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AUTHENTICATION

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

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GROWER SUMMARY

Headline

- Young plants were the predominant source of bacterial soft rot in cyclamen, although extent of infection varied with batch and links between infection and conditions in transit are suspected (particularly exposure of plants to condensation). None of the products tested were effective at controlling these infections once found and drenching with excess water prior to potting significantly increased levels of infection.
- Bacterial leaf spots on ivy and impatiens however were reduced by using preventative sprays of Cuprokylt FL. Amitar also had some effect against leaf spot of Impatiens.

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 and Cheiranthus
- *Pseudomonas* spp. causing leaf spots e.g. on Impatiens, Pelargonium and Primula.
- *Pectobacterium* causing soft rot e.g. on Cyclamen, Euphorbia, Pelargonium, Primula and Zantedeschia.
- *Agrobacterium* spp. causing crown gall of Dendranthema and other ornamentals.
- *Rhodococcus fascians* causing leafy gall e.g. on Pelargonium and Petunia.

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

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

This project aimed 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 were:

- A review of potential treatments for control of bacterial diseases.
- Greater awareness by growers of bacterial diseases and their management.

- An illustrated Factsheet on control of bacterial diseases on protected ornamentals.

Summary of the project and main conclusions

Review of potential treatments for control of bacterial diseases

A full review is given in the Year 1 Annual Report. In summary, there are very few approved products with proven bactericidal activity. Copper compounds have mostly been used to limit spread of bacterial leaf spots (*Xanthomonas* spp. and *Pseudomonas syringae* pathovars) but the level of control is limited, treatment can be phytotoxic and resistance can develop. Overseas, Serenade ASO (*Bacillus subtilis*) is recommended for control of fire blight (*Erwinia amylovora*) and some leaf spot diseases caused by *Pseudomonas syringae*.

Aliette 80WG (fosetyl Al) and potassium phosphite products applied regularly and at high doses have sometimes given moderate control of *Xanthomonas* leaf spot diseases. There is limited evidence for reduction of some bacterial diseases using products that induce Systemic Acquired Resistance (SAR), including the fungicides Amistar (azoxystrobin) and Signum (boscalid + pyraclostrobin). There is increasing interest in the use of specific viruses (phage) that infect and kill bacteria for control of bacterial diseases; a product based on phage was recently approved for use on ornamentals in the USA.

Occurrence of P. carotovorum in young cyclamen plants

Latent infection by *P. carotovorum* was detected in some batches of plug plants. During 2009 and 2010, only one batch out of 86 tested positive. In 2011, however, bacterial soft rot developed within a few days of receipt of some deliveries while plants were still in the plug trays. *P. carotovorum* was confirmed in 16 out of 17 lots of plug cyclamen cv. Halios sampled on 14 June 2011, including 4 out of 5 samples of visibly healthy plants. It is suggested that moisture and temperature conditions experienced during transport of young plants over long distances may induce development of bacterial soft rot.

Nursery sources of P. carotovorum

Samples of water from concrete pathways and cyclamen leaves tested positive for *P. carotovorum*. The presence of *P. carotovorum* in water droplets on leaves suggests the possibility of secondary disease spread from initial sources of infection. Samples of growing medium, dust from pathways, irrigation water, slime from irrigation lines, sand from beneath capillary matting and adult shore and sciarid flies, all tested negative for the bacterium.

Association of cyclamen bacterial soft rot with delivery batches of young plants

Cyclamen plants cv. Halios were assessed for bacterial soft rot in a growing-on test. In 2009, the disease was first observed 8 weeks after potting and eventually occurred in plants from four of five propagators (Table 1). Cumulative losses were significantly greater in plants from propagator C (9.7%) than other suppliers (nil to 1.2%). Losses to bacterial soft rot averaged over all propagators were greatest in the first two deliveries (9.2% and 6.0% respectively) than later deliveries (1.4 to 0.2%).

Sciarid fly were found associated with some of the early batches of plants and, after recognition of the problem, all deliveries were treated with Nemasys (*Steinernema feltiae*) for control of this pest. Possibly the high level of bacterial soft rot which developed in the first delivery may be associated with grazing damage to young plants by sciarid larvae that increased their susceptibility to infection by *P. carotovorum*. The effect of other factors, such as differences in leaf loss or occurrence of corm bruising at mechanical planting, cannot be discounted as influences on final losses to the disease. An experiment in 2010 on young cyclamen cv. Halios Flame showed that bruising the corm prior to inoculation with *P. carotovorum* greatly increased occurrence of bacterial soft rot over the following 10 weeks. Removal of leaves and addition of sciarid fly larvae prior to inoculation had no significant effect, although there was a trend to greater disease with addition of sciarid larvae.

In 2010, losses in plants of cv. Halios supplied by two propagators were monitored. All plants were treated at receipt for control of sciarid fly. Losses were slightly greater in the early deliveries and affected plants from one propagator more than the other (Table 1).

Table 1: Effect of propagator and delivery week on cyclamen bacterial soft rot, cv. Halios, grown on one nursery

Year and propagator	% bacterial soft rot in delivery week:				
	20	**22**	**24**	**26**	**28**
2009					
A	1	2	3	0	0
B	-	2	1	0	0
C	30	18	2	1	1
D	5	4	0	1	0
E	-	0	0	-	0
2010					
F	5	4	0	1	1
G	2	2	1	0	0

Effect of some foliar treatments on cyclamen bacterial soft rot

Six treatments were evaluated for control of cyclamen bacterial soft rot on a commercial nursery in 2009. Sprays of Amistar, Anthyllis growth stimulant (garlic extract), Cuprokylt (copper oxychloride), Farm Fos 44 + Silwet L77 (potassium phosphite + silicon wetter) and Signum were each applied up to five times from soon after potting, and Purogene (chlorine dioxide) was applied at every watering. None of the treatments reduced bacterial soft rot which ranged in severity from 14% plant collapse (untreated) to 8% (Cuprokylt). None of the treatments affected Botrytis severity or plant quality.

Effect of some integrated measures for control of cyclamen bacterial soft rot

The effect of an immersion treatment for sciarid control, different methods of potting and sprays of Cuprokylt FL (3 ml/L) and Serenade ASO (*Bacillus subtilis*) (10 L/ha) applied alone, in alternation and in mixture at half rate were evaluated on a nursery in 2011. The sprays were applied at potting and then weekly for 5 weeks, except for Cuprokylt FL alone which was applied 3 times at fortnightly intervals.

P. carotovorum was detected in plug plants immersed in water pre-potting and sampled immediately, and not in non-immersed plants. Symptoms of bacterial soft rot developed within 1 week of potting and the incidence of affected plants increased steadily over the course of the trial. *P. carotovorum* was confirmed in affected plants; no Fusarium wilt or other disease was found. At 1 week after the final sprays of Cuprokylt FL and Serenade ASO, the incidence of plants that had collapsed from bacterial soft rot ranged from nil to 19% (Table 2). Bacterial soft rot was greatly increased by immersing the plug plants in water at potting and slightly increased by spraying with Cuprokylt FL. Serenade ASO did not influence disease levels. Hand-potting did not reduce the disease compared with machine potting. At 13 weeks after potting the proportion of affected plants ranged from 30% to 71% (Table 2); differences between treatments at this time were not statistically significant, probably a reflection of secondary spread between plants during the trial period, masking the effects of treatments applied at and soon after potting.

Table 2: Effect of various treatments applied at and within 5 weeks of potting on occurrence of bacterial soft rot in cyclamen – 2011

Treatment		**Spray treatments after potting:**						**Mean % plants with bacterial soft rot**	
Potting	**Immersion in Nemasys**	**Wk 0**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**30 Aug**	**18 Oct**
1. Hand	Yes	-	-	-	-	-	-	6	39
2. Hand	No	-	-	-	-	-	-	0	30
3. Machine	Yes	-	-	-	-	-	-	10	47
4. Machine	No	-	-	-	-	-	-	2	31
5. Machine	Yes	Cup	-	Cup	-	Cup	-	15	53
6. Machine	Yes	Ser	Ser	Ser	Ser	Ser	Ser	6	46
7. Machine	Yes	Cup	Ser	Cup	Ser	Cup	Ser	19	71
8. Machine	Yes	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser	13	62

Cup – Cuprokylt FL spray; Ser – Serenade ASO spray; Cup/Ser – spray of Cuprokylt FL and Serenade ASO in mixture, each at half recommended rate.

Evaluation of treatments for control of Xanthomonas leaf spot of ivy

A greenhouse trial was done to assess the potential of five preventative treatments to control leaf spot on ivy caused by *Xanthomonas hortorum* pv. *hederae*. Foliar sprays of Cuprokylt FL (5 ml/L) significantly reduced the disease. Treatments with Farm-Fos 44, Aliette 80WG (3.75 g/L), Amistar (1 ml/L) or methyl jasmonate (a chemical used to induce SAR) failed to reduce the disease in comparison with untreated controls. Two applications (before and after inoculation) with Cuprokylt FL were more effective than a single preventative spray.

