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Project identification

1. Defra Project code: PH 0408 and variations
2. Project title:
   PHYTOPHTHORA RAMORUM & PHYTOPHTHORA KERNOVIAE : Conservation of rare Plant Species Threatened by Phytophthora ramorum and P.kernoviae
3. Contractor organisation(s):
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   Duchy College
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   TR14 0AB
4. Total Defra project costs (agreed fixed price): £ 27,000
5. Project: start date .... 01 April 2005
   end date .... 31 March 2009

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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.

The overarching aim of the work was to develop techniques that facilitate cloning as a conservation measure for rare, heritage or valuable plants. Since 2002, the plant diseases, *Phytophthora ramorum* and *Phytophthora kernoviae* have caused infection and the threat of eradication for numerous and ancient plants in Historic gardens. The disease outbreak of *Phytophthora spp.* was initially concentrated in Cornwall but infection has now spread throughout the UK. To mitigate against potential losses due to *Phytophthora spp.* a plant conservation programme of work was required. Conventional vegetative propagation (cloning) techniques were unsuitable because of the invariably old age of the mother plant (i.e. up to 160 years from propagation by seed) and the possibility of latent infection transfer. Preliminary investigations revealed that cloning could be carried out by using micropropagation techniques on young regenerating plant parts (i.e. new vegetative or floral tissue) under sterile laboratory conditions. Routine regeneration of old plant material required the development of species specific micropropagation protocols. The first group of plants for which protocols were developed was Rhododendron as they were initially the most severely *Phytophthora spp.* infected plants in Historic gardens. The plant conservation strategy developed in this project of cloning rare or old plants has been extended to explore micropropagation techniques for other *Phytophthora* infected genera such as Magnolia and Camellia.

The work was partitioned into four main Objectives to explore the various stages of regeneration of material from tissue culture in the laboratory, hardening off under protected conditions, through to successful re-introduction of maturing plants to historic 'host' gardens. More specifically the project addressed the following objectives, and detailed below each is a summary of the main findings.

Objective 01 To generate *Phytophthora spp.* pathogen free material that can be safely released into the environment.

The formulation of micropropagation protocols for the conservation of threatened Rhododendron species was achieved; to date 380 accessions have been successfully processed using the protocols established within this project. Material at different growth stages was received throughout the year and subjected to a range of decontamination techniques, using sodium hypochlorite as sterilent, according to the resilience of the tissues concerned. Plant material was routinely collected in a dry condition to aid successful decontamination, the dry surfaces also enabled surplus tissue to be placed in a cold store (4°C) for up to three months without deterioration. Two plant growth stages have proved more reliable for decontamination and regeneration; these are the floral bud, collected in January/February before the scale leaves have opened, and dormant terminal and/or axillary vegetative buds. Both these growth stages

have toughened scale leaves surrounding the growing area which can therefore be subjected to a harsh decontamination regime without damaging the delicate tissues within. Sterilization in 0.3% sodium hypochlorite solution for 20min, followed by rinsing in sterile distilled water resulted in optimum decontamination when the excised tissues were placed on an ericaceous nutrient jelly containing the plant growth regulators IAA, Thidiazuron and 2iP. Where both tissue types were available for use, a regeneration success rate of 95% was achieved.

Objective 02 To quantify the effects of nutrient deficit at selected stages of development on root and shoot growth.

The effects of the symbiotic relationship between rhododendron roots and mycorrhiza were explored to enhance the growth of selected rhododendron species which had not established rapidly following micropropagation. Ericoid mycorrhiza, *Hymenoscyphus ericae* and *Oidiodendron maius*, were inoculated into the seedling root zone of two recalcitrant Rhododendron species, *R. concinnoides* and *R. phaedropum*, which resulted in enhanced growth. Furthermore, when mycorrhiza were combined with increasing levels of nutrients, the growth rate increased 3-4 fold compared with plants established under control conditions.

Objective 03 To explore the conservation by micropropagation of other genera under threat from *Phytophthora spp.* infection.

