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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

- Novel plant protection products for the control of *Phytophthora* spp. on crops other than raspberry 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 rotting in *Rubus* spp., but it does not cause a wilt. It is likely that the resting spores of *Phytophthora* spp. 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 through spore-contaminated irrigation water from open reservoirs. Contamination can also occur from using growing medium that becomes contaminated prior to use, and from rooting-through if pots sit 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 (as of March 2012) use the soil fumigants Basamid (97% w/w dazomet) or chloropicrin pre-planting, although the chloropicrin EAMUs expire on 23 June 2013. However, commercial experience suggests that soil fumigation does not totally eliminate the pathogen from the soil. Unlike Verticillium wilt, no diagnostic tool is available to test soils for the presence of this pathogen before planting, so growers are often unsure as to the need to use a soil fumigant before planting.

Instead, growers tend to rely upon two soil applications of a fungicidal drench (spring and autumn) to protect plant roots from infection. An EAMU for SL 567A (44.7% w/w metalaxyl-M) is available (2195 of 2007) but resistance to metalaxyl has been reported in other Phytophthora species such as *Phytophthora infestans* (the cause of potato blight). For

several years, an EAMU for the potato blight fungicide Shirlan (fluazinam) has been used with some success in cane fruit. Paraat (500 g/kg dimethomorph) is a locally systemic product which has also been used more recently in the UK. There is always a greater chance of resistance developing in pathogens where products have only one mode of action, so chemical companies are developing mixtures to avoid this. As none of the currently approved fungicides gain full control of this pathogen, alternative products would be beneficial to the industry.

The aim of this project 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, for evaluation in an inoculated trial alongside the industry standard Paraat. The newly identified products are Fenomenal (fenamidone + fosetyl-aluminium), Hortiphyte (potassium phosphite), Ranman Twinpack (cyazofamid), Resplend (ametoctradin + dimethomorph) and Prestop (*Gliocladium catenulatum*). 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* demonstrated in HDC project SF 99. Ranman Twinpack and Resplend are approved for use against the closely related potato blight pathogen, *Phytophthora infestans*. The fourth candidate, potassium phosphite, a water-soluble fertiliser, has been widely reported to give control of *Phytophthora* spp., with strong evidence of this given in project SF 99. The fifth product, Prestop, is a biopesticide which is approved for use on protected cane fruit crops and has an EAMU for use against root pathogens on outdoor cane fruit.

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

Efficacy testing is being carried out in a crop of potted raspberries. It is not possible to use naturally infected existing plantations as the root rot is likely to be in patches/sections of row, and at various stages of development. Planting into an infected field would give similarly patchy infection. Several products will be tested under Experimental Approval, and this requires destruction of the fruit.

In 2011, work was done to develop a method of reliably producing *P. rubi* infection in potted raspberry plants that would mimic the speed and severity of symptom development in commercial crops. Isolates of *P. rubi* from different plantations (with different chemical histories) were sought for use in the inoculation testing. Unfortunately, although a total of 19 stools from 18 crops were processed, and *Phytophthora* spp. were detected in roots from three stools, no *P. rubi* was isolated. Therefore a stored isolate was used instead to develop the inoculation method.

Eight methods of inoculation were examined on container-grown plants using Polka, a variety known to be susceptible. Forty 5 litre pots of raspberries were grown in sphagnum peat compost in a polytunnel at ADAS Boxworth. The pots were drip irrigated to keep them moist and favour the movement of the motile zoospores of the pathogen. Potential inoculation methods were considered and a procedure using 10 mm diameter plugs of agar culture-plate (as used by Scottish raspberry breeders for variety resistance screening) was selected. The optimum depth of inoculum placement and the number of agar plugs required was investigated using permutations of depth and quantity, to give six treatments. Another two treatments used pieces of naturally infected raspberry roots as inoculum. Inoculation was carried out at the end of July 2011 with one pot per treatment in each of four replicate blocks. Although some leaf wilting was recorded, this occurred without any significant differences between the inoculated and uninoculated treatments. When the canes were cut down in January 2012, some staining was recorded under the epidermis in eight pots, but no Phytophthora was isolated.

It is possible that wilt symptoms failed to develop in 2011 because conditions were not favourable for infection. Work elsewhere indicates that infection of raspberry roots is favoured by temperatures below 25°C coupled with high moisture. New cane production in 2012 is being monitored as the disease may have developed slowly in the inoculated stools at temperatures around 10°C over the winter, with either poor emergence, or the development of “crooked” shoots, anticipated in spring 2012. The maximum of eight agar

plugs of *P. rubi* per pot did not cause unnaturally premature wilting of canes, and so this density should be acceptable for future tests.

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 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. 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 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 preliminary stage of the study.

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 seeks to identify novel products, including at least one biological, and to test their efficacy as preventative drenches in inoculated pot tests.

Methods

Objective 1 – Identification of candidate products for root rot control

Literature was examined, including a 2005 review (SF 66) on novel products for soft fruit and the recent experimental work on fungicides for the control of crown rot of strawberries (*Phytophthora cactorum*) (Berrie, 2011).

Plant protection product industry representatives were contacted to gain up-to-date information on substances most likely to reduce or eliminate root infection by *Phytophthora* spp.. Product selection considerations include the ease of grower use, cost implications and meeting any requirements for the granting of an EAMU (such as existing use under protection). A provisional short-list of Fenomenal, Resplend, Prestop, Ranman Twinpack and potassium phosphite and the standard Paraat was agreed with the Soft Fruit Panel in 2011 during the consideration of this project proposal and information was particularly sought on these products.

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

A preliminary experiment was done to identify a *P. rubi* inoculation method and inoculum concentration that resulted in reliable disease development. The trial diary is given in Appendix 1.

Collection of raspberry stools with root rot for P. rubi isolation

Raspberry stools with wilting resembling that caused by *Phytophthora* spp. were collected from across Britain in order to obtain a range of isolates of *P. rubi* (Table 1). One reason for multiple isolates was that some of the plantations could have *P. rubi* which had developed resistance to metalaxyl, and such isolates should be included in the fungicide efficacy testing in Year 2.