Evaluation of treatments for control of Pseudomonas leaf spot of impatiens

A greenhouse trial assessed the potential of six preventative treatments to control leaf spot on impatiens caused by *Pseudomonas syringae* pv. *syringae*. None of the treatments prevented development of leaf spot symptoms following artificial inoculation. Sprays of Cuprokylt FL (5 ml/L) reduced the incidence of infections by around 40%; Amistar (1 ml/L), Biosept (0.5 ml/L) and an experimental application of methyl jasmonate reduced the incidence of infections by 20%. Applications of Farm-Fos 44 (10 ml/L) and Aliette 80WG (3.75 g/L) were ineffective.

Evaluation of disinfectants for control of bacteria

Experiments were done to compare the activity of seven disinfectants against three bacterial pathogens (*Pectobacterium carotovorum, Pseudomonas syringae* and *Xanthomonas hortorum* pv. *hederae*). Products were: Biosept (grapefruit seed extract), bleach (sodium hypochlorite), Fam-30 (iodophor), Hortisept (quaternary ammonium compound), Menno-Florades (organic acid), Sanprox P (peroxygen) and Virkon S (peroxygen). All disinfectants completely inhibited bacterial growth in an agar plate test, at the manufacturers recommended rate, except for Biosept on *Ps. syringae*. Mypex-type matting and concrete were more difficult to disinfect than glass and aluminium. Sanprox P at 1% and Virkon S at 1% fully disinfected all surfaces of all three bacteria after 0.5 hour. *Xanthomonas hortorum* pv. *hederae* was most persistent, surviving exposure to 0.8% Hortisept, 1% Menno-Florades, 0.8% Fam-30 and 0.05% Biosept for 24 h on concrete.

Aspects of leafy gall (Rhodococcus fascians)

Leafy gall was diagnosed on a commercial crop of trailing petunia in summer 2010. The causal agent was identified as *Rhodococcus fascians*, although the bacterium differed slightly from the reference type strain. Cuttings taken from healthy parts of plants with symptoms were found to carry infection, suggesting that systemic infections can be spread through cuttings. Other plants (e.g. Hebe) were also found to be infected and may act as reservoirs of infection on the same nursery.

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.

In 2007 and 2008, several UK nurseries growing poinsettia 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 well received by 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 consult the factsheet produced as part of this project and follow the guidance provided about prevention and action required to minimise damage when outbreaks occur.
2. A slimy soft rot of cyclamen, usually originating in the upper part of the corm, is a good indication of bacterial soft rot caused by *Pectobacterium carotovorum*. Leaf petiole blackening is not a reliable indicator of the disease.
3. Work in this project strongly indicates that waterlogging of young cyclamen plants can greatly increase losses to bacterial soft rot. Do not immerse plug plants in water or drench or irrigate plants to excess such that plants become waterlogged.
4. Work in this project showed that *P. carotovorum* may be present in plug cyclamen plants and bacterial soft rot may develop within a few days of receipt on a nursery. Inspect each delivery of plants carefully on arrival; if disease symptoms are found, have a sample of plants tested to determine if *P. carotovorum* or other pathogens are present. The effect of plug plant transport conditions (particularly condensation) on occurrence of cyclamen bacterial soft rot warrants investigation but it would be advisable to remove packaging around trolleys of delivered plants on receipt to minimise exposure to wetness/high humidity and to carry out particularly thorough inspections on batches where excess condensation is observed inside the plastic film packaging.
5. Bruising of young, cyclamen corms greatly increases the potential for bacterial soft rot. Check that corms are not visibly damaged where mechanical transplanting is used.
6. There is circumstantial evidence that sciarid fly in young cyclamen may be associated with subsequent increased levels of bacterial soft rot. Check young plants arriving on a nursery for sciarid fly and take measures to control damage to corms caused by sciarid larvae.
7. Due to the lack of approved products with proven bactericidal activity, it is recommended that plants affected by bacterial diseases are removed promptly from a crop.
8. Foliar sprays of copper oxychloride (eg Cuprokylt FL) applied preventatively can significantly reduce bacterial leaf spot of ivy (*Xanthomonas hortorum* pv. *hederae)* and Impatiens (*Pseudomonas syringae* pv. *syringae*). Sprays of Amistar (1 ml/L) slightly reduced the latter disease. Use of Cuprokylt FL on protected ornamentals (maximum rate 3 ml/L) is currently permitted under the Long Term Arrangements for Extension of Use of Pesticides; higher rates are permitted on outdoor crops. Use of Amistar is permitted under a Specific Off Label Approval (SOLA 0443/09). Check the label and approval status before use on a crop. Test treat a small batch of plants first to check for crop safety and/or deposit.

9. Before laying out a new crop known to be susceptible to a bacterial disease, consider disinfection of standing areas and pathways. Concrete and Mypex-type matting are more difficult to disinfect than glass or plastic; peroxygen products at the manufacturers' recommended rates were more effective than other products we tested on these surfaces.
10. Do not take cuttings from petunia plants with symptoms of leafy gall; work in this project showed that visibly healthy shoots on affected plants can be infected systemically with the causal bacterium.

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.

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. Experimental work in this project is being 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 adhere 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 certain bacterial diseases, caused by species of *Pseudomonas, Ralstonia* and *Xanthomonas*. The potential of using novel chemical treatments for prevention and control of bacterial soft rot in cyclamen and bacterial leaf spots of various ornamentals warrants investigation in order to reduce losses and secure more reliable quality of important pot plant species.

In the first year we:

- Listed a range of chemical and biological interventions with potential for control of bacterial diseases of protected ornamental crops;
- Devised artificial inoculation procedures for production of bacterial soft rot (*Pectobacterium carotovorum)* in cyclamen and bacterial leaf spot (*Xanthomonas* sp.) of ivy;
- Evaluated treatments for control of bacterial soft rot in cyclamen.

In the second year we:

- Investigated sources of *P. carotovorum* entering a cyclamen crop, including young plants, water and beds where plants were stood (new objective);
- Evaluated some chemical and biological protective treatments in the laboratory against two bacterial species.

Specific objectives in the third year were:

- Evaluate effect of some damage treatments to cyclamen on bacterial soft rot;
- Monitor the effect of propagator and delivery week on losses to bacterial soft rot in cyclamen;
- Evaluate some integrated treatments for control of cyclamen bacterial soft rot;
- Evaluate available chemical disinfectants for efficacy in reducing populations of *Pectobacterium*, X*anthomonas* and *Pseudomonas* species on four types of surface;
- Evaluate some chemical and biological preventative treatments in the laboratory against a *Pseudomonas* leaf spot;
- Investigate a possible outbreak of leafy gall in trailing petunia (new objective).

1. Effect of damage to cyclamen on occurrence of bacterial soft rot

Introduction

In 2009 the incidence of bacterial soft rot that developed in commercial batches of cyclamen affected by sciarid fly at the plug plant stage, appeared to be negatively associated with control measures taken against this pest (Year 1 Annual Report). Plants not treated at the plug stage developed the greatest bacterial soft rot, and those soaked for 15 minutes before potting in a Nemasys (*Steinernema feltiae*) suspension for control of sciarid larvae developed the least. Plants damaged by sciarid fly larvae grazing on the corm surface and leaf petioles may be more susceptible to infection by *Pectobacterium carotovorum*. The aim of the current experiment was to determine if occurrence of bacterial soft rot in cyclamen corms is exacerbated by sciarid fly larval damage to young plants. Additionally, the effects of creating wound sites on the corm by removal of a leaf and corm bruising were investigated. It is commonly observed that leaf breakage occurs on plants potted by machine; it is suspected that corms transplanted by machine may be bruised.

Materials and methods

Site and crop details

Two experiments were done in a glasshouse at ADAS Boxworth. Plug cyclamen plants cv. Halios Flame in 84 cell trays were obtained from a commercial supplier, and potted into Levington M2 compost in 13 cm pots with the top portion of the corm visible. Plants were placed pot thick on capillary matting in plastic gravel trays and watered by hand. Plants were inoculated with a standard suspension of *P. carotovorum* on 27 May 2010.

Treatments

In the first experiment there were eight treatments arranged in two glasshouse compartments; the sciarid larval damage treatment was separated from others in order to minimise the risk of interference between plots (Table 1.1).

