Project title:	Detection and control of downy mildews on ornamentals
Project number:	PC230
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Key words: Downy mildew, *Plasmopara obducens*, *Peronospora violae*, pansy, seed, impatiens, detection, fungicides, crop safety, control.

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

AUTHENTICATION

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

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Headline

Criteria required for the infection of pansies by *Peronospora violae* have been identified, which will allow fungicide efficacy testing against downy mildew on pansies to take place. The previously developed PCR primers and probes have been shown to detect latent infections of downy mildew in pansy leaves and have indicated the presence of downy mildew DNA in some seed lots. Tests to date have not shown this DNA to be viable in terms of establishing plant infections.

Background and expected deliverables

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)).

According to the scientific literature, *P. obducens* 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. In reality, the disease is much more widespread than this and in 2008, also caused problems in South Africa, Australia and Japan. The full extent of its distribution in the UK is not clear as it may also occur on natural relatives e.g. *Impatiens noli-tangere* 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 civic displays in parks etc. (S. Coutts, personal communication). This is highly relevant and important with respect to the potential for pathogen introduction, subsequent disease spread and the future successful implementation of various control measures.

In the original UK outbreaks in 2003, it is suspected that *Impatiens* raised from seed or, more likely, as imported vegetative transplants (unrooted or rooted cuttings) may have provided the initial infection source of the disease, though this has not been confirmed. Over-wintering or resting spores (oospores) of the pathogen have previously been reported on/in seed in India, but their absence in early UK outbreaks indicated perhaps that the pathogens potential to survive over-winter under UK conditions was low. However, in 2008 the situation in the UK changed considerably when resting spores were found in infected stem tissues of *I. walleriana* for the first time. Given this new finding, the potential risk of carry-over between seasons on plant debris incorporated into the soil has increased significantly. The potential for seed-borne transmission via this route is also potentially increased though it must be emphasised that, as yet, oospores have not been found associated with seed-lots of *I. walleriana* or other species and cultivars in the UK.

In situations where resting spores (oospores) have been incorporated with infected plant debris into the soil there is an increased risk of direct infection in outdoor planting schemes. However, how big this risk is, relative to directly sown seed crops, remains unknown though it is considered that systemic infection from germinating oospores in the soil to established plants from 6-packs is relatively low but anyone repeat planting in situations where the disease was present the previous season should take extra care.

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 potentially lead to long-distance dissemination of the pathogen in plants that appear disease-free on delivery, as well as via potential contamination in consignments of seed.

Unfortunately when the project was commissioned, further occurrences of impatiens downy mildew throughout Europe remained low and this made it very difficult to progress as the pathogen involved is an obligate organism and therefore cannot be retained in artificial culture. In order to ensure progress could be maintained within the project, it was therefore agreed to use another host-specific downy mildew on an ornamental crop as a model. Downy mildew of pansy (*Peronospora violae*) was identified as an alternative 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, in theory, also be applicable for use with a *Plasmopara* species on impatiens, providing the PCR primers originally developed are capable of detecting a broad range of downy mildew species. The return of impatiens downy mildew to the UK in 2007 and on a much larger scale in 2008 has meant that the project work has been split between the two diseases.

In order for the industry to take responsibility for the overall management of downy mildew, it is necessary for growers to have at their disposal effective techniques for the detection of early (latent) infections of downy mildew on both seed and young propagation material and also access to a range of approved crop protection products with demonstrated activity and crop safety against *Plasmopara obducens, Peronospora violae* and other downy mildew fungi in ornamental crops such as *Peronospora tabacina* in Nicotiana, *Peronospora parasitica* in Cheiranthus/Erysimum and *Peronospora antirrhini* in 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.

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 safety and efficacy of a range of fungicides for effective disease prevention and control.
3. To develop an effective integrated GFP strategy which minimises the resistance risk and which can be quickly adopted by seed-houses, propagators/plug raisers, growers and commercial end-users of plants

Summary of the project and main conclusions

Outbreak monitoring and isolate collection

Pansy plants infected by downy mildew were collected throughout the growing season, largely from local garden centres. These isolates were used in the development of plant infection protocols. A number of impatiens plants heavily infected with *P. obducens* were sent to the laboratory from commercial growers via CSL & STC diagnostic laboratories or 'Plant Clinics'. One of the infected impatiens plants sent in had discolouration in the stem tissues; this was shown to result of aggregations of resting spores (oospores) of *P. obducens*. This was the first record of *P. obducens* oospores on impatiens grown in the UK and may have significant repercussions in terms of pathogen persistence and spread due to the potential for longer-term (over-wintering) survival of the pathogen outdoors under UK conditions.

Culturing/isolate maintenance

Fresh *P. violae* sporangia were produced on pansy leaves showing symptoms of downy mildew when incubated in a moisture chamber at 15°C in the dark for 20hrs. Leaf wetness was required for sporulation; high humidity alone was not sufficient. No sporulation occurred when leaves were incubated in the light or where stomata were covered by water. Germination rates between 30 and 40% were achieved for fresh sporangia incubated at 5°C in the dark; germination rates reduced as the temperature increased or if spores were incubated in the dark. Old sporangia (i.e. those collected from infected plants on arrival in the laboratory) exhibited an absence of or very limited germination under the same conditions.

For *P. obducens*, germination of 'old' sporangia was not achieved and as yet it has not been possible to induce fresh sporulation in the laboratory.

Plant infection

Infection of pansy plants by *P. violae* was observed following inoculation with freshly produced sporangia, provided that inoculated plants were kept at in the dark at 5°C for 20hrs to allow germination of sporangia. Downy mildew symptoms were first expressed after 10 days when plants were kept in the laboratory (mean daily temp 16°C) and 20 days when kept in the glasshouse (mean daily temp 8°C). Summing the mean daily temperature between inoculation and expression of symptoms for plants kept in the laboratory and plants kept in the glasshouse gave a thermal time of 170-degree days.

Detection of Peronospora violae and Plasmopara obducens

Seed

Of the commercial impatiens seed samples tested, 31% gave a Ct value indicating the presence of downy mildew DNA in the sample. This rate of DNA detection was lower than for the pansy seeds tested in the first year of the project, where 66% of the samples contained downy mildew DNA. To date it has not been possible to produce downy mildew symptoms in plants grown from any of the seed lots containing downy mildew DNA. As a result it is difficult to determine whether the DNA present is viable or indeed of any significance in terms subsequent downy mildew infection. Further tests are being carried out to try and express symptoms in plants grown from seed containing downy mildew DNA. Microscopic examination of seed lots positive for downy mildew DNA did not reveal the presence of any spores (sporangia or oospores) or mycelia in the samples.

Seedlings

DNA analysis of pansy and impatiens cotyledons and roots grown from seed lots with a range of downy mildew levels, generally, showed that for both hosts if downy mildew DNA was detected in a seed then it would also be detected in the plant material. However, downy mildew DNA was detected more often and at higher levels in root material than in the cotyledons. The significance of these findings are still not known as these plants did not express symptoms of downy mildew despite the presence of pathogen DNA.

