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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

Occurrence of bacterial soft rot (*Pectobacterium carotovorum*) in cyclamen was found to be associated with particular batches of plants; incidence of the disease was greatest in batches where sciarid flies were present at potting and were not adequately controlled. Cuprokylt FL as a foliar spray significantly reduced bacterial leaf spot (*Xanthomonas hortorum pv. hederae*) on ivy.

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*.

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 the control of bacterial diseases on protected ornamentals.
- Sound data on the potential benefits of resistance inducers and phage therapy for the control of bacterial diseases on ornamentals.
- Potential benefits to growers of reduced losses through the use of biological or chemical intervention, subject to regulatory approval where required.

Summary of the project and main conclusions

Occurrence of P. carotovorum in young cyclamen plants

Little direct evidence was found to support the hypothesis that cyclamen bacterial soft rot arises from latent infection by *P. carotovorum* in plug plants supplied by specialist propagators. Out of 22 batches of cyclamen cv. Halios Flame Mix supplied in 2009 by five propagators between weeks 19 and 28 (mid-May to mid-July), no visible symptoms of soft rot were found at plant receipt and only one batch tested positive for the bacterium in a laboratory test on macerated young plants, where *P. carotovorum* was recovered at a low level from one of five sub-samples. This hypothesis will be re-examined in 2010 by visual examination and testing of a greater number of plants.

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

Around 100 cyclamen plants cv. Halios Flame Mix from each of the 22 batches supplied to a nursery by five propagators between weeks 19-28 were assessed for bacterial soft rot in a growing-on test. Plants were potted into 13 cm pots and assessed every 2 weeks up to marketing for bacterial soft rot; the disease was confirmed by examination of collapsed plants for corm soft rotting and smell typical of the disease. Collapsed plants were removed from the trial as they were assessed. Bacterial soft rot was first observed 8 weeks after

potting of the first delivery and at that time affected plants from just one propagator. Losses increased with time and eventually occurred in plants from four of the five suppliers (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 delivered and, after recognition of the problem, deliveries were all treated with Nemasys (*Steinernema kraussei*) for control of this pest, applied as a soak from week 23. It is suggested that 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 an influence on final losses to the disease and are being investigated in current work.

Table 1: Effect of propagator and delivery week on cumulative losses to bacterial soft rot in 22 batches of cyclamen cv. Halios Flame Mix grown on one nursery – 2009

Propagator	Number of plants with bacterial soft rot (of 96) by delivery week					Mean (%)
	19-20	21-22	23-24	25-26	27-28	
A	1	2	3	0	0	1.1
B	-	2	1	0	0	1.2
C	30	18	2	1	1	9.7
D	5	4	0	1	0	1.8
E	-	0	0	-	0	0
Mean (%)	9.2	6.0	1.4	0.4	0.2	

Nursery sources of P. carotovorum

Samples of irrigation water, slime from irrigation lines and sand from beneath capillary matting, taken from a nursery where bacterial soft rot was present, all tested negative for *P. carotovorum.*

Early symptoms of bacterial soft rot in cyclamen

In July and October 2009, samples of cyclamen plants with different suspect symptoms of bacterial soft rot were tested for *P. carotovorum* by laboratory tests. The bacterium was recovered from 2 out of 13 samples tested in July and from 11 out of 22 samples tested in

October. The most reliable early symptom of *P. carotovorum* infection of cyclamen was found to be a slimy, malodorous rot, usually originating in the upper part of the corm. Although petiole blackening, usually with associated wilting and yellowing of leaves, was found quite commonly in the crop, *P. carotovorum* was rarely recovered from the petiole or corm of plants with this symptom.

Evaluation of treatments for control of bacterial leaf spot of ivy

A literature review in year 1 identified a number of chemical treatments with reported protective value against bacterial infections in ornamentals. A greenhouse trial was established to assess the potential of five preventative treatments to control leaf spot on ivy caused by *Xanthomonas hortorum* pv. *hederae*. Foliar sprays of copper oxychloride as Cuprokylt FL at 0.5% significantly reduced the disease. Treatments with potassium phosphite (Farm-Fos 44), fosetyl-aluminium (3.75 g per L Aliette 80WG), azoxystrobin (1 ml per L Amistar) or 10 mM methyl jasmonate failed to reduce development of leaf spot in comparison with untreated controls. Two applications (before and after inoculation) with copper oxychloride (0.5% Cuprokylt FL) were more effective than a single preventative spray.

