

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 experiments were carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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PRACTICAL SECTION FOR GROWERS

Background and Objectives

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 chloerae*. With the aim of reducing losses to this disease this project was set up to evaluate existing and novel fungicides for the control of downy mildew of lisianthus, 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 the biology and control of downy mildew in Lisianthus was completed as well as trials at HRI Stockbridge House demonstrating the efficacy of a number of fungicides against *P. chloerae* infections in the propagation phase. The second year (2001-2002) is reported here, and focused on evaluating the efficacy and crop safety of a range of fungicides for use for post-planting control of downy mildew in Lisianthus.

Main Results and Conclusions

Symptoms of the disease

Photographs in Figure A show typical symptoms of downy mildew disease in Lisianthus. Early symptoms of the disease can be seen as chlorosis and curling of the leaves and the shoot tips. As the disease becomes more severe, a white fluffy growth can be seen on the leaves and stems; these are the spores of the fungus. In severe cases, the plants will appear stunted with some leaf yellowing.

Figure A Photographs of *Peronospora chloerae* downy mildew symptoms in lisianthus.

A: Sporulation on young leaves in a mature plant.



B: Early symptoms in a young plant; chlorosis and curling leaves/shoots.



C: Early symptoms in a mature plant; chlorosis and curling or 'hooked' shoot tips.



Fungicide trial

Seven fungicide spray programmes were compared for downy mildew control in a lisianthus crop after planting out. The plants were inoculated with the disease and spray programmes were evaluated against an untreated control. The plants were planted out in early October 2001 and the trial was completed in March 2002 using the varieties Ventura Rose and Ventura White.

The treatments are detailed in Table A below. Treatments 2 to 6 were applied at 14 day intervals, starting on the second day after planting, until the appearance of the first downy mildew symptoms when the spray interval was reduced to 7 days. All sprays were applied to run-off using a flat fan nozzle.

Table A Details of fungicide programmes evaluated for the control of downy mildew of lisianthus

Treatment number	Treatment title	Spray programme
1	Untreated control	None
2	Protectant programme A	Amistar, Amistar, Filex, Filex
3	Protectant programme B	Aliette, Aliette, Filex, Filex
4	Protectant programme C	Horti 303 at every application
5	Protectant programme D	Aliette, Aliette, Amistar, Amistar
6	Protectant programme E	Fubol Gold WG, Amistar, Aliette, Filex (followed by Amistar, Aliette, Filex)
7	First symptoms programme A	Aliette, Amistar, Aliette, Amistar (sprays at 7 day intervals)
8	First symptoms programme B	Aliette, Amistar, Aliette, Amistar (sprays at 14 day intervals)

All fungicide treatment programmes significantly reduced disease compared to untreated controls. The most effective spray programmes were:

- Treatment 2. Protectant programme A: Amistar, Amistar, Filex, Filex
- Treatment 4. Protectant programme C: Horti 303 at every application
- Treatment 6. Protectant programme E: Fubol Gold, Amistar, Aliette, Filex
(followed by Amistar, Aliette, Filex).

Horti 303 was used alone as it is a new experimental product and it was important to gauge its performance alone. Whilst promising, this product is not yet registered for use in the UK but could prove to be a useful product for growers of lisianthus once approval is secured.

Whilst Aliette performed well in spray programmes, starting a spray programme with it appeared to reduce the level of disease control achieved.

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.

Plant damage due to fungicide treatment was not observed with any of the fungicide spray programmes assessed.

In the first experiment in this study a rapid, randomly distributed and widespread epidemic occurred at the two leaf stage in seeds planted in high grade hygiene conditions, in the absence of any obvious source of infective inoculum, and without the generation of secondary (conidial) inoculum. This indicates that it is highly likely that *P. chloreae* can initiate epidemics from seed-borne infection. Further work is necessary to determine the prevalence of seed-borne and of latent infection and measures to control such infection may significantly reduce the incidence of the disease post-planting.

