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

AUTHENTICATION

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

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GROWER SUMMARY

Headline

None of six foliar treatments (Amistar, Anthyllis, Cuprokylt, Farm-Fos 44 + Silwet-L77, Purogene and Signum) applied to cyclamen from one month after potting significantly reduced bacterial soft rot caused by *Pectobacterium carotovorum*.

Background and expected deliverables

A number of genera of bacterial pathogens cause disease in pot plants:

- Seed-borne *Xanthomonas* spp. e.g. on Begonia, Pelargonium, Cheiranthus and Lavandula
- *Pseudomonas* spp. causing leaf spots e.g. Camellia, Magnolia, Lonicera, Prunus and Canna.
- *Pectobacterium* and *Dickeya* spp. causing soft rot and stem wilts e.g. on Chrysanthemum, Cyclamen, Dahlia, Dianthus, Dieffenbachia, Euphorbia, Hyacinth, Kalanchoe, orchids, Pelargonium, Primula, Sedum, and Zantedeschia.
- *Agrobacterium* spp. causing crown gall of a range of ornamentals including chrysanthemum, roses, Euonymus, Prunus and many others.
- *Rhodococcus fascians* causing leafy gall on geranium.

Bacterial diseases causing significant losses on individual nurseries in recent years include cyclamen bacterial soft rot *(Pectobacterium carotovorum*), poinsettia bacterial leaf spot (*Xanthomonas axonopodis* pv. *poinsettiicola*), wallflower bacterial wilt (*Xanthomonas campestris* pv. *campestris*) and geranium bacterial wilt *Ralstonia solanacearum*.

Many bacterial diseases are favoured by high temperature and humidity and with global warming they may become a more significant problem if they are active over longer periods during the year. Others, such as bacterial soft rots on fleshy tissues (e.g. corms) have been persistent problems for many years.

Some control is possible through crop management, including avoiding high temperatures, waterlogged growing media, and mechanical and pest damage. Good nursery hygiene can also reduce the risk of persistent bacterial disease problems.

At present there are no chemical controls recommended for bacterial diseases other than copper fungicides, which provide limited protective control.

There is opportunity to make use of recent developments elsewhere in bacteriology to improve the control of bacterial diseases of protected ornamentals in the UK, particularly the areas of induced host-resistance, phage therapy and accurate detection and quantification of bacteria.

This project aims to assess the benefit of some chemical and biological interventions that could increase the options available to growers for management of bacterial diseases.

The expected deliverables are:

- Greater awareness by growers of bacterial diseases and their management.
- An illustrated Factsheet on control of bacterial diseases on protected ornamentals.
- Sound data on the potential benefit of resistance inducers and phage therapy for control of bacterial disease of ornamentals.
- Potential benefit to growers of reduced losses through use of biological or chemical intervention, subject to regulatory approval where required.

Summary of the project and main conclusions

A review of treatments with efficacy against bacterial plant diseases revealed a scarcity of approved products with proven bactericidal activity. Copper compounds have mostly been used to limit spread of leaf spot pathogens (*Xanthomonas* spp. and *Pseudomonas syringae* pathovars) but the level of control is limited, they can be phytotoxic and resistance can develop. Use of antibiotics such as streptomycin, oxytetracyclin or kasugamycin is not permitted.

The effects on bacterial diseases of growth promoting phosphonate products, such as fosetyl-Al (eg Aliette 80WG) and phosphorous acid, have been quite widely tested on ornamentals. Moderate efficacy with relatively high doses and regular applications has been

achieved inconsistently, especially for controlling spread of *Xanthomonas* leaf spots on various ornamental species.

Recent research has concentrated mainly on plant activators that induce systemic acquired resistance (SAR) such as acibenzolar-S-methyl (marketed as Bion in Europe) and methyl jasmonate. The rate of soft rot development in Calla Lily (caused by *Pectobacterium (Erwinia) carotovorum*) was reduced by Bion but completely inhibited after spraying leaves with methyl jasmonate. The efficacy of other plant activators marketed in the USA, such as harpin (Actiguard) and laminarin (Vacciplant) has yet to be tested on bacterial pathogens of ornamentals. There is also current interest among agrochemical companies in exploiting the induction of Systemic Acquired Resistance (SAR) by strobilurin and related fungicides such as Amistar (azoxystrobin) or Signum (boscalid + pyraclostrobin) to provide some protection against a range of fungal, viral and bacterial plant pathogens

Finally, there is revived interest in the use of specific viruses (phage) that infect and kill bacteria to replace chemical control of bacterial plant pathogens. Successful control of bacterial blight of geraniums by foliar applications of a mixture of phages against *Xanthomonas hortorum* pv*. pelargonii* strains has been demonstrated. Phages are available to evaluate control of a range of bacterial pathogens including *Pectobacterium carotovorum* and *Ralstonia solanacearum*.

The project has developed reliable methods for production of cyclamen plants affected by bacterial soft rot *(Pectobacterium carotovorum)* and ivy plants affected by bacterial leaf spot *(Xanthomonas hortorum)* in preparation for subsequent experiments on disease control.

Six treatments applied as foliar sprays were evaluated for control of cyclamen bacterial soft rot on a nursery with a history of the disease. The treatments were Amistar, Anthyllis growth stimulant (garlic extract), Cuprokylt (copper oxychloride), Farm-Foss 44 + Silwet L77 (potassium phosphate + silicon-based wetter), Purogene (chlorine dioxide) and Signum.
None of the treatments significantly influenced incidence of the disease.

Financial benefits

UK cyclamen production is around 16 million plants per year (4-6 million large-flowered and 10-12 million mini-cyclamen) valued at around £16 million (industry estimate, 2008). Assuming an average of 5% of plants are lost to bacterial soft rot (*Pectobacterium carotovorum*), the potential savings to growers by introduction of effective control measures would be worth around £800,000/annum.

Several UK nurseries growing poinsettia have recently suffered losses caused by *Xanthomonas* leaf spot, affecting young plants from at least two different suppliers. Severely affected plants are unmarketable, others require more labour to remove affected leaves and product will also be downgraded. This disease is currently notifiable to PHSI. Information on treatments that prevent and/or reduce spread of this disease is therefore likely to be important for growers.

Action points for growers

1. Several potentially very damaging diseases of pot plants are caused by bacteria including soft rot of cyclamen and a leaf spot of poinsettia. Growers should make sure that they can recognise symptoms of potentially damaging bacterial diseases.

2. Due to the lack of approved products with proven bactericidal activity, it is suggested that affected plants are removed promptly.

SCIENCE SECTION

Introduction

Bacterial soft rot caused by *Pectobacterium carotovorum* (formerly *Erwinia carotovora*) has been a consistent problem in UK cyclamen crops for many years and sporadically causes widespread and serious losses. The disease is exacerbated by high temperatures and was obvious in some crops in 2006 with losses of 15-20%. Affected plants develop leaf yellowing and collapse within a few days; the corm develops a soft, wet rot and plants do not recover. Both large-flowered and mini-cyclamen may be affected. Although the risk of bacterial soft rot can be reduced to a degree through crop management practices, at present there is no known chemical treatment to prevent this disease and losses are substantial. The uncommon bacterium *Erwinia chrysanthemi,* which can also cause a soft rot of cyclamen corms, has been confirmed in a number of hosts in the UK recently. The identity of bacteria associated with cyclamen bacterial soft rot in some UK crops will therefore be examined to determine the prevalence of *P. carotovorum* and *E. chrysanthemi*.

Bacterial leaf spot of ivy caused by *Xanthomonas hortorum* pv. *hederae* has occurred in the UK for many years and is occasionally damaging. The disease can be spread by taking visibly healthy cuttings contaminated with *X. hortorum* pv. *hederae* from infected stock plants. Further spread between plants can occur by water splash. More recently, bacterial leaf spot of poinsettia, a notifiable disease caused by *Xanthomonas axonopodis* pv. *poinsettiicola,* has occurred on a few nurseries in the UK. It is probable that outbreaks arose from symptomless infection in young plants or cuttings received from overseas. It was agreed that experimental work in this project would be done on *Xanthomonas* leaf spot of ivy rather than *Xanthomonas* leaf spot of poinsettia, as with the latter disease there is greater cost in experimental work due to the necessity to work to stringent plant health conditions. Results on control of *Xanthomonas* leaf spot of ivy will inform development of a strategy for control of *Xanthomonas* leaf spot of poinsettia.

