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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

This project formed the major part of the Defra matched funding for the EU project "Risk analysis for *Phytophthora ramorum*, a recently recognised pathogen threat to Europe and the cause of sudden oak death in the USA" (RAPRA). The main objective of the PH0308 project was to develop risk management strategies for control of natural outbreaks of *Phytophthora ramorum* in the UK and to contribute to the assessment of risk.

Epidemiology of *Phytophthora ramorum* in relation to risk and policy

This work contributed towards work package 3 of the RAPRA project. Studies were undertaken to determine how environmental factors, in particular humidity, water potential and temperature, affected sporulation and survival in *P. ramorum*. *In vitro* tests using a panel of ten isolates (5 EU and 5 US), examined the effects of humidity, water potential and temperature regimes ranging from 38 to 100%, 0.97 to 1 and 0 to 30°C respectively. Differences in humidity had most effect on sporangial production and zoospore germination, whereas sporangial germination was less sensitive to changes in water availability. Maximum levels of sporulation and zoospore germination occurred at 100% humidity or water potentials of 1. Temperature optima for sporulation and germination ranged from 20 to 30°C depending on the conditions of the experiment. Chlamydospores survived treatment at temperatures between 0 and 30°C for at least 2 months but died after short periods at high (40°C) or low (-25°C) temperatures.

Management of outbreaks of *P. ramorum* through experimentation

This work contributed towards work package 6 of the RAPRA project. Fungicides were tested using a panel of 17 isolates obtained from across Europe (12 isolates) and the USA (5 isolates). The efficacy of six fungicides (Amistar, Filex, SL 567A, Dithane 945, Ranman and Elvaron Multi) was tested through *in vitro* experiments against a population of *P. ramorum* to establish baseline sensitivity measurements and to investigate variations in tolerance. Agar-plate assays were used to test the effect of fungicides on mycelial extension, and an optical densitometry technique was used to determine effects on zoospore/sporangial germination. Fungicides were also tested for efficacy against zoospore motility. Of the products tested metalaxyl-M (as SL 567A) was consistently the most effective fungicide against both mycelial growth and zoospore germination of *P. ramorum*. *In planta* work indicated that metalaxyl-M applied as a protectant treatment prevented lesion development on detached rhododendron leaves and when applied as an eradicant stopped further lesion development within 1 day of treatment application. *P. ramorum* could not be recovered from leaves where the fungicide had been 100% effective i.e. no lesion had developed (protectant applications) or there was no further lesion development (eradicant applications), indicating that metalaxyl-M had killed the fungus and was not merely fungistatic. Where less effective products such as Amistar or Elvaron Multi were applied as protectants the onset of lesion

development was not delayed, indicating the fungicide tested had not caused latency of infection.

Results also showed that a reduction in sensitivity to metalaxyl-M had already developed in a number of isolates. Some of the most sensitive isolates were sourced from natural outbreak situations whereas those with reduced sensitivity came from nurseries, where they were more likely to have been exposed to metalaxyl-M. Some *P. ramorum* isolates showing low *in vitro* sensitivity to metalaxyl-M could not be fully controlled using a field rate application of SL 567A in a detached leaf assay, particularly when application timings were not optimal for control, indicating the loss of sensitivity could result in reduced efficacy of the product in controlling disease outbreaks on nurseries. There was evidence to suggest that robust applications of formulated mixtures containing reduced rates of metalaxyl-M (compared to SL 567A) would not be effective against these isolates. Results also showed that Consento (fenamidone/propamocarb hydrochloride) was highly effective as a protectant treatment against isolates with resistance to metalaxyl-M. These, and results from previous experiments, suggest that products containing metalaxyl-M (SL 567A, Fubol Gold, Folio Gold and Epok) used in conjunction with Consento could form the basis of a spray strategy for the control of *P. ramorum*, if policy were to change, which would both control the disease and minimise the risk of further resistance development.

Pathogenicity studies

This work contributed towards work package 2 of the RAPRA project. Eight *Rhododendron* species/hybrids of economically rare value were selected to determine their susceptibility to *P. ramorum*: *R. megacalyx*, *R. Johnnie Johnston*, *R. dalhousie* hybrid, *R. ponticum rubra*, *R. morvah* ('Elliottii x Wattii'), *R. elliotii*, *R. nattallii* and *R. macabeum*. A ninth species, *R. catawbiense* (cunninghams white), was included as a susceptible control. The sensitivity of each *Rhododendron* species and hybrid was tested using five *P. ramorum* isolates from different locations (3 EU and 2 US). Susceptibility was examined using a detached leaf assay where the inoculum was introduced as a zoospore suspension. All the *Rhododendron* species and hybrids tested were shown to be susceptible to *P. ramorum*. It would therefore be unadvisable to use these varieties in future planting schemes where *P. ramorum* is known to be present. It would also be prudent to protect the established bushes of these rare plants by removal of inoculum sources of *P. ramorum* from infected sites in the near vicinity.

Root infection studies were done on rhododendron & viburnum using a variety of inoculation approaches. Results suggested that roots were not a primary pathway for systemic infection of above ground parts of these ornamental species, though asymptomatic colonisation of roots was demonstrated.

P. ramorum inoculum thresholds

A single zoospore of *P. ramorum* is sufficient to infect and cause lesions on the susceptible genera *Rhododendron*, *Viburnum*, *Kalmia* and *Pieris*. *Camellia* and *Leucothoe* were less sensitive to the organism as they required a threshold of 10,000 zoospores before an infection established. No major differences were noted between isolates from different geographical locations.

European pest risk analysis for *P. ramorum*

This work contributed towards work package 8 of the RAPRA project. The new EU pest risk analysis (PRA) for *P. ramorum* is being developed in collaboration with other RAPRA partners. The new UK datasheet (<http://defraweb/planth/pram/pram.pdf>) draws upon the RAPRA results and is an output from this project. Work contributing the development of contingency plans will be reported in due course as part of the wider RAPRA project.

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Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
- the scientific objectives as set out in the contract;
 - the extent to which the objectives set out in the contract have been met;
 - details of methods used and the results obtained, including statistical analysis (if appropriate);
 - a discussion of the results and their reliability;
 - the main implications of the findings;

- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

Scientific Objectives

The work supports the following objectives of the EU-funded RAPRA project:

1. To establish the level of susceptibility (to both European and American isolates) of tree and non-tree species of significant environmental and economic value to the EU. (RAPRA work package 2)
2. To enhance and extend the research carried out on the epidemiology of *P. ramorum* to fully elucidate the risks of the pathogen spreading and establishing in Europe. (RAPRA work package 3)
3. To investigate aspects of risk management through the evaluation of existing and new active ingredients for the control of *P. ramorum* in ornamentals. Aspects of eradication, containment and fungicide control will be addressed, whilst minimising disruption to free trade. (RAPRA work package 6)
4. To bring together new information on epidemiology and control chemicals in order to develop coordinated management practices of immediate strategic and tactical relevance for dealing with *P. ramorum*.
5. To contribute to a European Pest Risk Analysis for *P. ramorum* and provide information to underpin and advise UK and EU plant health policy and legislation. (RAPRA work package 8)

Materials and Methods

1. Spore production

1.1. Sporangia

A single inoculum plug of *P. ramorum* was removed from the culture collection and grown on 10% V-8 agar at 20°C under day light bulbs (12h light/12h dark regime) until the colonies reached the edge of the agar plates. Plates were then flooded with 5 mL of sterile distilled water (SDW) and the sporangia removed from the agar surface using a sterile plastic rod. Fresh 10% V-8 agar plates were inoculated with 100 µL of the resulting sporangial suspension and incubated under the previously described temperature and light regime for 3 days. Plates were flooded with 10 mL SDW, sporangia removed from the agar surface using sterile plastic rods and sporangia were counted using a haemocytometer.

1.2. Zoospores

To induce synchronous zoospore release, three-day-old plates containing sporangia were flooded with 15-20 mL SDW, chilled at -20°C for 5 min and then returned to 20°C for 1 h. Plates were checked for zoospore release and the spore suspension filtered through Whatman No 113V filters (retention size >30 µm) to remove any spent or full sporangia. Zoospores were counted using a haemocytometer and the spore suspension adjusted to 10⁴ spores mL⁻¹. Vigorous shaking of the zoospore suspension ensured synchronous encystment.

1.3. Chamydospores

Cellophane disks (~8 cm diameter) were boiled in deionised water for 20 min to remove any plasticisers present. Disks were sterilised at 121°C for 15 minutes, placed onto the surface of 10% V-8 agar plates and inoculated in the centre with a single inoculum plug of *P. ramorum* (cork borer N°3) taken from the leading edge of a seven day old colony. Plates were sealed with parafilm and grown in the dark (to prevent production of sporangia) at 20°C for one month.

2. Epidemiology of *Phytophthora ramorum* in relation to risk and policy (Work package 3)

Experiments to quantify important epidemiological thresholds used ten *P. ramorum* isolates, five from the EU and five from the USA (Table 1).

Table 1. *P. ramorum* isolates tested to measure epidemiological relationships and fungicide sensitivity.

Isolate Code	Country of origin	Mating Type	Original Host
P1376	UK	A1	<i>Viburnum tinus</i>
P1577	Germany	A1	<i>Rhododendron catawbiense</i>
P1578	UK	A1	<i>R. grandiflora</i>
CC47	UK	A1	<i>Rhododendron</i> species
2046	Belgium	A2	Unknown
P1403	USA	A2	<i>Vaccinium ovatum</i>
P1579	USA	A2	<i>Quercus agrifolia</i>
1728	USA	A2	<i>Lithocarpus densiflorus</i>
1741	USA	A2	<i>Lithocarpus densiflorus</i>
1755	USA	A2	<i>Lithocarpus densiflorus</i>

2.1. Sporulation

2.1.1. Effect of humidity

The effect of humidity on sporangial production was assessed using all ten isolates of *P. ramorum* (Table 1) under five different humidity regimes (100, 93, 85, 62 and 38%). Humidity within the chambers was altered by using different glycerol:water ratios (Table 2).

Table 2. Volumes of glycerol used to generate humidity regimes

Relative Humidity (%)	Glycerol (mL L ⁻¹ water)
100	0:1000
93	250:750
85	350:650
62	600:400
38	830:170

Humidity chambers were created by securing four plastic bottle tops (30 mm) upside down to the base of a 90 mm Petri dish using a small blob of Vaseline. The space surrounding the secured tops was filled with 15 mL of a glycerol solution, and 1 mL of molten 10% V8 agar was added to each bottle top (Figure 1).

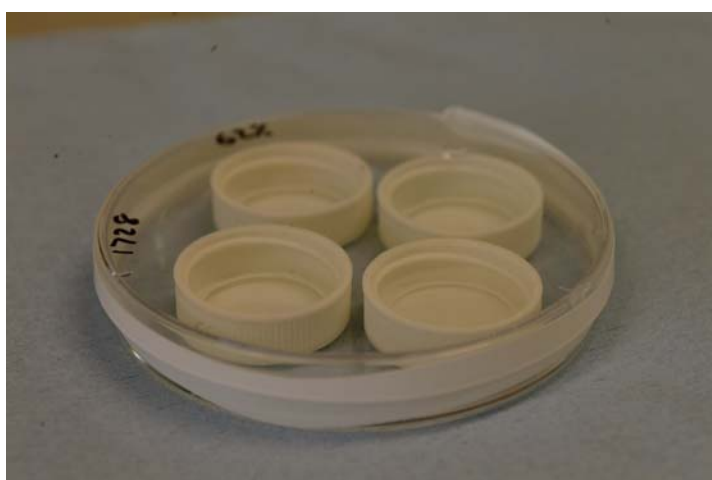


Figure 1. Humidity chamber set up used to measure the effect of humidity on sporulation.