The micropropagation of magnolia species was partially successful in that a protocol was devised where material could be propagated in aseptic conditions which resulted in shoot proliferation. Rooting has not been accomplished as yet. Research showed that dormant winter vegetative buds, which are enclosed by protective ‘perules’, could survive harsh decontamination regimes of an initial scrubbing with 0.5% sodium hypochlorite followed by soaking for 20min in a weaker 0.3% solution and provide sterile material for propagation. The excised buds were grown and proliferated on a nutrient jelly containing the plant growth regulators IAA and BAP. Softer summer ‘water shoots’ were unreliable in their decontamination regime. Other genera investigated for micropropagation were Camellia, Oaks and Conifers. The selected plant tissue was decontaminated successfully, but proved difficult to micropropagate, and continuing research effort is required to develop protocols that are as routinely successful as those for Rhododendron species.

Objective 04 To evaluate the performance of cloned rhododendron material on return to original habitat location.

A number of propagules were returned to host gardens for growing on. Three Cornish gardens have received small modules but one garden, however, has found the maintenance of these important plants difficult, so replacements will be returned at a later date when they have grown larger. A plant propagation centre which previously received rooted modules now has the facilities to wean off plantlets rooted in sterile culture. Micropropagated rhododendrons from early accessions have started to reach maturity and two cloned plants from separate gardens flowered under protection for the first time this year; both flowering true to type. Matured plants have been reinstated in these gardens in spring 2009, too early to evaluate their performance.

Summary main implications of the work

To date 16 gardens have had material processed successfully for 380 rhododendron accessions. There has been partial protocol development for Magnolia, Camellia and other woody species, and this work is ongoing. We continue to work with gardens in Cornwall and have extended the rare plant conservation programme to the wider UK and are currently working with 6 gardens processing 163 of their Rhododendrons.

Objective 01 GENERATION OF PATHOGEN FREE RHODODENDRON MATERIAL

- A high level of successful decontamination of rhododendron material can be achieved using a combination of dormant floral and vegetative buds.

Objective 02 ROOT AND SHOOT NUTRIENT SUPPLY

- The addition of selected ericoid mycorrhiza can have a beneficial effect on the establishment of rhododendron seedlings.

Objective 03 CONSERVATION OF OTHER GENERA

- Vegetative magnolia buds can be successfully decontaminated with subsequent proliferation. Rooting has not yet been achieved.
- Protocols have been developed for the successful decontamination of Camellia, Oak and

Conifer shoots but suitable formulations for tissue development have not been identified.

Objective 04 PERFORMANCE OF REINTRODUCED CLONED MATERIAL

- The performance of reintroduced cloned material has not yet been evaluated because micropropagated Rhododendron material has only sufficiently matured for reintroduction during spring 2009.

Future work

- Investigate whether there is an optimum stage in the development of the Rhododendron floral bud that results in maximum shoot production under micropropagation.
- Continue to investigate the use of mycorrhiza to enhance the growth and establishment of cloned Rhododendron propagules after weaning.
- Explore the use of micrografting *in vitro* as a successful technique to root Magnolias.
- Extend the conservation of rare species to more parks and gardens within the UK.
- Continue to evaluate the performance of reintroduced cloned material.
- Develop protocols for more rare or ‘at risk’ plants.

The micropropagation of historic and rare Rhododendrons has extended beyond Cornwall through the help of organisations such as the National Trust and RHS Rhododendron, Camellia and Magnolia Groups. At present there are seven gardens with historic collections of Rhododendrons under micropropagation at Rosewarne, with the expectation of more in subsequent years as the project continues. Further material from four of the 16 Cornish gardens is under micropropagation, as historically important plants continue to be identified at these sites.

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).

1. AIMS AND OBJECTIVES

The overarching aim was to provide significant and practical benefits to the management of *Phytophthora ramorum* and *Phytophthora kernoviae* by the conservation of rare and valuable plants using techniques of micropropagation. More specifically the work was split into four main objectives, which have been met in full or are ongoing (Table 1).

Table 1. Objective number, description and outcome.

Objective Number	Description	Progress
01	To generate *phytophthora spp.* pathogen free material that can be safely released into the environment	Met in Full
02	To quantify the effects of nutrient deficit at selected stages of development on root and shoot growth	Ongoing
03	To explore the conservation of other genera by micropropagation under threat from *Phytophthora spp.* infection	Ongoing
04	To evaluate the performance of cloned rhododendron material on return to original habitat location	Ongoing

2. INTRODUCTION

The historic gardens of Cornwall contain a unique heritage of plant species introduced from the mid 1800s onwards by plant hunters such as Sir Joseph Dalton Hooker and Frank Kingdom Ward. Cornwall's mild climate was ideal, enabling plants such as rhododendrons, magnolias, and camellias to be grown outside and these have now become the mainstay of Cornish gardens. Recently these historically important introductions and subsequent hybrids have become threatened by the fungal diseases *Phytophthora ramorum* and *P. kernoviae*. As these diseases are currently regarded as non-endemic any infected material has to be destroyed. (Defra, 2008). The Rare Species Laboratory of Duchy College has responded to the *Phytophthora* problem by proposing to clone rare plants at risk from infection to prevent permanent loss of this historically and botanically important material. The plants initially most at risk from infection were rhododendrons.

