2012 with Paraat (dimethomorph) used as the industry standard. Drenches of Paraat, HDC F181, HDC F182, HDC F183, Serenade ASO, and Prestop were carried out on 3 October 2012 on cv. Polka modules which were planted into 5 L pots of ericaceous growing medium in May 2012. Plants were grown in a Spanish tunnel with individual irrigation/feed drippers with the timing and volume adjusted to keep the growing media continuously wet. There were ten pots per plot and four replicate blocks of treatments. Each plant pot was placed in a saucer.

Drench treatments were applied twice by pouring 500 ml onto the pots. Artificial inoculation was done either a week later in 2012, or a month after drenching in 2013. Inoculation using mycelial plugs of *P. rubi* buried in the growing media was carried out on the two occasions in October 2012 and again in April 2013, using the isolate SCRP3333, FVR11, IMI355974.

In the first cropping year, no wilting or stem staining developed in the fruiting canes before their removal in January 2013. The re-grown crop was monitored throughout 2013 for wilting of the new shoots. There were no significant treatment differences in wilting. However, the inoculated untreated control had 48% of pots per plot with some symptoms of wilting. Product HDC F182 had the lowest incidence and severity of wilt, when assessed in September 2013. By October, in all the treatments except HDC F183, wilt severity was lower than in the inoculated untreated control.

Destructive assessments were made in January 2014. Most of the roots were found around the face of the rootball. In both the Paraat and HDC F182 treated plants, a smaller proportion of root area was dark brown compared to those in the inoculated untreated control plots (Figure 1). However 33% of the root ball surface was still affected. Other

treatments did not differ significantly from the inoculated untreated control plots (which had dark brown roots over 47% of the root ball surface). Lighter brown roots nearer the pot surface (Figure 2) were also present and were probably naturally tanned. Both these and the darker roots lower down were confirmed to contain Phytophthora in inoculated pots. Pythium was also present in these roots and was also present in the roots in the upper and lower root-zone of the uninoculated control pots. No external stem browning was seen and although some internal stem base staining was recorded, this was seen throughout the plots, without treatment differences and no Phytophthora was detected in samples. There was no phytotoxicity.

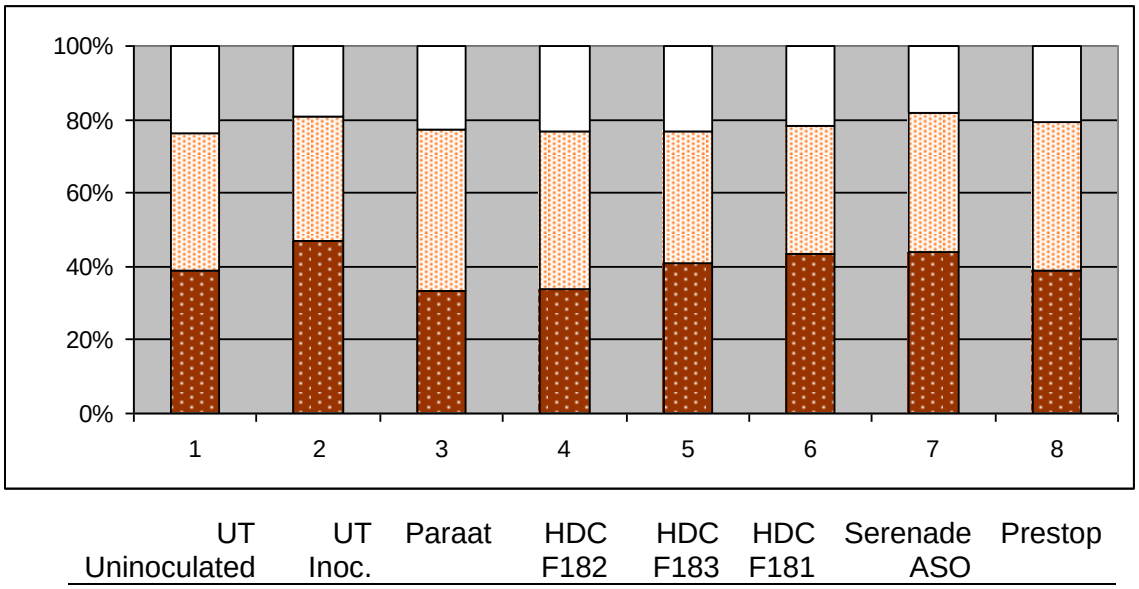


Figure 1: The mean proportion of root ball surfaces taken by dark rotted, lighter and white coloured roots (base to top of bar chart) in January 2014 showing a smaller area of dark brown roots where Paraat or HDC F182 had been drenched twice (P<0.05, Lsd 9.0%)

Figure 2:
Inoculated untreated two year old pot prior to destructive assessment in January 2014
Cane bases were examined for internal infection but the brown colour seen was not confirmed as Phytophthora.
Paler brown roots towards the top of the pot tested positive for both Phytophthora and Pythium. New white roots were growing.
Darker brown, rotted roots visible towards the pot base tested positive for both Phytophthora and naturally present Pythium.

Financial benefits

Effective treatments will reduce crop loss and extend the life of the plantation. Increasing the range of products available to growers for control of Phytophthora root rot via potential EAMUs, would increase the types of active ingredients used and reduce the chance of fungicide resistance developing. This will be particularly important if all soil fumigation products are withdrawn from the industry. Products effective on raspberry are likely to have an effect on other Phytophthora species such as those affecting strawberry and many ornamental species. Growers will be advised initially to drench small areas of their varieties in case of phytotoxicity not seen in the cv. Polka tested in the current work.

There has been interest in the two biological plant protection products to help the industry comply with the EU Sustainable Use Directive for reduced pesticide use. Neither gave significant root rot reduction and so growers should not expend money using them widely without first getting a better understanding of the conditions they might perform well under.

Action points for growers

- Determine the source and aim to stop the initial introduction of *P. rubi* into the crop.
- Utilise lateral flow devices to test roots to confirm symptoms are Phytophthora and thus ensure that the appropriate cultural or chemical control measures are used.
- Fungicide drenching against Phytophthora species needs to continue, but complete control is unlikely.
- Be aware that a molecular diagnostic test for *P. rubi* in soil is under development to facilitate decision making on soil versus container growing and tolerant variety selection based on inoculum thresholds.
- An EAMU should be sought for HDC F182 as it was found efficacious in the current work. This will widen the modes of action available to growers for *P. rubi* control.
- HDC F182 requires testing at different rates and application intervals in soil grown crops that have tested positive for *P. rubi* to seek to obtain the best efficacy.
- Do not discount the use of biological plant protection products; more understanding is needed of the host-pathogen-biological control agent relationship and how to utilise beneficial micro-organisms.

SCIENCE SECTION

Introduction

Raspberry root infection by *Phytophthora rubi* leads to root rot, wilt and cane death. Crop loss occurs in field-grown crops, but can also occur in container-grown crops. Losses can still occur on sterilised soil and in field or container grown crops using the current range of fungicide drenches. The current project sought to identify novel products, including at least one biological, and to test their efficacy as preventative drenches in inoculated pot tests. This has become increasingly important since one of the two products used in the UK for the control of raspberry root rot (Aliette, fosetyl-aluminium) lost authorisation for use after October 2012. This has left Paraat (dimethomorph) as the principal alternative, thereby increasing the risk of pesticide resistance developing through repeated use of a single active.

Methods

Objective 1 – Identification of candidate products for root rot control

A provisional short-list of HDC 181, HDC F182, HDC F183, Prestop (*Gliocladium catenulatum*), Ranman Twinpack (cyazofamid) and the standard Paraat (dimethomorph) was agreed with the Soft Fruit Panel in 2011. Subsequently, the Panel agreed to the substitution of the Ranman Twinpack with Serenade ASO (*Bacillus subtilis* strain QST 713) (with on-label approval for *Botrytis* on protected strawberries) as the latter was more likely to be able to be used in future on protected raspberry by growers as a recent Extension of Authorisation for Minor Use (EAMU number 0663 of 2012) allows it use as a once per year drench at 10 L/ha on outdoor cane and bush fruit against *Phytophthora.* An EAMU (0499 of 2012) had previously been obtained for its use as an annual drench against *Phytophthora* on trees in amenity situations and forest nurseries. A product containing the same *Bacillus subtilis* strain (Serenade Soil) is approved in the USA against *Verticillium* in fruit and vegetable crops and research in 2012 had been done by the manufacturers to evaluate its control of *Phytophthora* and *Fusarium* in the soil.

Objective 2 – Evaluation of products for control of P. rubi in raspberry

A preliminary experiment using bare-root raspberry cv. Polka was commenced in April 2011 to determine a *P. rubi* inoculation method and inoculum concentration that resulted in reliable disease development. The experimental methods used to establish and assess this work were reported up to the end of January 2012 in the Year 1 report and these were utilised in the main trial in Year 2. Six methods of inoculating pots using cultures were

examined: three densities of 10 mm diameter agar plugs of 3 week old *P. rubi* (4, 6 or 8 plugs) and two inoculation depths (60 mm and 120 mm). Another two methods utilised naturally infested raspberry roots. The results of assessments up to 25 January 2012 were reported in Year 1, with no wilt attributed to *Phytophthora*. The plants were allowed to grow up again in Spring 2012, however there was little root browning and no differences between the treatments were apparent.

