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The results and conclusions in this report are based on a number of glasshouse-based experiments and a review of literature. The conditions under which these experiments were carried out and the results 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.

Authentication

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

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PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

This project has initially identified a number of novel and experimental fungicides for the control of downy mildew (*Peronospora chlorae*) on *Lisianthus* plants and this should be of particular benefit to the cut flower industry to help improve disease control.

If this project is successful it could lead to enhanced crop quality through improved crop protection strategies, increased production efficiency through improved product quality and improved team-working, co-operation and trust between propagator and grower groups.

Background and objectives

Lisianthus is a relatively new, up and coming, flower crop and is perceived to have great potential in regenerating the UK cut flower industry. Unfortunately, in the last few years, the fungal pathogen *Peronospora chlorae*, cause of downy mildew in *Lisianthus*, has caused significant economic losses to the crop.

Diseased plants exhibit variable, and at times, unusual symptoms not entirely typical of the classical downy mildew diseases. Symptoms include chlorosis, necrosis and collapse of seedlings during propagation with little or no visible indication of sporulation; these symptoms appear more consistent with some form of phytotoxicity (chemical damage) than downy mildew. On older plants, more characteristic leaf yellowing symptoms are usually evident with purple-brown fungal growth on the undersurface of leaves.

The problem has continued over the last 3-4 years despite use of various fungicides by growers and it has been suggested that much of the observed infection in commercial crops has arisen during propagation, possibly a result of earlier seed-borne infection (Yang & Hsieh, 1998). Several other downy mildew fungi are reported to be seed-borne (Ahmad & Majumder, 1987; Corbiere *et al*, 1995, Inaba *et al*, 1983; Sadhana-Srivastara *et al*, 1988; Smith Y Price, 1997; Zad,

1989). The problem has been reported elsewhere in Europe and beyond and it is interesting to note that Sakata in Japan now offer a metalaxyl seed treatment for *Lisianthus* (ie a fungicide treatment aimed at downy mildew). Unfortunately, however, this is currently only being made available for the Far East market and not in the UK.

The host range for *P. chlorae* is limited and restricted to a few members of the Gentianaceae. The only reports of downy mildew on wild hosts in the UK have been on Yellow-Wort (*Blackstonia perfoliata*) (Avon, 1956 & East Anglia, 1981), though Lesser Centuary (*Centaurium pulchellum*), a common weed in Holland, is considered a possible host for the disease (T. Brokenshire, *pers. comm.*). The relative uncommon occurrence of Gentian weed hosts in the UK and the likelihood of physiological specialisation would suggest that weed hosts are unlikely to be responsible for the persistence of the disease over recent years.

Currently, growers have little option but to use fungicides preventatively or to resort to high volume fungicide applications once the disease occurs in a crop and this is particularly difficult to undertake as the crop matures. Also, the occurrence of phytotoxicity symptoms in the crop and the presence of fungicide deposits on leaves at harvest must be minimised. Where approved fungicides have been used they have largely been ineffective in controlling established outbreaks particularly under conditions conducive to the disease. Unfortunately, there are few products approved for use on this crop and growers rely heavily on the Long Term Arrangements for Extension of Use. Unless a product has been approved for use under protection it cannot be used legally on this crop due to Operator Safety restrictions. Further work is clearly needed to identify those products most effective against this, and other *Lisianthus* pathogens, in order to establish the effective rates and timing to minimise the risk of crop damage whilst optimising disease control. At the same time it is necessary to consider developing an integrated strategy to minimise the risk of fungicide resistance developing in pathogen populations.

The primary objectives of this project are:-

1. To evaluate existing and novel fungicides for control of downy mildew in *Lisianthus* in propagation and post-planting.
2. To produce an HDC Factsheet to improve the industry's knowledge of the epidemiology and control of this important disease in *Lisianthus.*

Summary of results

The literature on downy mildew diseases of ornamental crops caused by *Peronospora* species was reviewed. The biology and epidemiology of such disease, from spore germination through infection, latency and sporulation to spore discharge and dispersal is described. Favourable environmental conditions for different stages in the life-cycle are tabulated. High humidity and leaf wetness are critical.

During the first year of this project, valuable practical information has been gained from experiments on the effectiveness of a range of fungicides towards *Lisianthus* downy mildew (*P. chlorae*). During a glasshouse trial set up in the propagation phase of production, high levels of downy mildew infection were achieved providing a stern test for the fungicide treatments. Four fungicides Trustan (oxadixyl + cymoxanil + mancozeb), Invader (dimethomorph + mancozeb), Amistar (azoxystrobin) and SL567A (metalaxyl-M) significantly reduced the level of downy mildew infection during this trial. None of the treatments provided complete control. Trustan was most effective, reducing disease severity by 83% at 7 weeks after sowing.

Two fungicides (SL567A and Trustan) applied prior to seedling emergence significantly reduced the percentage seedling germination of one of the cultivars grown, Picotee Pink though not Malibu Dark Blue. No symptoms of phytotoxicity were seen on any of the other treatments. The two cultivars were equally susceptible to infection and development of downy mildew.

At the termination of the trial, seedlings treated with Amistar, Invader and SL567A all had significantly higher foliage dry weights compared with the untreated control. Although Trustan

treated plants had a higher dry weight than the control, this was not statistically significant. This is most likely due to the poor level of germination leading to a lower overall dry weight per plot.

Conclusions

Literature review

- The biology and epidemiology of downy mildew disease of ornamentals caused by *Peronospora* species is summarised.
- *Peronospora* species generally can infect their hosts over a wide range of temperatures. Moisture is critical for infection to occur. Temperature influences the latent period.
- *P. chlorae* causes a wide range of symptoms on *Lisianthus*: leaf necrosis and death of seedlings, leaf yellowing, stem twisting and distortion.
- Infection by *P. chlorae* is restricted to *Lisianthus* and a few weed hosts in the Gentianaceae, including Yellow Wort and probably Lesser Centuary.
- The disease is spread though the air by conidia, possibly over long distances as has been documented for cucumber downy mildew.

Fungicide evaluation

- Downy mildew (*Peronospora chlorae*) was effectively established in the propagation trial at HRI Stockbridge House through the introduction of 'infector plants' and provided a high level of mildew infection leading to stern test of novel fungicides under evaluation.
- Four fungicides (Trustan, Invader, Amistar and SL567A) significantly reduced the level of downy mildew infection during propagation.
- Application of two fungicides, SL567A and in particular Trustan, during the early stages of propagation resulted in a reduction in the levels of germination of *Lisianthus* seedlings. This reduction was greater with one of the two cultivars used in the trial, Picottee Pink.
- There was no difference in the levels on downy mildew infection between the two *Lisianthus* cultivars, Picotee Pink and Malibu Dark Blue, in this trial.
- Three fungicides Amistar, Invader and SL567A consistently increased the dry weight of *Lisianthus* seedlings after 10 weeks from sowing.

- Of all the fungicide treatments evaluated, Trustan reduced the extent and severity of downy mildew infection throughout the trial. However, treatment with Trustan at sowing reduced the level of germination by 20% compared to the control.
- No phytotoxicity symptoms were observed under any of the other fungicide treatments during this trial.

Action points for growers

1. The results from this project clarify the wide range of symptoms of downy mildew in *Lisianthus*. Growers should familiarise themselves with the various symptoms so that the disease can be spotted at an early stage. [The HDC is preparing a series of pest and disease identification cards for cut flowers including downy mildew of *Lisianthus*. These will be available to HDC members in early 2002].

2. The evaluation of oomycete fungicides in the propagation stage of *Lisianthus* plant production should hopefully allow growers to use a range of effective products on the crop in the future thereby 'ringing the changes' and minimising the risk of fungicide resistance developing.