Action Points

- The most effective spray programmes for downy mildew control of lisianthus that are immediately applicable are treatments 2, 4 and 6 as shown in Table A above with applications at a 14 day interval until appearance of first symptoms when this is reduced to 7 days.
- Whilst no phytotoxicity was observed in the trial reported here, a small-scale on-site nursery test of any new treatments is advisable before adopting them widely. Growers should also be aware that varieties may respond differently as regards crop safety.
- Delaying spray programmes until the first downy mildew symptoms are visible will reduce the efficacy of control programmes. However, if this approach is used, a shorter spray interval (7 days) will give the best results.
- Aliette is a valuable fungicide to be used as part of a spray programme but should not be used at the start of the spray schedule.
- Circumstantial evidence from this trial has provided strong indications that downy mildew disease of lisianthus may be seed borne. Growers should seek assurances from their propagator and seed supplier that reasonable measures have been taken to control the disease at these stages.

Anticipated Practical and Financial Benefits

Downy mildew epidemics can rapidly become very destructive in lisianthus crops and financial losses to individual growers to this disease have been very high. For example in 1998, total losses were estimated to be near to 40% (Lisianthus study group). Assuming an annual crop value of £2 million, even with average annual losses to the disease of 10-20%, the economic loss to the industry is likely to be in the region £200,000-400,000 *per annum*. Effective disease control will reduce these losses.

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 chloerae*. With the aim of reducing losses to this disease this project was set up to evaluate existing and novel fungicides for the control of *P. chloerae*, 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 the biology and control of downy mildew in Lisianthus was completed as well as trials at HRI Stockbridge House demonstrating the efficacy of a number of fungicides against *P. chloerae* infections in the propagation phase. The second year (2001-2002) is reported here, and was concerned with evaluating the efficacy and crop safety of a range of fungicides for use for post-planting control of downy mildew in Lisianthus.

Materials and Methods

Preliminary trial

The experiment that has been designated in this report as the 'main trial' was the second trial to be run with a full lisianthus crop. The circumstances of the first trial need to be outlined here; (a) to explain the lateness of the main trial and (b) because some of the results of this initial work throw up some useful aetiological evidence, especially relating to the occurrence of seed-borne *P. chloerae* infection in lisianthus.

Initial plans included the raising of plants from seed on site to avoid the possibility of infection during the propagation phase and the potential confounding effects this might have on fungicide regimes and distribution of disease in the post-planting trial. Seeds of cultivars Dark Malibu Blue and Kyoto Picotee Pink were sown in brand new plug trays (416 cell trays, 538x309x88 mm with 14x14mm cell size, Styropack (UK) Ltd.) and placed on a heated bench maintained at 21°C in propagation glasshouse E1 at HRI Efford on 12th April 2001. Immediately prior to the experiment, glasshouse E1 was stripped down, thoroughly washed, capillary mats all replaced and all surfaces were treated with Jet 5 sterilant. The resultant plants suffered widespread and severe downy mildew symptoms and on 11th June it was decided not to use them for the post-planting fungicide trial. Full details of this epidemic and its implications are described in the results section.

As a consequence of losing the in house-raised plants it was necessary to replace them at short notice with plug plants which were ordered from Florensis. The short notice of this order meant that only one variety was available in the numbers required for the trial work. The variety was Kyoto Yellow. The plants had been sown w/c 9th April 2001 and were planted in HRI Efford glasshouse E3 on 20th June 2001, following the same trial design and plan described in the 'main trial' section of this report. The crop was grown to final harvest on 24th September 2001. Unfortunately, despite having large numbers of infected seedlings on site, it proved impossible to induce sporulation in any of these plants. In

addition, no UK growers were able to supply any infected material until early September. Requests were made to the members of the lisianthus study group, but the disease was not present on any of the members' nurseries. Finally a small quantity of infective inoculum was collected on 11th September, but it was decided that the crop was too far advanced to carry out a meaningful/useful fungicide trial. The trial was therefore terminated and the main trial set up instead.