The main product efficacy experiment used modules (i.e. plants grown in trays in growing media) of cv. Polka and commenced on 16 May 2012. As in 2011, the plants were potted-on into 5 L pots of Ericaceous peat growing media, stood in saucers and placed in a 20 m by 6.5 m polythene clad tunnel with doors (Polytunnel 7) at ADAS Boxworth (**Figures 3** & **4**). Plants were stood in two rows either side of a training wire, with four wires down the tunnel length. Plots contained 10 pots, with five pots per plot each side (adjacent to each other) of the training wire. The 10 pots in a treatment plot were arranged in the rows within 1.5 m, with 0.5 m between plots (i.e. 2 m per plot). Once the plants reached 0.5 m in mid-June their tips were removed to obtain branching, although subsequently most of these were removed to aid distinguishing and thus assessment of individual plants and because fruit yield recording was not required. Irrigation, connected to a Dosatron and a liquid feed solution (Sangral Select 3-2-6) after the first five weeks, was set up with one dripper per pot. Irrigation frequency and duration of bursts was modified throughout the year according to the changing water demand from the plants, seeking to keep the growing media continually moist without leaving the pots standing in water. A temperature and humidity logger was placed on the central wire in the centre of the experiment above a pot so that the stems grew around it. Another, temperature only, logger was buried half way down a central uninoculated pot. The use of pesticides to control pests and disease was avoided where possible, with biological control of vine weevil, aphids, two-spotted spider mite and caterpillars being used instead.

Efficacy treatments

Treatments (**Table 1**) to be evaluated for their efficacy against *P. rubi* for the main experiment (Years 2 and 3) were applied on 3 October 2012, when fresh root and shoot growth was still occurring. Inoculation followed a week later when the temperature was within the range for optimal development of *P. rubi*. It was later agreed with the Soft Fruit Panel that, in keeping with commercial practice, a repeat series of drench applications were to be made in Spring 2013.

Replicate 1	
Plot	Treatment
1	4
2	2
3	3
4	6
5	7
6	5
7	8
8	1

Replicate 2	
Plot	Treatment
9	3
10	4
11	6
12	2
13	8
14	1
15	5
16	7

Replicate 3	
Plot	Treatment
17	1
18	3
19	7
20	8
21	4
22	2
23	6
24	5

Replicate 4	
Plot	Treatment
25	5
26	6
27	1
28	8
29	2
30	7
31	4
32	3

Figure 3: Randomised Blocks of plots of 10 potted cv. Polka plants, with eight treatments / replicate row. May 2012 to January 2014, Polytunnel 7 at ADAS Boxworth

Figure 4: Raspberries in an ADAS Boxworth polytunnel, showing canes coming into flower on 30 August 2012, three months after potting cv. Polka plug plants. Five pots per plot stood each side of the training wires. Replicates 1 to 4 seen from left to right

There were six treatments plus two untreated controls (one uninoculated) (**Table 1**) and four replicate blocks, in total 32 plots (**Figure 3**). The four plots of 10 pots across the tunnel width thus covered 13 m² (i.e. 2m plot length x 6.5 m tunnel width) of the tunnel, including central and side pathways. Therefore, for the use of drench calculations 3.25 m² of ground "belonged" to each plot (including pathway). The target dose was the maximum label rate per hectare allowed per application. Where products were being used experimentally and specified a rate/ha it was agreed with the pesticide suppliers that the entire fungicide dose available for 3.25 m² would be given to the 10 pots in a plot, i.e. the paths will not be drenched and the pots will receive their area allocation instead. All other treatments used the same calculation.

Fungicides were poured across the moist compost surface using a calibrated container. A volume of 500 ml was used (i.e. 10% of pot volume) after it was found to wet the compost in the pot to the point of drain-out, as required. HDC F182 and HDC F183 were used under Administrative Experimental Approval COP 2012 1844, and Serenade ASO under COP 2011 00274 and so destruction of the fruit, and finally the plants, treated with these products was required.

Table 1. Six chemical and biological treatments drenched on potted raspberries on 3 October 2012 and 12 March 2013.
All treatments except Treatment 1 were inoculated with *Phytophthora rubi* at least a week after each product application date.

Treat-ment number	*P. rubi* agar plugs	Product & & FRAC group	Active ingredient	Rate of product to be added to water	Application volume	Approval status and rate on approved crop All products to be used in this experiment as a drench to the compost
T1	N	None		-	500 ml per 5 L pot	Water only
T2	Y	None		-	500 ml per 5 L pot	Water only
T3	Y	Paraat (standard) **40**	dimethomorph	0.1 g / plant	500 ml per 5 L pot	Approved for minor use on raspberry (indoor and outdoor) as a drench at 1g / plant in a minimum 200 ml water/pot. The strawberry rate of 0.1g to keep within the maximum 3 kg/ha label rate for both crops.
T4	Y	HDC F182	not disclosed	not disclosed	500 ml per 5 L pot	AEA COP 2012/01844 Used at the maximum rate/ha for container crops.
T5	Y	HDC F183	not disclosed	not disclosed	500 ml per 5 L pot	AEA COP 2012/01844 Rate agreed with chemical company.
T6	Y	HDC F181	not disclosed	not disclosed	500 ml per 5 L pot	Label rate for the crop in Autumn 2012, trebled in Spring 2013.

Table continued on next page.

Table 1 continued. Drench treatments used on 3 October 2012 and 12 March 2013 over raspberry pots.

Treat-ment number	*P. rubi* agar plugs	Product & FRAC group	Active ingredient	Rate of product to be added to water	Application volume	Approval status and rate on approved crop All products to be used in this experiment as a drench to the compost
T7	Y	Serenade ASO	*Bacillus subtilis*	0.32 ml / plant	500 ml per 5 L pot	AEA COP 2011 00274. Experimental rate agreed with BASF based on the maximum of 10 L product/ha applied as a spray.
T8	Y	Prestop	*Gliocladium catenulatum*	Add 5 g to 1 L water to make a 0.5% solution then apply this diluent at 500 ml per 5 L of compost in pot	500 ml of 0.5% solution per pot	EAMU for use against root pathogens on outdoor cane fruit, full label recommendation on protected crops. Used at recommended drench rate of maximum volume of 100 L of 0.5% solution /1000 L compost.

Inoculation of the main trial

P. rubi is relatively slow growing and so plates were used at 21 days old after incubation in the dark at 20°C to produce mycelium that nearly filled the 90 mm diameter agar plate. Agar plates (thickly poured) were of P5ARP (cornmeal + antibiotics) and inoculated using a single central disc of *P. rubi*. The isolate used was of *Phytophthora fragariae* var. *rubi* SCRP333, FVR11, IMI355974, ATCC 90442 collected from raspberry from Scotland in 1985 (supplied by D. Cooke, James Hutton Institute), as used in the preliminary experiment. The isolate was re-confirmed as *P. rubi* by Fera using molecular diagnostics in September 2012. Two other old isolates were received from Fera in time to use for inoculation, but were not used. These plates had been sub-cultured directly from the culture collection and when they were examined the mycelial growth was white (as for *P. rubi*), but the colonies were much more floccose than the SCRP isolate and under the microscope there were abundant hyphal cross-walls (septae) which should not have been present. Molecular diagnosis subsequently confirmed that *P. rubi* DNA was present (dead or alive). It is likely that the slopes had become overgrown with a contaminant during storage. A raspberry stool assessed to have *Phytophthora* root rot was sent to Fera in February 2012 to see if a fresh isolate could be obtained, but although DNA of *P. rubi* was detected it was not possible to obtain a culture of the pathogen from either the roots or the stem base.

Attempts at obtaining some fresh *P. rubi* isolates were continued in May 2012 with the collection of stools from eight raspberry farms across the UK with histories of *Phytophthora* root rot from Project SF 130. A further eight pots of raspberries with root rot symptoms from one plantation (Robert Irving pers. comm.) were taken to Fera in February 2013, in a further attempt to obtain fresh *P. rubi* isolates.

It was concluded from the preliminary trial in Year 1 that no more than eight 10 mm diameter plugs would be used in the Year 2 work, buried half way down each pot, as it was not known if more than this would cause more root rotting than was required. At the first inoculation of the main trial, five months after potting, holes were made with a dibber 50 mm in from the pot edge to 50 mm deep. A hole was made on each side of the pot, including the pots that were to remain uninoculated. It was intended to make four holes per pot on a single day and place two 10 mm diameter agar culture plugs in each, however contamination of some of the *P. rubi* agar plates meant that only half the inoculation points were made on 10 October 2013, and the remaining inoculations were made into holes at different positions on 31 October 2013 once more *P. rubi* cultures had grown. Agar plugs 10 mm wide were cut with a cork borer from the 21 day old *P. rubi* plates, with half the plugs

from just inside the leading edge of the colony and the other half of the plugs from older mycelium nearer the centre of the dish. Pots each received some older and younger aged mycelium. The hole was refilled within half an hour of adding the inoculum using the same Ericaceous growing-media (moistened) as used for potting. Because of a difficulty in obtaining sufficient culture plates the number of plugs differed between the replicates, with Replicates 1 and 2 having six, Replicate 3 having five and Replicate 4 having four *P. rubi* plugs buried in the growing media.

It was agreed with the HDC that the plants would be re-drenched in Spring as commonly done in commercial practice, and that inoculation would follow to mimic the infection that can develop as microbial pathogens resume growth and multiplication with the warmer weather. The drenches were carried out on 12 March 2013. On 12 April 2013, two holes were dibbed on opposite sides of each pot and 5 inoculum discs put in each hole. Smaller, 5mm and 6 mm, plugs were used than previously so that they dropped further down the inoculation hole. The holes were re-filled with growing media and watered to seal the hole and prevent the plugs drying up. Uninoculated agar plugs were dibbed into Treatment 1 pots before starting work with the *P. rubi* agar plugs.