Collection of raspberry material from commercial plantations commenced before the official start date of this project and they are continuing while symptoms are visible on growing canes in the field. Stools were dug up and posted, unwashed, for next day delivery. Tissue processing was started on the day of delivery, or if this was not possible the material was wrapped to prevent drying and kept in a cold store at 4°C overnight.

Procedures for isolation of Phytophthora spp. from stem base and root samples

A number of isolation techniques for *Phytophthora* are given in Erwin and Ribeiro (1996), each method having various modifications according to the laboratory carrying out the work. The isolation methods used in the current work are given below. The method for plating roots on agar has previously been used successfully by the Project Leader in commercial trials at Boxworth with *Phytophthora cinnamomi* infected conifers.

Isolation from roots

Roots were rinsed of soil or growing-media and then flushed for up to 24 hours under running tap water in order to remove tannins from the roots. Tannins can suppress mycelium growth from the roots onto agar. The roots were placed in a net bag floating in a beaker of tap water under a running cold tap (with sufficient flow to just give a steady stream of water onto the roots). Roots of a range of sizes were then cut up and pieces from the transition between rotted and healthy tissue were selected for isolation. The root pieces were cut into 5-10 mm length sections and dipped for 5-10 seconds in 75% ethanol in order to surface sterilise them, and blotted on sterile filter paper before being placed on agar. Some pieces were also plated without surface sterilising. Isolations were made onto either V8 or, more frequently, the Oomycete selective agar P_5ARP (particularly where the roots were in an advanced stage of rot and likely to be hosting secondary fungi). White/colourless colonies (potential *Phytophthora* spp.) were then sub-cultured after about four days growth before colonies touched each other on the isolation plates. Coloured colonies of e.g. *Fusarium* spp. were recorded from the original plates without sub-culturing.

Phytophthora spp. can be difficult to isolate onto agar from their host and so a phytophthora 'baiting' technique was also used for the samples collected in 2011. A Golden Delicious apple was wiped with 75% ethanol and then a semi-circular flap (with the opening nearest the stalk) was cut just under the skin. A triangular area of flesh was then cut from the core-side of the opening. A pinch of about twenty untrimmed and unsterilized running-water rinsed root pieces were pushed into the hole. The flap was then sealed shut with a strip of masking tape. A slot was filled with roots on either side of the apple face. The apple was

placed on a Petri dish base so that it stood with the stalk upwards when placed in a transparent polythene grip-seal bag. The sealed bag was then placed in an incubator at 18°C under a daily 16 hour light cycle. After a week, the apple flesh was sliced through around the root pocket to find brown rot streaks. These areas of rot were then surface sterilised (as previously with the root isolations) and plated onto P_5ARP.

The remaining rinsed roots were placed, without surface sterilising, into Petri dishes of sterile pond water in order to induce sporangia. Sporangia are not usually produced in agar plates. Plates were incubated at room temperature in ambient light for two to three days. If no sporulation developed then the water was drained off and replaced to remove any metabolites which could inhibit sporulation. Distinction was possible between the sporangia of *Pythium* spp. and *Phytophthora* spp. with the aid of CAB/IMI keys.

Pythium and *Fusarium* species can cause primary infection of roots but are frequently also found in plant tissue as secondary colonisers.

Isolation from stems

Isolations following 75% ethanol surface sterilisation were made onto agar. The bark was removed to find any areas of brown streaking and thick slivers of tissue from large stems, or transverse sections from narrower stems, were plated out.

Lateral flow device use

Both phytophthora and pythium Lateral Flow Devices (LFDs) (from Forsite Diagnostics) were used when colony characteristics and morphology under the microscope indicated that phytophthora might be present. A square of mycelium on agar was cut from the suspect colony and placed in the extraction buffer, shaken, and then drops put in the LFD well. In a few cases washed roots were also tested directly using a phytophthora LFD kit.

Plant clinic samples of stools BX11/92, 93,100,101,102 received in summer 2011 were potted-up in Ericaceous peat compost and left outside over winter, as isolation of *P. rubi* from root may be more difficult between April and October when roots have been in warmer conditions and extended water saturation of the compost is less likely.

Isolates of P. rubi already in collections

In addition to seeking to obtain fresh isolates of *P. rubi* the MycoBank International Mycological Association collection www.mcyobank.org was searched and there was a single isolate of *Phytophthora fragariae* var. *rubi* (now named *Phytophthora rubi*) (CUP

62528) collected in Scotland deposited by SCRI (now the James Hutton Institute or JHI) in 1993 available, and also a 1991 isolate FVR67 (deposit CBS 109892) deposited in the Netherland's www.cbs.knaw.nl also by SCRI. The age of the isolates meant that there was a possibility that they would have lost virulence. The James Hutton Institute (previously SCRI) was contacted directly and a plate of *P. rubi* SCRP3333, FVR11, IMI355974 received.

There were no isolates of *P. rubi* available in storage at the Food and Environment Research Agency (Fera) in York as they use molecular techniques for diagnosis of *Phytophthora* root rot rather than culturing, nor were any held at either East Malling Research or at Science and Advice for Scottish Agriculture (SASA) in 2011. *Phytophthora* spp. do not survive well on agar in refrigerated culture collections without re-subbing onto new agar (ideally at about six-monthly intervals, whereas fungal mycelium can survive several years without refreshing) and this may have contributed to the lack of stored isolates.

Potting-up and husbandry

On 20 March 2011, 120 bare-root cold-stored stools of cv. Polka had their roots soaked in water before planting in 5L pots of Erin Ericaceous sphagnum peat compost. No module plants (the preferred option) could be obtained by the time the project funding was confirmed in April 2011. The pots were stood on saucers on woven ground-cover material in a polythene tunnel at ADAS Boxworth. They were arranged in four replicate blocks across the width of the tunnel. Each pot was placed to one side or the other of horizontal wires to which the floricanes were trained. Floricanes were not thinned. A temperature logger was buried in the pot, and another suspended at 1 m in the crop.