Twenty cellophane disks (~8 cm diameter) were boiled in deionised water for 20 min to remove any plasticizers present. Disks were sterilised at 121°C for 15 minutes and placed onto the surface of 10 % V-8 agar plates. For each *P. ramorum* isolate (Table 1) two agar plates were inoculated with five inoculum plugs (cork borer N°3) taken from the leading edge of a seven day old colony; inoculum plugs were placed in a circle 2 cm in from the edge of the Petri dish. Plates were sealed with parafilm and grown in the dark (to prevent production of sporangia) at 20°C for three to five days. After incubation, the inoculum plugs were removed and 1cm² sections were cut from within the mycelial growth and the sections examined to ensure the absence of sporangia. For each isolate and humidity one cellophane section was placed, mycelial side up, into each of the four bottle tops contained within a humidity chamber. The humidity chambers were incubated, unsealed, at 20°C under a 12h day 12 h night light regime for 3 days. Following incubation the cellophane sections were removed from each humidity chamber and placed in separate 1 mL eppendorf tubes containing 500µL of sterile distilled water. Any sporangia present on the cellophanes were removed by vigorous shaking of the tube for 15 seconds followed by 15 seconds vortex mixing. Sporangia were counted using a haemocytometer and the total number of sporangia produced calculated.

2.1.2. Effect of temperature and humidity

The effect of humidity and temperature on sporangial production was assessed using two *P. ramorum* isolates [CC47 (EU) and 1755 (US) Table 1] under five different humidity regimes (100, 93, 85, 62 and 38%) and six temperatures (0, 5, 10, 20, 25 and 30°C). The humidity chamber set up and assessments were as described in section 2.1.1 with four replicate counts taken for each humidity, temperature and isolate.

2.2. Germination

2.2.1. Effect of water potential

The effect of water potential on germination and germ tube extension of sporangia and zoospores were assessed using the *P. ramorum* isolates described in Table 1 at four water potentials (0.998, 0.990, 0.980 and 0.970). A 2% tap water agar was used as the base medium, and was amended with sucrose to give the water potentials as outlined in Table 3.

Table 3. Amount of sucrose added to adjust agar water activity levels

Water Activity	Wt Sucrose (g per litre)
0.998	23.43
0.99	70.29
0.98	164.01
0.97	281.16
0.96	421.74

Three replicate plates were set up for each water potential, isolate and spore combination. Each replicate plate was spread inoculated with 100 μ L of a spore suspension. Plates were incubated at 20°C for 24 hours after which 100 spores were assessed for germination. The germ tube length was measured on 10 germinating spores. A spore was classed as germinated if the length of the germ tube was equal to or greater than that of the spore.

2.2.2. Effect of temperature and water potential

The effect of water potential and temperature on sporangial and zoospore germination/germ tube extension was assessed using two *P. ramorum* isolates of [CC47 (EU) and 1755 (US) Table 1] at four water potentials (0.998, 0.99, 0.98 and 0.97) and six temperatures (0, 5, 10, 20, 25 and 30°C). The water potential method and assessments were as described in section 2.2.1 with three replicate plates for each water activity, temperature, isolate and spore combination.

2.2.3. Effect of humidity

The effect of humidity on germination and germ tube extension of sporangia and zoospores were assessed on the isolates described in Table 1 under five humidity regimes (100, 93, 85, 62 and 38%). Humidity was altered using the glycerol:water ratios outlined in Table 2.

The set up of the chambers was as described in section 2.1.1 except that a cover slip (18mm x 18 mm) was placed inside the inverted bottle tops rather than 1 mL agar. Twenty microlitres of a suspension containing 10^4 sporangia mL^{-1} (produced as described in section 1.1) was pipetted onto each cover slip.

Each humidity chamber was sealed with insulation tape and incubated at 20°C for 24 hours. On three of the four cover slips 100 sporangia were assessed and categorised as germinated, empty (where zoospores had been released) or full (where no zoospores had been released and sporangia had not germinated). Where zoospores had been released from the sporangia 100 were assessed for germination, of these the length of the germ tube was measured on 10. A spore was classed as germinated if the length of the germ tube was equal to or greater than that of the spore. The fourth cover slip was inverted onto PARPH media and incubated at 20°C for 48 hours to assess propagule viability – growth of *P. ramorum* from under the cover slip indicated viability.

2.2.4. Effect of temperature and humidity

The effect of humidity and temperature on germination and germ tube extension of sporangia and zoospores were assessed using one *P. ramorum* isolate [CC47 (EU)] under five humidity regimes (100, 93, 85, 62 and 38%) and six temperatures (0, 5, 10, 20, 25 and 30°C). The humidity chambers were set up and assessments were as described in section 2.1.1 with three replicate counts taken at each humidity, temperature, *P. ramorum* isolate and spore combination.

2.3. Effect of temperature on chlamydospore survival

Cellophane disks (~8 cm diameter) were boiled for 20 min in deionised water (to remove any plasticisers present), autoclaved at 121°C for 15 minutes and finally placed onto the surface of 10 % V-8 agar plates. The surface of each cellophane was inoculated with an inoculum plug (cork borer N^o3) taken from the leading edge of a seven day old colony of *P. ramorum* (CC47) and incubated in the dark at 20°C until the mycelial growth had reached the edge of the cellophane. Each cellophane surface was checked microscopically for chlamydospore production and 30 plates were placed into each of six incubators held at -25, 0, 5, 15, 30 and 40°C.

Chlamydospore viability was examined after 2, 24, and 48 hours; 1, 2 and 3 days; 1, 2, and 3 weeks; and 1 and 2 months (-25°C and 40°C treatments were sampled after 2 and 24 hrs only). For each time interval and temperature treatment, three cellophanes were removed and the chlamydospores removed by gently scraping the surface of the cellophane using a round edged scalpel blade. The chlamydospores were suspended in 25 mL SDW, blended for 15 sec using a hand blender (to break down any mycelial fragments), centrifuged at 500g for 2.5 min and the supernatant removed. The resultant pellet was resuspended 1 mL SWD.

Chlamydospore viability was assessed in two ways; firstly by assessing germination on agar and secondly using a viability stain (LIVE/DEAD® BacLight™ Bacterial Viability Kit). Germination was assessed by spreading 100 µL of the 1 mL chlamydospore suspension onto PARPH. Plates were incubated at 20°C for 48 hours after which 100 spores were assessed; a spore was classed as germinated if the length of the germ tube was at least equal to that of the spore. Chlamydospore viability was assessed using the stain by adding 100µL of the chlamydospore suspension into a 0.5 mL microtube, and combined with 1.5µl of component A and 1.5µl of component B from the viability kit (the two components were mixed thoroughly before adding to the chlamydospores suspension). The chlamydospores/stain mixture was kept at room temperature in the dark for 15 minutes. Twenty microlitres of the stained chlamydospore suspension was trapped between a microscope slide and an 18mm² coverslip and 100 chlamydospores examined using fluorescence microscopy. Spores fluorescing green were recorded as viable and those fluorescing red as dead (Figure 2).



Figure 2. Staining of chlamydospores using LIVE/DEAD® BacLight™ bacterial viability stain; viable spore fluoresce green and dead spores fluoresce red.

3. Management of outbreaks of *P. ramorum* through experimentation (Work package 6)

Fungicides were tested using a panel of isolates obtained from across Europe and the USA. This panel was composed of the core isolates described in Table 1 plus those described in Table 4.

Table 4. Isolates of *P. ramorum* (additional to those described in Table 1) used during fungicide efficacy studies.

Isolate code	Country of origin	Mating type	Original host
CC70	UK	A1	<i>Rhododendron ponticum</i>
CC54	UK	A1	<i>Kalmia</i> spp.
CC76	UK	A1	Isolated from soil
2163	Czech Rep.	A1	Unknown
1648	Holland	A1	<i>Rhododendron</i> spp.
1649	Holland	A1	<i>Viburnum</i> spp.
1650	Germany	A1	<i>R. catawbiense</i>

The efficacy of a range of fungicides was tested through *in vitro* experiments (Table 5) against populations of *P. ramorum*, to establish baseline sensitivity measurements and to investigate variations in tolerance. Agar plate assays were used to test the effect of fungicides on mycelial extension, and an optical densitometry technique was used to determine effects on zoospore and sporangial germination. Fungicides were also tested for efficacy against zoospore motility.

Table 5. Fungicides tested for efficacy against *P. ramorum* isolates.

Fungicide	Active ingredient
Amistar	Azoxystrobin (250 g l ⁻¹)
Filex	Propamocarb hydrochloride (800 g kg ⁻¹)
SL 567A	Metalaxyl-M (480 g l ⁻¹)
Dithane 945	Mancozeb (800 g l)
Ranman	Cyazofamid (400 g l)
Elvaron Multi	Tolyfluanid (505 g l)

3.1. Effects of fungicides on mycelial growth *in vitro* (agar plate assays)

Variation in fungicide sensitivity within the *P. ramorum* population was assessed using 17 isolates (Tables 1 and 4) and six fungicides (Table 5) tested at 0, 1, 10, 100 and 1000 ppm. A V-8 agar base medium was amended with fungicide to provide the fungicide concentration series. Three replicate plates for each fungicide concentration and controls (0 ppm) were inoculated with a 5 mm agar plug taken from the leading edge of a 7 day old culture. Plates were sealed with parafilm and incubated at 20°C for 7 days. Colony diameters were measured after 7 days and the EC₅₀ values calculated from the dose response curves. The EC₅₀ was defined as the fungicide concentration at which growth of the fungus was inhibited by 50% compared to growth on the untreated controls.

Experiments by other workers within the RAPRA project showed that a number of isolates exhibited reduced sensitivity to metalaxyl-M. These isolates along with a number of sensitive isolates (Table 6) were tested using V-8 agar amended with metalaxyl-M at concentrations of 0.0001, 0.001, 0.01, 0.1, 1 and 10 ppm.

Table 6. *P. ramorum* isolates used to evaluate reduced sensitivity to metalaxyl-M.

Isolate code	Country of origin	Mating type	Original host
1578	UK	A1	<i>Viburnum tinus</i>
1650	Germany	A1	<i>Rhododendron. catawbiense</i>
CC70	UK	A1	<i>Rhododendron ponticum</i>
CC47	UK	A1	<i>Rhododendron spp.</i>
1653	UK	A1	<i>R. catawbiense</i> cv. <i>Everestianum</i>
1657	Germany	A1	<i>R. catawbiense</i>
1658	Germany	A1	<i>R. catawbiense</i> cv. <i>Boursault</i>
1659	Germany	A1	<i>Rhododendron grandiflorum</i>
1664	Germany	A1	<i>Viburnum bodnantense</i>

3.2. Detached leaf assay

3.2.1. Efficacy of fungicides against *P. ramorum* infections

Three fungicides, SL 567A, Amistar and Elvaron Multi (Table 2) were tested using a detached-leaf assay at the manufacturer's recommended rates - 1.3 L ha⁻¹, 1.0 L ha⁻¹ and 3.4 kg ha⁻¹ respectively.

Four application timings were tested (fungicide applied 7 or 4 days before inoculation or 4 or 7 days after inoculation). For each fungicide treatment and timing, three leaves of a similar age were sprayed. Sprays were applied to the abaxial leaf surface (underside) in a water volume equivalent to 200 L ha⁻¹ using a battery powered ('lunch-box') sprayer (model LBP18; S/N3081) set at 2 bar, with a 1 m boom and three twin fan nozzles (Tee Jet XR 1100VK Yellow). Control leaves were sprayed with an equivalent volume of water.

A zoospore suspension comprising spores from three *P. ramorum* isolates (CC47, P1577 & P1376; Table 1) was used to inoculate the detached leaves. Zoospores were produced as described in section 2.5 and adjusted to give a zoospore concentration of 1x10⁶ zoospore mL⁻¹. Leaves were inoculated with 50 µl of the zoospore suspension applied to the abaxial leaf surface. The inoculum was applied equidistant between the leaf edge and the leaf midrib.

