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Contents

Page No.

Grower Summary 1

Science Section

Introduction 6

Materials and Methods 8

Results and Discussion 12

Conclusions 18

References 19

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Grower summary

Headline

Molecular techniques, previously developed and used successfully for the detection of 'latent' downy mildew in distorted roots of red beet, have been successfully used for the detection of downy mildews in ornamentals, particularly impatiens (*Plasmopara obducens*) and pansy (*Peronspora violae*). In addition, early tests with seed samples of various ornamental species susceptible to downy mildew, whilst interesting, require further validation.

Background and expected deliverables

Downy mildew of impatiens, caused by a *Plasmopara* sp., was first reported in the UK in June 2003 causing considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. The identity of the fungus was subsequently confirmed as *P. obducens.* Emergency statutory action was immediately taken by the Plant Health & Seeds Inspectorate (PHSI) under Article 22 of the Plant Health Order 1993 and the downy mildew pathogen on impatiens was declared notifiable. This emergency legislation required the industry to notify PHSI of any suspect cases of the disease and, where the pathogen was confirmed, statutory action was taken to destroy the infected plants and to quarantine (for a pre-determined time period) the remaining apparently disease-free stock. If the pathogen was subsequently found to have spread to adjacent stock this would also be destroyed. The cost of such crop loss had to be met by the individual grower, as government compensation for crop loss was not available. Subsequently, a cost-benefit analysis of management options for the control of impatiens downy mildew was undertaken in which it concluded: "the most cost-effective option for government would be for the industry to police itself in the future as regards this disease". This was a view supported by the industry and in

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May 2005, Defra announced that statutory action would no longer be taken and that the industry would take on responsibility for management of the disease through implementation of an industry code of practice (Good Horticultural Practice (GHP)) that was produced by the HDC and NFU and distributed to industry in 2005.

The pathogen is reported to occur in North America and parts of Asia and Europe, including Denmark, Finland, the Netherlands, Germany the Czech Republic and Lithuania in the EU and Romania and Russia in the rest of the EPPO region, and may be much more widespread than reported. The full extent of its distribution in the UK is not known and, whilst it has not yet been found, it may also occur on natural relatives in the wild. Commercial trade of impatiens, valued at around £40M/annum, is complex and involves seed houses, specialist propagators, including for cutting-raised material (often overseas), growers, the retail trade including garden centres & mail order enterprises and municipal production for park displays etc (S. Coutts, personal communication). This is highly relevant and important with respect to the potential for pathogen introduction, subsequent disease epidemiology and the future successful implementation of various control measures.

In the UK outbreaks in 2003, *Impatiens* raised from seed and/or vegetative transplants may have originally become infected through seed transmission, or, later via air-borne infection of seedlings, or as older plants, either under glass or outdoors. No over-wintering spores have been observed in outbreaks in the UK but these have been observed in harvested seed of *Impatiens* spp. in India. Given the potential to over-winter as oospores, and infect through seed or by infecting plants directly, establishment of this disease in outdoor plantings is possible. Infected seed is reported to give rise to systemically infected plants and a long latent period between infection and appearance of symptoms has also been observed. This could lead to long-distance spread of the disease in plants that appear disease-free, as well as with consignments of seed.

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Fortunately, for the bedding plant industry at least, occurrences of impatiens downy mildew have been scarce within Europe over the last two years. In order to ensure progress could be maintained within this project, it was proposed to use downy mildew of pansy (*Peronospora violae*) as a model test system for the development of the culturing techniques and detection methods as this disease is endemic within UK grown pansies and continues to cause considerable losses and requires prophylactic fungicide application for control. Because downy mildews are host specific pathogens, the detection methods developed for pansy ought to be entirely applicable for use on impatiens.