Conventional propagation methods for rhododendrons were unsuitable due to the age of the specimens. Low rooting success from cuttings was anticipated (Gardiner, 2002), furthermore infected propagules might transfer the disease on reinstatement to the garden environment. A method of propagation in sterile culture was therefore required that could be used on older plants and which would facilitate early detection of the presence of *Phytophthora* species. A range of micropropagation techniques were therefore developed, from Spring 2005, to successfully conserve important rhododendron germplasm and produce disease-free rooted plantlets for eventual reintroduction to original locations.

3. MATERIALS AND METHODS

3 01 TO GENERATE PHYTOPHTHORA RAMORUM PATHOGEN FREE MATERIAL THAT CAN BE SAFELY RELEASED INTO THE ENVIRONMENT

Different parts of the rhododendron plant have been used for shoot regeneration in sterile culture ranging from shoot tips (Anderson, 1975) to floral tissues such as pedicels and ovary bases (Meyer, 1982), ovaries (Dai *et al.*,1987), stamens (Shevade and Preece 1993), and more recently ovary and pedicel (Tomsone and Gertnere, 2003). A range of micropropagation techniques were developed, from Spring 2005, to successfully conserve important rhododendron germplasm and produce disease-free rooted plantlets for eventual reintroduction.

Material for micropropagation from potentially infected Rhododendrons was collected *in situ*. For each accession between 3 and 6 stems of up to 15cm length were usually collected either by the owners or head gardeners in order to select the most important species or cultivars. The leaves were cut off and material placed in sealed polythene bags and brought or posted triple wrapped to the laboratory. Great care was taken to minimise the potential risk of disease transmission of *Phytophthora spp*. in the handling, movement and storage of these samples between garden and laboratory and particularly in the disposal of waste material throughout processing.

At the beginning of the micropropagation programme in 2005, vegetative shoots were used as the initiation material. These were selected *in situ* just before bud burst in spring (April/May) and the older wood sprayed with a solution of 0.1% NaOCl. Each shoot was enclosed within a perforated flower sleeve to give some protection to the developing bud without encouraging high humidity and possible increased fungal activity.
After bud burst, the juvenile growth together with approximately 10cm of older stem was removed from the parent plant, sealed in a polythene bag and brought to the laboratory. Here the older leaves were reduced in number and length and the old wood disinfected by scrubbing with a solution of approx. 0.3% NaOCl, followed by rinsing off and drying with tissues. Stems were then shortened and placed in flower food solution in natural light for 2-3 weeks to develop further. The young growth of 2-3cm length was then removed and washed in distilled water containing a few drops of surfactant for 15min and transferred to a sterilizing solution of 0.2% NaOCl and agitated for 20min. After thorough rinsing in sterile distilled water, shoots were re-cut and placed in sterile containers of Anderson's rhododendron media (Anderson 1978) containing the plant growth regulators IAA, an auxin, and 2iP, a cytokinin. All explants were maintained at a temperature of 22°C under fluorescent tubes (16hrs duration/day giving PAR of 24µmol/m²/sec) in a controlled environment room.

At a later date, July, young shoots were taken from another garden where they were at a more mature stage of growth. These were de-leafed, trimmed to 3cm length, washed under running water for 30min and sterilized in a stronger solution of 0.25% NaOCl for 20min. It was necessary to work with shoots from three Rhododendrons 'condemned' in the autumn (September) because of *Phytophthora* infection; a higher concentration (0.3%) was used as it was thought that the matured stems could withstand this stronger sterilant.