Sangral liquid feed was added in the irrigation water at each irrigation session using a Dosatron diluter once the five week feed content of the growing media was expected to have been exhausted. The irrigation dripper in each pot was placed towards the pot centre, with water delivered throughout daylight hours to replace the water lost by plant transpiration. The water draining through the peat into the pot saucers from around the dripper was drawn back up to moisten any drier areas of the pot. The irrigation was scheduled to keep the peat growing-medium moist at all times, without the pots standing in water and becoming anaerobic. The zoospores of *Phytophthora* spp. have been recorded as negatively geotrophic and so may move in the free water towards the top of the pots.

When top-up watering was needed on warm days this was carried out into the pot saucers to avoid flushing out the zoospores.

All pots were given two drench applications with biological control nematodes (Nemasys L) to protect against root damage by vine weevil larvae. Two-spotted spider mites were managed using applications of phytoseilus predatory mites. No insecticides, fungicides or herbicides were applied.

Inoculation method selection and development

There are many different methods available to inoculate growing-media or soil with *Phytophthora* spp. (Erwin and Ribero, 1996). Methods include the production of zoospores in liquid, with one technique being described for the inoculation of strawberry plants with *Phytophthora cactorum* (Berrie, 2011). A zoospore procedure was formerly used with *P. rubi* at the Scottish Crops Research Institute (now James Hutton Institute) requiring several stages of production and large volumes of suspensions. Zoospore suspensions can cause symptoms within three weeks, and so, where such a severe screen is not required, the use of agar plugs is likely to be more satisfactory (Kennedy and Duncan, 1991). Since the 1990's the more easily managed use of agar plugs (Kennedy and Duncan, 1991) has been carried out (with four to six plugs per 10 inch pot) with equivalent results to zoospore suspensions for variety resistance screening. Sufficient plugs need to be used so that areas of root zone do not escape inoculation (David Cooke, James Hutton Institute, pers. comm.).

Another method of inoculation with artificial media was initially investigated in this project based on experience with *Phytophthora cinnamomi*, but abandoned after repeating the culturing procedure once. *P. rubi* did not grow in 18°C incubated bags of sterilised Vermiculite moistened with diluted V8 juice.

Naturally infected root pieces (which had tested positive for *Phytophthora* spp. using LFDs) were also included as inoculation treatments to compare with the use of agar cultures.

There should be several cycles of the disease, with the agar and root pieces initially producing sporangia, leading to the infection of root tips and development, particularly in the phloem. As roots rot then further sporangia should be produced to cause further infection of the root system.

Production of agar plates for direct inoculation

Ten plates of the isolate of *Phytophthora fragariae* var *rubi* SCRP3333, FVR11, IMI355974, ATCC 90442 collected from raspberry from Scotland in 1985 (supplied by D. Cooke, James Hutton Institute) were made on V8 agar. The plates were incubated for 10 days at 20^{o}C in the dark. This produces a white felt-like aerial growth with a colony about 50 mm wide in the 90 mm Petri dish. Cultures were also grown on Lima bean agar, and produced a 25 mm felt-like colony in the same period. Sections of the culture plates were floated in sterile distilled water to induce sporulation and sporangia were seen using both agars. V8 plates were used in subsequent work. Confirmation that the isolate was a *Phytophthora* sp. was made by using a *Phytophthora* spp. specific lateral flow device and by examining for sporangia in floats of the colony in sterile pond water.

Preparation of naturally infected root material for use as inoculum

Roots were taken from raspberry stools collected from protected crops of cv. Octavia and cv. Glen Fyne at Sunclose Farm, Cambridgeshire in May 2011. The roots from stools gave positive lateral flow device (LFD) test results for *Phytophthora* spp. but, although material was plated onto P_5ARP agar (selective for pythium and phytophthora*)* and damp chambered in slots cut in apples to allow isolation from rotting developed in apple tissue (to obtain fewer secondary fungi than directly from roots), it was not possible to isolate any *Phytophthora* spp.. No other roots were tested by LFD at ADAS Boxworth before isolation.

Roots with browning were sampled from a range of root diameters. The roots were shaken free of excess compost or soil and then chopped into pieces of no more than 10 mm. The pieces from a cultivar were shared between four dishes so that each of the four pots would have samples from the same roots. Either fine roots plus a piece of tap root of cv. Glen Fyne or five pieces of cv. Octavia root were used per hole for Treatments 9 and 10, respectively.

Preliminary inoculation test experiment

Eight methods of inoculation were examined for their ability to cause phytophthora root rot in container-grown raspberry cv. Polka (Table 1). Three densities of 10 mm diameter agar plug (4, 6 or 8 plugs) and two inoculation depths (60 mm and 120 mm) were examined. Inoculation within the compost was done as, although zoospores are active towards the compost surface, the mycelium on the agar may keep moister when deeper in the pot.

Treatments 3, 5 and 7 used a shallower burial depth than the 120 mm, which was nearly at the bottom of the pot. A depth of 20 to 30 mm was used in published work (with 15 mm agar discs), but as this was carried out in 0.5 L pots in a controlled environment room at 15-20°C (Kennedy and Duncan, 1991) it was considered that it would be too shallow for work with sunlit pots in a polytunnel in the present work, when the top would probably be quite warm and less moist at times during the day.

Treatments 9 and 10 used naturally infected dirty roots to provide an indication of the speed and severity of pot infection which could occur in commercial crops via contamination with infected debris. The treatment method might also provide a suitable inoculation method.

