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:	Annual report, March 2013
Previous report:	Annual report, March 2012
Key staff:	Erika Wedgwood Tracey Evans Sarah Mayne
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

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 Tim O'Neill
Horticulture Research Manager
ADAS

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

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.

CONTENTS

GROWER SUMMARY

Headline

- Novel plant protection products for the control of *Phytophthora* spp. have been identified with potential to control raspberry root rot

Background and expected deliverables

Soil-borne *Phytophthora rubi* (previously known as *Phytophthora fragariae* var. *rubi*) can infect raspberry and cause wilting leading to the death of otherwise long-lived plants. Other species of *Phytophthora* can also cause root rot, but *P. rubi* causes the most common and serious form of rot (Kennedy and Duncan, 1991). Sections of row and their fruit yields are lost for the remainder of the crop's life as the soil contamination means that any replacement plants are also likely to succumb to infection. *Phytophthora ideae* has now also been found causing root rotting in *Rubus* spp., but it does not cause a wilt. It is likely that *Phytophthora* spp. resting spores 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 through the soil. The motile zoospores produced move in irrigation water. Once a plant becomes infected the pathogen multiplies and neighbouring plants become affected as zoospores spread. Although some crops are grown in substrate, plant losses still occur through the contamination of the substrate (such as by spore-contaminated irrigation water from open reservoirs), from using growing medium that becomes contaminated prior to use, and from rooting-through if pots are stood directly on woven ground-cover. The disease can also spread from infected, but initially symptomless plants, introduced to the crop.

Where fruit crops are soil-grown, growers can still (for now) use the soil fumigants Basamid (97% w/w dazomet) or chloropicrin pre-planting. The chloropicrin EAMUs (previously SOLAs) expire on 23 June 2013 and authorisation may be continued as a 120 day approval. Soil fumigation does not appear to totally eliminate the chance of plants with *P. rubi* wilt from appearing in a crop. There is currently no commercial soil test for *P. rubi* (although the molecular research on detection and quantification which could lead to a test is being carried out in Project SF 130). Growers not wishing to disinfest soil with a fumigant (possibly because a pre-planting *Verticillium dahliae* Harris test has proved negative) will not know the risk of *P. rubi* infection and could suffer if it is present.

Growers usually apply fungicide drenches biannually to raspberry crops against *Phytophthora* root rot, either as a soil-applied drench or via the drip irrigation. SL567A (44.7% w/w metalaxyl-M) use under EAMU 2195 of 2007 is possible but resistance to

metalaxyl has been reported in other crops such as to *Phytophthora infestans* in potato. The potato blight fungicide, Shirlan (fluazinam) has been used through an EAMU for several years. Paraat (500 g/kg dimethomorph) is a locally systemic product introduced more recently to the UK. There is always a greater chance of resistance developing in pathogens where products have only a single mode of action and thus chemical companies are developing mixtures. *Phytophthora* root rot can still be reported from drench-treated plantations. Alternatives to the currently used products would be beneficial to the industry.

The aim of the current work is to identify new drench treatments that protect raspberries from root infection by *P. rubi.*

Specific objectives are:

- 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* root rot in raspberry.
- To provide information to growers and the relevant chemical companies on any products that have efficacy and to seek co-operation within the industry for work towards the production of EAMUs.

Summary of the project and main conclusions

Objective 1 – Identification of candidate products for root rot control

Five products with potential efficacy against *Phytophthora* root rot in raspberry were identified in Year 1 of the project. The evaluation of these products was commenced in 2012 in an inoculated trial alongside the industry standard Paraat (dimethomorph). The newly identified products include Fenomenal (fenamidone + fosetyl-aluminium), Resplend (ametoctradin + dimethomorph), Prestop (*Gliocladium catenulatum*) and an experimental product. Fenomenal has recently been registered in the UK for use on outdoor strawberries against red core and crown rot, with its efficacy against *Phytophthora cactorum* shown in HDC project SF 99. Resplend 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 protected cane fruit and an EAMU for outdoor crops. Ranman Twinpack (cyazofamid) was initially selected in 2011, but removed from the final selection because gaining extension of use from potato is not very likely. After consultation with the HDC Soft Fruit Panel and the product's suppliers (then BASF) a second biological product, Serenade ASO (*Bacillus subtilis* strain QST 713) was introduced following