In following years other plant parts were trialled in order to increase the level of successful decontamination. The floral bud, being encased in a tight covering of leaf sheaths, provided a relatively sterile explant compared with exposed shoots. Floral buds were collected from a number of gardens between February and April in the following year and consisted of a range of developmental stages from very tight bud up to partial bud burst. The accessions were picked on only one date from each garden, hence the variability in the stages of bud development. On receipt, samples were placed in a cold store at 4°C until processed. Only half of the plant material received for micropropagation was utilized initially. The surplus was stored for later use if the initial decontamination regime was unsuccessful. To prepare the floral bud for sterilization, outer leaf scales were carefully prised off until nearing the florets. The whole floral bud was then scrubbed with a solution of 0.5% NaOCl together with bacterial hand wash before removal from the stem which was replaced in the cold store for possible future use. The bud was surface sterilized in 0.3% NaOCl for 20min before rinsing with sterile distilled water. It was carefully dissected and individual florets excised with as much pedicel as possible. These were soaked for 15min in an anti-oxidant solution of ascorbic acid and citric acid together with the plant growth regulator TDZ which acts as a cytokinin. Florets were placed with the pedicel in Anderson's rhododendron media (Anderson, 1984) supplemented with IAA, 2iP and TDZ.

During the winter of 2008/9, dormant vegetative buds from fresh or stored shoots were treated as per the floral buds. Surplus cold stored shoots consisted of terminal buds or lateral buds after removal of the floral bud. A layer of outer scale leaves were removed before sterilization and a further layer removed before soaking. Buds were then placed in Anderson's media (Anderson, 1978) together with the plant growth regulators of before.

3 02 THE EFFECTS OF NUTRIENT DEFICIT AT SELECTED STAGES OF DEVELOPMENT ON ROOT AND SHOOT GROWTH

While the majority of rhododendron propagules have been successfully weaned off into glasshouse conditions there were a small number of accessions where the transition from controlled conditions *in vitro* has had limited success. This may be because most plants growing in their natural environment form symbiotic relationships with mycorrhizal fungi: the fungus benefits from a supply of carbohydrate from its host and in return supplies the plant with extra nutrients and water from the soil. This association may also be linked to plant health, through improved resistance to pathogens or ensuring a plants' fitness to its environment (Smith & Read, 1997). Work was undertaken to explore the impact of selected mycorrhiza on improving the establishment success of rhododendrons.

Two Rhododendron species, *R.concinnoides* and *R. phaedropum*, were chosen which had previously shown poor seedling establishment. Seeds were sterilized in a 0.3% NaOCl solution for 20min before rinsing with sterile distilled water and placement on sugarless Anderson's Rhododendron media (Anderson, 1975). They were germinated in a controlled environment at 22°C under fluorescent lights with 16hrs/day illumination (24µmol/m²/sec. PAR). After 24 days the germinated seeds were laid onto moist peat in sterile lidded containers, 15 seedlings per pot, and returned to the controlled environment.

Solutions of two ericoid mycorrhiza sourced from CABI European Regional Centre (Bakeham Lane, Egham, Surrey. UK), *Hymenoscyphus ericae* and *Oidiodendron maius* (Plates 1, 2), were prepared from freeze dried cultures as directed for later inoculation onto the seedlings. After 13 weeks growth, individual seedlings of the two

rhododendron species were transplanted into a 70:30 mix of peat and fine bark in P84 modules. The growing media also compared two fertilizer regimes (Osmocote Exact Mini and Osmocote Start) against a fertilizer free control. Five seedlings were used for each treatment, there being 18 treatments in total. 10ml of the prepared mycorrhizal inocula or distilled water control was then pipetted into individual modules. These were placed in a propagator under similar growing conditions and gradually acclimatised to glasshouse conditions. Plants were watered and misted as required and 2ml diluted Maxicrop applied to all fertilized modules on four occasions. Seedlings were harvested 16 weeks after inoculation.

Plate 1. *Hymenoscyphus ericae*
(McClymont, 2008).

Plate 2. *Oidiodendron maius*
(McClymont, 2008).

3 03 CONSERVATION THROUGH MICROPROPAGATION OF OTHER GENERA

The conservation through micropropagation of other threatened species has had limited success, due to the difficulty of creating suitable culture formulations. Magnolia and Camellia were the first 'other genera' to be investigated for protocol development due to their observed susceptibility to *Phytophthora ramorum/kernoviae*. (http://www.defra.gov.uk/planth/newsitems/suscept.pdf).