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 per 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 were unmarketable, others required more labour to remove affected leaves and product was also 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 be able to recognise symptoms of potentially damaging bacterial diseases.

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

3. 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 (e.g. treat plants with Nemasys).

4. A slimy soft rot of cyclamen corms, usually originating in the upper part, is a good indication of bacterial soft rot caused by *Pectobacterium carotovorum* (see Figure 1 below). Leaf petiole blackening is not a reliable indicator of the disease.

5. Foliar sprays of copper oxychloride (eg Cuprokylt FL) can significantly reduce bacterial leaf spot of ivy caused by *Xanthomonas hortorum* pv. *hederae.* Use of Cuprokylt FL on protected ornamentals is currently permitted under the Long Term Arrangements for Extension of Use of Pesticides; check the approval status before use on a crop and test spray a small batch of plants first before more widespread use.

Figure 1 Bacterial soft rot symptoms in cyclamen

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 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 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 warrants investigation in order to reduce losses and secure more reliable quality of this 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

Specific objectives in the second year were:

- Investigate sources of *P. carotovorum* entering a cyclamen crop, including young plants, water and beds where plants are stood (new objective);
- Evaluate some chemical and biological protective treatments in the laboratory against two bacterial species;
- Evaluate available chemical disinfectants for efficacy in reducing populations of *Pectobacterium, Xanthomonas* and *Pseudomonas* on four types of surface.

1. Sources of *Pectobacterium carotovorum* on a nursery

Introduction

Occurrence of bacterial soft rot of cyclamen in the UK varies greatly between nurseries and years. One possible reason for this is that the disease originates with young plants supplied to a nursery by specialist cyclamen propagators. Alternatively, or additionally, the causal bacterium may persist on a nursery in the water supply, or on surfaces on which plants are stood. The main aim of this work was to investigate young plants as possible sources of *P. carotovorum* on one production nursery with a history of the disease. Additionally, water, growing medium and sandbeds on the nursery were tested for the bacterium.

Materials and methods

Site and crop details

The work was done on a pot plant nursery in Lincolnshire in 2009. Plug plants of large-flowered cyclamen cv. Halios Flame Mix were potted into 13 cm pots. Plug plants were machine-potted within a few days of their arrival. The growing medium was a nursery-specified mix consisting of light peat (30%), black peat (30%), clay (10%) and soil (10%). The experiment was located in a new (2008) glasshouse on the site which had grown one crop of cyclamen previously. Plants were grown on the floor on plastic-backed capillary matting with perforated white top over a sandbed. Plants were watered from overhead by sprinklers for around 6 weeks and, after spacing, watering was by hand onto the matting between plants. The crop was grown unheated until 1 October and thereafter a minimum temperature of 14ºC was used. No fungicides or other treatments were applied to plants. Irrigation water (a mix of rainwater and Anglian water) was treated with chlorine dioxide at 1%. Rainwater was stored in a covered tank. Biosept (a plant stimulant containing grapefruit oil, natural plant extracts and other essential oils) at 0.05% was applied as a high volume spray once at 7 d after potting.

Treatments

1. Plug plants supplied by propagator A on 5 occasions between weeks 19-28
2. Plug plants supplied by propagator B on 5 occasions between weeks 19-28
3. Plug plants supplied by propagator C on 5 occasions between weeks 19-28
4. Plug plants supplied by propagator D on 5 occasions between weeks 19-28
5. Plug plants supplied by propagator E on 5 occasions between weeks 19-28

Each propagator supplied two trays of plants (around 150 plants) at each delivery date. A sample of 50 plants was directly tested for *P. carovotorum* and 96 plants were used in a growing-on test.

Sampling and testing of plug plants for *P. carotovorum*

For each delivery from each propagator, 50 plants were selected by pulling out every third plant from the plug trays. These were placed into new plastic bags, 10 plants per bag, labelled with propagator code and delivery week, and posted to Fera within 24 h.

After washing off loose growing medium, the roots and corm of each bulk of 10 plants were macerated in phosphate buffer, solids were allowed to settle and the supernatant liquid was plated onto a selective medium for pectolytic bacteria. Plates were examined for typical colonies after incubation for 2-5 days at 25ºC.

Other samples tested for *P. carotovorum*

Potted cyclamen from the nursery crops with suspected early symptoms of bacterial soft rot were collected on 2 July and 2 October 2009 and tested for *P. carotovorum* at Fera. Samples of nursery irrigation water, slime from within irrigation pipes and sand from beneath capillary matting on the floor were also tested using the method described above.