Main trial

Trial design and treatments

Lisianthus plants of cvs. Ventura White and Ventura Rose were planted on 11 October 2001 in split plots in a randomised block comprising 8 treatments x 4 replicates in glasshouse E3 at HRI Efford. Applications of fungicides were made using an Oxford Precision Sprayer with a flat fan nozzle sprayed to run-off (see Table 1 for application schedule). The 8 treatments were as follows:

1. Untreated control
2. Protectant programme A:
Amistar, Amistar, Filex, Filex
3. Protectant programme B:
Aliette, Aliette, Filex, Filex
4. Protectant programme C:
Horti 303 at every application
5. Protectant programme D:
Aliette, Aliette, Amistar, Amistar
6. Protectant programme E:
Fubol Gold WG, Amistar, Aliette, Filex (followed by
Amistar, Aliette, Filex)
7. First symptoms programme A (sprays at 7 day intervals)
Aliette, Amistar, Aliette, Amistar
8. First symptoms programme B (sprays at 14 day intervals)
Aliette, Amistar, Aliette, Amistar

In all treatments except 7 and 8, spray programmes commenced 2 days after planting. Sprays were applied at approximately 14-day intervals until the first visual symptoms of downy mildew were seen in untreated plots. Spray intervals were then reduced to 7 days except in treatment 8 where the spray interval was fourteen days. In treatments 7 and 8, spray programmes were started immediately symptoms were seen in the untreated plots. The sequences of sprays in the treatment list above were repeated after the fourth spray in all treatments except treatment 6, where the repeat sequence was Amistar, Aliette, Filex. This is because the specific off label approval (2446/2001) for Fubol Gold WG on

protected lettuce only allows sprays in the first 3 weeks after planting. Of the chemicals assessed in the first year trials at HRI Stockbridge House, several were not included in the trial reported here for various reasons. Invader, Ripost and Bion were not included as their use on protected crops is not permitted. F279 was not included as it is a chemical from the same group (strobilurins) as Amistar but was less effective than Amistar in the first year trials. A new formulation was included (Treatment 4 - Horti 303), produced by Hortichem Ltd (now Certis Europe Ltd), that has very recently given good results against both grape downy mildew (*Plasmopara viticola*) and potato blight (*Phytophthora infestans*). This product was included in the trial because if it is demonstrated to be effective against a wide range of oomycetes in horticultural crops, it will be highly likely to be taken through registration. SL 567A was not included, as similar activity to Fongarid was anticipated and the latter is currently permitted on protected crops.

All spray programmes tested deployed at least 2 different fungicide chemical groups, except for treatment 4 which consisted of continuous applications of Horti 303. The treatments concentrated on using Aliette and Amistar, which have performed well in other studies against downy mildews on hebe (*P. grisea*) and on rose (*P. sparsa*), as well as on lisianthus seedlings (see year 1 report), and can be legally used on protected Lisianthus crops. Amistar was applied at a maximum of 50% of any of the spray programmes and, as advised by its producers Syngenta, sprays were deployed in treatments 2 and 5 in blocks of 2 (see list above).

Inoculum production and inoculation

Pots containing 5 infected plants bearing sporulating downy mildew were introduced into the crop on 20th November 2001 (7 days after the first fungicide application to treatments 2-6). 40 infector pots were placed at regular intervals on the pathways between and surrounding the plots as shown in the trial plan (Figure 1). The infector pots had been infected with downy mildew in glasshouse E1, the original source of the inoculum being West End Nurseries, Leiston, Suffolk. This was collected on 11th September 2001 and incubated under high humidity conditions. Generation of sufficient infector material proved difficult and a range of conditions were tried before successful widespread infection was achieved. These included using a light sensor-driven misting unit to maintain continuous surface wetness at 21°C, misting for periods of 8h per day from 1800 hrs to 0200 hrs and keeping capillary mats permanently wet using pulsed trickle irrigation and no misting. The last treatment maintained the screen-measured RH at >90% and proved successful in generating sporulating lesions in sufficient quantity.

