Project Number:	PC 230a and PC230b
Project Title:	a) Control of downy mildew (Plasmopara obducens) an economically important foliar disease on impatiens b) Source of downy mildew (Plasmopara obducens) infection on impatiens
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AUTHENTICATION

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

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GROWER SUMMARY

Headline

Protectant spray programmes were the most effective in controlling downy mildew infections in impatiens. For curative activity, drenches (where available) were more effective than foliar sprays. Several effective and crop safe products have been identified but the introduction of metalaxyl-M resistant isolates in 2011 highlights the need to review and target programmes according to current infections. Oospores of *P. obducens* have been shown to overwinter under UK conditions and, as a result, could pose a threat to impatiens planted in soils contaminated by oospores.

Background and expected deliverables

Downy mildew of impatiens caused by *Plasmopara obducens* was first reported in the UK in June 2003 and caused considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. Initially emergency statutory action was taken by the Plant Health & Seeds Inspectorate (PHSI) and the downy mildew pathogen on impatiens was declared notifiable. This was revoked in 2005 on the proviso that the industry took on responsibility for management of the disease through implementation of an industry code of practice (Good Horticultural Practice (GHP). Between 2004 and 2006, the disease was not reported in commercial crops but reappeared at low-moderate levels in 2007, however, in 2008 and 2011 the disease was once again widespread and damaging. In 2008, the disease was principally found late-season in municipal and other outdoor plantings, whereas in 2011 the disease was first noticed in the early spring on nursery premises and was associated with imported vegetative material.

In the original UK 2003 outbreak, it was suspected that impatiens raised from seed or imported vegetative transplants (unrooted or rooted cuttings) may have provided the initial infection source of the disease, though this was not confirmed at the time. Over-wintering or resting spores (oospores) of the pathogen had been previously reported on/in seed in India but were not reported in the early UK outbreaks, suggesting that the pathogen's potential to over-winter under UK conditions was low. However during 2008, oospores were found in stem tissues of *I. walleriana* grown in the UK (Turner *et al*, 2009). Given this, the risk of disease carry-over between seasons on plant debris incorporated into soil has increased significantly; although the reality of this occurring has not been

demonstrated under UK conditions. 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 within seed-lots of *I. walleriana* or other species and cultivars in the UK.

Work carried out in a previous HDC project (PC 230) and the current projects (PC 230a and PC 230b) addressed a number of the key issues that will allow the industry to take responsibility for the overall management of downy mildew on impatiens; namely the development of techniques for the detection of the pathogen on both seed and young propagation material, identification of effective crop protection products to control downy mildew of impatiens and investigating the potential of seed-borne infections and overwintering of oospores as inoculum sources for disease development under UK conditions.

There are 2 main aims to projects PC230a and 230b:

1. To evaluate the efficacy of a range of fungicides for control of infection by *P. obducens* on impatiens (PC230a).
2. To gain a better understanding of the epidemiology and biology of impatiens downy mildew, especially in relation to the risk posed by potential sources of infection, in particular from seed-borne inoculum and oospore survival (PC230b).

Summary of the project and main conclusions

Fungicide efficacy testing

Fungicide efficacy: Results from the initial laboratory-scale efficacy tests, using the isolate collected in 2009, indicated that a range of different products were effective against impatiens downy mildew, particularly when applied in advance of infection i.e. as a protectant application. Where available, soil drench applications appeared to have better curative activity than the foliar sprays. The wide range of active ingredients showing effective protectant control of the disease is encouraging, as this means that spray programmes could be identified which do not rely on a single mode of action or active ingredient, thus reducing the risk of resistant populations developing and persisting.

Spray timing: Products identified with potential for the control of downy mildew symptoms caused by *P. obducens* were further examined to determine the appropriate time period

between applications. The results highlighted a number of products (including some experimental) which provided good protective activity when applied up to seven days prior to infection (as a protectant application) and as a result have the potential to be used as part of a weekly fungicide programme for the prevention of downy mildew infection of impatiens. These included Fubol Gold, Subdue, Paraat, Karamate Dry Flo, Rose Tonic (potassium phosphate) and Valbon. The two experimental products that showed promise were HDC F32 (fenamidone and fosetyl-al) and Percos. However, a lack of products that showed good curative activity has also been highlighted. This potentially could lead to control problems should infected material arrive on a nursery.

Baseline sensitivity data: Concentrations of active ingredient which reduced infection by *P. obducens* isolates by an average of 50% compared to the control (EC_{50}) were determined for the products Fubol Gold and Subdue. These products carry the greatest risk of development of resistance due to containing metalaxyl-M. EC_{50} values of 108 and 0.34 ppm for total active ingredient in Fubol Gold (metalaxyl-M + mancozeb) and Subdue (metalaxyl-M) respectively were established for the *P. obducens* isolate collected in 2009. EC_{50} values could not be established for the *P. obducens* isolates collected in 2011 as no control was achieved even following the application of full rate Fubol Gold (EC50>645ppm) and Subdue (EC50>56 ppm). These tests indicated that all the isolates collected from UK nurseries during 2011 were resistant to metalaxyl-M, and potentially suggested they had a common source.

Glasshouse-scale programme trials (on both impatiens and pansy downy mildew): To validate the small-scale laboratory experiments carried out in this and a previous project two large scale replicated trials were established, one on impatiens and the other on pansy/viola. In each trial 19 spray programmes (as both preventative and curative) were trialled alongside an untreated control. Both trials identified a number of spray programmes with good preventative activity; generally based around products containing metalaxyl-M. In both trials, curative spray programmes (where the programme was started after the onset of disease) were generally less effective than preventative programmes (where the programme was started in advance of introduction of the disease).

Detection of latent impatiens downy mildew infection

The downy mildew primers and probes first used in HDC project PC230, and which proved capable of detecting latent infections of downy mildew in pansy leaf tissues, have also been shown to detect latent infection of impatiens caused by *P. obducens*. 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.

Testing for seed-borne transmission of impatiens downy mildew

As a result of the tests carried out in this project no evidence was found to suggest that downy mildew of impatiens caused by *P. obducens* is seed-borne.

Over winter survival of Plasmopara obducens oospores

Experiments were set up to examine the survival of *P. obducens* oospores over the 2010 winter period. The use of the LIVE/DEAD® BacLightTM bacterial viability stain indicated that oospores were able to survive what was a particularly harsh winter period, with only a 5% drop in viability (100% down to 95%) between September 2010 and June 2011. Oospore viability could not be confirmed by either of the direct germination tests used in this study.

As a difference in metalaxyl-M resistance had been shown between isolates of *P. obducens* collected pre-2011 (metalaxyl-M sensitive) and those collected in 2011 (metalaxyl-M resistant) it seemed that, through resistance testing of isolates collected infections in the wider environment, it might be possible to determine whether oospores had overwintered and then gone on to cause infections during 2011. As a result a number of requests were made during September and October 2011 for infected plant material to be sent to Fera for testing.

Four downy mildew infected plants were received, three from 'back gardens' in Leeds, York and Coventry, and the fourth from a growers hanging basket located in Cambridge. There was no previous history of impatiens downy mildew at any of the four locations and all four were late season infections. All four infections were shown to be caused by the

metalaxyl-M resistant strain introduced in 2011 suggesting that they were caused by airborne sporangial inoculum.

Financial benefits

It is still not possible to predict the full financial benefits from this project. However, the impatiens downy mildew pathogen is aggressive and, under favourable environmental conditions, can cause significant economic losses. Therefore, if the project is successful in helping the industry to reduce the risk from the disease it will be of considerable economic benefit. If we assume a conservative retail cost per plant of 25p with approximately 94.5 million plants produced by the industry annually (97% from seed raised and 3% from vegetative material) then we have an industry value of £23.5M/annum for the production of *I. walleriana* alone (Davis, pers. comm.). If we estimate 10% plant losses of impatiens may be incurred due to the disease, then the financial benefit in impatiens alone could be as high as £2.4M/annum (in years where disease severity is high). Assuming losses also occur due to downy mildew infections in other crops e.g. pansy and viola, then the gross economic benefit of this R&D could be much higher.

Action Points for Growers

- Ensure any starting plant material (vegetative cuttings or seed) is disease free. If possible avoid importation of vegetative cutting material.
- If unrooted or rooted cutting material is imported, check it immediately on receipt, ideally prior to transfer to the glasshouse. If there are signs of downy mildew infection reject the cuttings immediately and seek confirmatory diagnosis using available Plant Clinic services.
- Isolate and clearly label vegetative cutting and seed crops, including those from different suppliers to allow containment of outbreaks and also traceability should future problems arise. This is particularly important with the identification of metalaxyl-M resistant isolates in 2011.
- For seed crops 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, especially late in the day, as this is likely to encourage infection. If it is necessary to water from overhead systems then do this early, on days when solar radiation levels will ensure the leaves have a chance to dry out quickly.

- Be aware of criteria for sporulation and infection and check susceptible crops regularly, making 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 protectant fungicide programme on the crop, ensuring a range of products with different modes of action is included to minimise the risk of resistance development. Consider also the need for fungicides active against other important pathogens especially on pansy & viola e.g. black root rot (*Thielaviopsis basicola*) & leaf-spot (*Ramularia* spp.).
- Following the introduction of metalaxyl-M resistant isolates to the UK in 2011 care should be taken when compiling fungicide programmes. Consider producing programmes that are not based around metalaxyl-M.
- Prior to use of any fungicide for the first time treat a few test plants prior to widespread use on the crop to ensure freedom from phytotoxicity.
- 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 with developments in fungicide approvals and changes to the Long Term Arrangements for Extension of Use (LTAEU) arrangements.
- Remove diseased plants and associated debris in floral displays and gardens as soon as possible. Do not leave them to decay *in situ* as this will allow resting spores (oospores) to contaminate the soil and infect impatiens planted the following year.
- Avoid planting impatiens into beds where the disease has previously been identified.

Future work

- The introduction of metalaxyl-M resistant *P. obducens* to the UK means that further efficacy studies are required to determine alternative effective spray programmes and also identify possible new active ingredients to replace metalaxyl-M.
- Monitoring the sensitivity of *P. obducens* isolates involved in any future outbreaks to metalaxyl-M (from both nurseries and parks/gardens) to help determine the

geographical spread and persistence of the metalaxyl-M resistant isolate in the wider environment.

- This project has indicated that *P. obducens* oospores are able to survive the harsh conditions encountered during the winter of 2010. Work is required to determine the length of time these structures are able to survive in soil and whether they are capable of re-infection, especially semi-mature plants from 6-packs or similar.
- Potential sources of inoculum in wild or introduced impatiens plant species need to be established, e.g. can *Impatiens noli-tangere* (a native Impatiens species to the UK) or Himalayan (Indian) balsam (*I. glandulifera*) as an introduced 'nuisance' species of Impatiens become infected by *P. obducens* and harbour inoculum, which may then act as an annual and persistent infection source to commercially grown impatiens.
- Are other cultivated *Impatiens* species susceptible to the disease e.g. *I. hawkeri* (New Guinea types) and could sources of resistance be transferred to *I. walleriana* to prevent future economic losses due to downy mildew.

SCIENCE SECTION

PC 230 A) Control of downy mildew (*Plasmopara obducens*) an economically important foliar disease on impatiens

PC 230 B) Source of downy mildew (*Plasmopara obducens*) infection on impatiens

Introduction

Downy mildew on *Impatiens walleriana*, was first reported in the UK by STC (McPherson & Finlay, pers. comm.) in June 2003 causing considerable economic damage to commercial crops and municipal plantings, especially, though not exclusively, in the South of England. STC provided a presumptive identification as *Plasmopara obducens* and, as it was considered unusual and a potential first UK record, Plant Health were notified, The identity of the fungus was subsequently confirmed as *P. obducens* by CSL (now Fera) (Lane *et al.*, 2005). 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 was undertaken (Jones, 2009) which concluded that: “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), McPherson and Brough, 2009).

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 or introduced relatives of Impatiens found in the wild e.g. *Impatiens noli-tangere and I. glandulifera*; however, in limited observations to date no such findings have been made. Commercial trade of impatiens is complex and involves seed houses, specialist propagators (including overseas), growers, the retail trade (including garden centres & mail order enterprises) and municipal production for civic displays in parks etc. (S. Coutts, pers. comm.). This chain of production 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, for the first time, resting spores were found in infected stem tissues of *I. walleriana* (Turner *et al*, 2009). 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) maybe incorporated into the soil in infected plant debris there is a potential increased risk of direct infection to subsequent outdoor planting schemes. However, how big this risk is, relative to directly sown seed crops, remains unknown, but repeat planting in situations where the disease was present the previous season should be avoided where possible.

Work carried out in the HDC projects PC 230, PC 230a and PC 230b addressed a number of the key issues that will allow the industry to take responsibility for the overall management of downy mildew on impatiens; namely the development of techniques for the detection of the pathogen on both seed and young propagation material, effective crop protection products to control downy mildew of impatiens and evaluate the potential of seed-borne infections and overwintering of oospores as inoculum sources for disease development under UK conditions.

Materials and Methods

1.1. *Plasmopara obducens inoculum production*

Unless otherwise stated the *P. obducens* isolate collected during project PC 230 (i.e. a metalaxyl-M sensitive isolate) was used during these tests. This isolate was from an Impatiens c.v. DeZire various colours sent to Fera on the 10th August 2009 (isolate 1). The isolate was maintained through fortnightly sub-culturing using the impatiens variety Accent White as the host. These infector plants were grown in a glasshouse maintained at a constant 20°C, with 12hr day period.

Inoculum production for the tests was started two weeks prior to the proposed inoculation date. Leaves bearing freshly produced sporangia of *P. obducens* were removed from the infector plants. The sporangia were washed from the leaves using sterile distilled water (SDW) and the concentration of sporangia established using a counting chamber. The sporangial concentration was adjusted to 10^4 sporangia mL^{-1} and two week old impatiens seedlings inoculated to run-off with the sporangial suspension. The inoculated plants were incubated for approximately 18hrs in the dark at 5°C; plants were incubated in propagator tops to prevent drying out of inoculum. Plants were then transferred to a glasshouse and grown on for 10-14 days. During this period plants were watered to the bottom to ensure sporangia were not prematurely produced. Sporangial production was initiated by wetting the upper surface of leaves the evening before they were required and incubating overnight in a propagator top, again to prevent drying of leaf surface.