Experiment design and statistical analysis for growing-on test

The experiment was a fully randomised design with five replicates (delivery dates) of five treatments (plant suppliers). The target delivery dates were weeks 19, 21, 23, 25 and 27. Each plot consisted of 96 plants arranged initially in 12 adjacent trays of 8 pots, and after spacing as a square of 6 x 16 plants at 26 cm from pot centre to centre. Blocks were located in different parts of the glasshouse according to space available at the time. The exception was supplier D, where 87 plants were supplied at delivery 4 (week 25). Results were examined by regression analysis using the GLM.

Disease assessment

Plants were examined once every 2 weeks and the numbers of collapsed plants recorded; all collapsed plants were removed from the crop. Bacterial soft rot of the corm was diagnosed by its appearance and smell as described in Year 1.

Results

Occurrence of *P carotovorum* in young plants

No symptoms of bacterial soft rot were observed in the 22 batches of plants supplied by the five propagators at delivery onto the nursery (3 of the expected 25 batches were not

received). When tested at Fera, *P. carotovorum* was confirmed in just one sub-sample (10 plants) from one propagator at one delivery date (week 24) (Table 1.1).

Table 1.1 Detection of *P. carotovorum* in cyclamen plug plants, cv. Halios Flame Mix, from five propagators – 2009

Propagator	Delivery period (week number)				
	19-20	21-22	23-24	25-26	27-28
A	Nil	Nil	+	Nil	Nil
B	NS	Nil	Nil	Nil	Nil
C	NT	Nil	Nil	Nil	Nil
D	Nil	Nil	Nil	Nil	Nil
E	NS	Nil	Nil	NS	Nil

NS – no sample; NT – not tested; + *P carotovorum* confirmed.

Occurrence of bacterial soft rot in cyclamen (growing-on test)

Bacterial soft rot was first observed on 2 July 2009, at 8 weeks after potting of the first delivery, and affected plants from one of the five propagators (propagator C). Losses increased steadily with time and eventually occurred in plants from all the propagators except propagator E (Table 1.2). Cumulative losses up to the point of marketing were greatest in plants supplied by propagator C (9.7%), significantly greater ($P < 0.001$) than in batches from the other propagators, which ranged from nil to 1.2% (Table 1.3). When data for all propagators was examined by delivery week, the incidence of bacterial soft rot was greatest in the first delivery (9.2%), moderately high in the second delivery (6.0%), and low in the three remaining deliveries (0.2–1.4%; Table 1.4). Differences in disease incidence according to delivery week were statistically significant ($P < 0.001$).

Nursery records of losses of 13 cm cyclamen by propagator and delivery week were also examined (Table 1.5). The majority of losses were considered by nursery staff as likely due to bacterial soft rot, though some were considered due to fusarium wilt or drought. The greatest proportion of plants lost (4.3%) was from propagator C, consistent with the replicated trial results, although this value was only slightly greater than propagator B (3.9%) where a low incidence was recorded in the replicated trial.

Association of *P. carotovorum* with different symptoms

P. carotovorum was recovered from 2 out of 13 cyclamen collected on 2 July 2009 and from 11 out of 22 samples collected on 2 October 2009 (Tables 1.6 – 1.7). The most common symptom found on 2 July was plants with one or a few blackened petioles, usually of a lower leaf, sometimes extending into the leaf blade and with associated leaf yellowing and wilting.

Corm staining and rotting was also found in a few plants. *P. carotovorum* was recovered from one blackened petiole and one rotting corm. On 2 October 2009, the most common symptom was corm rotting and collapsed plants. Corm rot nearly always appeared to start at the top of corms, around the base of leaf petioles. *P. carotovorum* was recovered from 8 out of 11 plants tested with this symptom, and from one plant each with a rot of the corm top, red-brown staining in the corm and black staining in the corm (Table 1.7). A range of other bacteria were also recovered (see Table 1.7), none of which are reported to cause soft rotting in cyclamen. *Botrytis cinerea* and *Fusarium oxysporum* were also found associated with rotting corms, and the latter fungus with red staining in the corm. Samples were also tested for virus and none was found.

Recovery of *P. carotovorum* from sand, water and irrigation pipe slime

No *P. carotovorum* was recovered from samples of sand from beneath matting, irrigation water, or slime from irrigation pipes.