Recently, various novel chemical treatments have been demonstrated to provide some control of some bacterial diseases, caused by species of *Pseudomonas, Ralstonia* and *Xanthamonas*. The potential of using novel chemical treatments for prevention and control of bacterial soft rot in cyclamen warrants investigation in order to reduce losses and secure more reliable quality of this important pot plant species.

Specific objectives in the first year are:

- To list a range of chemical and biological interventions with potential for control of

bacterial diseases of protected ornamental crops;

- To devise artificial inoculation procedures for production of bacterial soft rot (*Pectobacterium carotovorum)* in cyclamen and bacterial leaf spot (*Xanthomonas* sp.) of ivy.

One objective from the second year was also examined in the first year. Work on this objective was brought forward to Year 1 due to the opportunity to do work in a naturally infected crop of cyclamen.

- To evaluate on a commercial nursery some treatments that appear to show promise for control of bacterial soft rot of cyclamen.

1. Review of potential treatments for control of bacterial diseases

1.1. Bactericides

Bacterial diseases of ornamentals are difficult to control due to lack of approved, efficacious and non-phytotoxic bactericides. Antimicrobials for prophylactic treatment of bacterial diseases of plants are limited in availability, use, and efficacy, and therapeutic use is largely ineffective (Benson *et al*., 2001; Chase, 1987). The only products currently approved specifically for use as bactericides in the UK are those based on copper oxychloride. Some products approved for use as fungicides or growth promoters may also have antibacterial activities. In general there has been little research activity in developing new bactericides due to the relatively low perceived market value (compared with fungicides, insecticides and herbicides) and the uncertainty of acquiring registration.

In some countries, including the USA, antibiotics, such as streptomycin, oxytetracyclin or kasugamycin, have been approved for control of bacterial plant pathogens. The use of antibiotics is usually heavily regulated and is in decline because of the development of plasmid-borne resistance and the risk that it can be transmitted to human and animal pathogens (Huang & Burr, 1999; Chiou & Jones, 1995). Most applications of these products were on fruit trees to control fireblight (*Erwinia amylovora*) and leaf spots (*Pseudomonas syringae*), and there is only limited information regarding use on greenhouse ornamentals.

Ornamental plant producers have mostly had to rely on the use of copper compounds to control bacterial plant diseases (Burr, 2001). The most frequently used copper compounds are copper oxychloride, $CuCl_2.3Cu(OH)_2$, basic copper sulphate, $CuSO_4.3Cu(OH)_2$ and copper hydroxide, $Cu(OH)_2$. Copper oxide, copper oxyquinolate and ammoniacal copper sulphate have also been used as bactericides. Copper products are usually less effective than antibiotics for controlling bacterial diseases and are more phytotoxic, especially under high humidity, and may also leave unsightly residues. The application rates and growth stages at which copper products can be used are therefore limited. Some plant pathogenic bacteria have also developed resistance to copper (Cooksey, 1994; Scheck *et al*., 1996).

McFadden & Morey (1962) stated that copper sulphate applied weekly to naturally infected plants of poinsettia provided “adequate control” of bacterial blight caused by *Xanthomonas axonopodis* pv. *poinsettiicola*. Miller & Seymour (1973) found that treatments of streptomycin or copper hydroxide with mancozeb both gave excellent control of the pathogen on poinsettia in their experiments. Copper hydroxide has also been recommended for use in controlling this disease (Miller, 1998).

New compounds against bacterial diseases of ornamentals have been tested where available but few if any have shown activity equal to or surpassing that of the copper or antibiotic compounds. Particular interest has been shown in the phosphonates (e.g. fosetyl aluminium, potassium phosphite and phosphorous acid), which have been marketed as growth promoters or foliar fertilisers with added potential to control some fungal and bacterial diseases. They are systemically translocated in both xylem and phloem of plants.

Fosetyl-Al (eg Aliette 80WG) has been evaluated in greenhouse and field trials for the control of several bacterial diseases caused by *Erwinia chrysanthemi*, *Pseudomonas cichorii* and several *Xanthomonas* pathovars on a number of ornamental host plants (Chase, 1993). Variable levels of control of different xanthomonads were achieved with preventative applications of Aliette 80WP at rates similar to those labeled for control of pythiaceous fungi. For example, Aliette 80WP reduced severity of leaf spotting on ivy caused by *Xanthomonas hortorum* pv. *hederae* to a similar degree as cupric hydroxide (Kocide 101 77WP). However, better control of *Erwinia chrysanthemi* and *Pseudomonas cichorii* was achieved with copper than with fosetyl-Al. The same treatments failed to control *Pseudomonas syringae* on impatiens (Chase, 1987b and 1989). Fosetyl-Al is currently recommended in the USA to prevent spread of *Xanthomonas* leaf spot pathogens on a wide range of hosts including *Anthurium*, *Dieffenbachia*, *Euphorbium pulcherrima, Ficus, Hedera*, *Schefflera*, *Spathiphyllum* and *Syngonii* (Simone *et al*., 1994; Miller, 1998). The main problems reported with the use of fosetyl-Al are inconsistency of results, moderate efficacy and the high dose and number of applications needed to obtain reliable disease control (Moragrega *et al*., 1998).

Other treatments, found to reduce spread of *X. hortorum* pv. *hederae* on ivy, are the contact fungicide fluazinam from ISK Biosciences Corporation, and daily applications of a quaternary ammonium disinfectant (Greenshield) from Whitmire (Chase, 1987b and 1989). Fluazinam has also been found to control *Pseudomonas syringae* on peach in the USA (Ritchie Pollard, 1996).

Norman *et al*. (2006) screened various products with reported bactericidal activity (including fosetyl-Al (Aliette), benzothiadiazole (Actigard), copper sulfate (Cuprofix), copper hydroxide (Kocide 2000), copper pentahydrate (Phyton 27), copper salts (Camelot), hydrogen peroxide (Zerotol), furfuraldehyde (Multiguard), alkyl dimethyl benzyl ammonium chloride (Timsen), oxolinic acid (Starner), *Bacillus. subtilis* (Serenade, Companion), and potassium salts of phosphorous acid (K-Phite)) for efficacy in protecting geranium plants (*Pelargonium hortorum*) from *Ralstonia solanacearum* infection. Most of the bactericides slowed disease progress but were not able to protect the plants from infection and subsequent death.

However, potassium salts of phosphorous acid were found to be effective in protecting plants from infection when applied as a drench. Phosphorous acid (H_3PO_3) was found to inhibit *in vitro* growth of *R. solanacearum* and is thought to protect plants from infection by acting as a bacteriostatic compound in the soil. The plants, however, were not protected from above-ground infection on wounded surfaces. Other phosphorous-containing products commonly used in the industry, such as phosphorus pentoxide (P_2O_5) and phosphoric acid (H_3PO_4), did not protect plants from bacterial wilt infection.

In Italy, the product DF100, based on grapefruit seeds, is recommended by the cyclamen propagator Lazzeri for use against bacterial soft rot of cyclamen. However, no information on efficacy to support this recommendation was found.

With the exception of copper products, none of the chemicals mentioned above are approved in the UK specifically to control any bacterial pathogen but general use of some may be permitted. Products based on copper oxychloride (Cuprokylt, Cuprokylt FL and Headland Inorganic Copper) are variously approved for control of bacterial black rot (*Xanthomonas campestris*) and spear rot (*Pseudomonas fluorescens*) on broccoli and other brassicas; bacterial canker (*Pseudomonas syringae* pv. *morsprunorum*) in cherry and plum and bacterial storage rots (*Pseudomonas* spp.) in onions and leeks. None currently has a label approval or SOLA for use on ornamentals. The use of antibiotics such as streptomycin has never been permitted in the UK, and copper hydroxide is only permitted for use as a disinfectant for plastic containers. However, the use of fosetyl-Al would be permitted as Aliette 80WG® has an on-label approval for protected pot plants but it recommends that the tolerance of ornamentals is checked before large-scale treatments are carried out. While the long-term extension of use arrangement remains in place for non-edible crops (products used under this arrangement are currently under review and the arrangement will be discontinued in the future), the use of mancozeb would also be permitted but this would be at the grower's own risk. It should be noted that the use of certain chemicals might be considered unacceptable to the horticultural industry due to the spray deposits that would be left on the leaves. The other major practical concern for growers would be the risk of phytoxicity associated with chemical treatment. Any damage could affect saleability and cultivars can vary in their sensitivity to chemical treatment.