3. Growers must note that several of the experimental fungicides evaluated in this work are **not** currently approved for use under protection and therefore cannot legally be applied to *Lisianthus* crops. These include, Invader, Trustan and SL567A. Additional work will be required via the HDC SOLA programme and other means, to secure the use of the most effective fungicides under protection. Expensive residue studies are not necessary on ornamental crops. Fungicides which can be legally used on protected *Lisianthus* for downy mildew include: Aliette (fosetyl Al), Amistar (azoxystrobin), Bravo 500 (chlorothalonil), Favour (metalaxyl + thiram), Filex (propamocarb HCI), Fongarid (furalaxyl) and Karamate Dry Flo (mancozeb). Of the products tested during the propagation phase Amistar was the most effective, reducing disease severity by 61%. The currently registered fungicides Favour and Fongarid are not being supported in the EU Review and approvals will cease from July 2003.

4. Growers should remember that where fungicides are approved on ornamentals either via the Long-term Arrangements for Extension of Use (2000) or by a Specific Off-Label Approval, use of the product is at their own risk and the manufacturers carry no liability for poor performance or crop safety.

Future work (2001 to 2003)

Up to 8 fungicides will be evaluated for the control of downy mildew in an experimental *Lisianthus* crop over a 13 week period during June to August 2001. Various spray programmes will be evaluated involving 4 applications at 7 to 14 day intervals.

Evaluations on a commercial holding will be conducted in year 3 (2002) to ensure that recommended treatments are safe, practical, effective and economically viable. A detailed grower factsheet providing guidelines for the control of downy mildew on *Lisianthus* will be prepared by the end of the project.

Practical and financial benefits from the study

Lisianthus production in the UK and elsewhere in Europe is expanding rapidly and it has been estimated that some 5-6 million plants were produced during 1998/99. At an average price/stem of between 25-40p this puts the farm gate value of the UK crop is in the region of £2 million/annum. Due to the crop's increasing popularity the production area is expected to rise considerably over the coming years.

Previously, where downy mildew has occurred, growers have sometimes been unable to control the disease and crops have been rapidly destroyed. Financial losses to individual growers has therefore been very high. Even assuming average annual losses to the disease of 10-20%, the economic loss to the industry is likely to be in the region of £300,000-400,000 per annum. In 1998 for example, losses were significantly higher, estimated to be nearer to 40% (*Lisianthus* Study Group members, *pers. comm.*). The potential financial benefits to the *Lisianthus* industry

are therefore particularly attractive if effective control of the disease can be achieved in the future.

SCIENCE SECTION

1. Introduction and objectives

Lisianthus is a relatively new, up and coming, flower crop although unfortunately, in the last few years, the fungal pathogen *Peronospora chlorae*, cause of downy mildew in *Lisianthus*, has caused significant economic losses to the crop. Infected plants exhibit an unusual chlorosis and dieback, not at all characteristic of downy mildew infection, with little or no sporulation. On close examination by microscopy, however, seedling leaves have been found to be heavily contaminated with oospores of an oomycete fungus. It has been suggested that this could be *P. chlorae*. If the identity of these oospores can be confirmed it suggests very early infection, possibly even via the seed (Yang & Hsieh, 1998; Brokenshire, *pers. comm*.).

After crop establishment, downy mildew infection is expressed by more characteristic symptoms with obvious purple-brown sporulation of the fungus. However, experience by many growers suggests that the rate of disease spread is very rapid under these conditions and control is all too difficult with those fungicides currently available for use under protection.

Objectives of year 1 (2000/2001)

1. To undertake a worldwide literature review on published information regarding the optimum conditions for infection with various downy mildew pathogens.

2. To determine the potential of fungicides applied as seed treatments or pre-/post-emergence drenches to minimise infection during the propagation period of *Lisianthus* production.

3. To evaluate the efficacy and crop safety of a range of existing and novel oomycete fungicides for the control of *P. chlorae* during propagation.

1. Review of literature on downy mildew diseases

2.1 Introduction

Downy mildews are primarily diseases of the foliage, although, as will be seen later, they are also capable of affecting other plant parts. Depending on the stage of growth and the resistance status of the host plant, coupled with the prevailing environmental conditions, the fungus may sometimes colonise the interior tissues of the plant (leaves, stems, roots, etc) extensively, in what is known as a systemic infection.

The downy mildews are closely related to the root-rotting fungal pathogens *Pythium* and *Phytophthora*. They have very little in common with the powdery mildews, apart from the fact that they affect leaves and sometimes produce similar symptoms.

Pythium and *Phytophthora* are sometimes referred to as the 'water moulds' as they thrive in wet soil conditions, and their infective spores (zoospores) actually swim in water films in order to reach the roots of their host plants. The downy mildews are very similar to their relatives in terms of the conditions they require for spore production and infection.

In many of the downy mildews, the infective spore type is still a swimming zoospore. The zoospores are produced in structures known as zoosporangia. It is these zoosporangia that are commonly known as the 'spores' of the mildew and are visible on the affected leaves - the zoospores they produce are tiny, and cannot be seen with the naked eye.

However, some of the downy mildews, particularly those in the genus *Peronospora*, do not produce zoospores. The spores they produce on the leaves are known as conidia rather than zoosporangia, and the conidia germinate directly by producing a germ tube, which then penetrates the host. This method of germination is found in most of the foliar diseases (e.g. *Botrytis*, powdery mildews, rusts) and is thought to be more advanced evolutionarily than the production of zoospores.

Many of the downy mildews affecting ornamental plants belong to the genus *Peronospora,* including Lisianthus downy mildew, *Peronospora chlorae.* However, although these mildews are slightly more advanced, they have not lost their requirement for wetness in order to infect plants.

2.2 Symptoms

The symptoms caused by downy mildews vary according to the specific mildew and its host plant, but there are two general symptom types:

- Symptoms resulting from systemic infection.
- 'Local lesions', usually resulting from secondary spread of the fungus.

2.2.1 Symptoms due to systemic infection

Many downy mildews are capable of invading the internal parts of the plant, causing marked deviations from normal growth habits. Whether a plant becomes systemically infected is dependant on such factors as its age, its growth stage, the environmental conditions and the point on the plant at which infection occurs. Some plants may be systemically infected from the seedling stage, whilst in others the infection becomes systemic at a later date.

Systemically infected plants are often stunted and distorted with pale foliage. Profuse sporulation of the fungus may occur on both leaf surfaces, or even over the entire plant. In some cases, however, there may be no sporulation on the surface of the plant (although resting spores or oospores of the pathogen are produced in large numbers in internal tissues). This can make it difficult to identify downy mildew as the cause of the problem. Affected plants may eventually shrivel and die. If the systemic invasion occurs when a plant is older the lower leaves may remain healthy, but leaves produced following the invasion are of the distorted, pale type. A good example of a plant which often suffers from systemic downy mildew infection is marguerite.

2.2.2 Local lesions

These lesions are what most people would regard as the 'classic' symptoms of downy mildew. They result from infection by spores produced from systemic or local infections of other plants, or of the same plant, and which spread through the air. Areas of the leaf develop which are often discoloured on the upper surface and become covered by a 'downy' or felty growth on the undersurface, consisting of sporulation of the fungus. Occasionally, sporulation may occur before any leaf discolouration has developed. Depending on the host plant, local lesions may be quite angular and bordered by the leaf veins, or they may gradually enlarge to affect a large proportion of the leaf area. Local lesions may also develop on stems, petioles, flower parts, etc.