1.2. *Detection of latent impatiens downy mildew infection caused by P. obducens.*

Three replicate pots were sown with approximately 20 seed of Impatiens (c.v. Accent white) and grown for two weeks at which point one seedling was removed from each pot and placed in three separate DNA extraction bags each labelled T -1. The remaining seedlings were inoculated to run off with a *P. obducens* sporangial suspension containing 10^4 sporangia mL^{-1}; one further seedling was removed from each pot and again placed in three separate extraction bags labelled T0. The inoculated seedlings were incubated in a propagator top at 5°C in the dark for 20 hrs at which point a further seedling was removed from each pot and placed in three separate extraction bags labelled T1. The remaining seedlings were grown on in a glasshouse maintained at 20°C for 8 days with seedlings removed and placed in appropriately labelled extraction bags 2, 4, 6, 7 and 8 days after

inoculation. Immediately after sampling all leaves were placed at -20°C until required for DNA analysis. After the final sampling all leaves were analysed for the presence of *P. obducens* DNA (see the first year report from HDC project PC 230 for details of primers and probes, DNA extraction and DNA analysis from leaf material).

1.3. *Efficacy of fungicides against the impatiens downy mildew pathogen P. obducens.*

1.3.1. *Fungicide efficacy testing - glasshouse (small scale) (Fera)*

A total of 17 fungicides (and related products) (16 as foliar sprays and six as drench applications) were tested for efficacy against the impatiens downy mildew pathogen *Plasmopara obducens* (Table 1). All products were applied according to the manufacturer's recommended rate. Foliar sprays were applied to run off and soil drenches were applied at volumes corresponding to 10% of the pot volume (with the exception of BAYF5388 which was applied at 100 mL L^{-1} of compost). Both foliar and drench treatments were applied at two timings: two days pre- or two days post-inoculation. Control plants were either sprayed or drenched with an equivalent volume of SDW.

Table 1. Fungicides used in efficacy testing against *Plasmopara obducens*.

Product	Active ingredient	Application type	Application rate (L^{-1})
Amistar	Azoxystrobin (250 g L^{-1})	Foliar spray	0.5 mL
HDC F32	Bayer experimental product	Foliar spray/ drench	2.25 g 1 g
HDC F33	Fenamidone + fosetyl-al	Foliar spray	1.25 mL
Percos	BASF experimental product	Foliar spray	0.4 mL
Epok	BASF experimental product	Foliar spray	0.4 mL
Fubol Gold	Fluazinam (400 g kg^{-1}) + metalaxyl-M (200 g kg^{-1})	Foliar spray/ drench	0.19 mL 0.25 mL
Karamate DryFlo	Mancozeb (640 g kg^{-1}) + metalaxyl-M (40 g kg^{-1})	Foliar spray	0.95 g
Olympus	Mancozeb (750 g kg^{-1})	Foliar spray	0.9 g
Previcur Energy	Azoxystrobin (80 g L^{-1})+ Chlorothalonil (400 g L^{-1})	Foliar spray	1.25 mL
Revus	Propamocarb-HCL (530 g L^{-1}) + fosetyl-al (310 g L^{-1})	Foliar spray/ drench	1.25 mL 1.66 mL
Rose tonic	Mandipropamid (250 g L^{-1})	Foliar spray	0.3 mL
Signum	Potassium phosphite	Foliar spray/ drench	10 mL 10 mL
Stroby	Boscalid (267 g kg^{-1}) + pyraclostrobin (67 g kg^{-1})	Foliar spray	0.75 g
Subdue	Kresoxim-methyl (500 g kg^{-1})	Foliar spray	0.15 g
Paraat	Metalaxyl-M (480 g L^{-1})	Drench	0.12 mL
Valbon	Dimethomorph (500 g kg^{-1})	Foliar spray/ drench	0.15 g 0.2 g
Vitomex	Benthiavalicarb (7.5 g kg^{-1}) + mancozeb (700 g kg^{-1})	Foliar spray	0.8 g
	Phosphonic acid + derivatives	Foliar spray	2.0 mL

Fresh inoculum was produced and plants inoculated as described in section 2.1. For each fungicide and control treatment, three replicate impatiens plants (6 weeks old) were inoculated with the sporangial suspension to run-off, ensuring that both the abaxial and adaxial surfaces of the leaf were covered. Plants were transferred to a propagator top and incubated at 5°C in the dark for 20h and before being placed in a glasshouse maintained at 20°C. After 14 days, leaves on all plants were wetted over night and then assessed for the presence of *P. obducens* sporangia.

1.3.2. *Effect of application timing on fungicide efficacy – glasshouse (small scale) (Fera)*

To investigate the effect of application timing on fungicide efficacy the tests described in 2.3.1 were repeated using application timings of 2 and 7 days pre- and post-inoculation and 14 days pre-inoculation. The fungicides applied at a particular timing were selected based on their efficacy at the previous application timing. Three replicate impatiens plants (6 weeks old) were used for each fungicide treatment, control and application timing.

Inoculum production, inoculation, fungicide application and assessment methodologies were as described in section 2.3.1.

1.3.3. *Baseline sensitivity testing*

Baseline sensitivity testing to metalaxyl-M (as Fubol Gold (foliar treatment) and Subdue (soil drench)) was originally to be carried out on isolates of both impatiens and pansy downy mildew (max of 10 isolates of each), however, a the limited incidence of pansy downy mildew and an outbreak of impatiens downy mildew in spring 2011 led to studies focusing on baseline testing of *P. obducens* isolates only. A total of 15 isolates from 11 nurseries were tested with all but one isolate (isolate 1 collected 2009) collected during the spring/early summer 2011.

Initial studies were carried out on isolate 1 using the method described in 2.3.1. using a 2 day pre-inoculation treatment of Fubol Gold or Subdue at full, 1/2, 1/4, 1/8 and 1/16 rates on 6 week old plants. As no disease was observed on any fungicide-treated plants, the methodology was altered to a seven day pre-inoculation fungicide treatment of two week old seedlings. Two sets of fungicide dilutions were used for each fungicide treatment (Table 2) with a lower dilution series used for isolate 1 and higher rates used for the remaining isolates. Treatments were replicated 3 times, with 10 seedlings per pot

assessed for disease symptoms 9 days after inoculation. For the Fubol Gold treatment plants were sprayed to run-off (2 mL product/pot) and for the Subdue soil drench treatment 40 mL product was applied per pot. Following inoculation, plants were grown on in the glasshouse for 10 days at which point the upper surface of the leaves were wetted overnight to encourage sporulation. Disease levels were assessed by counting the number of seedlings (out of 10) showing sporulation. The level of disease control compared to the untreated seedlings was calculated and plotted for each isolate/treatment combination. From these graphs the concentration of total active ingredient giving 50% disease control (EC_{50}) was calculated.

Table 2. Fungicide dilution rates used to calculate the EC_{50} values for metalaxyl-M as Fubol Gold and Subdue for 15 isolates of *Plasmopara obducens*.

	Fungicide application rate (compared to full recommended rate)	
	Fubol Gold	Subdue
Isolate 1	1/2, 1/4, 1/8, 1/16, 1/32	1/50, 1/100, 1/500, 1/1000, 1/5000
All other isolates	Full, 1/2, 1/4, 1/8, 1/16	Full, 1/5, 1/10, 1/50, 1/100

1.3.4. *Efficacy study – Glasshouse (large scale) (STC)*

Two large, fully replicated glasshouse trials were carried out at STC to investigate the performance of a range of fungicides and biological control products. The products were used singly, but with repeated applications, or as part of various 2 or 3 spray programmes (outlined in Table 3). The trials were carried out on impatiens (Carnival mixed) in the spring of 2010 and on pansy (Supreme Deep Blue) & viola (Sorbet Sunny Royale) during autumn of the same year. These times of year have been found to provide environmental conditions best suited to the development and spread of downy mildew in a range of crops e.g. cool and moist.

Both trials were arranged in a split plot arrangement on 4 mobile benches in a glasshouse compartment (Appendix 1, Figure A1). Half the benches received treatment applications prior to inoculation (protectant treatments) with the remaining half benches treated following inoculation (eradicant treatments). Each plot was comprised of ten 6-pack trays giving 60 plants/plot in the impatiens trial and four x 6-pack trays of both pansy and violas per plot in the pansy/viola trial. All treatments were applied as sprays using a Hozelock hand sprayer and a water volume of 1500L ha^{-1}. The application rate of each treatment is outlined in Table 4.

Table 3. Details of treatments applied to impatiens and pansy/viola trials.

Treatment	Spray timings		
	Spray 1* (2-3 days post planting)	Spray 2* (7-14 days post planting)	Spray 3* (1-2 days pre-dispatch)
1. Untreated control	-	-	-
2. Best Practice Programme 1.	Subdue	Karamate	Previcur Energy
3. Best Practice Programme 2.	Previcur Energy	Karamate	Subdue
4. Best Practice Programme 3.	Fubol Gold	-	Amistar
5. Best Practice Programme 4.	Previcur Energy	-	Fubol Gold
6. Straight Prog. 1	Serenade	Serenade	Serenade
7. Straight Prog. 2	Karamate	Karamate	Karamate
8. Straight Prog 3.	Amistar	Amistar	Amistar
9. Straight Prog. 4.	Signum	Signum	Signum
10. Straight Prog. 5	Revus	Revus	Revus
11. Straight Prog. 6	Paraat	Paraat	Paraat
12. Straight Prog. 7	Epok	Epok	Epok
13. Straight Prog. 8	Stroby	Stroby	Stroby
14. Exp. 2 Spray Prog 1	Subdue	-	Previcur Energy
15. Exp. 2 Spray Prog 2	Signum	-	Fubol Gold
16. Exp. 2 Spray Prog 3	Fubol Gold	-	Paraat
17. Exp. 2 Spray Prog 4	Stroby	-	Fubol Gold
18. Exp. 3 Spray Prog. 1	Subdue	Stroby	Paraat
19. Exp. 3 Spray Prog. 2	Previcur Energy	Fubol Gold	Serenade
20. Exp. 3 spray Prog. 3	Epok	Paraat	Revus

* Spray intervals were based on 7-14 days, but adjusted slightly to take account of weather patterns and infection potential. After last spray the plants were held for a shelf-life period of 2 weeks to assess the persistence of the fungicide application in terms of post-dispatch protection from downy mildew. **IT IS IMPORTANT TO NOTE THAT SEVERAL OF THE FUNGICIDES LISTED HERE ARE EXPERIMENTAL AND NOT CURRENTLY APPROVED FOR COMMERCIAL USE.**

Table 4. Treatment application rate details.

Product	Active ingredient	Application rate/ha	Product volume/L
Amistar	Azoxystrobin	1.0L	0.67ml
Epok	Fluazinam + metalaxyl-M	0.375L	0.25ml
Fubol Gold	Mancozeb & metalaxyl-M	1.9kg	1.27g
Karamate	Mancozeb	1.8kg/1000L water	1.8g
Paraat	Dimethomorph	0.3kg	0.2g
Previcur Energy	Propamocarb-HCl + fosetyl-aluminium	2.5L	1.66ml
Revus	Mandipropamid	0.6L	0.4ml
Serenade	*Bacillus subtilis*	10L	6.6ml
Signum	Pyraclostrobin + boscalid	1.35kg	0.9g
Stroby	Kresoxim-methyl	0.3kg	0.2g
Subdue	Metalaxyl-M	12.5ml/100L	0.125ml

Inoculum was introduced into both trials through the use of 'infector' plants. Plug plants (impatiens or pansy) with an existing downy mildew infection or which had already been exposed to downy mildew were potted-on into 6-packs and introduced into the crop by placing them in an even distribution along the central area of each bench (Figure 1). For 2-3 nights following introduction of the 'infector' plants the entire crop was irrigated overhead, ensuring the upper leaf surface was wet, and covered with polythene overnight, to encourage sporulation, spore germination and infection. The isolate of *P. obducens* used was collected in 2009 (isolate 1 in section 2.3.3.).

Figure 1. Image of impatiens trial (top) and pansy/viola trial (bottom) showing 'infector' plants down the centre of the benches.

On the pansy/viola trial the crop was re-infected with a *Peronospora violae* sporangial suspension to try and revive infection following a period of hot and sunny weather which 'dried-up' the already initiated infection in the crop. Following inoculation the crop was covered with polythene for two nights to increase the chances of infection.

Disease assessments were carried out on each of the trial crops over the duration of the study. Individual plants were scored for presence or absence of downy mildew sporulation or leaf symptoms (no sporulation). See Table A1 in Appendix 1 for a crop diary of each trial.

One week following the final application of products to the Impatiens crop a decision was made to investigate the persistence of the treatment effects in a commercial scenario e.g. planting out in an amenity site following sale from the propagation nursery. To this end two 6-packs from each replicate/treatment were removed from the glasshouse and planted out into a field site at STC for monitoring (48 plants/treatment for each type of spray programme). The plots were irrigated using an overhead (irrigator) to maintain moisture during a period of increasing temperatures.

1.4. *Potential for seed borne infections caused by Plasmopara obducens*

As part of the previous project (PC 230) a large number of impatiens seed of different varieties were tested for the presence of downy mildew pathogen DNA. From this, 17 'infected' seed lots, with a range of pathogen DNA levels, were selected and 200 seed from each lot sown in plug trays and grown on for seven weeks. During this period plants were watered from the top as late in the day as possible and regularly covered overnight with polythene sheets and assessed for sporulation; any sporulating leaves were removed to prevent spread of the disease. All plants were grown in a clean glasshouse free from downy mildew infected plant and any potentially sporulating plants were assessed away from the remaining test plants to try and prevent cross contamination.

At the end of the test, five plants from each batch of 200 were analysed for presence of pathogen DNA using the TaqMan PCR assay validated in PC 230 to try to correlate any presence of *P. obducens* DNA in the plant to disease expression.

1.5. *Over winter survival of P. obducens oospores*

1.5.1. *Oospore production and extraction*

Oospore-rich plant material was produced by infecting impatiens plants with *P. obducens* and growing the plants in conditions known to prevent sporangial production on the leaves i.e. by ensuring that the leaves did not become wet overnight. Dark streaking of stem material, indicating oospore production, generally occurred four to five weeks after infection (Figure 2).