All control and treated leaves were incubated in a moist chamber at 20°C. The length and breadth of leaf lesions were measured after 14 days incubation and the control achieved was calculated for each treatment and timing as:

$$\text{Control (\%)} = (\text{untreated lesion} - \text{treated lesion} / \text{untreated lesion}) \times 100$$

To test the viability of *P. ramorum* following fungicide treatment, leaf lesions were plated onto P₅ARPH agar immediately after the 14-day disease assessment. Where no lesions were present the area inoculated was tested. Plates were incubated at 20°C and assessed for growth of *P. ramorum* after 7 days.

3.2.2. Efficacy of fungicides against *P. ramorum* isolates with decreased metalaxyl-M sensitivity

The isolates showing decreased sensitivity to metalaxyl-M were tested to indicate whether this might lead to disease control problems in the field.

Four fungicides, SL567A, Ranman, Fubol Gold [a.i. mancozeb (640 g l⁻¹) + metalaxyl-M (40 g l⁻¹)] and Consentio [a.i. Fenamidone (75 g l⁻¹) + propamocarb hydrochlorite (375 g l⁻¹)] were tested in a detached leaf assay using three replicate rhododendron leaves per treatment; all leaves tested were of a similar age. The fungicides were applied according to the manufacturer's recommended rate (SL567A 1.3 L ha⁻¹, Fubol Gold 1.9 L ha⁻¹, Consentio 2 L ha⁻¹ and Ranman 0.2 (A) and 0.15 (B) L ha⁻¹) at two preventative application timings (7 or 4 days before inoculation). Fungicides were applied to the adaxial leaf surface (upperside) in a water volume equivalent to 200 L ha⁻¹ using a battery powered 'lunch-box' sprayer (model LBP18; S/N3081) set at 2 bar, with a 1 m boom and

three twin fan nozzles (Tee Jet XR 1100VK Yellow). Control leaves were sprayed with an equivalent volume of water.

Five *P. ramorum* isolates (1650, 1659, 1653, 1658 and 1664) were tested. Leaf surfaces were damaged (score <5mm) either four or seven days after fungicide treatment at a point equidistant between the leaf edge and the leaf midrib. A drop of water placed over the damaged area and an inoculum plug (cork borer N°3), taken from the leading edge of a seven day old colony, placed on top of it. All control and treated leaves were incubated in a moist chamber at 20°C. The length and breadth of lesions were measured after 14 days incubation and the control achieved by each treatment and timing was calculated as,

$$\text{Control (\%)} = (\text{untreated lesion} - \text{treated lesion} / \text{untreated lesion}) \times 100$$

3.3. Effects of fungicides on spore germination in vitro (photometric assays)

The effect of fungicides on sporangial germination of 17 *P. ramorum* isolates was determined by a photometric technique adapted from Pijls *et al.* (1994), which uses optical densitometry to measure the amount of spore germination. The fungicides were tested at 100, 10, 1, 0.1, 0.01, 0.001 and 0.0001 ppm, and are detailed in Table 5.

Dilutions of each fungicide were prepared in a glucose-peptone growth medium (GPM) and 100 µL of each added to the appropriate wells of a 96 well flat-bottomed microtitre plate. Three replicate wells were set up for each fungicide concentration; 100 µL of unamended GPM was used in the control wells. Zoospores were produced using the method described in section 1.2, adjusted to 10^4 spores mL⁻¹ and 150 µL of the spore suspension pipetted in each treatment well; 150 µL GPM was added to the control wells.

Absorbance was read across all wells on the plates at 405 nm immediately after the addition of spores and then after 48 h incubation at 20°C. The absorbance readings were used to calculate the percentage inhibition by comparison to the control. Dose response curves were plotted and the EC₅₀ values determined.

3.4. Effects of fungicides on zoospore motility

Zoospores were produced as described in section 1.2, but were released in GPM rather than SDW. Zoospore concentration was determined using a haemocytometer and adjusted to give 2×10^4 zoospores /mL by diluting with GPM.

The effectiveness of six fungicides (Table 5) against zoospore motility of 10 isolates (Table 1) was tested using a method adapted from Matheron & Porchas (2000). For each isolate 100 µl of fungicide diluted to 0.002, 0.02, 0.2, 2, 20, 200 and 2000 ppm was added to each of 4 replicate wells on a 96-well plate; 100 µl of GPM was added to a further 4 control wells. The zoospore suspension was gently agitated before adding 100 µl of the suspension to each test and control well. This gave final fungicide concentrations of 0.001, 0.01, 0.1, 1, 10, 100 and 1000 ppm and a concentration of 1×10^4 zoospores mL⁻¹. An initial examination was made using a microscope at x10 magnification and a baseline photometric reading taken at 450 nm prior to incubating the plates at 20°C. Plates were removed from the incubator and examined every 30 minutes and the time taken for the zoospores to stop moving was recorded.

4. Susceptibility of important rare *Rhododendron* species and hybrids to *P. ramorum*.

4.1. Selection of plant material

Following discussion with head gardeners/horticulturalists, eight *Rhododendron* species/hybrids of with economic rarity value were selected to determine their susceptibility to *P. ramorum*. These were *R. megacalyx*, *R. Johnnie Johnston*, *R. dalhousie* hybrid, *R. ponticum rubra*, *R. morvah* ('Elliottii x Wattii'), *R. elliottii*, *R. nattallii* and *R. macabeanum*. A ninth species *R. catawbiense* (Cunninghams White) was included as a susceptible control.

4.2. Production of inoculum

Zoospores were produced as described in section 1.2 and all inoculations used 10^4 spores mL⁻¹ in a 100 µl drop of sterile distilled water (SDW). Five isolates from different locations (Table 7) were used in each study.

Table 7. Isolates of *P. ramorum* used during the susceptibility of important rare *Rhododendron* species and hybrids study

CSL Reference	Origin	Mating type	Host
1659	EU	A1	<i>R. catawbiense grandiflorum</i>
2034	USA	A2	<i>Rhododendron</i> sp.
2266*	EU	A1	<i>Rhododendron</i> sp
2268	EU	A1	<i>Rhododendron</i> sp
2269	USA	A2	<i>Rhododendron</i> sp

* Type isolate (BBA 9/95)

4.3. Leaf inoculation and assessment.

Four healthy detached leaves of each *Rhododendron* species/hybrid under test were placed in a damp box, two with leaf adaxial surface uppermost and the other two with abaxial surface uppermost. Each leaf was divided into six sections; the midrib dividing wounded (three scrapes with a scalpel blade over inoculation point) and unwounded inoculation points (Figure 3). Unwounded and wounded inoculation points nearest the leaf tip were negative control SDW with no zoospores. The remaining unwounded points were inoculated with different *P. ramorum* isolates and replicated on the wounded side. A lid was placed on the incubation box and incubated at 20°C under day light bulbs (12h light/12h dark regime).

Leaves were inspected 0, 2, 4, 7 and 14 days post inoculation and scored according to an index of susceptibility: 0 = No visible lesion, 1 = Lesion ≤5mm diameter, 2 = Lesion >5mm diameter.

To compare susceptibility between rhododendrons, the overall susceptibility score was calculated using the following formula:

$$\Sigma (au \times 1) + (aw \times 1.5) + (bw \times 2) + (bu \times 2.5) \text{ for upper and lower leaf surfaces (all isolates)}$$

where a= Total susceptibility index 1, b= Total susceptibility index 2; w = wounded, u = unwounded.

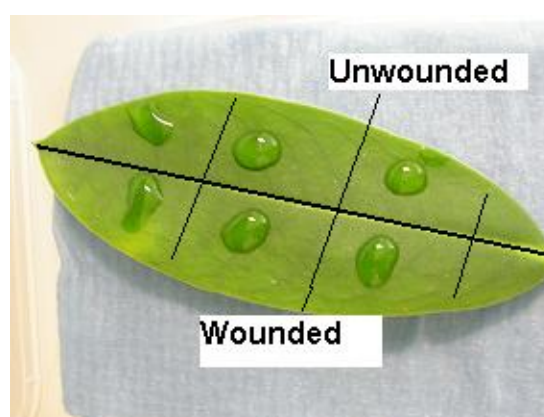


Figure 3. Rhododendron leaf inoculation test showing the three unwounded leaf inoculation points above midrib, three below midrib wounded.

5. *P. ramorum* inoculum thresholds on ornamentals

Four *P. ramorum* isolates (1659, 2266 (EU) and 2034, 2269 (US)) were used to inoculate leaves from seven ornamental plants - *Camellia japonica*, *Kalmia latifolia*, *Leucothoe* sp., *Pieris formosa*, *Rhododendron catawbiense* "Cunninghams white", *Syringa vulgaris* and *Viburnum tinus*.

Unwounded leaves were inoculated with a single 100 µl drop of zoospore suspension (10^4 , 10^3 , 10^2 and 10^1 zoospores per ml) applied in the middle of the leaf as close to the midrib as possible (SDW was used for control leaves). A small black mark was made on the leaf near the inoculation point to identify where the inoculation had taken place. Walls of the damp chamber were moistened using SDW sprayed from an atomizer. The chamber was closed and sealed with plastic wrap. Inoculated leaves were incubated at 19°C, 12-hour day/night cycle for one day. After which time the lid of the chamber was removed and the inoculation drop allowed to dry out. The chamber was then re-moistened as before with SDW and closed for seven days. Following incubation, lesion size was recorded to the nearest 0.1 cm. The lesion was removed from a representative number of each host, leaves, surface sterilised in 70 % ethanol for 30 s, rinsed with five washes of SDW and a small piece of leaf tissue from the leading edge of the lesion plated onto P₅ARP[H] medium. Plates were examined after 5 days to confirm the presence of *P. ramorum*.

If no lesion was observed at the highest inoculum dosage (i.e. 1×10^4 zoospores per ml) tested, the experiment was repeated with 10 fold zoospore inoculum density increase until a lesion was found to a maximum of 1×10^6 zoospores per ml.

6. Root infection studies

Root infection studies were done on rhododendron & viburnum using a variety of inoculation approaches. Methodologies for these studies are reported in the RAPRA final report, but essentially involved three types of inoculation: zoospore suspensions applied in to the saucer of pots; zoospore suspensions applied to the surface of the growing media in the pot, around the stem base; zoospore suspension applied to the surface of the growing media but away from the stem base.

Results and discussion

1. Epidemiology of *Phytophthora ramorum* in relation to risk and policy (Work package 3)

1.1 Sporulation

1.1.1 Effect of humidity

The effect of humidity on sporulation of isolates of *P. ramorum* is shown in Figure 4. No difference was evident between the US and EU isolates in the minima, maxima and optima humidity levels for sporulation. Sporulation was suppressed at levels up to and including 62% relative humidity, and optimal at 93% or above.

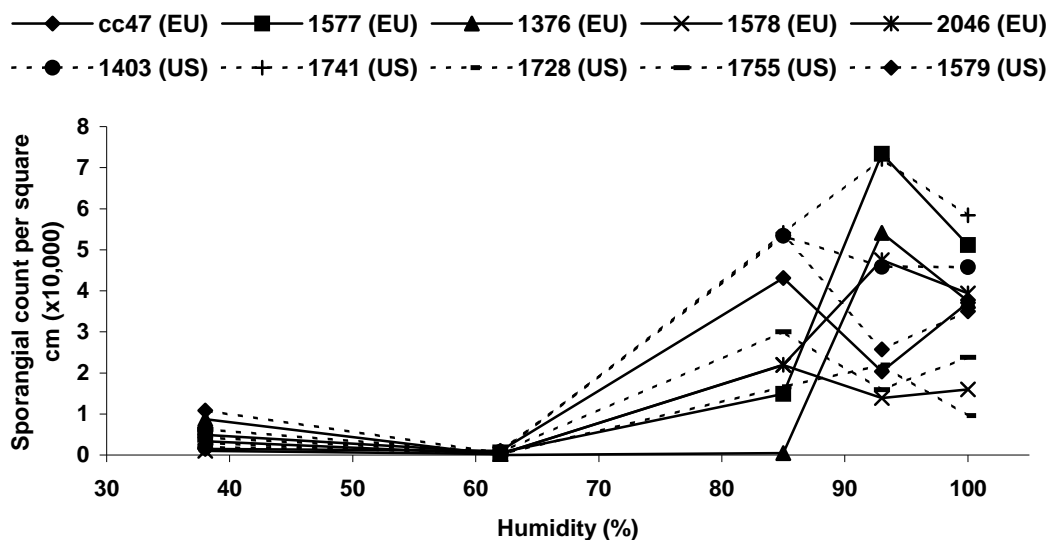


Figure 4 Effect of humidity on production of sporangia by *P. ramorum*.