2.3 Environmental conditions

In this and the following sections the emphasis will be on *Peronospora* species, although occasional reference will be made to *Bremia lactucae*. This downy mildew causes serious problems on lettuce (particularly protected crops) and has, therefore, been studied extensively. Strains of the fungus also affect a number of ornamental plants in the genus Compositae (e.g. *Osteospermum, Gazania, Gaillardia*). Like *Peronospora,* the spores of this downy mildew also germinate directly to produce a germ tube.

Tables 2.1 and 2.2 summarise the information known about a number of the downy mildews commonly affecting ornamentals grown as cut flower, pot or bedding plant crops. It is possible to make some generalisations regarding the environmental conditions that are conducive to infection, spore production and spread of these diseases.

2.3.1 Temperature and moisture/humidity

Spore germination and infection

Free moisture on the surface of the host plant is an essential requirement for infection by downy mildews. This free moisture is needed for the spore to germinate and produce a germ tube, which then penetrates the host. Penetration of leaves may occur through the stomata, but often

direct penetration through the cuticle and surface cell layers occurs. Once the fungus is inside the plant it is protected somewhat from adverse environmental conditions.

The optimum temperature and duration of the wet period will vary between the various downy mildews. For a given mildew, the optimum temperature will often also vary according to the duration of the wet period. In some cases germination and infection will occur in as little as two hours if the temperature and wetness criteria are satisfied. For downy mildew of tobacco (*Peronospora tabacina* - also known as blue mould), the optimum temperature for spore germination is 15°C, with a minimum of 3.5°C and a maximum of 30°C (Schiltz, 1981).

The amount of infection occurring will also vary according to the amount of disease inoculum, i.e. the number of spores. It can be seen that if there are very large numbers of spores, significant levels of infection may occur, even under temperature and wetness criteria that allow only a small percentage of the spores to successfully germinate and infect.

Incubation and latent periods

The incubation period is the time from the start of infection to the first appearance of symptoms, whilst the latent period is the time from the start of infection to the production of the first spores.

The duration of the incubation period and the latent period once again vary between downy mildews, and within a given downy mildew at different temperatures. As stated previously, the fungus is protected to a certain extent from low humidities once it is growing within the plant tissue, but in some mildews the incubation and latent periods may still be considerably shorter at higher relative humidities; in others there is little difference at different humidities. These periods may also vary according to the amount of inoculum present.

As an example of the variation discussed above, the latent period in *Bremia lactucae* may last from five to fourteen days (Crute & Dixon, 1981).

Sporulation

Sporulation in most downy mildews requires moist, dark conditions after a period of light. Once again the optimum conditions required for sporulation vary between mildews.

The light and moisture requirements for sporulation in tobacco blue mould (*Peronospora tabacina)* at 20°C have been studied in some detail (Schiltz, 1981). At this temperature, the amount of sporulation produced on tobacco leaves following their exposure to darkness increased with the duration of the previous period of light (photoperiod), up to a photoperiod of thirteen hours.

After a thirteen-hour photoperiod, the minimum moist dark period for inducing sporulation was two hours. Maximum sporulation was induced after five hours of darkness. Sporulation also occurred if the start of the dark period was dry, but only if this was followed by a moist dark period. The sporulation process (i.e. spores fully formed) was completed eleven hours after the start of the dark period when the previous photoperiod was equal to or longer than thirteen hours.

Sporulation in downy mildews requires high relative humidity (often optimal at around 100% r.h.), but does not usually require the free water that is necessary for spore germination - in fact, in some mildews free water will prevent sporulation or cause abnormalities in the spores.

Downy mildews are able to sporulate for several successive nights on the same lesion. This means that large numbers of spores could be produced during the lifetime of a single, large lesion.

In many downy mildews, infection occurs more readily, and sporulation of the fungus is more profuse, on younger as opposed to older foliage. Symptoms on older leaves are sometimes restricted to small necrotic or chlorotic flecks, with little or no production of conidia. In some mildews, however, these small flecks may contain large numbers of resting spores (oospores).

Spore release

This phenomenon has also been studied extensively in *Peronspora tabacina.* Whilst studies on other *Peronospora* species have been more limited, the information obtained suggests that they behave in a similar manner.

There are two main methods of spore release in *P. tabacina*:

1) Physical disturbance of the leaves or spores (primarily by the wind or by rain-drops falling onto the leaves, but also by man, equipment or anything that causes leaf movement). Even very light rain is enough to cause spore release. If there is a lengthy period of rain, spore release is greatest at the beginning of the rainfall and steadily declines. If there are scattered showers, there is a period of spore release at the beginning of each shower.

   In breezy or windy conditions, more spores are released if there is an increase in air movement than if the airflow is steady (even at quite high wind speeds). Both this and the effect of scattered showers show that it is increases in mechanical stimulus that result in spore release, rather than a steady stimulus.

2) An effect of decreasing humidity. As the humidity drops the stalks (conidiophores) on which the spores are produced begin to dehydrate and twist, a feature known as hygroscopic movement. These movements are enough to release the spores.

Spore dispersal

Long-distance spread of air-borne conidia has been reported for some downy mildews. In the case of both cucumber downy mildew (*Pseudoperonospora cubensis*) and tobacco blue mould (*Peronospora tabacina*) in the USA, the disease first appears each year in the southern states, and then spreads via wind-borne conidia into the Atlantic coastal states (Cohen, 1981; Schiltz, 1981). When it was first introduced into Europe in the 1960's, tobacco blue mould progressed across the continent at distances of up to 400 kilometres per month, again thought to be due to air-borne conidia.

2.4 Periodicity of spore release, and its effect on disease development.

As has been described above, downy mildews require a period of darkness (and high humidity) for spore production to occur. Given such conditions, spores will then usually be produced within a few hours. These spores are, therefore, normally present by dawn, when the increasing temperature and decreasing humidity will trigger spore release by the twisting of the conidiophores. Given a dry day, spores are generally present in the air in greatest quantity in the early morning. Numbers then normally decline through the day, as the spores fall back down to earth.

If, however, there is a period of rainfall or an increase in wind speed during the day, this may trigger a further episode of spore release.

We have already seen also that downy mildew spores will only germinate in the presence of free water. If the spores are released during a dry day, and deposited on dry leaf surfaces of the host plant, they will not be able to germinate immediately.

There are, in fact, a number of ways in which new infections with a downy mildew could occur in an outdoor situation:

1) Spores are released in the morning of a dry day and deposited on dry leaf surfaces, so that they cannot germinate immediately. Germination and infection could occur in any dew deposited the following night, but the infection efficiency is likely to be quite poor. This is because there will be a decline in the viability of the spores during the preceeding hours of dryness.

2) Spores are released on a dry morning, but rain occurs during the day. The efficiency of infection in this case would depend on the length of the period of leaf wetness caused by the rain. This will be determined firstly by the length of the period of rain, and the conditions following it (if sunny conditions follow the rain, the leaves will dry more quickly than if conditions remain overcast). The second factor will be the time of day at which the rain

occurred (if it is late in the day then many spores may still have become unviable due to the preceding hours of dryness).

3) There is an overlap of the early morning spore release with the persistence of dew. In these conditions spore germination could occur immediately in the water film caused by the dew, and there will have been little or no decrease in viability of the spores. The infection efficiency will depend on how long the morning dew persists.

4) Spores are released by rain during the day, and immediate germination and infection occurs in the resulting leaf wetness. This will give high infection efficiency with evening rain or lengthy periods of daytime rain, both of which will result in a prolonged period of leaf wetness.

Because Lisianthus is grown as a protected crop in the UK, there will obviously be differences in the way infection occurs. Conditions will be influenced greatly by grower practices such as irrigation regimes and ventilation. This will be discussed in more detail in the separate section on Lisianthus downy mildew.