Cane assessment at time of pruning-out in January

Final assessments of stems for browning under the epidermis that was likely to be caused by *Phytophthora* was carried out after leaf drop during the winter husbandry procedure of spent cane removal in the preliminary and main experiments.

In the preliminary experiment, on 25 January 2012, the epidermis was removed with a scalpel from compost level to 100 mm up the cane base to examine for staining underneath the epidermis. Several mature canes were present in each pot. Samples of internally stained tissue were taken from the leading edge of any staining for isolation on agar and for *Phytophthora* spp. LFD testing from each treatment.

In the main experiment, damage of the lower stem tissue by removing the epidermis from ground level in January 2013 was avoided as the old canes were to be cut back to two buds about 60 mm above the pot surface and cropped again throughout 2013. The cane growth in 2012 originated from the original plug plant and so there was a single cane per pot. The lower cane was examined externally on 25 January 2013 for any browning that could be distinguished from maturation and the stem cross-section was examined when the spent cane was removed. If any browning was seen then the epidermis was stripped from the upper, cut-off, cane section to look for *Phytophthora* staining symptoms.

Foliage assessments

Plants were monitored for leaf and cane wilting, and the yellowing that could precede it, throughout the preliminary (inoculation technique) and main (efficacy) experiments after inoculation. Records were made of the proportion of leaves and/or number of canes affected and photographs and descriptions made of the symptoms.

Plants were assessed for any phytotoxicity following drenching. Symptoms such as yellowing, necrosis, distortion or stunting were looked for and would be assessed in the most appropriate way following standard operating procedures. Descriptions and photographs of any suspected symptoms were made and incidence and severity recorded. Nil symptoms were also reported.

Assessment dates are given in the diary in the Appendix and alongside the results.

Destructive assessment of Spring growth, and roots.

At the termination of the preliminary experiment on 11 May 2012 the new shoots were assessed for the presence of any browning or leaf wilting. The root ball of each stool was then knocked out of each pot. Root rot was assessed on all the roots around the outside of the root ball, before cutting down through the root ball from top to bottom twice (quartering the root ball) and examining the roots inside. Care was taken to not include roots as rotted that were just naturally tanned. The appearance of roots in the uninoculated pots was used as a guide and also, when uncertain, the surface of roots was scraped to see the inner tissue and confirm whether it was white and not brown / rotted. The number of rotted root lengths visible was assessed.

At the termination of the main experiment on 7 January 2014, the surface area taken by rotted roots around the outside of each root ball was assessed and also the proportion of the surface with white roots. Golden stained roots were also present which were usually not rotted, although when some were scraped they had internal staining, but as it was impossible to check every root, roots of this colour were all recorded together. The root ball was halved vertically for assessment. The epidermis was removed from the cane bases to record staining. Brown roots and stem staining were tested for the presence of *Phytophthora* spp. with lateral flow devices (LFDs).

Results

Objective 1 – Identification of candidate products for root rot control

Six products were selected for application to the main trial in Autumn 2012. Details were given in Table 1.

Objective 2 – Evaluation of products for control of P. rubi in raspberry

Isolates for inoculation with P. rubi

Details of the infection recorded in specific plants samples are given below (**Table 2**) and showed that Phytophthora root rot is commonly seen by ADAS advisors. Two plants were sent from some sites and one plant had obvious wilting likely to have been caused by Phytophthora root rot and the other plant further along the row that had symptoms just visible. Nineteen raspberry stools showing wilting were collected from commercial plantations in 2011 and one in February 2012, but although oospores resembling those of *P. rubi* were seen it was not possible to obtain an isolate of *P. rubi*. No isolate was obtained by Fera from potted raspberries delivered by ADAS in February 2013. No fresh isolates have since been obtained by early 2014.

Molecular (Taqman) tests were completed as part of project HDC SF 130 but as they were for a *Phytophthora fragariae* and *P. rubi* primer set they may not be entirely specific (James Woodhall, Fera, pers. comm.). Oospores (thick walled resting spores) were seen in the roots of most of the plants, although some were smaller and rounder than the large lens-shaped ones usually produced by *P. rubi.* In several cases e.g. 9466, 9703 (**Table 2**) the oospores were typical of *P. rubi* and the lateral flow device (LFD) test (based on antibody detection of any *Phytophthora* species) and the Taqman all confirmed positives. The only positive LFD tests not confirmed by the Taqman (9704 and 9719) were “weak” indications (faint blue lines) which are normally accepted as being positive. Phytophthora oospores of a species other than *P. rubi* were present in sample 9704.

PCR confirmed *P. rubi* in isolates from the Fera culture collection (CC2106 and CC1218) received in September 2012. The third sample in the collection was dead. When sub-cultured a white colony grew but all the mycelium was cross-walled (Oomycetes have no cross-walls in growing hyphae) and the colony morphology was incorrect for *P. rubi*. It was likely that the DNA of *P. rubi* detected was from dead mycelium. A contaminant had taken over during the long term storage under water (one isolate was from 2003) and so no Fera isolates were able to be used. The isolate SCRP3333 of *P. rubi* received from the James

Hutton Institute culture collection was thus used in both the preliminary inoculation method experiment, which ran between March 2011 and May 2012, and in the main efficacy test which started in May 2012 and ran until the end of January 2014.

Table 2. Results of Fera laboratory tests on raspberry stools sampled from soil-grown commercial plantations in May 2012 for Project SF 120

Fera laboratory ID code	**Variety**	**Location or sample ID & ADAS sampler**	**Diagnosis by oospores in roots**	**Identification by LFD or Taqman**
21209493	Glen Clova	"Plant 1, sample A".	No oospores seen	LFD +ve
21209494	Glen Clova	"Plant 1, sample B".	No oospores seen	LFD not done
21209466	-	"Bad plant". R. Irving	Oospores typical of *P. rubi*	LFD +ve
21209467	-	"Good plant". R. Irving	Oospores smaller but still in *P. rubi* range (overlap with other species)	LFD +ve Taqman +ve
21209703	Glen Clova	Essex, "Plant A". J. Allen	Oospores typical of *P. rubi*	LFD +ve Taqman +ve
21209704	Glen Clova	Essex, "Plant B". J. Allen	*Phytophthora* oospores not typical of *P. rubi*	LFD weak +ve Taqman -ve
21209719	Glen Clova	Surrey, "Plant A". J. Allen	No oospores seen	LFD weak +ve Taqman -ve
21209720	Glen Clova	Surrey, "Plant B". J. Allen	Oospores not typical of *P. rubi*	LFD +ve Taqman +ve
21211050	Octavia	Kent, Farm 1. H. Roberts.	Small/round oospores not typical of *P. rubi*, but also two large/lens-shaped oospores morphologically typical of *P. rubi*	LFD not done Taqman positive
21211051	Glen Ample	Kent, Farm 2 H. Roberts	Visibly infected, small/round oospores not typical of *P. rubi*	LFD not done
21211224	-	Cornwall, PYO site. C. Nicholson	No oospores seen	Taqman positive
21211226	-	Cornwall, Farm site. C. Nicholson	Mixture of small/round oospores not typical of P. rubi and large/lens-shaped oospores typical of *P. rubi*	LFD not done Taqman positive
21213088	-	Devon, PYO site. C. Nicholson	Oospores typical of *P. rubi*	LFD not done Taqman +ve

Assessments of Phytophthora in stems and roots

Preliminary experiment; April 2011 to May 2012

Results of the preliminary inoculation experiment were given in detail in the Year 1 report. No wilting occurred on the floricanes. By the destructive assessment in Spring 2012 new shoots were growing up but had not wilted. A few brown roots were seen on the exterior circumference of the root balls of the inoculated pots but no *Phytophthora* was isolated from them. No brown roots were seen inside quartered root balls.

First cropping year of efficacy experiment; May 2012 to January 2013

Assessments were carried out on 27 September 2012 before the first treatment drenches and inoculation. There was a small amount of leaf wilting (mean 1.5% of leaves) and leaf discolouration (mean 1.5% of leaves). However, these were spread equally across the trial.

After the main trial was drenched on 3 October 2012 leaf discolouration had increased a little to a mean 2.6% of leaves when assessed on 19 October (between the two October inoculation dates). There were no treatment differences. Yellowing was greater ($P<0.01$) in Replicates 3 and 4 (2.9%) than 1 and 2 (2.3%), and may have been related to differences in feed uptake from the drip irrigation provided for each row. There was no other leaf damage that could have been phytotoxicity, nor any differences in vigour.

The plants were checked again for leaf wilting and phytotoxicity on the fruiting canes on 30 November 2012 and 11 December 2012, and none seen before leaves dropped for the winter.

The floricanes were cut back on 25 January 2013 to giving as long as possible for any wilting or cane browning to develop. Plants usually had a either one or two floricanes, with two more often present in Replicate 1 to give a mean of 1.7 canes/pot ($P<0.001$; data not shown), but there was no difference between treatments. There was no difference visible in plant vigour between plots and no phytotoxicity. There was no obvious external browning on the cane bases. Each cane was examined for internal stem staining as it was cut down to a few centimetres above the pot surface. No staining, which could be caused by the invasion of *P. rubi* into the stem, was seen. Root assessment was not carried out until pot destruction in January 2014.

