Project Title	Hardy nursery stock: manipulation of copper in irrigation water as a component of integrated crop protection
Project number:	HNS 142
Project leader:	John Atwood, ADAS Boxworth
Report:	Final report, October 2009
Key staff:	John Atwood – Overall Project Manager Tim O'Neill – Joint Project Leader (Plant Pathology) Erika Wedgwood, Plant Pathologist Chris Dyer – ADAS Statistician Olga Grant – East Malling Research
Location of project:	East Malling Research & Robin Tacchi Plants Ltd, Illington, Norfolk
Project coordinator:	John Adlam, Dove Associates, Dickleborough, Norfolk. Nick Dunn, Frank P Matthews, Tenbury Wells, Worcs
Date project commenced:	1 March 2006
Date completion due:	31 October 2009
Key words:	Bacillus, Carex Evergold, Chamaecyparis lawsoniana 'Chilworth Silver', compost tea, copper, Choisya 'Sundance', disease, electromagnetic, ioniser, Phytophthora cinnamomi, Hedera helix, Helleborus orientalis, Iris foetidissima, Prunus laurocerasus, Prunus lusitanica, Pseudomonas syringae, Pythium, Trichoderma, Vinca minor, Xanthomonas

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the Horticultural Development Council..

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.

John Atwood	
Senior Horticultural Consultant	
ADAS	

Signature Date

Report authorised by:

Dr T O' Neill Horticulture Research Manager ADAS

Signature Date

CONTENTS

Grower Summary	1
Headline	1
Background and expected deliverables	1
Summary of the project and main conclusions	2
Phytophthora root rot	2
Pythium root rot study	2
Bacterial leaf spot study	3
Moss and liverwort	4
Compatibility with biological control agents	4
Performance of the Aqua Hort unit and application	4
Financial benefits	5
Action points for growers	6
Science Section	8
Introduction	8
Materials and methods1	10
Trial layout1	10
Crop details1	10
Copper ioniser treatment1	11
Compost tea treatments 1	11
Other pesticide applications 1	11
Assessments1	12
Plant/disease assessments1	12
Liverwort assessments1	12
Compost tea viability 1	13
Copper in drainage water 1	13
Aquahort Copper ioniser performance1	14
Results and discussion 1	15
Phytophthora root rot1	15
Pythium root rot1	16
Pseudomonas leaf spot1	17
Liverwort control1	19
Compost tea viability2	20
Performance of the Aquahort unit2	21

Conclusions	22
Phytophthora root rot	22
Pythium root rot	22
Bacterial leaf spots	22
Moss and liverwort	23
Compatibility with biological control agents	23
Operational issues	23
Technology transfer	24
References	
Appendix 1. Nursery observation layout	25

GROWER SUMMARY

Headline

Adding copper to irrigation water (using The Aqua Hort system) reduced plant losses due to *Phytophthora* root rot in *Chamaecyparis lawsoniana* and *Prunus lusitanica*.

Background and expected deliverables

Control of root diseases caused by species of *Phytophthora* and *Pythium* continues to present problems for nursery stock growers particularly in the conifer and herbaceous perennial sectors. Bacterial leaf spots such as those caused by *Xanthomonas hederae* and *Pseudomonas syringae* are also a particular problem on *Hedera* and evergreen *Prunus* respectively, both major landscape lines. A nursery survey (HNS 71) also identified many other plants that are often affected by *Pseudomonas leaf spot diseases*, including the important lines *Philadelphus* and *Spiraea*. Since that study there have been an increasing number of reports from nurseries of bacterial leaf spot problems on a range of herbaceous crops. Follow-up work (HNS 91) did not identify a satisfactory control measure and control of bacterial leaf spots in nursery stock remains a problem. Individual nurseries have lost production in excess of £75,000 due to these diseases. The industry loss is estimated to be in excess of £1.5m per annum.

Water is the key means for spread of these diseases on a nursery. Dispersal of *Phytophthora* and *Pythium* species occurs by movement of zoospores in water, including films of water at the base of pots, water splash, or in recycled drainage water. Bacteria causing leaf spot diseases are spread by water splash and in films of water on leaf surfaces.

The regular addition of copper to irrigation water could substantially limit spread of *Phytophthora* and *Pythium* species and bacteria that cause leaf spots. With copper fungicide sprays, treatment is often applied infrequently and occasions are likely to occur when plants are wet and insufficient copper is present to achieve pathogen control. The use of electromagnetic water treatment with copper ions is claimed to enhance the activity of the copper (Goldsworthy *et al.*, 1999) and has been adopted by pot plant growers in Denmark for control of a range of root infecting fungi (Pedersen, 2003). The main experience so far has been with "ebb and flow" irrigation systems. At the start of this project there were only two installations, in Holland and Denmark, on nursery stock with overhead irrigation, however further installations have since been made in Holland and Germany. Recently, mini portable ionising water treatment units have become available, suitable for smaller scale growers, or treatment of specific crop batches (e.g. "Aqua-Hort Mini", Aqua-Perl Denmark

ApS). Larger, more costly, units are also available, including units with plate electrodes suitable for installation in reservoirs or storage tanks.

The expected deliverables from this work include:

- An evaluation of the Aqua Hort copper ioniser system for the control of *Phytophthora* and *Pythium* root rots and bacterial leaf spot, compared with fungicide and biological treatments, and the control of liverworts and moss.
- An assessment of the compatibility of the Aqua Hort copper ioniser system with biological control systems used in normal commercial practice.