1.2. Induction of systemic acquired resistance (SAR)

Recent research on ornamentals and other crops suggests there is opportunity for some control of various bacterial diseases through the use of chemicals that induce host

resistance.

Pre-inoculation of plants with certain chemicals can stimulate plant cellular defence mechanisms such that when plants are subsequently challenged by a pathogen the plant is resistant. When the resistance is expressed at a distance from the leaf initially challenged, the phenomenon is known as Systemic Acquired Resistance (SAR) (Kessman *et. al.*, 1994; Jones, 2001). SAR inducers are attractive because they do not act directly on the pathogen, which is therefore less likely to develop resistance to them. Plant activators such as 1,2,3-benzothiadiazole (or acibenzolar-S-methyl; marketed as Actigard in the USA or Bion in Europe) and probenazole (Yoshioka *et al*., 2001; Nakashita *et al*., 2002) which induce systemic resistance in plants (Sticher *et al*., 1997; Vallad & Goodman, 2004) can be used to control bacterial leaf pathogens. Louws *et al.* (2001) have shown that Acibenzolar-S-methyl can be integrated as a viable alternative to copper-based bactericides for field management of bacterial speck, caused by *P. syringae* pv. *tomato*, particularly where copper-resistant populations predominate. Actigard was also used to control *P. syringae* pv. *tabaci* on tobacco in field trials (Cole, 1999) and *Xanthomonas axonopodis* pv. *allii* on onion (Lang *et al*., 2007). Induction of resistance to *Pectobacterium (Erwinia) carotovorum* soft rot in calla lily was achieved by pre-treating with Bion which slowed down the rate of soft rot development (Luzzatto *et al*., 2007). However the soft rot was completely inhibited by pre-treating leaves by spraying with 10 mM methyl jasmonate. This effect was attributed to elicitation of resistance brought about by accumulation of free phenolics, suggesting priming of bioactive polyphenols as a principal factor in the calla lily defence against *P. carotovorum*.

Soil application of the systemic insecticide imidacloprid produced season-long control of citrus canker caused by *Xanthomonas citri* subsp. *citri* (Francis *et al.,* 2009). Imidacloprid breaks down within plants into a compound closely related to the systemic acquired resistance inducer isonicotinic acid. Soil applied imidacloprid and some other SAR inducers reduced canker lesions by up to 70% compared with untreated inoculated plants. Imidacloprid is marketed in the UK (eg as Intercept 70WG) for control of glasshouse whitefly on pot plants.

SAR is also induced when the bacterial protein harpin (obtained from the fireblight pathogen *Erwinia amylovora*) is used to elicit the plant hypersensitive response. Harpin is the active ingredient of Messenger, which is marketed in the USA and has been reported to increase resistance to a wide range of pathogens in several crops (Jones, 2001). Messenger and Bion treatments were not as effective as copper treatments in trials against *Pseudomonas cichorii* in tomato (Ustun *et al*., 2005) and *Xanthomonas axonopodis* pv. *vitians* on lettuce (Bull & Koike, 2005). Bion treatments against *Ralstonia solanacearum* increased resistance in moderately resistant but not in susceptible tomato varieties whereas Messenger

treatments had no effect (Pradhanang *et al*., 2005).

Another SAR inducer, with the active ingredient laminarin extracted from seaweed, is registered in the USA and traded as Vacciplant. It has been found in some trials to increase resistance to gram negative plant pathogenic bacterial pathogens such as *Erwinia amylovora* and *Pseudomonas syringae* pv. *tomato* (Sobolewski *et al*., 2007).

There is currently interest among agrochemical companies in exploiting the induction of SAR by strobilurin and related fungicides such as Amistar (azoxystrobin) or Signum (boscalid + pyraclostrobin) to provide some protection against a range of fungal, viral and bacterial plant pathogens (Turecheck *et al.*, 2006). For example, Herms *et. al.,* (2002) showed increased resistance of tobacco to *Pseudomonas syringae* pv. *tabaci,* as well as to *Tobacco mosaic virus*, in response to pre-treatment with pyraclostrobin.

1.3. Phage therapy

Bacteriophages are viruses that infect and kill bacteria. The increased potential for the use of phages to replace chemicals in control measures for bacterial plant diseases was recently reviewed by Jones *et al*. (2007). With the first registration in the USA of a phage to control a plant disease (tomato spot caused by *Xanthomonas vesicatoria*), there is renewed interest in this technology within integrated management of a wide range of diseases. Phage treatments have been identified with activity against *Agrobacterium tumefaciens* (Stanier *et al*., 1967), *Xanthomonas campestris* pv. *pruni* (Civerolo & Keil, 1969), *X. oryzae* (Kuo *et al.*, 1971), *Ralstonia solanacearum* (Tanaka *et al*., 1990), *Erwinia amylovora* (Svircev, 2006), *Xanthomonas vesicatoria* (Jones *et al*., 2006), *Xanthomonas citri* (Balogh, 2006) and *X. axonopodis* pv*. allii* (Lang *et al*., 2007). A range of phages with activity against *Pectobacterium* (*Erwinia*) *carotovora* isolates from potato have been collected in the UK (Elphinstone, unpublished). Work is ongoing to assess the activity of these against a wider range of host plants.

The only report found on control of bacterial disease in ornamentals using phage therapy describes the reduction of incidence of bacterial blight of geraniums with foliar applications of a mixture of phages against *Xanthomonas hortorum* pv*. pelargonii (*Flaherty *et al.,* 2001*)*. Sixteen phages were evaluated for ability to lyse *Xanthomonas* strains isolated from around the world, and then mutants were developed from five phages that exhibited the broadest host range and included in the mixture that was used for disease control. Foliar applications of the phage mixture applied daily significantly reduced spread of disease. The disease

incidence was reduced by 50% or more in phage-treated plots compared to the control, and was significantly less than in plots treated with the recommended bactericide.

A range of products that appear to show useful potential for control of *P. carotovorum* on cyclamen and *X.hortorum* pv*. hederae* on ivy will be evaluated in year 2.

2. Evaluation of inoculation procedures to cause bacterial soft rot in cyclamen

2.1 Introduction

In order to evaluate potential treatments for control of cyclamen bacterial soft rot, it is useful to have a method for reliable production of disease symptoms by artificial inoculation with the causal bacterium. Two experiments were done to determine the effect of inoculum level, inoculation site and post-inoculation incubation on development of bacterial soft rot caused by *P. carotovorum*.

2.2 Materials and methods

Crop and site details

Plug cyclamen plants cvs Miracle White (Experiment 1) and Laser Pink (Experiment 2) obtained from a UK plant propagator were grown in Levington M2 compost in 11 cm pots. Plants were placed on capillary matting in plastic gravel trays and watered by hand as required onto the plants for 6 weeks and then onto the matting between plants. A liquid feed (75 mg/L nitrogen, 50 mg/L phosphate and 150 mg/L potassium) used at every watering was prepared by dissolving 6 g ammonium nitrate with 8.5 g of mono-ammonium phosphate and 35 g of potassium nitrate in 1 L of water and diluting this 1 in 100 with water. The plants were grown on the concrete floor of glasshouse compartment 3 at ADAS Boxworth. The house was shaded and set to a minimum temperature of 10ºC with ventilation as required. A crop diary is given in Appendix 1. No fungicides or insecticides were applied.

Inoculation

Plants were inoculated with an isolate of *P. carotovorum* obtained from the National Collection of Plant Pathogenic Bacteria (NCPPB), originally obtained from infected cyclamen in Italy. Bacteria were grown on plates of potato dextrose agar (PDA) for 48-72 h and a suspension prepared in sterile distilled water (SDW). The concentration of cells was determined by measuring the optical density of the suspension using a spectrophotometer and reference to a calibration curve prepared previously. The concentration was adjusted as required by dilution with SDW.

For experiment 1, the inoculum concentration ranged from nil to 10^7 cells / mL. For experiment 2, a concentration of 10^7 cells / mL was used throughout.

Plants were inoculated by drench treatment with 50 mL of a cell suspension poured over the plant crown or into the growing medium at the edge of pots, or by soaking roots in a cell suspension for 15 minutes. Where stated, trays of plants were loosely enclosed in a polythene bag for 48 h after inoculation to create a high humidity around the plants.