In order for the industry to take responsibility for the overall management of downy mildew, it is necessary to have at their disposal effective techniques for the early (latent) detection of the pathogen on both seed and young propagation material and also a range of approved crop protection products with demonstrated activity against *Plasmopara obducens*, *Peronospora violae* and other downy mildew fungi in ornamental crops. Any recommended spray programme must also take due regard of the resistance risk with certain pathogen groups e.g. phenylamides and ensure a range of products with different modes of action is available to minimise the selection of tolerant and/or resistant strains in the pathogen population. Baseline sensitivity data would be valuable in this regard.

There are 3 main aims to this project:

1. To develop a sensitive molecular method for detection of downy mildews, especially *Plasmopara obducens* and *P. violae* in seed/young plants.
2. To evaluate the efficacy and safety of a range of fungicides for control of the disease using an integrated strategy to minimise resistance risk.
3. To develop an effective integrated GFP strategy which minimises the resistance risk and which can be quickly adopted by seed-houses, plug raisers, growers and commercial end-users of plants

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Summary of the project and main conclusions

Outbreak monitoring and isolate collection

A range of isolates from different pansy and impatiens varieties have been collected during the first year of the project. A proportion of each batch of the infected material was frozen as a potential source of inoculum for use later in the project. The remaining material was used to carry out infection studies.

Spore germination

Studies tested the effect of different spore treatments (freezing, washing and presence of leaf material) on germination of *P. violae* at a wide range of temperatures. Very low levels of germination (0-2%) were observed and no conclusions could be drawn on the effects of temperature and spore treatments. Experimental work to develop robust protocols for induction of spore germination is continuing.

Plant infection

A number of different methods have been tested for establishing uniform and consistent infections on host plants (required in order to carry out efficacy testing of fungicides). Currently, none of the methods used has been successful in producing plants infected by either *Peronospora violae* or *Plasmopara obducens*. To date, the work has been carried out solely under laboratory conditions, which may be less favourable for plant infection i.e. too dry compared to the glasshouse environment. Future infection studies will be conducted in glasshouses/poly tunnels to try to provide conditions more favourable to disease development.

Detection of Peronospora violae and Plasmopara obducens

Leaf material

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A number of pansy and impatiens leaf samples with symptoms of downy mildew were analysed using a primer set and probe developed in a previous project to detect *Peronospora* species on red beet (FV 228). The results of the analysis indicated that DNA of both *Peronospora violae* and *Plasmopara obducens* could be reliably in infected leaf material.

Seed

A total of 62 seed samples (50 pansy, 9 nicotiana, 2 wallflower and 1 antirrhinum) were analysed using the *Peronospora* primer set and probe. Of the seed samples tested, 60% gave a result which indicated the possible presence of downy mildew pathogens on/in the seed. It is important to note that these are preliminary results in the assay validation process and, as such, it is too early to use the data as confirmation of seed-borne downy mildew. Further verification of the specificity of the molecular assay is being carried out.

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Financial benefits

It is too early to predict the likely financial benefits from this project. However, the pathogens responsible for downy mildew are aggressive and, under favourable environmental conditions, can cause significant economic losses. Therefore, if the project is successful and is able to help reduce the risk from the disease it will be of considerable economic benefit to growers.

Action Points for Growers

- Check susceptible crops regularly and where infected plants are found remove them immediately by carefully placing them in a plastic bag *in situ* to avoid dispersing spores to other plants.
- Ensure adequate air circulation around plants to minimise prolonged periods of leaf wetness by better spacing and by increasing the ventilation in the glasshouse. Additionally, if possible, avoid overhead watering as this is likely to aggravate the disease. If it is necessary to water from overhead then try and do this early, on days when solar radiation levels will ensure the leaves have a chance to dry out quickly.
- Remove leaf and other plant debris at end of season to minimise the risk of carry-over of the disease and maintain effective weed control in and around the growing areas.
- Where infected material is found notify the project team and, if possible, submit a sample for R&D purposes.
- Maintain an effective fungicide programme on the crop, ensuring a range of products with different modes of action are included to minimise the risk of resistance development. Consider also the need for fungicides active against other important pathogens e.g. black root rot (*Thielaviopsis basicola*) & leaf-spot (*Ramularia* spp.).