1.1.2 Temperature and humidity

The combined effect of temperature and humidity on sporulation is shown in Figure 5. Sporulation by both isolates at temperatures below 20°C and above 25°C was minimal. The temperature optimum for the EU isolate was 25°C whereas the optimum for the US isolate was 20°C. Sporangial production by each isolate under each humidity regime increased with increasing temperature up to the optimum temperature and then decreased. Optimal sporulation occurred at 100% humidity. Overall, increasing temperature was more influential on sporulation compared to increasing humidity. However at the temperature optimum, a more significant increase in sporulation was measured for the US isolate than the EU isolate.

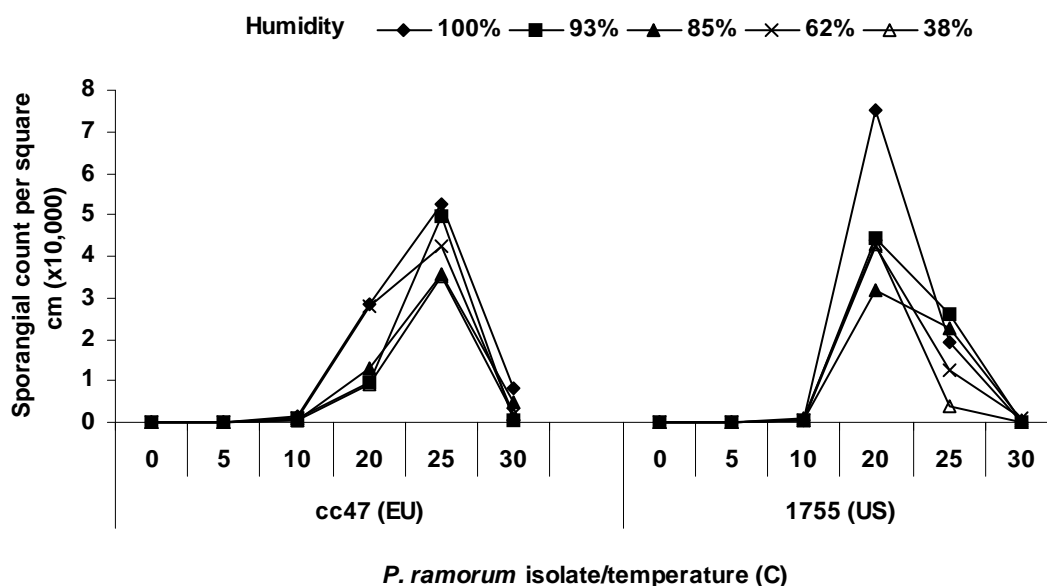


Figure 5 Combined effect from temperature and humidity on production of sporangia by *P. ramorum*.

1.2 Germination

1.2.1 Effect of water potential

Levels of germination remained consistent at all water potentials tested, indicating that water potential has no effect on zoospore germination in the range tested (Figure 6)

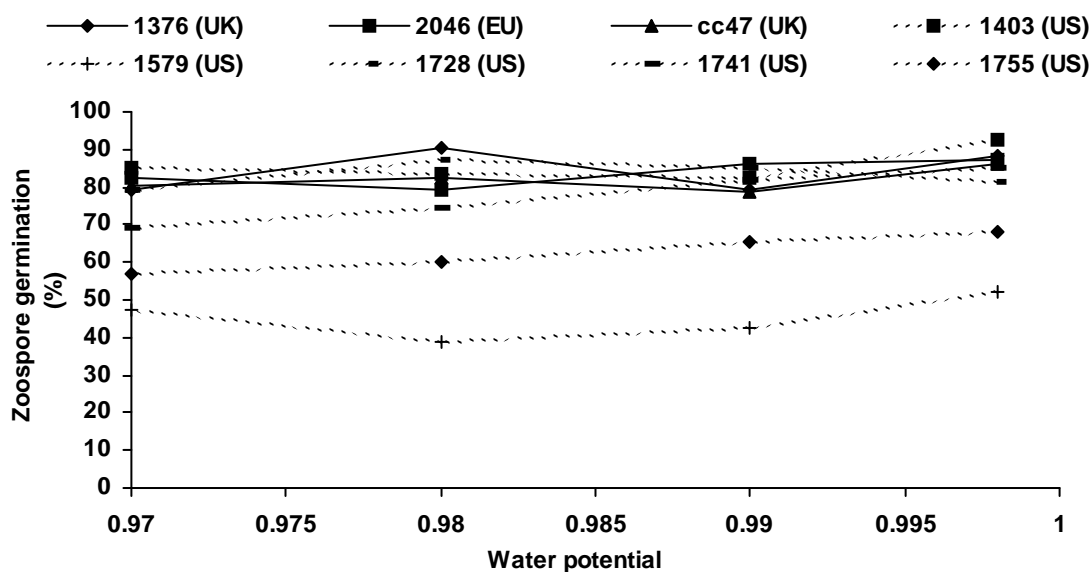


Figure 6 Effect of water potential (sucrose) on zoospore germination

Germ tube elongation was minimal at water potential of 0.97 and increased with increasing water potential to a maximum at 100% water availability (Figure 7).

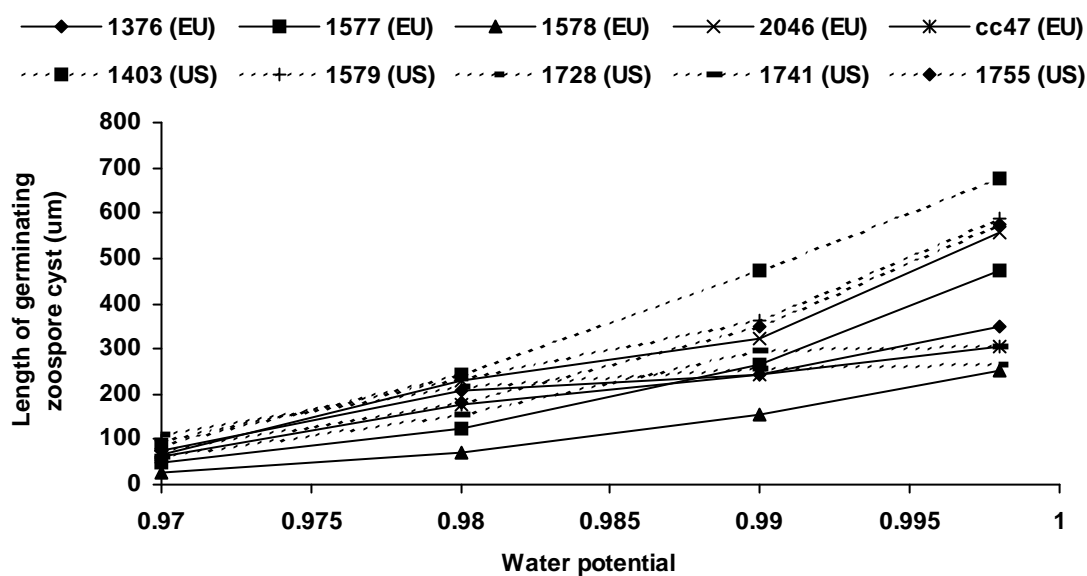


Figure 7 Effect of water potential (adjusted using sucrose) on zoospore germ tube elongation.

Sporangial germination increased with increasing water potential for all isolates (Figure 8).

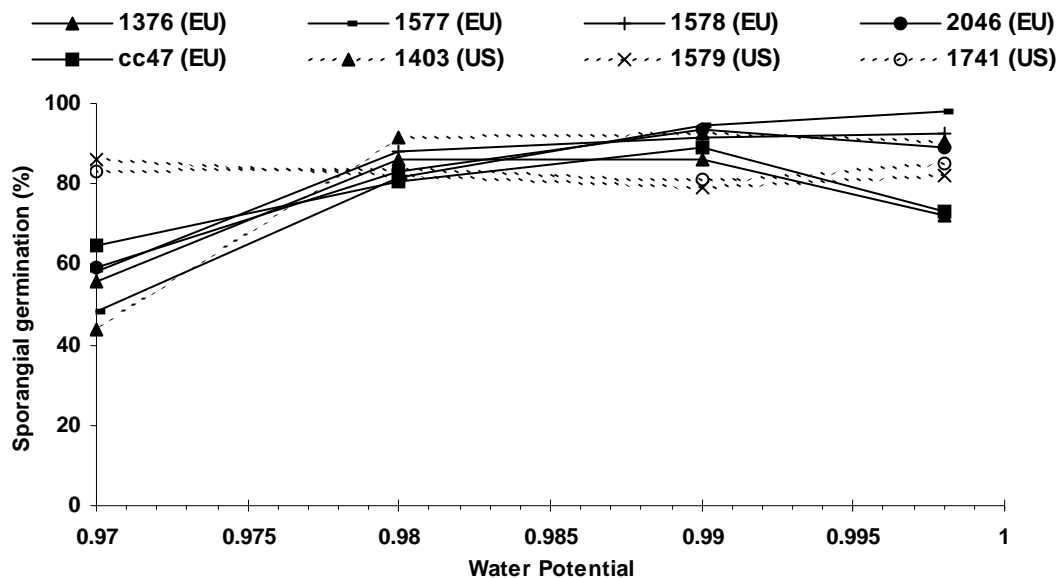


Figure 8 Effect of water potential (adjusted using sucrose) on sporangial germination

1.2.2 Effect of temperature and water potential

The effects of combined temperature and water potential treatments on sporangial germination and sporangial germ tube length are shown in Figures 9 and 10. Figures 11 and 12 show the effects of the same treatments on zoospore germination and germ tube development. These graphs illustrate that optimal germination and germ tube development occurred under conditions of high water potential (>0.99) at temperatures between 20 and 25°C.

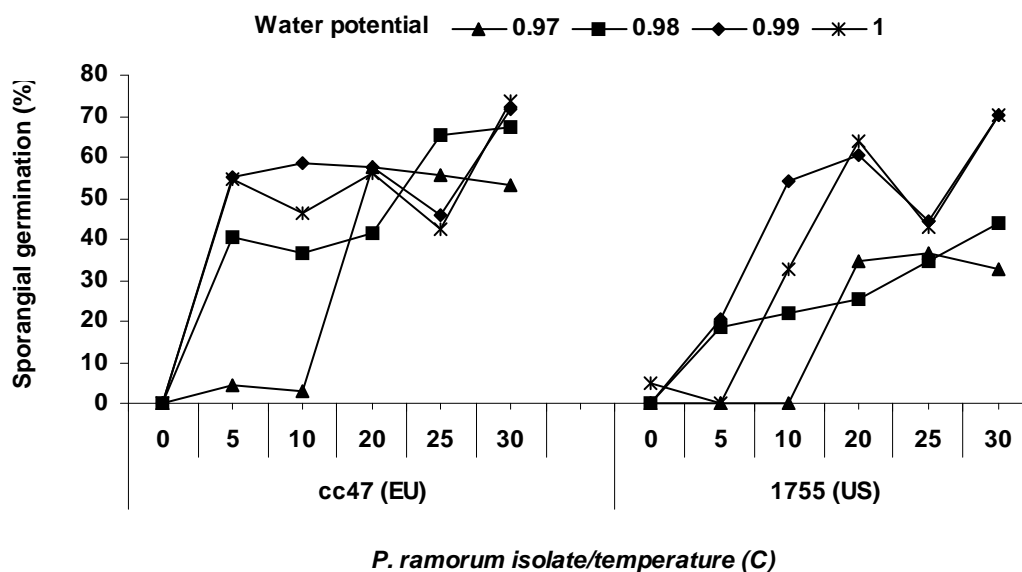


Figure 9 Effects of temperature and water potential on sporangial germination of two *P. ramorum* isolates.