Latent infection

The downy mildew primers and probes have proved capable of detecting latent infections of downy mildew in pansy leaf tissues. Levels of downy mildew DNA were

shown to increase in the leaf material until the point when symptoms were expressed. This approach could therefore potentially be used in the future to 'screen' vegetative cutting material for the presence of the pathogen, though further validation would be required to develop it as a commercial service.

Crop Safety

15 products (Table 1) were used in replicated trials to indicate product safety on both pansy and impatiens crops during 2008. Product safety has been tested on young module-raised and semi-mature (in 6-pack) pansies and semi-mature (in 6-pack) impatiens that were flowering at the point of application. Initial work on young module raised Impatiens was unsuccessful and will be repeated in early 2009. Each product was applied to each crop at standard (1xN) and twice standard (2xN) rate. Spray and/or drench applications were applied as indicated in Table 1.

In general all the products tested appeared to be relatively crop-safe on established plants, with little visually obvious or consistent distortion or scorching of the foliage or flowers. However, whilst not immediately visible in the crop 'by eye' the in-crop measurements did detect a statistically significant reduction in plant height following application of some of the products in at least one of the pansy cultivars. Treatments that caused a reduction in plant height included Vitomex spray (both 1N and 2N rates), Shirlan spray (2N rate only), Revus drench (2N rate only), Epok drench (2N rate only), the Subdue & Paraat drenches and the experimental product from Syngenta applied as a spray. In the case of the latter 3 products the effect was only observed at the 1N, and not at the 2N, rate so this questions the significance of the data..

Growers considering using any of the products listed above (noting that some of them are either experimental or can only be used on outdoor crops) should proceed with extreme caution and test treat a few plants of different cultivars on their own nursery prior to widespread crop use. It is also worth noting that whilst several of the products listed can be used at the current time on protected ornamentals via the Long Term Arrangements for Extension of Use (LTAEU), recently announced changes to these arrangements will impact on their future availability. From the 1st June 2009 several active ingredients (for full list evaluated see http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Appendix_1_Active_substances_excluded_from_the_LTAEU_from_1_June_2009.doc) have been evaluated and where possible uses have been transferred to Specific Off Label Approvals (SOLAs). In some cases, especially in protected ornamentals, this has not

been possible due to concerns regarding operator or worker safety and growers or their advisers need to keep abreast of the latest situation to avoid application of non-approved products after the 1st June 2009. The remaining active substances not included in this first evaluation will be processed over the next 5 years as the active

Table 1. Fungicides and application used in pansy and impatiens crop safety trials.

Product	Active ingredient	Application type	Application rate (mls or gms per litre)		Permitted 'commercial' use~	
			1xN	2xN	Protected crops	Outdoor crops
Subdue	metalaxyl-M	Drench	0.12 ml	0.24 ml	Yes (On-Label)	Yes (On-Label)
Amistar	azoxystrobin	Drench & spray	0.66 ml	1.32 ml	Yes (via SOLA 0443/09)	Yes (via SOLA 0443/09)
Bayer Exp.	imidazolinone gp. + ethyl-phosphonate	Drench & spray	3.0 g	6.0 g	No (experimental product)	No (experimental product)
Previcur Energy	propamocarb-HCL + fosetyl-al	Drench & spray	1.66 ml	3.32 ml	Yes (SOLA 2667/08)	Yes (SOLA 2667/08)
Epok	fluazinam + metalaxyl-M	Drench & spray	0.25 ml	0.5 ml	No	Yes (via extended LTAEU)
Revus	mandipropamid	Drench & spray	0.4 ml	0.8 ml	No	Yes (SOLA 2867/08 via LTAEU transfer)
Shinkon	amisulbrom	Spray	0.33 ml	0.66 ml	No	Yes (via extended LTAEU)
Rose tonic*	potassium phosphate	Drench & spray	10 ml	20 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)
Fubol Gold	metalaxyl-M + mancozeb	Spray	1.25 g	2.5 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Olympus	azoxystrobin + chlorothalonil	Spray	1.66 ml	3.32 ml	No	Yes (via extended LTAEU)
Stroby	kresoxim-methyl	Drench & spray	0.2 g	0.4 g	Yes (On-Label)	Yes (On-Label)
Shirlan#	fluazinam	Drench & spray	0.26 ml	0.52 ml	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Paraat	dimethomorph	Drench & spray	0.2 g	0.4 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Syngenta Exp.	carboxylic acid amide+dithiocarbamate	Spray	1.66 g	3.32 g	No (experimental product)	No (experimental product)
Vitomex	phosphonic acid + derivatives	Spray	2.66 ml	5.32 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)

* Not applied to pansy
\# Potential for skin sensitisation in vulnerable individuals. This product should not be applied in situations where unprotected workers are handling plants.
~ In many cases, subject to growers own risk.

ingredients come up for re-registration. For further details of what Extension of Use arrangements remain after the 1st June 2009 see: http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/2nd_phase_product_list_27Jan2009_Web_version.xls

Financial benefits

It is still not possible to predict the full financial benefits from this project. However, the pathogens responsible for downy mildew are aggressive and, under favourable environmental conditions, such as those seen in 2008, they can cause significant economic losses. Therefore, if the project is successful and can help the industry to reduce the risk from the disease it will be of considerable economic benefit. If we assume an industry value of £40M/annum for Impatiens alone (Coutts, pers com) and in a year like 2008 estimate that some 10% plant losses of Impatiens were incurred (this includes potential lost trade in subsequent years due to poorly performing plants in civic displays etc) the financial benefit in Impatiens alone could be as high as £4M/annum (in years where disease severity is high). Assuming losses occur due to downy mildew in other crops e.g. pansy & viola the gross economic benefit of this R&D could be much higher.

Action Points for Growers

- Try to ensure any starting plant material (vegetative cuttings or seed) is disease free.
- Isolate and clearly label vegetative cutting and seed crops, including those from different suppliers to allow traceability should future problems arise.
- Ensure adequate air circulation around plants to minimise prolonged periods of leaf wetness by better spacing and by increasing the ventilation in the glasshouse. Avoid overhead watering as this is likely to aggravate the disease. If it is necessary to water from overhead then do this early, on days when solar radiation levels will ensure the leaves have a chance to dry out quickly.
- Check susceptible crops regularly and make arrangements for any suspicious plant material to be sent for diagnosis. 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. Destroy any infected plants either by burial at landfill or via incineration.

- 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.) on pansy & viola.
- Practice good nursery hygiene, clean up crop debris between crops and at the end of the season to minimise the risk of carry-over of the disease and maintain effective weed control (including 'volunteer' Impatiens plants) in and around the growing areas. Use appropriate disinfectants responsibly to help minimise potential carry-over of inoculum.
- Where infected material is found notify the project team and, if possible, submit a sample for R&D purposes.
- Keep abreast of developments with changes to the Long Term Arrangements for Extension of Use (LTAEU) arrangements.

Science Section

Introduction

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communication). This is highly relevant and important with respect to the potential for pathogen introduction, subsequent disease spread and the future successful implementation of various control measures.