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- Refer to the HDC/NFU Code of Practice – Grower guidelines for the prevention of Impatiens Downy Mildew *(Plasmopara obducens)* and the HDC factsheet published in March 2004, 05/04 Impatiens downy mildew for further information.

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Science Section

Detection and control of downy mildews on ornamentals

Introduction

Downy mildew of impatiens, caused by a *Plasmopara* sp., was first reported in the UK in June 2003 (McPherson & Finlay, personal communication) causing considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. The identity of the fungus was subsequently confirmed as *P. obducens* by CSL (Lane *et al*, 2004). Emergency statutory action was immediately taken by the Plant Health & Seeds Inspectorate (PHSI) under Article 22 of the Plant Health Order 1993 and the downy mildew pathogen on impatiens was declared notifiable. This emergency legislation required the industry to notify PHSI of any suspect cases of the disease and, where the pathogen was confirmed, statutory action was taken to destroy the infected plants and to quarantine (for a pre-determined time period) the remaining apparently disease-free stock. If the pathogen was subsequently found to have spread to adjacent stock this would also be destroyed. The cost of such crop loss had to be met by the individual grower, as government compensation for crop loss was not available. A cost-benefit analysis of management options for the control of impatiens downy mildew has been undertaken (Jones, 2004) in which it concludes: "the most cost-effective option for government would be for the industry to police itself in the future as regards this disease". This was a view supported by the industry and in May 2005, Defra announced that statutory action would no longer be taken and that the industry would take on responsibility for management of the disease through implementation of an industry code of practice (Good Horticultural Practice (GHP)).

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The pathogen is reported to occur in North America and parts of Asia and Europe, including Denmark, Finland, the Netherlands, Germany the Czech Republic and Lithuania in the EU and Romania and Russia in the rest of the EPPO region, and may be much more widespread than reported. The full extent of its distribution in the UK is not clear as it may also occur on natural relatives in the wild; however, in limited observations to date no such findings have been made. Commercial trade of impatiens, valued at around £40M/annum, is complex and involves seed houses, specialist propagators (often overseas), growers, the retail trade including garden centres & mail order enterprises and municipal production for park displays etc (S. Coutts, personal communication). This is highly relevant and important with respect to the potential for pathogen introduction, subsequent disease epidemiology and the future successful implementation of various control measures.

In the UK outbreaks in 2003, *Impatiens* raised from seed or as imported vegetative transplants may have been infected either through seed transmission, or, could have become infected as seedlings, or as older plants, either under glass or outdoors. No over-wintering spores (oospores) have been observed in disease outbreaks in the UK though they have been reported in harvested seed of *Impatiens* spp. in India. Given the potential to over-winter as oospores, and infect through seed or by infecting plants directly, establishment of this disease in outdoor plantings is possible. Infected seed is reported to give rise to systemically infected plants and a long latent period between infection and appearance of symptoms has also been observed. This could lead to long-distance spread of the disease in plants that appear disease-free on delivery, as well as via potential contamination in consignments of seed.

Occurrences of impatiens downy mildew have been scarce within Europe over the last two years and in order to ensure progress could be maintained within the project it was proposed to use another host-specific downy mildew on an ornamental crop instead. Downy mildew of pansy (*Peronospora violae*) was

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accepted as an alternative model test system as this disease is endemic within UK grown pansies and continues to cause considerable economic losses requiring prophylactic fungicide application for control. Because downy mildews are host specific, the detection methods developed for pansy should hopefully be applicable for use with a *Plasmopara* species on impatiens, providing the PCR primers developed are capable of detecting a broad range of downy mildew species.

In order for the industry to take responsibility for the overall management of downy mildew, it will be necessary to have at their disposal effective techniques for the early (latent) detection of the pathogen on both seed and young propagation material and also a range of approved crop protection products with demonstrated activity against *Plasmopara obducens*, *Peronospora violae* and other downy mildew fungi in ornamental crops such as Nicotiana & Antirrhinum. Any recommended spray programme must also take due regard of the resistance risk with certain pathogen groups e.g. phenylamides and ensure a range of products with different modes of action is available to minimise the selection of tolerant and/or resistant strains in the pathogen population. Baseline sensitivity data would be valuable in this regard.