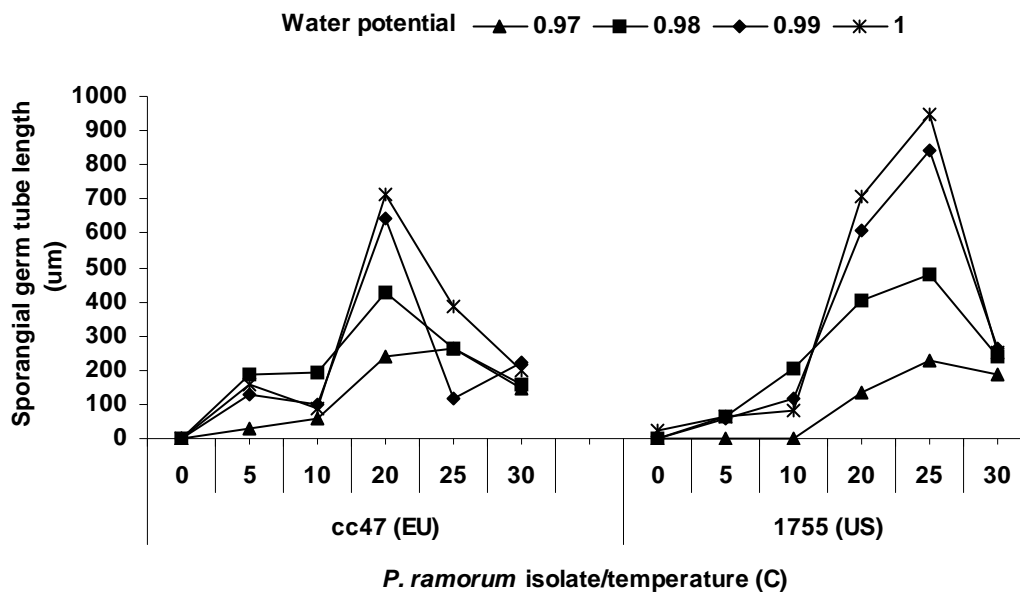


Figure 10 Effects of temperature and water potential on sporangial germ tube length of two *P. ramorum* isolates

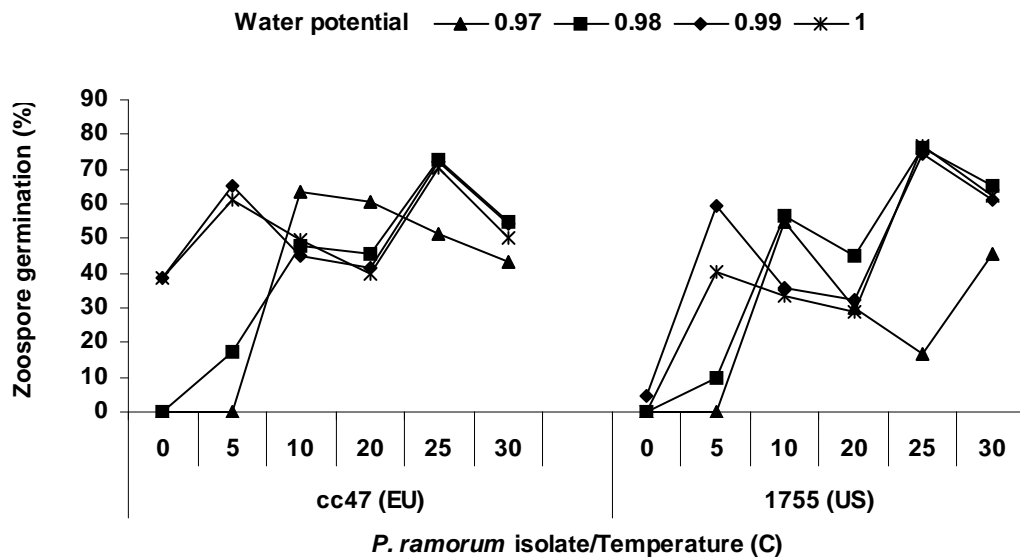


Figure 11 Effect of temperature and water potential on zoospore germination of two *P. ramorum* isolates.

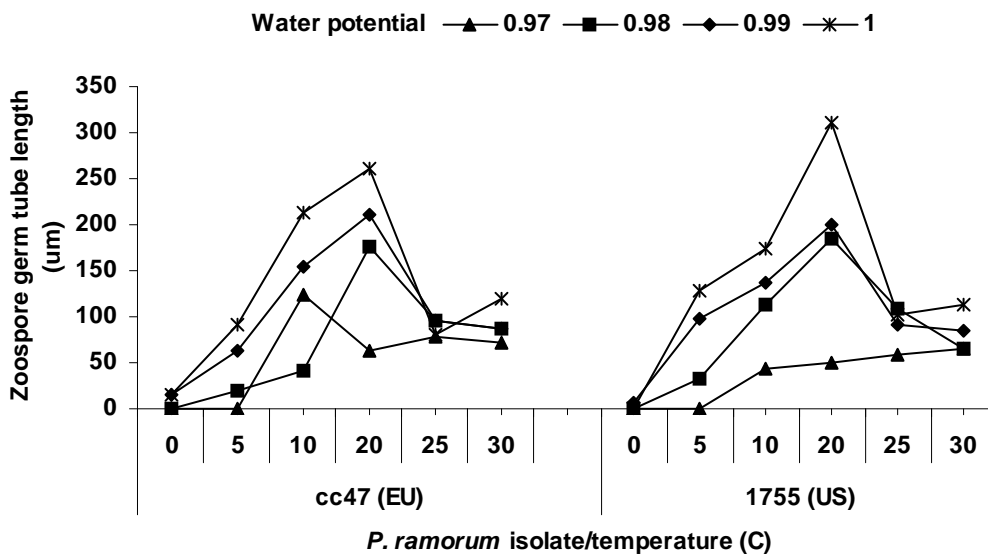


Figure 12 Effect of temperature and water potential on zoospore germ tube length of two *P. ramorum* isolates.

1.2.3 Effect of humidity

Reduced humidity levels did not significantly affect sporangial germination of EU or US isolates (Figures 13 and 14). For the purposes of presentation, results have been expressed as sporangial germination only; this includes sporangia that have germinated either by the production of mycelia or by the release of zoospores.

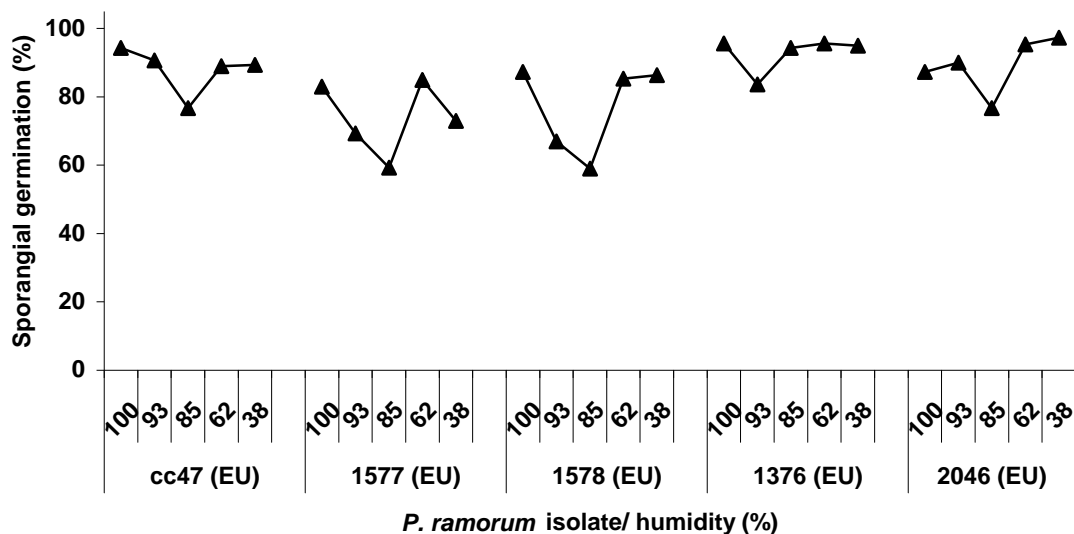


Figure 13 Effect of humidity on sporangial germination of EU isolates of *P. ramorum*.

For the majority of the isolates tested, levels of zoospore germination were above 70% at humidity levels of 85% and above (Figures 15 and 16). Germ tube development was more sensitive to humidity levels with limited germ tube elongation occurring at humidity levels below 85% (Figures 15 and 16).

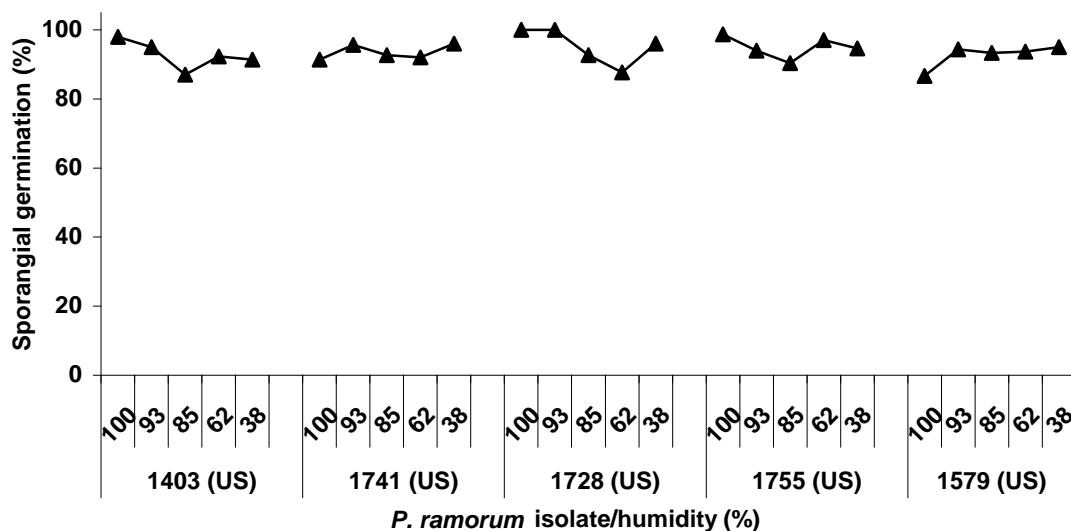


Figure 14. Effect of humidity on sporangial germination of US isolates of *P. ramorum*.