Table 1. Inoculation treatments tested examined as methods for producing phytophthora root rot in container-grown raspberry - 2011

Treatment code	Inoculation details Plug number, diameter and burial depth. All had four holes dibbed
1	Uninoculated control with four holes made to 120 mm
2	Uninoculated control with four holes made to 120 mm
3	6 x 10 mm diameter *P. rubi* agar deposited at 60 mm
4	6 x 10 mm diameter *P. rubi* agar deposited at 120 mm
5	4 x 10 mm diameter *P. rubi* agar deposited at 60 mm
6	4 x 10 mm diameter *P. rubi* agar deposited at 120 mm
7	8 x 10 mm diameter *P. rubi* agar deposited at 60 mm
8	8 x 10 mm diameter *P. rubi* agar deposited at 120 mm
9	4 x 120 mm holes with infected roots of container raspberry (cv. Glen Fyne)
10	4x 120 mm holes with infected roots of soil-grown raspberry (cv. Octavia)

Forty pots, arranged in four replicate blocks, were allocated for the test, with treatments randomised within the blocks (Figure 1). Analysis of variance was used to compare the results between treatments, using a mean of the two uninoculated treatments. Where there were too few symptoms recorded (e.g. of cane browning) the results were examined without analysis.

A further 80 pots were retained without any treatments (but otherwise maintained in the same way in the polytunnel) ready for use when any isolates were obtained from the field-collected wilted stools.

Inoculation of pots in preliminary experiment

Inoculation was carried out on 27 July 2011 once the bare root cold-stored stools had produced two or three primocanes (so that the oldest was about a metre tall) and the original floricane had been pruned off. Once the stools had produced new roots, susceptible tissue and metabolites would be present to stimulate zoospore infection after release from

sporangia in the agar culture. Inoculation took place in late summer when usually night temperatures were above 15°C and the day temperatures below 25°C. The optimum temperature range for zoospore infection by the related *P. fragariae* var. *fragariae* is 10-17°C (EPPO data sheet contract 90/399003).

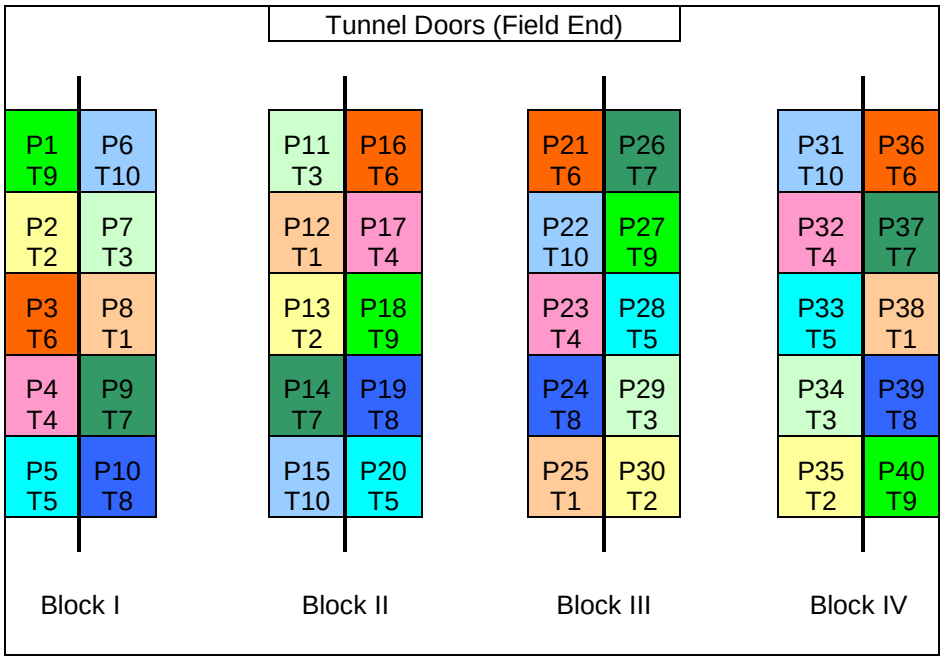


Figure 1. Layout of plots (P1 to P40) in inoculation experiment

All inoculation holes were made with a dibber (with a point 25 mm at the top, narrowing to 5 mm at the bottom) at one of two depths. Holes were made to 120 mm, 60 mm or 40 mm (the latter two holes having a 10 mm wide top). Four holes were made at equal spacing about 50 mm in from the pot edge (keeping away from the outside of the pot to reduce the amount of conducted heat received from the pot sides). Agar plugs 10 mm wide were cut from just inside the leading edge and from the centre of 10 day old culture plates and each plug dropped into the hole so that it was stood on edge in the hole. Pots received an equal share of agar from the inner and outer (younger) colony zones. 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.

Uninoculated pots had holes made to 120 mm (to repeat the possible root damage likely to occur in the most deeply inoculated pots), but did not have any inoculum material placed in the hole before they were re-filled.

Assessments

Above- ground plant parts assessment

Regular observations were made of the pots and, when wilting was seen, records per pot were made of:

- The number of canes growing per pot (as an indication of vigour)
- The number of leaves wilted
- % leaf chlorosis or necrosis

Records were taken on 11 October and 4 November 2011.

A final assessment of stems showing browning was carried out after leaf drop in January 2012, six months after inoculation, before the cutting-down of fruited canes. The epidermis was removed with a scalpel from compost level to 10 cm up the cane base to examine for staining underneath. Samples of internally stained tissue were taken from the leading edge for isolation and for LFD testing from each treatment. This enabled matching of any visual stem darkening symptoms assessed with the presence of *Phytophthora* spp.

Root assessment

Stools will only be knocked out of pots at the termination of the experiment as the canes may become damaged.

- Root rot will be assessed if possible without washing the growing media from the roots. Care will be taken not to include roots as rotted that are just naturally tanned. Selected roots will be scraped to confirm that inner tissue is white or brown/rotted. The % of root area rotted will be assessed.