The primary aims of the proposed project are to investigate techniques for the early detection and control of downy mildew in order to provide the industry with appropriate tools for effective disease management. The objectives will be to develop a sensitive molecular method for detection of the downy mildew fungus in seed/young plants and to evaluate the efficacy and safety of a range of fungicides for control of the disease using an integrated strategy to minimise resistance risk.

Materials and Methods

Outbreak monitoring and isolate collection

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To ensure sufficient isolates of *Peronospora violae* and *Plasmopara obducens* were available to the project the industry was monitored for the development of outbreaks; this was done mainly via the diagnostic clinics at the Central Science Laboratory (CSL) and Stockbridge Technology Centre (STC). The first case of impatiens downy mildew in the UK for a number of years was detected in May 2007 and an HDC alert was produced which included a request for further infected plant material to be sent to either CSL or STC. This resulted in a number of additional samples being received for pathogen isolation and storage.

Isolate maintenance

Storage

From each infected pansy or impatiens sample that arrived at the laboratory, up to 10 infected leaves (depending on the level of infection) were removed and placed in a 50 mL centrifuge tube which was labelled with the date, plant type, variety/colour (if known) and origin of infected material. The plant material was then stored at -25°C until required. Freezing was chosen as the method of storage as it has proven successful with other downy mildew pathogens, e.g. *Bremia lactucae* on lettuce.

Germination studies

Germination studies were carried out on *P. violae* isolates to determine whether freezing was likely to be a viable option for storage of downy mildew isolates. Spores on infected leaf material were subjected to four different treatments prior to the germination tests: (i) leaves were frozen at -25°C for 3 weeks and then washed from the leaves, (ii) fresh spores were washed directly from leaves, (iii) fresh spores were washed direct from leaves and washed twice by centrifugation at 1000g for 150 seconds and (iv) spores were washed direct from leaves and a piece of fresh healthy leaf placed in with the spore suspension. Each spore suspension was divided into five aliquots of 1 mL and one aliquot placed at 0, 5,

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10, 15, 20 or 30°C. Levels of spore germination were measured after 24 and 48 hours by assessing 50 spores either for the production of a germ tube or release/differentiation of zoospores.

Plant infection

P. violae and *P. obducens* are obligate plant pathogens and as such cannot be cultured on substrates other than host plant material. Therefore in order to bulk-up inoculum to carry out future fungicide efficacy testing, methods were required which produced uniform and consistent infection of pansy and impatiens plants by the specific downy mildew pathogens. Reviews of the literature did not reveal any published methods for the artificial inoculation and infection of either pansies or impatiens with *P. violae* or *P. obducens* respectively. However, methods were cited for other downy mildews, including those found on basil [*Peronospora* sp. (Garibaldi *et al*, 2007)] and roses [*Peronospora sparsa* (Aegerter *et al*, 2003)]. Based on the methodologies described for infection of basil and rose, a range of methods, primarily based on duration of leaf wetness and temperature, were developed for the infection of pansy and impatiens plants.

Plants were inoculated using one of four methods: (i) spores were washed from diseased leaves and the resulting spore suspension sprayed directly onto both abaxial (lower) and adaxial (upper) leaf surfaces (spore concentrations applied varied between 10^4-10^5 spores mL^{-1}, with approximately 2.5 mL of spore suspension applied to each plant), (ii) spores were washed directly from infected leaves and then the spores washed twice (by centrifugation at 1000g) prior to inoculation onto the plant, (iii) spores were by tapped directly from infected leaves onto a wet leaf, and (iv) spores were tapped directly from infected leaves onto a dry leaf surface and the plant immediately misted with water. Following inoculation, plants were placed into moisture chambers (Figure 1) and the duration of leaf wetness maintained for 2, 4, 6 or 18 hours. Plants were removed from the

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chambers following the required duration of leaf wetness and the leaves allowed to air dry.