information on the activity of this product in soil against *Phytophthora* spp. and the issuing of EAMUs for the drench treatment of outdoor raspberries, and for trees in amenity situations and forest nurseries, against *Phytophthora* root rot. The fifth candidate was a chemical product shown to give control of the *Phytophthora* species causing crown rot in strawberries (Project SF 99).

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

Drenches of Paraat, Fenomenal, Resplend, Serenade ASO, Prestop and an experimental product coded HDC F142 were carried out on 3 October 2012 on cv. Polka modules potted in May 2012. After investigating a number of different root inoculation techniques in 2011, the selected method of burial of mycelial plugs of *P. rubi* in potted raspberry plants was carried out a week later. An isolate from a Scottish culture collection (SCRP3333, FVR11, IMI355974) was used after it had not proved possible to obtain a fresh isolate from the rotted roots of a series of samples of field-collected raspberry plants.

No wilting or stem staining developed in the fruiting canes before their removal in January 2013. The crop will be monitored throughout 2013 for wilting of the new shoots, with destructive assessment, including of the roots, due in January 2014.

Financial benefits

Effective treatments will reduce crop loss and extend the life of the plantation. Increasing the range of products available to growers against *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 sterilisation 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. The selected products will probably need to be tested on a field scale before being approved for use. After this, growers will still be advised initially to drench small areas of their varieties in case of phytotoxicity.

The confidence of growers to plant into soil without sterilisation, to save expenditure, will be increased. Growers who might otherwise plant in growing media to avoid *Phytophthora* root rot on a field with a history of this disease might be able to return to soil use with the application of a novel fungicide or biofungicide drench directly after planting. This could save growers the need for the materials associated with container production, including for some growers the expense of having to use mains water because their borehole water has too much chloride.

If the newly available biological control product Prestop, proves effective, then this may help the industry comply with the EU Sustainable Use Directive for reduced pesticide use.

Action points for growers

There are no grower action points at this stage as the efficacy testing is ongoing. However, the prevalence of *Phytophthora* root rot in sampled soil-grown raspberry plantations indicates that growers would benefit from being able to utilise a soil diagnostic test to guide planting decisions. Greater use could also be made of already commercially available lateral flow devices to test whether or not wilting seen is caused by *Phytophthora* root rot and thus ensure that the appropriate cultural and chemical control measures are used.

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 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 ingredient.

Methods

Objective 1 – Identification of candidate products for root rot control

A provisional short-list of Fenomenal (fenamidone + fosetyl-aluminium), Resplend (ametoctradin + dimethomorph), Prestop (*Gliocladium catenulatum*), Ranman Twinpack (cyazofamid), an experimental product HDC F142 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). This substitution was made because 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.* It had previously obtained an EAMU (0499 of 2012) for 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 has 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 potted 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 inoculation using cultures were examined: three densities of 10 mm diameter agar plugs of three week old *P. rubi* (four, six or eight 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*.

Once the canes were removed from the preliminary experiment in January the pots were covered with fleece to provide temperatures in the range of 10-17°C (the optimum temperature range for oospore germination and infection by zoospores of closely related *Phytophthora* root rot of strawberries. They were also kept well watered in order to produce early growth in the pots to stimulate zoospore production. The destructive assessment of fruiting canes and roots on 11 May 2012 has not been previously reported. Young shoot browning and leaf wilting was assessed and the root ball examined externally and internally for root rot. Isolations were made from tissue with potential *Phytophthora* symptoms.

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. 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 for the Year 2, efficacy, experiment 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 agreed with the Soft Fruit Panel that, in keeping with commercial practice, a repeat series of drench applications will be made in spring 2013.