In the original UK outbreaks in 2003, it is suspected that *Impatiens* raised from seed or, more likely, as imported vegetative transplants (unrooted or rooted cuttings) may have provided the initial infection source of the disease, though this has not been confirmed. Over-wintering or resting spores (oospores) of the pathogen have previously been reported on/in seed in India, but their absence in early UK outbreaks indicated perhaps that the pathogens potential to survive over-winter under UK conditions was low. However, in 2008 the situation in the UK changed considerably when resting spores were found in infected stem tissues of *I. walleriana* for the first time. Given this new finding, the potential risk of carry-over between seasons on plant debris incorporated into the soil has increased significantly. The potential for seed-borne transmission via this route is also potentially increased though it must be emphasised that, as yet, oospores have not been found associated with seed-lots of *I. walleriana* or other species and cultivars in the UK.

In situations where resting spores (oospores) have been incorporated with infected plant debris into the soil there is an increased risk of direct infection in outdoor planting schemes. However, how big this risk is, relative to directly sown seed crops, remains unknown though it is considered that systemic infection from germinating oospores in the soil to established plants from 6-packs is relatively low but anyone repeat planting in situations where the disease was present the previous season should take extra care.

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Unfortunately when the project was commissioned, further occurrences of impatiens downy mildew throughout Europe remained low and this made it very difficult to progress as the pathogen involved is an obligate organism and therefore cannot be retained in artificial culture. In order to ensure progress could be maintained within the project, it was therefore agreed to use another host-specific downy mildew on an

ornamental crop as a model. Downy mildew of pansy (*Peronospora violae*) was identified as an alternative 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, in theory, also be applicable for use with a *Plasmopara* species on impatiens, providing the PCR primers originally developed are capable of detecting a broad range of downy mildew species. The return of impatiens downy mildew to the UK in 2007 and on a much larger scale in 2008 has meant that the project work has been split between the two diseases.

In order for the industry to take responsibility for the overall management of downy mildew, it is necessary for growers to have at their disposal effective techniques for the detection of early (latent) infections of downy mildew on both seed and young propagation material and also access to a range of approved crop protection products with demonstrated activity and crop safety against *Plasmopara obducens, Peronospora violae* and other downy mildew fungi in ornamental crops such as *Peronospora tabacina* in Nicotiana, *Peronospora parasitica* in Cheiranthus/Erysimum and *Peronospora antirrhini* in 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

To ensure sufficient isolates of *Peronospora violae* and *Plasmopara obducens* were available to the project, requests for plant material were publicised to growers and 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) and garden centres local to CSL.

Culturing/isolate maintenance

Spore production

Results from work carried out in the first year of the project showed that sporangia of *P. violae* were not robust and that unless conditions were favourable for infection, they did not survive for long. In fact zero or very low levels of sporangial germination were detected in studies carried out in year 1 of the project. As it was impossible to determine the age of the spores collected from plants showing symptoms in plant clinic and garden centres, the focus in year two switched to the production of fresh spores to determine whether germination rates could be improved. As the literature suggests leaf wetness or high humidity is one trigger for sporulation for a number of other downy mildew pathogens the effect of relative humidity in inducing sporulation of both pathogens was investigated using detached leaves. Leaves showing symptoms of downy mildew were detached from the plant and placed abaxial (lower) side up on absorbent tissue paper saturated with sterile distilled water (SDW) in a large Petri-dish (13.5 cm diameter). The lid was placed on the dish and leaves incubated either in the light or in the dark at 5 or 15°C for between 20 and 24 hrs.

Sporangial germination

A comparison of germination rates between freshly produced and 'old' sporangia of *P. violae* was carried out. Spores were washed directly from leaves and the sporangial suspensions incubated at 5 or 15°C either in the light or in the dark. Sporangial germination was measured after 18 hrs. A sporangium was recorded as germinated if the germ tube was longer than the sporangium.

Oospore germination studies - impatiens

Through the course of 2008, oospores of *P. obducens* were identified in both leaf and stem tissue of downy mildew infected impatiens plants. These were used to try and initiate fresh sporangial production for the infection of impatiens. Methods were based on those described by Rossi & Caffi (2007) for germination of *Plasmopara viticola* oospores and included submerging dried or frozen oospores of *P. obducens* under water while floating impatiens leaf discs over them.

Plant infection studies

P. violae and *P. obducens* are obligate plant pathogens and as such cannot be cultured on substrates other than host plant material. Thus, to bulk-up inoculum in order to carry out fungicide efficacy testing, methods were required which produced a uniform and consistent infection of plants.

Work in the first year of the project had been unsuccessful in producing methodologies for the maintenance/production of inoculum of *P. violae* or *P. obducens* in either pansy or impatiens plants respectively. Work on infection studies continued in the second year of the project, primarily using *P. violae*, and were based on results obtained during the sporangial production and germination studies carried out within this project and methodologies described for infection of plant material i.e. basil and roses, with downy mildew pathogens (Garibaldi *et al*, 2007, Aegerter *et al*, 2003).

Fresh *P. violae* sporangia were produced by detaching leaves showing symptoms of downy mildew from the pansy plant and placing them abaxial side up on absorbent tissue paper saturated with SDW in large Petri-dishes (13.5 cm diameter). The lid was placed on the dish and leaves incubated in the dark for between 20 and 24 hrs. Fresh sporangia were harvested in SDW and spore concentrations adjusted to between 10^4 and 10^5 sporangia mL^{-1}. Ten pansy plants were spray inoculated with the sporangial suspension to run-off, ensuring that both the abaxial and adaxial surfaces of the leaf were inoculated. Plants were transferred into a propagator top and incubated at 5°C in the dark for 20hrs. Following removal of plant from the 5°C incubator five leaves were removed at random and examined under the scanning electron microscope (SEM). Plants were split into two batches of five, and one batch grown on in the laboratory (average temp 18°C) and the other in the glasshouse (average temp 8°C). A second set of five leaves was examined under the SEM 48 hrs after inoculation. Both batches of pansies were examined daily for the development of downy mildew symptoms.

Detection of *Peronospora violae* and *Plasmopara obducens* DNA in seed and plant material

In the first year of the project, the suitability of the primer sets for detection of *Peronospora violae* and *Plasmopara obducens* was established using leaves and

seed from pansies, and leaves from impatiens (see the first year report for details of the primer set used). Seed from cheiranthus, nicotiana and antirrhinum, suspected of being infested with *Peronospora parasitica*, *Peronospora tabacina* and *Peronospora antirrhini* respectively, were also tested and downy mildew DNA detected. In the second year validation continued using impatiens seed and seedlings of impatiens and pansies. In addition, pansy leaves artificially inoculated with *P. violae* were analysed to determine whether latent infections could be detected by the primer sets.

Downy mildew DNA detection in seed

Impatiens seed lots (ranging in variety and flower colour) were obtained from several different suppliers and tested for the presence of *P. obducens* DNA using protocols developed in year 1 of the project.

Downy mildew DNA detection in seedlings

Twelve pansy and twelve impatiens seed lots, with a range of cycle threshold (Ct) values (and hence DNA levels), were selected for further testing to determine whether DNA in the seed was detected in the plant and whether this resulted in symptom expression.