Assessments

The crop was monitored 3 times per week after the infector plants were introduced, to search for first symptoms of downy mildew, the signal to initiate the spray programmes for treatments 7 and 8. Assessments of the incidence and severity of foliar downy mildew symptoms were carried out on all trial plants at the end of the trial. In addition, a visual assessment of phytotoxicity was carried out on all plants at the end of the trial. This was done by direct observation of the foliage for signs of scorch or leaf spotting, and by comparative measurement of plant heights.

Crop diary

11/10/01	Crop planted
13/11/01	First spray treatments applied (treatments 2-6)
20/11/01	Infector plants in pots placed in the crop
09/01/02	Disease first seen in unsprayed plots
21/02/02	Final spray treatments applied
08/03/02	Foliar disease symptoms and phytotoxicity assessed

Statistical analysis

A row-column design ((4x4)/2 Trojan design (Edmondson, 1998)) for four replicates of eight treatments was used. The design was proposed to allow for possible spatial variability across both rows and columns.

Official recognition (trials certificate)

The study described here was undertaken in accordance with the PSD guidelines for Official Recognition of Efficacy Testing Organisations.

Certificate No. ORETO 041;

Date of issue: 10 March 1998,

Expiry date: 31 December 2002.

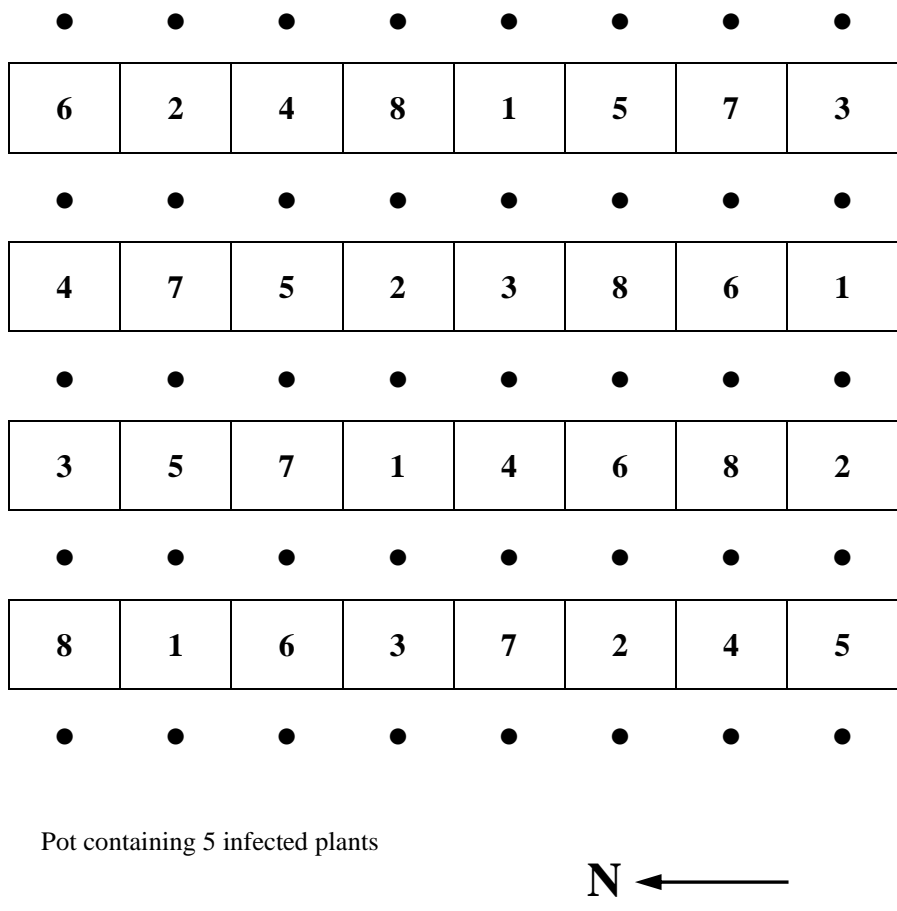
Table 1a: *Spray application schedule*

Date	Treatment application
13/11/01	Treatments 2-6
28/11/01	Treatments 2-6
14/12/01	Treatments 2-6
27/12/01	Treatments 2-6
9/01/02	Treatments 2-8
17/01/02	Treatments 2-7
23/01/02	Treatments 2-8
30/01/02	Treatments 2-7
7/02/02	Treatments 2-8
13/02/02	Treatments 2-7
21/02/02	Treatments 2-8