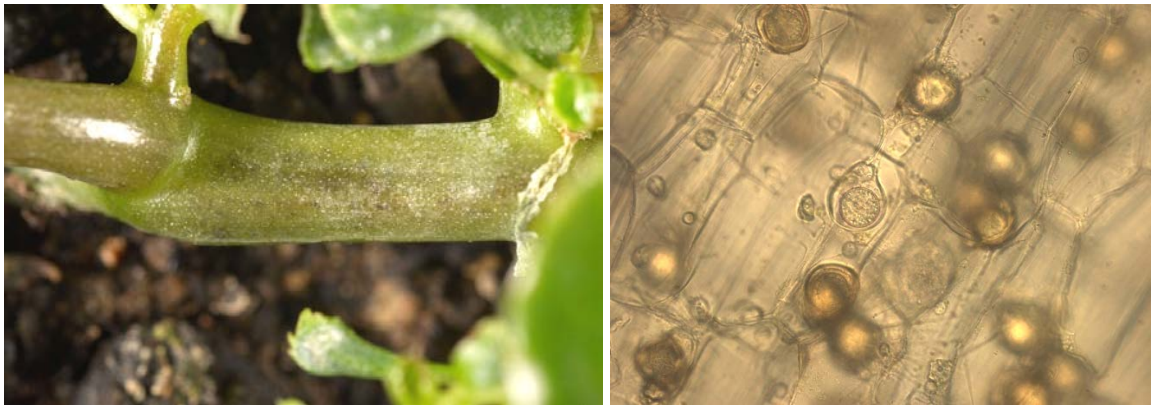


Figure 2. Dark streaking of impatiens stem tissue (left) indicating the presence of *Plasmopara obducens* oospores (right).

Oospores were extracted from the stem material by finely grinding stems in 3-5 mL SDW. The resultant suspension was initially passed through a 53 µm pore size filter followed by a 15 µm filter. The filters were then washed in 50 mL SDW. As the average size of a *P. obducens* oospore is 27 µm oospores passed through the first filter and are collected on the second. The oospores were harvested from the second filter in 1 mL of SDW.

1.5.2. *Viability testing*

A number of methods were used to determine oospore viability. These included both the use of vital stains and direct germination tests. Methods used for the germination tests were based on those reported for other *Plasmopara* species e.g. *P. viticola* and *P. halstedii*.

Direct germination tests

Test 1 - based on a method described by Spring and Zipper (2000) for *P. halstedii.*
Oospores were extracted as described in section 2.5.1. and resuspended in sterile distilled water containing rifampicin (10 ppm) to give a concentration of $1x10^3$ oospores per mL. Sixty to 80 mL of this suspension was pipetted onto a cavity slide and covered with a cover slip. The slides were then placed in a plastic box and kept in a saturated atmosphere at a temperature between 15 and 20°C. Slides were examined regularly for germination and additional diluents (water plus rifampicin) added as required.

Test 2 - based on a method described by Wong *et al*. (2001) for *P. viticola.*

Six 10-mm diameter leaf discs, taken from glasshouse grown impatiens, were floated abaxial side (lower side) down, on a suspension containing 20 mL of distilled water and 5 crushed stems rich in oospores. One disc was removed daily from the surface of the water and placed on moist tissue in a moisture chamber with the abaxial side exposed. The moisture chamber was incubated at room temperature (between 15 and 20°C) and the leaf discs examined daily for signs of sporangial production.

Viability staining

The LIVE/DEAD® BacLightTM bacterial viability stain was used to determine oospore viability. The stain utilises a mixture of a green and a red-fluorescent nucleic acid stain. These stains differ both in their spectral characteristics and in their ability to penetrate healthy cells, with a living cell fluorescing green and dead cells fluorescing red. The stain was applied to oospore suspensions according to the manufacturer's instructions.

1.5.3. *Survival experiment*

Twenty nylon bags were filled with approximately 10 impatiens stem fragments containing high numbers of oospores (10 bags with dried fragments and 10 with fresh fragments) which were tied and laid on the soil surface (Figure 3). Nylon was chosen as it would not interfere with environmental influences on the oospores and would allow rapid and easy recovery of fragments from the soil surface. Two bags (one containing dried and the other fresh fragments) were recovered at intervals between September 2010 and August 2011 and oospore viability assessed using methods established in 2.5.2. Meteorological data were collected thought the test period.

Figure 3. Nylon bags containing impatiens stem fragments containing oospore of *Plasmopara obducens* - October 2010.

1.5.4. *Oospore survival – monitoring infected garden plants.*

As a difference in metalaxyl-M resistance had been shown between isolates of *P. obducens* responsible for downy mildew infections pre-2011 (metalaxyl-M sensitive) and those responsible for the disease in 2011 (metalaxyl-M resistant) it seemed it could be possible to determine whether oospores had survived to cause infection during 2011. This could be done through testing *P. obducens* isolates that caused IDM infections in the wider environment (park gardens etc) during 2011 for sensitivity to metalaxyl-M, if an isolate could be controlled by metalaxyl-M then it was likely to be one that had survived as oospores the soil whereas if no control of the isolate was achieved then the disease was likely to be caused by the isolate introduced this year.

As a result a number of requests were made during September and October 2011 for downy mildew infected impatiens planted in the wider environment to be sent to Fera for testing. Samples that arrived at the laboratory were moist chambered overnight to encourage fresh sporulation. From this fresh sporulation a sporangial suspension was produced and used to inoculate two pots of impatiens each consisting of 10 two week old seedlings. One of the pots was then treated with a full rate Subdue soil drench as described in section 2.3.1. and the other with an equivalent amount of water. Plants were incubated overnight (as described previously) and transferred to a glasshouse kept at 20°C. After 8-10 days both the upper leaf surface of both sets of seedlings were moistened over night to encourage sporulation and then assessed for disease.

Results

2.1 *Detection of latent infection caused by P. Obducens*

TaqMan results are often presented as a mean cycle threshold (Ct) value, with the Ct value representing the number of amplification cycles after which fluorescence, indicating the presence of target 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 any Ct value obtained in this experimental work has been subtracted from 40 so that the resultant value increases with an increasing level of DNA.

Tests to determine whether the primers and probes could detect latent downy mildew infection in impatiens leaf material were carried out using a seedling assay. Seedlings inoculated with fresh *P. obducens* sporangia were, following an initial spore germination step, incubated at 20°C and DNA levels analysed over a period of 8-days. No *P. obducens* DNA was detected from impatiens seedlings sampled prior to inoculation (T -1, Figure 4). The presence of sporangia/zoospores on the seedling material (T0, Figure 5a) produced an increase in the level of *P. obducens* DNA. This was followed by a further increase in *P. obducens* DNA at the T1 sampling, possibly related to germination and production of a germ tube by the zoospores (Figure 5b). *P. obducens* DNA levels in the two day sample dropped slightly compared to those detected at T1; this drop was similar to that seen in a similar study carried out on *Peronospora violae* in HDC project PC 230. Levels of DNA increased steadily between four and seven days, during the period of latent build-up. The levels of *P. obducens* DNA then increased dramatically once sporulation occurred at the 8 day sampling time.

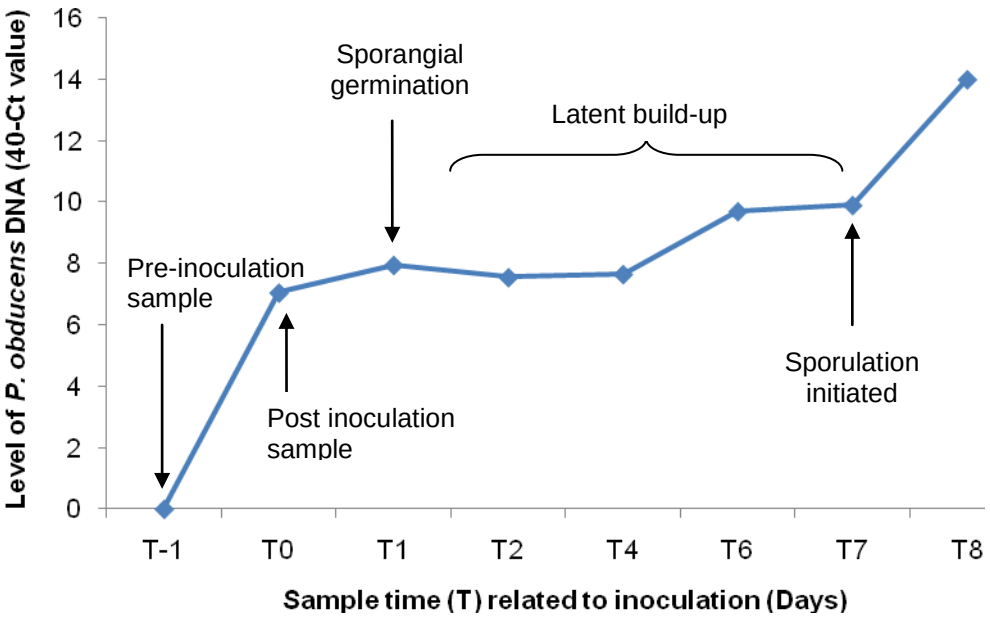


Figure 4. Detection of latent build-up of *Plasmopara obducens* DNA following inoculation of impatiens with sporangia.

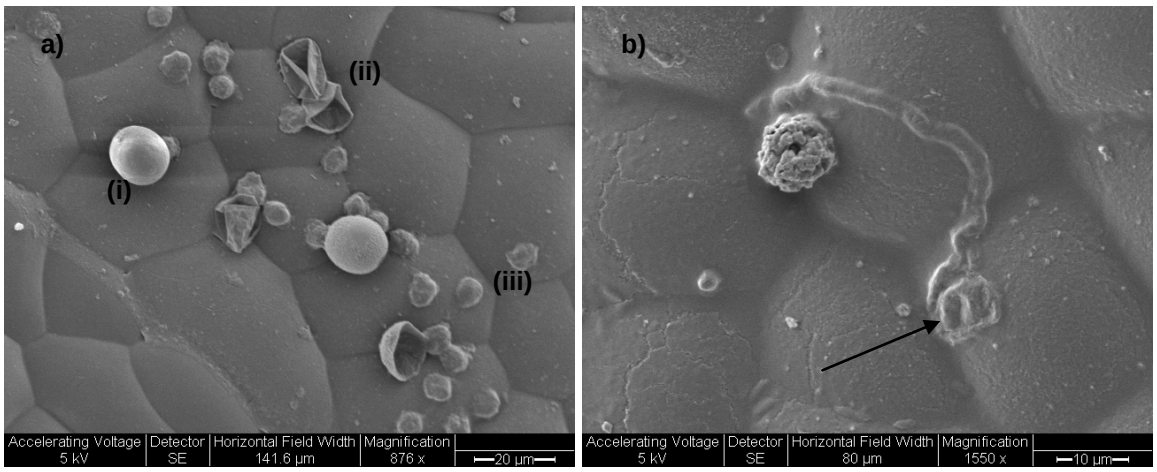


Figure 5. Electron micrographs of *Plasmopara obducens* on impatiens leaf surface a) showing full sporangia (i), collapsed empty sporangia (ii) and zoospores (iii), and b) a germinating zoospore (arrowed).

2.2 Efficacy of fungicides against the impatiens downy mildew pathogen *P.obducens*

2.2.1 Fungicide efficacy testing – glasshouse (small scale) (Fera)

Sixteen products were tested for efficacy against *P. obducens* when applied as foliar sprays. In these initial tests sprays were applied either as a protectant treatment (2 days before plant inoculation) or a curative treatment (2 days after inoculation). When applied as a protectant, all products caused a significant reduction in disease (Figure 6a), with the

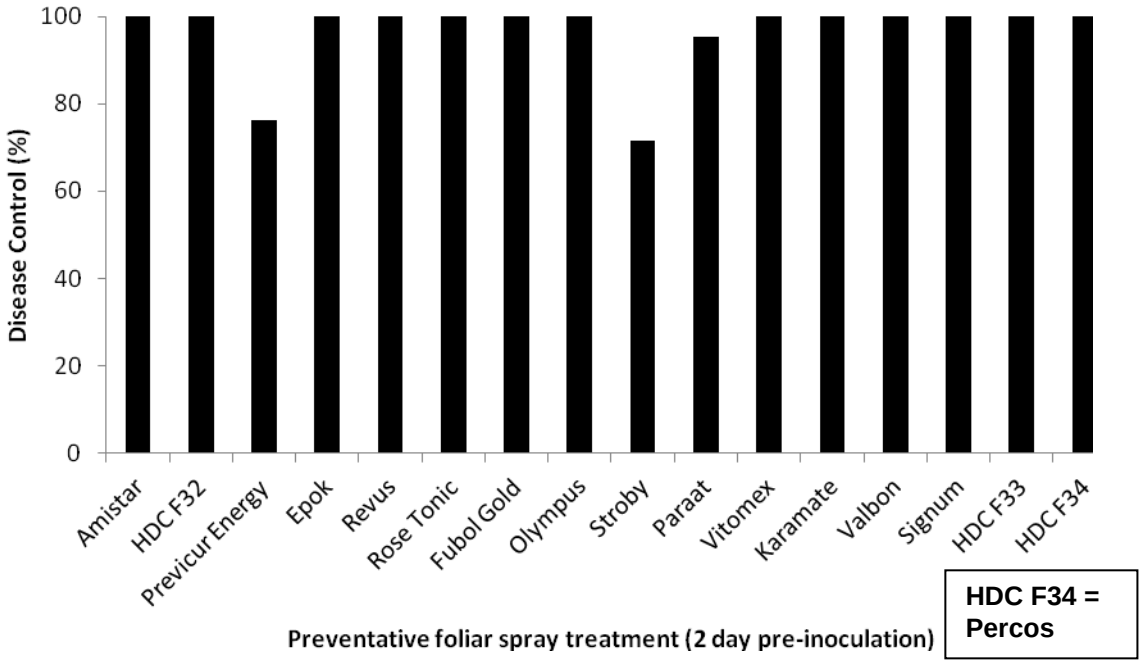
level of control ranging from 100 to 71%; with 13 of the 16 treatments providing complete control. Products were less effective when applied as a curative treatment (Figure 6b). As with the protectant treatments all products resulted in some level of disease control with the level of control ranging from 89 to 14%. The level of control achieved in the curative test was significant for all but three of the products trialled (Karamate Dry Flo, Valbon and Amistar). The best performing products in the curative foliar spray tests were Fubol Gold, Rose Tonic, HDC F33, Olympus and Stroby.