Confirmation of phytophthora infection in stems and roots

Stem samples of tissue were taken for isolation following 75% ethanol surface sterilization to remove contaminants on the surface. Non-selective potato dextrose agar (PDA) was used in order to detect the full range of pathogens which could cause rotting or brown staining e.g. *Fusarium* spp., *Verticillium* spp. or *Cylindrocarpon destructans* as well as *Pythium* spp. and *Phytophthora* spp., with *Botrytis cinerea* or cane blight also possible on stems. Each plant number was noted on the agar plates in order to be able to relate the results back to the whole plant assessments.

The colour and growth pattern of any phytophthora – like colonies (white, slightly floccose with a roseate growth pattern and a fern-like branched colony margin) on the agar plates were compared with that of the original isolate of *P. rubi* used in the agar inoculated pots.

pythium and phytophthora LFD tests were used on some samples where it was not certain from the morphology which oomycete had been isolated.

The roots of the plants were not destructively sampled for assessment in 2011, but this will be carried out after any symptoms develop in the canes produced in 2012. The pots were covered with fleece after destructive assessment of the fruiting canes in January 2012 and kept well watered. This was done in order to produce early growth in the pots to stimulate zoospore production and 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).

Results

Objective 1 – Identification of candidate products for root rot control

The product Fenomenal (60 g/kg fenamidone + 600 g/kg fosetyl-aluminium has recently been approved for use as a drench, dip and foliar spray on outdoor strawberries against *Phytophthora* spp. and *Pythium* spp. (already having had approval on protected and outdoor container and field grown ornamentals). It gave significant control of strawberry crown rot in HDC Project SF 99 (Berrie, 2011). Fosetyl-Al has already been approved for use on raspberries, but residue data is not available for fenamidone / Fenomenal use on raspberries (Richard Meredith, Bayer Crop Science (BCS) pers. comm.). Fenamidone is being 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 .

With the loss of Aliette (fosetyl-aluminium) Fenomenal, with the systemic properties of both fenamidone and fosetyl-Al and their individual benefits, respectively, of killing zoospores and priming the defence mechanism of plants against fungal elicitors (Peter Newman, BCS, pers. comm.) is a good candidate for efficacy testing in raspberries. There is no residue data yet for Fenomenal on raspberry, but the manufacturers have said that they will not start that work until they have established what level of efficacy is likely to be achieved and how best to use the product on raspberry. Raspberry is much more of a perennial crop than strawberry, so the approach (and results) could differ. Work on product efficacy in raspberry would thus be worthwhile (Richard Meredith of BCS, pers. comm.)

The potato blight (*Phytophthora infestans*) products Infinito (62.5 g/L fluopicolide + 625 g/L propamocarb hydrochloride), Resplend (300 g/L ametoctradin + 225 g/L dimethomorph)

Valbon (17.5 g/kg benthiavalicarb + 700 g/kg mancozeb) and Revus (250 g/L mandipropamid) were tested against the standard SL567A (465.2 g/L metalaxyl-M) in the Defra project PH0604 (Wedgwood, 2012) as foliar sprays against *Phytophthora ramorum.* SL567A, Resplend, Infinito and Valbon significantly reduced *P. ramorum* on leaves. Revus gave no significant reduction.

Project SF 99 on strawberry presented information of products with efficacy against *Phytophthora* spp. and evaluated eight fungicides, the plant resistance stimulators potassium phosphite and chitosan as well as Prestop and Serenade ASO against *Phytophthora cactorum* (strawberry crown rot, which is closely related to *P. rubi*). Revus, Ranman A (cyazofamid) and Fenomenal were as effective at reducing crown rot incidence as the standards Aliette and Paraat (dimethomorph) (Berrie, 2011). Resplend was not tested and so it will be tested in the current project, having proved more effective than Revus against *P. ramorum* (Wedgwood, 2012).

There is ongoing work in the USA within the IR4 minor use programme for crops such as raspberry, and it possible that these relatively new potato blight products will be tested in the USA and so provide information to help towards decisions on registrations in the UK.

Valbon may be unsuitable as a drench as it has minimal systemic movement and mainly works as a protectant by contact activity (Richard Meredith, BCS, pers. comm.). Infinito has systemic activity, but also principally works by contact action and is recommended for foliar use against stem and tuber blight as a protectant (Richard Meredith BCS, pers. comm.). The mancozeb component both Valbon and another potato blight fungicide, Invader (7.5% w/w dimethomorph + 66.7% w/w mancozeb), is being queried under European pesticide legislation (Roma Gwynn, Rationale, pers. comm.) and so these products may not have a long term future. The fungicide Revus, with provisional approval in the UK on potatoes, uses a new active, mandipropamid, and this has approval for spray or irrigation application against phytophthora blight and downy mildew on vegetables and grapes in North America.

The dimethomorph component in Resplend 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 has been shown to have up to 72 hours curative activity (Simon Townsend, BASF, pers. comm.) Ametoctradin (also known as Initium) 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). Ametoctradin has no residue data for soft fruit, but Resplend is considered worth screening for activity against raspberry phytophthora root (S. Townsend, pers. comm.). A formulation of ametoctradin + metiram is being developed for use on grapes (S. Townsend pers. comm.) and residue and toxicity data could be used towards the use of ametoctradin on raspberries.

In the UK, biological products such as Wormcast Pro-T and mycorrhizal treatments such as endoRoots (normally applied to turf and so available for application to large areas) have also been reported to produce healthy root growth in the presence of pathogens. Revive, containing the bacteria *Bacillus subtilis,* is used by compost incorporation to aid root health in containerised ornamentals. Evidence for disease control by these products is not available and in the current project effort will be focused on products already known to have fungicidal activity.

A number of alternative microbial products with potential against the oomycetes *P. cactorum* (strawberry crown rot), *P. fragariae* var. *fragariae* (strawberry red core) and *P. rubi* (raspberry root rot) were reviewed by Fitzgerald (2005) in HDC Project SF 66. The bacterial products Rhizostar (*Serratia plymuthica*), Mycostop (*Streptomyces griseoviridis*) Actinovate (*Streptomyces lydicus*), the fungal antagonist *Gliocladium virens* (in SoilGard), arbuscular-mycorrhizal fungi in Vaminoc-S and the oomycete antagonist *Pythium oligandrum* (in Polyversum) were available principally as soil drenches for ornamentals. The German product Rhizostar, as a root dip, was the only material considered to warrant further investigation for phytophthora control, but it only gave an 18% reduction in plants with crown rot in trials.