Table 1b: *Application rates of fungicides used in schedule (see Table 1a):*

Application rates	
Fungicide	Rate per litre
Amistar	0.8 ml
Aliette	1.5 g
Filex	1.0 ml
Horti 303*	Experimental
Fubol Gold WG	1.33 g

Figure 1 Trial layout showing treatment numbers and infector plant positions.



Each row contains a complete replicate of the treatments.

Each pair of columns (1&2, 3&4, 5&6, 7&8) contains a complete replicate of the treatment

Figure 2 Photograph of trial layout



Results and Discussion

Possibility of seed-borne P. chlorae infection

In the preliminary trial >40% losses of seedlings to downy mildew occurred in both cvs. Dark Malibu Blue and Picotee Pink. The seeds were sown on 12th April and the first symptoms were observed on 14th May. To avoid applying fungicides, the problem was initially dealt with by rogueing out plants showing symptoms. Symptoms consisted initially of chlorosis and in advanced stages tip scorch of affected leaves, often followed by plant death. Formation of conidia and conidiophores was not observed and attempts to induce sporulation using high RH chambers were not successful. *Peronospora chlorae* infection was diagnosed following leaf clearing and identification of oospores (Hall, 1994) which were formed in high numbers in many affected leaves. Despite the rogueing treatment, disease had reached 30% incidence in both cultivars by 21st May. Filex treatments were applied to the seedlings on 24th May and on 6th June. However, without sign of recovery, the epidemic continued to progress, and once the incidence of disease had exceeded 40% (11th June), it was decided to use bought-in plants instead, and attempt to use affected seedlings to generate conidial inoculum for the main experiment.

Such a rapid, randomly distributed and widespread epidemic, in the absence of an obvious source of infective inoculum, and without the generation of secondary (conidial) inoculum, is strong circumstantial evidence for the occurrence of seed-borne infection. In addition, the rogueing-out of affected individuals and, later, treatments with Filex, both appeared to have no impact on the progress of disease. The presence of seed-borne infection, even at low levels, could have a strong impact on disease in final crops, although its relative importance still needs to be demonstrated. Tackling seed-borne disease may significantly reduce overall incidence of disease. However, it was not possible to induce conidial sporulation in the affected seedlings in the study reported here, which might preclude such material as primary inocula. Nevertheless, where seedlings survive, seed-borne infections may remain 'latent' until later in the plants' life before the development of conidial sporulation. In addition, the lack of sporulation in the work reported here may reflect the wider lack of disease expression seen in the spring/summer of the 2001 season.

Impact of fungicide programmes on disease post-planting

Assessments of phytotoxicity

No obvious signs of phytotoxicity, such as leaf spotting, curling or scorch, were observed in any of the eight treatments throughout the trial. Another possible impact of chemical treatments would be on plant heights. The timing of this trial meant that at its conclusion the range of plant heights was more variable than a summer crop, and there did not appear to be any treatment effects on the mean plant heights (Figure 3). The variation in plant heights recorded also masked any possible effects of downy mildew infection on plant heights, although only severe infections in younger plants would be expected to have a significant stunting effect. Of the two cultivars assessed, Ventura Rose plants were consistently taller than Ventura White plants (Figure 3).