Summary of the project and main conclusions

Phytophthora root rot

Three years of experiments were carried out. The first two years of experiments were carried out at East Malling Research using newly potted *Chamaecyparis lawsoniana* plants with infector plants irrigated with either plain water or electromagnetic copper (E Cu) treated water. Other treatments included a fungicidal control and a biological control (*Trichoderma*). For the final year, commercial blocks of plants were treated on a nursery in an unreplicated observation comparing E Cu treated irrigation with irrigation with plain water.

The E Cu water treatment was effective in reducing a high level (8%) of *Phytophthora* root rot in *Chamaecyparis lawsoniana* 'Elwoodii' in the year 1 experiments to 1.5%. Although these results could not be confirmed in the year 2 experiments, due to the failure of the disease to establish, results from the nursery observations in year 3 indicated a good level of control, this time in naturally infected *Prunus lusitanica* "Myrtifolia" reducing plant losses from 2.5% to 0.7%.

Pythium root rot

The first two years of experiments were carried out at East Malling Research using newly potted *Aquilegia* plants in year one and *Choisya* in year two. Infector plants were used and plots were irrigated with either plain water or E Cu treated water. Other treatments included a fungicidal control and a biological control (*Bacillus*). In the third year, commercial blocks of plants were treated on a nursery in an unreplicated observation comparing E Cu treated irrigation with irrigation with plain water

Results from the replicated experiments in year 1 were inconclusive because the infected *Aquilegia* test plants (year 1) also suffered copper phytotoxicity. In the year 2 study on *Choisya*, there was a small reduction in root death from both the E Cu and *Bacillus* treatments. However, the levels of disease were low and the differences were not significant. The most interesting results came from the unreplicated nursery observations in year 3 where a high level of *Pythium* infection in *Helleborus orientalis* was effectively controlled and plant quality improved. It should be noted that the E Cu treated plots also received more liquid feed than the control plots. This was necessary because the Aqua Hort ioniser required a minimum water EC (electrical conductivity) of at least 1000 μ S/m³. However all plants were freshly potted so differences in foliar nutrition are unlikely to have influenced quality at this stage.

Bacterial leaf spot

The first two years of experiments were carried out at East Malling Research using newly potted *Prunus laurocerasus* plants in year one and *Hedera helix* in year two. Infector plants of *Pseudomonas syringae* (for the *Prunus*) and *Xanthomonas hortum hederae* were used and plots were irrigated with either plain water or E Cu treated water. Other treatments included a copper fungicide control and a biological control (either compost tea or green waste mulch). For the final year, commercial blocks of *Prunus laurocerasus* cultivars were treated on a nursery in an unreplicated observation comparing E Cu treated irrigation with irrigation with plain water

Initial (year 1) work on *Prunus laurocerasus* indicated a slight reduction in *Pseudomonas* leaf spot in E Cu treated plants, however infection levels were low. Much higher infection levels were noted in the year 3 nursery observation and a good level of control was noted, reducing infection from up to 30% to 5% or less. The disease was not completely eradicated though. It should be noted that the E Cu treated plots also received more liquid feed than the control plots so some of the improvement in plant quality may have resulted from an improvement in nutrition. The overall control level was however much better than normally achieved on the nursery where control measures are based on 2 -3 weekly sprays of copper fungicide.

Xanthomonas leaf spot was present in the *Hedera* study in year 2 but at a relatively low level. Population numbers of *Xanthomonas* bacteria per leaf were quite variable, but showed some reduction where the E Cu treatment had been applied. The total number of leaf spots was reduced by 20% where the E Cu treatment was used, but these differences were not statistically significant.

Moss and liverwort

A natural infestation of liverwort started to develop in two plant batches in the year 3 nursery observation, moss was not present. The E Cu treatment appeared to delay the infestation, compared with the control. However with a heavy infestation pressure (as in the year 1 experiment) the E Cu treatment failed to give a significant control of moss or liverwort. In general, the experience of other nurseries is that liverwort and moss still remain a problem even when using E Cu irrigation. However, previous studies (HNS 93c) have shown copper to have some efficacy in control of liverwort. Therefore whilst E Cu irrigation might help to control liverwort it would appear that other measures need to be taken as part of an integrated control strategy.

Compatibility with biological control agents

Biological control treatments were included in the experiments. This included *Trichoderma* for *Phytophthora* control, *Bacillus* for *Pythium* control and compost tea or green waste mulches for bacterial control.

Compatibility with microbial and invertebrate biological control agents was quite good with the E Cu treatment. When the growing media was analysed there was no significant reduction in microbial populations of *Trichoderma* and compost tea microbes, but a slight (not statistically significant) reduction in *Bacillus* populations. It was not possible to test the effect on functionality of the microbial biological control agents because they were not significantly effective in the control of any of the target diseases. There were no adverse effects noted on *Phytoseilus persimilis* mites in the nursery observation and control of two spotted spider mite was unaffected.

Performance of the Aqua Hort unit and application

The main problem with the Aqua Hort unit has been the copper ion output being lower than the target set on the equipment and the need to ensure adequate conductivity in the water supply for the equipment to work effectively. This was achieved through supplementing the water supply with liquid feed.

Two sets of equipment were used during the course of this study. In year 1 a small portable unit, the AquaHort Mini was used. This unit achieved 2 ppm Cu when set at 3 ppm. In years 2 and 3 a larger capacity 4 rod electrode unit was used. The larger capacity unit did not seem to be so well suited to small plot work and in year 2 output averaged 1 ppm compared with a (lower) target of 2 ppm. However the same unit gave a higher output 1.8 –

2.4 ppm when used under commercial conditions in year 3 with the water supply supplemented with liquid feed to give a conductivity of around 1600 μ S/m³. The manufacturers recommend an EC of > 1000 μ S/m³ for this type of unit.