MAGNOLIA: Initiation and Proliferation. Early work in 2006 focussed on micropropagating from summer water shoots. Shoots were collected and rinsed under running water for 20min before sterilization in 0.15% NaOCl solution and placement in a nutrient jelly of half strength Murashige and Skoog (1962) together with plant growth regulators BAP(0.5mg/l) and IAA(0.05mg/l).

Dormant winter buds from both water shoots and floral buds were collected during winter 2008/2009 and, if not used immediately, cold stored as before. The dormant magnolia bud, whether floral or vegetative, has successive layers of covering tissue (perules) separated by a short length of stem. A similar decontamination technique as used for the Rhododendron floral buds was applied. The exterior covering was treated with a harsh decontamination regime of surface scrubbing with 0.5% NaOCl and antibacterial hand wash before rinsing and washing in a 0.3% NaOCl solution for 20min. The outer perule was carefully opened to expose a clean inner bud and perule covering. This was dissected out and placed on half strength Murashige and Skoog (1962) media together with the plant growth regulators as previously BAP(0.5mg/l) and IAA(0.05mg/l).

Rooting. Shoots from the earlier proliferating buds were placed in the rooting media devised by Chaidaroon et.al.(2004), with the addition of the plant growth regulator IBA(4mg/l). An indirect method of rooting may be by micrografting. This is a technique that has been used successfully for other genera, such as Citrus sp. (Navarro, 1988). Seeds which will form the rootstock are germinated in sterile conditions, in order to confirm their disease free status. When these are large enough the seedling is decapitated and micropropagated shoots forming the scion material are placed on the decapitated tip.

To explore the technique of micrografting, a range of magnolia seeds were therefore collected. These were cleaned, sterilized in 0.3% NaOCL for 20min, rinsed, and placed on tissue in a sealed polythene bag in a cold store for 8 weeks as part of the dormancy breaking regime. They were sterilized further in 0.35% NaOCl for 30min prior to planting in sterile vermiculite, damp peat or sugar free Anderson's (1975) ericaceous media.

CAMELLIA: These are notoriously difficult to regenerate in micropropagation conditions. Young shoots were taken from old Camellias growing *in situ*, and surface sterilized with 0.3% NaOCl for 20min. These were placed in a range of nutrient media including Murashige and Skoog (1962), Samartin and Gonzalez (1986) and Vieitez *et al.(*1989). In 2008, young shoots from camellias grown commercially under protected conditions were prepared as before and again placed in a range of nutrient media.

Many 'old' gardens also contain rare, champion or unique varieties of other trees and shrubs that are susceptible, more specifically two other related genera were investigated for their potential to regenerate under micropropagation conditions.

OAK: Dormant woody stems of Quercus sp. were soaked in 0.1% NaOCl solution for 30min, before rinsing and air drying. They were stood in small jars of flower food at an average temperature of 25°C under artificial lights until shoots emerged. These were removed and sterilized in 0.15% NaOCl solution for 15min, rinsed and placed on the media of Vieitez et al, (1985).

CONIFER: Shoots of *Matasequoia glyptostroboides, Arthrotaxis cupressoides*, *A. laxifolia* and *Taiwania cryptomeriodes* were collected after the young growth had developed and sterilized with 0.2% NaOCl for 20min before placing in half strength Murushige and Skoog (1962) media together with plant growth regulators BAP and IAA as before.

3 04 EVALUATION OF PERFORMANCE OF CLONED RHODODENDRON MATERIAL ON RETURN TO ORIGINAL HABITAT LOCATION

Cloned material at different stages of growth development has been returned to four gardens for growing on. In September 2007, 280 weaned propagules of Rhododendron cultivars 'Morvah', 'Johnnie Johnston' and 'Creek's Cross' were produced; a quantity of these cultivars were returned to the original garden in February 2009 for replanting. Nine different liner sized accessions and 18 accessions in large cell modular trays were returned to a second garden in September 2007 for growing on. Larger plants have been planted out around the gardens (April 2009). Four rhododendron accessions in P24 modules totalling 77 plantlets were returned to a third garden (October 2008) to be grown on. One accession from a fourth garden, totalling 15 modules has also been returned.

4. RESULTS

4 01 GENERATION OF PATHOGEN FREE MATERIAL

A successful result was indicated by both the absence of fungal, bacterial or algal contamination of the nutrient jelly and the initiation of shoots. If contamination was present it would be visible within a three week period for young vegetative shoots but might take up to two months to appear with other plant tissues due to the slow expansion of the petals/scale leaves.