Table 1.2: Effect of propagator and delivery week on cumulative losses to bacterial soft rot in cyclamen cv. Halios Flame Mix – 2009

Propagator	**Number plants affected (of 96) in plants delivered in week[a]:**				
	19-20	**21-22**	**23-24**	**25-26**	**27-28**
A	1	2	3	0	0
B	NS[b]	2	1	0	0
C	30	18	2	1	1
D	5	4	0	1	0
E	NS	0	0	NS	0

[a] Final assessments at point of marketing, were week 37 (deliveries 19-20 and 21-22), week 39 (deliveries 23-24 and 25-26) and week 44 (delivery week 27-28).

[b] NS – no sample supplied.

Table 1.3: Effect of propagator on cumulative losses to bacterial soft rot in cyclamen cv. Halios Flame Mix – 2009

Propagator	**Mean % cyclamen affected (standard error)**
A	1.1 (0.4)
B	1.2 (0.7)
C	9.7 (1.3)
D	1.8 (0.6)
E	0.0 (0.0)
Significance (13 df)	<0.001

Table 1.4: Effect of delivery week on cumulative losses to bacterial soft rot of cyclamen cv. Halios Flame Mix – 2009

Delivery week	Mean % cyclamen affected (standard error)	Sciarid control measures
19-20	9.2	None
21-22	6.0	None/Drench
23-24	1.4	Drench/Soak
25-26	0.4	Soak
27-28	0.2	Soak
Significance (13 df)	<0.001	

Table 1.5: Nursery losses of 13 cm cyclamen (all varieties) to bacterial soft rot and other disease or damage, grouped according to propagator – 2009

Propagator	Number plants Grown	% losses to soft rot and other problems[a]
A	1,310	2.9
B	1,773	3.9
C	1,951	4.3
E	1,464	3.3

[a] Up to week 40. No plants were grown from propagator D.

Table 1.6: Recovery of *P. carotovorum* from cyclamen plants with various symptoms at trial site nursery – sampled 2 July 2009

Symptom type	Propagator	Part of plant isolated from	*Pectobacterium* recovered
Black petiole	C	Outer corm	Nil
Black petiole and leaf	C	Black petiole	Nil
		Healthy corm	Nil
Black petiole and corm	A	Corm	Nil
Black petiole, soft patches in corm	E	Soft corm	Nil
Top		Vascular discolouration	Nil
Black petiole, spongy black corm	C	Petiole leading edge	Nil
Black petiole, corm gone	C	Black petiole	Yes
Black petiole base, yellowing	C	Black petiole base	Nil
leaves, corm OK	C	Yellow leaf	Nil
Stain at corm top	E	Corm stain	Nil
Stain at corm base	C	Corm stain	Nil
Mottled corm, rotting	A	Rotting corm	Yes

Discussion

Factors associated with soft rot

Sciarid fly larvae were evident in some plants when received on the nursery, especially plants from propagator C at delivery week 19. Nemasys was applied for control of sciarid, as a drench in weeks 22-23, and a soak (plug trays were immersed in a Nemasys suspension for 15 minutes) for all subsequent deliveries. No control measure for sciarid was applied in weeks 19-21.

A relatively high level of bacterial soft rot occurred in plants which received no control for sciarid, a lower level in those which received a Nemasys drench and least in those which received a Nemasys soak (Table 1.4). This suggests that damage to petioles or corms caused by grazing from sciarid larvae may predispose young plants to bacterial soft rot. Alternative causes for the large differences in incidence of bacterial soft rot with different batches of plants cannot be excluded. This includes loss of leaves at mechanical planting, which may be greater according to plant size, or other factor. A wound site created by loss of a leaf, or damage to a leaf, would be much more susceptible to infection by *P. carotovorum* than intact tissue. Another possible factor is the length of the cropping cycle; plants received in weeks 19-21 were on the nursery for around 18 weeks while those received in weeks 27-28 were on the nursery for 16 weeks. Differences in duration of cropping on the nursery and duration of high temperatures experienced may influence the level of symptomatic bacterial soft rot. However, this would not account for differences in the incidence of bacterial soft rot between suppliers.

Sources of *P. carotovorum*

There was little evidence from this study that plug cyclamen plants received on a nursery were already infected with *P. carotovorum*. Further work will be done in 2010 to investigate the possible association of *P. carotovorum* with young cyclamen plants, including inspection of a large number of trays at receipt for suspicious symptoms.