2.5 Long-term survival of downy mildews

2.5.1 Mycelium

A number of downy mildews are known to survive for considerable periods as mycelium in plants or plant parts. Amongst the *Peronospora* mildews, these include *Peronospora destructor*, which can survive in onion bulbs, *P. farinosa*, which can overwinter in sugar beet roots, and *P. tabacina*, which can survive as mycelium in tobacco plants.

2.5.2 Conidia

Downy mildew conidia are not particularly resilient, but given an appropriate set of environmental conditions could, in theory at least, survive for a considerable length of time. Populer (1981), summarises the results of work done in Australia in the 1960's, which showed that one per cent of conidia of *Peronospora tabacina* was still able to germinate after being stored in the laboratory at constant temperature and low humidity for 100 days. He also describes further work in Germany, in which conidia of *P. tabacina, P. parasitica* and *P. farinosa,* when stored in dry soil or dust in an open shed, were still infective over several weeks in summer, and several months in winter.

2.5.3 Oospores

Oospores, or resting spores, are produced by many, but not all, of the downy mildews. They may be produced in most parts of the plant (leaves, stems, roots, etc), and can also sometimes contaminate seed (see below). They are often formed in response to conditions that are unsuitable for the production of conidia, on older or senescing leaves, or towards the end of the growing period of their host. The spores are generally released into the soil from the senescing plant material. Oospores of *Peronospora* species are often capable of surviving for many years, with those of *P. destructor* reported to last for up to 25 years (Viranyi, 1981).

In many cases, whilst oospores can be found in large numbers in affected plants, it has been difficult to germinate them and prove that they are capable of initiating new infections. In the case of some crops, however, such as peas, sunflowers, tobacco and vines, infection via oospores is known to occur.

2.5.4 Seed

Contamination of seed with downy mildew has been noted for a number of crops, such as sorghum, maize, grasses, peas, beans, beet and sunflower. Seed-borne infection of crops which are grown as ornamentals has been noted for *Mecanopsis* and *Papaver* (affected by *Peronospora arborescens*) and *Clarkia* (affected by *Peronospora arthurii*). Seed-borne infection has also been noted in blue mould of tobacco (*Peronospora tabacina*) although is not thought to be particularly significant – species of *Nicotiana* are, of course, grown as ornamentals. Seed contamination by downy mildews may range from the presence of oospores on the surface of the seed to a more deep-seated infection by mycelium and/or oospores.

Infections arising from contaminated seed are often systemic. In the case of sunflowers (affected by the downy mildew *Plasmopara halstedii*), plants grown from contaminated seed often look healthy, but sporulation is produced on a high proportion of the roots (Sackston, 1981). Oospores produced in this way contaminate the soil and then cause systemic infection of subsequent crops. The infected but symptomless plants arising from infected seed may subsequently also produce infected seed themselves.

Table 2.1: Environmental conditions conducive to some of the common downy mildews affecting ornamental plants.

Downy mildew species	Ornamental hosts affected	Environmental conditions required
Peronospora antirrhini	Antirrhinum	Production of conidia: Minimum temp. : 7°C Optimum temp. : 10°C Maximum temp. : 22°C Humidity : High Germination of conidia: Optimum temp. : 13°C Water film required.
Peronospora chlorae	Lisianthus	General favourable conditions: High humidity
Peronospora dianthi	Dianthus *(annual species)*	General favourable conditions: High humidity
Peronospora grisea	Hebe	General favourable conditions: High humidity
Peronospora parasitica	*Stocks, wallflowers, aubrietia*	Production of conidia: Temp. range : 8-16°C Darkness required Germination of conidia: Temp. range : 8-12°C Humidity : High Infection: Temp. range : 8-25°C Humidity : High
Peronospora sparsa	Rosa	Germination of conidia: Minimum temp. : 4°C Optimum temp. : 18°C Maximum temp. : 27°C Leaf wetness: four hours in free water Overall disease development: relatively low temperatures and 90-100% relative humidity.
Peronospora tabacina (P. hyoscyami f. sp. t*abacina)*	Nicotiana	Production of conidia: Minimum temp. : 1-2° Optimum temp : 15-23°C Maximum temp. : 30°C Humidity : 95% for 3 hours Darkness : minimum 1.5hrs Release of conidia: increase in temp. and insolation; decrease in relative humidity. Germination of conidia: Minimum temp : 3.5°C Optimum temp. : 15°C Maximum temp. : 30°C Most favourable overall conditions: average temp. of 20°C (16°C night/ 24°C day), high relative humidity, overcast, moving air.

Table 2.1 (continued)

Downy mildew species	Ornamental hosts affected	Environmental conditions required
Peronospora violae	Viola *(mainly pansy)*	General favourable conditions: High humidity
Bremia lactucae	Gaillardia, Gazania, Osteospermum	Production of conidia: Temp range : 4-20°C Humidity : >95% Darkness : 6 hours Release of conidia: decrease in relative humidity. Germination of conidia: Temp. range : 0-21°C Optimum temp. : 10°C Requires free water

Table 2.2: Further details of some of the common downy mildews affecting ornamental plants.

Downy mildew species	Ornamental hosts affected	Oospores produced ?	Spores causing infection	Seed transmission?	Race structure	Resistance in host?	Other information
Peronospora antirrhini	Antirrhinum	Yes	Conidia, oospores	Possibly by oospore-contaminated seed; not confirmed	N/k	Varietal differences in susceptibility noted	Causes systemic and local infections. Mainly a disease of seedlings and young plants.
Peronospora chlorae	Lisianthus	Yes	Conidia (role of oospores unknown)	Not known	Not known	Varietal differences in susceptibility noted	
Peronospora dianthi	Dianthus *(annual species)*	Yes	Conidia (oospores suspected but not confirmed)	Not known	Not known	Not known	Mainly systemic infection.
Peronospora grisea	Hebe	Yes	Conidia (role of oospores unknown)	Not known	Not known	Varietal differences in susceptibility noted	
Peronospora parasitica	*Stocks, wallflowers, aubrietia*	Yes	Conidia (oospores suspected but not confirmed)	Yes (but probably insignificant)	Yes	Varietal differences in susceptibility noted	Systemic or local infection. Fungicide resistance to phenylamides recorded.
Peronospora sparsa	Rosa	Yes	Conidia (role of oospores unknown)	Suggested but not proven	Not known	Varietal differences in susceptibility noted	Can be systemic. May overwinter in host as dormant mycelium.
Peronospora tabacina (P. hyocyami f.sp. *tabacina)*	Nicotiana	Yes	Conidia, oospores (occasionally)	Yes (but probably insignificant)	Yes	Yes – bred into plants as part of control strategy	Systemic or local infection. Overwinters as conidia or oospores, or in plants as mycelium.
Peronospora violae	Viola *(mainly pansy)*	Yes	Conidia (role of oospores unknown)	Not known	Not known	Varietal differences in susceptibility noted	
Bremia lactucae	Gaillardia, Gazania, Osteospermum	Yes	Conidia, oospores	Yes (but probably insignificant)	Yes	Yes – bred into plants as part of control strategy	Systemic or local infection. Fungicide resistance to phenylamides recorded.

2.6 Lisianthus downy mildew (*Peronospora chlorae)*

As can be seen from the sparse entries in Tables 2.1 and 2.2 for *Peronospora chlorae*, little is known about Lisianthus downy mildew in terms of its method of transmission or the precise environmental conditions that are optimal for infection.

The symptoms of the disease are very variable. Established plants tend to show 'typical' downy mildew symptoms of chlorosis and downcurling of affected leaves, accompanied by profuse purplish-brown sporulation (consisting of conidiophores and conidia) on the underside. Severely affected leaves eventually wither. The attack is often most severe on the younger leaves (as has been discussed previously for downy mildews on some other crops, leaves tend to become less susceptible to attack as they age). The fungus also causes brown lesions on the stem, which can lead to bending of the stem if they are extensive. These lesions tend not to produce conidia but are often full of oospores, as are senescing leaves and, occasionally, roots.