Information on microbial control was provided in a 2009 gap analysis of biopesticides for the soft fruit industry (Gwynne, 2009). In this report, the root disease control product Trianum (*Trichoderma harzianum* T-22) was noted as being exempted from UK pesticide registration, with the product Serenade ASO (1.34% w/w *Bacillus subtilis* strain QST 713) being registered as a pesticide. Serenade 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 for Minor Use (0499 of 2012) has recently been granted 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* spp.. It also has an Extension of Authorisation for Minor 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 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. Prestop, unlike Serenade, is already available for cane fruit growers to use as a drench against root pathogens, but information is lacking on its efficacy in this crop and so testing this product would be advantageous.

Naturally occurring saprophytic micro-organisms can suppress a range of plant pathogens (Noble & Roberts, 2003 www.wrap.org.uk). Microbe-plant interactions in plant growth promotion and disease control have been reviewed (Bernard, 2009 www.escholarship.org/). In plantations given fungicide drenches, and in the peat and coir composts used in containers, little natural disease suppression will occur and so biopesticide application may be worthwhile.

Potassium phosphite, a plant nutrient, has 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. Fosetyl-aluminium probably gains its activity by forming phosphite which primes plant cells to defend themselves against fungi. Phosphite is then slowly oxidized in soil to become phosphate salts which act as a fertilizer. The product Farmfos (28% w/w P_20_5), at twice the normal 5 L / ha rate for use as a foliar feed, gave a significant reduction in the incidence of strawberry plants with crown rot in peat grow-bags (Berrie, 2011). Another product, Hortiphyte (30% w/w P_20_5), is also available. The cost of potassium phosphite use is much less than that of fungicides, possibly because the manufacturers of the latter need to recover the costs of product development and registration. In 2012, for example, Hortiphyte at £6 / L applied at 10 L /ha would cost £60 / ha, whereas Paraat at £83.64 / kg applied as a drench at 3 kg/ha would cost £251 / ha, and

Fenomenal at £60 / kg applied as a drench at 3 kg / ha would cost £180 / ha (Robert Irving, ADAS, pers. comm.).

The effectiveness of products will principally depend on their ability to stop the pathogen zoospores or hyphae before they enter the roots, although products that are also able to enter the roots will provide a second line of defence.

In 2011, a four-year HortLINK project (HL01109, HDC project CP 77) co-funded by Defra called SCEPTRE (Sustainable Crop & Environment Protection – Targeted Research for edibles) www.hdc.org.uk was started to test conventional, biological and salts on a selection of weeds, pests and diseases of edible crops. Efficacy information on products being investigated for downy mildew on brassicas (with three experimental coded products found effective in 2011) will be able to be used to indicate of potential candidates for the control of other oomycete species such as *Phytophthora* spp. on other crops. Work on the control of strawberry crown rot is being done in the HortLINK SCEPTRE project (HL01109, HDC CP 77) in 2012.

The products Resplend, Ranman Twinpack and Fenomenal, all without UK Approval on cane fruit, will need to be tested under Administrative Experimental Approval (AEA) (requiring either crop destruction or fruit destruction for five years) in this project. This, and the need to provide uniform inoculation of test plots has meant that the work will be carried out in pots, but the results are expected to be relevant to field crops. The Year 2 experiment will be conducted according to EPPO guidelines in order to generate information on efficacy and residues for potential EAMUs. Field work could follow once the primary screening is carried out and might receive support from the manufacturers of the product(s) in order to gain CRD product approval for use on raspberries.

Integrated control of raspberry root rot

Other methods of controlling raspberry root rot have been investigated elsewhere, including using clean stock (with testing required for certified stock), ridging to keep root systems above water, mulching which may help shed water from the ridges and the exploitation of host resistance. By using these cultural measures it was suggested that fungicide use might be reduced (McNicol, 1997).

An HDC project is commencing in 2012 to develop a quantitative molecular test for *P. rubi* in soil and this could target plantations requiring control treatment.

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

Collection and isolation of phytophthora

Nineteen raspberry stools showing wilting were collected from commercial plantations to enable *P. rubi* isolation. The variety collected, the county of origin and the results from both stem and root culturing on agar and root incubation in apples baits are shown in Table 2.

The cultivars Octavia and Glen Ample were frequently found with wilting, believed to be caused by phytophthora, although one stool was found to have verticillium. No samples were permitted to be collected from wilting primocane varieties that were being grown in plantations under a production agreement with the plant supplier.

Table 2. Raspberry samples processed for *P. rubi* isolation from stems and roots - 2010/11