Six products were tested as drench applications for efficacy against *P. obducens*, again initial tests were carried out to examine protectant and curative activity either through application 2 days pre or 2 days post inoculation of impatiens. Results from these initial drench tests (Figure 7a and b), unlike those for the foliar spray tests, indicated that all but one of the products tested (Previcur Energy) performed equally well as a curative or as a protectant treatment, with four of the six products (Subdue, Epok, Rose Tonic and HDC F32) giving 100% control in both tests. Previcur Energy did not provide any control in these tests when applied as a protectant, but did provide significant control when applied as a curative.

No phytotoxicity effects were apparent from any of the treatments, confirming the results reported in the final report for PC 230.

Results from the initial laboratory-scale efficacy tests, using the isolate collected in 2009, have therefore indicated that a range of products were effective against impatiens downy mildew, this was especially true when the products were applied as a protectant. Soil drench applications appeared to have better curative activity that the foliar sprays. The wide range of active ingredients showing effective protectant control of the disease is encouraging as this means effective spray programmes, could be identified which do not rely on a single mode of action or active ingredient could, in theory, be developed thus reducing the risk of resistant populations developing.

a)



b)

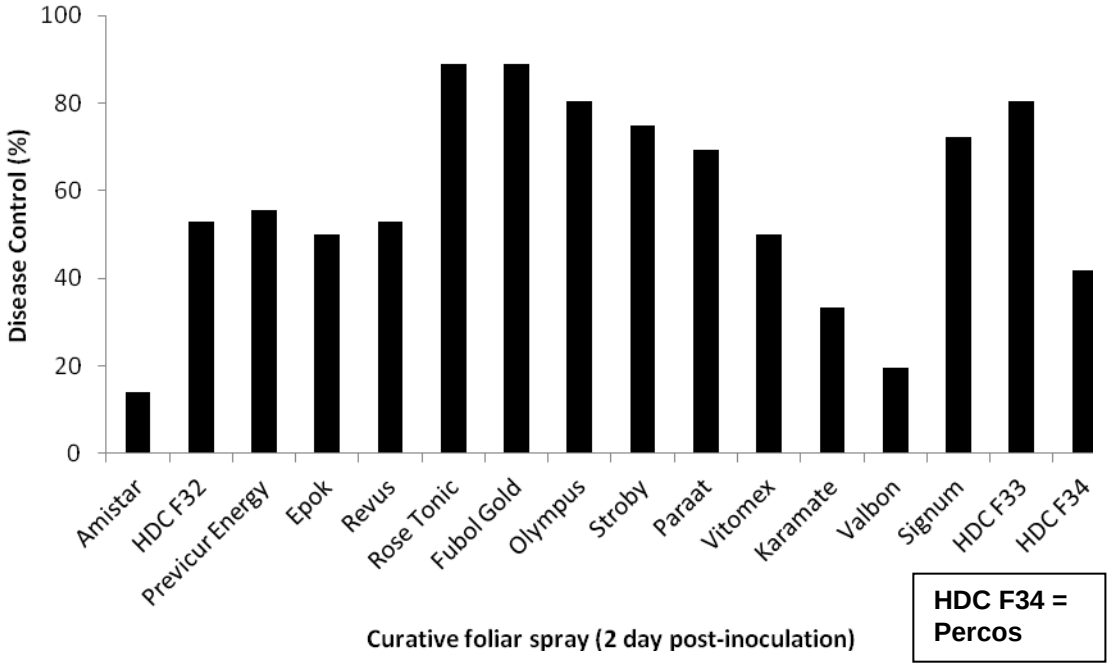
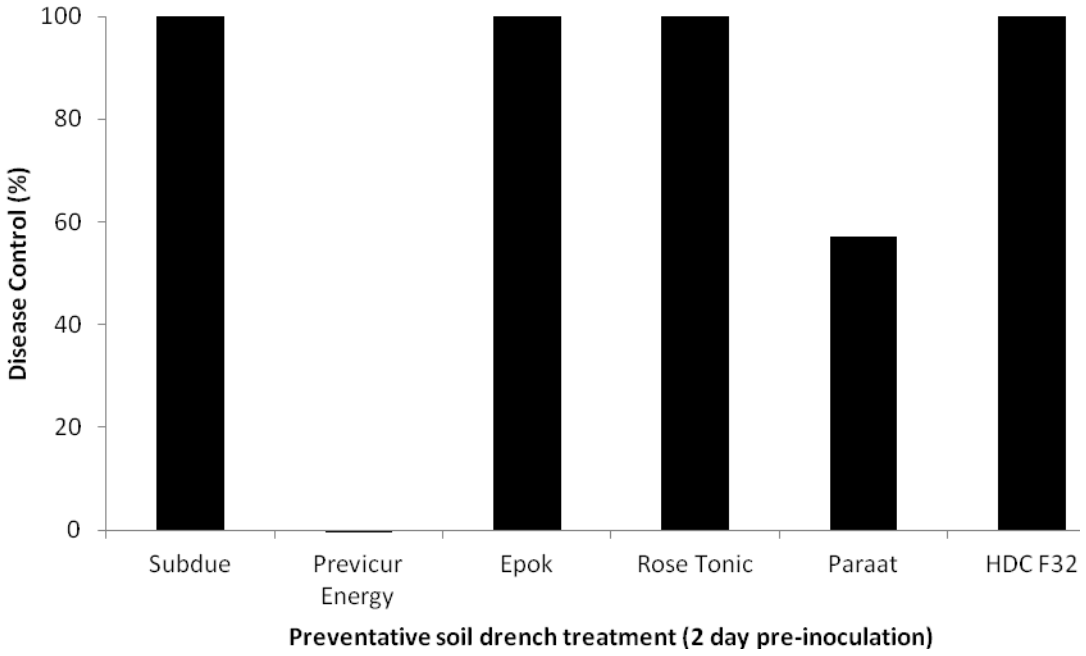


Figure 6. Efficacy of foliar spray applications against *Plasmopara obducens* on impatiens applied as a) preventative treatments (2 days pre-inoculation) and b) curative treatments (2 days post-inoculation).

a)



b)

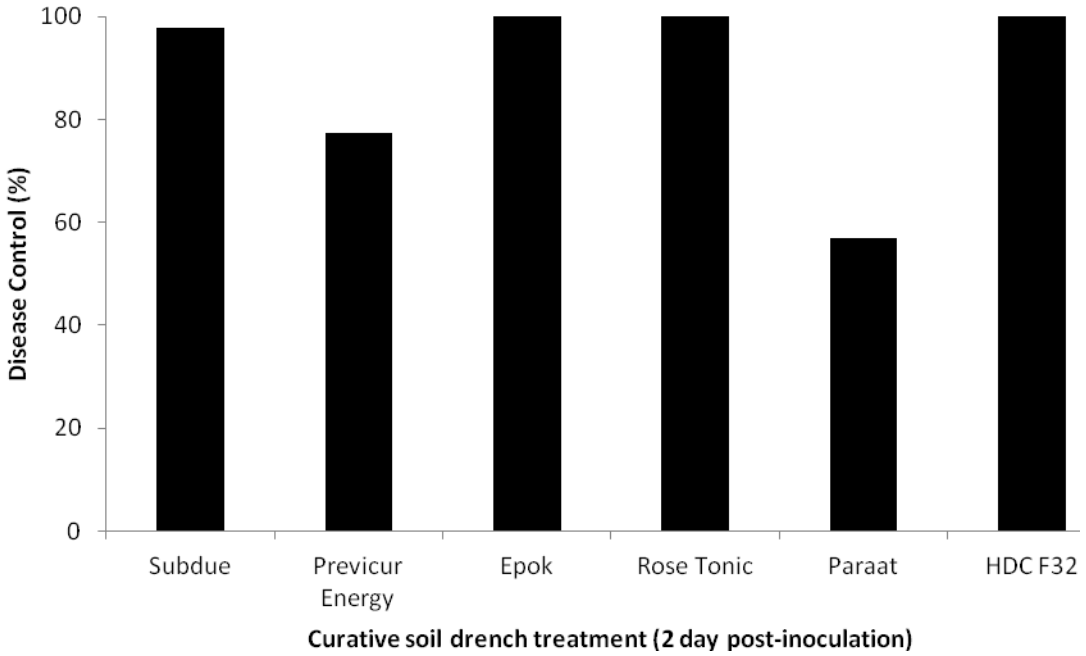


Figure 7. Efficacy of soil drench applications against *Plasmopara obducens* on impatiens applied as a) preventative treatments (2 days pre-inoculation) and b) curative treatments (2 days post-inoculation).

2.3 *Effect of application timing on fungicide efficacy – glasshouse (small scale) (Fera)*

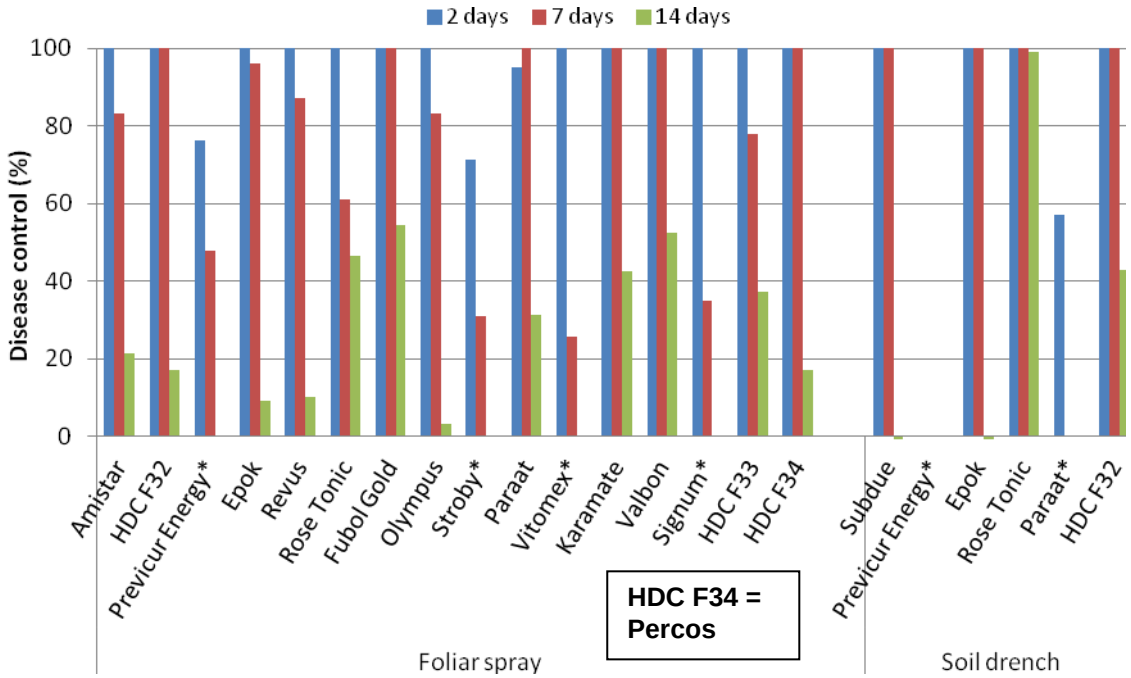
Products shown to have potential for the control of downy mildew symptoms caused by *P. obducens* were further examined to determine the appropriate time period between applications for each treatment. Timings were evaluated for 16 foliar sprays and six drenches (Figure 8) as both protectant and curative treatments at the 2 and 7 day time intervals. For the 14 day treatment the number of products assessed was reduced to 12 foliar sprays and four soil drenches applied as protectant treatments only.

Six foliar sprays (Fubol Gold, Paraat, Karamate, Valbon, HDC F32 and Percos) and four soil drench treatments (Subdue, Epok, Rose Tonic and HDC F32) when applied as a protectant gave 100% control of downy mildew even when treatments were applied seven days prior to the infection of plants with *P. obducens* (Figure 8a). A further five foliar treatments (Amistar, Epok, Revus, Olympus and HDC F33) gave a significant level of downy mildew control ($p = 0.05$) at the seven day protectant timing, although the level of control was reduced compared to the two day pre-inoculation treatment (from 100% to between 95 and 80%). The remaining foliar applications (Previcur Energy, Rose Tonic, Stroby, Vitomex and Signum) provided some control at the seven day pre-infection timing, but the control achieved was not significant compared to the untreated control plants. No treatment gave significant disease control when applied 14 days prior to infection.

As with the protectant treatments the level of disease control achieved with curative treatments reduced as the time between inoculation and product application increased (Figure 8b). No curative treatment gave 100% control of disease where the window between infection and treatment was greater than two days. Where the gap between infection and treatment was seven days, Fubol Gold, Rose Tonic, Epok and the experimental product HDC F32 applied as foliar sprays gave the greatest level of curative disease control (64, 52, 55 and 50% respectively); however this level of control was not significant. No curative soil drench treatment was effective where the gap between infection and treatment was greater than two days.

These tests have indicated a number of products (including some experimental) that provide good protective activity when applied up to seven days prior to infection and as a result have the potential to be used as part of a weekly fungicide programme for the prevention of downy mildew infection of impatiens. However, a lack in curative activity in these products has also been highlighted, which could lead to control problems should pre-infected plant material e.g. vegetative cuttings be delivered to a nursery.