Symptoms on young seedlings range from chlorosis, through to necrosis and collapse of the plant (these latter symptoms are often more suggestive of a phytotoxic reaction to a chemical application than an effect of downy mildew). There is often no production of conidia in these cases, but affected tissue will again contain oospores. Such early attack has led to the suggestion that the infection could have arisen from seed contaminated by the fungus (Yang and Hsieh, 1998), but this has not been proven. The symptoms are certainly suggestive of systemic infection.

Peronospora chlorae affects some other members of the plant family Gentianaceae, to which *Lisianthus* belongs. As well as gentians themselves, this family includesYellow-wort (*Blackstonia perfoliata*), on which downy mildew has been reported in the UK (in Avon in 1956 and East Anglia in 1981). Another member of the family that occurs in the UK is Lesser Centaury (*Centaurium pulchellum*). This plant was associated with outbreaks of the disease in Holland in 1983 (Hobolth & Loschenkohl, 1987). However, weed hosts in the family Gentianaceae are relatively uncommon in the UK, and there is also the possibility (although this is not confirmed) that the fungus could exist as different races which do not spread from one

species to another. It thus seems unlikely that weed hosts are responsible for the widespread and damaging outbreaks of the disease in the UK in recent years.

There has been little or no study into the precise environmental conditions favouring such aspects as production, germination and infection of the host plant by the conidia of *Peronospora chlorae*. Outbreaks in Denmark have been associated with damp conditions (Hobolth & Loschenkohl, 1987), whilst a severe outbreak in Italy occurred when the maximum relative humidity (measured weekly) was at or around 100% (Buonocore & Pane, 1995). The temperature fluctuated during this period from below 10°C to around 40°C. Work in China (Yang & Hsieh, 1998) showed that the conidia of the fungus germinated at temperatures ranging from 8°C to 32°C. Circumstantial evidence from UK outbreaks of the disease also suggests that, like most downy mildews, the fungus is favoured by conditions of high humidity and leaf wetness.

Despite this lack of precise knowledge of the environmental conditions favourable to *Peronospora chlorae*, it is possible to speculate on the effects that various grower practices will have on the disease, given the knowledge available on other members of the Peronosporaceae (outlined in earlier sections). 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, of course, not known for *P. chlorae*. In most cases, production of spores 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 (the accompanying increase in solar radiation (insolation) is also thought to play a part in spore release of some mildews).

It follows, therefore, that anything that can be done to reduce or prevent leaf wetness will reduce the possibility of spores germinating and infecting the plant, whilst a reduction in the overall relative humidity may also help prevent or reduce spore production. As *Lisianthus* is grown under protection in the UK, it is possible to manipulate the environment by ventilation (or heating and ventilation during colder weather) to lower the humidity and prevent leaf wetness.

Other factors such as plant spacing, the use of fans and the positioning of heating pipes to improve airflow through the canopy will also help in this respect.

If overhead watering of plants is carried out towards the end of the day, then high humidity during the hours of darkness is likely to result, and this will trigger the production of large numbers of spores. If any leaf wetness persists into the following morning, these spores could immediately infect the plants. It may also be unwise to overhead irrigate plants too early in the morning (particularly if the weather is overcast), as this watering is likely to trigger the release of any spores that have been produced the previous night, and to provide conditions suitable for infection if leaf wetness persists for any length of time. The aim should be, if overhead irrigating, to do so at a time when the leaf surfaces will dry out rapidly, and humidity will quickly return to levels which are unsuitable for the fungus.

Even though the precise role of oospores in the infection cycle of *P. chlorae* is not known, it is a sensible precaution to destroy any affected material. It has been shown for some other downy mildews that the conidia can also survive for extended periods (days or even weeks) given an appropriate set of environmental conditions (generally, low relative humidity).

In some downy mildew diseases (e.g. blue mould of tobacco, downy mildew of cucumber), investigations have been undertaken into the prevention of spore production by manipulating wavelengths of light, or interrupting the dark period during the night. Exposing tobacco plants to blue wavelengths of light, or providing intermittent flashes of light during the night can hinder spore production in *Peronospora tabacina* and some other mildews. In Israel, some cucumber crops are grown in polytunnels clad with blue film to aid reduction of downy mildew (Reuveni & Raviv, 1997). Similar investigations may be worthwhile with *Peronospora chlorae.*

3. Evaluation of crop safety and efficacy of fungicide treatments

3.1 Introduction

Whilst there are numerous fungicides with potential activity against downy mildew, including *Peronospora chlorae*, little work has been done to identify the most effective against this disease in *Lisianthus* crops. The work in the first year is focusing on controlling the disease in the propagation phase of plant production as well as assessing phytotoxicity symptoms on the plants.

The objective of this first experiment was to screen a range of fungicides with the potential to control *P. chlorae* and assess their crop safety towards *Lisianthus* plants in the propagation phase of production.

3.2 Materials and Methods

Plant material and trial location

Two Lisianthus cultivars were used in this study, Picotee Pink (Hamer Seeds) and Malibu Dark Blue (Colgrave Seeds). The trial was located in propagation house F14, HRI Stockbridge House.

Experiment design and analysis

The trial was laid out in an incomplete Trojan Square design, with 4 replicates of each of the 10 treatments listed below, as advised by Biometrics Department, HRI Wellesbourne. Each plot/replicate consisted of 200 *Lisianthus* plants sown by hand into polystyrene modular trays, of which there were 100 plants of each of the two *Lisianthus* varieties, known to be susceptible to downy mildew, Picotee Pink (Kyoto Series: Hamer Seeds) and Malibu Dark Blue (Colgrave Seeds).

Treatments

The following treatments and application rates listed below were included in this trial. The fungicide treatments were applied on three separate occasions, just after the point of seeding, at radicle emergence around 2 weeks from sowing and at 100% cotyledon emergence after about 4 to 5 weeks from sowing. Sprays were applied using an Oxford Precision sprayer. Rates of use are detailed in the table below.

Treatment		**Application timing**			
		Post seeding	**Post radicle emergence**	**Post-emergence**	**Approval status**
1	Untreated control Water applied to run-off	+	+	+	-
2	Fongarid (furalaxyl) – Standard 1.0g product/litre water	+	+	+	Approved
3	SL567A (metalaxyl-M) 1.3ml product/litre water	+	+	+	Not approved
4	Aliette (fosetyl-Al) 1.5g product/litre water	+	+	+	Approved
5	Filex (Propamocarb-HCl) 1ml product/litre water	+	+	+	Approved
6	Amistar (azoxystrobin) 0.8ml product/litre water	+	+	+	Approved
7	F279 (trifloxystrobin) 2.0ml product/litre water	+	+	+	Not approved
8	Bion (acibenzolar) 0.1g product/litre water	+	+	+	Not approved
9	Invader (dimethomorph + mancozeb) 2.0g Product/litre water	+	+	+	Not approved
10	Trustan (oxadixyl + cymoxanil + mancozeb) 6.5g (or 3.0kg) product/litre water	+	+	+	Not approved

The approval status 'Approved' indicates that the product has approval for use under protected cropping conditions for ornamental crops. Approval may be via on-label approval, or off-label approval under the Long Term Arrangements for Extension of Use (2000). It is important for Growers to check the approval status of any fungicide, before application to the crop.