Table 1.1: Treatments examined for their effect on development of cyclamen bacterial soft rot – 2010 (Experiment 1)

Damage to plug cyclamen plant	Inoculation with *P. carotovorum*	Glasshouse compartment
1. Nil	-	1
2. Two leaves removed	-	1
3. Nil	+	1
4. Two leaves removed	+	1
5. Nil	-	2
6. Sciarid larvae added	-	2
7. Nil	+	2
8. Sciarid larvae added	+	2

Leaf damage was done by pulling off two lower leaves at potting (treatments 2 and 4); a small petiole stub or scar was visible after leaf removal. Laboratory raised sciarid larvae (10 per plant) were placed onto the compost surface close to the corm immediately after potting (treatments 6 and 8). Plants were shaded with netting for 1 week afterwards. All plants in treatments 1-4 and 5 and 7 in Experiment 1 were drench treated with Nemasys (100 ml/plant) at the plug stage and again after potting to minimise the risk of damage to corms from natural infestation by sciarid fly.

In Experiment 2, corms were bruised by firmly clasping the sides of a corm with a pair of broad forceps in two directions at 90º to each other, or left unbruised. Plants with bruised corms were inoculated with *P. carotovorum* or left uninoculated (control).

Inoculation with P. carotovorum

At one week after leaf removal and addition of sciarid fly larvae, plants in Experiment 1 were drench inoculated (100 ml/plant) with a suspension of *P. carotovorum* (6 x 10^6 cells/ml) in sterile distilled water (SDW) that was poured over the crown of plants (27 May 2010). Control treatments were drenched with water alone. Treatments 2 and 3 in Experiment 2 were drench inoculated with *P. carotovorum*, as above, on 3 June 2010. This method of inoculation had been shown to induce symptoms of bacterial rot in previous experiments (Year 1, Annual Report).

Disease assessments

Plants were examined 2-3 times weekly for leaf yellowing and plant collapse to determine the first occurrence of bacterial soft rot. The numbers of collapsed plants was assessed at 2, 4 and 8 weeks after inoculation. The cause of plant collapse was determined by examination for the soft slimy corm rot typical of *P. carotovorum* infection.

Experiment design and data analysis

Treatments from both Experiments 1 and 2 were arranged in randomized blocks with fourfold replication. The design for Experiment 1 was not completely randomised, due to the need to keep the sciarid fly treatments in a separate glasshouse. There were 10 plants per plot. Results were examined by generalised linear modelling.

Results and discussion

Both sciarid fly (*Bradysia* spp.) and shore fly (*Scatella* spp.) were found associated with the plug plants when they were delivered. Plants that were not due to be inoculated with sciarid fly larva were immediately treated with Nemasys to control these pests.

Symptoms of bacterial soft rot first occurred at 2 weeks after inoculation in both experiments. In Experiment 1, at 11 weeks after inoculation, bacterial soft rot was present in all treatments inoculated with *P. carotovorum* and none of the uninoculated treatments (Table 1.2). Addition of sciarid larvae appeared to increase the incidence of bacterial soft rot, from 18% to 25% of plants, but the difference was not statistically significant. Leaf removal had no effect on disease incidence. The incidence of bacterial soft rot was greater in the undamaged plants of treatment 7 (18%) than in the same treatment in an adjacent compartment (8%); possibly this was associated with a temperature difference between the end compartment and a middle compartment, the higher temperature in the end compartment favouring development of soft rot.

In Experiment 2, corm bruising significantly ($P < 0.004$) increased bacterial soft rot (Table 1.3). Bacterial soft rot also developed at a low level on non-inoculated plants that had been bruised.

These experiments indicate that corm bruising as might occur at mechanised potting can increase the risk of bacterial soft rot. The effect of adding sciarid larvae to plug plants was unclear, possibly due to established infestation (and therefore a risk of existing damage in corms) when the experiment was set up. Removal of two lower leaves had no effect on the incidence of bacterial soft rot. This may indicate that infection of petiole bases by *P. carotovorum* is less successful than on damaged corm tissue, or possibly the small surface area did not come into contact with the bacterium at drench inoculation. A direct comparison of hand potting and machine potting on the incidence of bacterial soft rot in a commercial crop of cyclamen will be examined in another experiment (see section 3).

Table 1.2: Effect of de-leafing at potting and inoculation with sciarid larvae on occurrence of cyclamen bacterial soft rot – 24 August 2010 (Experiment 1)

Treatment	Pc drench[a]	Mean % plants affected after 11 weeks
1. Nil	-	0
2. Leaf damage	-	0
3. Nil	+	5 (2.8)
4. Leaf damage	+	8 (3.3)
5. Nil	-	0
6. Larvae added	-	0
7. Nil	+	18 (4.7)
8. Larvae added	+	25 (5.3)
Significance (31 df)		<0.001

[a] Drench inoculated with *P. carotovorum*; ( ) – standard errors

Table 1.3: Effect of mechanical bruising at potting on occurrence of cyclamen bacterial soft rot – 24 August 2010 (Experiment 2)

Treatment	Pc drench[a]	Mean % plants affected after 10 weeks
1. Bruised	-	13 (6.1)
2. Bruised	+	56 (9.1)
3. Nil	+	0
Significance (11 df)		0.004

[a] Drench inoculated with *P. carotovorum*; ( ) – standard errors

2. Monitoring for bacterial soft rot in cyclamen

Introduction

In 2009 the incidence of bacterial soft rot that developed in cyclamen was found to be associated with specific delivery batches. However, almost all plants sampled from trays and tested for *P. carotovorum* were found to be free of the bacterium. Previously, nursery staff have reported that some plug cyclamen plants were visibly affected by bacterial soft rot at delivery. The aims of the current experiment was to determine if there were any symptoms of bacterial soft rot at delivery among 800 trays of plug plants; to test suspect plants for the presence of *P. carotovorum*; and to determine the incidence of bacterial soft rot after potting in plants grown from 20 different batches.

Materials and methods

Site and crop details

Monitoring was done on a commercial nursery on cyclamen plants delivered from two propagators during 2010. Plants were potted into 13 cm pots and grown on the nursery following grower-standard practice. Plants were placed on plastic-backed capillary matting over sand and watered as required, initially from overhead and subsequently from below by watering the matting around plants.

Treatments

40 trays of plants (at 96 plants per tray) were examined on receipt and after potting up as detailed in Table 2.1. The same colours (Bright Scarlet, Purple/Violet, Light rose and White compact) were chosen from the two propagators; the names of the colours differed slightly between the propagators. Trays of four additional colours Halios Deep Rose, Halios Flame Mix, Halios Lilac and Halios Magenta, were examined at receipt on six deliveries. . These plants were not assessed after potting up

Table 2.1: Details of plug cyclamen plants examined for bacterial soft rot on receipt and during commercial production – 2010

Propagator	**Variety/colour**	**Delivery weeks**				
	(label description)	**20**	**22**	**24**	**26**	**28**
A	Bright scarlet	✓	✓	✓	✓	✓
	Purple	✓	✓	✓	✓	✓
	Light rose	✓	✓	✓	✓	✓
	White	✓	✓	✓	✓	✓
B	Bright scarlet	✓	✓	✓	✓	✓
	Violet	✓	✓	✓	✓	✓
	Light rose	✓	✓	✓	✓	✓
	White	✓	✓	✓	✓	✓

One tray assessed for each variety, at each delivery week

Disease assessment

Each tray of 96 plants was carefully examined for symptoms of leaf rot and/or corm collapse. Samples of plants were sent to Fera to check for *P. carotovorum* by isolation tests.

One tray of plants from each variety from each delivery week that had no evidence of bacterial soft rot was potted up and labelled so that the specific batch could be found again on the nursery. These plants were assessed for disease at intervals up to the point of sale, and the cumulative number of collapsed plants was recorded.

Experiment design and statistical analysis

Data was tabulated to show the effect of propagator, colour and delivery data on the incidence of bacterial soft rot at delivery and during crop production.

Results and discussion

Health of plug plants received

No symptoms typical of bacterial soft rot were found on any of the 64 trays of plug cyclamen plants examined (around 6,200 plants). Eleven samples of young plants with weak growth or other symptoms were sent to Fera to test for *P. carotovorum* (as described in Year 1 report). No *P. carotovorum* was isolated from any of the samples.

Development of bacterial soft rot

A total of 58 plants had collapsed due to bacterial soft rot (confirmed by symptom appearance) by week 40, around 1.5% of the total examined. The main losses occurred in plants delivered in weeks 20 and 22 (27 and 20 plants respectively) (Table 2.2). Overall, collapsed plants were found at nine of the 18 weekly inspections, with losses greatest in weeks 26, 32, 33 and 40.

Comparing the two propagators, plant losses were greater from propagator A (41) than B (17); comparing the four colours, losses were greater in white (24), than light rose (20), bright scarlet (10) and purple/violet (4). However, the majority of white compact losses were in plants from propagator A, so this could have been a propagator rather than a varietal effect. Full results are given in Table 2.3.

Overall losses in 2010 (1.5%) were less than those observed in a similar experiment on the same nursery in 2009 (3.4%). Losses were again greater in the first two deliveries than in later deliveries even though sciarid control treatment was applied to all plug plants on receipt in 2010. Possibly this apparent greater susceptibility of early delivered plants reflects weaker plants produced early in the year under reduced light or other sub-optimal growth conditions.