Efficacy of fungicide spray programmes

Disease was measured using counts of the total incidence of symptoms and score for symptom severity. Both measures of disease followed closely similar patterns indicating that where the incidence of disease was reduced by fungicide treatments, the severity in those plants that were affected was also reduced. The worst disease was seen in the untreated control plots and the most susceptible of the two cultivars used was consistently Ventura White (Figures 4 & 5), with more than 60% of plants showing symptoms of downy mildew as opposed to just over 20% for Ventura Rose in the untreated plots.

Complete control of downy mildew was achieved with three of the experimental fungicide programmes (Figures 4 & 5). These were: treatment 2 (Protectant programme A: Amistar, Amistar, Filex, Filex), treatment 4 (Protectant programme C: Horti 303 at every application) and treatment 6 (Protectant programme E: starting with Fubol Gold, followed by cycles of Amistar, Aliette, Filex). Whilst reasonable disease control was obtained with treatments 3 (Protectant programme B: Aliette, Aliette, Filex, Filex) and 5 (Protectant programme D: Aliette, Aliette, Amistar, Amistar) (Figures 4 & 5), none of the spray programme starting with Aliette applications achieved complete control. This result may be linked to the mode of action of fosetyl-aluminium, the active ingredient of Aliette, which controls pathogens indirectly by stimulating the host's defence responses. Whilst this approach has been successful in many other host species, especially in the control of downy mildews in rose and hebe (O'Neill & Bobbin, 2000; O'Neill *et al.*, 2002), the host response may not be so strong in lisianthus, and this active ingredient may not be an appropriate selection with which to start fungicide programmes in this crop.

Delaying the start of fungicide programmes until the first appearance of downy mildew symptoms reduced the efficacy of fungicide spray programmes, as did increasing the spray interval of these programmes from 7 to 14 days (treatments 7 and 8, Figures 4 & 5). Since both of the delayed start programmes (treatment 7 = First symptoms programme A: Aliette, Amistar, Aliette, Amistar – 7 day interval & treatment 8 = First symptoms programme B: Aliette, Amistar, Aliette, Amistar – 14 day interval) deployed Aliette as their first fungicide, a reduction in efficacy may be expected judging from the performance of treatments 3 and 5. However, there was significantly more disease in both treatments 7 and 8 than in treatments 3 and 5, showing that delaying the first spray until disease symptoms were seen still had a negative impact on efficacy against downy mildew (Figures 4 & 5).

Figure 3: Effect of fungicide spray programme on plant heights in lisianthus cultivars Ventura White and Ventura Rose.