Treatments

Two experiments were done in parallel. Experiment 1 investigated the effect of inoculum level applied as a drench treatment over the crown; Experiment 2 investigated the effect of inoculation site (main plots) and incubation humidity (split plots). Treatments were as follows:

Experiment 1: Effect of inoculum level

1. Control (water drench)
2. 10^2 cells/mL
3. 10^3 cells/mL
4. 10^5 cells/mL
5. 10^7 cells/mL

Experiment 2: Effect of inoculation site and incubation humidity

1. Root dip in water
2. Root dip in *P. carotovorum*
3. Growing medium drench with water
4. Growing medium drench with *P. carotovorum*
5. Crown drench with water
6. Crown drench with *P. carotovorum*

Experiment design and statistical analyses

Experiment 1: The experiment was a fully randomised design with four replicates of five treatments. Each plot consisted of a tray of eight plants. All trays of plants were enclosed in a polythene bag for 48 h after inoculation. Results were examined by regression analysis using generalised linear models.

Experiment 2: The experiment was a randomised block split-plot design with six inoculation treatments as main plots and two incubation treatments (high and low humidity) as split-plots. Each main plot consisted of a tray of six plants; each split plot contained three plants.

Results were examined by analysis of variance on three specific contrasts: inoculation vs. no inoculation; humid incubation vs. no humid incubation and inoculation site (dip vs. crown drench in growing medium drench) and two and three-way interactions.

Disease assessment

Plants were examined every 2 weeks after inoculation and the incidence of collapsed plants recorded. Bacterial soft rot of the corm was confirmed by its soft, mushy appearance and by the smell. All collapsed plants were removed. At the final assessment, all corms were cut transversely and examined for symptoms of bacterial soft rot or other disease in the crown.

2.3 Results and discussion

Experiment 1: Effect of inoculum level

All plants initially grew well but some plants began to collapse around two weeks after inoculation. There was a clear effect of inoculum level at this time, with 15-20% plants affected at inoculum levels of 10^2-10^5 cells/mL and 44% affected at the highest inoculum level, 10^7 cells/ml. One month later bacterial soft rot had caused collapse of 22-35% of plants at the lower inoculum levels and 67% at the highest (Table 2.1). Around 3% of uninoculated plants were affected. There was little change in the incidence of collapsed plants over the next eight weeks.

At the final assessment on 18 December (18 weeks after inoculation) no evidence of typical bacterial soft rot symptoms (grey watery staining) was found within the corms of surviving plants (Table 2.2).

Table 2.1: Effect of *P. carotovorum* inoculum level on occurrence of bacterial soft rot in the corm of cyclamen plants, cv. Miracle White – Cambridge, 2008

Treatment	Mean % plants with bacterial soft rot			
(cells/mL)	29 Aug	24 Oct	1 Dec	18 Dec
1. Nil	0.0 (0)	3.1 (3.1)	6.3 (4.5)	6.3 (4.5)
2. 10^2	15.6 (7.7)	28.1 (7.9)	28.1 (8.1)	34.4 (8.6)
3. 10^3	15.6 (7.7)	34.4 (8.4)	34.4 (8.6)	34.4 (8.6)
4. 10^5	15.6 (7.7)	21.9 (7.4)	25.0 (7.9)	25.0 (7.9)
5. 10^7	43.7 (10.3)	62.5 (8.6)	62.5 (8.8)	66.7 (8.8)
Significance	0.023	0.003	0.006	0.007

Plants inoculated by 50 mL crown drench on 14 August 2008. ( ) – standard error

Table 2.2: Effect of *P. carotovorum* inoculum level on occurrence of symptoms in corms of visibly health plants – December 2008

Treatment	Means % corms of surviving plants with:		
(cells/mL)	Grey watery staining (bacterial soft rot)	Brown staining	No symptoms
1. Nil	0	9.3 (4.9)	78.2 (8.3)
2. 10^2	0	12.5 (5.5)	53.2 (10.0)
3. 10^3	0	6.3 (4.1)	59.4 (9.9)
4. 10^5	0	6.3 (4.1)	68.7 (9.5)
5. 10^7	0	0 (0)	37.5 (9.7)
Significance	NS	NS	0.08

( ) – standard error

Experiment 2: Effect of inoculation site and incubation humidity

Relatively few of the inoculated plants developed symptoms of bacterial soft rot in this experiment. By 24 October, around 10 weeks after inoculation, less than 5% of plants had collapsed (Table 2.3). At the final assessment on 18 December the incidence of infection ranged from 0 to 8%. There were no significant differences between treatments (Table 2.4). Symptoms occurred at a low incidence in a few plants of both inoculated and uninoculated treatments.

The reason for low disease occurrence in this experiment is unknown. The same isolate of *P. carotovorum* was used as in Experiment 1, and at a high inoculum level (10^7 cells/mL). Even with a crown drench as used in Experiment 1, little disease developed. The varieties in the two experiments differed, but grower experience indicates that both mini- and large-flowered cyclamen are susceptible to bacterial soft rot. One possible explanation may be the age of the plants at potting. The mini-cyclamen plants used in Experiment 1 were kept in plug trays for several weeks before potting, whereas the large-flowered cyclamen were potted within 2 weeks of receipt. Possibly petioles had stretched in the plants kept longer in the plug trays and as a result there was more physical damage (broken leaves) at potting: damaged leaves are known to be susceptible to infection by *P. carotovorum*. Alternatively, the plants kept longer in plug trays may have been subject to dry/wet cycles in the growing medium which may have resulted in corm splits; these are also reported to be susceptible to infection by *P. carotovorum*.

Table 2.3: Effect of inoculation site and incubation humidity on occurrence of bacterial soft rot in the corm of cyclamen plants, cv. Laser Pink – Cambridge, 2008

Treatment			Mean % plants with bacterial soft rot	
Inoculation site	Inoculated	Incubation humidity	24 Oct	18 Dec
1. Root dip	No	Low	0	4.2
	No	High	0	8.3
2. Root dip	Yes	Low	0	4.2
	Yes	High	0	0
3. Compost drench	No	Low	0	0
	No	High	0	4.2
4. Compost drench	Yes	Low	4.2	4.2
	Yes	High	0	0
5. Crown drench	No	Low	0	0
	No	High	0	4.2
6. Crown drench	Yes	Low	4.2	4.2
	Yes	High	0	0

Table 2.4: Analysis of variance of effect of inoculation and incubation conditions on bacterial soft rot in cyclamen

Factor	Df	F probability
Block	1	0.123
Inoculation (yes/no)	1	0.146
Inoculation site (root, compost, crown)	2	0.261
Inoculation x Inoculation site	2	0.662
Humid incubation (yes/no)	1	0.630
Humid incubation x inoculation	1	0.274
Humid incubation x inoculation site	2	0.344
Humid incubation x inoculation x inoculation site	2	0.450
Residual	18	-

3. Evaluation of inoculation procedures to cause bacterial leaf spot in ivy

3.1 Introduction

An experiment was devised to determine the effect of inoculation method and post-inoculation incubation conditions on development of bacterial leaf spot on ivy (*Hedera* sp.). The objective was to devise a method for reliable production of disease symptoms by artificial inoculation with the causal bacterium, *Xanthomonas hortorum* pv. *hederae*.

3.2 Materials and methods

Crop and site details

Rooted ivy cuttings cv. Ester obtained from a UK grower in Lincolnshire were grown in Levington M2 compost in 9 cm pots. Plants were placed on capillary matting in plastic gravel trays and watered by hand onto the plants until inoculation, and then onto the matting between plants. The trays of plants were placed on the concrete floor of glasshouse compartment 1 at ADAS Boxworth. The house was shaded and set to a minimum temperature of 10ºC. At 2 weeks after inoculation, the whole trial area was covered with perforated polythene suspended around 50 cm above the crop to create a more humid environment. A crop diary is given in Appendix 2. No fungicides or insecticides were used on plants. The mean 24 h temperature during the experiment was around 23 - 25°C (Appendix 3). The maximum relative humidity on uncovered pants ranged from 60 – 100% RH until 30 May when the whole trail was covered with perforated polythene; thereafter the maximum relative humidity was around 100% (Appendix 3).

Inoculation

Plants were inoculated with an isolate of *X. hortorum* pv*. hederae* (isolate N939) from the NCPPB. Bacteria were grown on plates of nutrient agar for 48-72 h and a suspension prepared in SDW. A milky suspension was used to inoculate plants (approx. 10^8 cells/mL). A dilution series of this suspension was plated onto nutrient agar and the actual concentration determined by colony counts.

