

Project title: Lisianthus (*Eustoma*): Control of downy mildew (*Peronospora chlorae*)

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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of this 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

I declare that this work was done under my 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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Signature..... Date.....

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SCIENCE SECTION

Introduction

Lisianthus (*Eustoma*) is a relatively new and 'up and coming' cut flower crop with increasing popularity in the UK. Unfortunately, UK production of this crop has suffered significant economic losses in the last few years as a result of downy mildew epidemics caused by the fungal pathogen *Peronospora chlorae*. In order to reduce losses to this disease, this project was set up with the objectives to evaluate existing and novel fungicides for the control of *P. chlorae*, both in the propagation and the post-planting phases of crop development, and to provide grower guidelines for the use of the most effective and crop-safe chemicals.

In the first year of this project (2000-2001), a literature review on downy mildew diseases of ornamental crops (including lisianthus) caused by *Peronospora* species was carried out, with the biology and epidemiology of these diseases described. Trials at HRI Stockbridge House demonstrated the efficacy of a number of fungicides against *P. chlorae* infections in the propagation phase. In year 2 (2001-2002), trials were undertaken at HRI Efford to identify effective fungicide programmes for post-planting use. In addition, observations from a disease outbreak in one trial have provided further circumstantial evidence to support the hypothesis that *P. chlorae* can initiate epidemics of lisianthus downy mildew from seed-borne infection.

While evidence from the UK and elsewhere suggests that, like most downy mildews, *P. chlorae* is favoured by conditions of high humidity and leaf wetness, there is a lack of precise knowledge of the environmental conditions conducive to pathogen development. As lisianthus is grown under protection in the UK, there is potential to manipulate the environment by ventilation/heating, plant spacing, improved airflow, and timing and duration of overhead watering, for example to reduce relative humidity and leaf wetness duration.

Knowledge available on other members of the Peronosporaceae can be used to identify the environmental factors that are most likely to influence stages in the development of *P. chlorae*. In most cases, for example, spore production on the leaves of affected plants requires a combination of high humidity and a period of darkness. As a result of this, sporulation is usually present on the leaves in the morning. Spore release then generally occurs as a result of a decrease in humidity and an increase in temperature (and possibly solar radiation). Without fail, leaf wetness is required for spore germination and infection to occur, although the precise length of the minimum wet period varies between mildews and is not known for *P. chlorae*.

In order to provide guidelines to growers on practices that would minimise the development of lisianthus downy mildew, studies proposed for year 3 of the project were to determine the precise environmental conditions (temperature and leaf wetness) favouring infection of lisianthus by the conidia of *P. chlorae*.

Studies on lisianthus downy mildew can be problematic for the following reasons:

- *P. chlorae* is an obligate pathogen such that it can only survive on live host tissue and cannot be cultured. Therefore maintenance of the disease on lisianthus plants is necessary in order to provide inoculum for experimental work.
- It is difficult to guarantee that plants to be used in infection studies are healthy at the beginning of infection studies due to the possibility of latent (symptomless) infection.

Literature and knowledge review (update)

The literature review conducted in Year 1 was updated using references obtained from a recent literature search (2000-2003) and with information obtained from industry contacts.

Environmental conditions

Although circumstantial evidence suggests that development of lisianthus downy mildew is favoured by conditions of high humidity and leaf wetness, there is still negligible literature available on the environmental conditions that are optimal for *P. chlorae* infection, symptom development, spore production and release.

Detailed work has recently been published on the environmental factors affecting rose downy mildew caused by *Peronospora sparsa* on rose (Aegerter, 2003). Optimal temperatures for infection and colonisation of rose leaves were 15-20°C and 20-25°C, respectively. At optimal temperatures, infection required only 2 h of leaf wetness, although disease severity increased significantly with an increasing duration of leaf wetness up to 10 h. Infection of leaves occurred at temperatures as low as 5°C with 8 h of leaf wetness. The latent period of infection varied from 4 to 7 days.

Potential for disease forecasting

Although downy mildew diseases tend to occur only sporadically, growers often apply fungicides routinely because losses are potentially devastating. For these reasons and because downy mildew is apparently limited by some environmental conditions, it can be a good candidate for forecasting. Disease warning or advisory systems have been developed to predict downy mildew occurrence in a number of crops including onion (e.g. de Visser, 2001; Wright *et al.*, 2002), grapes (Madden *et al.*, 2000) and roses (Aegerter, 2003), in order to optimise fungicide spray timing. For rose downy mildew, the optimum forecasting model incorporated three weather variables calculated as cumulative totals over the previous 10 days: i) hours of leaf wetness when temperatures were less than 20°C, ii) hours between 15 and 20°C, and iii) hours when temperature exceeded 30°C. However a simpler model based only on cumulative number of hours of leaf wetness, showed that the critical number of hours of leaf wetness for disease development was an average of 8.4 h per day over 10 days (Aegerter, 2003).