(a)



(b)

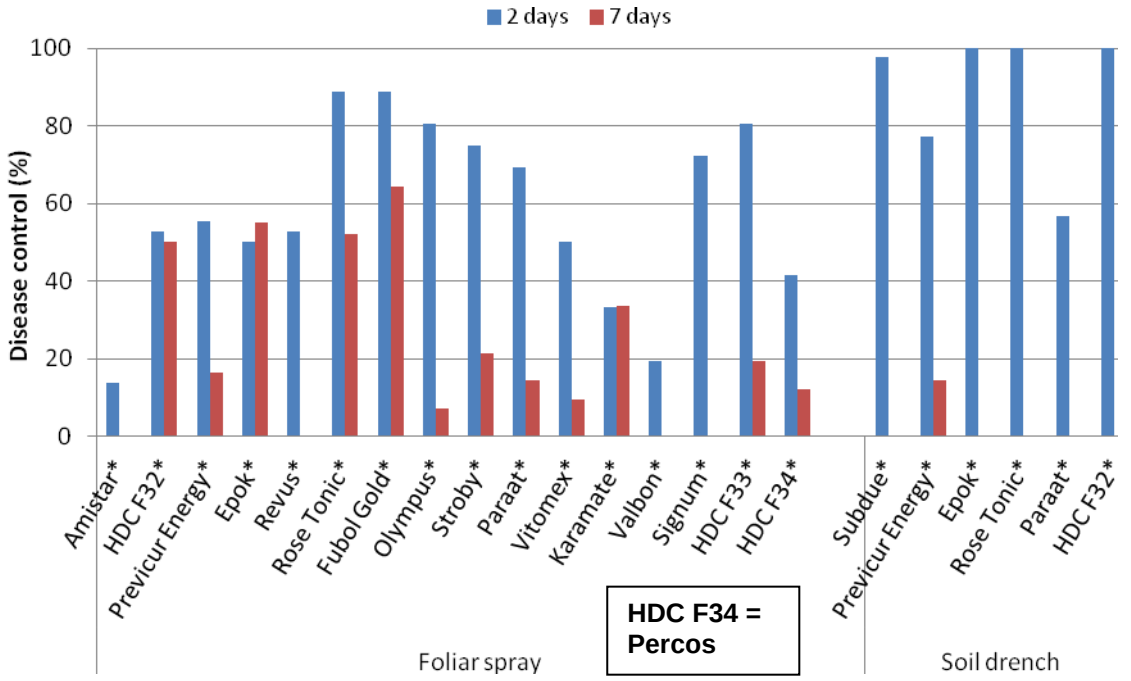


Figure 8. The effect of (a) preventative and (b) curative fungicide application timings on impatiens downy mildew symptoms caused by *Plasmopara obducens*. Products marked with * were not tested at the 14 day timing.

2.3.1 Baseline sensitivity testing

Baseline sensitivity testing was undertaken for a total of 15 isolates from 11 nursery outbreaks. Fourteen of the isolates were collected during the spring/early summer of 2011 and these were compared to a baseline isolate collected in 2009. Tests were carried out using Fubol Gold (metalaxyl-M + mancozeb) foliar spray and Subdue (metalaxyl-M only) soil drench applications to determine the effective concentration giving a 50% reduction in disease symptoms compared to the control (EC_{50}). Results for each isolate are expressed as an EC_{50} of the total active ingredient.

The EC_{50} values obtained for the baseline isolate were 0.34 and 188 ppm for Subdue and Fubol Gold respectively (Table 5). For the remaining isolates an EC_{50} value was only confirmed for isolate 15 treated with Fubol Gold (546 ppm), the EC_{50} values for all other isolates for both Fubol Gold and Subdue could not be determined as they were above the full application rate for the product. Some control of isolates 2-12 was achieved using full rate Fubol Gold, however there was no disease control following a full rate Subdue treatment (Figure 9). The original EC_{50} tests were carried out using a seven day pre-inoculation treatment, however a similar lack of disease control was obtained when plants were inoculated with isolates 2, 8, 12 or 14 and treated with full rate Subdue on the same day; this 'same day' test gave 100% control of isolate 1. The superior performance of Fubol Gold compared to Subdue against these isolates suggests that the control achieved following treatment with Fubol Gold was a result of the activity of the mancozeb component in the formulation.

The results outlined here indicate that resistance to metalaxyl-M had appeared in the *P. obducens* isolates collected during 2011. The development of resistance does not seem to have affected the fitness of the isolate as, if anything, the isolates appeared to be more aggressive than the 'sensitive' baseline isolate collected in 2009. The development of resistance to metalaxyl-M in *P. obducens* will undoubtedly cause problems for the control of impatiens downy mildew in the future, as the previously most effective systemic component in recommended control programmes is now effectively rendered redundant and the efficacy of the other alternative actives against the metalaxyl-M resistant isolates has not yet been established. However, the true extent of the problem will only be fully revealed over the coming years as we gain a clearer understanding of the potential of the metalaxyl-M resistant strain(s) to over-winter in the UK. In reality, through good hygiene (removal of all infected plant debris followed by disinfection etc) it should be possible to

minimise the reoccurrence of the metalaxyl-M resistant isolate on nurseries where there have been problems this year; however the introduction of the isolate into the wider environment through planting of infected plants into parks and gardens may cause problems for the industry in the future.

Table 5. EC_{50} results for foliar (Fubol Gold) and soil drench (Subdue) applications. EC_{50} values are for total active ingredient.

Isolate	Nursery	Total a.i. EC_{50} (ppm)	
		Fubol Gold*	Subdue*
1[#]	1	188	0.34
2	2	>645 (13%)	>56 (0%)
3	3	>645	>56 (0%)
4	2	>645 (43%)	>56 (0%)
5	2	>645 (47%)	>56 (0%)
6	4	>645	>56 (0%)
7	5	>645 (20%)	>56 (0%)
8	5	>645 (47%)	>56 (0%)
9	6	>645 (23%)	>56 (0%)
10	7	>645 (37%)	>56 (0%)
11	7	>645 (47%)	>56 (0%)
12	8	>645 (47%)	>56 (0%)
13	9	>645	>56 (0%)
14	10	>645 (23%)	>56 (0%)
15	11	546	>56 (0%)

* Figures in parenthesis indicate the level of control achieved where an EC_{50} was not obtained.

baseline isolate from 2009

Figure 9. Comparison of downy mildew sporulation on control plants (left) and full rate Subdue treated plants (right) inoculated with a metalaxyl-M resistant isolate of *Plasmopara obducens* collected during 2011.

2.3.2 Efficacy study – glasshouse (large scale) (STC)

Impatiens trial

An interim assessment was carried out 13 days after the application of the first protectant spray. At this point, infection had started to develop on all control plants and also on plants where Previcur Energy, Serenade or Stroby had been applied as the first protectant treatment (Appendix 1, Table A2). Plants treated with products containing metalaxyl-M (Subdue, Epok or Fubol Gold), mancozeb (Karamate or Fubol Gold), mandipropamid (Revus), dimethomorph (Paraat) or a strobilurin (Amistar or Signum) remained free from infection at this point in the study.

The first full assessment of the trial was carried out on the 2nd June 2010 (Figures 10 and 11). By this time, all the protectant fungicides and 2 out of the 3 eradicant treatments had been applied. Downy mildew disease levels, as assessed by either sporulating or symptomatic plants, showed that infection was generally lower where protectant

treatments were applied. The lower level of disease in the control plots in the protectant treatment trial compared to those on the eradicant treatment trial is likely to be due to the reduction in inoculum in the adjacent comparative treated plots. There were five protectant programmes where no sporulating plants were observed T2, T7, T10, T12, and T20 (Figure 10). At this stage, all of the eradicant programmes had reduced the incidence of symptoms compared to the untreated control programme (T1). However, symptoms were particularly reduced following the application of programmes T2 (Subdue/Karamate), T4 (Fubol Gold only), T12 (Epok x 2), T14 (Subdue only) and T18 (Subdue/Stroby).

The final disease assessment on this crop was carried out on the 28th June 2010. By this date, 2 x 6-pack module trays from each treatment had been removed for planting out in the field site and therefore this assessment was carried out on 3 of the remaining 6-packs/treatment (72 plants in total). The plants were assessed for sporulation only (Figure 12). A similar incidence of sporulation was observed in the untreated plants in both the protectant and eradicant programmes. In both the eradicant and protectant treatments a number of the products/programmes resulted in plants with a low level, or no sporulation present e.g. T10 (Revus x 3), T12 (Epok x 3), T2 (Subdue/Karamate/Previcur Energy), T4 (Fubol Gold/Amistar), T15 (Signum/Fubol Gold), T16 (Fubol Gold/Paraat), T18 (Subdue/Stroby/Paraat) and T20 (Epok/Paraat/Revus). Other treatments were effective but only in one or other of the treatment regimes e.g. T9 (Signum x 3) showed good control in the eradicant part of the trial, but much poorer control of sporulation in the protectant area. A number of the treatments did not provide much control of sporulation in either parts of the investigation e.g. T6 (Serenade x 3), T7 (Karamate x 3), T8 (Amistar x 3), T11 (Paraat x 3), T13 (Stroby x 3) and T17 (Stroby and Fubol Gold) and for some of these treatments this represents a significant alteration to the results seen during the second assessment. This assessment was carried out approximately 4 weeks after the final protectant application and 3 weeks after the final eradicant products were applied. It is possible that the treatment effects may have worn-off, allowing plants to become re-infected in the glasshouse by a second or third infection cycle of spore release. However, where crops remained free of sporulation there are some clear indications of good efficacy.

The infected plants which were transplanted to the field were monitored over an additional 4 week period. The plants in the untreated plots in each treatment regime deteriorated rapidly, and were soon followed by the remaining treatment plots. No clear indication of product persistence was determined. However, the field area remained available for an interim period for oospore survival studies.

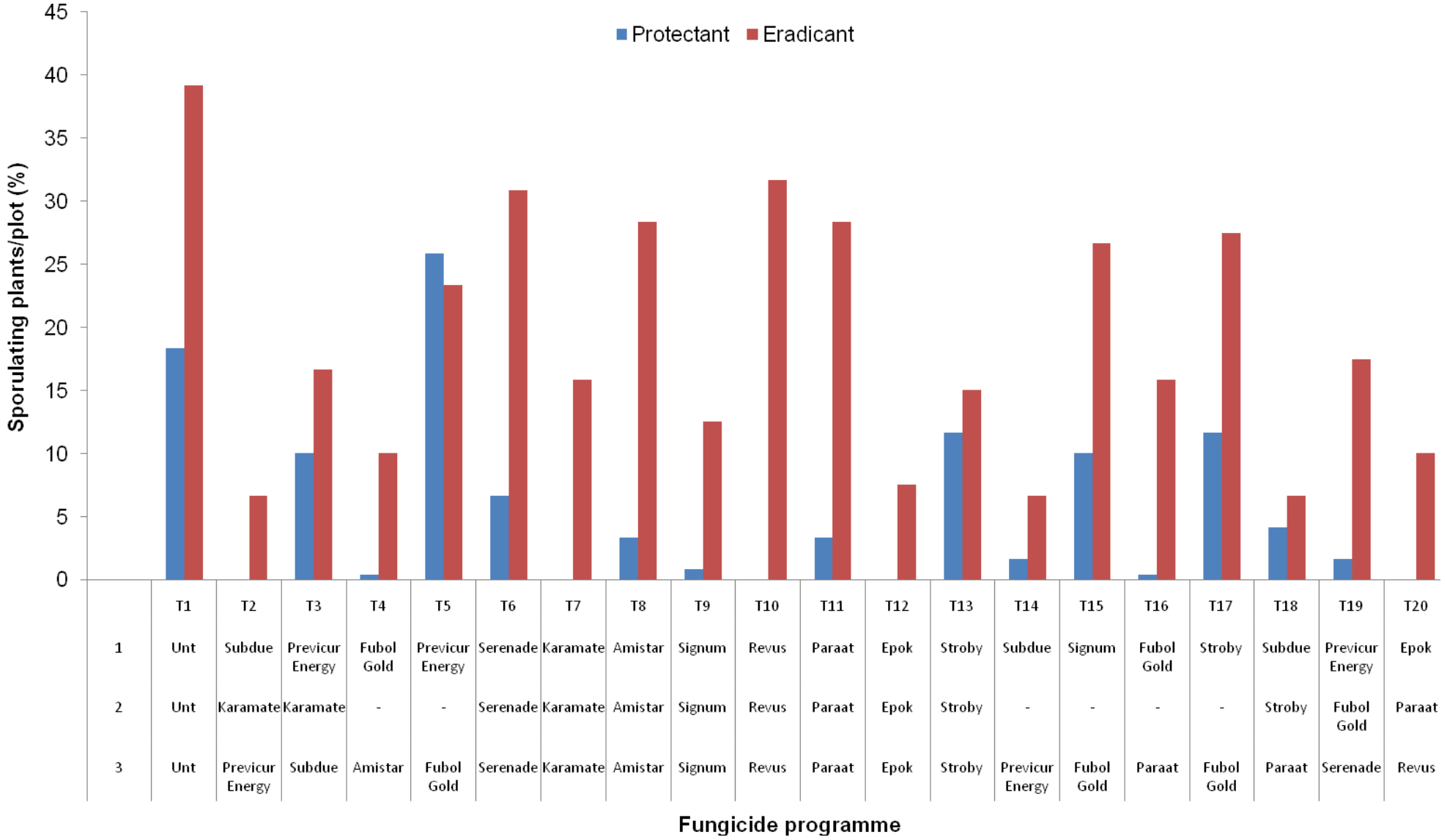


Figure 10. Effect of fungicide programme on impatiens downy mildew infection (% sporulating plants) as assessed on 2nd June 2010.