Clear plastic sandwich boxes (17x11x6cm) were filled to a depth of 2cm with vermiculite and two filter papers (9 cm diameter) placed on the vermiculite surface. The filter papers and vermiculite were moistened and sterilised at 121°C for 15 min. For each seed lot, 50 seeds were sown onto one of the filter papers (two seed lots per sandwich box) and the seeds left to germinate and grow to the cotyledon stage (Figure 1). For each set of seedlings the leaf/stem material was separated from the roots and DNA extraction/analysis carried out on both.

Figure 1. Sandwich box set up for germination of impatiens and pansy seed lots.

Detection of latent infection

Sixty-four pansy leaves were detached from plants, and divided into four sub-sets of 14 leaves. The remaining eight leaves were placed in extraction bags labelled T -1. Leaves from each sub-set were placed abaxial side up on moistened absorbent tissue paper in Petri dishes (13.5 cm diameter) and inoculated to run off with a suspension of fresh *P. violae* sporangia containing 10^4 sporangia mL^{-1}. Two leaves were removed from each sub-set and placed in extraction bags labelled T0. All sub-sets were then incubated at 5°C in the dark for 20 hrs and then two further leaves removed from each sub-set and placed in extraction bags labelled T1. At this point in the experiment all leaves had still been treated the same. To test differences in development of disease, two of the sub-sets were transferred to an incubator at 15°C whilst the remaining two sub-sets were left at 5°C. Leaves were removed and placed in appropriately labelled extraction bags 4, 6, 8, 10 and 14 days after inoculation. Immediately after sampling all leaves were placed at -20°C until required for analysis. After the final sampling all leaves were analysed for the presence of *P. violae* DNA (see the first year report for details of DNA extraction and DNA analysis from leaf material).

Crop Safety

A series of replicated crop safety experiments were carried out on both pansy and impatiens at STC during 2008.

The first experiment was carried out in July on 3 pansy cultivars of varying ages in order to provide safety data of possible effects on young foliage, flowers and plants in

bud. F1 'Red with Blotch', a batch of larger older plants, which were already flowering, were tested alongside two batches of young plants ('Yellow Turbo' and 'Blue Turbo'), which were bought in as young plugs and potted into 6 packs prior to the commencement of the trial. One 6-pack represented a 'plot' and 4 replicate 6-packs per treatment were used. The trial was laid out in a fully randomised block design and maintained in an ambient glasshouse with venting for the duration of the trial.

A second experiment was carried out in October 2008 using Impatiens (Expo mixed). Plants were raised from seeds and planted on into 6 packs and allowed to flower before the trial started. Due to a relatively poor germination rate it was not possible to undertake separate trials on both plug plants and established semi-mature plants. A decision was taken to evaluate the various products on plants in 6-packs initially with subsequent work on plug plants to be repeated at a later stage.

Treatments

A total of 15 products were included in the crop safety studies (Table 1). Metalaxyl-M (Subdue) and metalaxyl-M + mancozeb (Fubol Gold) were included as standard drench and spray treatments and used alongside the water control for comparison purposes. The remainder of the 'novel' products included were chosen either for their known performance against other related oomycete fungi e.g. *Phytophthora infestans*, cause of blight in potato. Two experimental products were included which the manufacturers thought had the potential to offer some control of downy mildew, and also two less conventional 'unregistered' products (potassium phosphate & phosphonic acid+derivatives). The majority of the products chosen for this study were already available and could be used on both outdoor and protected crops, in some cases via On-Label Approval, through Specific Off Label Approvals (SOLAs) or via the Long Term Arrangements for Extension of Use (LTAEU)[1].

Products were applied either as a drench or foliar spray depending on their label recommendation or potential use. Drenches were applied at 20ml/module whilst spray treatments used a water rate of 1500 L ha^{-1} using an Oxford Precision

[1] It is important to note that the industry is currently in a transitional and confusing period with respect to what products can be legally used on outdoor and protected ornamental crops. The existing arrangements apply until 1st June 2009 but after this time the Long term Arrangements for Extension of Use do not necessarily apply, though it does depend on the active ingredient under consideration. For full details of the transitional arrangements see http://www.pesticides.gov.uk/approvals.asp?id=2634

Knapsack sprayer at a constant 2 Bar pressure. Products were applied at the normal label(1 x N) and twice label (2 x N) rates.

Assessment

Following application, the plants were monitored for visual signs of phytotoxicity e.g. stunting, scorch, and leaf or flower distortion. A full destructive assessment was carried out approximately 10 days post-application. During this assessment measurements of plant height and fresh weight were recorded and the number of plants displaying any potential phytotoxicity symptoms was scored and photographed. The data from both trials was analysed using Agricultural Research Manager Software (ARM).

Table 1. Fungicides and application used in pansy and impatiens crop safety trials.

Product	Active ingredient	Application type	Application rate (mls or gms per litre)		Permitted 'commercial' use~	
			1xN	2xN	Protected crops	Outdoor crops
Subdue	metalaxyl-M	Drench	0.12 ml	0.24 ml	Yes (On-Label)	Yes (On-Label)
Amistar	azoxystrobin	Drench & spray	0.66 ml	1.32 ml	Yes (via SOLA 0443/09)	Yes (via SOLA 0443/09)
Bayer Exp.	imidazolinone gp. + ethyl-phosphonate	Drench & spray	3.0 g	6.0 g	No (experimental product)	No (experimental product)
Previcur Energy	propamocarb-HCL + fosetyl-al	Drench & spray	1.66 ml	3.32 ml	Yes (SOLA 2667/08)	Yes (SOLA 2667/08)
Epok	fluazinam + metalaxyl-M	Drench & spray	0.25 ml	0.5 ml	No	Yes (via extended LTAEU)
Revus	mandipropamid	Drench & spray	0.4 ml	0.8 ml	No	Yes (SOLA 2867/08 via LTAEU transfer)
Shinkon	amisulbrom	Spray	0.33 ml	0.66 ml	No	Yes (via extended LTAEU)
Rose tonic*	potassium phosphate	Drench & spray	10 ml	20 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)
Fubol Gold	metalaxyl-M + mancozeb	Spray	1.25 g	2.5 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Olympus	azoxystrobin + chlorothalonil	Spray	1.66 ml	3.32 ml	No	Yes (via extended LTAEU)
Stroby	kresoxim-methyl	Drench & spray	0.2 g	0.4 g	Yes (On-Label)	Yes (On-Label)
Shirlan#	fluazinam	Drench & spray	0.26 ml	0.52 ml	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Paraat	dimethomorph	Drench & spray	0.2 g	0.4 g	Yes (via extended LTAEU)	Yes (via extended LTAEU)
Syngenta Exp.	carboxylic acid amide+dithiocarbamate	Spray	1.66 g	3.32 g	No (experimental product)	No (experimental product)
Vitomex	phosphonic acid + derivatives	Spray	2.66 ml	5.32 ml	Yes (not a registered fungicide)	Yes (not a registered fungicide)

* Not applied to pansy

\# Potential for skin sensitisation in vulnerable individuals. This product should not be applied in situations where unprotected workers are handling plants.