Clinic code	Collected	Cultivar	Source	Results from stem and root isolations
BX10/88	16/08/10	Chermanus	Cambs (trough)	No pathogens isolated. Brown colony.
BX10/89	16/08/10	Octavia	Cambs	Probable pythium. Brown colony.
BX10/93	19/08/10	Tulameen	Cambs	No pathogens isolated.
BX10/94	19/08/10	Autumn Bliss	Cambs	No pathogens isolated.
BX10/101	22/09/10	-	Norfolk	No pathogens isolated
BX11/41	27/04/11	Octavia	N. Cambs	Pythium +LFD. Phytophthora-like +LFD initially but culture swamped.
BX11/51	25/05/11	Glen Fyne	Cambs (trough)	Pythium. Roots +LFD phytophthora.
BX11/52	25/05/11	Octavia	Cambs	Pythium. Also phytophthora-like but -LFDs pythium and phytophthora. Roots +LFD phytophthora.
BX11/53	25/05/11	Chermanus	Cambs	Pythium.
BX11/72	14/07/11	Minerva	Essex	No pathogens isolated.
BX11/77	22/07/11	Glen Ample	Kent	No pathogens isolated.
BX11/78	22/07/11	Glen Ample	Kent	No pathogens isolated.
BX11/92	22/08/11*	Octavia	Medway	Phytophthora-like but –LFD and pythium +LFD. Fusarium.
BX11/93	22/08/11*	Octavia	Medway	Phytophthora-like but -LFD. Fusarium. Roots fusarium & pythium.
BX11/100a	08/08/11*	Glen Ample	Yorkshire	Phytophthora-like from stem but –LFD, and + for pythium.
BX11/100b	08/08/11*	Glen Ample	Yorkshire	Verticillium.
BX11/101	08/09/11*	Glen Ample	Yorkshire	Pythium +LFD.
BX11/102	08/09/11*	Glen Ample	Stafford-shire	Pythium.
Fera 21201522	13/02/12	-	Oxford-shire	roots had DNA + for *P. rubi*, but no culture obtained from stem or roots.

BX11/92, 93, 100, 101, 102 were potted-up and put outside over winter before sampling

A few of the isolates resembled *Phytophthora* spp., but the LFD tests on these were negative and usually positive instead for *Pythium* spp.. No *Phytophthora* spp. were isolated at ADAS Boxworth from any of the 18 stools collected with wilting leaves. Although BX11/41 initially recorded a positive LFD for *Phytophthora* spp. the culture was a mixture with faster-growing *Pythium* spp. and a phytophthora isolate alone could not be obtained (Table 1). A selection of isolates from the various stools, (principally *Pythium* spp.), were placed in the ADAS culture collection.

In the absence of fresh isolates, the *P. rubi* inoculation methodology test was therefore carried out with the isolate SCRP3333 of *P. rubi* received from the James Hutton Institute. This preliminary test did not require a mixture of isolates from plantations with different fungicide use histories as there was no fungicide efficacy testing to be conducted. 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. Further stools are to be sent to Fera in April 2012.

Assessments of phytophthora wilting in inoculated plants

In October 2011, 11 weeks after inoculation, some leaf wilting (three or four lower leaves per plant) was observed in four of the pots and recorded on 11 October 2011. Two pots were of Treatment 6 and the others in Treatments 3 and 4, but there were insufficient pots affected to be able to draw any conclusions.

In November 2011, although some wilting and yellowing/necrosis of the leaves on the fruiting canes was seen, there were no significant differences ($P>0.05$) between the inoculated treatments, and none had more wilt than the uninoculated (Table 3). It is possible that natural senescence was occurring. Treatment 6 had slightly (not significantly) more leaves with symptoms in November than the other treatments, but these pots were more exposed to the sun and wind than other pots. During the experiment it was found that although the drippers provided the same volume of water per pot, the individual demand of pots differed and had to be made up by hand-watering.

In January 2012, after leaf drop, some staining was recorded when the canes were examined for any darkening towards the base of the cane and the tissue under the epidermis removed. One cane was stained in each of pots 5, 10, 16 and 29 (all different treatments) and two to four canes in each of plots 15, 19, 20 and 35 (all different treatments). However, when the tissue was plated onto agar three pots were found to have botrytis, two also having alternaria. Three other pots had only alternaria and two had

fusarium. Therefore cane infection by phytophthora moving up from the stool was not confirmed. The number of primocanes was compared as a record of stool vigour, but there were no significant treatment differences (P>0.05) (Table 3).

Table 3. Leaf wilting, yellowing and necrosis of plants (4 November 2011) and numbers of canes produced (25 January 2012)

Treatment code	No. *P. rubi* agar plugs & burial depth	4th November 2011			25 Jan 2012
		% of leaves wilting	% of leaves with yellowing	% of leaves with necrosis	Number of canes / pot
1+2	No *P. rubi*	5.00	0.36	3.16	3.87
3	6 at 60 mm	8.00	1.12	1.68	4.00
4	6 at 120 mm	7.50	0.53	0.53	2.75
5	4 at 60 mm	5.00	2.00	0.38	3.00
6	4 at 120 mm	11.25	1.77	8.88	3.25
7	8 at 60 mm	6.25	0.33	1.63	3.25
8	8 at 120 mm	2.50	0.30	0.50	3.50
9	*120 mm	5.00	1.05	1.65	1.75
10	*120 mm	6.25	0.18	2.78	2.50
Mean		6.17	0.80	2.43	3.17
Lsd between T 3 -10		6.191	1.446	8.071	3.879
Lsd between UT & T3 -10		5.362	1.252	6.990	3.359
F pr. (28 d.f.)		P = 0.254	P = 0.092	P = 0.527	P = 0.955