The availability of chemicals with some curative activity (e.g. metalaxyl) in some of the crop examples above, allows some flexibility in timing fungicide applications once a disease warning has been issued.

Sources of inoculum

While there is increasing circumstantial evidence from growers and researchers to suggest that epidemics of lisianthus downy mildew may be due to seed-borne inoculum of *Peronospora chlorae*, there is no published work to confirm this as yet. It is known that Dutch plant raisers (Balls and Florensis) use lisianthus seed that has been fungicide-treated but information on the products used is not available (R. Geater, pers. comm.).

Fungicide update

Results from the fungicide trials carried out in years 1 and 2 are provided in Annual Reports for Years 1 and 2, and are summarised in the Conclusions section below. Additional notes on specific fungicide use for lisianthus downy mildew are as follows:

- Fongarid (furalaxyl), formerly approved for use during lisianthus propagation, was revoked in July 2003.
- Amistar (azoxystrobin) was found to be effective in year 1 and 2 trials for reducing downy mildew in both propagation and post-planting. Guidelines have been provided in the project factsheet to avoid development of pathogen resistance to this product.
- Fubol Gold WG (mancozeb and metalaxyl) was found to be effective for control of lisianthus downy mildew control when used in a protective post-planting fungicide programme (year 2). Approval for this use is extrapolated from a protected radish SOLA (1610/01), which does not restrict time of application, apart from a 14 d harvest interval.
- Horti 303: In year 2, a protectant spray programme using an experimental product, Horti 303 (Certis Europe Ltd) gave complete control of lisianthus downy mildew post-planting, with no phytotoxic effects. This product has recently given good results against both grape downy mildew and potato blight, and is currently being registered for potatoes in UK. It is anticipated that a Specific Off-Label Approval for use of the product on ornamentals may follow (P. Hingley, Certis, pers. comm.).

Experimental work undertaken in year 3 of the project (2002/2003)

Experiment 1. (From 07.10.02 to 04.11.02)

Objective

To obtain sufficient inoculum of *Peronospora chlorae* on lisianthus plants to use in subsequent controlled environment experiments.

Methods

Spores were washed from the leaves of four lisianthus plants with sporulating downy mildew lesions using sterile distilled water (SDW). The resulting spore suspension (approximately 10^4 spores/ml) was used to inoculate eight visibly healthy lisianthus plants (var. Malibu Rose) in 7 cm diameter pots, by spraying to run-off with a hand-held mister. The inoculated plants were placed in a glasshouse (mean temperature 12°C). A polythene bag was placed over each pot during the night to increase relative humidity and prolong leaf wetness duration, and then removed during the day. The plants were watered by hand as necessary.

Spore suspension remaining after inoculation (10 ml) was frozen for use in a future experiment.

Results

Downy mildew lesions with sporulation on the undersides of the lisianthus leaves were observed 28 days after inoculation but the lesions were too few to provide sufficient inoculum for controlled environment experiments.

Experiment 2. (From 07.11.02 to 31.12.02)

Objectives

- To continue to build up inoculum for subsequent experiments.
- To determine whether spore suspension that had been frozen then thawed could be used to successfully infect lisianthus plants.

Methods

All leaves with sporulating lesions from Experiment 1 were removed and washed in SDW. Spore suspensions of 1×10^4 spores per ml and 1×10^3 spores per ml were made and each used to inoculate twelve visibly healthy lisianthus plants var. Malibu Rose, by spraying to run-off with a hand-held mister (10 ml per plant). The remaining spore suspension (10 ml) was frozen.

Spore suspension that had been frozen on 04.11.02 (in Experiment 1) was thawed and observed microscopically. The spore suspension (1×10^4 spores/ml) was used to inoculate eight visibly healthy lisianthus plants var. Malibu Rose by spraying to run-off with a hand-held mister (10 ml per plant).