Replicate 1	
Plot	Treat-ment
1	4
2	2
3	3
4	6
5	7
6	5
7	8
8	1

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

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

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

Figure 1. Arrangement of plots of 10 cv. Polka plants per plot (in two rows of five pots) from May 2012 to January 2014 in Polytunnel 7 at ADAS Boxworth

There were six treatments plus two untreated controls (one uninoculated) (Table 1) and four replicate blocks, making a total of 32 plots (Figure 1). There were four plots of 10 pots across the tunnel width, thus covering 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.

Fenomenal and Resplend 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 is required.

Table 1. Chemical and biological treatments used on 3 October 2012 in the main, efficacy, experiment. All except T1 were inoculated after treatment.

Treat-ment number	*P. rubi* added	Product & FRAC group	Active ingredient	Rate of product to be added to water (20 L for 40 plants)	Application volume	Approval status and rate on approved crop All products to be used in this experiment as a drench to the compost
1*	N	None		-	500 ml per 5 L pot	Water only
2	Y	None		-	500 ml per 5 L pot	Water only
3	Y	Paraat (standard) **40**	dimethomorph	0.1 g / plant (3.8 g in 20 L)	500 ml per 5 L pot	Approved for minor use on raspberry (indoor and outdoor) as a drench at 1g per plant in a minimum of 200 ml water per pot. The strawberry rate of 0.1g was used however after consultation with BASF to keep within the maximum label rate for both crops of 3 kg/ha. One application/yr 90 days before harvest.
4	Y	Fenomenal **33 + 11**	fenamidone + fosetyl-aluminium	0.1 g / plant (3.8 g in 20 L)	500 ml per 5 L pot	AEA COP 2012/01844 Approval only on outdoor strawberries against red core and crown rot, with 100 ml diluent (75 g product / 100 L water). However to reach the maximum of 3 kg product/ha in container crops a slightly higher rate than 0.075g was used.
5	Y	Resplend **45 + 40**	ametoctradin + dimethomorph	0.025 ml / plant (1.0g in 20 L)	500 ml per 5 L pot	AEA COP 2012/01844 Approval only as potato fungicide at a maximum of 0.8 L / ha. Rate agreed with BASF.
6	Y	HDC F142	not disclosed	1.5 ml / plant (60 ml in 20 L)	500 ml per 5 L pot	3 ml / L of water used at a volume to wet the compost.

Table 1. contd. Drench treatments used on 3 October 2012 in the main, efficacy, experiment

Treat-ment number	*P. rubi* added	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
7	Y	Serenade ASO	*Bacillus subtilis*	0.32 ml / plant (12.8 ml in 20 L)	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.
8	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 (100 g in 20 L)	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 per 1000 L compost

Inoculations

It was concluded from Year 1 that no more than eight 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.

P. rubi is relatively slow growing and so plates were used at 21 days old after incubation in the dark at 20°C when the mycelium had nearly filled the 90 mm 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.

At inoculation, 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 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. Inoculation was completed between 15.30 and 17.00 h on each day. Because of the difficulty of 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.

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 both of the experiments.

In the preliminary experiment (as reported for 25 January 2012 in the previous annual report), as the plants were to be destroyed not long after, 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 for isolation and for *Phytophthora* spp. LFD testing from each treatment.

In the main experiment, as the old canes were to be cut back to two buds about 60 mm above the pot surface and cropped again in 2013, the lower stem tissue was not damaged. The plants had been pruned to a single cane. On 25 January 2013, the lower cane was examined externally for any browning that could be distinguished from maturation and the stem cross-section 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.

Destructive assessment of spring growth, and roots in preliminary (inoculation) experiment

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 rootball 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 rootball from top to bottom twice (quartering the rootball) 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 it was white, not brown / rotted. The number of rotted root lengths visible was assessed.

Any brown roots were surface sterilised (by a 10 second dip in 75% ethanol) and plated-out on non-selective potato dextrose agar (PDA). Each plant number was noted on the agar plates in order to be able to relate the results back to the whole plant assessments. Stem isolation would have been carried out similarly, but was not required as no primocane staining was seen.