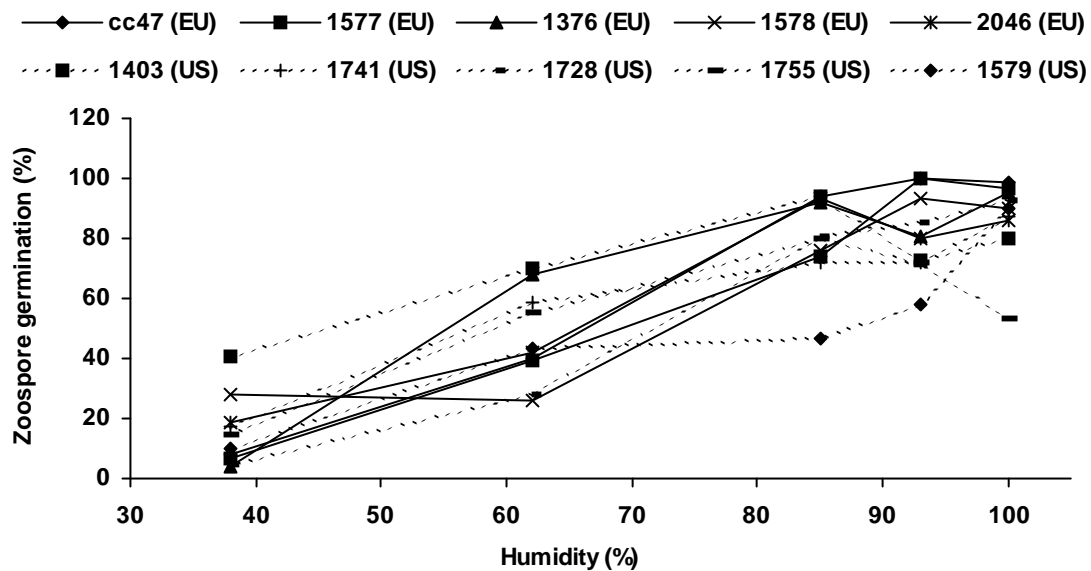


Figure 15 Effect of humidity on zoospore germination.

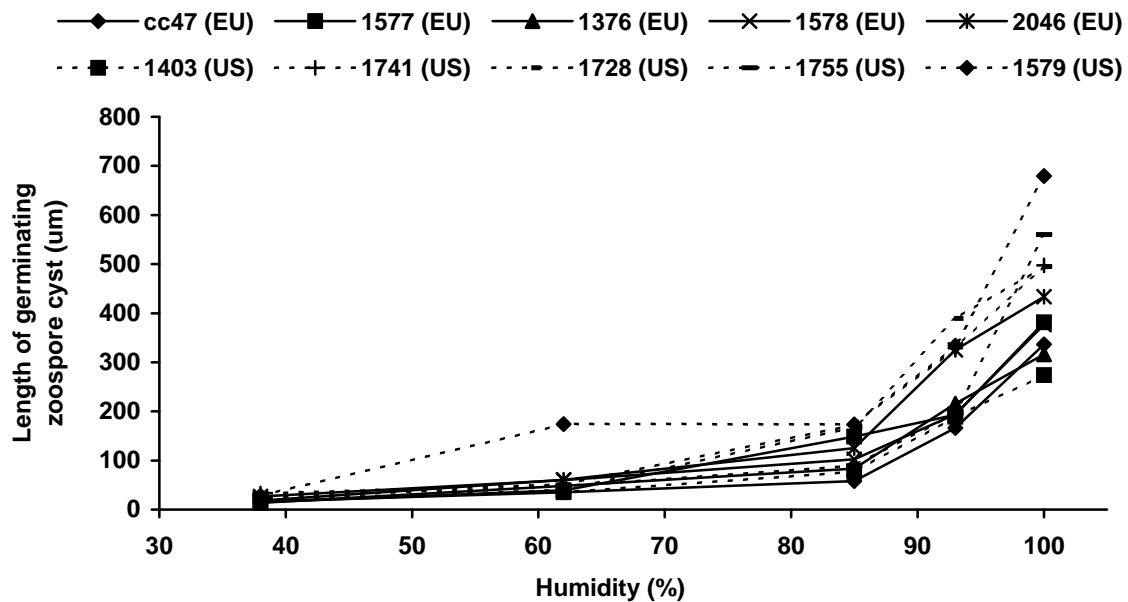


Figure 16. Effect of humidity on zoospore germ tube length.

1.2.4 Effect of temperature and humidity

The effect of combined temperature and humidity regimes on sporangial germination is shown in Figure 17. As in previous experiments, temperature was found to be more influential than humidity on sporangial germination.

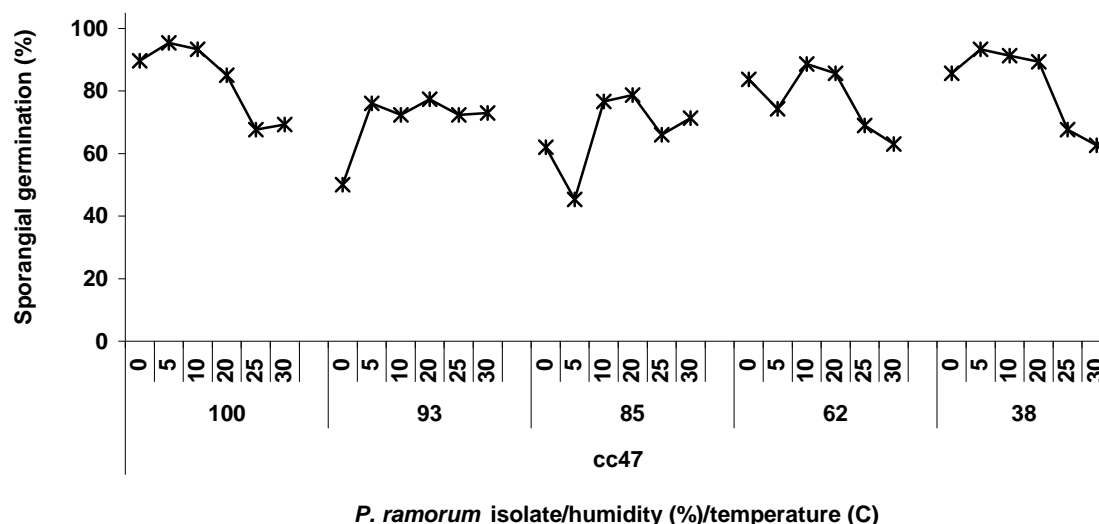


Figure 17. Effects of temperature and humidity on sporangial germination for *P. ramorum* isolate cc47

Experiments on the effects of combined temperature and humidity treatments on zoospore germination and germ tube development showed that humidity levels of 100% were critical for zoospore germination and germ tube elongation and that optimum temperatures were between 20 and 30°C (Figure 18 and 19).

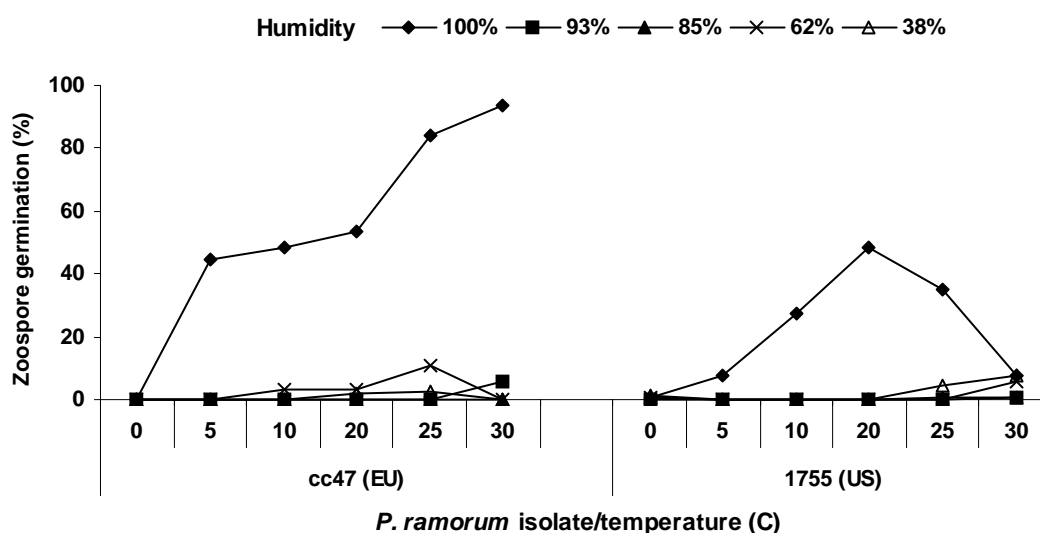


Figure 18. Effects of temperature and humidity on zoospore germination for *P. ramorum* isolates cc47 and 1755.

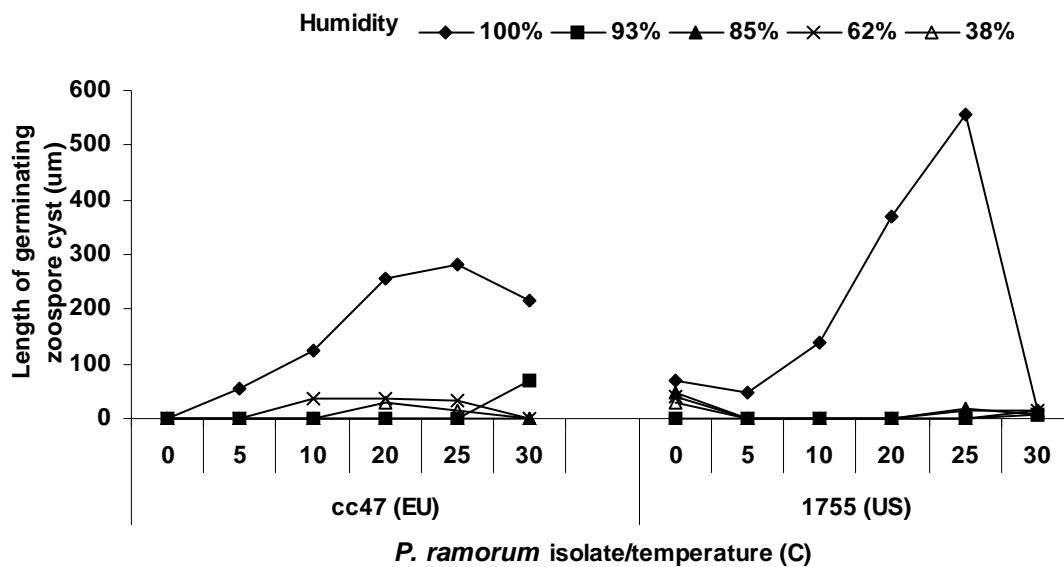


Figure 19. Effects of temperature and humidity on zoospore germ tube length of two *P. ramorum* isolates.

1.3 Effect of temperature on chlamydospore survival

Chlamydospores survived at all treatment temperatures except -25°C and 40°C for the duration of the experiment (Figures 20 and 21).