Plants were inoculated by stab inoculation onto leaf lamina using a needle (2 leaves/plant) or by spray inoculation over the whole plant (20 mL/plant). Inoculated leaves were tagged. Controls were stab or spray inoculated with SDW. Trays of plants were loosely enclosed in a clear plastic bag for 0, 24, 48 or 96 h after inoculation.

Treatments

	Inoculation method	Treatment	High RH incubation
1.	Stab	SDW	Nil
2.	Stab	Xhh	Nil
3.	Stab	Xhh	96 h
4.	Spray	Xhh	Nil
5.	Spray	Xhh	24 h
6.	Spray	Xhh	48 h
7.	Spray	Xhh	96 h
8.	Spray	SDW	Nil
9.	Spray	SDW	96 h

SDW – Sterile Distilled Water; Xhh – *Xanthomonas hortorum* pv. *hederae*

Experiment design and statistical analysis

The experiment was a fully randomised design with four replicates of nine treatments and five plants per plot. Each plot consisted of a tray of five plants. Results were examined by GLM using regression analysis for % leaves affected and by ANOVA for % leaf area affected.

Disease assessment

Plants were examined at 2 and 4 weeks after inoculation and the number of bacterial leaf spots on each plant was counted.

3.3 Results and discussion

The suspension used to inoculate plants contained $1.1x10^8$ bacterial cells/mL,a concentration close to that recommended for incoculation work. This magnitude of cell concentration was found to result in leaf spot symptoms in ivy in previous experiments in controlled environment cabinets at Fera. In the work described here, bacterial leaf spot symptoms were first observed 10 days after inoculation as small (1-2 mm diameter) purplish-coloured spots. At 14 days after inoculation, symptoms were visible on both stab and spray-inoculated plants in all treatments. All leaves that were stab-inoculated with *X. hortorum* pv. *hederae* developed leaf spot symptoms. No symptoms were observed on uninoculated plants (treatments 1, 8 and 9, Table 3.1) at this time. The duration of high humidity incubation (0-96 h, treatments 4-7) after spray inoculation had no significant effect on disease incidence, with a low level of bacterial leaf spot (from 5% to 15%) in all treatments.

At 28 days after inoculation, a majority of leaves inoculated with *X. hortorum* pv*. hederae* showed bacterial leaf spotting (Table 3.1). The three control treatments (T1, T8 and T9) again showed nil or virtually nil symptoms. The size of leaf spots had increased from the earlier assessment up to 5 mm diameter, and the colour had darkened to black. Bacterial streaming was observed from the edge of spots when a sample was examined microscopically, confirming that leaf spotting was associated with bacterial infection. On plants spray-inoculated with *X. hortorum* pv*. hederae,* the incidence and severity of spotting was significantly greater (P<0.001) on leaves incubated at high humidity for 24-96 h than on leaves that were incubated at ambient humidity after inoculation.

Table 3.1: Effect of inoculation method and post-inoculation incubation conditions on development of bacterial leaf spot in ivy – Cambridge, 2008

Inoculation	Inoculum*	High RH incubation	Mean % leaves affected after:		Mean % leaf area affected after
			14 days	28 days	28 days
1. Stab	SDW	Nil	0 (0.0)	5 (4.2)	0.0
2. Stab	Xhh	Nil	100 (0.1)	100 (0.0)	2.4
3. Stab	Xhh	96 h	100 (0.1)	100 (0.0)	2.8
4. Spray	Xhh	Nil	5 (2.7)	40 (9.2)	0.7
5. Spray	Xhh	24 h	15 (4.3)	74 (8.6)	3.0
6. Spray	Xhh	48 h	15 (4.3)	75 (8.3)	2.5
7. Spray	Xhh	96 h	5 (2.7)	65 (9.0)	1.8
8. Spay	SDW	Nil	0 (0.0)	0 (0.0)	0.0
9. Spray	SDW	96 h	0 (0.0)	0 (0.0)	0.0
Significance (24 df)			<0.001	<0.001	<0.001
LSD					1.51

* *Xanthomonas hortorum* pv. *hederae*

( ) – standard error.

4. Effect of some foliar treatments on cyclamen bacterial soft rot

4.1 Introduction

A number of fungicides and nutritional products are reported to have some effect against one or more bacterial diseases (see section 1). The objective of this work was to determine if six treatments reported to give some control of bacterial diseases give any control of cyclamen bacterial soft rot when used as foliar sprays. Additionally, the effect on disease spread of leaving a plant affected by bacterial soft rot among visibly healthy plants for 6 weeks was investigated.

This work was brought forward from year 2 due to the substantial losses occurring to this disease commercially and the offer of a site with a recent history of severe losses on which to do an experiment.

4.2 Materials and methods

Crop and site details

The experiment was done on a nursery in Lincolnshire where bacterial soft rot of cyclamen has caused significant losses in recent years. Plug plants of the large-flowered cyclamen Halios Flame Mix supplied by Florensis (Netherlands) were potted in week 28 into 13 cm pots. The growing medium was a nursery-specified mix consisting of light peat (30%), black peat (30%), perlite (10%), clay (10%) and soil (10%). The experiment was located in a new glasshouse on the site; the cyclamen were the first crop in the house. Plants were grown on the floor on plastic-backed capillary matting with perforated white top.

Plants were initially watered from overhead, for around 6 weeks, and subsequently by hand-irrigation onto the beds between plants. Nemasys (*Steinermema feltiae*) was applied to the plug plants before potting for control of sciarid fly. The crop was grown unheated until 1 October and then grown at a minimum temperature of 14^{o}C. No fungicides or other chemical treatments were applied to plants except as specified treatments (below). A crop diary is given in Appendix 4.

Treatments

1. Untreated
2. Cuprokylt (copper oxychloride) at 3 g/L
3. Amistar (azoxystrobin) at 1 mL/L
4. Signum (boscalid + pyraclostrobin) at 1.8 g/L
5. Farm-Fos 44 (potassium phosphate) at 10 mL/L + Silwet-L77 (silicon-based wetter) at 0.6 mL/L (foliar feed)
6. Anthyllis growth stimulant (garlic extract with high sulphur content) at 4 mL/L
7. Purogene (chlorine dioxide) at 0.85 ppm active ingredient water treatment
8. Untreated – infector plant left in plots for 6 weeks

All treatments were applied as high volume sprays at 1,000 L/ha (100 mL/m^2) using a knapsack pressurised sprayer with a 02F100 nozzle at 2 bar pressure, except for Purogene which was applied with every watering by nursery staff. Treatments 2, 4, 5 and 6 were each applied on five occasions at 14 day intervals from one month after potting; treatment 3 was applied on three occasions, the maximum spray number permitted at the time, from the same date.

Consultation with PSD in July 2008 and HSE in August 2008 (ref: HPHS-7H3ER6) confirmed that, at present, where chlorine dioxide is used to treat irrigation lines, the drainage water can be used on ornamental plants. A data package for chlorine dioxide has been submitted by a company for evaluation as a type 2 product under the Biocidal Products Directive (BPD). Until a decision has been made whether or not to include it on Annex I of the BPD, UK Legislation applies. The conditions of use of chlorine dioxide to disinfect water in irrigation lines may change if and when it is listed under the BPD.

Experiment design and statistical analysis

The experiment was a fully randomised design with four replicate blocks of seven treatments and eight replicates of the untreated control. Each plot consisted of 48 plants, with 47 visibly healthy plant arranged around one 'infector' plant. The plants were initially pot thick in six trays of eight plants and were removed from trays and spaced diagonally at 26 cm (centre to centre) in week 36. The central infector plants in treatments 1-7 were removed one week after establishment of the experiment; those in treatment 8 were removed after six weeks. Results were examined by ANOVA and by regression analysis using GLM as appropriate.

Disease assessment

The experiment was examined once every 2 weeks and the numbers of collapsed plants recorded. All collapsed plants (except for infector plants) were removed from the crop. Bacterial soft rot of the corm was diagnosed by appearance and smell as described

previously.

A sample of affected plants were examined at Fera to confirm the cause of plant collapse. Isolations were made from the margin of rots and bacterial colonies were identified by a Fatty Acid Profile (FAP) test.

Ten plants in each plot, consisting of two runs of five plants at diagonals, were assessed for grey mould (*B. cinerea*) on 4 December using the scale below and a mean disease severity was calculated.