Plants were incubated at temperatures between 5 and 20°C and examined daily over a period of four weeks for development of downy mildew symptoms. In an additional set of experiments on impatiens, plants were replaced in the moist chamber each evening to produce alternate periods of dry and wet and the temperature varied to give a day temperature of 20°C and a night temperature of 14°C.

Figure 1. Moisture chamber containing a pansy plant inoculated with a spore suspension of *P. violae*.

Detection of *Peronospora violae* and *Plasmopara obducens*

The PCR primer set and probe developed in a previous project for detection of downy mildew (*Peronospora farinosa* f.sp. *betae*) in red beet (FV 228) was examined to determine whether they would be suitable for the detection of *Peronospora violae* and *Plasmopara obducens*. The primers were tested and validated using leaves and seed from pansies, and leaves from impatiens. In

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addition seed from cheiranthus, nicotiana and antirrhinum, suspected of being infested with *Peronospora parasitica*, *Peronospora tabacina* and *Peronospora antirrhini* respectively, were also tested.

DNA extraction

DNA was extracted from both leaves and seed using the Wizard Magentic DNA purification system for food, in combination with CTAB buffer and a Kingfisher ML machine.

Leaves

Impatiens and pansy leaves collected as described in Section 2.1 were used to test and validate the PCR assay. DNA was extracted as follows.

For each sample 2-3 infected leaves were placed in an appropriately labelled double mesh grinding bag and 4 mL of CTAB soil extraction lysis buffer added. An additional grinding bag was prepared, using healthy leaves, as an extraction negative control. The sample, and negative control, was ground for approximately 15 seconds using a Homex flat bed grinder and the resulting liquid transferred into a 2 mL centrifuge tube. The lysate was separated from the debris by centrifugation at 10,000 rpm for 5 minutes. 1mL of the lysate was transferred to a fresh 2 mL centrifuge tube, which contained 250 µL Buffer B (Wizard Magnetic DNA purification system for food) and 750 µL of precipitation solution (Wizard kit). The tube was briefly vortex mixed and centrifuged at 13,000 rpm for 10 minutes. 750 µL of the supernatant was transferred to a clean 2 mL cenrifuge tube and 50 µL of Magensil beads (Wizard kit) and 600 µL of isopropanol added. The sample was vortex mixed and incubated for 5 minutes at room temperature with occasional mixing by inverting the tube. DNA was retrieved using the 'gDNA' program on the Kingfisher with an incubation period of 5 minutes at 65ºC. DNA could then either be kept at 4ºC for immediate use or stored at -25ºC for later use.

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Seed

Pansy seed lots (ranging in variety and flower colour) were obtained from several different suppliers and tested for the presence of *P. violae*. In addition, a number of wallflower, nicotiana and antirrhinum seed samples were also collected and tested for the presence of *Peronospora* species (*P. tabacina*, *P. parasitica* and *P. antirrhini* respectively).

For each batch of pansy seed, 0.2 g (approximately 130 seeds) was weighed into an appropriately labelled 5 mL capped transport tube containing one 7/16 inch ball bearing and 2 mL of CTAB soil extraction lysis buffer (see appendix I), with 2% antifoam B added. A second tube was prepared (minus the seed) as an extraction negative control. The sample, and negative control, was ground for 2 min using a Kleco 96 grinding machine; if required a further 2 mL of lysis buffer (with antifoam) was added and the sample ground for an extra 30 seconds. The ground sample and control was transferred from the 5 mL tubes to individual 2 mL centrifuge tubes, which contained 250 µL Buffer B (Wizard Magnetic DNA purification system for food) and 750 µL of precipitation solution (Wizard kit). From this point, the procedure used was as described in Section 2.3.1.1.