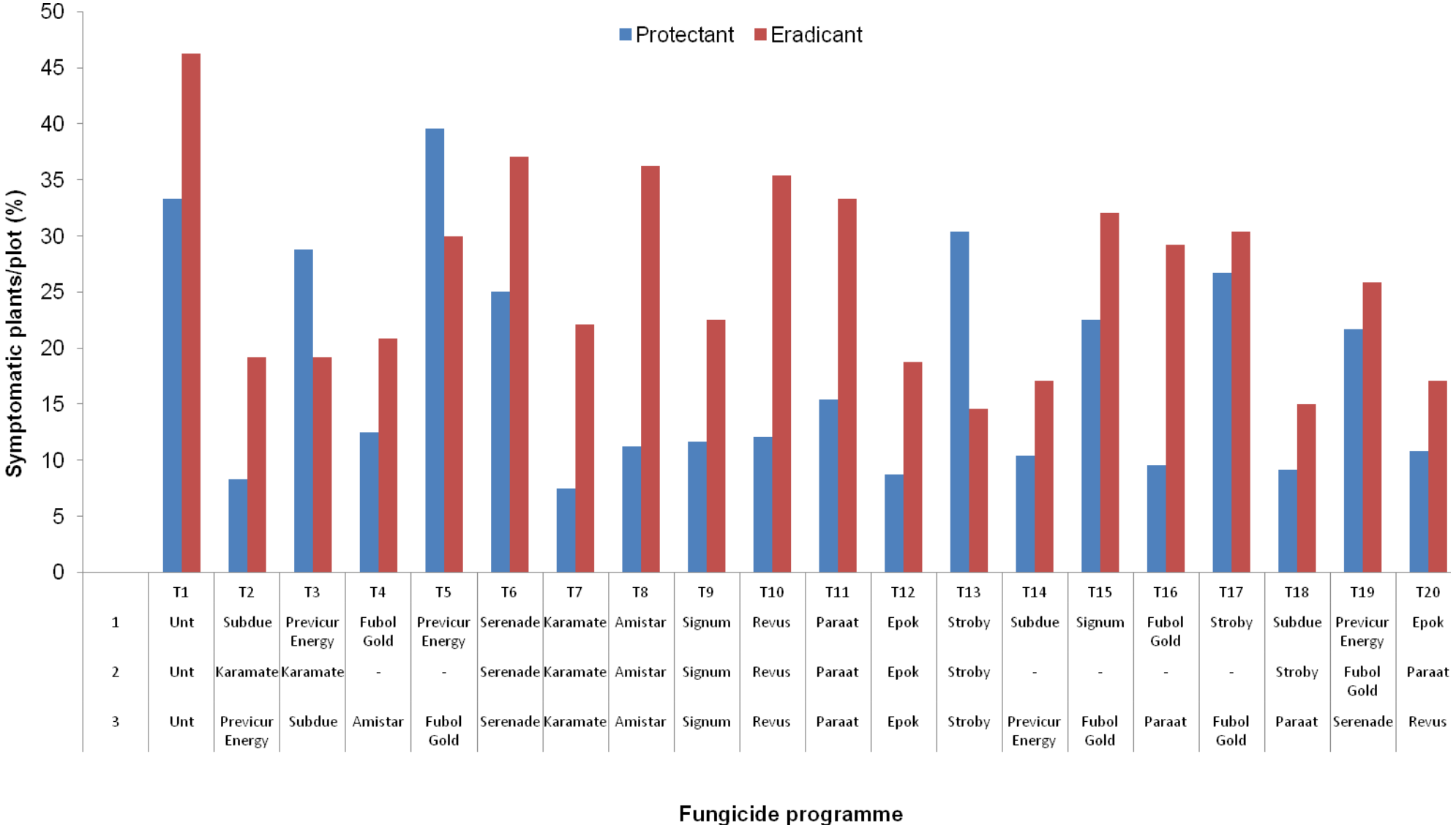


Figure 11. Effect of fungicide programme on impatiens downy mildew infection (% symptomatic plants) as assessed on 2nd June 2010.

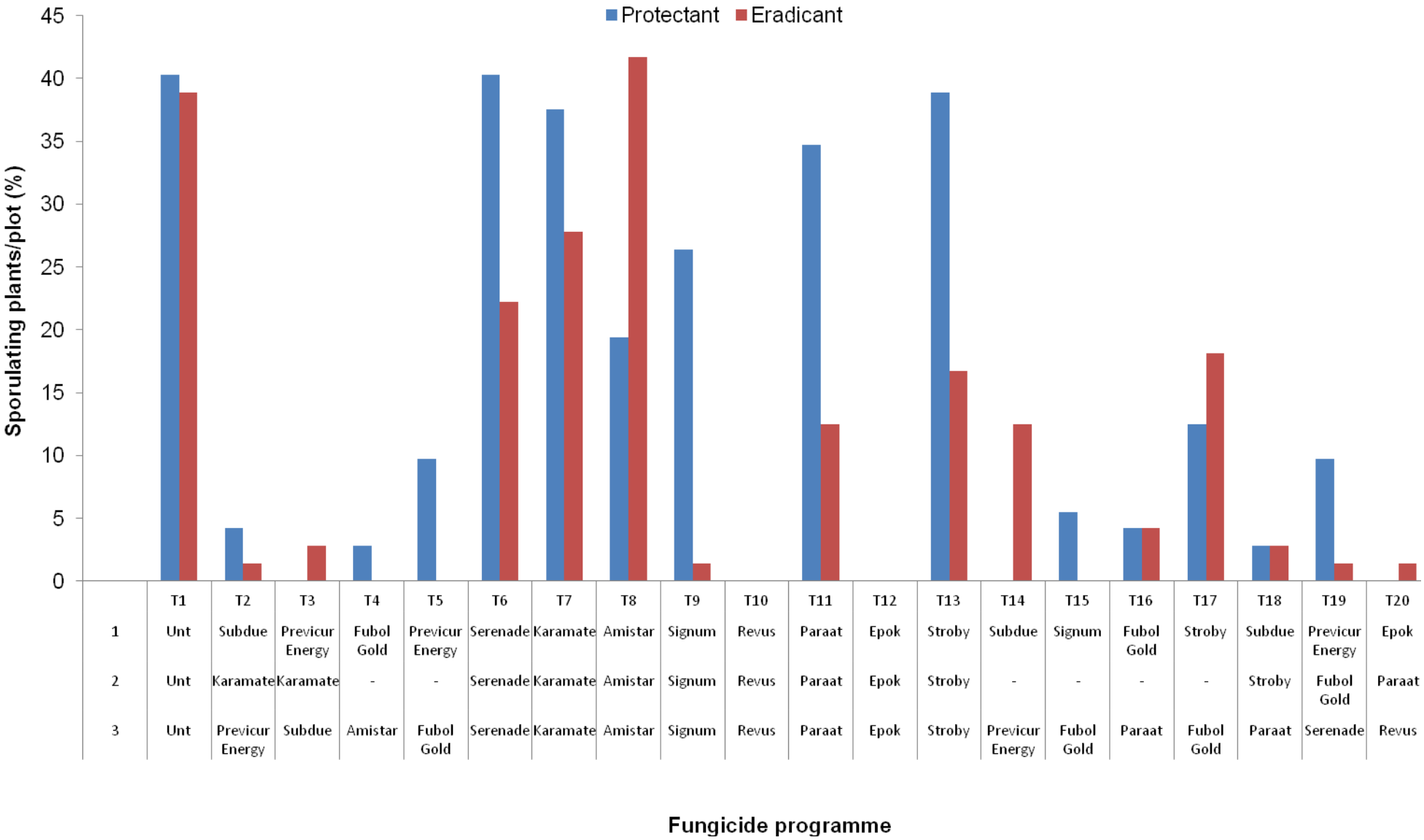


Figure 12. Effect of fungicide programme on impatiens downy mildew infection (% sporulating plants) as assessed on 28th June 2010.

Pansy and viola trial

Sporulation in the crop plants was observed on the 11th October and the first disease assessment was carried out on that date. Very low levels of infection were observed on the untreated plants in the protectant part of the trial had which had received 2 fungicide applications by this date (Figure 13). In the 'eradicant' part of the trial, where fungicide applications had not yet been applied, low levels of infection were observed across the trial area (Figure 13). Following this assessment a period of unseasonably warm weather occurred and downy mildew sporulation 'dried up'. A spore suspension was prepared and applied to all plants in the trial on the 14th October, followed by foliar wetting-up and covering for 2 nights to re-induce sporulation.

A second disease assessment was carried out on the 27th October when sporulation had re-occurred (Figure 14). In the 'protectant' area approximately 50% of the untreated pansy plants showed downy mildew sporulation, with a similar level of infection in T17 (Stroby only applied at this stage), and those plants treated with Serenade (T6). Many treatments provided good or excellent control of infection at this stage. In the 'eradicant' section of the trial, downy mildew sporulation was more widespread with approx 70% of the untreated plants showing infection. The lowest levels of infections (between 7 and 14%) were observed where metalaxyl-M (either as Subdue, Fubol Gold or Epok - T2, T4, T12, T14, T16, T18 and T20) had been applied. The majority of other treatments resulted in between 30 and 50% of plant infected/plot.

The final disease assessment on 17th November 2010 was carried out approximately 18 and 9 days after the final protectant and eradicant applications respectively. Higher levels of infection were observed in the 'protectant' area at this assessment than had been observed in earlier assessments and this may be due to the slightly longer interval between the last spray application and the assessment date (Figure 15). Several of the treatments where products containing metalaxyl-M were used early in the spray regime (T12, T14, T16, T18-20) still provided a good level of protection. A similar treatment effect was observed in the 'eradicant' treated plants, although it was apparent that some products had greater effect when used in a 'protectant' capacity than as an eradicant, whilst Paraat provided better eradicant efficacy.

Disease trends were similar in both the pansy and viola trials, although levels of infection were generally greater in the pansy trial.

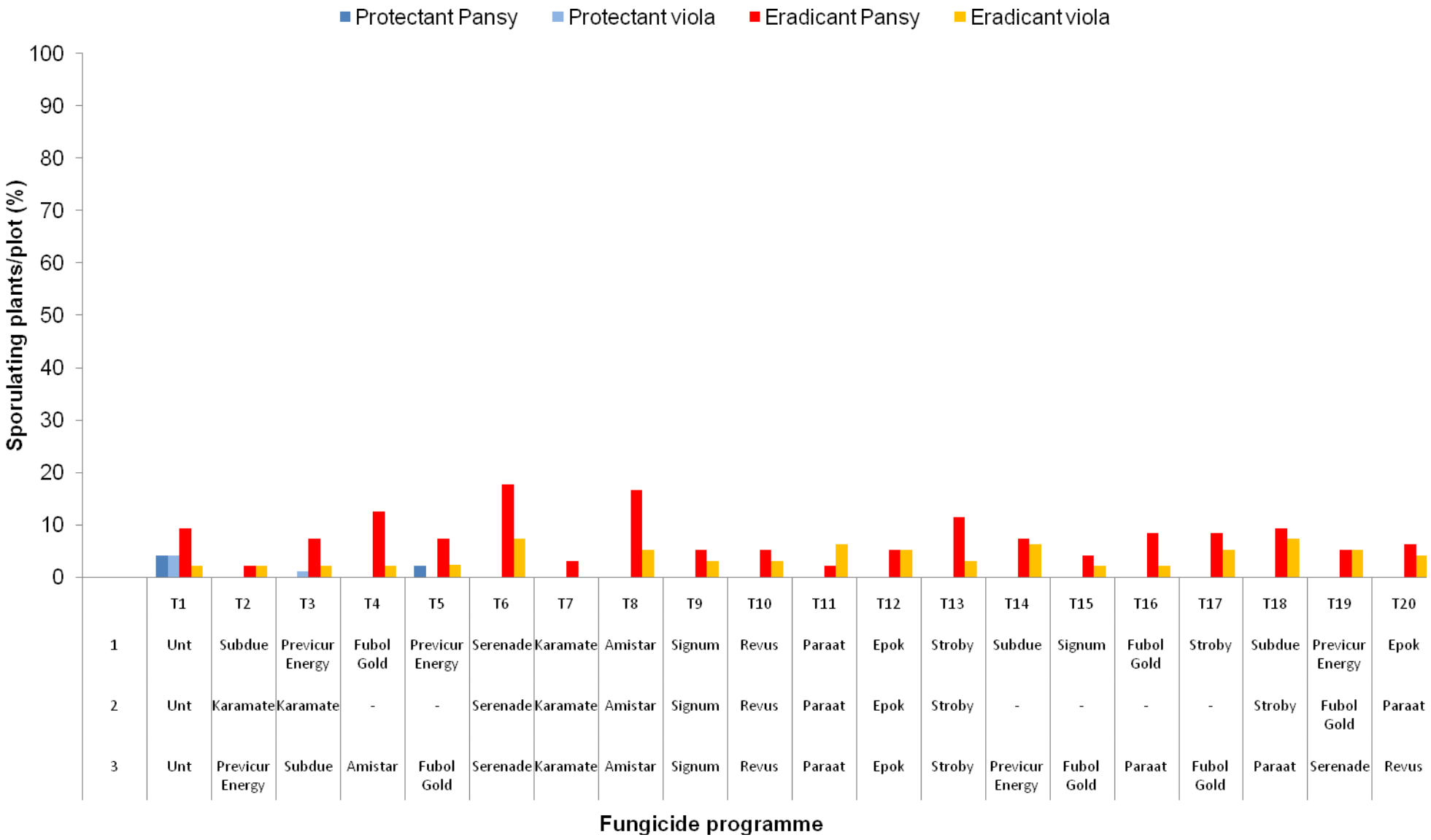


Figure 13. Effect of fungicide programmes on pansy/viola downy mildew infections (% sporulating plants) as assessed on 11th October 2010.