A large difference between propagators occurred in 2010, as in 2009. This may reflect differences arising from different methods of plant production, treatments applied pre-delivery and/or conditions experienced during transport, for example.

Although overall there appeared to be a difference in incidence of bacterial soft rot between the four colours, with white and light rose suffering greater losses than the other two, numbers are relatively small and firm conclusions should not be drawn on this observation alone.

Table 2.2: Development of cyclamen bacterial soft rot after potting in five deliveries of young plants – 2010

Delivery	**Number of plants[a] removed in week number**																	
week	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
20	3	0	1	4	0	0	1	0	0	11	3	4	0	0	0	0	0	0
22	0	0	0	3	0	2	0	0	0	2	5	0	0	0	0	0	0	8
24	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
26	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
28	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	4

[a] Out of 768, comprising 2 propagators x 4 colours x 96 plants (1 plug tray).

Table 2.3: Effect of propagator, flower colour and delivery week on cumulative incidence of cyclamen bacterial soft rot – 2010

Propagator (A or B) and flower colour		**% plants collapsed up to week 40 according to delivery week**				
		20	**22**	**24**	**26**	**28**
A	Bright scarlet	5	0	0	2	0
	Purple/violet	0	2	0	0	0
	Light rose	4	6	0	3	1
	White	10	6	0	0	2
B	Bright scarlet	2	1	0	0	0
	Purple/violet	0	0	1	0	1
	Light rose	5	1	0	0	0
	White	1	4	1	0	0
Total		27	20	2	5	4

3. Integrated treatments for control of cyclamen bacterial soft rot

Introduction

Plant tissues with physical damage are generally more susceptible to infection by *P. carotovorum*. Earlier work in this project indicated that sciarid larvae damage to young plants and corm bruising may increase risk of the disease in cyclamen. The only two products with evidence of activity against bacterial diseases and currently approved for use on cyclamen are Serenade ASO (*Bacillus subtilis*) and Cuprokylt FL (copper oxychloride). In an experiment in Year 1, none of several treatments tested alone gave control of cyclamen bacterial soft rot. The aim of this experiment was to test the efficacy of some integrated treatments combining reduced risk of physical damage to corms with use of Cuprokylt FL and/or Serenade ASO applied at and immediately after potting. Additionally, opportunity was taken to further examine batches of plug cyclamen soon after arrival on a nursery for occurrence of latent *P. carotovorum*, and to further examine possible sources of *P. carotovorum* on a production nursery.

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 (the Netherlands) were potted in week 29 into 13 cm pots. The growing medium was a nursery specified mix consisting of light peat (40%), black peat (30%), perlite (10%), clay (10%) and soil (10%). 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. The crop was grown unheated until 1 October, and then at a minimum temperature of 14ºC. No fungicides or chemical treatments were applied to plants except as specified treatments (see below). The experiment was terminated in week 42 when plants were flowering and ready for sale. A crop diary is given in Appendix 1.

Treatments

Treatments compared i) the effect of machine potting (nursery standard) with hand potting, in the knowledge that machine potting sometimes results in broken leaves, thereby providing an entry point for infection; ii) immersion of plug plants in Nemasys, for control of sciarid fly, with no immersion, following work in 2010 that indicated an increased risk of bacterial soft rot in plants infested by sciarid fly larvae; and iii) the effect of post-planting high volume sprays of Cuprokylt FL (copper oxychloride) and Serenade ASO (*Bacillus subtilis*), two products with evidence for control of bacterial diseases. The high volume sprays were applied immediately after potting, and then at weekly (Serenade ASO) or fortnightly (Cuprokylt FL) intervals for five weeks. Cuprokylt FL (3 ml/L) and Serenade ASO (10 L/ha) were applied alone, in alternation, and in a

mixture with each product used at half the above rates. Application rate and spray interval of the individual products was at label recommendations. Cuprokylt FL is currently permitted on protected ornamentals under the Long Term Arrangements for Extension of Use; Serenade ASO is permitted under SOLA 0246/09; both uses are at growers' own risk. Treatments are listed below:

Table 3.1: Treatments examined for effect on bacterial soft rot of cyclamen, cv. Halios Flame Mix 2011

Potting	Immersion in Nemasys	Sprays after potting					
		Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5
1. Hand	Yes	-	-	-	-	-	-
2. Hand	No	-	-	-	-	-	-
3. Machine	Yes	-	-	-	-	-	-
4. Machine	No	-	-	-	-	-	-
5. Machine	Yes	Cup	-	Cup	-	Cup	-
6. Machine	Yes	Ser	Ser	Ser	Ser	Ser	Ser
7. Machine	Yes	Cup	Ser	Cup	Ser	Cup	Ser
8. Machine	Yes	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser	Cup/Ser

Cup – Cuprokylt FL; Ser – Serenade ASO

The immersion treatment was done when plants were in the plug trays. A tray of plants was fully immersed in a suspension of Nemasys for around 2 seconds, removed and allowed to drain. Machine and hand potting were done by trained nursery staff. Post-planting sprays were applied at 1,000 L/ha using a knapsack pressurised sprayer with 02F100 nozzles at 2 bar pressure.

Experimental design and statistic analysis

The experiment was a randomised design with four replicate blocks of eight treatments. Each plot consisted of 48 plants, initially placed in six adjacent trays of eight plants. At spacing, the plants were removed from the trays and spaced diagonally at 26 cm (centre to centre). Results were examined by ANOVA and regression analysis as appropriate. Specific contrasts were done to determine the overall effects of immersion vs non-immersion at potting, and Cuprokylt FL vs no Cuprokylt FL spray treatment post-potting.

Disease assessments

The experiment was examined weekly for 8 weeks and then fortnightly. All collapsed plants were removed from the crop at each visit and the cause of collapse determined by examination of the corm for symptoms of bacterial soft rot, Fusarium wilt or other disease.

At the final assessment in week 42, the number of surviving plants per plot was counted.

Monitoring for P. carotovorum

Samples of plants, water and insects were collected from the nursery at intervals and sent to Fera to test for *P. carotovorum*, using the methods described in previous reports. Briefly, plants were macerated in buffer and a dilution series was streaked onto a soft rot bacterium semi-selective medium (CVP). Plants with suspected symptoms of soft rot were tested separately. Suspected colonies of *P. carotovorum* were confirmed by fatty acid profile analysis. The samples collected were as follows:

14 June	Cyclamen plug plants (suspected bacterial soft rot)
19 July	Plug plants and growing medium used in the trial, taken at potting
26 July	Sciarid flies
2 Aug	Sciarid and shore flies
16 Aug	Water from glasshouse floor and Nemasys drench tank
23 Aug	Water from glasshouse floor and cyclamen leaves (commercial crop close to the trial)

Plant growth

An assessment of plant growth was done on 30 August, 6 weeks after potting. Top growth was assessed on a 1-5 scale and root growth was assessed as % root ball covered by roots. Ten plants per plot were examined.

Results

Bacterial soft rot

No symptoms of bacterial soft rot were observed in plug plants at potting.

Plant collapse due to bacterial soft rot was first observed one week after potting. A sample of 10 collapsed plants was sent to Fera and *P. carotovorum* was confirmed in all of them. Further collapsed plants were found at each visit for the duration of the trial. Symptoms of the collapsed plants were typical of bacterial soft rot. No Fusarium wilt was found.

At 6 weeks after potting (1 week after the final sprays of Cuprokylt FL and Serenade ASO), plants affected by bacterial soft rot were found in all treatments except the untreated control, treatment 3, where plants were hand potted, not immersed in water at the plug plant stage and not treated with Cuprokylt FL or Serenade ASO (Table 3.2). The number of plants with bacterial soft rot was significantly ($P = 0.007$) increased by treatments 3, 5, 7 and 8. Treatments were grouped to compare the effects of specific components of treatments at this time (Table 3.3). Brief immersion of plug plants in a tank of water containing Nemasys ($P = 0.002$), and sprays of Cuprokylt FL ($P < 0.001$) significantly increased bacterial soft rot, by x13 and x3

respectively. There was no evidence that machine potting increased bacterial soft rot compared with hand potting (T3 compared with T1, T4 compared with T2).

An assessment was done on plant growth at 1 week after the final spray to determine if any treatment, and especially Cuprokylt FL, caused damage or affected growth and thereby increased susceptibility to bacterial soft rot. No differences were observed in the amount of top growth (Table 3.4). No stunting of roots or other crop damage was observed.

The numbers of plants developing bacterial soft rot continued to increase with time. The trial was concluded on 18 October, 13 weeks after potting, when plants were in flower and ready for sale. The mean number of surviving plants per plot at this time ranged from 14.0 (29%) in treatment 7 to 33.8 (70%) in treatment 2 (Table 3.4). The greatest numbers of surviving plants were in treatments 2 and 4, plants which were not immersed in water at the plug plant stage. Although the pattern of treatment effects at the end of the trial reflected that recorded at 6 weeks after potting, differences between treatments were not statistically significant (P = 0.077).