Use pesticides safely – Always read the label

Crop Diary

Lisianthus seed sown:	21 August 2000
Fungicide application 1 (Sowing/seeding):	25 August 2000
Fungicide application 2 (Radicle emergence):	11 September 2000
Seedling emergence:	13 September 2000
Infector plants added to trial area:	15 September 2000
Seedling emergence:	28 September 2000
Disease assessment (% plants infected):	10 October 2000
Fungicide application 3 (Cotyledon stage):	10 October 2000
Infector plants added to trial area:	10 October 2000
Trial termination	
Disease assessment (% plants infected):	27 October 2000
Disease level scored (0-3):	27 October 2000
Dry weight:	31 October 2000

Method

Untreated *Lisianthus* seed was sown in modular trays with Levington F2 compost that was previously sieved to remove larger lumps and mixed with about a 20% vermiculite mix and then propagated according to standard commercial practice. The seed/seedlings were then treated with the experimental fungicide treatments at three stages during propagation or left untreated as a control.

Growing environment

The glasshouse environment was maintained at 20^0C to represent commercial growing conditions. The trays were covered for the first 2 weeks from sowing until the radicles emerged.

Collection and maintenance of *Peronospora chlorae* isolates

Infected plant material received from commercial nurseries in the UK was sampled for *Peronospora chlorae*. A number of isolates were used to inoculate live Lisianthus plant material and maintained in an isolated polythene tunnel at Stockbridge House. The plants were maintained under day/night temperatures of $16^{O}C/10^{O}C$ and were covered in a polythene tent at night. Young plants, grown at Stockbridge House, were placed beside infected ones to maintain the stock mildew culture for this experiment.

Inoculation

The trial was inoculated through the introduction of Lisianthus plant material infected with *P. chlorae* to allow natural infection of downy mildew into the treated plants. Polythene sheeting was laid over the plant material at night to increase the relative humidity. In addition, to encourage infection and allow natural dissemination of downy mildew spores, the plants were watered from above to maintain leaf surface moisture.

Assessments

During the trial, (13 September and 28 September) the seedling emergence under each treatment regime was assessed for each cultivar. On 10 October and 27 October, the number of plants with symptoms of *P. chlorae* infection was recorded and the percentage of total plants per cultivar calculated. In addition, the level and extent of any phytotoxicity symptoms in each treatment was noted.

On the termination of the trial on 27 October, the severity of downy mildew infection of 20 individual seedlings of each cultivar per treatment plot were scored (0-3) on the extent of the symptoms:

0 = no *P. chlorae* sporulation on the plant tissues
1 = low level of *P. chlorae* sporulation on the underside of the cotyledons
2 = high level of *P. chlorae* sporulation on the undersides of the cotyledons
3 = sporulation of *P. chlorae* on both sides of the cotyledons

The disease index, expressed in the results section was calculated from the severity score assessments as follows:

$$\frac{1(\text{No in category 1}) + 2(\text{No in 2}) + 3(\text{No in 3})}{\text{No of plants assessed}} \times \frac{100}{3}$$

The range of this index was, therefore, zero (no infection) to 100 (high level of infection).

Finally, the seedling foliage from each treatment and cultivar was cut off and placed in an oven at 70^{o}C for 48 hours to record the dry weights.

Statistical analysis and storage of data

The data from this experiment was analysed by the Biometrics Department, HRI Wellesbourne.

The raw data from these experiments will be archived for a period of not less than 5 years. Access to the data can only be made through a designated Archivist.

Official Recognition and Quality Assurance

The experiments reported were conducted in accordance with the guidelines for 'Official Recognition of Efficacy Testing Organisations in the United Kingdom' (Certificate Number ORETO 020) as outlined by the Pesticide Safety Directorate (''RD Ref. 2400/2996) and HRI's Standard Operating Procedures (SOPs).

A specific quality assurance audit was not undertaken during this work.

3.3 Results and Discussion

During this trial, conducted in the propagation phase of *Lisianthus* plant production, a high level of downy mildew infection was achieved through the introduction of naturally infected plant material to the trial area, with *P. chlorae* sporulation on up to 70-80% of the seedlings per plot. This provided a stern test for the evaluation of the treatment fungicides to control this pathogen.

Two *Lisianthus* cultivars, both of which were known to be susceptible to downy mildew infection, were used in this experiment. Levels of germination of these two cultivars, recorded on 13 September and 28 September, in the control treatment, where only water was applied, showed no significant difference in percentage germination between the two cultivars (Table 3.1 and 3.2). However, the germination counts recorded on these two assessments (Table 3.1 and 3.2) indicate that two fungicide treatments, SL567A and in particular Trustan, applied at the time of sowing and radicle emergence, resulted in significant reductions in levels of germination of one of the two cultivars; Picotee Pink. Overall, the levels of germination of the cultivar Malibu Dark Blue were, in general, higher than Picotee pink under the control and the fungicide treatments, although these differences were not significant.

On 10 October, 4 weeks from emergence, after an assessment for the presence of downy mildew symptoms on the seedlings, the percentage of plants infected with *P. chlorae* was calculated (Table 3.3). Three fungicides, SL567A, Amistar and Trustan completely controlled the development of downy mildew symptoms. All of the other fungicides, except Aliette, resulted in a significant reduction in the presence of downy mildew development on the seedlings. There was no significant difference in the susceptibility of the two cultivars used in this trial.

Approximately 2 weeks later a further assessment of the extent of downy mildew revealed that all of the treatment plots showed some degree of infection (Table 3.4). The level of downy mildew in the untreated controls reach high levels with between 73.2% - 78.0% of all emerged seedlings displaying sporulation of *P. chlorae*. Three fungicides, Trustan, Invader and Amistar significantly reduced the level of downy mildew symptoms on both of the *Lisianthus* cultivars.

There was no significant differences in the level of infection between the two cultivars, Picotee Pink and Malibu Dark Blue.

The degree of infection was scored on 20 random seedlings per plot and the treatment means are displayed in Table 3.5. Again three fungicides, Trustan, Invader and Amistar reduced the level of mildew infection significantly on both *Lisianthus* cultivars. The fungicide SL567A reduced the level of mildew on the Picotee Pink but not on the Malibu Dark Blue.

Finally, at the termination of the trial, the dry weight of the plant foliage was recorded (Table 3.6). When the dry weight measurements for each treatment were meaned, three treatment fungicides, Amistar, Invader and SL567A all resulted in a significant increase in the levels of dry weight compared to the untreated control. Fongarid, Bion and Trustan resulted in a significant increase in foliage dry weight of Malibu Dark Blue but not Picotee Pink seedlings.

In assessments on the extent as well as the degree of downy mildew infection, the fungicide Trustan gave the most significant reductions in downy mildew on both cultivars used in this trial. However, when recording the foliage dry weight the levels for Trustan treated plants were low due to the poor germination levels recorded (Tables 3.1 and 3.2). Application of Trustan after seedling emergence did not further reduce the development of *Lisianthus* seedlings. The only other fungicide that significantly affected the levels of germination was SL567A, although this was limited to one of the *Lisianthus* cultivars.

There was no sign of any other phytotoxicity symptoms observed on the seedlings during the experiment which was conducted for 7 weeks after seedling emergence. However, with a heavy downy mildew infection, it is difficult to confirm that the fungicide application was not partly related to the reduction in plant growth and development.

At earlier disease assessments SL567A completely controlled downy mildew symptoms. Towards the end of the propagation phase, this metalaxyl-based product did not control the development of the disease. A possible explanation for this ineffectiveness could be due to the development of strains of *P. chlorae* insensitive to metalaxyl in the pathogen population

introduced on the infector plants. However, although the presence of such strains is suspected, there is currently no published data on the occurrence of resistance in the populations of *P. chlorae* towards metalaxyl or other phenylamide fungicides.