The progression of research techniques developed since the Final Report PH0316 (Conservation of rare plant species threatened by *Phytophthora ramorum* in SW England) has improved decontamination rates considerably. The use of developing floral buds illustrated in plate 3 produced a high level of success (65%) in its own right, but if dormant vegetative buds are processed simultaneously, the success rate increases considerably (95%; Table 2). It has been found that stored material can be processed successfully up to 3 months after receipt, provided that there is no moisture present to encourage fungal and bacterial development. This has enabled accessions to be processed on further occasions until the decontamination regime is successful.

It was observed that vegetative bud regeneration occurred from the excised base and further regeneration could occur if, after approximately 6 weeks, individual scale leaves were pulled away and transferred into more jelly (Plate 4). This could be a useful technique where plant material is limited in quantity or floral tissue not available. From one garden site, of the 33 accessions processed in January 2009, 32 are in propagation from floral or vegetative buds or both. Similarly, 49 of the 52 accessions received from another garden are in culture.

Table 2. Decontamination rates using a range of Rhododendron propagation material

Tissue growth stage	Accessions processed	Successful sterile cultures	Success (%)
Bud burst-April/May (2004)	25	4	16
Young growth (2004)	25	5	20
Mature shoots (2004)	3	0	0
Floral buds (2005-8)	380	247	65
Floral & vegetative buds combined (2009)	85	81	95

Plate 3. Florets with developing shoots.

Plate 4. Vegetative bud with developing shoots.

4 02 ROOT AND SHOOT NUTRIENT SUPPLY

Analysis of above ground fresh material of *R. concinnoides* produced significant results (Figure 1), not only with regard to mycorrhizal inoculation, but specifically with the plants reaction to inocululation under different fertilizer regimes. Increased levels of fertilizer inhibited plant growth in uninoculated controls of *R. concinnoides*, supporting the findings of Moore-Parkhurst & Englander (1982). Similar overall results were achieved with *R. phaedropum*. Both mycorrhizal strains conferred a beneficial effect on the fresh weight of the host plants under increasing levels of fertilizer application.

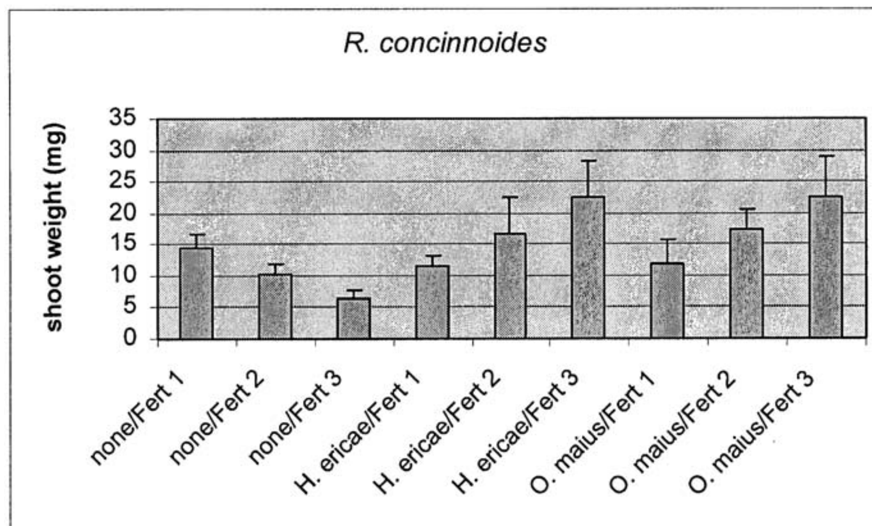

Figure 1. Fresh weight of *R. concinnoides* seedling shoots 16 weeks after mycorrhizal inoculation under 3 different fertilizer regimes (McClymont, 2008).