Table 3.2: Effect of treatments applied at potting and post-planting sprays on cyclamen bacterial soft rot – 2011

Potting	Immersion in Nemasys	Sprays after potting	Mean no. plants (of 48) with bacterial soft rot at intervals (week) after potting						
			1	2	3	4	5	6	7
1. Hand	Yes	-	0	0.5	0.8	2.0	2.3	2.8	5.3
2. Hand	No	-	0	0	0	0	0	0	0
3. Machine	Yes	-	0	0.3	0.8	2.3	3.8	4.8	8.0
4. Machine	No	-	0	0	0	0	0.3	0.8	1.8
5. Machine	Yes	Cup	0	1.5	3.0	5.0	5.8	7.0	8.5
6. Machine	Yes	Ser	0	0.3	0.8	1.8	2.3	2.8	7.0
7. Machine	Yes	Cup/Ser	0.8	1.8	2.5	4.3	7.0	9.3	14.5
8. Machine	Yes	Cup+Ser	0.3	1.3	2.3	4.3	4.3	6.0	9.5
Significance (21 df)			NS	0.046	NS	NS	0.011	0.007	0.016
LSD			-	1.29	-	-	3.86	4.75	7.40

Table 3.3: Effect of immersion of plug plants at potting and Cuprokylt FL spray treatment on cyclamen bacterial soft rot at 6 weeks after potting (30 August 2011)

Treatment contrast	Treatment numbers	Number of replicates	Mean number of plants affected (of 48)	F pr.
No immersion	2, 4	8	0.4	0.002
Immersion	1, 3, 5-8	24	5.5	
No Cuprokylt FL	1-4, 6	20	2.2	<0.001
Cuprokylt FL	5, 7, 8	12	7.5	

Table 3.4: Effect of treatments applied at potting and post-planting sprays on plant growth and number of surviving plants

Potting	Immersion in Nemasys	Sprays after potting	Plant growth score (30 Aug) (1 – 5)	Number surviving non-collapsed plants out of 48 (18 Oct)
1. Hand	Yes	-	2.5	29.2
2. Hand	No	-	2.8	33.8
3. Machine	Yes		2.8	25.2
4. Machine	No	-	3.0	33.2
5. Machine	Yes	Cup	2.5	22.5
6. Machine	Yes	Ser	2.8	26.8
7. Machine	Yes	Cup/Ser	3.0	14.0
8. Machine	Yes	Cup+Ser	2.5	18.0
Significance (21 df)			NS	NS
LSD			-	-

Monitoring for P. carotovorum

Trays of plug cyclamen plants cv. Halios from the Netherlands received at the nursery on 10 June showed symptoms of bacterial soft rot by 13 June, while still in the plug trays. Plants of four colours were sampled from seven trays, selecting two to five plants with obvious soft rot (mushy corm), possible soft rot (soft petioles) and visibly healthy from the same tray. No trays completely free of soft rot symptoms were found. *P. carotovorum* was confirmed in 16 of the 17 sets of plants sampled (Table 3.5). Four out of five samples of visibly healthy plants tested positive for the bacterium. The level of infection, as indicated by the number of bacteria recovered, was generally greatest in plants with obvious soft rot but some of the samples with possible soft rot and visibly healthy were also heavily infected. Although no count on the distribution of affected plants was done, it appeared that plants with obvious soft rot were more common towards the edges of plug trays than in the centre.

The cyclamen plug plants used in the trial were sampled on 19 July 2011, the day the trial was set up, and tested for *P. carotovorum*. Sub-samples consisted of 10-11 plants. Two out of six samples tested positive for the bacterium (Table 3.6); these were both from trays of plants that had been immersed in a suspension of Nemasys in water. The four samples of plants that were not immersed all tested negative.

A sample of the growing medium collected on 19 July 2011 tested negative for *P. carotovorum.*

A sample of water collected on 16 August from the water tank used to immerse plug plants was found to be free of *P. carotovorum*. A sample of water collected from a puddle in the trial glasshouse on the same day (1 week after potting) had a low level of *P. carotovorum* (Table 3.7). Samples of water collected from puddles in the glasshouse one week later had relatively high levels of the bacterium, up to 6000 cfu/ml (Table 3.7). It is considered that these levels, while not high enough to cause soft rot without further multiplication, are significant as an indication of water as a source of infection, especially on plants that are damaged and wet. Interestingly, a low level of *P. carotovorum* was also detected in one of three samples of water pipetted from the surface of cyclamen leaves.

No *P. carotovorum* was detected in a sample of sciarid and shore flies collected from the nursery on 2 August. It was not possible to test a sample collected one week earlier due to breakage of the sample tube in the post and loss of the flies.

Table 3.5: Recovery of *P. carotovorum* from trays of plug cyclamen plants in which one or more plants had symptoms of bacterial soft rot – sampled 14 June 2011

Plug tray	Plant colour	No. plants in sample	Symptoms	*P. carotovorum* detected	Relative level
1	Light rose	2	Obvious rot	Yes	High
1	Light rose	2	Possible rot (soft petioles)	Yes	Medium
1	Light rose	3	Healthy	No	-
2	Light rose	2	Obvious rot	Yes	High
2	Light rose	3	Possible rot	Yes	Medium
2	Light rose	2	Healthy	Yes	Medium
3	Magenta	5	Obvious rot	Yes	High
3	Magenta	3	Possible rot	Yes	High
3	Magenta	5	Healthy	Yes	Medium
4	Magenta	2	Possible rot	Yes	High
5	White	5	Obvious rot	Yes	High
5	White	5	Possible rot	Yes	High
5	White	5	Healthy	Yes	High
6	Fantasia Violet	3	Possible rot	Yes	Low
6	Fantasia Violet	5	Healthy	Yes	Low
7	Fantasia Violet	2	Obvious rot	Yes	High
7	Fantasia Violet	2	Possible rot	Yes	Low

Table 3.6: Recovery of *P. carotovorum* from plug plants of cyclamen cv. Halios Flame Mix sampled 19 July 2011, immediately before potting for the integrated control trial

Plug plant treatment	Symptoms	Number of plants	*P. carotovorum* detected	Relative level
1. None	None	10	No	-
2. None	None	10	No	-
3. None	None	10	No	-
4. None	Possible	11	No	-
5. Nemasys immersion	None	10	Yes	Low
6. Nemasys immersion	None	10	Yes	Low

Table 3.7: Results of tests for *P. carotovorum*

Date collected	Sample type	Sub-sample	*P. carotovorum* detected	Count (cfu/ml)
16 Aug	Immersion tank	-	No	-
	Path water	-	Yes	44
23 Aug	Path water	1	Yes	6000
		2	Yes	1900
		3	Yes	1550
	Leaf surface water	1	Yes	4
		2	No	0
		3	No	0

Discussion

Infection in young plants

The detection of *P. carotovorum* in plug plants sampled on the day of potting suggests that plants were already infected when they arrived on the nursery, or became infected in the few days the plants remained in the trays or when they were immersed in water containing Nemasys. It is possible that the water used in the immersion treatment had been contaminated with *P. carotovorum* from a few plants with bacterial soft rot, during earlier treatment of plants for a commercial crop, and subsequently this water contaminated plants used in the experiment. However, a sample of water taken from the bottom of the immersion tank, albeit one week after the experiment was set up, tested negative for *P. carotovorum*. An alternative explanation is that many of the plants already contained latent infection by *P. carotovorum* at the time they were immersed in water, and that the thorough wetting of the plants, especially the crowns, triggered multiplication of the bacterium and development of soft rot symptoms. This latter explanation is supported by a) the detection of *P. carotovorum* in visibly healthy plug plants in a delivery from the same supplier a few weeks previously and

b) the speed and extent of soft rot development in plug plants immersed at potting – some plants collapsed within just 1 week.

Further work is required to investigate the effect of drenching plants with water on the development of bacterial soft rot. Earlier work in this project has recorded a significant effect of delivery batch on occurrence of the disease. It is possible that the duration of transport of plug plants from propagation nursery to production nursery, and the conditions experienced by plants during this period, affect occurrence of bacterial soft rot. Specifically, it is suggested that condensation may occur on plants during transport and this may increase occurrence of the disease. Discussion with propagators and growers indicated that plug plants from the continent can be on a lorry for 3-5 days, and that in most cases the temperature in the lorry is not controlled. Water will be transpired by plants and, combined with temperature changes in a lorry, this is likely to result in condensation on plants.

Treatment effects

There was no evidence from this experiment that any damage to plug plants caused by machine potting, compared with hand potting, was sufficient to increase the level of bacterial soft rot.