Table 3.1: Assessment of percentage germination of Lisianthus seedlings on 13 September 2000.

Treatment		Germination (%)[1]		
		Picotee Pink	Malibu Dark Blue	Treatment Mean
T1	Inoculated control, water	82.2 (65.7)	84.0 (67.2)	83.1 (66.5)
T2	Fongarid	75.0 (60.2)	93.5 (76.2)	84.2 (68.2)
T3	SL567A	68.0 (55.6)	87.5 (70.2)	77.8 (62.92)
T4	Aliette	78.5 (63.1)	89.8 (72.0)	84.1 (67.5)
T5	Filex	79.0 (62.9)	88.2 (71.6)	83.6 (67.3)
T6	Amistar	80.5 (64.3)	86.5 (69.4)	83.5 (66.9)
T7	F279	79.5 (64.1)	85.5 (69.1)	82.5 (66.6)
T8	Bion	81.5 (65.2)	85.2 (68.3)	83.1 (66.7)
T9	Invader	88.5 (70.9)	87.2 (70.5)	87.6 (70.7)
T10	Trustan	46.0 (42.7)	72.0 (58.4)	59.0 (50.5)

	Compare 2 different treatment means	
	Significance	(***)
	SED (57 df)	(5.62)
	Compare different cultivars per treatment	
	Significance	(NS)
	SED (57 df)	(3.98)

[1] Data in parenthesis has been transformed by angular transform prior to analysis of variance

Table 3.2: Assessment of percentage germination of Lisianthus seedlings on 28 September 2000.

Treatment		Germination (%)[1]		
		Picotee Pink	Malibu Dark Blue	Mean
T1	Inoculated control, water	80.8 (64.9)	83.0 (66.5)	81.9 (65.7)
T2	Fongarid	73.8 (59.4)	93.8 (76.2)	83.8 (67.8)
T3	SL567A	69.2 (56.4)	92.2 (74.2)	80.8 (65.3)
T4	Aliette	79.8 (64.1)	88.8 (71.1)	84.2 (67.6)
T5	Filex	79.0 (62.9)	88.8 (72.3)	83.9 (67.6)
T6	Amistar	86.0 (69.1)	86.5 (69.6)	86.2 (69.3)
T7	F279	81.8 (66.0)	88.2 (71.3)	85.0 (68.7)
T8	Bion	77.2 (62.2)	90.0 (72.3)	83.6 (67.2)
T9	Invader	91.0 (73.1)	88.0 (71.4)	89.5 (72.3)
T10	Trustan	51.5 (45.9)	76.0 (61.3)	63.8 (53.6)

	Compare 2 different treatment means	
	Significance	(**)
	SED (57 df)	(3.97)
	Compare different cultivars per treatment	
	Significance	(NS)
	SED (57 df)	(5.61)

[1] Data in parenthesis has been transformed by angular transform prior to analysis of variance

Table 3.3: Assessment of downy mildew infection: percentage of plants infected with *P. chlorae* on 10 October 2000.

Treatment		Percentage of plants infected with *P. chlorae* (%)[1]		
		Picotee Pink	Malibu Dark Blue	Treatment Mean
T1	Inoculated control, water	14.0 (19.9)	17.3 (24.0)	15.6 (22.0)
T2	Fongarid	4.3 (10.1)	1.6 (6.0)	2.8 (8.0)
T3	SL567A	0 (0)	0 (0)	0 (0)
T4	Aliette	8.9 (17.1)	11.4 (16.7)	10.2 (16.9)
T5	Filex	5.0 (11.0)	6.8 (19.5)	6.0 (15.2)
T6	Amistar	0 (0)	0 (0)	0 (0)
T7	F279	2.2 (7.9)	11.4 (15.5)	7.0 (11.7)
T8	Bion	3.4 (7.7)	4.1 (10.9)	3.8 (9.3)
T9	Invader	0.3 (1.5)	0 (0)	0.1 (0.8)
T10	Trustan	0 (0)	0 (0)	0 (1.9)

	Compare 2 different treatment means	
	Significance	(***)
	SED (57 df)	(3.41)
	Compare different cultivars per treatment	
	Significance	(NS)
	SED (57 df)	(4.82)

[1] Data in parenthesis has been transformed by angular transform prior to analysis of variance

Table 3.4: Assessment of downy mildew infection: percentage of plants infected with *P. chlorae* on 27 October 2000.

Treatment		Percentage of plants infected with *P. chlorae* (%)[1]		
		Picotee Pink	Malibu Dark Blue	Treatment Mean
T1	Inoculated control, water	73.2 (66.4)	78.0 (72.6)	75.6 (69.5)
T2	Fongarid	81.2 (71.2)	84.8 (77.1)	83.0 (74.2)
T3	SL567A	62.0 (59.7)	78.5 (66.8)	70.2 (63.3)
T4	Aliette	86.2 (78.0)	93.8 (82.5)	90.0 (80.2)
T5	Filex	81.2 (75.0)	80.0 (75.1)	80.6 (75.1)
T6	Amistar	52.0 (45.3)	34.2 (34.5)	43.1 (39.9)
T7	F279	49.5 (90.0)	87.8 (78.9)	93.6 (84.5)
T8	Bion	74.2 (64.5)	89.0 (79.6)	81.6 (71.5)
T9	Invader	36.8 (35.0)	42.8 (36.9)	39.8 (36.0)
T10	Trustan	16.25 (17.3)	11.2 (14.2)	13.8 (15.8)

	Compare 2 different treatment means	
	Significance	(**)
	SED (57 df)	(9.53)
	Compare different cultivars per treatment	
	Significance	(NS)
	SED (57 df)	(13.48)

[1] Data in parenthesis has been transformed by angular transform prior to analysis of variance

Table 3.5: Assessment of downy mildew infection: severity of *P. chlorae* symptoms expressed as a Disease Index (0-100) on 27 October 2000.

Treatment		Disease Index; 0 = no disease and 100 = high level of infection/disease		
		Picotee Pink	Malibu Dark Blue	Treatment Mean
T1	Inoculated control, water	75.4	90.0	82.7
T2	Fongarid	82.9	83.3	82.7
T3	SL567A	55.0	71.2	63.1
T4	Aliette	87.5	88.3	87.9
T5	Filex	72.9	80.0	76.4
T6	Amistar	41.2	23.3	32.3
T7	F279	84.2	70.8	77.5
T8	Bion	83.7	87.9	85.8
T9	Invader	37.1	43.7	40.4
T10	Trustan	11.67	16.7	14.2

	Compare 2 different treatment means	
	Significance	***
	SED (57 df)	9.63
	Compare different cultivars per treatment	
	Significance	NS
	SED (57 df)	13.6

Table 3.6: Assessment of plant dry weight at the termination of the trial on 31 October 2000.

Treatment		Foliage dry weight (g)		
		Picotee Pink	Malibu Dark Blue	Mean
T1	Inoculated control, water	1.5	1.6	1.6
T2	Fongarid	1.6	3.0	2.3
T3	SL567A	2.7	4.9	3.8
T4	Aliette	1.6	2.2	1.9
T5	Filex	1.5	2.4	2.0
T6	Amistar	3.7	5.0	4.3
T7	F279	1.7	2.2	1.9
T8	Bion	1.1	2.7	1.9
T9	Invader	3.6	4.6	4.1
T10	Trustan	1.9	3.2	2.5

	Compare 2 different treatment means	
	Significance	***
	SED (57 df)	0.49
	Compare different cultivars per treatment	
	Significance	NS
	SED (57 df)	0.69

4. Conclusions

Literature review

- The biology and epidemiology of downy mildew disease of ornamentals caused by *Peronospora* species is 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. chlorae* causes a wide range of symptoms on *Lisianthus*: leaf necrosis and death of seedlings, leaf yellowing, stem twisting and distortion.