It is a significant disadvantage, where good quality low EC water is available, to have to add liquid feed merely to ensure operation of the ionizer. However this issue is being addressed by the development of a plate electrode model which is designed to float on the irrigation reservoir (Figures 1 and 2). The manufacturers advise that these models are suitable for operation in low EC water.

Analysis of drainage water from a nursery site where the Aqua Hort system has been operational for 2 years showed that there was a small accumulation (0.19 ppm) of copper in the water from capillary sandbeds and only a minimal level (0.01 ppm) in drainage water from overhead irrigated beds.

In conclusion, the Aqua Hort system has good potential for control of *Phytophthora* and *Pythium* root rots when used with overhead irrigation. Control of *Psudomonas* leaf spot also appeared good but these results were solely from an unreplicated observation.

The Aqua-Hort system did not affect the establishment of *Trichoderma* when used as a control agent for *Phytophthora* control but caused a slight reduction in *Bacillus* when used for *Pythium* control. Analysis of compost tea microbes did not reveal any loss of fungal or bacterial populations following treatment, but this was from a single sample.

There are some issues concerning the lower than target copper ion output of the Aqua Hort units which should be considered before the smaller (rod electrode) units can be fully recommended, particularly where water supplies have a low electrical conductivity (EC). In practice it would appear that continental nurseries are now opting for the higher output plate electrode models which are effective at a range of water ECs.

Financial benefits

The Aqua Hort unit proved effective for the control of *Phytophthora* root rot in *Chamaecyparis lawsoniana* 'Elwoodii' and *Prunus lusitanica* "Myrtifolia".

• It is known that some nurseries have lost in excess of £75,000 of stock from *Phytophthora* infection in a year.

- With a purchase price of around £6,000 for a 10 m³/hr unit suitable for a medium to large nursery, and assuming the cost is written off over 10 years @ 6%, plus running costs, the annual cost to the business is approximately £1,300.
- There are likely to be many nurseries who lose in excess of £1,300 stock to *Phytophthora* alone each year.
- The plate electrode units are £1,900 more expensive than the equivalent capacity rod electrode models but will work in low EC water.

Further benefits could accrue from the use of the Aqua Hort unit for control of *Pythium* root rot and bacterial leaf spots. However the evidence is less secure, derived largely from the unreplicated nursery observation so no financial benefits are estimated at this stage.

Action points for growers

- The Aqua Hort system shows potential for control of *Phytophthora* and *Pythium* root rots in hardy nursery stock, when used with overhead irrigation, and would be cost effective where nursery losses to these diseases amount to > £1300 per year.
- *Pseudomonas* leaf spot appeared to be well controlled in unreplicated observations.
- Some care should be taken with use on herbaceous plants as *Aquilegia* suffered copper toxicity damage to the foliage.
- The system appears to be compatible with the use of *Trichoderma*, compost tea and invertebrate biological control agents such as *Phytoseiulus*, but caused a small reduction in activity of *Bacillus*.
- There was no build up of copper in the drainage water following 2 years of overhead irrigation on plastic ground cover beds but a small accumulation in capillary beds.
- Sites with low EC water will have to add liquid feed to increase the EC to over 1000 μ S/m³, which is environmentally undesirable, or use the more expensive plate electrode models (Figures 1-3)



Figure 1. Plate electrode unit.



Figure 2. Plate electrodes in unit

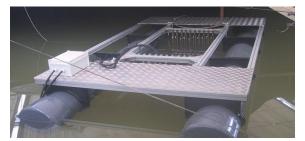


Figure 3. Floating plate electrode unit for use in large reservoirs or storage tanks.

SCIENCE SECTION

Introduction

Control of root diseases caused by species of *Phytophthora* and *Pythium* continues to present problems for nursery stock growers particularly in the conifer and herbaceous perennial sectors. Bacterial leaf spots such as those caused by *Xanthomonas hortum hederae* and *Pseudomonas syringae* are also a particular problem on *Hedera* and evergreen *Prunus* respectively, both major landscape lines. A nursery survey (HNS 71) also identified many other plants that are often affected by *Pseudomonas leaf spot diseases* including *Philadelphus* and *Spiraea*; both are important lines. Since that study there have been an increasing number of reports from nurseries of bacterial leaf spot problems on a range of herbaceous crops. Follow-up work (HNS 91) did not identify a satisfactory control measure and control of bacterial leaf spots in nursery stock remains a problem. Individual nurseries have lost production in excess of £75,000 due to these diseases. The industry loss is estimated to be in excess of £1.5m per annum.

Water is the key means for spread of these diseases on a nursery. Dispersal of *Phytophthora* and *Pythium* species occurs by movement of zoospores in water, including films of water at the base of pots, water splash, or in recycled drainage water. Bacteria causing leaf spot diseases are spread by water splash and in films of water on leaf surfaces.

The regular addition of copper to irrigation water could substantially limit spread of *Phytophthora* and *Pythium* species and bacteria that cause leaf spots. With copper fungicide sprays, treatment is often applied infrequently and occasions are likely to occur when plants are wet and insufficient copper is present to achieve pathogen control. The use of electromagnetic water treatment with copper ions is claimed to enhance the activity of the copper (Goldsworthy *et al.*, 1999) and has been adopted by pot plant growers in Denmark for control of a range of root infecting fungi (Pedersen, 2003). The main experience so far has been with "ebb and flow" irrigation systems. At the start of this project there were only two installations, in Holland and Denmark, on nursery stock with overhead irrigation, however further installations have since been made in Holland and Germany. Recently, mini portable ionising water treatment units have become available, suitable for smaller scale growers, or treatment of specific crop batches (e.g. "Aqua-Hort Mini", Aqua-Perl Denmark ApS). Larger, more costly units are available including units with plate electrodes suitable for installation in reservoirs or storage tanks.