Second cropping year of efficacy experiment; January 2013 to January 2014

On 12 April 2013, 4 weeks after fungicide drenches were given to supplement the first drenches of the same products in October 2012, there were significant (P<0.01; **Table 3**) differences in the growth of the new canes between treatments. The developing shoots were from 100 mm to 200 mm tall. Plant vigour differences were seen in both the height of the canes (and the number of expanding leaves) and the number of shoots which had emerged to cover the top of each pot. Pot foliar ground coverage as viewed from above was assessed separately, as well as comprising part of the vigour assessment, but the ranking of these values was generally similar across the treatments (**Table 3**).

Table 3. Height and density of young canes scored as vigour (1 = poor to 10 = strong) and the mean % coverage of the pot surface by shoot growth when viewed from above on 12 April 2013.

Mean	**Treatments**								**LSD**
Measure	1	2	3	4	5	6	7	8	**(df)**
	UT Uninoc.	UT Inoc.	Paraat	HDC F182	HDC F183	HDC F181	Sere-nade	Pre-stop	**F pr.**
Vigour index 1-10	6.5	7.3	5.7	6.6	6.5	**8.6**	7.2	**8.1**	1.25 (21) 0.002
% Pot foliage coverage	68.00	65.75	61.01	73.29	60.57	**87.79**	67.88	74.62	12.132 (21) 0.003

It was clearly visible within the crop that plants after HDC F181 were the most vigorous with a good ground cover (significantly more than inoculated untreated plots of Treatment 2), although the Vigour Index 8.6 was not significantly greater than that of Prestop. The dose rate of HDC F181 had been increased since the first drench. Paraat and HDC F183 had the lowest vigour of all the fungicide treatments (Indices 5.7 and 6.5, respectively) although this did not differ from the uninoculated untreated plants of Treatment 1, and so probably represents the baseline vigour. The cover of shoots in HDC F183 was noticeably low. Any differences were not visible after a week as warmer weather increased all growth.

A significant difference (P<0.001; data not shown) had also developed in the new primocane growth between the replicate blocks, with stools in Replicate 1 being less vigorous (Index 5.6) than those in the other replicates (mean Index 7.6). No phytotoxicity such as scorching or distortion was seen on 12 April 2013, and the October 2012 *P. rubi* inoculation had not resulted in any wilting.

Wilting of leaves was seen and assessed on 5 September 2013 and was less (not significantly) severe in pots which had either been left uninoculated or given HDC F182 in Autumn 2012 and Spring 2013. Wilt in other treatments was more severe, with more pots affected than HDC F182, more similar to the 32% of uninoculated untreated pots (**Table 4**).

Table 4. Wilting Index for floricanes (0 = none, 1 = uncertain, 2 = slight, 3 = severe) and the mean % of pots per plot with any foliar symptoms on 5 September 2013.

Mean	**Treatments**								**LSD**
Measure	1	2	3	4	5	6	7	8	**(df)**
	UT Uninoc	UT Inoc.	Paraat	HDC F182	HDC F183	HDC F181	Sere-nade	Pre-stop	**F pr.**
Wilt 0-3 Index (severity)	**0.458**	1.025	0.611	**0.450**	0.653	0.639	0.825	0.675	0.542 (21) 0.431
% with Wilt (incidence)	32.8	47.5	48.6	**26.9**	42.3	36.1	37.5	30.0	32.75 (21) 0.821

Significantly (P<0.05; **Table 4**) more pots (58%) were wilted in Replicate 4 in September than the other replicates, with a gradation across the rows from Replicate 1 (24%). Replicate 4 crop row was along the south side of the tunnel. Necrotic areas on leaves which may have been from leaf desiccation unrelated to pathogen-caused root loss were given an Index of 1, distinct from plants with leaves which were becoming flaccid and more likely to have been caused by insufficient roots surviving to take up sufficient irrigation water.

By 3 October 2013, affected leaves were dying, rather than wilting, and new leaves were still being produced thus making the necrotic leaves less obvious. The wilt severity across the treatments was still low (**Table 5**). Except for the slightly greater wilt incidence and severity in HDC F183, the treatments were comparable with the uninoculated untreated and so the drenches may have provided some benefit. Incidence and severity of wilt increased significantly (P<0.001; data no shown) across the rows with Replicate 1<2<3<4 ranging from a mean16% to 75% of pots affected (grand mean 43%).

Table 5. Wilting Index for floricanes (0 = none, 1 = uncertain, 2 = slight, 3 = severe) and the mean % of pots per plot with any foliar symptoms on 3 October 2013.

Mean Measure	**Treatments**								**LSD (df) F pr.**
	1 UT Uninoc	2 UT Inoc.	3 Paraat	4 HDC F182	5 HDC F183	6 HDC F181	7 Sere-nade	8 Pre-stop	
Wilt 0-3 Index (severity)	**0.8**	1.4	**0.7**	**0.8**	1.2	**0.8**	**0.9**	**0.7**	0.48 (21) 0.063
% with Wilt (incidence)	**37.8**	52.5	**41.7**	**37.2**	61.4	**41.1**	**37.5**	**35.0**	24.73 (21) 0.350

Destructive assessments 7 January 2014

Destructive assessment of the experiment started on 7 January 2014 after leaf drop and was completed by the 10 January. It was necessary to use a separate assessor for each replicate in order to complete the work in the same week.

When the root balls were cut in half to assess the volume of the root mass and to see the extent of rotting it was seen that root ball (from two year's growth) formed a solid uniformly dark mass that filled the pot in all treatments. There were very few roots discernible individually within the growing media and those found were tanned, but those sampled did not appear to be rotted when scraped. It was impossible to readily determine the extent of any rotting inside the root ball. However, there were obvious differences on the root ball surface. Most of the current year's roots appeared to be forming a root mat growing around the ball next to the pot wall (**Figure 6**). Root ball halves were placed side by side to aid assessment of the proportion of the root ball surface area taken up by the different root colours that were visible (totalling 100%).

Three colours of root were recorded; white, dark golden (tobacco) brown (mainly in the top half of the pot) and dark chocolate roots (**Figure 7**). The dark roots were thinner and rotted and formed a band around the ball extending from the pot base. The golden roots were sometimes rotted when scraped, but it was impossible to distinguish them externally from healthy roots. The white roots looked like new growth and they were growing mainly from near the pot shoulders, but extended through the brown roots on the root ball surface.

At the start of the destructive assessments samples of roots were taken and shaken and crushed in buffer bottles and then drops of extract tested with both Pythium and

Phytophthora LFDs. *Pythium* spp. and *Phytophthora* spp. were confirmed in both the golden and the dark roots of some inoculated untreated pots. Pythium only was detected by the LFDs in both the upper root ball and lower root ball dark roots of uninoculated untreated pots (**Table 8**).

Significantly fewer (14%) dark brown roots were found in plots drenched with either Paraat or HDC F182 compared with the inoculated untreated plots. However, they still comprised 33% of the root ball (compared with 47% in the untreated) (**Table 6**). 33% was significantly less than those receiving either HDC F181 or Serenade ASO (43%). However, neither Prestop nor HDC F183 differed significantly from Paraat and HDC F182 (but they also did not differ from the inoculated untreated) in the proportion of dark brown roots. The uninoculated pots also had a significant proportion of their roots dark brown (39%) and this was probably caused by the Pythium detected or death from physiological causes. The extra 8% dark root rot in the inoculated was probably as a result of the *P. rubi* inoculation.

All treatments had the same proportion of white healthy roots, a mean 22% (**Table 6**), thus the greater proportion of golden roots present in Paraat and HDC F182 pots was because more of the golden roots had not become dark brown and rotted (**Figure 5**).

Table 6. The mean proportions of the root ball external surface area for each pot (100%) which were dark brown and soft, golden brown or white

Mean				**Treatments**					**LSD**
Measure	1	2	3	4	5	6	7	8	**(df)**
	UT Uninoc	UT Inoc.	Paraat	HDC F182	HDC F183	HDC F181	Sere-nade	Pre-stop	**F pr.**
% of root ball dark brown	38.88	46.93	**33.19**	**33.58**	41.25	43.35	43.75	39.13	9.017 (21) 0.049
% of root ball golden brown	37.62	33.66	44.08	42.67	35.67	34.58	38.20	40.30	7.162 (21) 0.058
% of root ball white	24.00	19.41	22.72	22.99	23.33	21.82	18.17	20.57	6.966 (21) 0.632

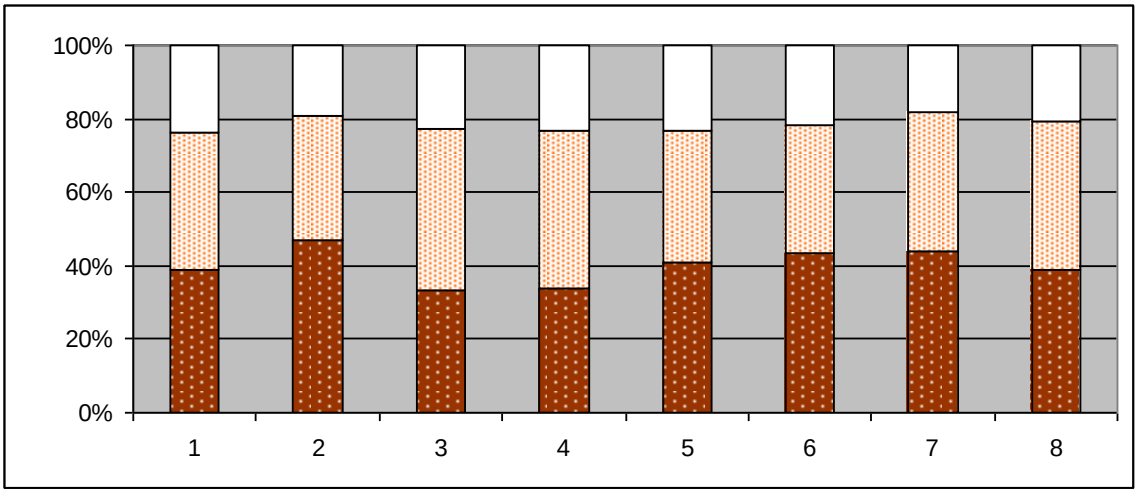


Figure 5. The proportion of the root ball surface taken by dark rotted, lighter and white coloured roots in January 2014 for the treatments given in Table 6

Figure 6: Root ball of an inoculated untreated pot showing dark rotted roots at the base which formed a greater proportion of the root ball than in uninoculated pots.