0. no sporulating botrytis;
1. sporulating botrytis on dead leaves (absent on green leaves), usually on growing medium surface (marketable after cleaning);
2. sporulating botrytis on 1-3 green or part-green leaves above the growing medium surface (marketable after cleaning);
3. sporulating botrytis on 4 or more green or part-green leaves above the growing medium surface; collapse of up to half of the plant (unmarketable);
4. collapse of more than half of the plant (unmarketable).

Plant quality

All remaining plants in each plot were assessed on 2 December for marketing grade (class 1, class 2 and out of grade). The diameter of the foliage of ten plants in each plot was measured, and the number of plants with no flowers was recorded.

Water test

Water samples were collected from the covered water storage tank (roof water) and from a tap inside the glasshouse on 22 August and 11 December and tested for pectolytic bacteria (*Pectobacterium carotovorum* and closely related species) by planting onto a selective agar at FERA.

4.3 Results and discussion

Bacterial soft rot

Plant collapse due to bacterial soft rot of the corm was first observed one month after the first application of foliar treatments, when 0-2.6% of plants were affected (Table 4.1). Two weeks later this had increased to 2.1-9.1% of plants. Bacterial soft rot at this time was least following treatment with Farm-Fos 44 + Silwet-L77 (2.1% of plants) and greatest in untreated plots where the central infector plant remained (9.1% of plants), although differences were

not statistically significant at P = 0.05. There was little increase in disease over the following month (Figure 4.1 - weeks 34 and 36), whereas over 70 plants collapsed in the subsequent month (Figure 4.1 - weeks 38 and 40). By week 48, high incidences of bacterial soft rot occurred in untreated plants (14.4%) and following Signum treatment (16.0%) and relatively lower levels following treatment with Cuprokylt (9.6%) and Farm-Fos 44 + Silwet-L77. Again, differences were not statistically significant. At the final assessment in week 50, seven plants were removed due to plant collapse, all had bacterial soft rot and five were also infected by fusarium wilt. The level of bacterial soft rot was only slightly greater than in week 48. Full results of interim assessments are given in Appendix 5.

P. carotovorum was confirmed in all of the collapsed plants tested. No *Erwina chrysanthemi*, a related bacterium that can also cause a soft rot of cyclamen corms, was detected.

The occurrence of plant collapse over time, summarised over all treatments, is shown in Fig 4.1. The greatest number of plants collapsed in weeks 38 and 40 (mid-September to early October), soon after plant spacing. Possibly the movement of plants at spacing, or greater exposure of pots to sun after spacing, influenced the development of the disease.

Mean daily glasshouse air temperature as recorded by the nursery (Appendix 6) ranged from around 7 to 27ºC and was generally in the range 15-20ºC. *P. carotovorum* is able to grow over this temperature range and is generally most active at 25-30ºC.

The source of *P. carotovorum* in this experiment is unknown. Water samples collected in August and December 2008 were all found to be free of pectolytic bacteria. Possible alternative sources are latent infection in the plug plants at receipt onto the nursery, the growing medium into which plants were potted, capillary matting and sand on which plants stood, and transmission to the plants by insects or other means from the environment. The possible occurrence of *P. carotovorum* on plug plants as received on a nursery and other sources will be investigated in year 2 of this project.

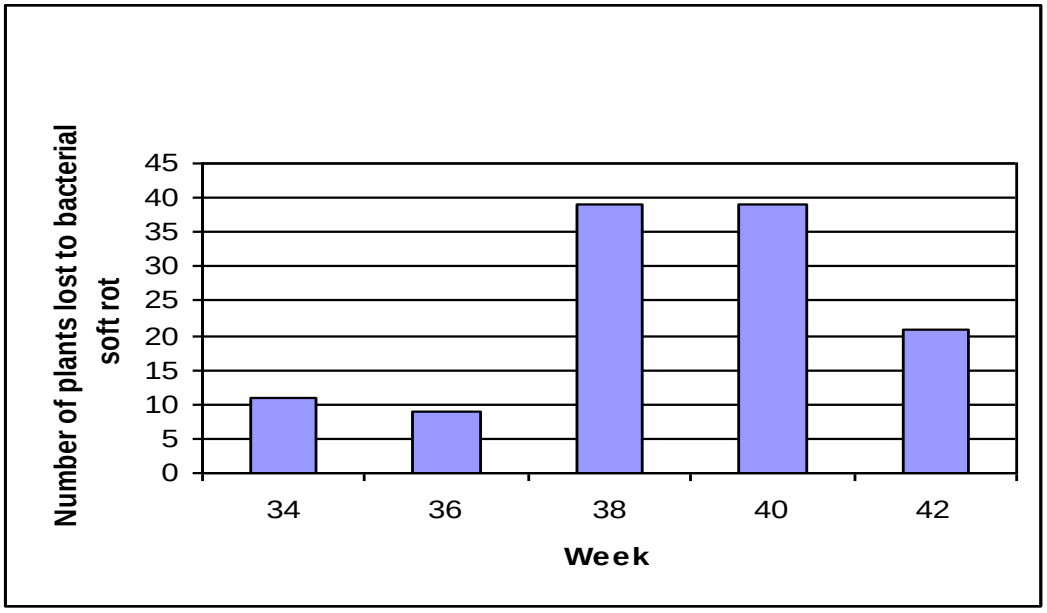

Figure 4.1: Losses per fortnight to bacterial soft rot (no. of plants) over the whole trial, (1,692 plants). Plants were potted in week 28 and the first treatments were applied in week 32.

Grey mould

Grey mould was assessed in week 48, two weeks before plant dispatch. Levels of grey mould were relatively low and there were no significant differences ($P > 0.05$) between treatments in mean disease severity or in the proportion of unmarketable plants due to grey mould (Table 4.2). Mean disease severity (0-4 index) was least following treatment with Signum (0.9) and greatest in the untreated plots (1.5).

Plant quality

Over 74% of all plants were classed as grade 1 or 2 (marketable). Foliar treatments for control of bacterial soft rot had no significant effect ($P>0.05$) on the proportions of remaining plants in each class (Table 4.3). Mean plant diameter was not significantly affected by treatment ($P>0.05$) (Table 4.3). It was significantly affected by block ($P<0.001$), with the diameter steadily decreasing from block 1 (24.3 cm) to block 4 (22.2 cm). Blocks were arranged lengthwise along a bed from the central pathway in the glasshouse so this effect was not due to proximity to the glasshouse wall. The proportion of plants without any flowers on 11 December ranged from 10% to 17% of plants and was not significantly affected by treatment.

Product approvals

Cuprokylt, Amistar and Signum were used in this experiment under the Long Term Arrangements for Extension of Use extant at the time of the work. Since 1 June 2009, SOLA 0443/09 permits the use of Amistar on protected ornamentals. There are no approvals for

use of Cuprokylt or Signum on protected ornamentals at present (June 2009).

Table 4.1: Effect of foliar treatments on bacterial soft rot in cyclamen – 2008

Treatment (number of applications)	Mean % cyclamen plants affected:			
	2 Sept (week 36)	16 Sept (week 38)	11 Dec (week 50)	% healthy plants remaining
1. Untreated	1.3	5.9 (1.9)	13.6 (3.1)	85.4 (3.1)
2. Cuprokylt (x5)	2.6	3.7 (2.3)	9.6 (3.5)	90.1 (3.5)
3. Amistar (x3)	1.6	7.5 (3.2)	15.4 (4.6)	84.6 (4.4)
4. Signum (x5)	0.5	5.3 (2.7)	18.6 (4.9)	81.4 (4.7)
5. FarmFos44 + Silwett-L77 (x5)	0	2.1 (1.7)	11.7 (4.1)	88.3 (3.9)
6. Anthyllis growth stimulant (x5)	1.0	6.4 (2.9)	12.8 (4.2)	86.7 (4.2)
7. Purogene treated water	1.0	6.4 (2.9)	13.8 (4.4)	85.6 (4.3)
8. Infector plant left in for 7 weeks	1.6	9.1 (3.4)	14.4 (4.4)	84.6 (4.4)
Significance	-	NS	NS	NS

( ) – standard error. Sprays were applied at 14 day intervals from 5 August.

Table 4.2: Effect of foliar treatments on botrytis in cyclamen – 2008

Treatment (number of applications)	Mean severity (0-4)	Mean incidence (%)	Index >1 (%)	Index >2 (%)
1. Untreated	1.5	84	53	13
2. Cuprokylt (x5)	1.1	67	33	10
3. Amistar (x3)	1.2	73	33	15
4. Signum (x5)	0.9	55	30	8
5. FarmFos44 + Silwett-L77 (x5)	1.2	80	30	5
6. Anthyllis growth stimulant (x5)	1.2	73	28	10
7. Purogene treated water	1.4	73	45	18
8. Infector plant left in for 7 weeks	1.5	83	45	15
Significance	NS	NS	NS	NS

( ) – standard error; 10 plants assessed per plot.