The commercial objective of this work is to evaluate a simple water treatment system compatible with current practice that results in improved control of *Pythium* and *Phytophthora* root rots, bacterial leaf spots and liverworts and moss.

The first two years of the project were carried out at East Malling Research with replicated experiments carried out on small plots in polytunnels irrigated with either plain water or electromagnetic water (E Cu). The crop/disease problems investigated in year 1 were *Phytophthora* root rot in *Chamaecyparis, Pythium* root rot in *Aquilegia, Pseudomonas* leaf spot in *Prunus*, and moss and liverwort control. In each case treatments were compared with an antagonistic fungal or bacterial biological control. In year 2, crop/disease problems investigated were *Phytophthora* root rot on *Chamaecyparis* again, *Pythium* root rot on *Choisya* and *Xanthomonas* leaf spot on *Hedera*. In these studies the E Cu treatments were again compared with the biological treatment, but in addition the biological treatment was combined with E Cu water treatment and checks were made on the effect of E Cu water on the viability of the biological treatment.

The results of the year 1 and 2 studies indicated that the E Cu treatment reduced *Phytophthora* infection in *Chamaecyparis*, slightly reduced root rotting due to *Pythium* in *Choisya* and reduced *Xanthomonas* leaf spot populations on leaves of *Hedera*.

For the final year of the project the equipment was moved to a nursery for testing under commercial conditions on a range of crops. For this part of the project, E Cu treated and untreated blocks were compared in an unreplicated observation. The main crop/disease problems observed were *Phytophthora* root rot and *Pseudomonas* leaf spot on *Prunus*, and *Pythium* root rot on *Helleborus*. For selected plant batches, a compost tea biological treatment was superimposed on E Cu treated and untreated areas to check for incompatibility. Biological pest control agents were introduced in both treated and untreated areas where pest infestation was noted to check for possible adverse effects of the E Cu treatment.

Materials and methods

In the third year of this project, observations were made at Robin Tacchi Plants, Illington, Norfolk to determine the effect of E Cu water treatment on a range of commercial nursery stock species.

Trial layout

The treated areas comprised one $(10 \times 75m)$ bay of a multibay polytunnel and two (each 10 x 75m) outside mypex covered standing beds. The control (untreated) area was an adjacent (10 x 75m) bay of the multibay polytunnel and an adjacent (10 x 75m) outside mypex covered standing bed (see Appendix 1 for layout)

A wide range of crops were grown in the polytunnel and a range of cultivars of evergreen *Prunus* were grown on the outside beds. The main crops that were monitored are shown below (Table 1).

Crop details

<u>Polytunnel</u>	No. of	Pot	Potting date
	plants	size	-
Aucuba japonica 'Crotonifolia'	100	3L	16/5/09
Carex 'Evergold'	1000+	2L	22/9/08
Choisya 'Sundance'	100	3L	16/5/09
Hedera helix 'Green Ripple'	100	3L	16/5/09
Helleborus orientalis	400	2L	7/8/08
Iris foetidissima	1000+	2L	2/7/09
Lavandula 'Blue Star'	100	3L	30/6/09
Osmanthus burkwoodii	1000+	3L	2/10/08
Prunus laurocerasus 'Interlo'	100	3L	16/5/09
Prunus lusitanica 'Myrtifolia'	1000+	3L	19/6/09
Viburnum tinus	100	3L	16/5/09
Vinca minor	100	3L	16/5/09
<u>Outside</u>			
Prunus laurocerasus 'Cherry Brandy'	1000+	2 & 3L	26/6/07-12/12/08
Prunus laurocerasus 'Etna'	1000+	3L	Not known
Prunus laurocerasus 'Otto Luyken'	1000+	3L	11/2/07-13/12/08
Prunus laurocerasus 'Piri'	1000+	3L	25/5/07-25/6/07
Prunus laurocerasus 'Rotundifolia'	1000+	3L	25/6/07-12/5/08
Prunus laurocerasus 'Zabelliana'	1000+	2 & 3L	20/4/07-12/5/08

Table 1. Crops monitored in the nursery observation

The crops of *Osmanthus, Prunus, Carex* and *Iris* were large commercial batches (1000+ plants) spread across treated and untreated areas. The other species were small batches with 50 plants each in treated and untreated area.

All plants were Robin Tacchi Plants' production stock and had been potted into a growing media comprising:

Peat	85%
Bark	15%
Lime	To adjust to pH5
Sincrocell 12 (14:8:13) CRF	4.0 kg/m ³
Multimix (12:14:24) compound	0.5 kg/m ³
Imidasect 5GR (imidacloprid 5%)	0.28 kg/m ³

Copper ioniser treatment

The AquaHort copper ioniser was installed at Robin Tacchi Nurseries on 24 March 2009. The ioniser was initially set at 1ppm, increased to 2 ppm on 20 April 2009 and increased further to 3 ppm on 7 May 2009. It remained set at 3 ppm for the duration of the trial. The conductivity of the water supply was 553 uS/m³ which was too low for efficient operation of the ioniser. It was decided to increase the conductivity of the water by adding a liquid feed using Universol 4.1.2 (N 23, 6 P_2O_5 , 10 K_2O) (Scotts Co.) 12.5 kg per 200 L stock diluted 2%. The dilution was reduced to 1.5% on 8 June 2009 since it appeared to function sufficiently well at the lower EC. From 1 September 2009, potassium nitrate (N 13, 46 K_2O) 12.5 kg per 200 L stock diluted 1.5% was used. The liquid feed was applied continuously with every irrigation. The untreated control areas also received a similar liquid feed. However in these areas feed was applied only once a week but at 2%.