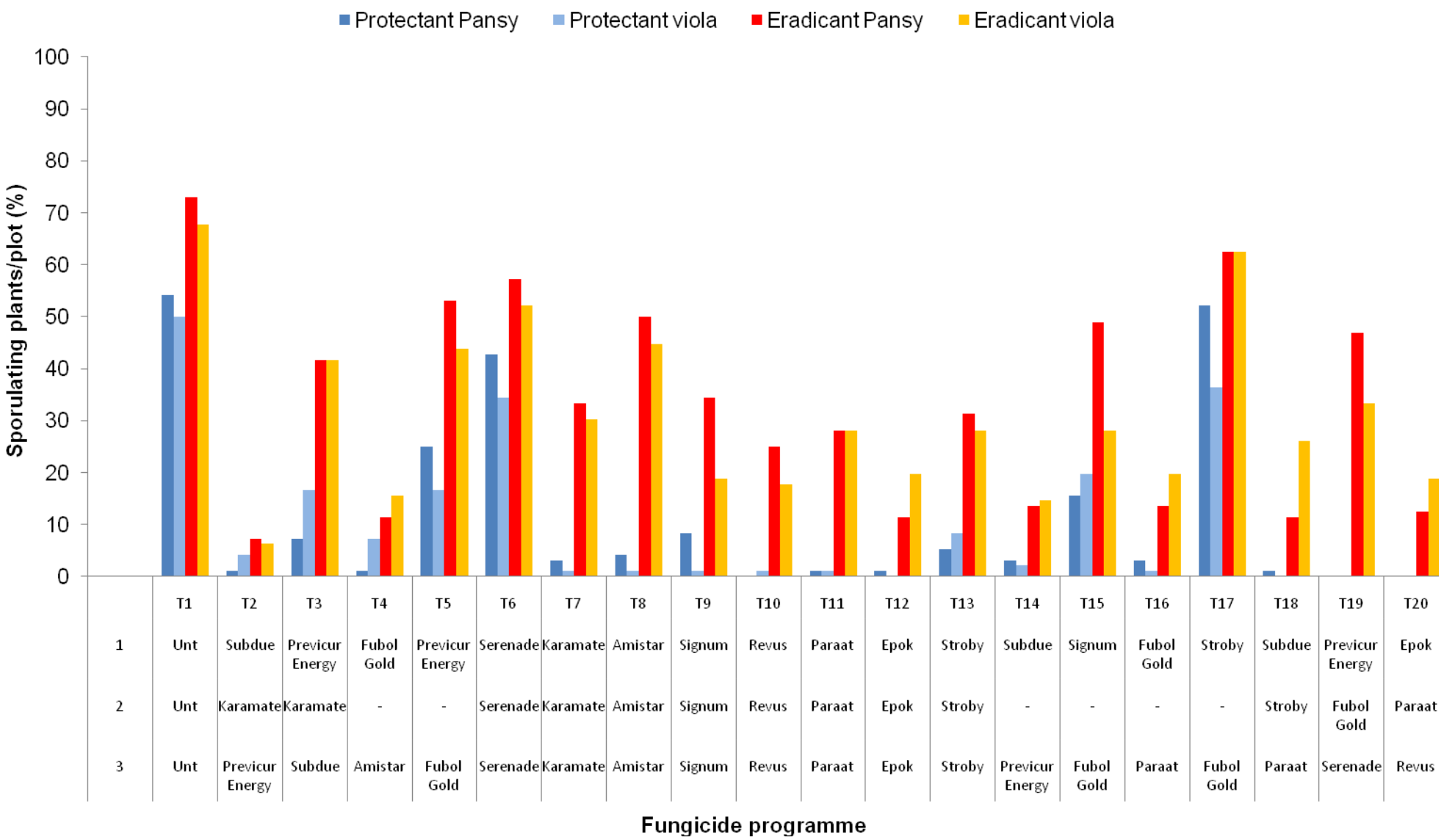


Figure 14. Effect of fungicide programmes on pansy/viola downy mildew infections (% sporulating plants) as assessed on 27th October 2010.

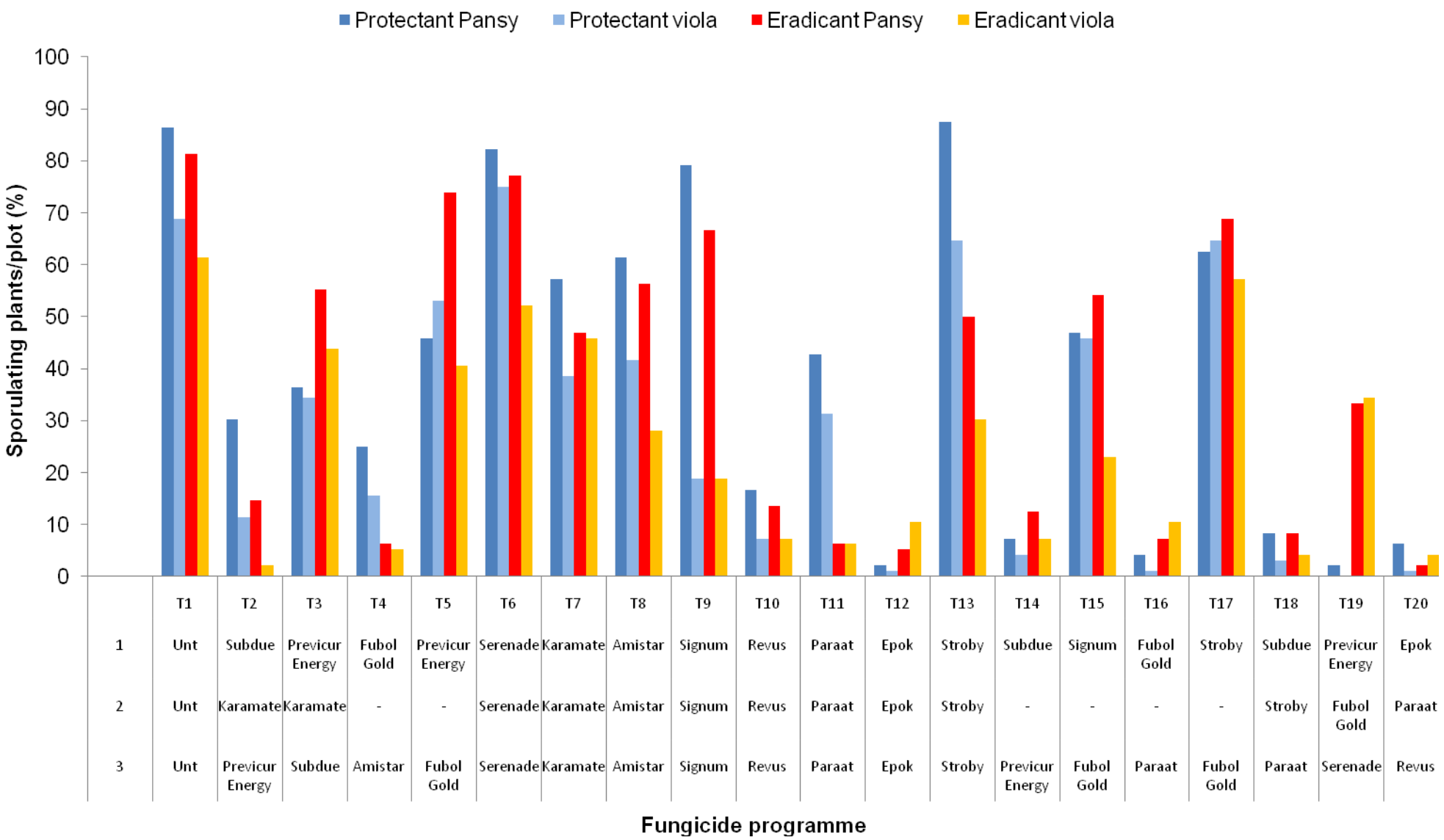


Figure 15. Effect of fungicide programmes on pansy/viola downy mildew infections (% sporulating plants) as assessed on 17th November 2010.

2.4 *Potential for seed-borne infections caused by Plasmopara obducens*

Seventeen seed lots were selected for testing to examine whether downy mildew infection of impatiens caused by *P. obducens* could be seed-borne. Of the lots selected, 12 had previously been shown (project PC 230) to have Ct values in a PCR assay lower than 40, which indicated the presence of *P. obducens* DNA; Ct values ranged between 31.6 and 39.5, with a higher DNA content in the lots with the lower Ct values. The remaining 5 lots, with Ct values of 40 (indicating that *P. obducens* DNA was not present in these seed lots), were used as comparative controls.

From each of the seventeen seed lots 200 seed were germinated and then grown on for 7 weeks. During this period plants were regularly subjected to conditions which have previously been shown to stimulate plant infection and sporulation. Despite this no downy mildew symptoms were observed on any of the plants.

PCR analysis of these plants generally indicated that low levels of *P. obducens* DNA was present in plants from seed-lots showing the higher levels of DNA. However, the results presented here do not indicate seed-borne infection is an important route for disease spread. The most likely route of entry to UK crops, as indicated in the 2011 outbreak and potentially in previous outbreaks also, is through the importation of infected cutting-raised material. If undetected, these infections can quickly spread to seed-raised material and cause severe economic loss.

2.5 *Over winter survival of P. obducens oospores*

Nylon bags containing stem material rich in *P. obducens* oospores were placed outdoors on 01/09/10. Bags were retrieved for viability testing on 7/10/10, 25/10/10, 29/11/10, 12/01/11, 10/02/11 and 16/03/11 giving exposure times of 37, 55, 90, 134, 163 and 197 days respectively. Whilst collecting the scheduled April sample it was noticed that many of the bags had been torn and much of the contents eaten. The limited amount of stem material remaining was bulked, suspended off the ground and analysed on 30/06/11 giving an exposure time of 303 days.

Meteorological data was collected from 01/09/10 to 30/06/11, with daily mean temperatures, daily rainfall and the minimum temperature below zero shown in Figure 16.

The summary data (Table 6) indicated that oospore batches 1 and 2 did not experience particularly harsh conditions with minimum temperatures of 3.7 and -2°C respectively. However, after this, the temperature dropped dramatically. In particular temperatures below 0 were experienced for a period equivalent to 21 days out of 35 in the period immediately following the retrieval of oospore batch 3.

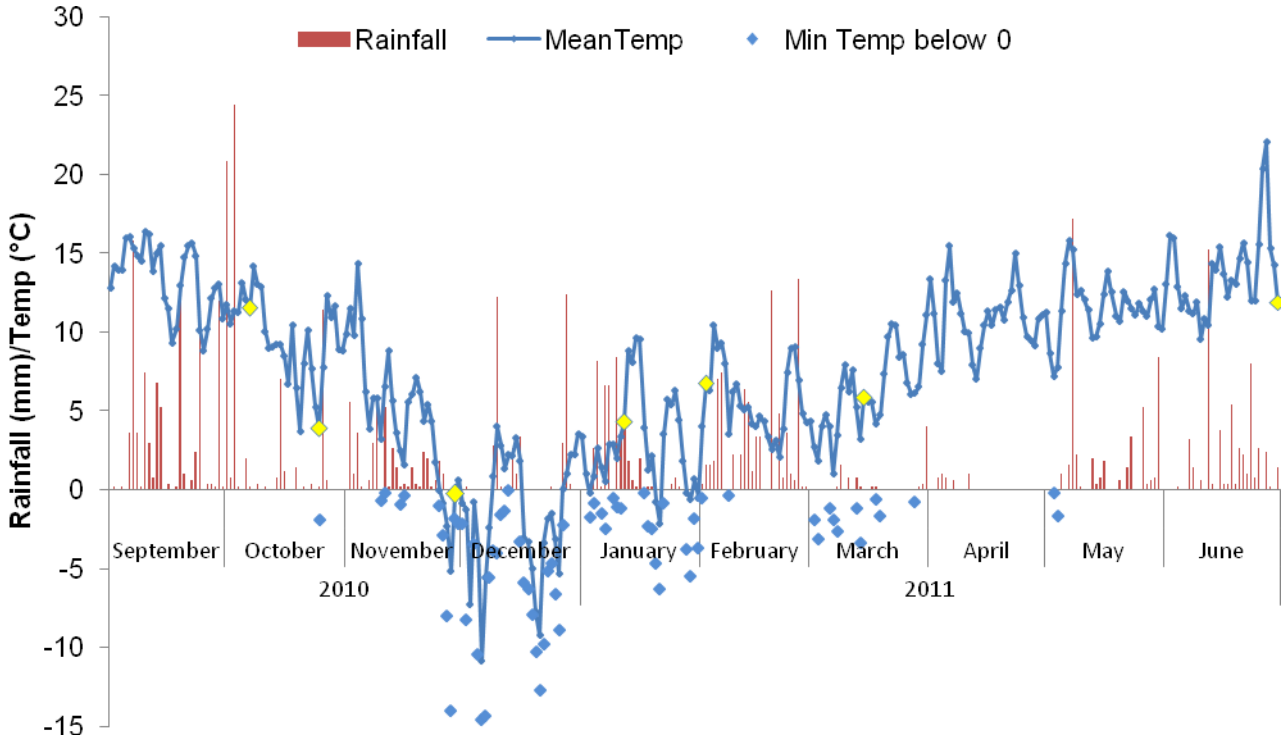


Figure 16. Meteorological conditions during period of oospore survival experiment. Yellow markers indicate date when stem material was collected for oospore viability testing.

Table 6. Summary meteorological data showing min, max and mean temperature, total rainfall and exposure time below 0°C for each batch of oospores in the survival study.

Oospore batch	Date retrieved	Total rainfall (mm)	Daily average Temperature (°C)			Exposure time (hrs) below 0°C
			Max	Mean	Min	
1	7/10/10	137	22.1	13.1	3.7	0
2	25/10/10	149	22.1	11.7	-2.0	4
3	29/11/10	201	22.1	9.4	-14.0	101
4	12/01/11	208	22.1	6.1	-15.2	596
5	10/02/11	309	22.1	5.9	-15.2	706
6	16/03/11	372	22.1	5.7	-15.2	748
7	30/06/11	481	29.4	7.7	-15.2	763

Direct microscopic examination of oospores indicated changes occurred in oospore appearance over time (Figure 17). The changes were mainly an increase in thickness of the inner oospore wall and a reduction in the size of the ooplast, however these changes in appearance may indicate oospore dormancy, and did not suggest any loss in oospore viability over the winter period.