Figure 7: Close up of root ball with dark brown rotted, golden brown and white roots. These were seen in both inoculated and uninoculated pots, with *Pythium* spp. detected in brown roots. Phytophthora spp. was also detected in the dark and golden roots of inoculated pots.

There was a mean of ten canes per pot. Young shoots had been allowed to grow up later in the year as it was anticipated that these might provide a good indication of the presence of infection by showing "shepherd's crook" Phytophthora wilt symptoms (whereas floricanes could die more slowly), however no young shoot wilt developed. During the destructive assessment all the stems were scraped with a scalpel to remove the epidermis around the cane diameter to 100 mm above the stool. Some browning was seen inside green stems (**Figure 8**), usually changing back to green about 200 mm up. However, although a mean 21% of canes per pot had lower staining there were no treatment differences (**Table 7**). Internal cane staining higher up was also checked if there was any external browning, because this might mean that another pathogen such as Botrytis had grown down the canes to also be seen lower down. A mean of 11% of upper canes per pot had this symptom, but there was no relationship (P=0.474; data not shown) to the treatments and the symptom may have been the result of cane abrasion on the training wires.

Table 7. The proportion of cane bases with internal browning in January 2014

Mean	**Treatments**								**LSD**
Measure	1	2	3	4	5	6	7	8	**(df)**
	UT Uninoc	UT Inoc.	Paraat	HDC F182	HDC F183	HDC F181	Sere-nade	Pre-stop	**F pr.**
% canes with browning	21.8	22.9	25.3	19.6	13.4	23.4	25.2	19.2	8.34 (21) 0.115

Samples of lower stems were taken for LFD testing for Phytophthora on 24 March 2014, taking slivers of brown tissue after stripping off the outer tissue. Both the inoculated untreated and the uninoculated untreated pots had canes (mainly thinner canes) that were drying off and some of the green canes had browning under the epidermis. Samples were taken separately from green and drier stems of the inoculated pots and no Phytophthora was detected in either tissue. Drier and greener stems from an uninoculated pot were sampled together and these too tested negative for Phytophthora (**Table 8**).

Figure 8: Epidermis removed from a lower stem base to show brown and green tissue. Samples tested were not positive for *Phytophthora* spp. and were present across the treatments.

Table 8. Positive or negative results of lateral flow device tests carried out on 7 January 2014 on roots from pots in Plot 14 (uninoculated) and Plot 12 (inoculated).

Treatment	Position, symptoms	Phytophthora LFD results	Pythium LFD results
T1 uninoculated & untreated	Upper roots, black and white	-ve	NT
	Upper roots, black and white	not tested	+ve but faint
	Lower roots, brown-black	-ve	+ve
	Lower stem internal browning	-ve	not tested
T2 inoculated with *P. rubi* & untreated	Upper roots, tan brown	+ve but faint	+ve
	Upper roots, tan brown	+ve but faint	not tested
	Lower roots, brown-black	+ve	+ve
	Lower roots, brown-black	+ve	not tested
	Lower stem internal browning on green cane	-ve	not tested
	Lower stem internal browning on drying cane	-ve	not tested

Discussion

The products selected for use in this project have been reported elsewhere as having good activity against “water-moulds” such as *Pythium* and *Phytophthora* spp.

The systemic product HDC F182 gained approval for use as a drench, dip and foliar spray on outdoor strawberries against *Phytophthora* spp. and *Pythium* spp. during the life of this project. It gave significant control of strawberry crown rot in HDC Project SF 99. The active components kill zoospores and prime the defence mechanism of plants. One component is used in other countries as a foliar fungicide on grapes and vegetables, having activity against downy mildews as well as *Phytophthora* spp. and *Pythium* spp. The manufacturers will use the information on product efficacy from the current project to decide whether to seek to obtain residue data to allow HDC F182 registration for raspberry. HDC F182 was as effective at reducing crown rot incidence in Project SF 99 as the standards Aliette 80WG and Paraat (dimethomorph). HDC F182 has given a significant reduction in root rot in the current project when symptoms were recorded 10 months after its use and there was an indication of a reduction of wilting at assessment four months earlier.

The potato blight (*Phytophthora infestans*) fungicide HDC F183 was tested against the standard SL567A (465.2 g/L metalaxyl-M) in the Defra project PH0604 (Wedgwood, 2012) as foliar sprays against *Phytophthora ramorum* and significantly reduced *P. ramorum* on leaves. One component is the active ingredient in Paraat (dimethomorph) and this latter product is known to be safe and effective on raspberries. Dimethomorph is locally systemic, moving in the xylem, and can be taken up by the roots. It controls all parts of the *Phytophthora* lifecycle, with the exception of zoospore motility, but with effectiveness against developing and germinating oospores. Dimethomorph has translaminar activity and up to 72 hours curative activity. The other component of HDC F183 belongs to a new class of chemistry and is a mitochondrial respiration inhibitor. It has been found to be effective as a preventative spray against potato blight and downy mildews (Gold *et al*., 2009), but there is no residue data for soft fruit. A formulation of this active ingredient is being developed for use on grapes and residue and toxicity data could be used towards its use on raspberries. This product did not have any significant benefit at the rate used in the current project.

A number of microbial products with potential for use against the Oomycetes *Phytophthora cactorum* (strawberry crown rot), *Phytophthora fragariae* var. *fragariae* (strawberry red core) and *P. rubi* (raspberry root rot) were reviewed in HDC Project SF 66. Only the German bacterial product Rhizostar (*Serratia plymuthica*) used as a root dip was considered to have potential, but it only gave an 18% reduction in plants with crown rot in trials. A number of biological products put forward by manufacturers are currently being tested in the UK on some edible crops against various pathogens within a HortLINK project (HL01109 - HDC project CP 77) sponsored by Defra called SCEPTRE (Sustainable Crop & Environment Protection – Targeted Research for Edibles). In 2012, three biological products were found to be effective against downy mildew and so could have activity against *Phytophthora* spp., and work in 2012 on the control of strawberry crown rot (*Phytophthora cactorum*) could also prove useful for *P. rubi* control.

The biopesticide Serenade ASO (1.34% w/w *Bacillus subtilis* strain QST 713) has approval for use against *Botrytis* on a range of protected and outdoor crops, including cane fruit (sprayed to just before run-off). There is information that the product is effective against soil-borne pathogens (Simon Townsend, BASF, pers. comm.) and an Extension of Authorisation (0499 of 2012) was granted in 2012 for the product's use as an annual drench of maximum dose 10 L / ha against *Phytophthora* on trees in amenity situations and forest nurseries.

Prestop (32% w/w *Gliocladium catenulatum*) is registered as a biopesticide in the UK for use as a spray or drench in protected edible and non-edible crops and outdoor strawberry

for the control of damping off and root diseases caused by *Pythium, Phytophthora, Rhizoctonia* and *Fusarium*. It also has Extension of Use (0564 of 2012) for outdoor crops including cane fruit. Both crop situations have maximum drench doses of 500 g product / 100 L water, but application is also approved as a spray to the soil and by incorporation in compost. There is no information concerning its effectiveness against *P. rubi.* The beneficial fungus works by competing for space with the pathogenic fungi and utilises enzymes to break down fungal cell walls (Fargro Technical Notes for Prestop www.fargro.co.uk).

Both Serenade ASO and Prestop gave some reduction in crown rot, *P. cactorum,* incidence (not statistically significant) when used in strawberries before inoculation (Berrie, 2011 (Project SF 99)). Both products were used by drenching peat grow-bags at planting with 100 ml of either of the suspensions per plant, with repetition a fortnight later. In the current project repeat applications were five months apart. Repeat application could be investigated in future if growers are likely to be willing to drench more frequently. The roots assessed were growing around the outside of the root ball and it is perhaps less likely that a biological product would be as active in this drier environment. Neither product reduced raspberry root rot at the application interval and dose rates used in this project.

A product with the same active ingredient as HDC F181 used at twice the normal 5 L / ha rate for use as a foliar feed gave significant reduction in crown rot incidence in Project SF 99. However, other than transient increase in plant vigour at three times the normal rate in the current project there was no benefit from its use in raspberries with root rot.