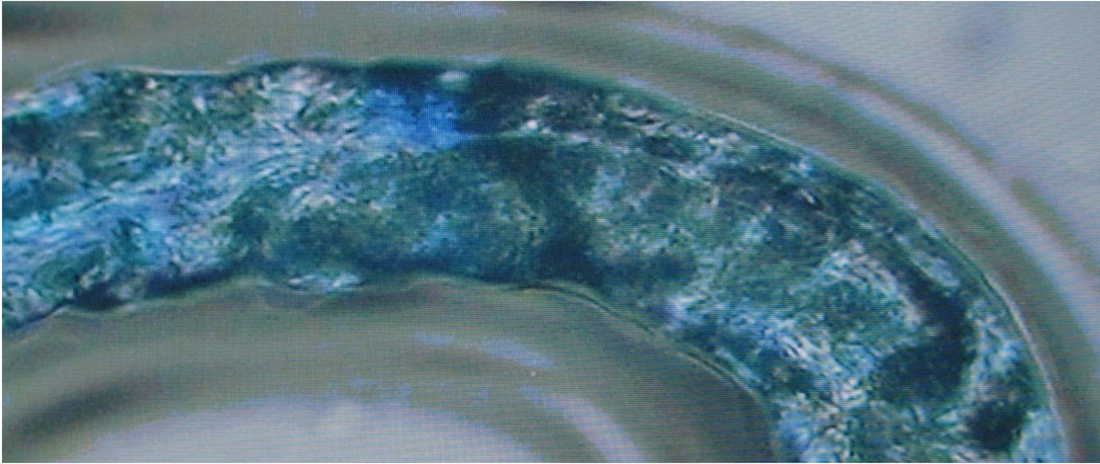

Plate 5. Detail of hair root of *R. phaedropum* seedling showing colonisation by *H. ericae* x400 (McClymont, 2008).

4 03 CONSERVATION OF OTHER GENERA

MAGNOLIA: Initiation and Proliferation. The use of summer water shoots for initiation in sterile culture was partially successful. Of the ten species/cultivars investigated 8 were initially successfully decontaminated. After further transfers only four have continued to develop and proliferate, producing shoot clusters which have been divided and proliferated many times. Unfortunately, using this technique, many buds were processed before sterilization was successful.

Winter buds were available over a longer period of time and fewer were needed before decontamination was successful. There is the added advantage that with careful observation, any cultures initiating contaminants after sterilization can have clean tissue successfully removed without the complete loss of the culture, due to the successive layers of tissue present at this time of year. As with the Rhododendron floral buds, however, there is a point of development where the perule begins to loosen and decontamination is unsuccessful.

Rooting: This has not been successful yet using some of the proliferated shoots originating from summer water shoots. Attempts to germinate a range of Magnolia seedlings in sterile culture have so far been unsuccessful due to the difficulty of sterilizing the seed and to a greater extent the notorious difficulty of breaking seed dormancy. Communications with Cornish Magnolia growers have indicated that preference would be given to directly rooted material rather than grafted because of the risk of the scion snapping off from the rootstock in windy conditions.

CAMELLIA: Shoots collected from old gardens were not successfully decontaminated without killing the tissue. Soft tip growth from protected material was successfully decontaminated but could only be persuaded to develop a little more extension growth, before necrosis eventually occurred.

OAK: The samples of *Quercus sp*. were successfully decontaminated, but unfortunately could not be maintained in culture for longer than three weeks.

CONIFER: Samples of Arthrotaxus sp. were successfully decontaminated and began to proliferate over several months, but then gradually declined before a suitable maintenance formula could be determined.

4 04 PERFORMANCE OF REINTRODUCED CLONED MATERIAL

It is too early to give a conclusive evaluation of the performance of micropropagated Rhododendrons. Clones have only begun to be reinstated recently (spring, 2009). Cloned material can be checked for its 'trueness to type'. One plant of R. 'Johnnie Johnston' has flowered undercover and proved to be a true clone of the original. A cloned R. decorum at Duchy College, grown on as a stock plant from floral tissue started in February 2006, flowered in April 2009 and is identical to the original.

There has been difficulty in one garden with the maintenance of propagules on their transfer from modules to larger pots with the consequence of few surviving. In September 2008 replacement plants were grown on in 1L pots, short term, in secure conditions. In March 2009 these were transferred to Duchy College and it is anticipated that some will be returned in autumn as robust plants for reintroduction or sale at the host garden.

Where good facilities and expertise are present, no difficulties have been encountered with returned material. A propagation centre, has taken rooted propagules in jelly and has successfully and routinely grown on cloned material for reintroduction once sufficiently mature to selected host gardens.

5. MAIN IMPLICATIONS OF THE WORK

5 01 GENERATION OF PATHOGEN FREE RHODODENDRON MATERIAL

- A high level of successful decontamination of rhododendron material can be achieved using a combination of dormant floral and vegetative buds.