Results

Objective 1 – Identification of candidate products for root rot control

Six products were selected for application in autumn 2012. Details were given in Table 1.

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

Collection and isolation of Phytophthora

This work was detailed in the previous annual report. Although 19 raspberry stools showing wilting were collected from commercial plantations in 2011 and one in February 2012 it was not possible to obtain an isolate of *P. rubi*. The success or otherwise of isolation of *P. rubi* from the potted raspberries taken to the Fera laboratories in February 2013 is not known at present, but if available in time any isolate will be utilised in a re-inoculation of the trial in March/April 2013, shortly after a second drench application of the plant protection products. 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 will run until the end of January 2014. A further stool was sent to Fera in February 2012, 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.

Results from Fera for stools and soils sampled in May 2012 by ADAS for Project SF 130 (Table 2) confirmed in June 2012 that most plantations diagnosed in the field by advisors were determined in the laboratory to have been infected by *P. rubi*. Identification was carried out on the basis of appearance of the oospores (resting spores) in a root preparation or positive lateral flow device (LFD) tests for *Phytophthora* spp.. Six farm sites were confirmed to have plantations containing *Phytophthora* spp. resembling *P. rubi*. The results

show that raspberry root rot is a problem for a number of commercial growers across England. The results of the molecular diagnosis (Taqman procedure) will be available in the annual report for SF 130. Unfortunately, no isolates were obtained by Fera from the material that could be used in the main inoculation experiment of the current project (SF 123). The stools and soils were retained in cold storage at Fera and although it has been proposed that more tissue is taken from these to see if isolates can be obtained, the accepted technique for isolation of *P. rubi* involves the use of freshly dug plants. Fresh plant samples are being sought by Fera for proposed research on the validation of the *P. rubi* detection work, the outcome of which would be a quick assay for *P. rubi* detection and an initial indication of how soil DNA levels affect disease development in the main raspberry varieties.

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 sent for Taqman
21209494	Glen Clova	"Plant 1, sample B".	No oospores seen	sent for Taqman
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 sent for Taqman
21209703	Glen Clova	Essex, "Plant A". J. Allen	Oospores typical of *P. rubi*	LFD +ve
21209704	Glen Clova	Essex, "Plant B". J. Allen	*Phytophthora* oospores not typical of *P. rubi*	LFD weak +ve
21209719	Glen Clova	Surrey, "Plant A". J. Allen	No oospores seen	LFD weak +ve sent for Taqman
21209720	Glen Clova	Surrey, "Plant B". J. Allen	Oospores not typical of *P. rubi*	LFD +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*	Not done
21211051	Glen Ample	Kent, Farm 2 H. Roberts	Visibly infected, small/round oospores not typical of *P. rubi*	sent for Taqman
21211224	-	Cornwall, PYO site.	No oospores seen	sent for Taqman

Fera laboratory ID code	Variety	Location or sample ID & ADAS sampler	Diagnosis by oospores in roots	Identification by LFD or Taqman
21211226	-	C. Nicholson Cornwall, Farm site. C. Nicholson	Mixture of small/round oospores not typical of P. rubi and large/lens-shaped oospores typical of *P. rubi*	Not done
21213088	-	Devon, PYO site. C. Nicholson	Oospores typical of *P. rubi*	Not done

Assessments of Phytophthora in stems and roots

Results of the preliminary experiment (reported in the Year 1 report) inoculated in late August 2011, up to and including the pruning-off of fruiting canes in January 2012, showed that no wilting or internal cane staining developed that could be attributed to *P. rubi* had developed in the canes. By the destructive assessment on 11 May 2012 primocane shoots were growing up but none had wilted. A few brown roots in some plots across inoculation treatments were noted on the exterior circumference of the root ball, but no *Phytophthora* was isolated from them. No brown roots were seen inside quartered root balls.