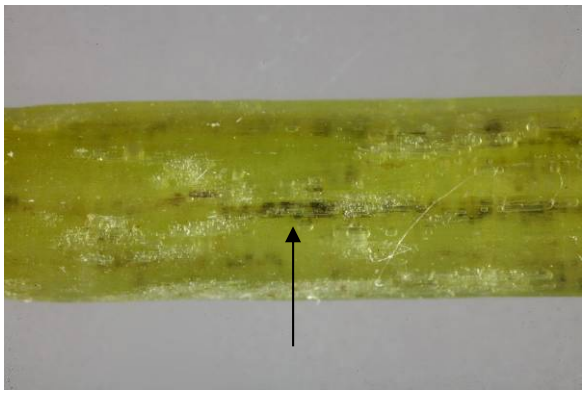
~ In many cases, subject to growers own risk.

Results and Discussion

Outbreak monitoring and isolate collection

Pansy plants infected by downy mildew were collected throughout the growing season from local garden centres; no additional plants were sourced via the plant clinics. Downy mildew isolates sourced from these plants were used in the development of plant infection methodologies. Numerous impatiens plants infected by *P. obducens* were submitted from growers and/or their consultants to Plant Clinics in both STC and CSL. All impatiens plants arriving at the laboratory were already severely infected and sporulating heavily with none having early symptoms; which would have allowed for the production of fresh sporangia. One of the infected impatiens plants showed discolouration of the stem material (Figure 2a), which was subsequently shown to be caused by aggregations of *P. obducens* oospores (Figures 2b and c). This was the first record of *P. obducens* oospores in the UK and may have repercussions for pathogen persistence over-winter and subsequent spread due to the longer-term survival of the pathogen.

a)



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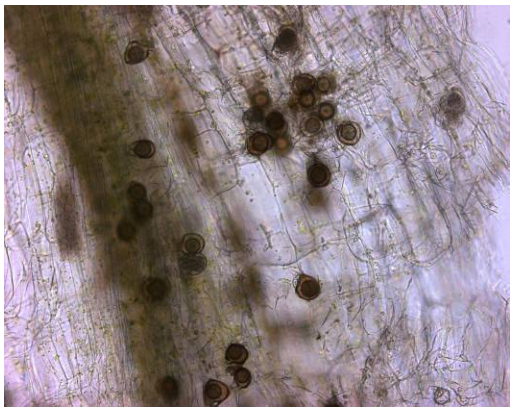


c)

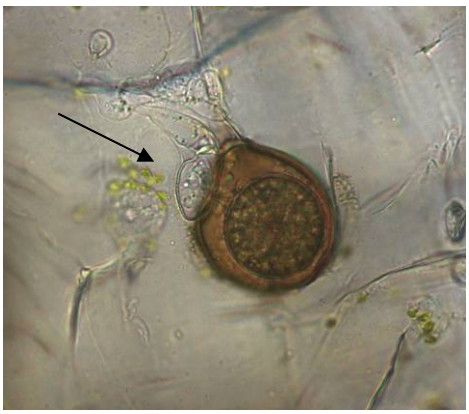


Figure 2. Impatiens stem symptoms caused by *Plasmopara obducens*. (a) stem discolouration (arrowed), (b) oospores of *P. obducens* in stem tissue, (c) close-up of *P. obducens* oospore with paragynous antheridium (arrowed).

Culturing/isolate maintenance

Spore production – *P. violae*

Fresh sporangia of *P. violae* were produced following incubation of leaves on wet tissue at either 5 or 15°C in the dark, with a larger numbers of sporangia produced at 15°C. No sporulation occurred when leaves were incubated in the light, indicating that on a nursery, it is likely that most sporulation occurs overnight at a time to coincide with increased likelihood of leaf wetness. Observations also indicated that high humidity alone was not sufficient to induce sporulation, but that leaf wetness was also required. However, sporulation did not occur in areas in direct contact with the water. Sporulation was seen on both the upper and lower leaf surfaces, but was greater on the lower surface due to the larger number of stomata through which the spore bearing conidiophores emerged (Figure 3).

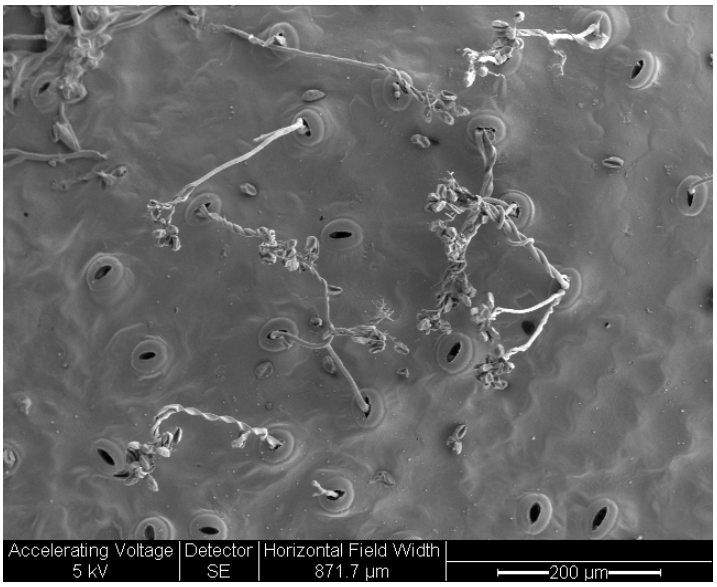


Figure 3. Spore bearing conidiophores of *Peronospora violae* emerging from stomatal openings on the lower surface of a pansy leaf.

Spore production - *P. obducens*

Incubation of impatiens leaves (either already sporulating or non-symptomatic leaves from infected plants) did not induce fresh sporulation of *P. obducens*.

Observations showed that leaves infected by *P. obducens* generally sporulated across the entire lower leaf surface, with no leaves showing non-sporulating symptoms. This is in contrast to symptoms on pansies caused by *P. violae* where sporulation generally only occurred on part of the leaf and non-sporulating symptoms

could be regularly identified. This difference may explain the lack of fresh sporulation seen following incubation of *P. obducens* infected leaf material compared to those caused by *P. violae*. In order to produce fresh sporangia of *P. obducens* it seems likely that infected impatiens plants will need to be sourced earlier in the infection process, before sporulation has occurred.

In the first year of the project it was demonstrated that freezing did not appear to be a viable option for the storage of *P. violae* sporangia. However, the spores, which were tested, were 'old' and these have also been shown not to germinate. In the third year, of the project freshly produced sporangia will be frozen to determine whether this affects their germination rate and whether freezing could be considered a viable storage method for *P. violae* inoculum/isolates.

Sporangial germination – *P. violae*

As in year 1, zero or very limited germination of 'old' sporangia was observed under any of the experimental conditions examined. However, germination of fresh sporangia was observed (Figure 4). Over several repeats of the experiment germination rates between 30 and 40% were observed when spores were incubated at 5°C in the dark. The germination rate reduced to between 6 and 10% when spores were incubated at 15°C in the dark, and to less than 1% when incubated at either 5 or 15°C in the light.