Compost tea treatments

Half of the *Carex* 'Evergold', *Iris foetidissima* and *Prunus Iusitanica* 'Myrtifolia' blocks also received regular applications of compost tea. Compost tea was prepared using a Microfarming Xtractor 100 Litre brewer using the Microfarming fungal dominant compost package. Mains water was warmed to glasshouse temperature, $15-20^{\circ}$ C. The brewer was run for 1 hr to remove chlorine and bring oxygen levels up to 6 ppm. A half of the additive package was mixed in warm water then added to 50 litres water in the brewer with 5 ml sunflower oil to prevent foaming. The brewer was run for 18 hours with the lid off. After brewing the solution it was allowed to settle for 10 minutes then filtered before use, applied at 60 L / 240 L to fully wet the foliage just to the point of run off every 14 days. From 14 July 2009 onwards a quarter strength brew was made and applied at 100 L / 240 L. Applications were made on 12 and 26 June, 11 July, 4, 19 and 28 August, 11 and 25 September 2009.

Other pesticide applications

Fungex (copper ammonium carbonate 8% w/w) was applied routinely to all *Prunus laurocerasus* crops at 5 ml / L to half of the untreated outside bed and to the untreated

tunnel bays. E Cu irrigated areas were **not** treated. Applications were made every 3 weeks from May. No other pesticide applications were made.

Assessments

Plant/disease assessments

Prunus lusitanica 'Myrtifolia' - Phytophthora root rot

Plants showing wilting and or severe foliage browning were counted in the treated and untreated areas on 16 July, 6 August and 28 September 2009 and the percentage with symptoms calculated. Between assessments dead plants were removed by nursery staff. On 6 August 2009 samples of root tissue were tested using a lateral flow device (Pocket Diagnostics, York).

Helleborus orientalis - Pythium root rot

Plants showing wilting and or severe foliage browning were counted in the treated and untreated areas on 28 September 2009 and the percentage with symptoms calculated. On 29 September 2009 samples of root tissue were tested using a lateral flow device (Pocket Diagnostics, York).

Prunus laurocerasus cultivars - Pseudomonas leaf spot

An overall assessment of percentage leaf area (including margins) spotted or dropped out was made for each batch of plants. To aid assessments a quadrat was placed over the crop and percentage leaf loss estimated. The plants were fairly open with most of the leaf surface visible from above, no attempt was made to look under the canopy. Assessments were made in both E Cu treated and untreated areas. For the E Cu untreated area separate assessments were made in the half of the bed treated with Fungex fungicide and the half completely untreated.

Samples were taken from *Prunus laurocerasus* 'Otto Luyken' in the untreated area on 28 September 2009 for confirmation of the identity of the leaf spotting bacteria. Samples were sent to FERA York for identification initially by fatty acid profile analysis and fluorescent *Pseudomonas* LOPAT testing. Identification of the pathovar was made by genetic sequencing.

Liverwort assessments

Assessments of percentage of pot covered by liverwort were made in batches of *Osmanthus burkwoodii* and *Prunus lusitanica* 'Myrtifolia' on 16 July 2009. Assessments were made by

taking a random line of 10 pots from the edge of each block (E Cu treated and untreated) and assessing the percentage cover in each pot.

On 6 August 2009 a batch of *Iris foetidissima* was assessed for the percentage of the pot covered by liverwort. An overall estimation was made for the treated and untreated areas.

Biological control of pests

To assess the compatibility of E Cu with a biological control agent, introductions of *Phytoseilus persimilis* (Phytosure BCP Ltd) were made to control a natural infestation of two spotted spider mite *Tetranychus urticae* on *Vinca major*. Introductions were made on 12 and 26 June, 14 and 28 July and 11 August 2009 using 3 mites per m².

Assessments were made on 16 July, 6 August and 28 September 2009, by inspecting 10 leaves showing symptoms of infestation randomly selected from plants in E Cu treated and E Cu untreated areas, counting the number of mites and eggs.

Compost tea viability

Samples of growing media (1.5 L) were taken from the *Iris foetidissima* blocks treated with compost tea, one sample from the E Cu treated area and one sample from the E Cu untreated area to check for microbial activity in the growing media. Samples were analysed for bacterial and fungal counts (Compost Foodweb Analysis, Laverstoke Park Farm, Hampshire).

Copper in drainage water

In order to check for a possible accumulation of copper in the standing beds and drainage water it was decided to test runoff water at a site where an AquaHort installation had been in operation for 2 years rather than at the experimental site which was only partly treated and had only run for 6 months. Drainage water was collected from two areas - overhead irrigated mypex beds and capillary sandbeds - at Golden Grove Nurseries Lincolnshire, and tested for copper in the water. Samples were collected on 20 July 2009 after light rainfall.

Aquahort Copper ioniser performance

Copper levels in the output from the copper ioniser were checked on 4 and 9 June, 16 July and 28 September 2009 using a hand test kit (VWR Aquaquant MERC1.14414.0001Copper Test Method). Further samples were taken for laboratory analysis at NRM Ltd, Bracknell.