DNA analysis

The DNA was analysed using an Applied Biosystems 7900HT Fast Real-Time PCR machine in combination with the *Peronospora* specific primers and probe. The temperature cycle consisted of denaturation (50°C) for 2 min, annealing (95°C) for 10 min and 40 amplification cycles with cycles alternating between 95°C for 15 sec and 60 for 1 min.

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Results and Discussion

Outbreak monitoring and isolate collection

A panel of isolates from different pansy and impatiens varieties were collected during the first year of the project. A proportion of the infected leaves were frozen for later use, either to help validate detection of downy mildew using the *Peronospora* primer set and probe or to provide a source of inoculum for the fungicide work planned later in the project. The remainder of the infected plant material was used during the plant infection studies.

Isolate maintenance

Germination study

Germination tests were carried out on *P. violae* spores subjected to different treatments and incubated at a range of temperatures (Table 1). Low levels of germination (0-2%) were observed under all treatments and temperatures, thus giving no clear indication of the factors affecting germination or the suitability of freezing as a storage option. No spores were seen to differentiate to produce zoospores; those that did germinate appeared to produce appressoria (Figure 1a) or appressoria with an infection peg (Figure 1b).

Table 1. Effect of temperature and spore treatment on the percentage germination of *Peronospora violae* spores.

Spore treatment	Percent Germination at Different Temperatures (°C)					
	0	5	10	15	20	30
Frozen spores	0	0	0	0	0	0
Fresh spores	0	0	2	0	1	0
Washed fresh spores	0	0	0	0	0	0
Spores + leaf	0	0	2	2	0	0

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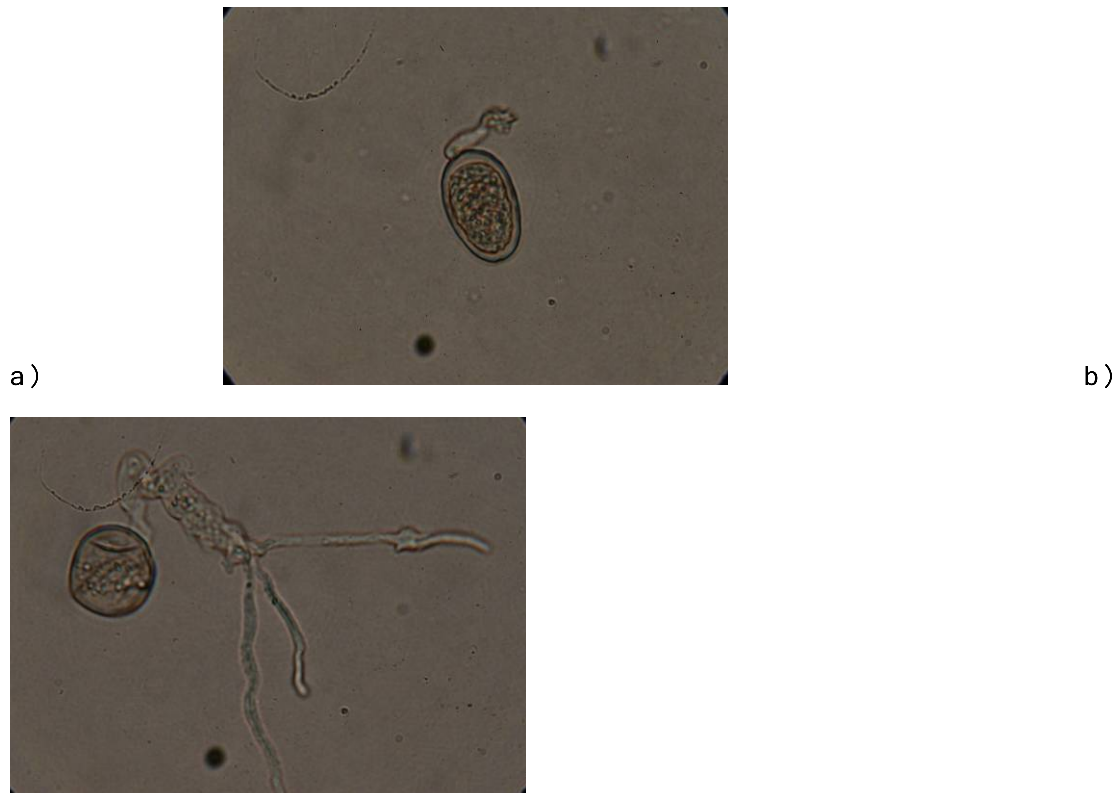
a)  b)

Figure 1. Germinating *Peronospora violae* spores showing structure resembling appressoria (a) and appressoria with infection peg (b).