The inoculated plants were placed on trays in a controlled environment cabinet (20°C) and were misted with distilled water (DW) 30 min and 180 min after inoculation. The plants were covered with polythene bags to maintain leaf wetness and high relative humidity. Three days

after inoculation the plants were transferred to a glasshouse (mean temperature 12°C). The plants were covered with polythene bags during the night and uncovered during the day.

Results

It was observed microscopically that many of the spores in the suspension that was frozen then thawed, appeared to be damaged (darker in colour and a more granular appearance than non-frozen spores).

All inoculated plants were monitored for 2 months but symptoms of downy mildew were not observed.

Experiment 3. (From 04.02.03 to 13.03.03)

Objective

No plants with symptoms of downy mildew were available either from previous experiments or from commercial nurseries, so a second attempt was made to infect lisianthus plants using spore suspension that had been frozen then thawed.

Method

Eight lisianthus plants (var. Malibu Rose) were each inoculated with 10 ml thawed spore suspension (from Experiment 2) by spraying to run-off with a hand-held mister. The spore concentration was between 10^3 and 10^4 spores/ml but as in experiment 2, the spores were dark and granular in appearance. Each plant was spray misted with DW and covered with a polythene bag. The plants were initially kept on a laboratory bench by a window (20°C) and were then moved to a controlled environment cabinet (12°C, 12 h day/12 h night). The plants were monitored for symptoms of downy mildew for over a month.

Inoculated leaves were cleared and stained using the following method, to check for the presence of oospores of *Peronospora chloreae* in leaf tissue. Folded tissue paper mats were soaked in 1:1 (v/v) mixture of Jet 5 (peroxyacetic acid) and 95 % ethanol and placed in 9 cm Petri dishes. Discs (5-10 mm) were cut using a cork borer from inoculated lisianthus leaves (six leaves per plant). The discs from each plant were placed on filter paper on top of the soaked tissue in a Petri dish, which was then sealed with parafilm and left on a laboratory bench (ambient light) until the following day. Cleared discs were then stained with cotton blue in lactophenol, and observed microscopically.

Results

No downy mildew symptoms were observed on the inoculated plants. No oospores were observed microscopically in cleared and stained leaf discs.

Discussion

Due to lack of viable inoculum of *Peronospora chloreae*, we were unable to proceed with experiments to determine infection conditions for lisianthus downy mildew. Difficulties in getting downy mildew symptom development to occur following inoculation of lisianthus plants, were similar to those experienced at HRI Efford in Year 2 of the project. Our initial attempts to produce inoculum (sporulating leaf lesions) were successful in Experiment 1, however, subsequent inoculations did not result in symptom development, despite the use of a range of incubation conditions, selected based on relevant literature and advice from HRI colleagues.

In order to reduce dependency on inoculum harvested fresh from sporulating lesions, attempts were made to infect lisianthus plants using spore suspension that had been frozen then thawed. No symptoms developed following use of this type of inoculum and microscopic examination suggested that spores were damaged by the freezing/thawing process.

A clearing/staining technique was used to check for latent infection (e.g. oospores) in inoculated leaf tissue, but no fungal structures were detected.

During the experimental work and subsequently in April and May 2003, regular attempts were made to source lisianthus plants with downy mildew to use for inoculum, but no growers had observed disease symptoms on their nurseries.

Overall conclusions (Years 1-3)

Literature and knowledge review

- The biology and epidemiology of downy mildew disease of ornamentals caused by *Peronospora* species has been summarised.
- *Peronospora* species generally can infect their hosts over a wide range of temperature. Moisture is critical for infection to occur. Temperature influences the latent period.
- *P. chloerae* causes a wide range of symptoms on lisianthus: leaf necrosis and death of seedlings, leaf yellowing, stem twisting and distortion.
- Infection by *P. chloerae* is restricted to lisianthus and a few weed hosts in the Gentianaceae, including Yellow Wort and probably Lesser Centaury.
- There is increasing circumstantial evidence, provided by both growers and researchers, which suggests that development of lisianthus downy mildew is due to seed-borne infection.
- There is little information available on the precise environmental conditions for different stages in the disease cycle of *P. chloerae*.

Fungicide evaluation

Fungicides approved for use as foliar sprays on protected lisianthus are shown in Table 1.