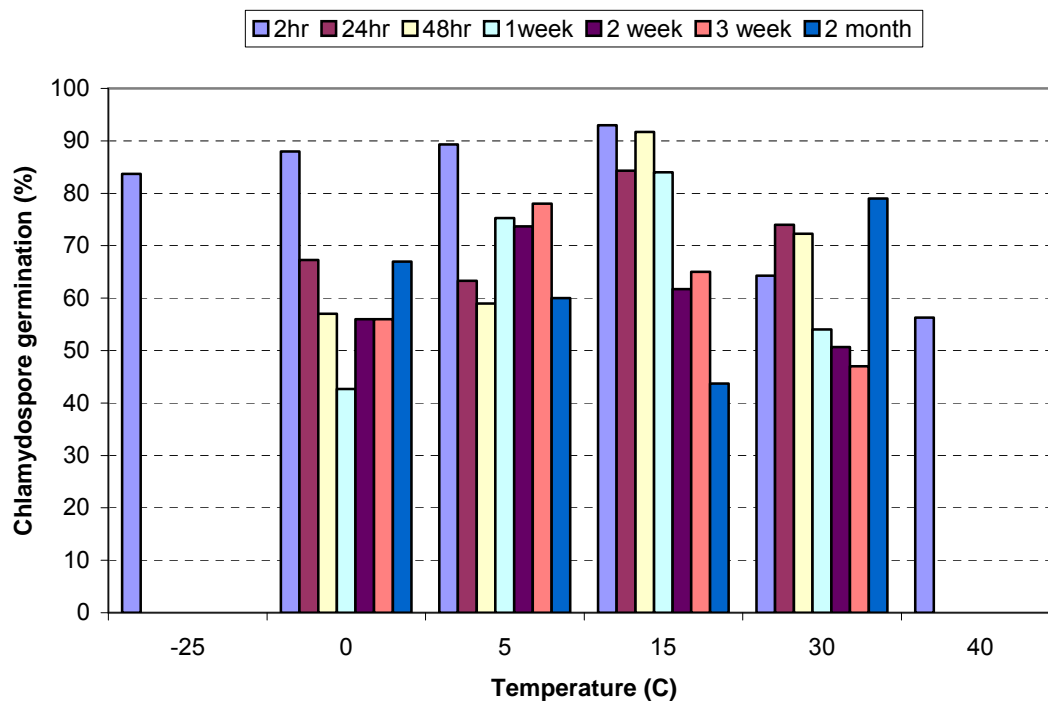


Figure 20. Effect of temperature on chlamydospore germination

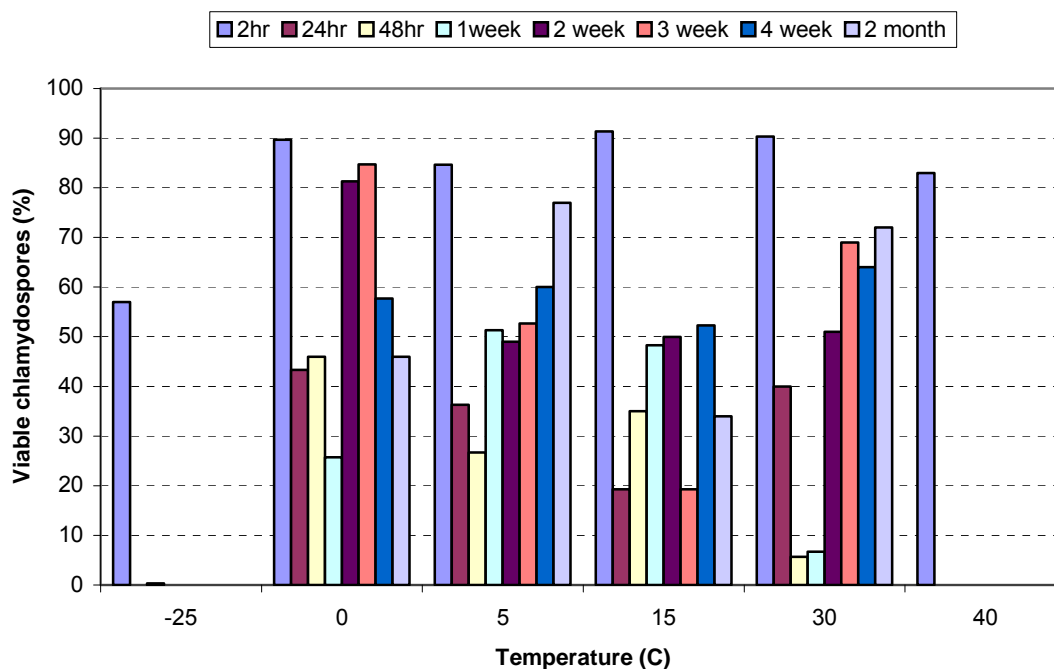


Figure 21. Effect on temperature on chlamydospore viability

2 Management of outbreaks of *P. ramorum* through Experimentation (work package 6)

2.1 Effects on mycelial growth

The sensitivity of *P. ramorum* isolates to the selected fungicides is presented in Annex 1 (Figures 1-6). For each fungicide the mean EC_{50} and the range across the isolates tested are shown in Table 8. Cyazofamid (Ranman) and metalaxyl-M (SL 567A) were the most effective against mycelial growth. However, variability in sensitivity across the range of isolates tested was also greatest for these two fungicides and data show evidence of reduced sensitivity to metalaxyl-M in one isolate (1650).

Table 8. EC_{50} values for fungicide tested against mycelial growth of *P. ramorum*

Fungicide	Active ingredient	EC_{50} on mycelial growth (ppm)	
		Mean	Range
Amistar	Azoxystrobin	11	1 - 24
Filex	Propamocarb hydrochloride	> 100	66 - >100
SL 567A	Metalaxyl-M	0.4	0.00005 - 6.7
Dithane 945	Mancozeb	57.7	39 - 73
Ranman	Cyazofamid	0.7	0.1 - >10
Elvaron Multi	Tolyfluanid	30.7	5 - 56

Further experiments using a selection of nine isolates, including some shown to be resistant to metalaxyl-M, demonstrated that EC_{50} values ranged from 0.004 (fully sensitive) to >10 (resistant) and that some isolates showed intermediate levels of sensitivity (Table 9 & Annex 1:Figure 7). These data indicate that three sub-groups could exist within the *P. ramorum* population worldwide. Some of the most sensitive isolates were sourced from natural outbreak situations, whereas those with reduced sensitivity came from nurseries, where exposure to metalaxyl-M is more likely.

Table 9. EC_{50} values for efficacy of metalaxyl-M against mycelial growth of resistant isolates

Isolate	Source	EC_{50} on mycelial growth (ppm)
CC47	UK (garden)	0.004
CC70	UK (woodland)	0.004
1657	Germany	0.005
1578	UK (nursery)	0.005
1650	Germany	2.7
1659	Germany	2.9
1653	UK (nursery)	3.7
1658	Germany	5.5
1664	Germany	>10

2.2 Effects on spore germination

Graphs showing the sensitivity of *P. ramorum* isolates to the selected fungicides are shown in Annex 1 (Figures 8-13). Azoxystrobin and metalaxyl-M were the most effective actives against zoospore germination (Table 10). Cyazofamid was intermediate in efficacy and mancozeb, propamocarb hydrochloride and tolyfluanid were the least effective. Considerable variation in sensitivity was found across the isolates tested, with some evidence that a number were significantly less sensitive to metalaxyl-M, cyazofamid and tolyfluanid.

Table 10. EC₅₀ values for fungicide tested against spore germination of *P. ramorum*

Fungicide	Active ingredient	EC ₅₀ on mycelial growth (ppm)	
		Mean	Range
Amistar	Azoxystrobin	0.09	0.0035-0.78
Filex	Propamocarb hydrochloride	49.1	0.05->100
SL 567A	Metalaxyl-M	0.03	0.0085->10
Dithane 945	Mancozeb	4.7	0.9-6.1
Ranman	Cyazofamid	18.1	0.07->100
Elvaron Multi	Tolyfluanid	1.9	0.38-3.3

2.3 Effects on zoospore motility

Graphs showing the sensitivity of *P. ramorum* isolates to the selected fungicides are shown in Annex 1 (Figures 14-19). All fungicides except propamocarb hydrochloride showed activity against zoospore motility (Table 11). The most effective active ingredients were azoxystrobin, mancozeb and tolyfluanid with EC₅₀ values of between 0.2 and 0.3 ppm. There was no evidence of resistance to metalaxyl-M in these tests, all isolates appeared equally sensitive.

Table 11. EC₅₀ values (ppm) for efficacy of fungicides against zoospore motility of *P. ramorum*

Fungicide	Active ingredient	EC ₅₀ on zoospore motility (ppm)	
		Mean	Range
Amistar	azoxystrobin	0.29	0.0007-1.92
Filex	propamocarb hydrochloride	>1000	-
SL 567A	metalaxyl-M	39	-
Dithane 945	mancozeb	0.2	0.02-0.37
Ranman	cyazofamid	3.4	0.41-3.95
Elvaron Multi	tolylfluanid	0.19	0.002-0.9

2.4 Fungicide efficacy against isolates of *P. ramorum* – detached leaf assay

Metalaxyl-M was the most effective of the protectant treatments applied to rhododendron leaves (Table 12). Lesions developed on control leaves within four days of inoculation. Where lesions developed following fungicide application, the onset of lesion development was not delayed, indicating that none of the fungicide applications caused latency of the infection. Isolations carried out to determine whether the fungicides were fungistatic or fungitoxic in activity showed that, where no lesion had developed, *P. ramorum* could not be re-isolated, indicating that the chemicals had killed the pathogen and were not fungistatic.

Table 12. Effect of protectant fungicide treatments on *P. ramorum* development (detached rhododendron leaves).

Fungicide	Active ingredient	Protectant treatment timing (% control)	
		7 days pre-inoculation	4 days pre-inoculation
Amistar	Azoxystrobin	0	28
SL 567A	Metalaxyl-M	100	100
Elvaron Multi	Tolyfluanid	0	4

When fungicides were applied to rhododendron leaves as eradicants (after the lesion development), only metalaxyl-M (SL 567A) halted lesion development (Table 13). Following treatment with SL 567A *P. ramorum* could not be cultured from lesions when re-isolations were made 14 days after inoculation, but was after treatment with Amistar or Elvaron Multi.

Table 13. Effect of eradicant fungicide treatments on *P. ramorum* development (detached rhododendron leaves).

Fungicide	Active ingredient	Eradicant treatment timing (% control)	
		7 days post-inoculation	4 days post-inoculation
Amistar	Azoxystrobin	0	0
SL 567A	Metalaxyl-M	98	100
Elvaron Multi	Tolyfluanid	0	31

2.5 Fungicide efficacy against metalaxyl-M resistant isolates of *P. ramorum* – detached leaf assay

A detached leaf assay was used to test the efficacy of fungicides in controlling five isolates of *P. ramorum*, which had previously shown differing levels of resistance to metalaxyl-M during *in vitro* testing. Two of the fungicides used, SL 567A and Fubol Gold, contained metalaxyl-M at 480 and 40g/L respectively. Figure 22 shows the effects of the treatments on the five isolates, which are presented in order of resistance level measured during the *in vitro* tests (most sensitive first).

Treatment of leaves with SL 567A as a protectant four days prior to inoculation resulted in more than 95% control of all the isolates tested, with 100% control achieved for isolates 1650 and 1653 (Figure 22). Generally, treatment with SL 567A seven days prior to inoculation resulted in reduced control compared to the 4 day treatment, with only one isolate completely controlled by the treatment (1653). The poorest level of control was for isolate 1664, where only 30% control was achieved. This isolate had the highest EC50 value and was the least sensitive isolate identified during the *in vitro* testing.

Fubol Gold applied four days prior to inoculation was completely ineffective against isolate 1664 (the least sensitive isolate to SL 567A) and was less effective than SL 567A against isolates 1658 and 1653 (92.6 and 58.1% control respectively). However, when Fubol Gold was applied seven days pre-inoculation the treatment increased disease control compared to the four-day treatment. This is in contrast to the results seen with SL 567A and may be due to the activity of mancozeb (the partner active) in Fubol Gold. At both application timings, Consento achieved greater than 95% control of all isolates, except 1659. Ranman did not show effective control against any of the isolates.

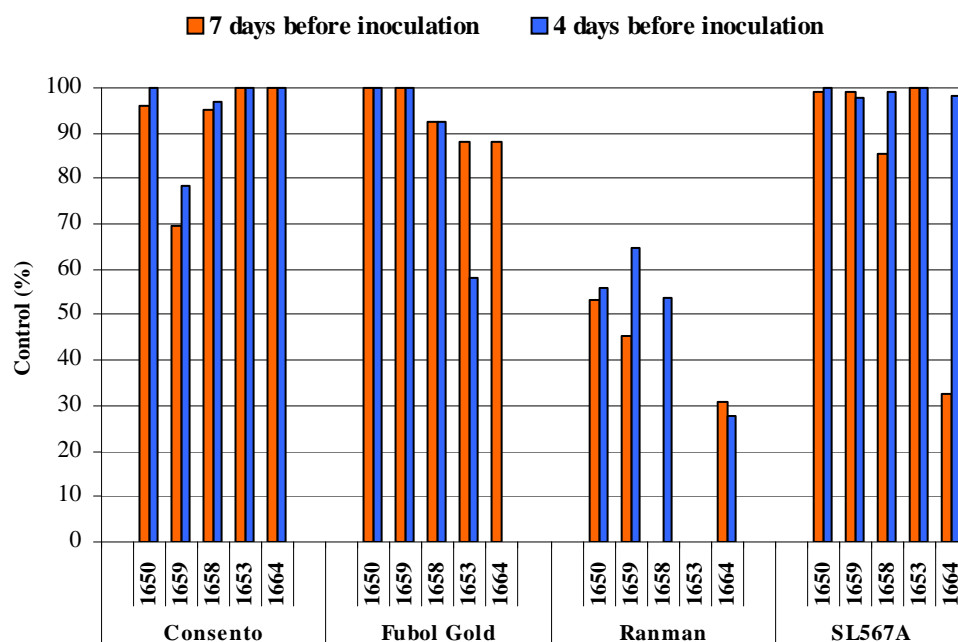


Figure 22. Effect of protectant fungicide applications against *P. ramorum* development in a detached rhododendron leaf assay (including isolates resistant to metalaxyl-M)

Detached leaf assays on the isolates showing reduced *in vitro* sensitivity to metalaxyl-M, indicated that reduced sensitivity of control might result in control failures on nurseries. In addition, robust applications of formulated mixtures containing reduced rates of metalaxyl-M (compared to SL 567A) might not be effective against these isolates. Results also show that Consento (fenamidone/propamocarb hydrochloride) was highly effective as a protectant treatment against isolates showing resistance to metalaxyl-M. These, and results from previous experiments, suggest that products containing metalaxyl-M (SL 567A, Fubol Gold, Folio Gold and Epok) used in conjunction with Consento could form the basis of a spray strategy for the control of *P. ramorum*, which would both control the disease and minimise the risk of further resistance development.