There was no evidence for irrigation water or sand below capillary matting being sources of *P. carotovorum* on a nursery with a history of bacterial soft rot in cyclamen.

Early symptoms of bacterial soft rot

Blackening of leaf petioles and corm tissue does not appear to be an early symptom of bacterial soft rot. Separate work by ADAS on consultancy samples suggest this symptom is likely caused by *Cylindrocarpon destructans*, a disease of cyclamen currently recognized more in mainland Europe than in the UK. The association of *F. oxysporum* with red-brown

staining in corms is expected as this is a typical early symptom of fusarium wilt in cyclamen caused by *F. oxysporum* f. sp. *cyclaminis*. Occurrence of *B. cinerea* on rotting cyclamen is also expected as this is one of the most common fungal pathogens found on cyclamen, occurring both as a primary pathogen and after damage caused by other reasons, including fusarium wilt and bacterial soft rot. The recovery of *P. carotovorum* from cyclamen with slight or extensive soft rot of the corm is also expected as this is the classic symptom of cyclamen bacterial soft rot caused by *P. carotovorum*. The conclusion from these tests is that the most reliable early symptom of *P. carotovorum* infection of cyclamen is a slimy, malodorous rot of the corm, usually progressing from the top downwards.

Table 1.7: Recovery of bacteria and other microorganisms from cyclamen plants with various symptoms suggestive of bacterial soft rot at the trial site – sampled 2 October 2009.

Symptom type	**No samples Tested**	**Number of samples from which microorganism recovered**			
		Pectobacterium carotovorum	**Other bacteria[a]**	***Botrytis cinerea***	***Fusarium* sp.[b]**
Fully soft rotting corm	11	8	8	2	1
Soft rot at corm top	1	1	1	1	0
Dry brown/red corm stain	6	1	6	0	2
Dry black stain in corm top	2	1	2	0	1
Healthy/cracked corm	2	0	2	0	0

[a] Other bacteria were *Panoea agglomerans, Pseudomonas putida, Pseudomonas fluorescens* type, *Agrobacterium/Rhizobium* and uncharacterised enterobactereacea.

[b] Three of the four *Fusarium* isolates were *F. oxysporum*; the other was unidentified.

2. 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 (Section 1). 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 kraussei*) 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 effect of creating wound sites on the corm by removal of a leaf was investigated. It is commonly observed that leaf breakage occurs on plants potted by machine.

Materials and methods

Site and crop details

The experiment was 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

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 2.1).

Table 2.1: Treatments examined for their effect on development of cyclamen bacterial soft rot – 2010

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; a small petiole stub or scar was visible after leaf removal. Sciarid larvae (10 per plant) were placed onto the compost surface close to the corm immediately after potting. Plants were shaded with netting for 1 week afterwards. The sciarid larvae were reared in the entomology laboratory. All plants in treatments 1-4 and 5 and 7 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.

Inoculation with *P. carotovorum*

At one week after leaf removal and addition of sciarid fly larvae, plants were drench inoculated (100 ml/plant) with a suspension of *P. carotovorum* (6×10^6 cells/ml) in sterile distilled water (SDW) that was poured over the crown of plants. Control treatments were drenched with water alone.

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*.

Experiment design and data analysis

Treatments were arranged in randomized blocks with fourfold replication. There were 10 plants per plot. Results will be examined by ANOVA or another method agreed with the biometrician.

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. This experiment is continuing and results will be reported in the Final Report.

3. 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. 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

Details of the plants examined on receipt and after potting up are shown in Table 3.1. Trays of four additional colours examined at receipt were: Halios Deep Rose, Halios Flame Mix, Halios Lilac and Halios Magenta. These plants were not assessed after potting up. In total, 24 batches of 10 trays were assessed at receipt and after potting, and 12 batches at receipt only.

Table 3.1: Details of cyclamen plug plant examined for bacterial soft rot at arrival on a nursery – 2010

Propagator	Variety/colour	Delivery weeks	Number of trays examined at each delivery
A	1. Halios Bright Scarlet/Red	20, 22, 28	10
	2. Halios Light Rose	20, 22, 28	10
	3. Halios White compact	20, 22, 28	10
	4. Fantasia Purple	20, 22, 28	10
B	1. Halios Bright Scarlet/Red	24, 26, 28, 30	10
	2. Halios Light Rose	24, 26, 28, 30	10
	3. Halios White compact	24, 26, 28, 30	10
	4. Fantasia Purple	24, 26, 28, 30	10

Disease assessment

Each tray of 96 plants was carefully examined for symptoms of leaf rot and/or corm collapse. The incidence of affected plants per tray was determined, and samples of affected and unaffected plants from the same tray were sent to Fera to check for *P. carotovorum* by isolation tests.

