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The results and conclusions in this report are based on a series of experiments 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

- The Aqua Hort system slightly reduced root rotting due to *Pythium* in *Choisya* and reduced *Xanthomonas* leaf spot populations on *Hedera* leaves.

Background and expected deliverables

Control of root diseases caused by species of *Phytophthora* and *Pythium* and bacterial leaf spot diseases caused by *Xanthomonas hederae* and *Pseudomonas syringae* continues to present major problems for nursery stock growers.

Water is the key means for spread of these pathogens on a nursery. Dispersal of *Phytophthora* and *Pythium* occurs by movement of zoospores in water (including films of water at the base of pots), by water splash, or in recycled drainage water. Bacterial leaf spots are spread by water splash and in films of water on leaf surfaces.

The regular addition of copper to irrigation water could substantially limit the spread of *Phytophthora*, *Pythium* and bacteria causing leaf spots. However, when using copper fungicide sprays to control leaf spot, treatment is often applied infrequently and there are occasions when plants are wet and insufficient copper is present to achieve pathogen control.

The use of electromagnetic water treatment with copper ions (E Cu) is claimed to enhance the activity of the copper and has been adopted by pot plant growers in Denmark for controlling a range of root infecting fungi. Initial results have been promising, but most experience has been gained with “ebb and flow” irrigation systems, and so far in the Netherlands and Denmark, there have only been two installations on nursery stock with overhead irrigation. Mini portable ionising water treatment units have recently become available, which are suitable for smaller scale growers, or treatment of specific crop batches (e.g. “Aqua-Hort Mini”, Aqua-Perl Denmark ApS). Previously, such units have been large and costly to install.

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.

- An assessment of the compatibility of the Aqua Hort copper ioniser system with biological control systems used in normal commercial practice.

- Development of guidelines for the use of the Aqua Hort copper ioniser system and its integration with biological systems on the nursery.

Summary of the project and main conclusions

Phytophthora root rot study

Chamaecyparis lawsoniana 'Chilworth Silver' plants, were potted from plugs, arranged around central 'infector' plants, and grown on for 21 weeks under different water treatment regimes with or without biological control measures, designed to reduce disease spread. Treatments are listed below:

Treatments

1.	Untreated water at each watering
2.	E Cu water at each watering, target 2 ppm Cu.
3.	*Trichoderma* as Trianum G granules 750 gm / m^3 incorporated at potting; untreated water for irrigation.
4.	*Trichoderma* as Trianum G granules 750 gm / m^3 incorporated at potting; E Cu water at each watering, target 2 ppm Cu.

No definite conclusions on disease control could be drawn as the *Phytophthora* inoculation failed to establish sufficiently to cause significant plant losses. Analysis of the growing media showed that the *Trichoderma* incorporation had established well and was unaffected by the E Cu copper treatment (Table 1).

Table 1. Mean *Trichoderma* colony forming units (Cfu)/g at 25ºC

Treatment	*Cfu/g*
1. Untreated water	0
2. E Cu treated water	1760
3. *Trichoderma* incorporation, untreated water	69500
4. *Trichoderma* incorporation, E Cu treated water	128012

Pythium root rot study

Choisya 'Sundance' infector plants, were potted from 9 cm pot liners into one litre containers, arranged around central 'infector' plants and grown on for 21 weeks under different water treatment regimes with or without biological control measures, designed to reduce disease spread. Treatments are listed below:

Treatments

1.	Untreated water at each watering
2.	E Cu water at each watering, target 2 ppm Cu.
3.	*Bacillus spp.* (as Revive™) drench after potting 2 mL / L applying 10% of pot volume; untreated water for irrigation
4.	*Bacillus spp.* (as Revive™) drench after potting 2 mL / L applying 10% of pot volume; E Cu water at each watering, target 2 ppm Cu.

Foliage dulling is a good indication of root death in growing crops of *Choisya.* Initially some foliage dulling was recorded on all plots. However, by September some recovery had taken place on both the *Bacillus* and E Cu water treatment plots. Only the untreated plots were still showing signs of foliage dulling (Table 2). By October, further recovery had taken place on the untreated plots and there were no significant differences between treatments.

There were indications that root death was reduced by all treatments, however differences were small and not statistically significant.

Table 2. Foliage dulling and root death of *Choisya* 'Sundance' – 2007

Treatments	*No of dull plants (per 14) (5 Sept)*	*Root death score* (7 Nov)*
1. Untreated water	1.50	2.66
2. E Cu treated water	0.00	2.46
3. *Bacillus spp.* drench, untreated water	0.00	2.09
4. *Bacillus spp.* drench, E Cu treated water	0.00	2.20

*Root death score: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

Thielaviopsis was prevalent in the Choisya samples taken during August, and *Phytophthora*, *Pythium* and *Thielaviopsis* were found in some samples taken during November following the conclusion of the experiment (Appendix 3).