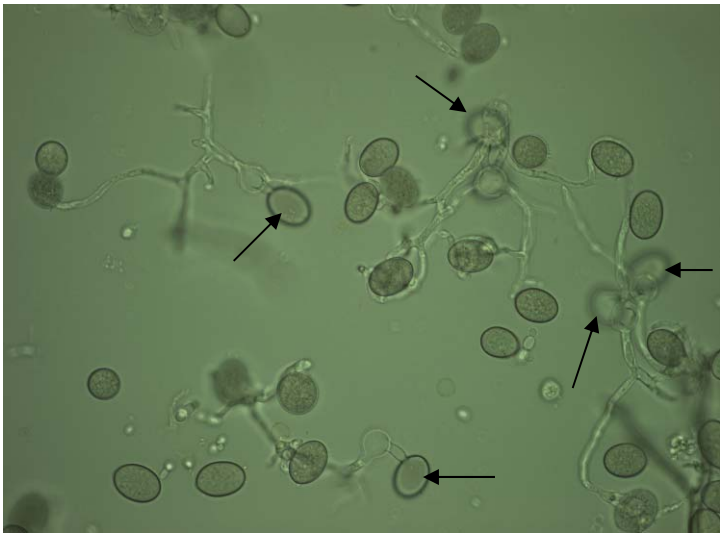


Figure 4. Germinating *Peronospora violae* sporangia. Arrows indicate germinated spores.

A number of processes/structures were associated with sporangial germination (Figure 5). On germination the sporangium (Figure 5 i) produced a short germ tube (Figure 5 ii) and lost its cytoplasmic content. The germ tube differentiated into a round vesicle structure (Figure 5 iii) from which a mycelium was produced (Figure 5 iv). On contact with a surface, in this case another sporangium, an appressorium was formed (Figure 5 v) which exerted pressure on the surface as indicated by the depression in the sporangial wall (Figure 5 vi).

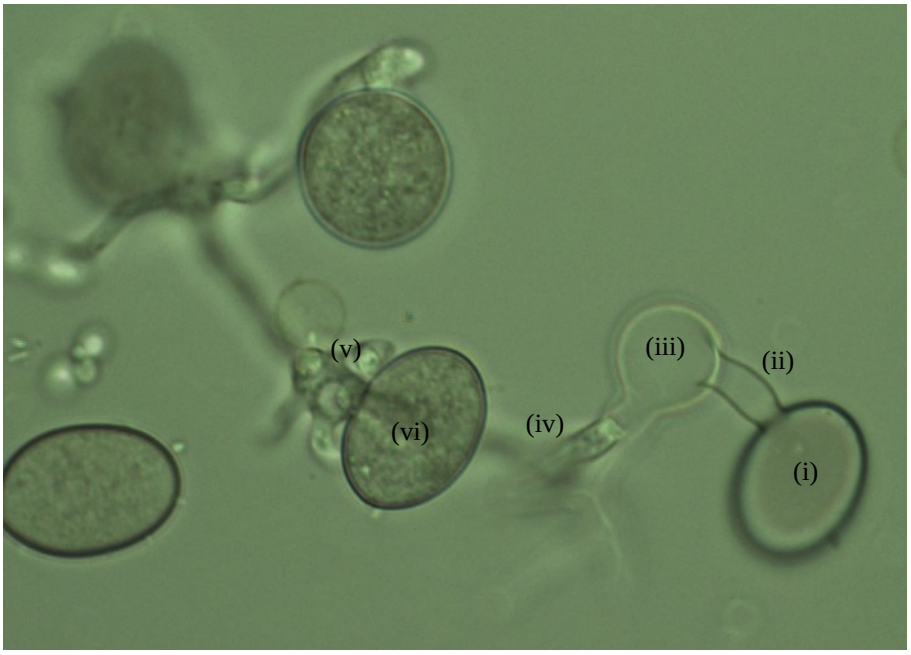


Figure 5. Structures associated with germination of a *Peronospora violae* sporangium. (i) germinating sporangium, (ii) short germ tube, (iii) vesicle, (iv) mycelium produced from vesicle, (v) appressorium and (vi) depression in sporangial cell wall resulting from pressure exerted by appressorium.

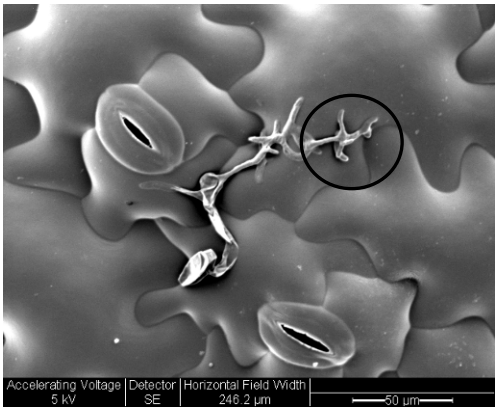
As was reported for *P. violae* in year 1, no germination of 'old' sporangia of *P. obducens* (those collected from infected plants on arrival at the lab) was seen. As no 'fresh' spores could be produced, further experimental work on spore germination could not be carried out for *P. obducens*.

Oospore germination studies

No germination of *P. obducens* oospores was observed under any of the conditions tested. No oospores of *P. violae* were observed.

Plant infection studies

Infection of pansy plants by *P. violae* was observed following inoculation with freshly produced sporangia. Examination of inoculated pansy leaf material under the scanning electron microscope immediately after the incubation at 5°C showed that sporangial germination had occurred (Figure 6a) and that the structures observed when sporangia germinated in water (Figure 5) could still be seen. It also appeared that infection occurred at the interface between cells (indicated by the area circled in Figure 6a). Repeating the observations 24 hrs later (Figure 6b) indicated that once infection had occurred no further growth of *P. violae* occurred on the leaf surface.

a) 

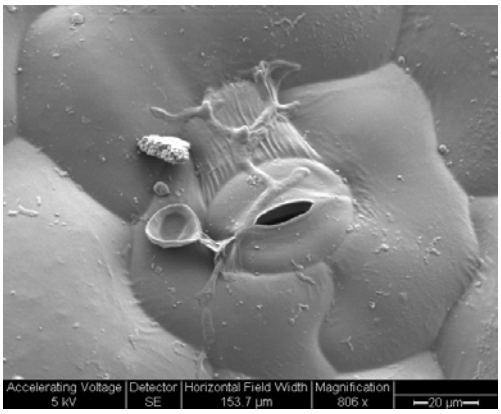
b) 

Figure 6. Scanning electron micrograph of germinated *Peronospora violae* sporangia on the surface of a pansy, (a) 24 hrs after inoculation (area circled indicates possible appressorium) and (b) 48 hrs after inoculation.

Daily observations of inoculated plants showed that symptoms appeared under laboratory conditions 10 days after inoculation (Figure 7), and 20 days after inoculation under glasshouse conditions. Summing the mean daily temperature during the period between inoculation and expression of symptoms for both laboratory and glasshouse conditions gave a thermal time of 170-degree days. The confirmation of protocols allowing the production of pansy material infected by *P. violae* now allows work on fungicide efficacy, baseline fungicide sensitivity and detection of latent infections to take place. The establishment of a thermal time from infection to symptom expression enables experimental progress to be monitored more effectively especially where experiments take place under glass and the temperature cannot be easily regulated.

Figure 7. Downy mildew symptoms expressed on pansies 170-degree days after inoculation with *Peronospora violae* sporangia.