3 Susceptibility of important rare *Rhododendron* species and hybrids to *P. ramorum*

All the rhododendrons tested were susceptible to *P. ramorum*, however, differences were evident between species (see Figure 23.)

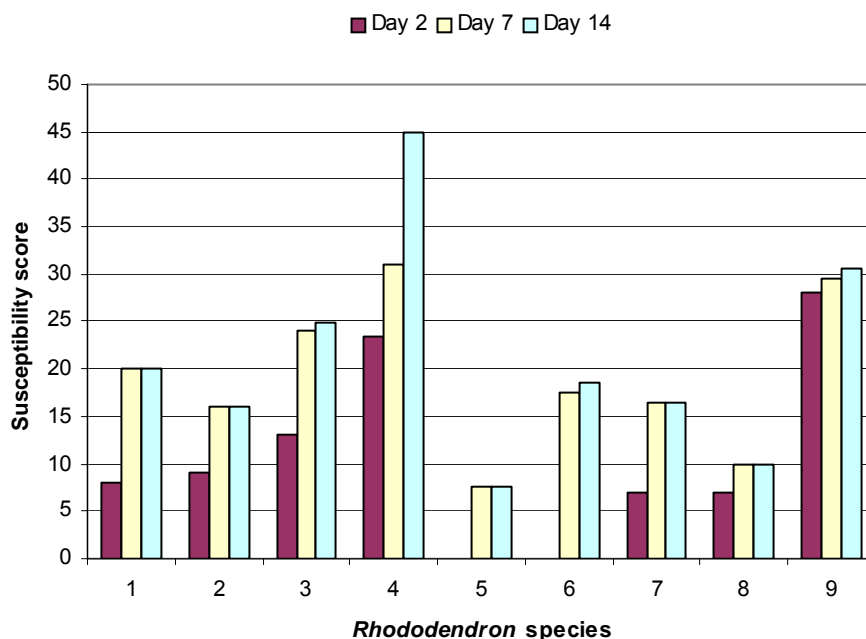


Figure 23. Susceptibility of *Rhododendron* species/hybrids to infection caused by *P. ramorum*.
 1 *R. Megacalyx*, 2 *R. Johnnie Johnston*, 3 *R. Dalhousie* hybrid, 4 *R. ponticum* rubra, 5 *R. Morvah*
 ('Elliottii x Wattii), 6 *R. elliotii*, 7 *R. nattallii*, 8 *R. macabeum*, 9 *R. catawbiense* (cunninghams white).

Visible lesions were present two days post inoculation for seven of the nine test rhododendrons, however after day 7, all nine rhododendrons showed symptoms. after 14 days the greatest level of disease was recorded on *R. ponticum* rubra.

4 *P. ramorum* inoculum thresholds

No lesions were evident from any of the negative control tests. All four isolates tested showed no obvious major differences on each host plant. Differences in the lowest concentration of *P. ramorum* zoospores to cause infection were observed between host plants (Figure 24).

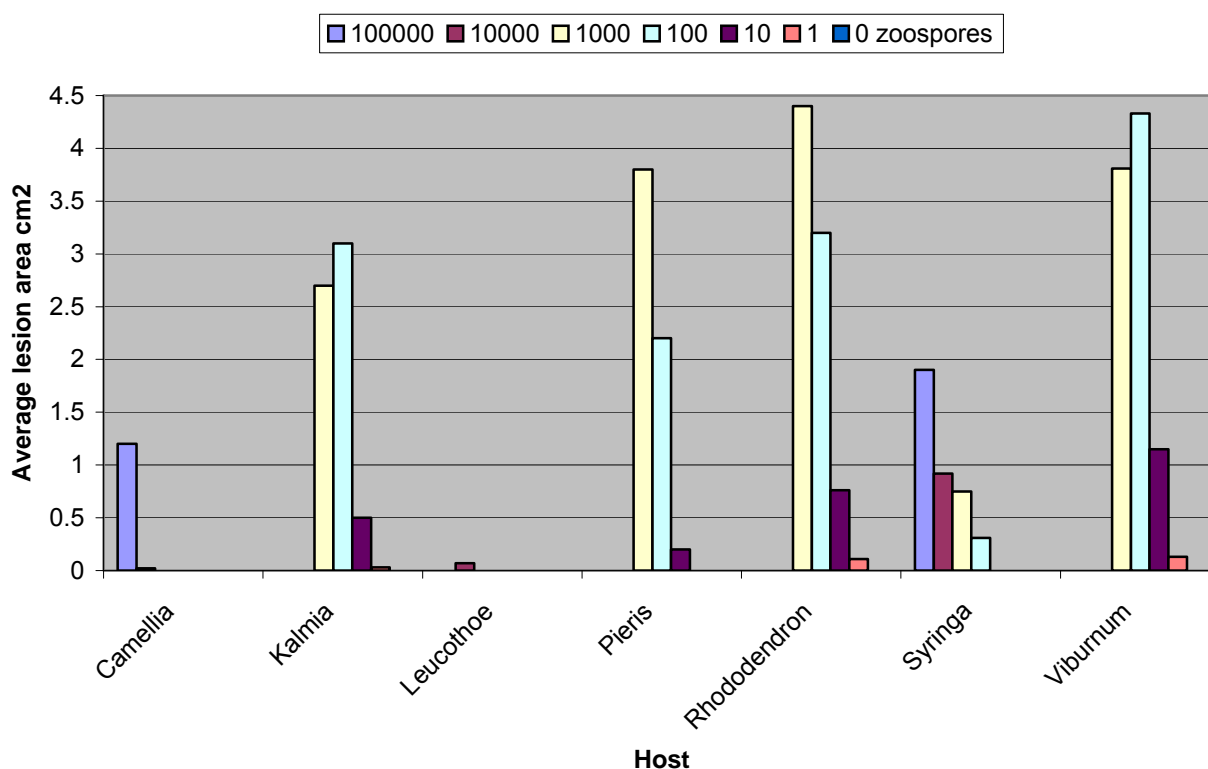


Figure 24. Average lesion area (cm²) following infection with *P. ramorum* isolates at various zoospore concentrations.

The most sensitive plants were *Kalmia*, *Pieris*, *Rhododendron* and *Viburnum* where lesions were observed following inoculation with a single zoospore. The largest lesion size at this zoospore rate was on *Rhododendron* and *Viburnum*. These findings reflect field observations where >95 % of nursery plants infected with *P. ramorum* are *Rhododendron* or *Viburnum*. A zoospore rate of 100 zoospores was required for lesion development on *Syringa* and 10,000 zoospores were required on *Leucothoe*. The least sensitive host plants tested were *Camellia* and *Leucothoe*, which required inoculation with at least 10000 zoospores before lesion development occurred. In general, average lesion area increased as zoospore levels increased.

5 Root infection studies

Results suggested that roots are not a primary pathway for systemic infection of above ground parts of these ornamental species, though asymptomatic colonisation of roots was demonstrated. See RAPRA report for more details.

6 European pest risk analysis for *P. ramorum*

The RAPRA PRA is being developed in collaboration with other RAPRA partners and is scheduled for autumn 2008. The UK-produced datasheet is an output from the RAPRA work and is available at:

<http://defraweb/planth/pram/pram.pdf>

Conclusions and policy implications of the research

Epidemiology of *Phytophthora ramorum* in relation to risk and policy

- Differences in humidity had most effect on sporangial production and zoospore germination whereas sporangial germination was less sensitive to changes in water availability.
- Maximum levels of sporulation and zoospore germination occurred at 100% humidity or water potentials of 1.
- Temperature optima for sporulation and germination ranged from 20 to 30°C depending on the conditions of the experiment.
- Chlamydospores survived treatment at temperatures between 0 and 30°C for at least 2 months but died after short periods at high (40°C) or low (-25°C) temperatures.

Management of outbreaks of *P. ramorum* through experimentation: Fungicide tolerance and development of tolerance.

- Robust baseline data on the *in vitro* sensitivity of a range of *P. ramorum* isolates to selected fungicide active ingredients has been established.
- The range of *in vitro* tests has illustrated that a range of fungicide modes of action are active against the pathogen. These could be utilised within control programmes to minimise the risk of further insensitivities developing should the restriction on the use of fungicides be lifted.
- Of the products tested, metalaxyl-M (as SL 567A) was consistently the most effective active against both mycelial growth and zoospore germination of *P. ramorum*.
- Products containing metalaxyl-M showed both protectant and potential eradicant activity.
- None of the fungicides used caused symptoms of phytotoxicity.
- A number of isolates have already developed reduced sensitivity to metalaxyl-M. Should fungicides become part of the management strategy they would need to be used within robust treatment programmes, which minimise the risk of developing resistance. Evidence in this project confirms that plant infections caused by these resistant isolates are difficult to control using metalaxyl-M.
- Consento could be a key product within spray programmes for both management of the disease and minimisation of resistance development.
- The most effective fungicides were lethal to the fungus and not fungistatic i.e. they killed the fungus rather than merely halting its development.
- There was no evidence from this work of fungicides causing latency in plant infections.

Inoculum thresholds, root infection and pathogenicity studies

- *Viburnum* and *Rhododendron* were the most sensitive, with infection and lesion development occurring with just a single zoospore. The susceptibility of these two plants was also evident as the inoculum dose increased; the average lesion area was consistently larger than all other host plants tested. These findings are reflected in the field where the vast majority (>95 %) of nursery samples found infected with *P. ramorum* are *Rhododendron* or *Viburnum*. Comparisons of the amount of inoculum needed to result in infection helps to inform the understanding of the risk to plants from *P. ramorum*. It could give a clearer indication of those susceptible genera most at risk when surrounded by infected plants producing high levels of inoculum.
- Resistance has already developed to metalaxyl-M in a number of isolates, particularly those originally collected from nursery situations. However, resistance was not evident in all tests and the effectiveness *in planta* cannot necessarily be predicted from these laboratory tests.

- All rare *Rhododendron* species/hybrids tested, were susceptible to *P. ramorum*. It is therefore unadvisable to use these varieties in future planting schemes where *P. ramorum* is known to be present. It would also be prudent to protect the established bushes of these rare plants by removal of inoculum sources of *P. ramorum* from infected sites in the near vicinity.
- Root infection studies suggested that roots are not a primary pathway for systemic infection of above ground parts of rhododendron and viburnum plants, though asymptomatic colonisation of roots was demonstrated.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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Venette, RC & Cohen, SD (2006) Potential climatic suitability for establishment of *Phytophthora ramorum* within the contiguous United States. *Forest Ecology and Management*, **231**: 18-26.