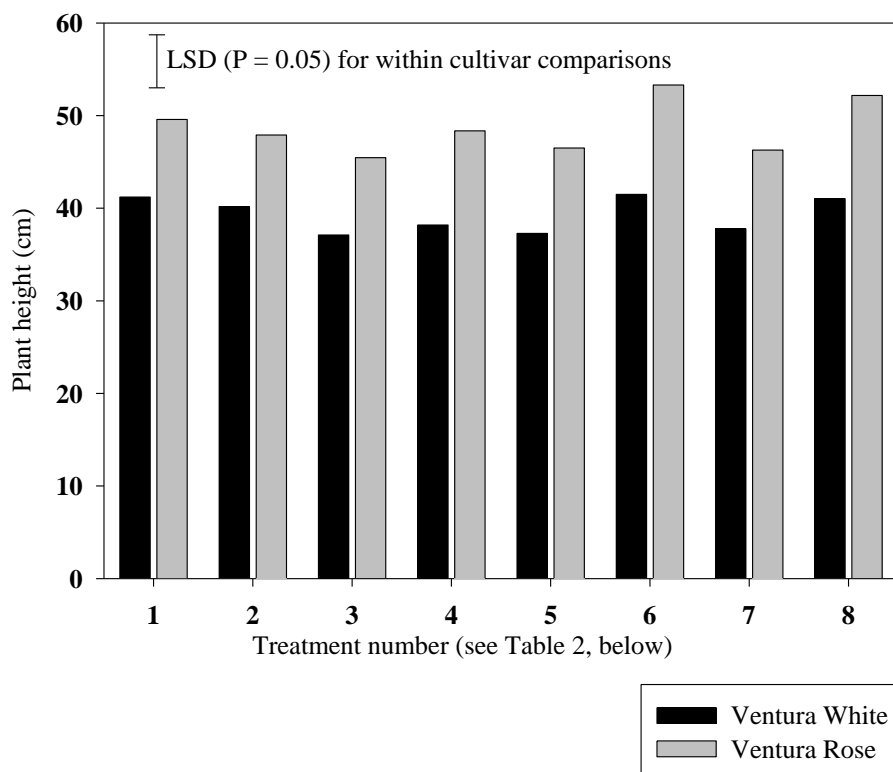


Table 2: Details of fungicide programmes

Treatment number	Treatment title	Spray programme (once four sprays applied, sequence was repeated)
1	Untreated control	None
2	Protectant programme A	Amistar, Amistar, Filex, Filex
3	Protectant programme B	Aliette, Aliette, Filex, Filex
4	Protectant programme C	Horti 303 at every application
5	Protectant programme D	Aliette, Aliette, Amistar, Amistar
6	Protectant programme E	Fubol Gold WG, Amistar, Aliette, Filex (followed by Amistar, Aliette, Filex)
7	First symptoms programme A	Aliette, Amistar, Aliette, Amistar (sprays at 7 day intervals)
8	First symptoms programme B	Aliette, Amistar, Aliette, Amistar (sprays at 14 day intervals)