Detection of *Peronospora violae* and *Plasmopara obducens*

Results from the primer set and probe developed to detect *Peronospora* species are usually presented as a mean cycle threshold (Ct) value, with the Ct value representing 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). However, for ease of interpretation in this report any Ct value obtained has been subtracted from 40 so that the resultant value increases with an increasing level of DNA.

Downy mildew DNA detection in seed

A total of 51 impatiens seed samples were analysed using the *Peronospora* primer set and probe (Table 2). Of the impatiens seed samples tested, 31% gave a Ct value that indicated the presence of downy mildew DNA in the sample. This level of DNA detection was lower than for the pansy seeds tested in the first year of the project where 66% of the samples contained downy mildew DNA.

To date it has not been possible to produce downy mildew symptoms in plants grown from any of the seed lots containing downy mildew DNA. As a result it is difficult to determine whether the DNA present is viable and indeed is of any significance in terms subsequent downy mildew infection. Microscopic examination of seed lots positive for downy mildew DNA did not reveal the presence of any spores (sporangia

or oospores) or mycelia in the samples. Further tests are being carried out to try to express symptoms in plants grown from seed containing downy mildew DNA.

Table 2. Detection of downy mildew (DM) DNA from impatiens and pansy seed lots using the primer set and probe developed to detect *Peronospora* species.

	Number of samples		DNA level (40-Ct value)	
	Tested	DM DNA positive	Range	Mean
Pansy	41	27	1.3-19.7	4.3
Impatiens	51	16	1.1-8.4	3.4

Downy mildew DNA detection in seedlings

Results from the downy mildew DNA analysis of pansy and impatiens cotyledons and roots grown from seed lots with a range of downy mildew levels are presented in Tables 3 and 4. Generally, the results showed that for both hosts, if downy mildew DNA were detected in a seed then it would also be detected in the plant material. However, downy mildew DNA was detected more often and at higher levels in root material than in the cotyledons. The significance of this is still not known.

Table 3. Detection of *Peronospora violae* DNA from pansy cotyledon and root material using the primer set and probe developed to detect *Peronospora* species.

Sample	DM DNA level (40-Ct value)		
	Seed	Cotyledon	Root
1	5.8	2.1	2.6
2	6.2	5.0	4.0
3	0.0	0.0	2.0
4	0.0	0.0	4.3
5	3.9	2.3	1.0
6	0.0	3.5	8.4
7	4.3	5.2	4.4
8	0.0	5.3	4.4
9	4.9	0.0	1.5
10	0.0	0.0	0.0
11	0.0	0.0	2.0
12	0.0	0.0	0.0
Samples containing DM DNA (%)	42	50	83
Mean DM DNA level	2.1	2.0	2.9

Table 4. Detection of *Plasmopara obducens* DNA from impatiens cotyledon and root material using the primer set and probe developed to detect *Peronospora* species.

Sample	DM DNA level (40-Ct value)		
	Seed	Cotyledon	Root
1	0.0	3.4	7.0
2	0.0	3.2	3.7
3	0.0	0.0	4.9
4	0.0	0.0	5.7
5	0.0	0.0	2.8
6	0.0	0.0	0.0
7	0.0	0.0	0.0
8	3.4	0.0	1.3
9	0.0	0.0	0.0
10	5.2	0.0	0.0
11	8.4	1.5	5.0
12	5.4	0.0	3.2
Samples containing DM DNA (%)	33	25	66
Mean DM DNA level	1.9	0.7	2.8

Detection of latent infections

Tests to determine whether the primers and probes could detect latent downy mildew infection in pansy leaf material were carried out using a detached leaf assay. Leaves inoculated with fresh *P. violae* sporangia were, following an initial spore germination step, incubated at 5 and 16°C and DNA levels analysed over a 14-day incubation period. No *P. violae* DNA was detected from pansy leaves taken directly from the plant (T -1, Figure 8). Following inoculation with sporangia (T0), the level of *P. violae* DNA increased three fold after spore germination at 5°C. After 4 days incubation at either 5 or 15°C the level of *P. violae* DNA detected at both temperatures dropped slightly compared to those detected at T0.

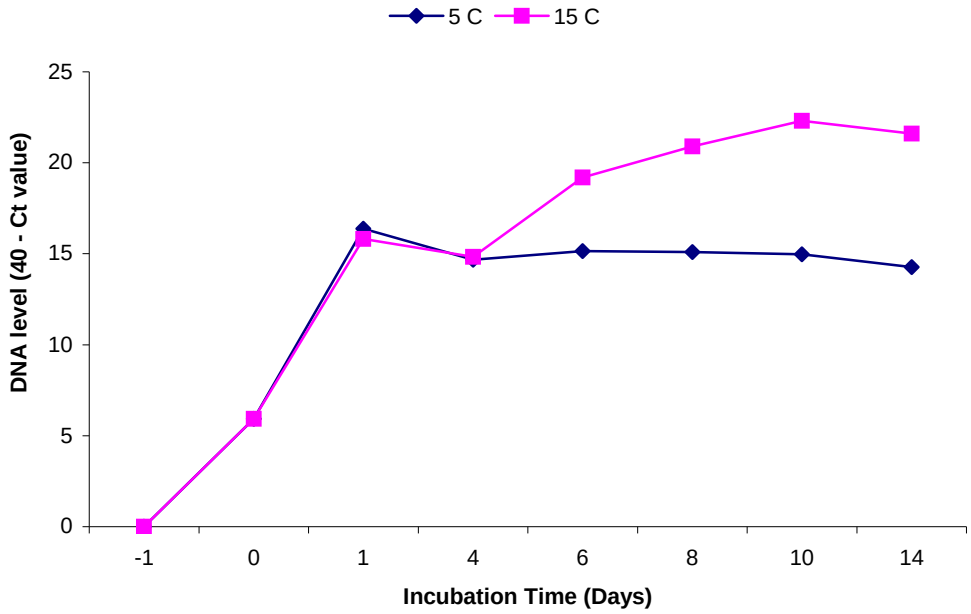


Figure 8. Detection of *Peronospora violae* DNA in from pre-symptomatic downy mildew infection of pansy leaves.

Between four and ten days the level of *P. violae* DNA increased in leaves incubated at 15°C. Symptoms were first seen on leaves incubated at 15°C after 10-days incubation; this timing equates to approximately 170 degree-days. No increase in *P. violae* DNA levels was seen after the initial increase seen as a result of sporangial germination. The successful detection of *P. violae* DNA in pre-symptomatic leaves suggests that the primers and probe are capable of detecting latent infections.

Crop safety

Pansy

Overall, there were no consistent visible phytotoxicity effects following application of any of the experimental or standard treatments. However, occasional plants were observed to show slight adverse symptoms either on the foliage or flowers and this was recorded (Figure 9). However, the absence of a consistent response affecting all treated plants in the different replicates suggests that the symptoms observed may have been unrelated to the applied chemicals. Statistical analysis of the data has been conducted and results are presented in Table 5.

a) 

b) 

c) 

Figure 9. (a) Pansy crop safety trial carried out at STC – July 2008; (b) bubbling of foliage apparent on many plants, irrespective of the applied treatment; (c) slight petal damage observed on flowers.