As germination studies will form a major part of the fungicide sensitivity work proposed for later in the project, these results highlight the importance of identifying the key factors affecting spore germination. Further experimental work in this area has already started.

Plant infection

None of the methods used within the project to date have been successful in producing new infections in host plants. So far the work has been carried out solely under laboratory conditions, which may less favourable for plant infection, e.g. too dry, compared to the glasshouse environment. Future infection studies will be conducted in glasshouses/poly tunnels to try to provide conditions more favourable to disease development. There is some evidence that certain flower colours may be more susceptible to infection than others and there also may be some varietal specificity with mildew isolates. To explore this, a range of pansy varieties/colours

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are continually being grown in order to ensure that isolates collected in the future can be inoculated onto plants of the same colour or variety.

Detection of Peronospora violae and Plasmopara obducens

Leaves

Six pansy and five impatiens leaf samples, infected by *P. violae* and *P. obducens* respectively, were analysed using the primer set and probe developed to detect *Peronospora* species on red beet (Table 2) and the results presented as the mean cycle threshold (Ct) value. The Ct value represents the number of amplification cycles after which fluorescence, and therefore DNA, was detected above a background level. **The Ct value is inversely proportional to the amount of target DNA, that is the lower the Ct value the more target DNA is present in the sample.** A value of 40 (the maximum number of amplification cycles) indicates a negative result (that is either no DNA was present or that the DNA present was non target and was not be detected). The Ct values obtained for both downy mildew infected pansy and impatiens leaves indicated that the primer set was highly sensitive for the detection of both pathogens. The Ct values obtained for *P. violae* (14.8 to 18.6) were lower than those obtained for *P. obducens* (20.6 to 22.7) suggesting that either the primer set was more specific to *P. violae* or that there was more *P. violae* DNA present in the infected leaf material. As the original primer set was developed to detect *Peronospora* species it is more likely that the former explanation is correct.

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Table 2. Detection of *Peronospora violae* and *Plasmopara obducens* from infected pansy and impatiens leaves using the primer set and probe developed to detect *Peronospora* species.

Sample Description	Mean Ct value
Pansy 1	18.6
Pansy 2	14.8
Pansy 3	16.4
Pansy 4	17.3
Pansy 5	16.2
Pansy 6	17.3
Impatiens 1	20.6
Impatiens 2	22.7
Impatiens 3	20.7
Impatiens 4	20.8
Impatiens 5	21.4
+ve control	14.8
-ve control	40.0
Pansy mottle symptom	33.1

Leaves showing symptoms of pansy mottle syndrome (Ref. Coutts & Bragg, *Pers Comm.*) were also analysed using the *Peronospora* primer set, as it has been hypothesised (M McPherson, *Pers Comm.*) that these may be an atypical downy mildew symptom arising from a systemic, potentially seed-borne, source. Analysis of the mottled leaves gave a Ct value of 33.1 (Table 2). This Ct value perhaps indicates that the mottle symptoms were caused by *P. violae*, with the amount of DNA (and therefore target pathogen) being present in the leaves at levels lower than in samples where typical downy mildew symptoms had been expressed.

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However, this result must be regarded as a preliminary observation only and significantly more investigative work will be required to confirm this interesting result.