Work in Year 1 of this project indicated that Cuprokylt FL may possibly have some effect against *P. carotovorum*, and work elsewhere indicates that Serenade ASO (*Bacillus subtilis*) can reduce fire blight caused by *Erwinia amylovora*. None of the four treatments using these products in the current trial reduced the incidence of cyclamen bacterial soft rot compared with untreated plants, even though treatments were applied up to six times at weekly intervals from immediately after potting. It appears likely that plants were already infected internally with the bacterium at or soon after potting, and consequentially any beneficial effects of subsequent sprays to protect plants from further infection were masked by the uncontrolled initial infection.

Immersion of plug plants in water at potting increased the incidence of bacterial soft rot developing within 6 weeks by 15-fold; and spray treatment with copper increased the disease incidence 3-fold. Possible reasons for immersion in water increasing soft rot include: the growing medium around plants remained wet for longer after potting, and wetness is known to favour infection and multiplication by bacterial pathogens; plants became contaminated from infested water; immersion in water results in a physiological change in plants and influenced susceptibility to bacterial soft rot/ability to resist infection. The adverse effect of copper oxychloride sprays was surprising and the reason for this effect is unknown. Possibly the copper treatment damaged plants such that infection by *P. carotovorum* occurred more readily; however, no visible damage or adverse effect on plant growth was seen. Possibly plants sprayed with Cuprokylt FL remained wet for longer than other plants and this increased risk of infection and disease development.

The pattern of treatment effects clearly visible at 6 weeks after potting was still evident at 13 weeks, although differences between treatments were not statistically significant. The incidence of collapsed plants was much greater at 13 weeks (30-71% of plants) than at 6 weeks (0-30% of plants). It is possible that there was

secondary spread of infection between plants which reduced the initial treatment effects; spray treatments ceased at 5 weeks after potting and so are unlikely to have influenced disease development in the latter stages of the trial. The recovery of *P. carotovorum* from water on the leaves of cyclamen plants in the crop indicates a possibility of water splash of the bacterium between treatments. Although sciarid and shore flies have been implicated in the spread of some plant pathogens, there was no evidence from our limited tests that they spread *P. carotovorum*; further testing is required to determine more conclusively the possible role of insects in secondary spread of bacterial soft rot of cyclamen.

The effect of a plug-plant immersion treatment in this experiment contrasts with nursery observations in 2010, when bacterial soft rot development was greatest in untreated plants and least in plants that had been immersed in a suspension of Nemasys at the plug stage. Possibly the reason that immersion treatment did not increase bacterial soft rot in 2010 was because there was nil or a very low population of *P. carotovorum* on or in plug plants in 2010, and there was a greater level present in 2011, sufficient to multiply and cause disease when wetted by immersion in water.

In conclusion, this experiment on control of cyclamen bacterial soft rot and a similar experiment in Year 1 of the project, show that it is very difficult to influence development of the disease after potting with the products tested. Efforts to control the disease are probably better targeted at preventing infection of young plants, including treatments during propagation and the conditions for transport of young plants.

4. Evaluation of treatments for control of *Pseudomonas* leaf spot on Impatiens

Introduction

Bacterial leaf spot of Impatiens has been recently observed in commercial crops at damaging levels. Plants in multipacks showed leaf margin spots, blackening and collapse. This disease of Impatiens and New Guinea Impatiens is caused by *Pseudomonas* species, most commonly *Pseudomonas syringae.* Infection is often associated with hydrathodes (water pores) at the leaf margin. Initial symptoms are small brown spots (3-5 mm diameter) near the leaf edge. These enlarge and develop a grey-black, greasy appearance. Adjacent areas yellow and leaves die, generally without wilting. Severely affected plants may lose most of their leaves. *Pseudomonas* leaf spot on Impatiens is generally of minor importance unless conditions are wet. Drips in polytunnels from condensation and overhead watering late in the day are likely to exacerbate the problem.

A literature review in year 1 identified a number of chemical treatments with reported protective value against bacterial infections in ornamentals. A glasshouse experiment was conducted in year 3 to assess the effect of a number of the selected preventative spray treatments on artificial leaf infection of Impatiens seedlings following inoculation with *Pseudomonas syringae* pv*. syringae.*

Materials and methods

Crop culture

Seedlings of Impatiens were transplanted into 10 cm pots in trays on capillary matting. Plants were watered from below (i.e. into the capillary matting) with a liquid feed (75 mg/L nitrogen, 50 mg/L phosphate and 150 mg/L potassium). Plants were grown in a heated shaded glasshouse. Average temperatures were maintained at 25 °C over the duration of the experiment.

Bactericide applications

After 5 weeks growth and one week before inoculation, the following spray treatments were applied to upper and lower leaf surfaces from hand held atomisers until run-off until just before run-off:

1. Untreated (sprayed with sterile distilled water)
2. Copper oxychloride (0.5% Cuprokylt FL, Unicrop) foliar spray
3. Potassium phosphite (1% Farm-Fos 44) + Breakthru wetter foliar application
4. Fosetyl-Al (3.75 g per L Aliette 80WG, Certis) foliar spray
5. Azoxystrobin (1 ml per L Amistar, Syngenta) foliar spray
6. 10 mM methyl jasmonate (Sigma-Aldrich) foliar spray

7. Grapefruit seed extract (0.05% Biosept 33 SL, Cintamani Poland) foliar spray

The same treatments were also repeated one week after inoculation. Plants were treated in five randomised blocks, each with two plants per treatment; there were four inoculation points on four leaves of each plant.

Inoculation

Pseudomonas syringae pv. *syringae* (isolate Fera 962204) was cultured on nutrient dextrose agar for 48 h and colonies were suspended in sterile distilled water (SDW). The concentration of bacteria in the suspension was then diluted in SDW to 10^7 cfu per ml according to its optical density ($OD_{\lambda=650nm}$), using previously prepared calibration curves. Seedlings were inoculated after 6 weeks growth by spraying the bacterial suspension in SDW at 10^7 cells per ml onto upper and lower surfaces of all fully-emerged leaves until the leaves were wetted but before run-off (approximately 15 ml per plant). Negative controls were sprayed with SDW. To encourage infection, one prominent leaf per plant was pricked with a sterile hypodermic needle immediately after spraying to create 10 small wounds to facilitate entry of the bacteria. Plants were observed weekly for development of symptoms of bacterial leaf spot.

% inoculation sites at which symptoms developed were determined from 16 inoculation sites per plant averaged over 10 plants per treatment.

Results and discussion

Small leaf spots (up to 0.5 mm diameter) were observed from 2 weeks after inoculation in untreated controls, but were only associated with leaves which had been punctured after inoculation (Fig 4.1). Similar spots developed around wounded inoculation sites in all treatments but the frequency of development of symptoms in the 6 weeks after inoculation varied between treatments (Table 4.1). The incidence of leaf spots was significantly reduced ($P<0.001$) by Cuprokylt FL, Amistar, grapefruit seed extract and methyl jasmonate. Cuprokylt FL provided the highest level of protection, although disease development still occurred at more than half of the inoculation sites.

Fig 4.1. Bacterial leaf spot caused by *Pseudomonas syringae* pv. *syringae* on Impatiens showing symptoms spreading from initial points of inoculation.

Table 4.1: Development of *Pseudomonas* leaf spot disease at wounded sites on inoculated Impatiens leaves after different preventative treatments compared with development on untreated leaves.

Treatment	**Sites at which symptoms had developed 6 weeks after inoculation**	
	Mean %	Standard error
1. Cuprokylt FL	53.5	6.0
2. Farm-Fos 44	86.3	3.9
3. Aliette 80WG	81.3	4.5
4. Amistar	69.4	5.3
5. Methyl jasmonate	69.4	5.3
6. Biosept	71.9	5.1
7. Sterile distilled water	89.6	6.3
Significance (55 df)	<0.001	

5. Evaluation of chemical disinfectants for efficacy in reducing populations of *Pectobacterium, Xanthomonas* and *Pseudomonas* on four types of surface

Introduction

Due to the lack of available products for use on plants to control bacterial diseases, the use of chemical disinfectants to prevent outbreaks is a key component in disease management strategies. For example, chemical disinfectants can be used on glasshouse surfaces and equipment after a disease outbreak to reduce bacterial populations in the environment and thereby reduce the risk of infection when a new crop is introduced. Previous work has shown that the type of surface can influence disinfectant efficacy. The aim of this work was to evaluate seven disinfectants for their efficacy in reducing populations of *Pectobacterium, Xanthomonas* and *Pseudomonas* on four types of surface (Mypex matting, glass, concrete and aluminium).