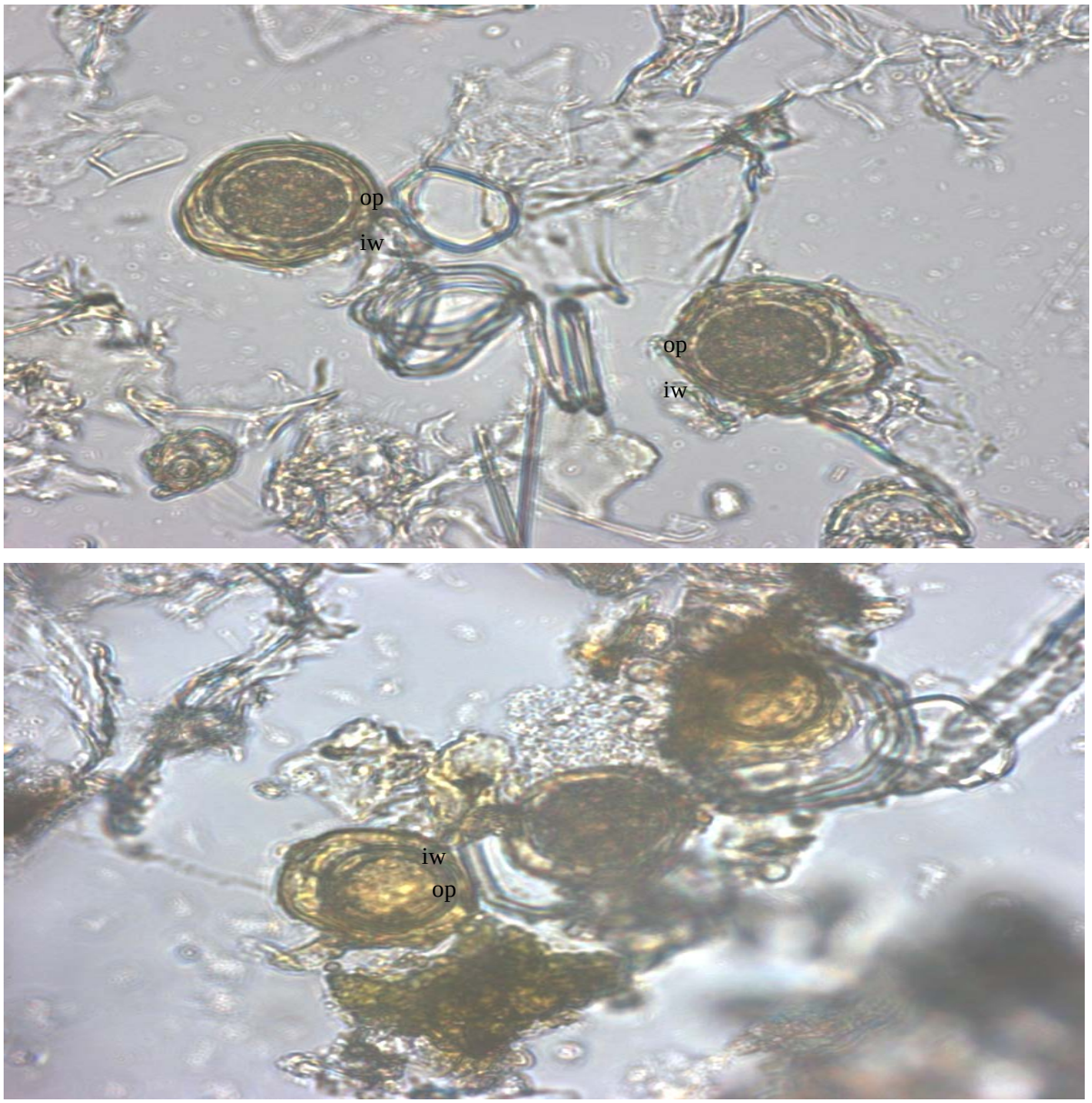


Figure 17. Changes in *Plasmopara obducens* oospore structure September '10 (top) to February '11. Inparticular showing a thickening of the inner oospore wall (iw) and a reduction in the size of the ooplast (op).

This observation was confirmed using the LIVE/DEAD® BacLightTM bacterial viability stain, where the percentage of viable spores observed gradually dropped from 100% in

batches 1 and 2 to 95% in batch 6. Generally, live spores fluoresced green but were surrounded by red fluorescence of the oogonium, whereas dead spores fluoresced only red (Figure 18).

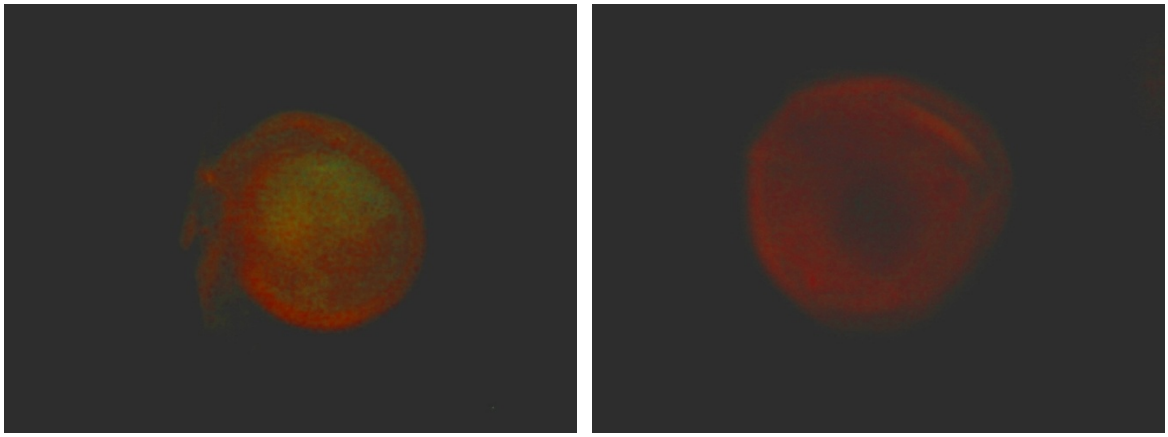


Figure 18. Staining of *Plasmopara obducens* oospores with LIVE/DEAD® BacLightTM bacterial viability stain, live spore (left) and dead spore (right)

Oospore viability was not confirmed by either of the direct germination tests used in this study. Again, this may indicate that spores were dormant and needed a stimulus not provided in these tests for germination to occur.

Overall results indicate that oospores remained viable during the winter period of 2010/11 which was the coldest for 30 years. Therefore oospore viability is unlikely to be adversely affected by winter conditions in the UK.

2.6 *Oospore survival – monitoring infected garden plants*

Four downy mildew infected plants were received at the laboratory for analysis. Three of the samples came from 'back gardens' in Leeds, York and Coventry, with all three gardens having no previous history of impatiens downy mildew. The fourth sample came from a growers hanging basket located in Cambridge, again there was no previous disease history on site, although a grower 'down the road' had reported disease problems. All four samples were as a result of late season infections.

All four infections proved to be caused by the metalaxyl-M resistant strain introduced in 2011. This result should come as no surprise as all the samples came from sites with no previous history of infection and as a result were unlikely to be exposed to infection caused by the metalaxyl-M sensitive strain. As the infections were all late season, this also

suggests that the infections did not result from plants bought with disease but were caused by airborne sporangial inoculum. A sample size of 4 was not sufficient to provide evidence that would support the theory that *P. obducens* could overwinter in the wider environment or help establish the distribution of the metalaxyl-M resistant strain. To do this further monitoring with a larger sample size would be required over the coming years.

Conclusions

- Small scale trials identified a number of products with differing active ingredients and modes of action (Fubol Gold, Subdue, Paraat, Karamate Dry Flo, Valbon, Rose Tonic and the experimental products HDC F32 and Percos) that potentially offer effective protection against the development of impatiens downy mildew. As a result there is the potential for the development of alternating spray programmes to provide effective control, which would also be commensurate with an effective resistance management strategy.
- None of the products tested provided any real curative activity towards impatiens downy mildew
- Subsequent large scale trials identified a number of such spray programmes which provided good preventative activity; though these were generally based around products containing metalaxyl-M. Again curative activity was not shown highlighting the importance of routine preventative sprays against this disease.
- When this work was conducted the isolate used in the study was sensitive to metalaxyl-M and hence the various products containing this active ingredient were highly effective.
- Resistance testing as a component of this project demonstrated that metalaxyl-M resistance was present in all *P. obducens* isolates collected during the 2011 outbreak of impatiens downy mildew. If the isolate is capable of over-wintering in the UK, or is re-introduced again on vegetative material in Spring 2012, then the fungicide programmes developed will need to be revised as one of the main active ingredients providing control was metalaxyl-M.
- No evidence was found to suggest that downy mildew of impatiens caused by *P. obducens* was seed-borne. Evidence from the 2011 outbreak clearly demonstrated that vegetative material was the source of *P. obducens* inoculum.
- Oospores of *P. obducens* appear to have the potential to over-winter in the UK. However, whether they have the potential to infect semi-mature transplanted plants in 6-packs (as opposed to direct drilled seed) is uncertain at this stage.

Future work

- The introduction of the metalaxyl-M resistant *P. obducens* isolate to the UK means that as a priority further work is required to evaluate alternative 'approved' spray programmes that exclude metalaxyl-M to determine whether can protect impatiens against infection from this aggressive and resistant isolate. At the same time, we need to further evaluate novel chemistry to try to identify alternative systemic fungicides with equivalent or better activity, to replace metalaxyl-M.
- Monitoring the sensitivity to metalaxyl-M of *P. obducens* isolates involved in any future outbreaks (from both nurseries and parks/gardens) would help determine the prevalence, persistence and geographical distribution of this aggressive metalaxyl-M resistant isolate in the wider environment.
- This project has indicated that *P. obducens* oospores are able to survive the harsh conditions encountered during the winter of 2010/11. Work is required to determine the length of time these structures are able to survive in soil and whether they are capable of re-infection, of both direct sown seed ('volunteers') and more importantly to semi-mature plants transplanted into civic display beds and public/private gardens from 6-packs or similar.
- Potential sources of inoculum in wild plant species need to be established, e.g. can *Impatiens noli-tangere* (a native Impatiens species to the UK) or Himalayan (Indian) balsam (*I. glandulifera*) as an introduced 'nuisance' species of Impatiens become infected by *P. obducens* and harbour inoculum, which may then act as an annual and persistent infection source to commercially grown impatiens.
- Are other cultivated impatiens susceptible to the disease e.g. *I. hawkeri* (New Guinea types) and are there sources of resistance that can be transferred to *I. walleriana* in the longer-term?

Technology transfer

- HDC News article November 2010
- Presentation of data to a BPOA meeting on 16th Feb 2011
- Presentation of data to a BOPP technical seminar on 15th Sept 2011

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APPENDICES

Figure A1. Trial Plan (bench layout & plot arrangement/size) for Impatiens and Pansy/viola trials.

[Note each trial included 4 benches of Impatiens or Pansy/Viola]

T1					T2				
T3					T4				
T5					T6				
T7					T8				
T9					T10				
T11					T12				
T13					T14				
T15					T16				
T17					T18				
T19					T20				
T1a					T2a				
T3a					T4a				
T5a					T6a				
T7a					T8a				
T9a					T10a				
T11a					T12a				
T13a					T14a				
T15a					T16a				
T17a					T18a				
T19a					T20a				

Light shaded part of bench represents protective spray programme (sprays applied prior to inoculum introduction). Dark shaded area of bench represents eradicant spray programme (sprays applied after the inoculum was introduced). Darkest shaded area shows individual plot size.

Table A1. Crop Diary for the impatiens and pansy/viola trials

Impatiens

29.4.10	Trays of plug plants potted-on into 6-pack trays using Levington's M2 compost.
7.5.10	1st Protectant spray application to 6-packs on ½ of each bench.
7.5.10	Infector plants in 6-packs introduced to middle section of each bench.
17.5.10	Sporulation of DM observed on a few plants in the 'eradicant' areas.
18.5.10	1st Eradicant spray and 2nd Protectant sprays applied.
20.5.10	Interim disease assessment carried out.
27.5.10	2nd Eradicant and 3rd Protectant sprays applied.
2.6.10	Full disease assessment carried out on all plants.
3.6.10	3rd Eradicant spray application.
10.6.10	2 x 6-packs/treatment/rep planted out in field to check persistence of products and potential oospore production.
28.6.10	Final disease and quality assessment carried out.

Pansy/Viola

17.9.10	Pansy & Viola plugs potted-on into 6-packs in Levington's M2 compost.
21.9.10	1st Protectant spray applied to 6-packs on ½ of each bench.
21.9.10	Infector plants in 6-packs introduced to middle section of each bench.
28.9.10	Infection observed on infector plants. Crop wetted and covered over night.
4.10.10	2nd Protectant spray applied.
5.10.10	B-nine growth regulator applied to all plants.
11.10.10	1st disease assessment carried out.
12.10.10	1st Eradicant spray applied to 'Eradicant' areas of each bench.
14.10.10	Downy mildew spore suspension prepared and applied to all plants.
27.10.10	2nd disease assessment carried out.
28.10.10	3rd Protectant and 2nd Eradicant spray applied.
8.11.10	3rd Eradicant spray applied.

Table A2. Impatiens trial interim disease assessment (20th May 2010).

Treatment No.	Treatment regime	Protectant spray programme*		Eradicant spray programme+	
		% plants with leaf symptoms	% plants with active sporulation	% plants with leaf symptoms	% plants with active sporulation
1	Untreated control	19.3	10.9	47.9	35.4
2	Subdue/Karamate/Prev Energy	0.0	0.0	-	-
3	Prev Energy/Karamate/Subdue	10.4	9.4	-	-
4	Fubol Gold/-/Amistar	0.0	0.0	-	-
5	Previcur Energy/-/Fubol Gold	25.5	16.7	-	-
6	Serenade/Serenade/Serenade	13.5	9.4	-	-
7	Karamate/Karamate/Karamate	0.0	0.0	-	-
8	Amistar/Amistar/Amistar	0.5	0.5	-	-
9	Signum/Signum/Signum	0.0	0.0	-	-
10	Revus/Revus/Revus	0.0	0.0	-	-
11	Paraat/Paraat/Paraat	0.0	0.0	-	-
12	Epok/Epok/Epok	0.0	0.0	-	-
13	Stroby/Stroby/Stroby	15.6	11.5	-	-
14	Subdue/-/Previcur Energy	0.0	0.0	-	-
15	Signum/-/Fubol Gold	0.0	0.0	-	-
16	Fubol Gold/-/Paraat	0.0	0.0	-	-
17	Stroby/-/Fubol Gold	5.7	3.6	-	-
18	Subdue/Stroby/Paraat	0.0	0.0	-	-
19	Prev Energy/Fubol Gold/Serenade	6.8	4.7	-	-
20	Epok/Paraat/Revus	0.0	0.0	-	-

**Interim disease assessment in protectant programme carried out 13 days after 1st spray application.*
+ Assessment of eradicant treatments not carried out at this stage, though a baseline infection level in the untreated control was determined at time of 1st spray application.
Data is the mean of 4 replicate blocks, each plot containing 10 x 6-packs with a total of 60 plants/plot.