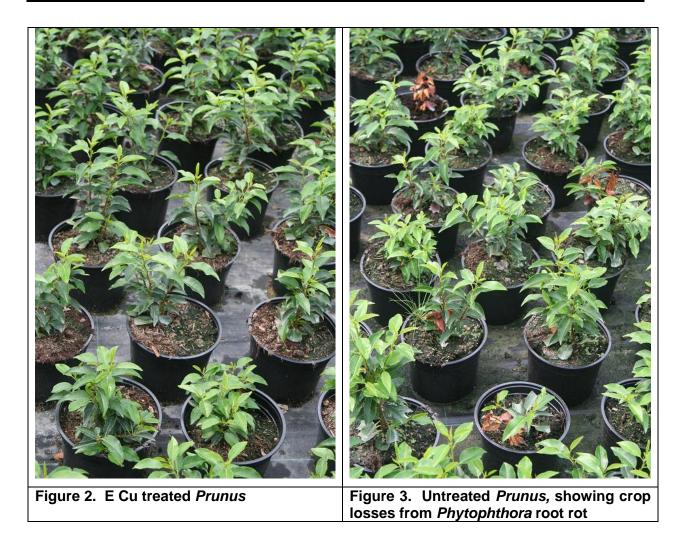
Results and discussion

Phytophthora root rot

The percentage of *Prunus lusitanica* 'Myrtifolia' showing symptoms of *Phytophthora* root rot was reduced by about two thirds in the E Cu treated area (Table 2, Fig. 3). The disease continued to infect plants at a low level throughout the summer. These results back up the findings of the year one experiment where treatment reduced infection in *Chamaecyparis lawsoniana* 'Elwoodii' from 8% to 1.5% infection.

Table 2. Percentage of *Prunus lusitanica* 'Myrtifolia' showing symptoms of *Phytophthora* root rot

Treatment	Date		
	16 July 2009	6 August 2009	28 September 2009
ECu treated	0	0.7	0.5
Untreated	0	2.5	1.3



Pythium root rot

Helleborus orientalis plants in the E Cu treatment area remained clear of *Pythium* root rot symptoms whereas plants in the untreated area had 6 per cent with severe symptoms (Table 3). In addition, plants in the treated area were much healthier looking with stronger growth (Fig. 4) compared with the untreated area (Fig. 5) and much better root development (Fig 6).

Table 3. Percentage of Helleborus orientalis showing symptoms of Pythium root rotrecorded 28 September 2009

	% of plants with symptoms
ECu treated	0
Untreated	6

Results in the year 1 experiment on *Pythium* root rot were inconclusive because of copper phytotoxicity on the test plants *Aquilegia*. There were however indications in the year 2 experiments that E Cu treatment slightly reduced root rotting due to *Pythium* in *Choisya*.

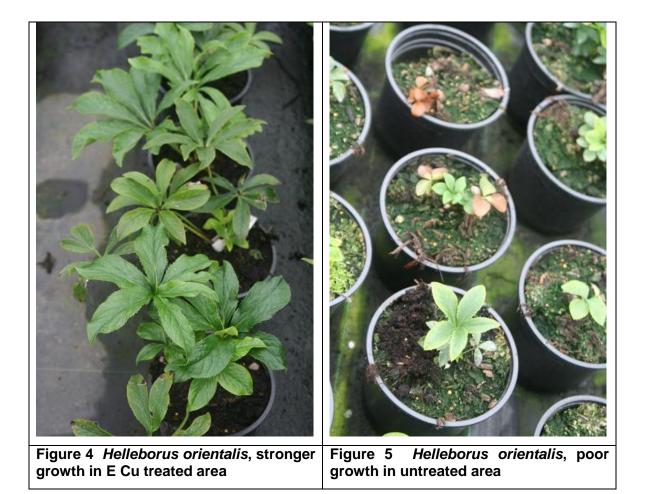




Figure 6Helleborus orientalis, goodFigure 7Helleborus orientalis, poorroot growth in E Cu treated arearoot growth in untreated area

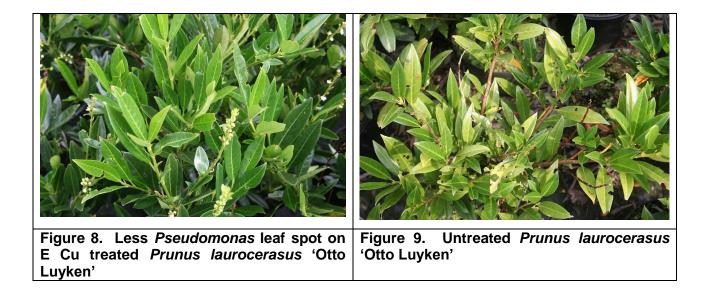
Pseudomonas and Xanthomonas leaf spots

The *Prunus laurocerasus* plants were much more heavily infected with *Pseudomonas* leaf spot (Fig. 9) than in the original experiment in year 1 where there was insufficient disease to test the system. Disease levels of up to 30% leaf area were recorded on 6 August 2009 in the worst batches in the untreated area, whereas the worst batch in the E Cu treated area was 5% infected (Table 4). Although there was some benefit from using the copper fungicide, the E Cu treatment was more effective. Analysis of a sample taken from leaf spots of the untreated *Prunus laurocerasus* 'Otto Luyken' confirmed infection by *Pseudomonas syringae* phylogroup 2 (FERA York). No *Xanthomonas* leaf spot occurred on the *Hedera helix* 'Green Ripple' in any of the beds.