Materials and methods

Seven approved disinfectants with distinct active ingredients were selected for laboratory efficacy experiments against the three bacterial pathogens *Pectobacterium carotovorum* (NCPPB 312), *Xanthomonas hortorum* pv. *hederae* (NCPPB 939) and *Pseudomonas syringae* pv. *syringae* (NCPPB 281). Activity was determined in Petri dish assays using the following products at their recommended rates:

- Sanprox P (peroxygen) at 1%
- Household bleach (approx 12% sodium hypochlorite) at 2%
- Virkon S (peroxygen) at 1%
- Hortisept (quaternary ammonium) at 0.8%
- Menno Florades (organic acids) at 1%
- Fam-30 (iodophor) at 0.8%
- Biosept (grapefruit seed extract) at 0.05%

Efficacy on different surfaces

Efficacy of these disinfectants was tested against all three organisms on four different surfaces (Mypex matting, glass, concrete and aluminium). Bacteria were collected by swabbing the surface of 24-hour cultures of Pectobacterium carotovorum (grown on nutrient agar), Pseudomonas syringae pv. syringae (grown on nutrient agar) and Xanthomonas hortorum pv. hederae (grown on nutrient dextrose agar) and evenly smearing each surface to deliver undiluted bacterial cells. Bacteria smeared onto the different surfaces were treated with each product applied to the surfaces from hand held atomisers until run-off at the recommended rate with surfaces allowed to air dry at room temperature (21 +/-3 C). Survival was measured either 30 min, 1 h or 24 h after treatment application. Bacteria were recovered using a sterile swab, dipped into sterile phosphate buffer (pH 7.2). After swabbing each surface, the same swab was used directly to

inoculate fresh plates of either nutrient agar (NA) A or nutrient dextrose (ND) agar. Plates were incubated for up to 5 days at 28 C and observed for bacterial growth.

Each treatment was replicated twice, with each replicate constituting a 5 cm^2 grid on the appropriate surface.

Agar plate test

High concentrations of bacteria (approximately 10^8 cfu/plate) were spread onto either nutrient agar or nutrient dextrose agar and disinfectants were applied to filter paper discs (6.2 mm diameter) at 3.5 µl/disc. Zones of inhibition were measured after 48 h incubation at 28ºC. Each product was tested at its recommended rate and at half, quarter and eighth rate. The mean of minimum and maximum zone of inhibition around each of 5 filter paper disks per treatment was calculated.

Results and discussion

Agar plate test

All the products were effective at the manufacturers' recommended rates against all three bacteria with the exception of Biosept on *Pseudomonas syringae (Appendix 2)*. When used at lower concentrations, Sanprox P and Virkon S were most potent (Table 5.1). Biosept and Menno-Florades were both less effective against *Pseudomonas syringae* than against *Pectobacterium carotovorum* or *Xanthomonas hortorum* at the dilute concentrations.

Sanprox P produced the largest inhibition zones of all the disinfectants tested against both *P. syringae* and *X. hortorum*. None of the products produced a large inhibition zone in growth of *P. carotovorum*.

Efficacy on different surfaces

Sanprox P and Virkon S were again the most effective, with no bacteria recovered from any of the surfaces (Table 5.2). Mypex-type matting and concrete were much more difficult to disinfect than glass and aluminium. This may be due to the porous nature of the surfaces of the matting and concrete, resulting in reduced contact of bacterium and disinfectant, or possibly to inactivation of the disinfectants by the matting and concrete, or from organic matter (e.g. dust) in their surfaces.

P. carotovorum and *X. hortorum* were notably more difficult to disinfect on matting and concrete than *Ps. hortorum*.

On the untreated glass and aluminium surfaces, no bacteria were recovered even after 0.5 hour exposure, suggesting that the bacteria rapidly die on these surfaces, likely due to air drying. On the concrete surface, all three bacterial species were recovered after 0.5 hour and *X. hortorum* was still viable after 24 hours. On the Mypex type matting surface, *P. carotovorum* and *X. hortorum* but not *Ps. syringae* were still alive after 0.5 hours.

Table 5.1: Activity of eight disinfectants against three plant pathogenic bacteria in an agar plate assay

Disinfectant and Dilution		**Bacterial growth (+) or inhibition (-)**		
		Pectobacterium carotovorum	***Pseudomonas syringae***	***Xanthomonas hortorum***
Bleach (2%)	1:1	-	-	-
	1:2	-	-	+
	1:4	-	-	+
	1:8	+	+	+
Biosept (0.8%)	1:1	-	+	-
	1:2	-	+	-
	1:4	-	+	-
	1:8	-	+	-
Fam-30 (0.8%)	1:1	-	-	-
	1:2	-	-	-
	1:4	-	-	+
	1:8	-	+	+
Hortisept (0.8%)	1:1	-	-	-
	1:2	-	-	-
	1:4	-	-	-
	1:8	+	+	+
Menno-Florades (1%)	1:1	-	-	-
	1:2	-	+	-
	1:4	-	+	-
	1:8	+	+	-
Sanprox P (1%)	1:1	-	-	-
	1:2	-	-	-
	1:4	-	-	-
	1:8	+	-	-
Virkon S (1%)	1:1	-	-	-
	1:2	-	-	-
	1:4	-	-	-
	1:8	-	-	-

Table 5.2: Activity of eight disinfectants against three plant pathogenic bacteria on glass (G), matting (M), concrete (C) and aluminium (A)

Disinfectant and exposure time (hours)		**Recovery of bacteria: + Yes; (+) trace; - No**											
		P. carotovorum				***Ps. syringae***				***X. hortorum***			
		G	**M**	**C**	**A**	**G**	**M**	**C**	**A**	**G**	**M**	**C**	**A**
Bleach	0.5	-	-	-	-	(+)	-	-	-	+	+	-	-
	1	-	-	-	-	-	-	-	-	-	+	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-
Biosept	0.5	-	+	+	-	-	-	+	-	-	+	+	-
	1	-	(+)	-	-	-	-	-	-	-	(+)	+	-
	24	-	-	-	-	-	-	-	-	-	(+)	+	-
Fam-30	0.5	-	+	+	-	-	-	+	-	-	+	+	-
	1	-	(+)	-	-	-	-	-	-	-	(+)	+	-
	24	-	-	-	-	-	-	-	-	-	(+)	+	-
Hortisept	0.5	-	+	+	-	-	-	-	-	-	+	+	-
	1	-	-	(+)	-	-	-	(+)	-	-	+	+	-
	24	-	-	-	-	-	-	-	-	-	+	+	-
Menno-Florades	0.5	-	+	+	-	-	-	+	-	-	+	+	-
	1	-	(+)	-	-	-	-	-	-	-	(+)	+	-
	24	-	-	-	-	-	-	-	-	-	(+)	+	-
Sanprox P	0.5	-	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-
Virkon S	0.5	-	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-	-
	24	-	-	-	-	-	-	-	-	-	-	-	-
Untreated	0.5	-	+	+	-	-	-	+	-	-	+	+	-
	1	-	(+)	-	-	-	-	-	-	-	(+)	+	-
	24	-	-	-	-	-	-	-	-	-	(+)	+	-

6. Investigation of leafy gall (*Rhodococcus fascians*) on Petunia

Introduction

Leafy gall caused by the bacterium *Rhodococcus fascians* causes growth abnormalities including production of an abnormal number of shoots, proliferation of buds in leaf axils at the stem base and misshapen, thickened leaves. Affected plants are unmarketable. In the UK it is an occasional problem most frequently found on Dendranthema, Pelargonium and Lathyrus, and less commonly on Antirrhinum, Dahlia, Dianthus, Petunia and Phlox. There are several outstanding questions about the disease, especially on how it spreads. Opportunity was taken to investigate some aspects of the disease on trailing petunia in summer 2010. Specifically, the aim was to determine if the causal bacterium was present on visibly healthy shoots taken as cuttings from plant affected in the crown; a possible indication of systemic infection. Additionally, shoots from visibly unaffected plants on the same bench as affected plants were also examined.

Materials and methods

Crop and sample details

Samples were taken from a commercial crop. Petunia plants were being grown in pots on a wire mesh bench and watered by hand from overhead. Symptoms were present on 10-20% of plants scattered throughout the crop, and appeared more common on some varieties than others. Samples consisted of 10 cuttings 2-4 cm in length taken from each plant. The base of cuttings was at least 5 cm from symptomatic tissue. Scalpel blades were heat sterilised between plants. A total of nine samples were collected:

1. Variety A, 10 cuttings from healthy plant
2. Variety A, 10 cuttings from plant with leafy gall
3. Variety A, 3 pieces of leafy gall tissue
4. Variety B, 10 cuttings from healthy plant
5. Variety B, 10 cuttings from plant with leafy gall
6. Variety B, 3 pieces of leafy gall tissue
7. Variety C. 10 cuttings from healthy plant
8. Variety C, 10 cuttings from plant with leafy gall
9. Variety C, 3 pieces of leafy gall tissue

Test for R. fascians

Samples were tested for *R. fascians* at Fera. The 10 cuttings or three pieces of leafy gall tissue from each plant were bulked to make a single sample. Tissues were macerated in phosphate buffer and the macerate was streaked on nutrient dextrose agar. Orange/yellow colonies with morphology expected of *R. fascians*

were identified according to fatty acid profile and 16S rRNA sequence comparisons with the reference type strain of *R. fascians* (NCPPB 3067).