As the pattern of distribution of foliar or flower distortion was not consistent e.g. possible phytotoxicity symptoms on low numbers of plants, or on applications at 1xN whilst nothing at 2xN, it is more indicative of random and sporadic leaf and foliar malformation that is not necessarily attributed to the applied fungicides. As the data-sets here are limited to a small number of cultivars and environmental conditions growers must be advised to test-treat any new chemicals on a small number of plants of the selected cultivars in the first instance to assure themselves that the product in question is safe to use.

Impatiens

A full assessment of the plants was carried out 7-10 days following application of the treatments. No significant difference between plant height or fresh weight was recorded. No visible symptoms of phytotoxicity were seen on any of the impatiens plants in the trial area.

Figure 10. Impatiens crop safety trial October 2008

It is also worth noting that whilst several of the products listed can be used at the current time on protected ornamentals via the Long Term Arrangements for Extension of Use (LTAEU), recently announced changes to these arrangements will impact on their future availability. From the 1st June 2009 several active ingredients (for full list evaluated see link) have been evaluated and where possible uses have been transferred to Specific Off Label Approvals (SOLAs). In some cases, especially in protected ornamentals, this has not been possible due to concerns regarding operator or worker safety and growers or their advisers need to keep abreast of the latest situation to avoid application of non-approved products after the 1st June 2009. The remaining active substances not included in this first evaluation will be processed over the next 5 years as the active ingredients come up for re-registration.

http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/Appendix_1_Active_substances_excluded_from_the_LTAEU_from_1_June_2009.doc)

For further details of what Extension of Use arrangements remain after the 1st June 2009 see:
http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/2nd_phase_product_list_27Jan2009_Web_version.xls

Table 5. Summary of analysis of pansy and impatiens assessment data

Treatment	Plant Height Effects				Fresh Weight Effects				Percentage plants with foliar distortion				Percentage plants with flower distortion			
	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps
Untreated D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	8	0	0	0	0	0
Untreated S	NS	NS	NS	NS	NS	NS	NS	NS	0	4	21	0	0	0	0	0
Subdue 1N D	NS	NS	NS	NS	NS	S	NS	NS	0	0	13	0	0	0	0	0
Subdue 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	17	17	0	0	0	0	0
Fubol 1N S	NS	NS	NS	NS	NS	NS	NS	NS	8	8	0	0	0	4	0	0
Fubol 2N S	NS	NS	NS	NS	NS	NS	NS	NS	4	4	4	0	0	0	0	0
Amistar 1N D	NS	NS	NS	NS	NS	NS	NS	NS	8	4	4	0	4	0	0	0
Amistar 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	13	8	0	0	0	0	0
Amistar 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Amistar 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	4	17	0	0	0	0	0
Olympus 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	13	0	0	0	0	0
Olympus 2N S	NS	NS	NS	NS	NS	NS	NS	NS	8	0	4	0	0	0	0	0
Fen & Fos 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Fen & Fos 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Fen & Fos 1N S	NS	NS	NS	NS	NS	NS	NS	NS	4	0	13	0	0	0	0	0
Fen & Fos 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Stroby 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Stroby 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Stroby 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Stroby 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
P. Energy 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	4	0	0	0	0	0
P. Energy 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	8	0	0	0	0	0
P. Energy 1N S	NS	NS	NS	NS	NS	NS	NS	NS	4	0	0	0	0	0	0	0
P. Energy 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	4	0	0	0	0	0
Shirlan 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Shirlan 2N D	NS	NS	NS	NS	NS	NS	NS	NS	25	0	0	0	0	0	0	0
Shirlan 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	13	0	0	0	0	0
Shirlan 2N S	NS	NS	NS	NS	NS	NS	S	NS	0	0	0	0	0	0	0	0

Table 5 continued. Summary of analysis of pansy and impatiens assessment data

Treatment	Plant Height Effects				Fresh Weight Effects				Percentage plants with foliar distortion				Percentage plants with flower distortion			
	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps	Red Blotch	Blue Turbo	Yellow Turbo	Imps
Epok 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Epok 2N D	NS	NS	NS	NS	NS	S	NS	NS	0	8	0	0	0	0	0	0
Epok 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Epok 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Paraat 1N D	NS	NS	NS	NS	NS	S	NS	NS	0	0	8	0	0	0	0	0
Paraat 2N D	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Paraat 1N S	NS	NS	NS	NS	NS	NS	NS	NS	4	0	0	0	0	0	0	0
Paraat 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	8	0	0	0	4	0	0
Revus 1N D	NS	NS	NS	NS	NS	NS	NS	NS	0	4	0	0	0	0	0	0
Revus 2N D	NS	NS	NS	NS	NS	NS	S	NS	0	0	0	0	0	0	0	0
Revus 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Revus 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Mandi & Manc 1N S	NS	NS	NS	NS	NS	NS	S	NS	0	0	0	0	0	0	0	0
Mandi & Manc 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	4	17	0	0	0	0	0
Shinkon 1N S	NS	NS	NS	NS	NS	NS	NS	NS	0	0	0	0	0	0	0	0
Shinkon 2N S	NS	NS	NS	NS	NS	NS	NS	NS	0	4	0	0	0	0	0	0
Vitomex 1N S	NS	NS	NS	NS	NS	NS	S	NS	0	0	21	0	0	0	0	0
Vitomex 2N S	NS	NS	NS	NS	NS	NS	S	NS	0	0	0	0	0	0	0	0

NS – No significant difference to untreated (Drench or Spray)

S – Significant difference to untreated (Drench or Spray) (P=0.05)

Conclusions

- Criteria required for infection and inoculum production of *P. violae* on pansies have been identified. Similar criteria still need to be identified for *P. obducens* on impatiens.
- Tests on a range of impatiens and pansy seed lots have indicated the presence of low levels of downy mildew DNA in both species. A higher percentage of pansy seed lots contained downy mildew DNA than impatiens.
- Downy mildew DNA was detected more frequently and at higher levels from seedling roots than from the cotyledons.
- Irrespective of the presence of pathogen DNA in seed-lots, downy mildew symptoms have to date not been produced in any pansy or impatiens plants grown from such seed lots which therefore questions the relevance (viability) of the pathogen DNA detected.
- Latent infections can be detected using the primers and probes developed for *Peronospora* species.

Future work

- Continue work to develop protocols for infection of impatiens with *Plasmopara obducens*, including work on spore germination.
- Determine the sensitivity of isolates of *P. obducens* or *Peronospora violae* to selected fungicides using *in planta* tests.
- Continue fungicide crop safety assessments on young impatiens plug plants, taking into account any consequences from the changes to the LTAEU arrangements.
- Generate baseline sensitivity data for a range of isolates to the selected fungicides.
- Develop and further refine a robust GFP strategy for the management and control of downy mildew in bedding plants

Technology transfer

The project has had input into the up dating the impatiens downy mildew fact sheet and the good horticultural practice guidelines. Results from the project were presented at the BPOA / HDC 'Disease Seminar' on the 18th February 2009 at HRI Warwick.

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