Cultivar	Date potted	<u>Nil (3b)</u>	<u>Cu</u> <u>fungicide</u> <u>(3a)</u>	<u>E Cu (1 & 2)</u>
Cherry Brandy	26/6/07 29/11/07 30/10/08 12/12/08	25 10	10	1
Etna	?		5	
Otto Luyken	? 11/2/07 25/6/07 24/4/08 25/6/08 5/9/08 12-13/12/08	30 30 10	20 20 5 15	1 2 0.5 1
Piri	25/5/07	15	2	
Rotundifolia	? 25/6/07 12/5/08			0 5 0
Zabelliana	? 20-23/4/07 25/6/07 3/4/08 12/5/08	5	5 5	0.5 0 0.5

Table 4. Effect of copper treatment on Pseudomonas leaf spot (percentage leaf areaspotted or dropped out) on Prunus laurocerasus cultivars on outdoor beds, 6 August2009

Where no assessments are indicated there were no plants from that potting date with that bed treatment.



Liverwort control

The establishment of liverwort *Marchantia polymorpha* L. was delayed in areas treated with E Cu irrigation (Tables 5-7, Figs. 10 and 11). The level of control achieved in this observation was much better than in the year 1 experiment where no significant effect was noted. This could be due to a much higher level of infestation pressure in the year 1 experiment.

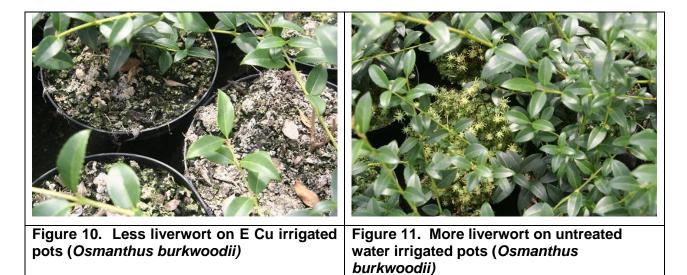


Table 5. Percentage liverwort cover on Osmanthus burkwoodii pots.Assessed 16July 2009

Treatment	% liverwort cover
Untreated	87
E Cu irrigated	10

Table 6. Percentage liverwort cover on Prunus lusitanica 'Myrtifolia' pots. Assessed16 July 2009

Treatment	% liverwort cover
Untreated	30
E Cu irrigated	1

Table 7. Percentage liverwort cover on Iris foetidissima pots. Assessed 6 August2009

Treatment	% liverwort cover
Untreated	75
ECu irrigated	10

Biological control of pests

An initial infestation of two *Tetranychus urticae* mites per leaf on *Vinca major* was noted in both treated and untreated areas on 16 July 2009. The initial infestation failed to develop in both areas and levels had dropped to 0.5 mites per leaf by 6 August 2009 and zero by 28 September 2009. There was no noticeable difference in control between the E Cu treated and untreated areas.

Other plant species monitored

Batches of *Aucuba japonica* 'Crotonifolia', *Carex* 'Evergold', *Choisya* 'Sundance', *Hedera helix* 'Green Ripple', *Iris foetidissima, Lavandula* 'Blue Star', *Osmanthus burkwoodii, Prunus laurocerasus* 'Interlo', and *Viburnum tinus* were monitored for pest and disease incidence in both E Cu treated and untreated areas. However no pest or diseases were noted and there were do differences in quality between plants in the two areas.

Compost tea viability

The results of testing for microbial activity in the growing media following compost tea applications with and without E Cu water treatment are shown in Table 8. The analysis results for both samples were classed as excellent for active bacterial and total fungal, good for total bacterial and low for active fungal (Laboratory Services and Research, Laverstock Park Farm, Hants.). There was no indication that the compost tea viability was reduced in the E Cu treated area.

Table 8.	Bacterial	and	fungal	counts	in	growing	media	following	compost	tea
applicatio	n									

Treatment	Active Bacterial (ug/g)	Total Bacterial (ug/g)	Active Fungal (ug/g)	Total Fungal (ug/g)
Untreated	42.6	201	13.90	490
E Cu irrigated	82.7	244	4.51	544

Monitoring of the plants in the compost tea treated and untreated areas did not reveal any differences.

Copper in drainage water

Samples taken from drainage water at Golden Grove Nurseries, Wigtoft, Lincolnshire, (Table 9) following two years of application of E Cu irrigation indicated that there was a small accumulation of copper in the drainage water from the capillary sandbeds but nothing from

the overhead irrigated, woven plastic groundcover beds. These results are much as expected.

Table 9. Co	opper (ppm) in	drainage water from	n two E Cu treated bed types
-------------	----------------	---------------------	------------------------------

Bed and irrigation	Copper (ppm)
Overhead irrigated groundcover beds	0.01
Capillary sandbed irrigation	0.19

Performance of the AquaHort unit

Copper levels in the E Cu irrigation water were monitored using a hand test kit (VWR Aquaquant MERC1.14414.0001Copper) or by laboratory analysis (NRM Bracknell). The manufacturers recommend that water samples are tested on site rather than by laboratory analysis as a delay in analysis can result in an inaccurate low reading. Results are shown in Table 10.