Index 1 = sporulating botrytis on dead leaves; Index 2 = sporulating botrytis 1-3 green leaves.

Table 4.3: Effect of foliar treatments on growth of cyclamen – 4 December 2008

Treatment (number of applications)	Mean % cyclamen plants			
	Class 1	Class 2	Unmarketable	Mean foliage diameter (cm)[a]
1. Untreated	60.1 (4.5)	15.9 (3.1)	9.9 (1.7)	23.1
2. Cuprokylt (x5)	59.9 (6.4)	17.7 (4.6)	11.9 (2.6)	22.6
3. Amistar (x3)	62.5 (6.3)	16.0 (4.4)	7.2 (2.0)	22.5
4. Signum (x5)	50.5 (6.5)	25.5 (5.3)	8.9 (2.3)	23.3
5. FarmFos44 + Silwett-L77 (x5)	61.5 (6.4)	19.3 (4.8)	7.8 (2.1)	23.6
6. Anthyllis growth stimulant (x5)	54.2 (6.50	20.3 (4.9)	12.0 (2.6)	23.6
7. Purogene treated water	67.2 (6.2)	9.0 (3.5)	8.9 (2.3)	24.2
8. Infector plant left in for 7 weeks	53.6 (6.5)	20.3 (4.9)	8.3 (2.2)	23.4
Significance (25 df)	NS	NS	NS	NS

( ) – standard error; [a] Mean of 10 plants.

Overall conclusions

1. Inoculation of potted cyclamen plants with *Pectobacterium carotovorum* by drenching with a cell suspension in water over the plant crown resulted in development of bacterial soft rot of the corm. Symptoms first occurred after around 2 weeks.

2. The proportion of plants developing bacterial soft rot of the corm increased with the concentration of inoculum applied. Using a 50 mL drench per plant, the proportion of plants developing bacterial soft rot increased from 25% to 67% as the inoculum increased from 10^5 cells/ml to 10^7 cells/mL.

3. Inoculation of potted ivy plants with *Xanthomonas hortorum* pv. *hederae* by stab and spray inoculation resulted in development of bacterial leaf spot. Stab inoculation was more successful than spray inoculation. Incubation at a high humidity for 24-96 h after spray inoculation significantly increased symptom expression.

4. *P carotovorum* was confirmed as the cause of cyclamen bacterial soft rot in a commercial crop. No *E. chrysanthemi* was detected.

5. None of six foliar spray treatments (Amistar, Anthyllis, Cuprokylt, Farm-Fos 44 + Silwet-L77, Purogene and Signum) applied to cyclamen from four weeks after potting significantly reduced bacterial soft rot.

6. None of the six foliar treatments affected plant size (mean foliage diameter) or the proportion of marketable plants

Technology transfer

Project meetings

Fungicide trial site meetings, 2 and 11 December 2009, Spalding.

Project progress meeting, 26 January 2009, CSL York.

Project progress meeting, 2 June 2009, CSL York

Publication

Controls for bacterial diseases in pot plants. *HDC News* **148**, p.7.

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Appendix 1: Trial diaries for cyclamen bacterial soft rot experiments – ADAS Boxworth

a. Experiment 1 – Inoculum levels

Date	Action
11/7/08	150 cyclamen plug plants cv. Miracle White potted into 13 cm pots using M3 compost
15/7/08	90 cyclamen plug plants cv. Miracle White potted into 13 cm pots using M3 compost
30/7/08	Streaked *Pectobacterium carotovorum* supplied by CSL onto PDA. Incubated at 20^0C
7/8/08	Calibration curve for *P carotovorum* determined.
14/8/08	Trial set up as per protocol. Inoculum prepared from 3 plates of *P. carotovorum* to give suspensions of 1 x 10^2, 1 x 10^3, 1 x 10^5, and 1 x 10^7 cfu for T2 –T5. Plants inoculated as a crown drench and covered for 48 hrs
21/8/08	First symptoms of bacterial soft rot seen in T4 and T5 plots
29/8/08	1st assessment of bacterial soft rot completed
5/9/08	2nd assessment completed
11/9/08	3rd assessment completed
19/9/08	4th assessment completed
25/9/08	5th assessment completed
10/10/08	6th assessment completed
24/10/08	7th assessment completed
1/12/08	8th assessment completed
18/12/08	Destructive assessment completed

b. Experiment 1 – Inoculation methods

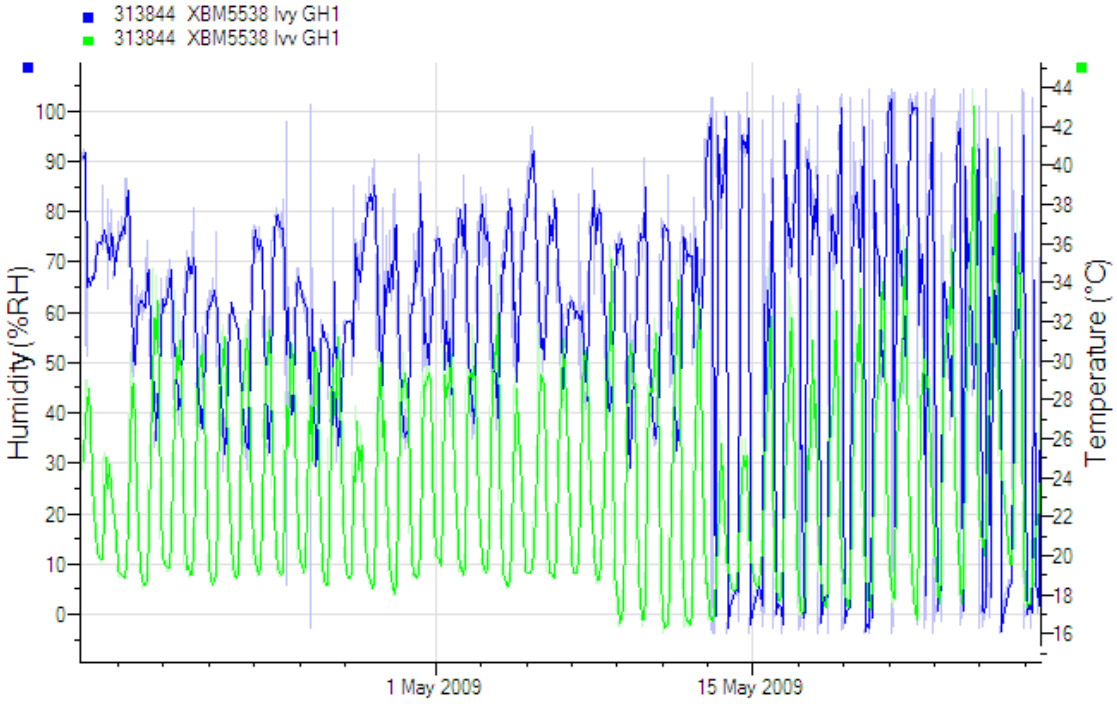
Date	Action
23/7/08	288 cyclamen plug plants cv. Laser Pink collected from propagator and placed into greenhouse
13/8/08	Trial set up as per protocol. Inoculum prepared from 4 plates of *P. carotovorum* to give a suspension of 1 x 10^7 cfu. Plants inoculated as a root dip, growing medium drench and crown drench, and controls inoculated by the same methods but with SDW. Half of each plot covered for 48 hours. Plants potted into M3 compost in 11cm pots.
28/8/08	First symptoms of bacterial soft rot seen.
28/8/08	1st assessment of bacterial soft rot completed

5/9/08	2nd assessment completed
11/9/08	3rd assessment completed
19/9/08	4th assessment completed
25/9/08	5th assessment completed
10/10/08	6th assessment completed
24/10/08	7th assessment completed
1/12/08	8th assessment completed
18/12/08	Destructive assessment completed