* Rotted roots of Glen Fyne or Octavia

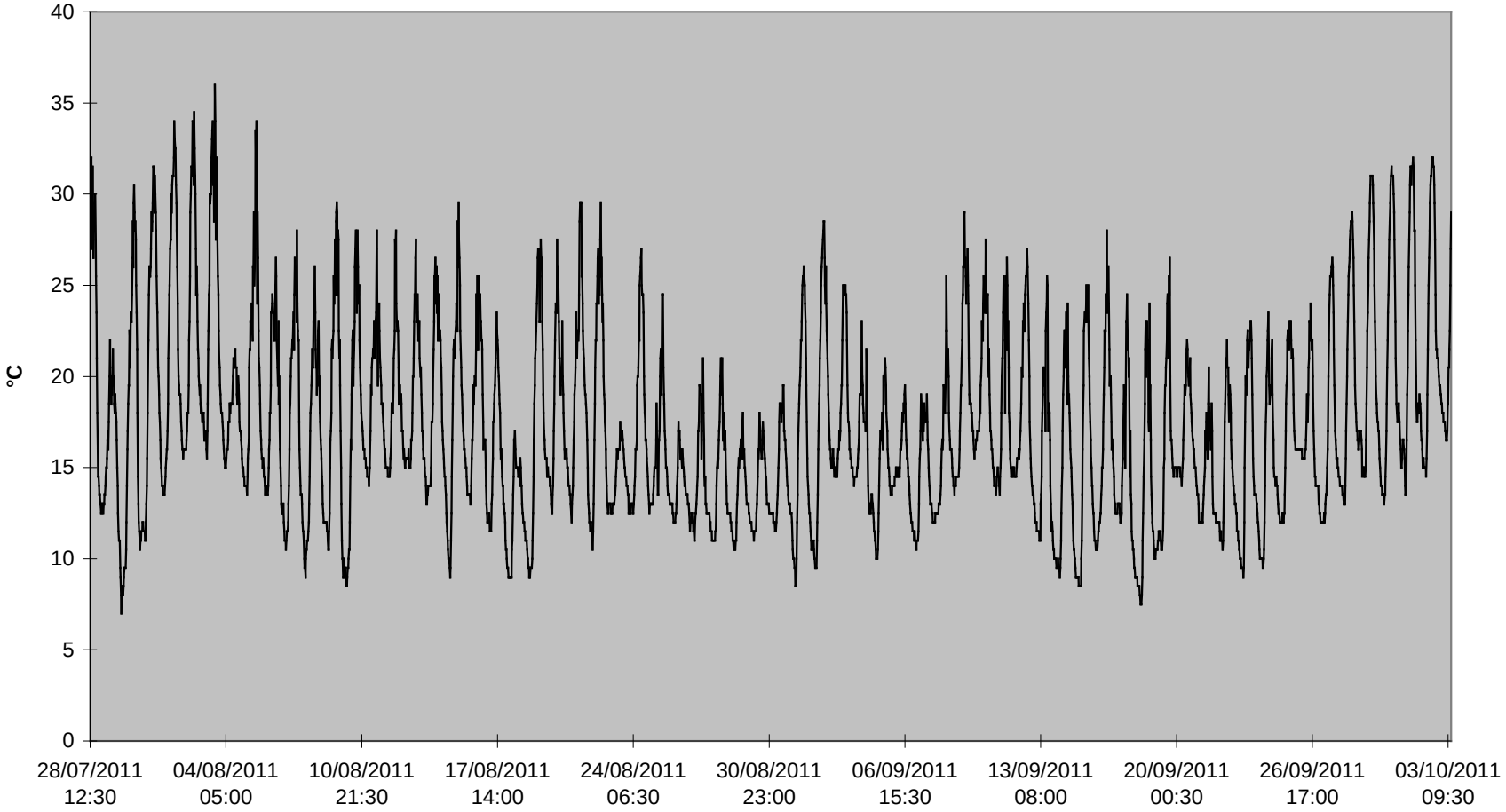
Table 3 gives results only when more than two out of four pots / treatment had symptoms.

There were two periods of unusually hot weather during August and October 2011 (Figure 2). Growing-media temperatures periodically above 25°C could have reduced zoospore infection ability and also have contributed to leaf wilting.

The 80 plants not used in the preliminary experiment remained unused as no fresh *P. rubi* isolates were able to be obtained for any additional experiments.

By 20 March 2012 new shoots were growing and so watering was increased, but no symptoms of wilting from root rot had yet developed.

Figure 2. Temperature in the growing-media of raspberry pots from July to October 2011



Discussion

A review was carried out of products not currently approved for use on raspberry, but having activity against *Phytophthora* spp. and thus having potential for use as a drench against *P. rubi*. The products to be used will be confirmed with the Soft Fruit Panel before commencing work in 2012.

No *P. rubi* was isolated from any of the 19 stools with suspect symptoms collected from commercial crops. This was an unexpected result as *Phytophthora* spp. from ornamentals have commonly been isolated from stems and roots at ADAS Boxworth and at Fera. *P. rubi* DNA was detected by Fera, but the organism might not have been alive. More samples are due to be collected and sent to Fera in April 2012. These may have mycelium in a more active state as the stools will just be starting to grow. With the use of the agar plate method the bulking up of the fresh isolates for inoculation is relatively quick and reliable and inoculation is still possible within the original timescale after arrival of the module plants in May 2012.

It is anticipated that the canes shooting in the polytunnel at Boxworth in March 2012 will start to show symptoms of stool infection, because in the related *P. fragariae* var. *fragariae* infection in strawberry more secondary inoculum is known to be produced, causing slow infection over winter when temperatures are around 10°C (EPPO contract 90/399003). However, temperatures reached above 30°C in August 2011 not long after inoculation, and in *P. fragariae* var. *fragariae* infection does not occur at 25°C (EPPO contract 90/399003). Oospores are usually formed by *Phytophthora* spp. as a resting mechanism when conditions are unfavourable. The growth of roots should stimulate oospore hatching and zoospore release.

Final (destructive) assessment of the main efficacy experiment, which will start in 2012, is due in November 2013 because of the known delay possible in producing symptoms. The preliminary work showed that the maximum density of plugs (eight per pot) did not cause too swift *P. rubi* symptom development for efficacy testing. Therefore, if no symptoms are seen in this trial by the end of April 2012 it is proposed, given that the inoculation by agar method is already utilised by the James Hutton Institute for resistance testing, that the most dense inoculation concentration used in the preliminary trial should be selected for use in 2012 rather than delay inoculation of the efficacy testing.

Conclusions

- Five products were identified as potential new treatments for the control of phytophthora root rot in raspberry: Fenomenal, Hortiphyte, Prestop, Ranman Twinpack and Resplend.
- Although 19 wilted raspberry plants, considered symptomatic of phytophthora, were collected from plantations across England during 2010 – 2012 no isolates of *P. rubi* were obtained. Sampling and isolation should be continued in April 2012.

Knowledge and Technology Transfer

No events or publications have been attended or produced by the Project Director during the period of this research.

Glossary

Technical terms have been explained within the text.

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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.