In the main trial, (drenched on 3 October 2012 and with inoculation completed on 31 October 2012), no plants showed phytotoxicity on 19 October. Some leaf discolouration, which was probably nutrition related, was visible throughout the plots. The plants were checked again for leaf wilting and phytotoxicity on the fruiting canes on 30 November 2012 and 11 December 2012, and no symptoms were seen. Leaves dropped for winter after this. When the floricanes were removed on 25 January 2012 no external or internal staining of the stem was seen. Root assessment will not be carried out until pot destruction in January 2014.

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 Fenomenal (60 g/kg fenamidone + 600 g/kg fosetyl-aluminium 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. Fenamidone kills zoospores and fosetyl-Al primes the defence mechanism of plants. Fenamidone 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. http://www.bayercropscience.com. The manufacturers will use the information on product efficacy from the current project to decide whether to seek to obtain residue data to allow Fenomenal registration for raspberry.

The potato blight (*Phytophthora infestans*) fungicide Resplend (300 g/L ametoctradin + 225 g/L dimethomorph) 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. The dimethomorph component in Resplend (300 g/L ametoctradin + 225 g/L dimethomorph) is the active ingredient in Paraat 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, with effectiveness against developing and germinating oospores. Dimethomorph has translaminar activity and up to 72 hours curative activity. Ametoctradin belongs to a new class of chemistry, the pyrimidylamines, and is a mitochondrial respiration inhibitor. It is effective as a preventative spray against potato blight and downy mildews (Gold *et al*., 2009). There is no residue data for ametoctradin on soft fruit.

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 (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 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). 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. Fenomenal was as effective at reducing crown rot incidence in this project (Project SF 99) as the standards Aliette 80WG and Paraat (dimethomorph). The potassium phosphite product Farmfos (at twice the normal 5 L / ha rate for use as a foliar feed) gave significant reduction in crown rot incidence, but it should be noted that this product is not approved for crown rot control. Another product, Hortiphyte (30% w/w P_20_5) is also available.

Fosetyl-aluminium probably gains its activity by forming phosphite which primes plant cells to defend themselves (systemic acquired resistance – SAR) against fungi. Phosphite is then slowly oxidized in soil to become phosphate salts which act as a fertilizer. Potassium phosphite, a plant nutrient, is believed to have activity against *Phytophthora* spp. when applied either as a root drench or foliar spray. Phosphite is transported in the xylem and phloem and reduces the production of viable zoospores.

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) 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 from the pathogen. *P. rubi* would then need to spread up into the canes to cause staining and it is likely this would take more than four months to show from main trial inoculation. Inoculation in the preliminary experiment was a bit later than intended because isolations were still being carried out to obtain fresh isolates (although isolations had been started before the official start of the project). Conditions for disease establishment would not have been ideal in July in the preliminary trial, but should have been favourable in the main trial in October. Any evidence of root rot by wilting of the new shoots and invasion of young roots in 2013 will be reported in the final report.

No products caused phytotoxicity as a drench to cv. Polka, including the product (Resplend) approved for foliar application to potato, and so all could be suitable for use on raspberry if shown to be efficacious. A re-drench is planned in spring 2013 (as most raspberry crops are drenched in autumn and spring) and will allow further phytotoxicity checks.

Conclusions

- Five products were identified as potential new treatments for the control of *Phytophthora* root rot in raspberry: Fenomenal, Resplend, an experimental product HDC F142, Prestop, and Serenade ASO.
- No phytotoxicity developed in the cv. Polka in the four months following autumn drenching of the fruiting canes.
- No fruiting canes developed *P. rubi* wilting following inoculation, but development can take months and the crop is intended to be monitored until January 2014.

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

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).

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

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. x10 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. 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	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	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 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 phytotox and 9=severe phytotox. 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 phytotox. 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 phytotox. Assessment scores will stay the same, but will not mean potential phytotoxicity.
31/10/2012	Completed 2nd inoculation used 3 discs 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
25/01/2012	Carried out cane internal staining assessment no disease found. Pruned back to 6 cm and cleaned up.
25/02/2012	A few pots have shoots just coming through compost.