5 02 ROOT AND SHOOT NUTRIENT SUPPLY

- The addition of selected ericoid mycorrhiza can have a beneficial effect on the establishment of rhododendron seedlings.

5 03 CONSERVATION OF OTHER GENERA

- Vegetative magnolia buds can be successfully decontaminated with subsequent proliferation. Rooting has not yet been achieved.
- Protocols have been developed for the successful decontamination of Camellia, Oak and Conifer shoots but suitable formulations for tissue development have not been identified.

5 04 PERFORMANCE OF REINTRODUCED CLONED MATERIAL

- The performance of reintroduced cloned material has not yet been evaluated because micropropagated Rhododendron material has only matured enough for reintroduction during spring 2009.

6. POSSIBLE FUTURE WORK

- Investigate whether there is an optimum stage in the development of the Rhododendron floral bud that results in maximum shoot production under micropropagation.
- Continue research of **5** 02 and investigate the use of mycorrhiza to enhance the growth and establishment of cloned Rhododendron propagules after weaning.
- Investigate the use of micrografting *in vitro* as a successful technique to root Magnolias.
- Extend the conservation of rare species to more parks and gardens within the UK.
- Continue to evaluate the performance of reintroduced cloned material.
- Develop protocols for more rare or ‘at risk’ plants.

7. IMPACT OF PH0408

Extension of micropropagation from Cornish gardens

There have been no new Cornish gardens requiring plant micropropagation, but four current client gardens have sent more material to be processed, the latter two as a result of the identification of additional historically important species and cultivars in their collections. There will be a number of new accessions from a fifth garden next year following an extensive Rhododendron identification programme taking place this year.

Extension of micropropagation as a conservation tool for Rhododendrons from gardens outside Cornwall.

- Following the formation of a propagation centre (by one garden body) that enables the growing on of micropropagated modules there, forty accessions have been received from that garden for micropropagation and return.
- After being approached as a possible source of funding, one Hampshire garden has funded the micropropagation of 20 of their most difficult to root rhododendrons.

- There are 30 accessions from a Staffordshire garden in micropropagation including one from a positive *Phytophthora spp.* test.
- Following on from the successful micropropagation of important material from one Cornish garden, 52 historically important rhododendron accessions identified from another garden that forms part of a restoration project have been received for micropropagation.
- Contact with the RHS Rhododendron, Camellia and Magnolia group has created funding for 13 rare hybrids to be micropropagated. This information was written up in the RHS Garden magazine. As a consequence there are 25 hybrids from a Scottish collection in micropropagation, plus two accessions from another Scottish Garden.
- Rhododendron plant material will be arriving later this year/next spring from additional Scottish and Welsh collections.

8. ADDITIONAL INFORMATION RELEVANT TO REPORT

Rhododendron micropropagation:

Cornish gardens – 16 gardens.

Non-Cornish gardens – 7 gardens.

A total of 540 accessions have been processed since the start of the conservation programme in August 2004 with an overall propagation success rate of 82.5%

Magnolia micropropagation:

From 6 gardens, a total of 21 accessions have been processed, with 9 species and cultivars in propagation.

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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.

PAPERS

Smith,R. and **Mulholland,B.** Use of Micropropagation for the Conservation of Rare Cornish Garden Plants at Risk from *Phytophthora ramorum*. Proc. Intl. Plant Prop. Soc. Vol. 58 (2008) – in press.

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Telegraph Gardening supplement – Saturday 21st March 2009.

Micropropagation work **filmed** for BBC Wales series 'A Year at Bodnant' – March 2009.

PRESENTATIONS and conference attendance

Paper presentation at International Plant Propagators Society, GB & Ireland Conference at Falmouth in October 2008.

Poster presentation at IPPS annual conference in Belgium, September 2006.

Presentation of research made to stakeholders meeting organised by Defra and held at Duchy College in September 2006.

Mulholland B.J. and Smith R. (2008) Knowledge Transfer and Heritage Plant Conservation. Invited presentation. Growing Success: Horticulture Business Perspectives, SCI Conference, London.

Attendance at Rhododendron Species Conservation Group Conference, at Helensburgh, Scotland, April 2009.

Attendance at Defra *Phytophthora* Workshop, Defra Innovation Centre, Reading UK. 5th March 2009.