Results and discussion

Both fatty acid analysis and 16S rRNA sequencing showed that isolates were closely related but not identical to the type strain of *R. fascians*. All isolates were pathogenic and caused leafy gall symptoms when inoculated onto healthy Petunia plants (Fig. 6.1). *R. fascian*s was not detected on cuttings taken from visually healthy (asymptomatic) plants but was detected in all samples taken from symptomatic tissue and in cuttings taken from healthy parts of 1 of 3 plants with leafy gall symptoms (Table 6.1). The results confirm that the disease can be spread by taking cuttings from infected plants. Further investigation, with larger sample numbers, will be required to assess the risk of taking cuttings from healthy plants maintained in the same environment as infected plants. Hebe but not lavender plants with suspected leafy gall symptoms, growing on the same nursery, were also found to be infected with the same pathogen strain.

Table 6.1: Occurrence of *R. fascians* in petunia samples – 2010

Variety	***R. fascians* confirmed:**		
	Cuttings from healthy plant	**Visibly healthy cuttings from leafy gall plant**	**Leafy gall tissue**
A	-	-	+
B	-	-	+
C	-	+	+

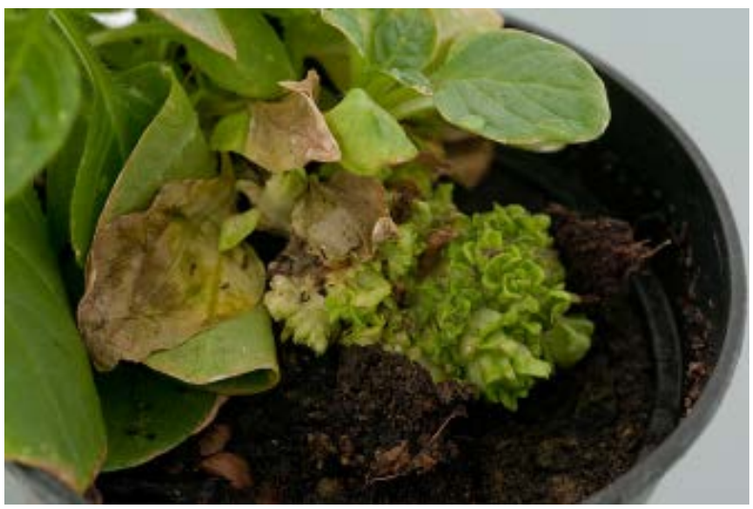

Fig. 6.1: Leafy gall symptoms induced after inoculation of healthy Petunia with isolates of *Rhodococcus fascians* from infected plants.

Project conclusions

Cyclamen bacterial soft rot (Pectobacterium carotovorum)

1. Cyclamen bacterial soft rot symptoms can be produced by drenching young plants with a suspension of *Pectobacterium carotovorum* over the crown; plant collapse first occurred in cv. Miracle White around 2 weeks after inoculation.
2. Nursery observations and associated isolation tests indicate that infection most commonly arises in the upper part of corms.
3. Individual batches of cyclamen supplied by different propagators and in different weeks, can differ greatly in the cumulative losses to bacterial soft rot by the time plants are ready for sale.
4. There is evidence to support the hypothesis that young cyclamen plants arriving on a nursery can sometimes be infected with latent *Pectobacterium carotovorum*, or become infected before potting. Occasionally, trays of plug plants can develop bacterial soft rot over a few days between receipt and potting.
5. Immersing trays of plug plants in water immediately pre-potting for just a few seconds can greatly increase occurrence of bacterial soft rot. Occurrence of bacterial soft rot in plug cyclamen plants at, or shortly after, receipt on a nursery may be triggered by condensation on plants related to long duration transport.
6. Nursery observations suggest that differences in incidence of bacterial soft rot that develop in different batches of cyclamen may also be associated with sciarid fly infestation and with control measures taken against this pest.
7. Bruising of young cyclamen corms greatly increases the potential for development of bacterial soft rots; there was no evidence that leaf removal on young plants increased the disease.
8. Spray applications of Cuprokylt FL (3 ml/L) and/or Serenade ASO (10 L/ha) applied weekly to cyclamen plants for up to 5 weeks for potting are unlikely to reduce bacterial soft rot in batches of plants where there is a high risk of the disease, as occurred in plants with probable latent infection and that were immersed in water pre-potting.
9. *P. carotovorum* can occur in puddles of water on a glasshouse floor and in water on cyclamen leaves, providing a potential source of infection for spread to healthy plants.

Ivy bacterial leaf spot (Xanthomonas hortorum pv. hederae)

1. Preventative application of Cuprokylt FL (5 ml/L) reduced bacterial leaf spot on ivy caused by *Xanthomonas hortorum* pv. *hederae.*

Impatiens bacterial leaf spot (Pseudomonas syringae pv. syringae)

1. A preventative application of Cuprokylt FL (5 ml/L) reduced bacterial leaf spot of Impatiens caused by *Pseudomonas syringae* pv. *syringae* by around 40%. Sprays of Amistar (1 ml/L), Biosept (0.5 ml/L) and methyl jasmonate (experimental treatment) slightly reduced the disease (by around 20%).

Chemical disinfectant activity against Pectobacterium, Xanthomonas and Pseudomonas pathovars

1. Type of surface influences disinfectant efficacy. Aluminium and glass surfaces were easier to disinfest from bacterial contamination than Mypex-type matting or concrete.
2. Sanprox P (hydrogen peroxide/peracetic acid) and Virkon S (peroxygen) were more effective than bleach (sodium hypochlorite), Hortisept (QAC), Menno-Florades (organic acid), Fam-30 (iodophor) and Biosept (grapefruit seed extract) at disinfesting Mypex-type matting and concrete of *P. carotovorum* and *X. hortorum* at the rates used in our work.
3. *Xanthomonas hortorum* pv. *hederae* was more difficult to disinfest on Mypex-type matting and concrete than *P. carotovorum* or *Pseudomonas syringae*, surviving exposure to several disinfectants for 24 hours.
4. All of the products detailed above were fully effective against all three bacteria, when used at the full recommended rate, in agar plate tests, with the exception of Biosept on *Pseudomonas syringae*.

Leafy gall (Rhodococcus fascians)

1. Symptomless systemic infection of Petunia by *Rhodococcus fascians* can occur; we found the bacterium in visibly healthy shoots on plants affected by leafy gall.

Technology transfer

<u>Project meetings</u>

Fera, York – 26 January 2009

Fera, York – 2 June 2009

Spalding – 2 July 2009

Spalding – 1 October 2009

Spalding – 14 December 2009

ADAS Boxworth – 24 August 2010

Spalding – 25 November 2010

Spalding – 18 October 2011

Articles

O'Neill, T. M. (2011). Taking a hard line against soft rot. *HDC News* 174:13-14.

O'Neill, T. M. and Elphinstone J (2010). Closing in on bacterial diseases. *HDC News* 163:20-21.

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

Presentations

Current problems and recent progress in cyclamen disease control. HDC Cyclamen Conference, Stratford on Avon, 19 October 2010 (Tim O'Neill)

Control options for bacterial disease control in pot plants. HDC/BPOA Poinsettia Meeting, Wellesbourne, 17 January 2012 (Tim O'Neill)

Factsheet

Bacterial diseases of protected ornamentals. Tim O'Neill, John Elphinstone and Andy Aspin.

Appendix 1: Cyclamen trial diary – 2011

Date	Assessment
14th June 2011	Collected plug plant samples (Florensis delivery #2)
19th July 2011	Collected plug plant and compost samples. Potted up plugs Applied spray 1 (potting spray, P)
26th July 2011	Spray P + 1 week applied Assessment on number of healthy plants remaining completed Sciarids collected
2nd August 2011	Spray P + 2 weeks applied Assessment on number of healthy plants remaining completed Collected sciarids and shore flies
9th August 2011	Spray P + 3 weeks applied Assessment on number of healthy plants remaining done
16th August 2011	Spray P + 4 weeks applied Assessment on number of healthy plants remaining done Samples of glasshouse water from on the plants and on the glasshouse floor and water from drench tank taken.
23rd August 2011	Spray P + 5 weeks applied Assessment on number of healthy plants remaining done 3 samples of glasshouse water on the plants and 3 samples from the glasshouse floor taken.
30th August 2011	Assessment on growth done Assessment on number of healthy plants remaining done
8th September 2011	Assessment on number of healthy plants remaining done Trial spaced out
4th October 2011	Assessment on number of healthy plants remaining done
18th October 2011	Assessment on number of healthy plants remaining done

Appendix 2: Summary of agar plate test data (Objective 5).