One tray of plants (around 100) from each batch assessed was potted up and labeled 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

This experiment is currently in progress.

4. Evaluation of chemical protective treatments against *Xanthomonas hortorum* pv. *hederae* on ivy

Introduction

A literature review in year 1 identified a number of chemical treatments with reported protective value against bacterial infections in ornamentals. Attempts to evaluate selected treatments against infection of cyclamen by *Pectobacterium atrosepticum* in year 1 were unsuccessful since disease failed to develop on inoculated plants, even in untreated controls. A glasshouse experiment was conducted in year 2 to assess the effect of a number of the selected preventative spray treatments on leaf infection of *Hedera helix* cuttings following inoculation with *Xanthomonas hortorum* pv. *hederae.*

Materials and methods

Crop culture

Rooted cuttings of *Hedera helix*, transplanted into 10 cm pots, were placed 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 ranged from 16.1 to 31.0 °C (mean = 20.7 °C) over the duration of the experiment.

Bactericide applications

After 3 weeks growth and one week before inoculation, the following spray treatments were applied to upper and lower leaf surfaces 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

A second batch of plants were treated similarly but also received a second treatment one week after inoculation. Plants were treated in 4 randomised blocks, each with 5 plants per treatment.

Inoculation

Xanthomonas hortorum pv. *hederae* (NCPPB 939) was cultured on ND for 48 hrs 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. Plants were inoculated after 4 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. After inoculation plants were covered with polythene bags to maximize humidity for 1 week. Thereafter plastic bags were removed and plants were observed weekly for development of symptoms of ivy bacterial leaf spot.

Results

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. Similar spots developed around wounded areas in all treatments but the frequency of development of symptoms in the 8 weeks after inoculation varied between treatments (Table 4.1).

Table 4.1: Development of *Xanthomonas* leaf spot disease at wound sites on inoculated ivy leaves after different preventative treatments compared with development on untreated leaves.

Treatment	Mean % of wounded sites at which symptoms developed	
	Single application	Two applications
Cuprokylt FL	45.0	15.0
Farm-Fos 44	94.3	89.5
Aliette 80WG	96.3	86.0
Amistar	68.8	80.0
Methyl jasmonate	85.0	86.0
Sterile distilled water	100.0	100.0
Standard deviation:	28.32	30.74

Cuprokylt FL provided the highest level of protection with significant control of disease development, especially after 2 applications. None of the other treatments significantly reduced the development of disease. Furthermore, the majority of lesions which did develop on Cuprokylt FL treated leaves were generally smaller than those on untreated leaves or leaves treated with any of the other products (Fig. 3.1).

a) Cuprokylt (2 applications)

b) Aliette (1 application)

c) Untreated inoculated controls

d) Uninoculated controls

Fig 3.1: Upper and lower surface views of *Hedera helix* leaves with different degrees of infection by *Xanthomonas hortorum* pv. *hederae* in response to wounding, inoculation and preventative bactericide applications.

5. Evaluation of available 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:

- Jet-5 (peroxygen)
- Household bleach (sodium hypochlorite)
- Virkon S (peroxygen)
- Menno Ter Forte (quaternary ammonium)
- Menno Florades (organic acids)
- Fam-30 (iodophor)
- Biosept (grapefruit seed extract)

Results and discussion

Experiments are currently in progress to establish efficacy of these disinfectants against all three organisms on four different surfaces (Mypex matting, glass, concrete and aluminium). Bacteria smeared onto the different surfaces were treated with each product at the recommended rate and at half of this rate. Survival is being measured by swabbing the treated surfaces and culturing the bacteria on NA and ND media at either 30 mins, 1 hour or 24 hours after treatment. Completion of these experiments is expected by the end of September 2010.

Conclusions

Year 2

1. There is little direct evidence to support the hypothesis that young cyclamen plants arriving on a nursery are already infected with latent *Pectobacterium carotovorum*, cause of bacterial soft rot, or show symptoms of the disease.

2. Individual batches of cyclamen supplied by different propagators and in different weeks, can differ significantly in the cumulative losses to bacterial soft rot by the time plants are sold.