Microbiological analysis of the growing media (Appendix 2) indicated that *Bacillus* was present in all samples including the untreated. Levels were higher where the *Bacillus* drench had been applied (treatments 3 and 4) but appeared to be reduced by the E Cu treatment, although there was some variability and the reduction was not statistically significant (Table 3).

Table 3. Mean *Bacillus* colony forming units (Cfu)/g (x1000)

Treatment	*Cfu/g (x1000)*
1. Untreated water	78
2. E Cu treated water	97
3. *Bacillus spp.* drench, untreated water	154
4. *Bacillus spp.* drench, E Cu treated water	104

Xanthomonas *leaf spot study*

Hedera helix along with infector plants, were grown on for 21 weeks under different water treatment regimes with or without biological control mulch treatments. Treatments are listed below:

Treatments

1. Untreated water at each watering
2. E Cu water at each watering, target 2 ppm Cu.
3. Composted green waste (Gardenscape) mulch 2.5cm depth applied to pot surface after potting. Untreated water at each watering.
4. Composted green waste (Gardenscape) mulch 2.5cm depth applied to pot surface after potting. E Cu water at each watering, target 2 ppm Cu.

Leaf spot levels were initially relatively low, declined through August then increased by November, reflecting the prevailing weather conditions. By the end of the experiment the E Cu copper treatment (2) had the lowest level of leaf spots (Table 4), however differences were relatively small and not statistically significant. The composted green waste treatment had no significant effect on leaf spot incidence.

Table 4. Leaf spot incidence in *Hedera helix*

Treatment	*Number of leaf spots per 15 plants (Nov 9)*
1. Untreated water	81.5
2. E Cu treated water	64.8
3. Composted green waste mulch, untreated water	76.5
4. Composted green waste mulch, E Cu treated water	83.0

The leaf spot was confirmed as *Xanthomonas hortum hedera*. Population numbers of *Xanthomonas* bacteria per leaf were very variable with no significant difference between treatments.

Performance of the Aqua Hort unit

The copper levels in the water treatments were monitored weekly and analysed for dissolved copper (mg/L). The Aqua Hort unit did not reach the target of 2 ppm Cu, averaging 1 ppm over the period of the experiment.

Because the water analysis for copper generally showed a level of less than the target, various measures were taken to try to increase the output of the Aqua Hort ioniser:

1. The water flow rate was adjusted to ensure it was not too fast for the capacity of the equipment.
2. The equipment was always run for 10 minutes before sampling and application.
3. In order to raise the EC of the water supply, a liquid feed of Scotts Agrolution 3:1:6, 13-5-28, (EC 1.1 mS/m^3 for 1 g/L) was applied at 0.5 g/L from 30 July to all treatments to raise the EC of the water from 520 uS/m^3 to approximately 1000 uS/m^3.
4. The setting of the equipment was raised from 2 ppm to 4 ppm.
5. The copper electrodes were cleaned.

The low output was surprising because a higher capacity machine was used in 2007 following similar problems in 2006. Although additional measures were taken to improve the output, it proved difficult to achieve a higher output. The manufacturers claimed that the low output was a result of too low an EC of the water. Further testing will be required before installation on a nursery site for the 2008 experiment.

In conclusion, the 2006 results showed the Aqua Hort system to have good potential for control of *Phytophthora* root rots when used with overhead irrigation and would be cost effective. Although it was not possible to confirm these results in 2007, this was due to a failure of the disease to develop rather than poor control.

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.

The benefits for using the Aqua Hort unit for the control of *Pythium* root rots and the reduction in *Xanthomonas* leaf spot remain to be proven.

Some issues concerning the low output of the Aqua Hort units need to be resolved before the unit can be fully recommended particularly where water supplies have a low electrical conductivity (EC).

Financial benefits

The Aqua Hort unit proved very effective in year 1 of the study for the control of *Phytophthora* root rot in *Chamaecyparis lawsoniana* 'Elwoodii'. These results could not be confirmed on *Chamaecyparis lawsoniana* 'Chilworth Silver' in year 2, because of the low incidence of the disease. However, if confirmed in the final year nursery experiment and in different growing systems, the potential benefits to growers who suffer persistent problems with *Phytophthora* root rots, not just on conifers but also broad-leaved shrubs and herbaceous plants, would be substantial.

- 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 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* each year.