Date tested	Copper (ppm)	Method	Comments	
9/4/09	0.74	Lab	loniser set at 1ppm	
6/5/09	1.75	Lab	loniser set at 2ppm	
9/6/09	2.4	Test kit	loniser set at 3ppm	
			Liquid feed at 1.5%	
16/7/09	0.8	Test kit	Ioniser set at 3ppm	
			No liquid feed	
16/7/09	2.4	Test kit	loniser set at 3ppm	
			Liquid feed re-instated	
28/10/09	1.8	Test kit	loniser set at 3ppm	
			Liquid feed continued	

Table 10. Copper (ppm) in E Cu treated irrigation water

The ioniser was initially set at 1 ppm, increased to 2 ppm on 20 April 2009 and increased further to 3 ppm on 7 May 2009. It remained set at 3 ppm for the duration of the trial. The conductivity (EC) of the water supply taken pre-treatment was 553 μ S/m³ which was thought to be too low for efficient operation of the ioniser. By adding liquid feed at 2% the EC was raised to around 2000 μ S/m³. However when the feed strength was subsequently reduced to 1.5% with an EC of around 1600 μ S/m³ this did not appear to affect the functioning of the ioniser. In spite of a more than adequate conductivity the level of copper in the E Cu water was slightly lower (at 1.8-2.4 ppm) than the target (3 ppm) set. Test results from the same ioniser used in 2008 were also lower than target. The unit operated better in 2009 under commercial conditions possibly due to longer irrigation cycles.

Conclusions

Phytophthora root rot

The E Cu water treatment was effective in reducing a high level of *Phytophthora* root rot in *Chamaecyparis lawsoniana* 'Elwoodii' in the year 1 experiments. Although these results could not be confirmed in the year 2 experiments, due to the failure of the disease to establish, results from the nursery observations in year 3 indicated again a good level of control, this time in infected *Prunus lusitanica* "Myrtifolia" reducing plant losses from 2.5% to 0.7%.

Pythium root rot

Results from the replicated experiments in year 1 were inconclusive because the infected *Aquilegia* test plants (year 1) also suffered copper phytotoxicity. In the year 2 study on *Choisya*, there was a small reduction in root death from both the E Cu and *Bacillus* treatments. However, the levels of disease were low and the differences were not significant. The most interesting results came from the nursery observations in year 3 where a high level of *Pythium* infection in *Helleborus orientalis* was controlled and plant quality improved.

Bacterial leaf spots

Initial (year 1) work on *Prunus laurocerasus* indicated a slight reduction in *Pseudomonas* leaf spot in E Cu treated plants, however infection levels were low. Much higher infection levels were noted in the year 3 nursery observation and a good level of control was noted, reducing infection from up to 35% to 5% or less. The disease was not completely eradicated though. It should be noted that the E Cu treated plots also received more liquid feed than the control plots so some of the improvement in plant quality may have resulted from an improvement in nutrition. The overall control level was, however, much better than normally achieved on the nursery where control measures are based on 2 -3 weekly sprays of copper fungicide.

Xanthomonas leaf spot was present in the *Hedera* study in year 2 but at relatively low level. Population numbers of *Xanthomonas* bacteria per leaf were quite variable, but showed some reduction where the E Cu treatment had been applied. The total number of leaf spots was reduced by 20% where the E Cu treatment was used, but these differences were not statistically significant. No *Xanthomonas* leaf spot occurred in year 3.

Moss and liverwort

Liverwort established in two plant batches in the nursery observation year 3 and the E Cu treatment appeared to delay the infestation of liverwort compared with the control: moss was not present. However, with a heavy infestation pressure in the year 1 experiment the E Cu treatment had failed to give a significant control of moss and liverwort. In general, the experience of other nurseries is that liverwort and moss still remain a problem even when using E Cu irrigation. Previous studies (HNS 93c) have shown copper to have some efficacy in control of liverwort. Whilst E Cu irrigation might help in a control strategy it would appear that other measures need to be taken as well.

Compatibility with biological control agents

Compatibility with microbial and invertebrate biological control agents was quite good with the E Cu treatment. There were no adverse effects on microbial populations of *Trichoderma* and compost tea microbes, but a slight (not statistically significant) reduction in *Bacillus* populations. It was not possible to test the effect on functionality of the microbial biological control agents because they were not significantly effective in the control of the target diseases. There were no adverse effects noted on *Phytoseilus persimilis* mites and control of two spotted spider mite was unaffected.

Operational issues

The main problems have been the copper ion output being lower than the target set on the equipment and the need to ensure adequate conductivity in the water supply for the equipment to work effectively.

Two sets of equipment were used during the course of this study. In year 1 a small portable unit, the AquaHort Mini, was used achieving 2 ppm Cu when set at 3 ppm. In years 2 and 3 a larger capacity 4 electrode unit was used. The larger capacity unit did not seem to be so well suited to small plot work and in year 2 output averaged 1 ppm compared with a lower target of 2 ppm. However the same unit gave a higher output 1.8 – 2.4 ppm when used under commercial conditions in year 3 with a water supply supplemented with liquid feed to give a conductivity of around 1600 μ S/m³. The manufacturers recommend an EC of > 1000 μ S/m³ for this type of unit.

It is a significant disadvantage where good quality low EC water is available to have to add liquid feed merely to ensure operation of the ionizer. However this issue has been addressed by the development of plate electrode models which can be free standing or designed to float in the irrigation reservoir. The manufacturers advise that these models are suitable for operation in low EC water.

Technology transfer

No technology transfer activities took place during the third year of the project.

References

Goldsworthy A, Whitney H & Morris E. 1999. Biological effects of physically conditioned water. *Water Research* **33**: 1618-1626.

Pedersen L. 2003. Afprøvning af AquaHort. Gartner Tidende 32: 4-5

Appendix 1. Nursery observation layout

Polytunnel

Outside Beds