3. Nursery observations suggest that differences in incidence of bacterial soft rot that develop in different batches of cyclamen may be associated with sciarid fly infestation and control measures taken against this pest.

4. A slimy malodorous soft rot of cyclamen corms, is a good indication of infection by *P. carotovorum*. Leaf petiole blackening is not a good indication of the disease.

5. Cyclamen corm soft rotting caused by *P. carotovorum* appears to start in the upper part of the corm.

6. No evidence was found to support the hypothesis that irrigation water or sand below capillary matting were sources of *P. carotovorum* on a nursery with a history of bacterial soft rot in cyclamen.

7. Preventative applications of copper oxychloride (Cuprokylt FL) but not of four other bactericide treatments, successfully reduced wound infections by *Xanthomonas hortorum* pv. *hederae* causing leaf spot disease on *Hedera helix.*

Technology transfer

Project meetings

Spalding – 2 July 2009

Spalding – 1 October 2009

Spalding – 14 December 2009

Article

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

APPENDICES

Appendix 1. Trial diary

Monitoring *P. carotovorum* for understanding and control of bacterial soft rot in Cyclamen

Date	Action carried out
7/5/2009	Visited nursery for sampling and collection of week 19 cyclamen plugs. Samples from propagators 1 and 4 sent to FERA for analysis.
21/5/2009	Visited nursery for sampling and collection of week 20 cyclamen plugs from propagator 3, and week 21 plugs from propagators 1, 2, 4 and 5. Samples from all 5 propagators sent to FERA.
28/5/2009	Visited nursery for sampling and collection of week 22 cyclamen plugs. Samples from propagator 3 sent to FERA.
4/6/2009	Visited nursery for sampling and collection of week 22 cyclamen plugs from propagator 5, and week 23 plugs from propagators 2 and 4. Samples sent to FERA. Previous weeks plugs planted up, no disease seen at this stage.
11/6/2009	Visited nursery for sampling and collection of week 24 cyclamen plugs. Samples from propagators 1 and 3 sent to FERA. Previous weeks plugs planted up, no disease seen at this stage.
18/6/2009	Visited nursery for sampling and collection of week 25 cyclamen plugs. Samples from propagators 1, 2 and 4 sent to FERA. Previous weeks plugs planted up, no disease seen at this stage. Samples of capillary matting, sand from under the beds, and potting material from the machine also taken and sent to FERA for analysis.
25/6/2009	Visited nursery for sampling and collection of week 26 cyclamen plugs. Samples from propagator 3 sent to FERA. Sciarid flies arrive on this delivery and Nemasys is applied for control as soon as plants received.
2/7/2009	Visited nursery for sampling and collection of week 27 cyclamen plugs. Samples from propagators 1 and 4 sent to FERA for analysis. 1st disease seen in week 20 plants from propagator 3 and week 21 plants from propagator 4.
9/7/2009	Visited nursery for sampling and collection of week 28 cyclamen plugs. Samples from propagators 2, 3 and 5 sent to FERA. Block 1 plants (from weeks 19 and 20) spaced as per nursery practice and placed into randomised trial plan.
23/7/2009	Block 2 plants (from weeks 21 and 22) spaced as per nursery practice and placed into randomised trial plan. Trial assessed.
6/8/2009	Trial assessed, bacterial soft rot now increasing in blocks 1 and 2.

7/8/2009	Block 3 and 4 plants (from weeks 23, 24, 25 and 26) spaced as per nursery practice and placed into randomised trial plan. Block 4 spaced earlier than anticipated due to rapid growth of plants. Trial assessed.
13/8/2009	Block 5 plants (from weeks 27 and 28) spaced as per nursery practice and placed into randomised trial plan. Trial assessed. Numbers of plants showing symptoms of bacterial soft rot from propagator 3 have doubled over the last week in blocks 1 and 2.
27/8/2009	Trial assessed. Again numbers of plants showing symptoms of bacterial soft rot from propagator 3 have doubled over the last week in blocks 1 and 2.
10/9/2009	Assessed blocks 3, 4 and 5 of trial. Blocks 1 and 2 have reached point of sale.
24/9/2009	Assessed block 5 of trial. All other plants have reached point of sale or been sold.
8/10/2009	Assessed block 5 of trial.
26/10/2009	Assessed block 5 of trial.
30/10/2009	Final assessment of block 5. Trial ended and results summarised.