Although reductions in root rotting due to *Pythium* on *Choisya,* and in the incidence of *Xanthomonas* leaf spot on *Hedera* were recorded from the use of the Aqua Hort system, differences were small and not statistically significant. At this stage no financial benefit can be predicted from the use of the Aqua Hort system for the control of these diseases,. However further work is planned during which it is hoped to test the system with greater disease pressure under nursery conditions.

Action points for growers

- The Aqua Hort system shows potential for control of *Phytophthora* root rots when used with overhead irrigation and would be cost effective.

- Some issues concerning the low output of the Aqua Hort units need to be resolved before the unit can be fully recommended.

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. *Phytophthora* root rot is listed as a major problem in the conifer R & D strategy and is a ** gap (HDC gap analysis) due to the limited number of control treatments available. Bacterial leaf spots such as *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* including *Philadelphus* and *Spiraea*; both are important lines. Follow-up work (HNS 91) did not identify a satisfactory control measure and control of bacterial leaf spots in nursery stock is a *** gap. 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* 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*, *Pythium* and bacterial 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. There have been only two installations, in Holland and Denmark, on nursery stock with overhead irrigation. 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). Previously, units have been large and costly to install.

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 results of the first years study indicated that there was no useful activity against moss or liverwort, so this part of the study was not continued.

The scientific objectives for the second year of the project were:

1. To determine the effectiveness of routinely irrigating plants with copper-ionised water in preventing the development of *Phytophthora* root rot, *Pythium* root rot and *Xanthomonas* leaf spot.
2. To compare the effectiveness of this copper treatment when combined with a biological treatment for each disease.

The technical objectives were:

1. To test the efficacy of electromagnetic copper (E Cu) water treatment in controlling the spread of *Phytophthora* root rot in *Chamaecyparis* 'Chilworth Silver', compared with a biological control (*Trichoderma*) applied alone and in combination.
2. To test the efficacy of E Cu water treatment in controlling the spread of *Pythium* stem and root rot in *Choisya* 'Sundance', compared with a biological control (*Bacillus* spp.), applied alone and in combination.
3. To test the efficacy of E Cu water treatment in controlling the spread of *Xanthomonas* leaf spot in *Hedera helix,* compared with a biological control (mulch of composted green waste), applied alone and in combination.

Materials and methods

In the second year of this project, experiments were done at East Malling Research to determine the effect of increased copper levels in the irrigation water and some biological treatments on control of *Phytophthora* root rot of Chamaecyparis, *Pythium* root rot of *Choisya* and bacterial leaf spot (*Xanthomonas*) of *Hedera*. The *Chamaecyparis* and *Choisya* were grown on benching in a polytunnel, the *Hedera* was grown outside on a gravel area.

Treatments for all three subjects were:

1. Irrigation with untreated water
2. Irrigation with E Cu treated water using an Aqua-Hort Mini copper ioniser with a target of 2 ppm Cu
3. Industry standard biological control, irrigated with untreated water
4. Industry standard biological control, irrigated with E Cu treated water using an Aqua-Hort Mini copper ionizer with a target of 2 ppm Cu

The experiment was laid out as a randomised complete block split-plot experiment with four fold replication. Within the experiment, three studies were run, each study with the four basic treatments above, with differences in the biological controls used according to the diseases. Results were examined by analysis of variance (ANOVA) or by a non-parametric test (e.g. Friedman's test) where conditions for ANOVA did not hold true.

The three studies were:

1. *Phytophthora* root rot control in Chamaecyparis.
2. *Pythium* root rot control in Choisya.
3. *Xanthomonas* leaf spot control on Hedera.

For studies 1 and 2, each plot consisted of 15 plants of *Chamaecyparis* 'Chilworth Silver' and 14 of *Choisya* 'Sundance' creating two sub-plots within a plot. Study 3 was located separately with each plot consisting of 12 plants of *Hedera helix*.

Phytophthora root rot study

Two hundred and forty *Chamaecyparis* 'Chilworth Silver' plants were potted from plugs into 9 cm pots (25/4/06) using a growing medium comprising: sphagum peat: sterilised loam 90:10 by volume + Osmocote Plus 12-14 month (4 kg/m^3), starter feed (14% N, 16% P_2O_5, 18% K_2O) at 0.5 kg/m^3, + wetter, + Intercept (imidacloprid) (280 gm/m^3). For plants in treatments 3 and 4, *Trichoderma* as Trianum G (750 gm / m^3) was added to the media at potting. Trianum G is reported to control *Phytophthora* root rots (Koppert Ltd). For each sub-plot, 15 healthy plants were grouped around one infector plant and placed in an isolation tray (90 cm x 30 cm with no holes). One tray of plants per plot was then placed in the trial area on benching in the polytunnel. The infector plants had been prepared by inoculating them with *Phytophthora cinnamomi* (identification confirmed by a PCR test at SCRI); plants showing initial symptoms of *Phytophthora* root rot were chosen for use as infector plants. Treatment details are given in Table 1.