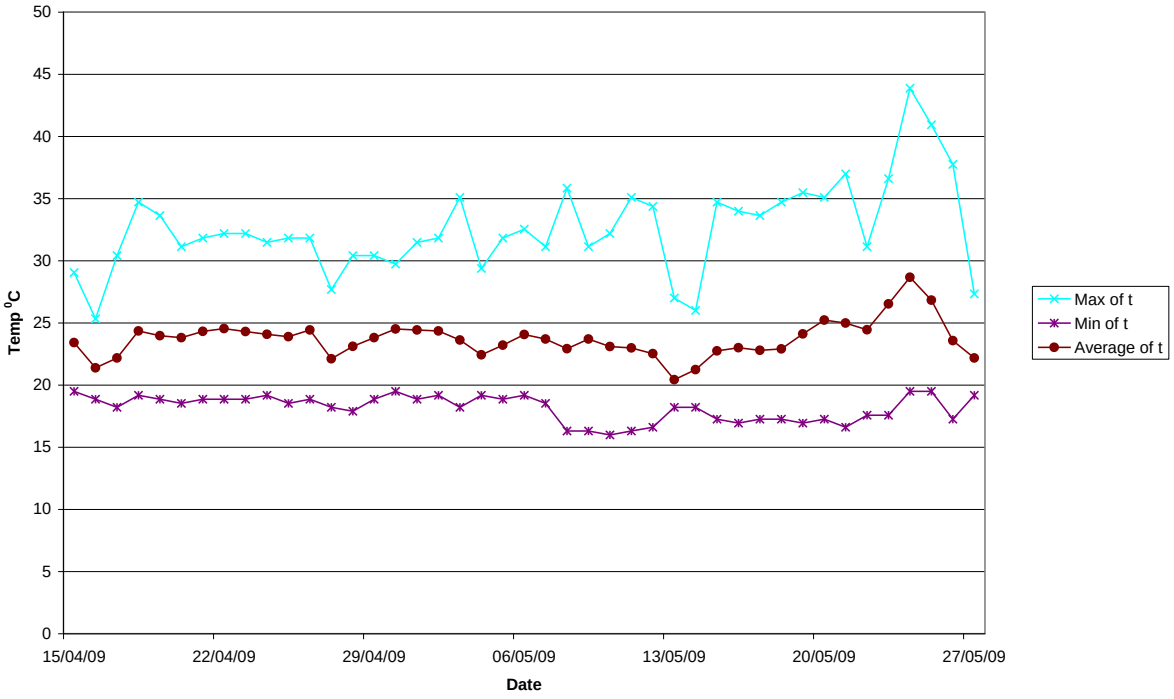
Appendix 2. Trial diary for ivy bacterial leaf spot, ADAS Boxworth

Date	Action
13/3/09	200 Hedera plants cv. Ester separated and potted into 9cm pots using M2 compost. Placed into GH1 at ADAS Boxworth.
14/4/09	Trial set up as per protocol and inoculated. Plates made up for plate counts to test inoculum level used. T3,T5,T6,T7 and T9 covered.
15/4/09	Covers removed from T5
16/4/09	Covers removed from T6
18/4/09	Covers removed from T3,T7, and T9. Plate counts completed, only low levels of cfu/ml present in inoculum. (100 cfu/ml)
28/4/09	Trial re-inoculated with 'thicker' suspension as no symptoms seen. Plates made up for plate counts. T3,T5,T6,T7 and T9 covered.
29/4/09	Covers removed from T5
30/4/09	Covers removed from T6
1/5/09	Plate counts completed, cfu/ml much higher.
2/5/09	Covers removed from T3,T7, and T9.
7/5/09	First appearance of leaf spots seen on T2 and T3 plants. Leaves misted to encourage disease.
12/5/09	Trial placed on capillary matting and covered with perforated polythene to raise humidity and encourage disease.
13/5/09	1st assessment completed.
27/5/09	2nd assessment completed.

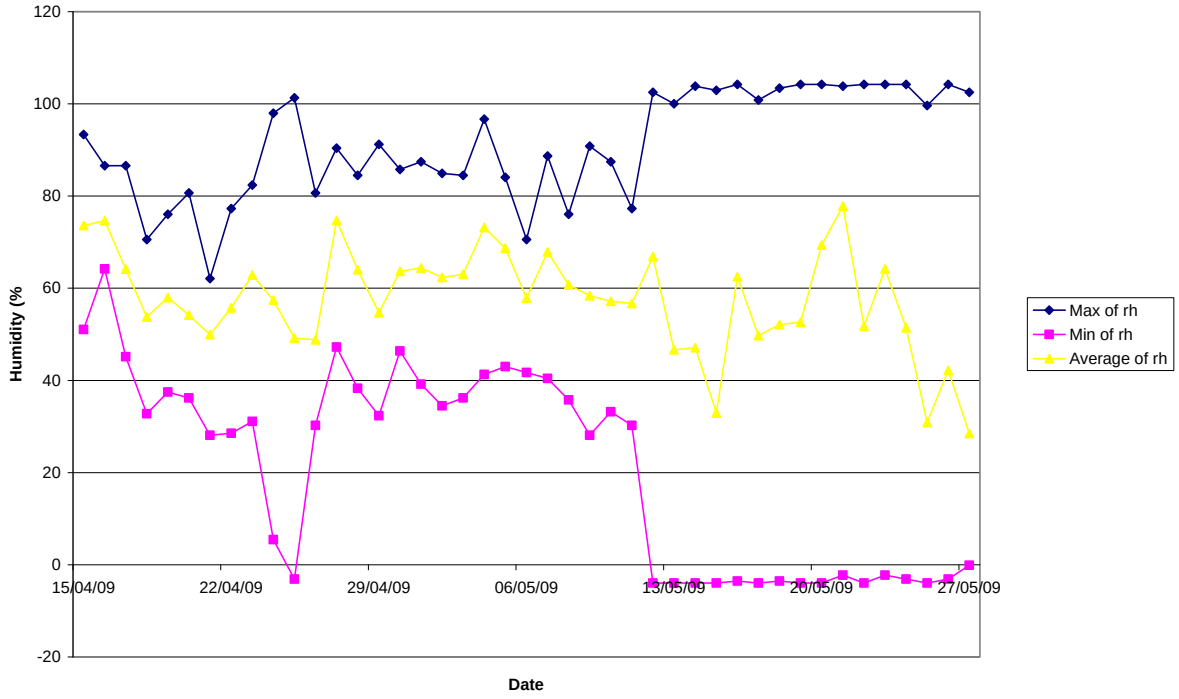
Appendix 3: Temperature and humidity records for ivy bacterial leaf spot



a) logger temperature/humidity records



b) Max, min and average temperatures.



c) Max, min and average relative humidity.

Appendix 4: Trial diary for cyclamen bacterial soft rot control trial - Spalding

Date	Action
01/8/08	Trial set up with plants still in trays, 47 healthy plants plus 1 infector in each plot.
05/8/08	1st spray applied.
12/8/08	Infector plants removed from all plots except treatment 9.
19/8/08	2nd spray applied
02/9/08	3rd spray applied.
15/9/08	4th spray applied.
25/9/08	Trial re-spaced as per nursery practice with plants 26 cm apart
30/9/08	5th spray applied; infector plants removed from T9 plots
2/12/08	Plant quality assessment
04/12/08	Grey mould assessment
11/12/08	Final disease assessment

Appendix 5: Interim disease assessments, nursery experiment

Effect of foliar treatments on bacterial soft rot in cyclamen – 2008 (interim assessments)

Treatment	Mean % cyclamen plants affected:			
	2 Sept	16 Sept	14 Oct	27 Nov
1. Untreated	1.3	5.9 (1.9)	6.3	14.4 (2.9)
2. Cuprokylt (x5)	2.6	3.7 (2.3)	4.7	8.0 (3.3)
3. Amistar (x3)	1.6	7.5 (3.2)	8.3	12.2 (4.0)
4. Signum (x5)	0.5	5.3 (2.7)	6.8	16.0 (4.5)
5. FarmFos44 + Silwett-L77 (x5)	0	2.1 (1.7)	4.2	10.1 (3.7)
6. Anthyllis growth stimulant (x5)	1.0	6.4 (2.9)	7.8	11.2 (3.9)
7. Purogene treated water	1.0	6.4 (2.9)	7.8	13.8 (4.2)
8. Infector plant left in for seven weeks	1.6	9.1 (3.4)	9.9	14.4 (4.3)
Significance	-	NS	NS	NS

( ) – standard error

Sprays were applied at 14 day intervals from 5 August. Purogene was applied at every watering to the end of October.

Appendix 6: Temperature and humidity records for fungicide trial – Spalding