The problem of not being able to isolate *P. rubi* should be considered in a wider context, because it seems likely that the pathogen has prolonged low activity periods. This could affect the timing of protectant treatments (timing them to immediately precede the higher activity periods) and possibly the timing of any future use of soil sampling to determine infestation levels. Chemical treatments are currently applied to commercial crops in Spring and Autumn (ensuring fruit harvest intervals are kept), but if biological products (with multiple applications allowed and no harvest intervals specified) prove to have efficacy then they could be deployed at what might be better times to kill the pathogens while they are active. Oospores are produced (and were seen) in the root tissue, to be released on root decay, and some are also likely to be produced by mycelium on the outside of roots and be readily detached into the soil. Oospore production is generally considered as a survival mechanism and so may have chemical or physical triggers to production. Any changes in seasonal abundance of *P. rubi* in the soil (as mycelium, resting spores or swimming zoospores) could affect the interpretation of results from any future pathogen threshold

research. Information on the epidemiology of the pathogen in open field as opposed to tunnelled crops of various covering periods is required. Based on laboratory techniques such as subjecting roots to running water and placing them in water to stimulate sporulation, and rapid chilling then warming cultures to stimulate and synchronise zoospore release, the effects of climate change on water flow through the crop, temperature changes and extreme sudden changes in the weather could all have a bearing on the pathogen and its control.

Both the preliminary and main experiments had strong fruiting cane growth and good root balls when inoculated with *P. rubi*. This was likely to have reduced the chance of wilt being seen as there would need to be a significant amount of root loss by the pathogen to sufficiently reduce the amount of water able to be taken up by the plant. *P. rubi* would then need to spread up into the canes to cause disruption of the vascular tissue and the staining takes several months to produce. Hot weather would not have been ideal for disease establishment in July 2012 when the preliminary trial was inoculated, but would have been favourable in the main trial in October 2012 and April 2013. That the inoculation was successful in the main trial was confirmed by the detection of Phytophthora (*P. rubi* cannot be confirmed specifically by LFD) in untreated roots and the 8% greater surface area of these root balls comprising a dark brown root rot compared with the uninoculated. There was also natural infection by Pythium and this was probably the principal contributor to the root rot towards the base of the pots because uninoculated pots had 39% rot. However, as pots receiving either Paraat or HDC F182 developed a smaller surface area of rotted roots (a difference of 6%) than uninoculated pots there was probably some control of Pythium. Control of Phytophthora by Paraat and HDC F182 occurred as rot was not as severe after their use as in the inoculated untreated plots (a difference of 14%). It should be noted that Pythium was probably the cause of such significant root loss in the current experiment, and so control of this pathogen is important.

The lack of a difference in cane staining internally, whether or not plants were inoculated, and the low incidence of wilt indicated that *P. rubi* did not visibly progress into the stool. It is possible that the browning seen was cane senescence starting at the older (lower) portion of the cane without disease being involved (particularly as the LFD tests for Phytophthora spp. were negative). The plants had a high water demand in their second year of fruiting and the aim to keep the growing media moist (to allow zoospore movement) without saturating it (causing anaerobic conditions) and encouraging any Pythium present to develop was not easily achieved, particularly as some pots were found to require more water than others. Pythium usually does not cause direct stem damage in woody plants.

No products caused phytotoxicity as a drench to cv. Polka. Products were applied to the peat-based growing medium around mature floricanes in Autumn. In the following Spring, the same drench products were poured around pruned-off floricanes with buds just starting to open and over the emerging spawn which had leaves starting to expand. Four weeks after application the HDC F183 (which is not approved for soil application) treated pots' Spring primocane growth vigour was not significantly different from that following Paraat use. One product, HDC F181, gave a marked temporary improvement in vigour, but this was not unexpected from its ingredients. Another product, Prestop, was biological and a short-lived growth boost four weeks after Spring application was noted, but it is not known whether the same stronger growth would have been seen in pots without *P. rubi*.

As part of the EC Sustainable Use Directive measures to reduce the use of pesticides need to be considered and growers should seek to reduce the chance of their plantations becoming contaminated by pathogens. With growers increasingly capturing run-off from soft fruit plantations in reservoirs and re-application to crops (Atwood, 2014) the development of a reliable method of detecting live Pythium and Phytophthora is required. HDC Project HNS PO/HNS 188 showed that baiting of Pythium and Phytophthora species from irrigation water run-off from ornamental plants is possible using pieces of apple in bags made from horticultural fleece. Baits attract and hold live zoospores of Pythium and Phytophthora. These can then be tested by either LFDs or molecular diagnostics which otherwise cannot discriminate live from dead pathogen material. This technique needs to be developed for use by soft fruit growers as the complication with *P. rubi* and the closely related *P. fragariae* is that their zoospores are only known to be baited by host plant roots (Schlenzig *et al*., 2005). More is becoming known about the chemical attractants and arrestants involved in zoospore infestation of tissue (Kong *et al*., 2010; Massola *et al*., 2012) and it is likely that with further research that baits could be developed for grower use for testing with either LFDs or in-field molecular assay equipment. Understanding more about the infection process could also have application in plant breeding and in the production of products that could confound zoospore host detection.

HDC Project SF 130 ran concurrently with the current project. Its aims were to develop a molecular test that could be used to identify *P. rubi* in soil destined for raspberry planting and to quantify the levels in order for follow-on work to look at soil inoculum thresholds tolerated by particular cultivars. A molecular, real-time PCR, test was developed for *P. rubi*. The sequencing work needed to develop the PCR test showed that *P. rubi* and *P.* fragariae are closely related and few sequence differences exist between the two species. One gene, the Cox1 gene, shows promise as a target for discriminating between the two species. Two

assays were designed, one for *P. fragariae* and another for *P. rubi.* However, further work is needed as initial assays had relatively low sensitivity and so were unsuitable for use for soil diagnostics. Container growing will continue to be the safer option if soil fumigation is not carried out, but it still carries a risk of Phytophthora contamination. The current project has identified a product other than Paraat the use of which in soil and container crops (if it becomes permitted by an EAMU) should reduce the chance of chemical resistance developing as the active ingredients differ. Further work on the quantitative molecular diagnostic test for *P. rubi* and elucidating cultivar resistance will be important for integrated crop protection so that preventative fungicide application in raspberries can be reduced.

Conclusions

- Five novel products with activity likely against Oomycetes were selected and tested as drenches against *P. rubi* for the control of Phytophthora root rot in raspberry: HDC F181, HDC F182, HDC F183, Prestop (*Gliocladium catenulatum*), and Serenade ASO (*Bacillus subtilis*).
- A May 2012 potted crop of cv. Polka was drenched in Autumn 2012 and Spring 2013 and inoculated with *P. rubi* after each application. Canes developed wilting in Autumn 2013. Foliar symptoms including leaf yellowing were not all related to root infection and there were no significant treatment differences, but HDC F182 had the lowest wilt incidence and severity in September.
- Roots of inoculated untreated pots were confirmed, in January 2014, to be infected by *Phytophthora* spp.. Natural colonisation by *Pythium* spp. had occurred in inoculated and uninoculated pots.
- By January 2014 a dark brown root rot comprised 47% of the root surface area in inoculated untreated pots. Pots drenched with Paraat (dimethomorph) and HDC F182 had 33% of their root surface area affected by this rot, significantly less than those receiving either HDC F181 or Serenade ASO (43%). Root rot reduction to 33% was probably as a result of some control of both Phytophthora and Pythium.
- Internal browning of cane bases was seen in all plots at the destructive assessment, but was unrelated to treatment.
- No phytotoxicity developed in cv. Polka following either of the two drench applications of each product.

Knowledge and Technology Transfer

Information on the project was provided for the HDC News Supplement HDC Soft Fruit Review 2012-2013, (page 15).

Glossary

Technical terms have been explained within the text.

References

Atwood, 2014. Water harvesting and recycling in soft fruit. A grower guide. HDC.

EPPO Data Sheets on Quarantine Pests *Phytophthora fragariae* prepared by CABI and EPPO under contract 90/399003 11pp. http://www.eppo.org/QUARANTINE/FUNGI/Phytophthora_fragariae/PHTFR_ds.pdf

Gold, R., Scherer, M., Rether J., Speakman, J., Navé, B., Levy, T., Storer, R. and Marris, D. (2009). Initium – An innovative fungicide of a new chemical class for the control of Oomycetes. BCPC Congress, November 9-11, 2009, Glasgow UK. www.bcpccongress.com

HDC CP 77, HortLINK HL01109 – Sustainable Crop and Environment Protection- Targeted Research for Edible (SCEPTRE). Year 2 Report, January 2013.

HDC Project SF 66. Review of biocontrol strategies and novel products for control of key pests and diseases in strawberry and raspberry. 2005.

HDC SF 99 - Sustainable control of crown rot (*Phytophthora cactorum*). Final report, 2011.

HDC SF 130 – Raspberry: detection and quantification of *Phytophthora rubi* in soil and in plant tissue. (April 2012 to March 2014).

HDC PO/HNS 188. Baiting and diagnostic techniques for monitoring Phytophthor spp. and Pythium spp. in irrigation water on ornamental nurseries.

Kennedy D.M. & Duncan J.M. (1991). Methods for assessing the resistance of raspberry genotypes to *Phytophthora* root rot. Plant Pathology 40, 387-394.