Seed

A total of 62 seed samples (50 pansy, 9 nicotiana, 2 wallflower and 1 antirrhinum) were analysed using the *Peronospora* primer set and probe (Table 3). In several cases the seed samples provided here were selected based on in observations of downy mildew infection in crops grown from the same seed batch. Of the seed samples tested, 60% gave a Ct value less than 40 possibly indicating the presence of *Peronospora* DNA in the seed sample. The Ct samples for all the positive samples were above 33, i.e. a relatively weak signal, suggesting that there was perhaps only a small amount of DNA present. It is important to note here that it is too early in the project to be able to fully interpret the meaning of these results. Further studies are currently being carried out to examine and quantify both the specificity and sensitivity of the test.

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Table 3. Detection of *Peronospora violae* from seed using the primer set and probe developed to detect *Peronospora* species in red beet.

Seed type	Sample	Mean Ct value	Seed type	Sample	Mean Ct value
Pansy	1	40.0	Pansy	32	36.1
Pansy	2	40.0	Pansy	33	40.0
Pansy	3	40.0	Pansy	34	35.8
Pansy	4	40.0	Pansy	35	40.0
Pansy	5	36.5	Pansy	36	37.9
Pansy	6	40.0	Pansy	37	40.0
Pansy	7	38.5	Pansy	38	40.0
Pansy	8	38.0	Pansy	39	40.0
Pansy	9	40.0	Pansy	40	40.0
Pansy	10	40.0	Pansy	41	37.3
Pansy	11	36.9	Pansy	42	39.6
Pansy	12	37.7	Pansy	43	40.0
Pansy	13	40.0	Pansy	44	40.0
Pansy	14	35.1	Pansy	45	34.5
Pansy	15	34.2	Pansy	46	26.5
Pansy	16	37.6	Pansy	47	36.7
Pansy	17	39.6	Pansy	48	35.4
Pansy	18	36.5	Pansy	49	38.6
Pansy	19	33.8	Pansy	50	37.7
Pansy	20	38.6	Wallflower	1	36.6
Pansy	21	35.7	Wallflower	2	36.9
Pansy	22	38.3	Nicotiana	1	38.5
Pansy	23	36.9	Nicotiana	2	37.4
Pansy	24	35.1	Nicotiana	3	40.0
Pansy	25	36.3	Nicotiana	4	40.0
Pansy	26	40.0	Nicotiana	5	40.0

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Pansy	27	40.0	Nicotiana	6	40.0
Pansy	28	39.3	Nicotiana	7	40.0
Pansy	29	37.2	Nicotiana	8	40.0
Pansy	30	33.9	Nicotiana	9	40.0
Pansy	31	36.5	Antirrhinum	1	38.3

Conclusions

- The selected PCR primers and probe were highly sensitive in the detection of both *Peronospora violae* and *Plasmopara obducens* in infected leaf material.
- Tests on seed from a range of different ornamental hosts produced Ct values of less than 40, indicating that low levels of downy mildew pathogen DNA may be present.
- In a single test on plants showing symptoms of pansy mottle syndrome, a positive Ct value was obtained, suggesting a possible causal relationship between downy mildew and pansy mottle symptoms. It is proposed that further work would be required outside the remit of this project to investigate this in more detail.

Future work

- Continue work to develop methods for infection of pansy or impatiens with *Peronospora violae* or *Plasmopara obducens*, including work on spore germination.
- Continue validation of assay for detection of downy mildew pathogens in seed, and determine whether it can detect latent infections in propagation material and module raised seedlings.
- Determine the sensitivity of isolates of *P. obducens* or *Peronospora violae* to selected fungicides using *in planta* tests.

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- Carry out full crop safety assessment on fungicides shown to be effective.
- Generate baseline sensitivity data for a range of isolates to the selected fungicides.
- Develop a robust GFP strategy for the management and control of downy mildew in bedding plants.

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Appendix 1

CTAB Buffer

120ml Sodium phosphate buffer pH8.0
2% Certyltrimethyl ammonium bromide (CTAB)
1.5M NaCl

Sodium phosphate buffer

1M Na_2HPO_4
1M NaH_2PO_4

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