- Infection by *P. chlorae* is restricted to *Lisianthus* and a few weed hosts in the Gentianaceae, including Yellow Wort and probably Lesser Centuary.

- The disease is spread though the air by conidia, possibly over long distances as has been documented for cucumber downy mildew.

Fungicide evaluation

- Downy mildew (*Peronospora chlorae*) was effectively established in the propagation trial at HRI Stockbridge House through the introduction of 'infector plants' and provided a high level of mildew infection leading to a stern test of novel fungicides under evaluation.

- Four fungicides (Trustan, Invader, Amistar and SL567A) significantly reduced the level of downy mildew infection during propagation.

- Application of two fungicides, SL567A and in particular Trustan, during the early stages of propagation resulted in a reduction in the levels of germination of *Lisianthus* seedlings. This reduction was greater with one of the two cultivars used in the trial, Picottee Pink.

- There was no difference in the levels on downy mildew infection between the two *Lisianthus* cultivars, Picotee Pink and Malibu Dark Blue, in this trial.

- Three fungicides Amistar, Invader and SL567A consistently increased the dry weight of *Lisianthus* seedlings after 10 weeks from sowing.

- Of all the fungicide treatments evaluated, Trustan reduced the extent and severity of downy mildew infection throughout the trial. However, treatment with Trustan at sowing reduced the level of germination by 20% compared to the control.

- No phytotoxicity symptoms were observed under any of the other fungicide treatments during this trial.

5. Technology transfer

Presentations at grower meetings

20-22 June 1999 Study visit to Holland with Lisianthus Study Group (Martin McPherson)

Spring 1999 Lisianthus downy mildew presentation to Lisianthus Study Group, HRI Wellesbourne (Tim O'Neill, Martin McPherson and Trevor Brokenshire).

30 November 2000 Lisianthus: Control of downy mildew (*Peronospora chlorae*). Presentation to the Lisianthus Study Group Meeting, HRI Wellesbourne (Andrew Jackson & Martin McPherson).

6. References

Ahmad, R & Majumder, SK. (1987). Seedborne nature, detection and seed transmission of sorghum downy mildew on sorghum. *Plant quarantine and phytosanitary barriers to trade in the ASEAN, 9-10 December, 1986.* 173-191; 29 ref. Selangor, Malyasia; ASEAN Plant Quarantine Centre and Training Institute.

Aloj, B. Scalcione, M. Nanni, B & Marziano, F. (1992). Studies of a new phytopathogen in Italy: Peronospora of Eustoma (Lisianthus) russelianum. *Annali dlla Facolta di Scienze Agrarie della Universita degli Studi di Napoli, Portici. 1990,* **24:** 45-52.

Boccardo, V. & Massone, I. (1994) La peronospora del Lisianthus in Liguria. *Colture Protette,* **11,** 95-96.

Buonocore, E. & Pane, A. (1995) Infezioni di *Peronospora chlorae* de Bary su Lisianthus in Sicilia. *Informatore Fitopatologico,* **45,** 31-34.

Cohen, Y. (1981) Downy mildew of cucurbits. Chapter 16 in: *The Downy Mildews*. Academic Press Ltd, London.

Corbiere, R. Molinero, V. Lefebvre, A & Spire, D. (1995). Detection of pea downy mildew (*Peronospora viciae*) in seed lots by ELISA. *EPPO conference on new methods of diagnosis in plant protection,* Wageningen, Netherlands, 25-28 Jan. 1994. Bulletin-OEPP. 1995, **25:** (1-2), 47-56.

Crute, I.R. & Dixon, G.R. (1981) Downy mildew diseases caused by the genus *Bremia* Regel. Chapter 20 in: *The Downy Mildews.* Academic Press Ltd, London.

Francis, S.M. (1981) *Peronospora antirrhini. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 685.* Commonwealth Mycological Institute, London.

Francis, S.M. (1981) *Peronospora sparsa. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 690.* Commonwealth Mycological Institute, London.

Francis, S.M. (1983) *Peronospora dianthi. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 763.* Commonwealth Mycological Institute, London.

Francis, S.M. & Berrie, A.M. (1983) *Peronospora grisea. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 766.* Commonwealth Mycological Institute, London.

Hall, G. (1989) *Peronospora hyoscyami* f. sp. *tabacina. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 975.* Commonwealth Mycological Institute, London.

Hall, G. (1989) *Peronospora violae. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, no. 977.* Commonwealth Mycological Institute, London.

Hobolth, L.A. & Loeschenkohl, B. (1987) A new fungal disease on Eustoma (Lisianthus).*Gartner Tidende,* **103(40),** 1143.

Inaba, T. Takahashi, K & Morinaka, T. (1983). Seed transmission of spinach downy mildew. *Plant Disease*. **67:** (10), 1139-1141.

Loeschenkohl, B. (1988) Fungal diseases in Eustoma. Preliminary studies. *Gartner Tidende*, **104(9),** 219-221.

Neergaard, P. (1979) *Seed Pathology.* Macmillan Press Ltd, London.

Populer, C. (1981) Epidemiology of downy mildews. Chapter 5 in: *The Downy Mildews.* Academic Press Ltd, London.

Reuveni, R. & Raviv, M. (1997) Control of downy mildew in greenhouse-grown cucumbers using blue photoselective polyethylene sheets. *Plant Disease,* **81,** 999-1004.

Sackston, W.E. (1981) Downy mildew of sunflower. Chapter 26 in: *The Downy Mildews.* Academic Press Ltd, London.

Schiltz, P. (1981) Downy mildew of tobacco. Chapter 27 in: *The Downy Mildews.* Academic Press Ltd, London.

Smith, I.M., Dunez, S., Leliot, R.A., Phillips, D.A. & Archer, S.A. (Eds). (1988) *European Handbook of Plant Diseases.* Blackwell Scientific Publications.

Srivastava, S. & Singh, L. (1988). Evidence of seed-borne nature of downy mildew fungus of balsam. *Seed Research*. **16:** (2), 254-255.

Smith, P.A. & Price, T.V. (1997). Preliminary study of seed transmission of downy mildew in some vegetable Brassica cultivars in Australia. *Australisian Plant Pathology*. **26:** 1, 54-59.

Viranyi, F. (1981) Downy mildew of onion. Chapter 21 in: *The Downy Mildews.* Academic Press Ltd, London.

Whipps, J.M. & Linfield, C.A. (1987) Occurrence of oospores of *Peronospora grisea* on *Hebe* species in West Sussex. *Plant Pathology*, **36(2),** 216-218.

Wicks, T J & Hall, B. (1990). Efficacy of demethomorph (CME 151) against downy mildew of grapevines. *Plant Disease.* **74**: (2) 114-116.

Wolcan, S. Ronco, L. Bo, E.D. Lori, G & Alippi, H. (1996). First report of diseases of Lisianthus in Argentina. *Plant Disease.* **80:** (2) 223.

Yang, HC & Hsieh, T. F. (1998). The occurrence and chemical control of downy mildew on Eustoma grandiflorum Salisb. *Plant Protection Bulletin Taipei*. **40:** (1), 37-48.

Yao, C L. Frederiksen, R A & Magill, C. W. (1990). Seed transmission of sorghum downy mildew: detection by DNA hybridisation. *Seed Science and Technology*. **18:** (2), 201-207.

Zad, S. J. (1989). Transmission of soybean downy mildew by seed. *Mededelingen-van-de-Faculteit-Landbouwwetenschappen, -Rijksuniversiteit-Gent.* **54:** (2b), 561-566.

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8 Appendix 1