Kong, P., Tyler, B.M., Richardson, P.A., Lee, B.W.K., Zhou, Z.S. and Hong, C. (2010). Zoospore interspecific signalling promotes plant infection by Phytophthora. BMC Microbiology, **10**:313. http://www.biomedcentral.com/1471-2180/10/313

Massola, N.S., Dalio, R., Fleishmann, F. and Osswald, W. (2012). A new method to quantify zoospore chemo-attraction. Poster 38. 6th IUFRO Working Party 7.02.09. "Phytophthora in Forests and natural Ecosystems. 9th to 14th September 2012, Cordoba, Spain. http://iufrophytophthora2012.org/

Schlenzig, A., Cooke, D.E.L. and Chard, J.M. (2005). Comparison of baiting method and PCR for the detection of Phytophthora fragariae var. rubi in certified raspberry stocks. EPPO Bulletin, **35**: 87-89.

Wedgwood, E. F. (2012). Defra Project PH0604 *Phytophthora ramorum* and *Phytophthora kernoviae*: Improved approaches for disease management in heritage gardens and parks. Annual Report for 2011. In: Research Key Findings www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/documents/prPkResearchJan12.pdf

Appendices

Appendix 1. Trial diary for the preliminary inoculation experiment in 2011 to 2012

Date	Action or observation
20/04/2011	Bare root cv. Polka ex Hargreaves potted in Ericaceous compost & put in polytunnel.
27/06/2011	A. Tinsley e-mailed to notify that no fresh *P. rubi* isolates have been isolated
29/06/2011	SCRP 3333 FVR11 IMI 2555974 *P. rubi* isolate from James Hutton Institute confirmed positive on *Phytophthora* LFD and sporangia later seen in pond water float.
14/07/2011	Drenched with Nemasys L against vine weevil.
15/07/2011	Vermiculite V8 bags made 1/7/11 have not grown *Phytophthora*. More agar plates inoculated. x 10 agar plates also made to inoculate directly in pots.
18/07/2011	2nd batch Vermiculite V8 bags inoculated with SCRP *P. rubi*
20/07/2011	Raspberry feeding increased. Pots are both wet & drier from same 4-way disc.
25/07/2011	Pots confirmed all at field capacity. Timings 7.05, 11.05, 15.05, 19.05, 23.05 for 5 mins on drippers.
27/07/2011	Agar plugs of SCRP isolate used to inoculate pots utilising dibber and 4 holes. 40 pot trial. Pot compost pH 7.
01/08/2011	Nemasys L drench against vine weevil.
15/08/2011	Watering increased. Drippers delivering 100ml/pot each after increase to 6 mins.
22/08/2011	Fruiting canes being tied-in. Fruit being removed (no yield required). No differences in vigour or wilting between plots. Vermiculite bag method abandoned as no sporangia have grown in test float set up 01/08/11ex. 2nd Vermiculite bags.
20/09/2011	Wind damage to trial, so some floricanes trimmed Block 1 T2; Block 2 T7; Block 3 T5 T3, T8, T1, T2; Block 4 T6,T7,T10,T4,T1,T8,T9.
10/10/2011	Removed drippers from the soil and laid them in saucer as delivering same water volume, but some pots are not becoming evenly moist.
04/11/2011	Assessed 40 pots for wilt. None related to root rot - plants with yellowing basal leaves were near the tunnel door. Spider mites have had 2x biological releases but are also yellowing some leaves.
25/01/2012	Floricanes assessed for external + internal browning & plated out. All cut to 2 buds and fleeced to encourage new shoot growth. Watering off, but pots kept will be kept moist by hand-watering.
13/02/2012	Some white isolates checked with LFD for *Phytophthora* but negative. Botrytis & Alternaria present.
20/04/2012	Fleeced plants now have 200 mm shoots, very dense. Look healthy. Fleece removed. Drippers put back on, but pots being kept wet by top-up watering twice a day by hand on hot days. Observations being made for “crooking” of new shoots from root rot.
11/05/2012	**Destructive assessment of preliminary trial**. Plants were examined for browning on stem and wilting of leaves, as well as browning of roots (outer and central). Where suspect browning was found, roots were surface sterilised, plated on PDA+S and stored in the incubator at 20 degrees. Data logger was downloaded.

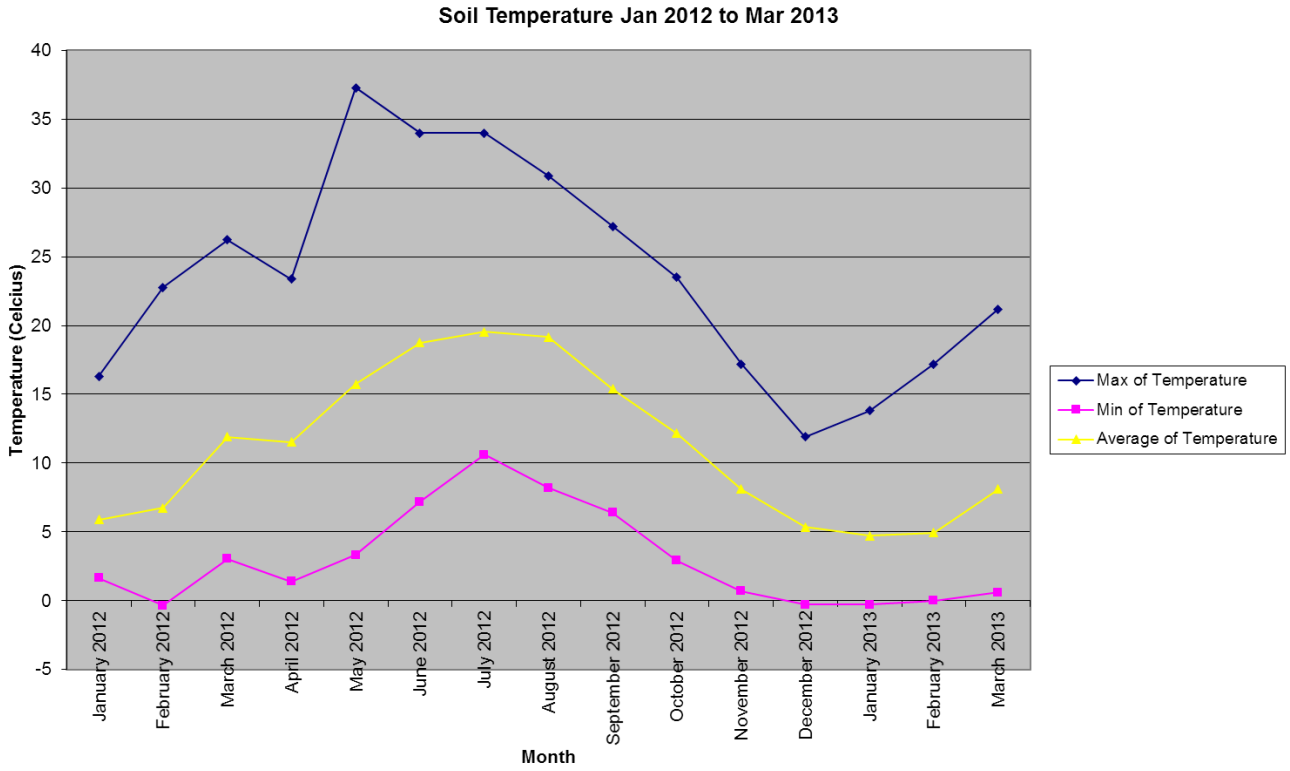
Appendix 2. Trial diary for the main, efficacy, experiment for 2012 to 2013

16/05/2012	Raspberry plugs cv. Polka ex Hargreaves arrived and stored in a cool temperature.
18/05/2012	**Set up main trial**. Raspberry plugs potted in Ericaceous compost in 5 L pots in saucers and placed in polytunnel 7.
22/05/2012	Plots and treatments labelled.
29/05/2012	Approx 10 plants failing to thrive, plot number and pot noted.
08/06/2012	Now only 4 plants failing to grow.
18/06/2012	Caned some plants as they were bending over but (not big enough to reach polytunnel wires).
20/06/2012	Set up irrigation. Feeding with Sangral Select 3-2-6, dilution is 1: 100 = 1kg to 10 L water.
25/06/2012	Original stems of modules allowed to grow to 50 cm then tips pinched out to encourage branching (and stimulate new cane growth from canes bases and from buds on main root). Tied up plants
01/07/2012	Tie up plants. Nemasys L drench against vine weevil.
9/06/2012	Sprayed Di Pel on all plots.
11/09/2012	Pots missing because failed to grow = 3 from Plot 24, and 1 from Plots 10, 11, 18, 25 & 31
12/09/2012	Laterals pruned back. Irrigation 3 mins at 8:00, 10:00, 12:00, 14:00, 16:00, 18:00h.
27/09/2012	Pre fungicide assessment carried out of wilting 1-5 index 1=least severe 5=most severe, 0=no wilting
03/10/2012	**Drench treatments applied** to plots. 20 L of each treatment made up and 500ml poured onto soil around stem for each pot. Raspberry drench was completed between 11:45-13:20. Soil and air temperature recorded. Data logger downloaded and re-started. Air temp was 24.2°C, relative humidity was 56.2%.
08/10/2012	Watering of pots by hand as variable amounts of uptake from automatic irrigation.
10/10/2012	**Inoculation.** Each pot 2 holes made to 5 cm deep. Uninoculated holes and agar blanks done first. No.8 (10mm) P5ARP agar plug using 3-week old *P. rubi* isolate plate confirmed by PCR. Inoculation of pots 11am to 3pm, cool with sun. Problem with contaminated plates so only used 2 agar plugs from outer edge of colony per pot + one inner Reps 1, 2, 3. Only 1 outer and 1 of either outer or inner Rep 4. Filled holes with more peat and given light water. More *P. rubi* plates made for further inoculation in 3wks time.
11/10/2012	3-4 pots have snapped stems.
17/10/2012	Broken stems due to ties not holding with fruit on. Pruned damage, removed fruit, re-tied. Replaced drippers in pots that were too wet, ready for irrigation to go back on.
18/10/2012	Some plants showing leaf yellow mosaic = likely nutrient deficiency so new feed will be given. Caterpillar spray given again as many leaves have feeding holes. Spider mites treated with BCA. Some green leaves coming off at petiole-not sure of reason (suspect might be an animal coming in the tunnel). Some leaf down-curl on young leaves but not treatment specific.
19/10/2012	**Phytotoxicity assessment** carried out on a 0-9 scale, with 0=no phytotoxicity and 9=severe phytotoxicity. Looked at untreated plots first to see what symptoms were naturally occurring. Leaf yellowing, spotting, curling and drooping all seen in untreated and treated plots so most plants score 0 for phytotoxicity Each plant was also given a discolouration score on a 0-5 scale, with 0=no discolouration and 5=severe discolouration. Many plants were discoloured potentially due to nutrient deficiencies. No plant collapse from potential root rot seen. Plants were assessed in the order from plants 1 and 2 at the top of the plot to plants 9 and 10 at the bottom of the plot.