Fig 4 Effect of fungicide pyraclostrobin on incidence of *P. blight* on rice

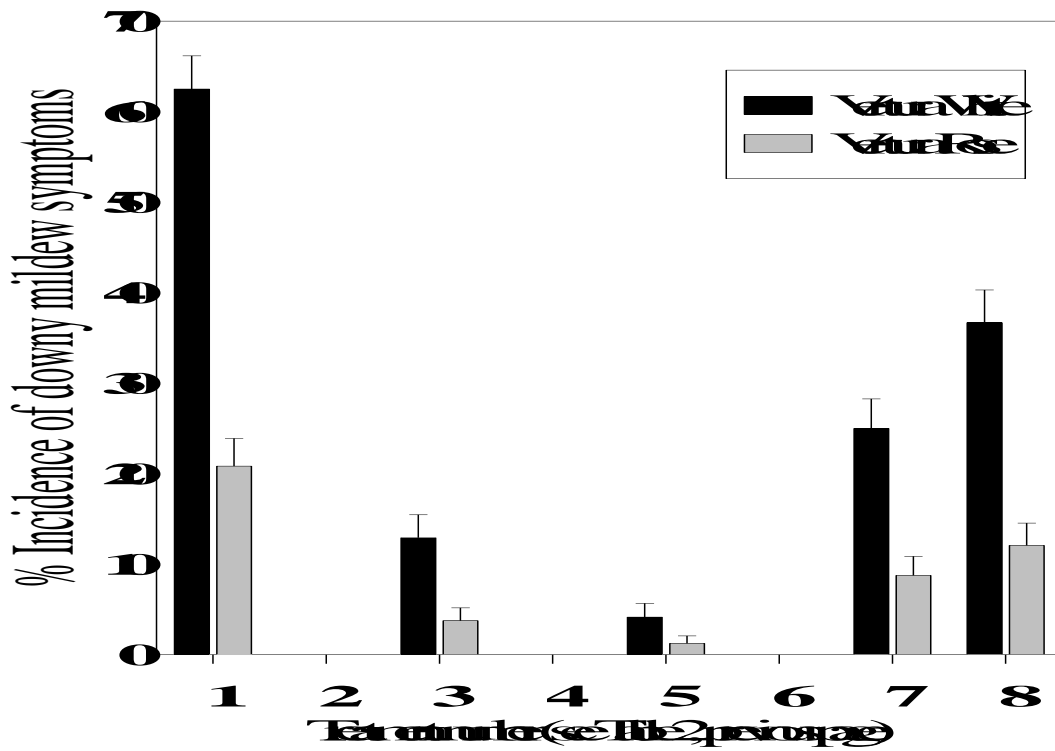
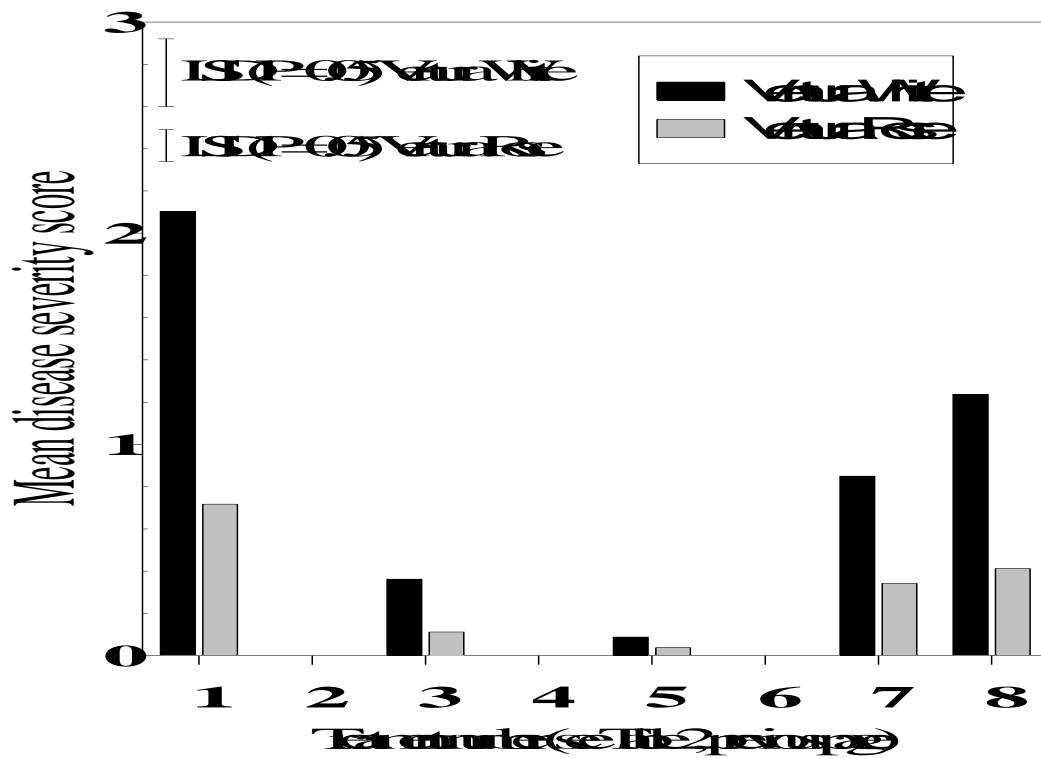


Fig 5 Effect of fungicide pyraclostrobin on severity of *P. blight* on rice



Conclusions

- It is highly likely that *Peronospora chloerae*, the causal agent of downy mildew in lisianthus, can initiate epidemics from seed-borne infection. Further work is necessary to determine the prevalence of seed borne and of latent infections. Nevertheless, measures to control such infection may significantly reduce the incidence of disease post-planting.
- All fungicide treatment programmes significantly reduced disease compared to untreated controls.
- The most effective spray programmes were:
 - Treatment 2. Protectant programme A: Amistar, Amistar, Filex, Filex
 - Treatment 4. Protectant programme C: Horti 303 at every application
 - Treatment 6. Protectant programme E: Fubol Gold WG, Amistar, Aliette, Filex
(followed by Amistar, Aliette, Filex).Horti 303 was used alone as it is a new experimental product and it was important to gauge its performance alone. Whilst promising, this product is not yet registered in the UK to allow its use for downy mildew control in lisianthus.
- Whilst Aliette performed well in spray programmes, starting a spray programme with Aliette appeared to reduce the level of disease control achieved.
- 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.
- Phytotoxicity was not observed with any of the fungicide spray programmes assessed in this study.

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