Table 1. Fungicides approved for use on protected lisianthus and found to be safe to the crop and to give control of downy mildew

Product and fungicide group	Active ingredient	Application rate per litre water	Comments and approval status
<u>Phosphonic acid</u>			
Aliette	Fosetyl-aluminium	1.5 g	Useful when used in a post-planting preventative spray programme. [Full approval]*
<u>Strobilurin</u>			
Amistar	Azoxystrobin	0.8 ml	Effective for propagation or when used in a post-planting preventative spray programme. Maximum of 3 sprays per crop [SOLA 1684/01]*.
<u>Carbamate</u>			
Filex / Proplant	Propamocarb hydrochloride	1.0 ml	Useful when used in a post-planting preventative spray programme. [Full approval]*
<u>Dithiocarbamate +phenylamide</u>			
Fubol Gold WG	Mancozeb and metalaxyl-M	1.33 g	Useful when used in a post-planting preventative spray programme. [SOLA 1610/01]*. Maximum of 3 kg/ha/year; 14 day harvest interval and 14 day spray interval*.

*Approved under Revised Long Term Arrangements for Extension of Use (2000)

During propagation

Amistar was shown to be particularly effective in reducing the severity of downy mildew during propagation (three applications). No phytotoxicity symptoms were observed following application of Aliette, Amistar or Filex to the cultivars tested (Picotee Pink and Malibu Dark Blue), and there were no adverse effects on seedling germination. Results are not available for Fubol Gold WG (mancozeb and metalaxyl-M) as it was not included in the propagation trial. However, metalaxyl-M applied as SL 567A (not approved) significantly reduced the level of lisianthus downy mildew during propagation with no phytotoxic effects, but also reduced germination in one cultivar (Picotee Pink).

Post-planting

The following protectant spray programmes gave complete control of the disease post-planting with no symptoms of phytotoxicity on the cultivars tested (Ventura White and Ventura Rose).

Amistar, Amistar, Filex, Filex, Amistar, Amistar, Filex, Filex	(8 sprays)
Fubol Gold WG, Amistar, Aliette, Filex, Amistar, Aliette, Filex	(7 sprays)

The following spray programmes also significantly reduced disease levels but were not as effective as programmes commencing with Amistar or Fubol Gold:

Aliette, Aliette, Filex, Filex, Aliette, Aliette, Filex, Filex	(8 sprays)
Aliette, Aliette, Amistar, Amistar, Aliette, Aliette, Amistar, Amistar	(8 sprays)

Fungicide timing was most effective when spray programmes commenced 2 days after planting and were applied at approximately 14 day intervals until the first visual symptoms of downy mildew were seen in control plots, when the spray interval was reduced to 7 days. Delaying spray applications until the appearance of the first visible symptoms of downy mildew reduced the efficacy of spray programmes and this was further reduced when the spray interval was increased from 7 to 14 days.

It should be noted that in a commercial situation, there is a maximum permitted application of 3.0 kg/ha/year for Fubol Gold WG; this equates to two sprays per year if using the maximum permitted dose of 1.5 kg/ha and a spray volume of 1,000 litres/ha. For Amistar, there is a maximum of three sprays per crop.

Use of high-volume fungicide sprays in a mature crop can result in unacceptable spray deposits on plants at harvest, however no obvious deposits were seen using Aliette, Amistar, Filex and Fubol Gold on cultivars Malibu and Picotee Pink in the trial.

A protectant spray programme (8 sprays) using an experimental product, Horti 303 (Certis Europe Ltd) also gave complete control of lisianthus downy mildew. This product has recently given good results against both grape downy mildew and potato blight, and is currently being registered for potatoes in UK. It is anticipated that a Specific Off-Label Approval for use of the product on ornamentals may follow.

Environmental conditions for infection

Due to lack of viable inoculum of *Peronospora chlorae*, we were unable to proceed with experiments to determine infection conditions for lisianthus downy mildew. Initial attempts to produce inoculum (sporulating leaf lesions) were successful, however, subsequent inoculations did not result in symptom development, despite the use of a range of incubation conditions.

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Technology transfer

1. Visit to Holland with Lisianthus study group (20-22 July 1999) (Martin McPherson)
2. Presentation to lisianthus study group, HRI Wellesbourne, Spring 1999 (Tim O'Neill, Martin McPherson)
3. Presentation to lisianthus study group, HRI Wellesbourne, Nov 2000 (Andrew Jackson, Martin McPherson)
4. Presentation at HDC Cut Flower meeting, HRI Kirton, 26 Sep 2002 (Tim O'Neill)
5. HDC Factsheet 12/03: Control of lisianthus downy mildew (Tim O'Neill, Kim Green and Tim Pettitt)
6. Various discussions with Ron Geater about the disease (Tim O'Neill and Kim Green)

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