29/10/2012	Photos taken from phytotoxicity assessment on 19.10.2012 were checked by EFW and it was decided that the symptoms were unlikely to be phytotoxicity. Assessment scores will be taken as physiological not toxicity.
31/10/2012	**Inoculation**. Inoculum supplemented. Used 3 agar discs of *P. rubi* on reps 1 & 2 (1 inside 2 outside colony) and 2 discs on reps 3 & 4 (1 inside 1 outside of colony plug). No sign of disease yet.
10/11/2012	No wilting from any disease.
19/11/2012	Put string along to hold stems. Fewer leaves on floor since door base blocked off.
30/11/2012	No leaf wilting. Pictures taken of some leaf marking, but EFW said likely wounding. Automatic watering off, keeping pots moist by hand.
11/12/2012	Now frosty outside. Water has been turned off, but compost still wet. Plants still have leaves, most still green, some colour differences but this does not relate to plot i.e. not likely to be phytotoxicity visible. No leaf collapse from *Phytophthora* wilt, but might not as compost wet so not short of water.
02/01/2013	Most of the leaves in the trial have wilted for winter.
08/01/2013	Weather mild. Pots moist as required. Leaves starting to drop off
25/01/2013	Carried out cane internal staining assessment no disease found. Pruned back to 6 cm and cleaned up.
25/02/2013	A few pots have shoots just coming through compost.
01/03/2013	Air temperature logger downloaded and restarted.
07/01/2013	Soil logger downloaded and replaced back buried in a pot in plot 14.
12/03/2013	Drenches carried out. Ensured all pots had been moist but without water in the saucers.
15/03/2013	No phytotoxicity seen. Most pots have young shoots just visible above the growing media. Buds on pruned-back floricanes have started to open. Pots moist.
20/02/2013	200 plates of SCRI *P. rubi* isolate subbed and incubated in the dark at 20 deg C
28/03/2013	No phytotoxicity. Shoots not growing much as it has been very cold.
02/04/2013	Snow then frost recently so water not on. Shoots growing as warm when sunny.
12/04/2013	**Inoculation** with *P. rubi* using 10 agar plugs per pot. Hand watered lightly to wash compost infill down around the agar plugs in each of the two dibber holes. Drippers and Dosatron feed diluter working.
12/04/2013	**Vigour** (1-10 index) based on shoot height and % ground cover. Variability within plots. Treatment 5 HDC F183 looked less vigorous (less ground cover) whereas HDC F181 looked very vigorous with high % ground cover. No disease wilting. No scorch. Shoots 10 -20cm high, the smaller still had leaves expanding and were redder.
18/04/2013	Pots are wet as required, without ponding in the saucers. Sangral being used at 1kg per 10 L of water in the stock tank and given at a dilution of 1:150.
19/04/2013	One collapsed shoot in Rep 2 T3. Differences in vigour seen last week have largely gone perhaps as a result of the fertigation being given.
26/04/2013	Most pots adequately moist, being hand watered as dripper delivery is not good. Shoots 45 cm tall and will need tying back. No further wilt.
03/05/2013	Irrigation pipes replaced and Heron timings on for 3 mins x9 a day i.e. 8.30, 10.00, 11.30, 12.30, 13.30, 14.30, 15.30, 16.30 and 17.30. Many pots were dry prior to this
09/05/2013	Water on so that saucers not full which is OK, but some not as moist as they could be. 50cm shoots will need cutting off. No disease, but sawflies have eaten the lower leaves and waiting to spray until the primocanes are removed. Some leaves were scorched by drying out.
10/05/2013	Janet Allen says to cut off the fruiting canes now to the ground so we don't get a summer crop and retain 3 or 4 of the primocanes which are coming from the soil. These should be sandwiched between double strings (clipped together at intervals to stop the canes slipping sideways. Any new ones coming after this can be kept trimmed off at the ground level. Don't cut the top of the shoots off if possible as this will make

	laterals come and by midsummer when the flowers come they will stop growing. At the end of June increase the potash and this will encourage earlier flowering to stress the plants and make disease likely. Water pots so they are saturated to get root rot.
21/06/2013	Saw some wilting on plots 1 and 8. A few leaves were dry scorched.
28/06/2013	Carried out overspray for caterpillars and cleared some new roots
02/07/2013	Removed new (30 cm) shoots from pots before spraying to leave those selected earlier. No rot/wilt seen on these shoots or on the main ones. Caterpillars dead, most damage on lower leaves. Fruit starting to come. Refilled the feed (same rate as for strawberry).
04/07/2013	Irrigation put up to 7mins per burst as pots have had saucers filled up by hand. Watering set until pots had water in the saucer as some pots looked drier (although heavy). Fruit starting to come. Pushed canes back behind the wire/string.
15/07/2013	Some discolouration of leaves.
23/07/2013	**Carried out assessment**, but very little disease present.
24/07/2013	No more sawfly, green fruit forming. Flowering stems need tying back. Some further spawn. Hot.
08/08/2013	Floricanes have been cut off so they don't bend over, together with most fruit. Reps 1 & 2 cut a bit short (hip height), other reps cut to top wire at chest height. Re-fixed logger under white cover as it had fallen on the floor. Overview of tunnel no obvious yellowing symptoms yet.
05/09/2013	**Carried out assessment** on disease symptoms. Plants in plot 32 were falling over a lot and plots 9, 17 and 25 (the opposite end to the car park, near the door) had scorch on the leaves possibly from the wind coming in through the open doors of the poly tunnel. Symptoms of disease were much more noticeable in block 4, even in plot 27 (treatment 1 - wasn't inoculated with the disease). Plants with no disease symptoms were given 0, those with wilting symptoms that were more slight or had scorched leaves were given a value of 1 and if the canes had become brown and leaves had wilted and died (severe symptoms) the plants were scored as 2. Data on excel sheet changed for analysis to a 0-3 index as it gave a better representation of the disease situation by separating plants into different categories of if they had necrotic patches (which might have been scorch) (1) or had slight symptoms of leaf collapse (Index 2).
10/09/2013	Two nights with temps down to 9 deg so senescence may start. Wound down sides/shut doors.
03/10/2013	**Disease assessment** completed on wilt symptoms. The same scoring was used as in the previous assessment. More of the leaves were dying off as opposed to wilting than in the last assessment. Amount of scorch of leaves had decreased.
12/12/2013	Leaves are still on some of the canes although plants in blocks 3 and 4 have more senescing leaves. Got data logger from plot 14 although light was no longer flashing. Looked at stem and root brown of one pot. There was a dark brown rotting at the base of the pot (about 3cm) likely to be Pythium, and then some other browning which could just be natural browning. Some of roots were still white. Scraped base of stems, some were green, one was brown and one that was dry that is uncertain. Delayed assessment due to lack of staff until early January.
07/01/2014	Leaves fell off over the Christmas period, but most floricanes still green. **Destructive scoring** method agreed with Study Director before commencing. All root balls sliced in half with a spade, however very few roots seem to be present and those seen were tanned and if surface-scraped then they were not rotted, thus scoring concentrated on the majority of roots – these were around the outside of the root ball. 3 root colours recorded white, dark golden (tobacco) brown and dark chocolate. The dark roots were thinner and rotted and formed a band around the ball extending from the pot base. The golden roots were sometimes rotted when scraped, but unable to distinguish externally from healthy roots. Halves of root ball placed side by side to aid % of root ball surface area comprised by different root colours. All stems scraped to remove epidermis around diameter to 10 cm up and some browning seen inside green stems. Internal staining higher also checked if any external browning. LFDs confirmed Pythium & Phytophthora in golden and dark roots of inoculated UT, and Pythium in dark roots of uninoculated UT. Assessments completed by 10 Jan.
13/01/2014	6 pots from Plots 12 and 13 (UT inoc & uninoc) kept and rest of trial destroyed.

Appendix 3. Minimum, maximum and mean temperatures in the growing medium of one raspberry pot recorded by a logger for the cropping years of 2012 and 2013



Growing media temperatures in cv. Polka pots from 7 March 2013 to 7 January 2014