Table 1. Treatment list for experiment on *Pythophthora* root rot control

1.	Untreated water at each watering
2.	E Cu water at each watering, target 2 ppm Cu.
3.	*Trichoderma* as Trianum G granules 750 gm / m^3 incorporated at potting; untreated water for irrigation.
4.	*Trichoderma* as Trianum G granules 750 gm / m^3 incorporated at potting; E Cu water at each watering, target 2 ppm Cu.

Irrigation treatments were maintained for 21 weeks from 11/06/07.

Assessments

Plants were visually assessed for foliar symptoms (foliage or stem browning, stunting or wilt) of *Phytophthora* root rot infection on 27/06/07, 25/07/07, 22/08/07, 02/09/07 and 31/10/07. At the final assessment the number of dead or dying plants, and vascular browning at the stem base, was recorded and the amount of root death was assessed by breaking up the root ball and scoring the amount of root death as follows:

Root death score: 1 = 0-10%, 2= 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100%

Samples of growing medium (50 ml from 5 plants per plot) for microbiological analysis were collected on (5/11/08). Samples were tested for total numbers of bacteria, fungi (moulds) and yeasts by standard methods by Eurofins, Wolverhampton (see Appendix 2).

After the experiment was completed, plant samples were taken for further analysis. A few discoloured or rotting roots were collected from five plants per plot. The roots were washed to remove compost and surface sterilised by ethanol dip. Five pieces of roots per plot were plated out onto a *Phytophthora* – selective agar medium (P5ARP) and five pieces were plated out onto a general fungal growth medium (PDA + Streptomycin). These plates were checked for *Phytophthora* after 7 days. The numbers of roots per plot that developed *Phytophthora* were recorded. Samples of the remaining roots were then floated in sterile pond-water (to stimulate sporangial production by *Phytophthora*) and examined for *Phytophthora* sporangia after 3-5 days.

Pythium root rot study

Two hundred and twenty four *Choisya* 'Sundance' plants were potted from 9 cm liners into one litre pots (30/05/07) using the growing medium as above. The plants in treatments 3 and 4 were drenched with *Bacillus* as Revive Liquid (2 ml/L) applying 10% of pot volume on 30/05/07. For each sub-plot, 14 healthy plants were grouped around one infector plant (plants with root rot) and placed in an isolation tray. One tray of plants per plot was placed in the trial area on benching in the polytunnel. Treatments are given in Table 2.

A mixture of fungi, including a *Pythium*, species identified as a *P.sylvaticum / P. ultimum* type (Tim Pettitt, pers comm) were isolated from poorly-growing *Choisya* plants obtained from a commercial nursery. 'Infector' plants were produced by mixing 16 agar plates containing *Pythium* spp. with distilled water (400 ml), breaking up the agar and applying this substrate equally between 16 one litre pots (25 ml per pot) containing *Choisya* plants, ensuring the agar was covered by growing media.

Table 2. Treatment list for experiment on *Pythium* root rot control.

1.	Untreated water at each watering
2.	E Cu water at each watering, target 2 ppm Cu.
3.	*Bacillus spp.* (as Revive™) drench after potting 2 mL / L applying 10% of pot volume; untreated water for irrigation
4.	*Bacillus spp.* (as Revive™) drench after potting 2 mL / L applying 10% of pot volume; E Cu water at each watering, target 2 ppm Cu.

Irrigation treatments were maintained for 21 weeks from 11/06/07.

Choisya plants were treated for two-spotted spider mite using *Phytoseiulus persimilis* predators on 05/09/07 and with Torq (fenbutatin oxide 50% w/w) at 5 mL / 5L applied to run off on 12/09/07, 24/09/07 and 11/10/07.

Assessments

Plants were visually assessed for foliar symptoms (foliage dulling, stunting or wilt) of *Pythium* root rot infection on 27/06/07, 25/07/07, 22/08/07,02/09/07 and 07/11/07. At the final assessment the number of dead plants was recorded and the severity of root rotting was assessed by breaking up the root ball and scoring the amount of root death as follows:

Root death score: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

The growing medium was sampled for microbiological analysis as described previously. After the experiment was completed, plant samples were taken for further analysis. A few discoloured or rotting roots were collected from five plants per plot. The roots were washed to remove compost and surface sterilised by ethanol dip. Five pieces of roots per plot were plated out onto two agar media as in the *Phytophthora* experiment described above. These were checked for *Pythium* after 3 days. The number of roots per plot that developed *Pythium* was then recorded. Samples of the remaining roots were floated in sterile pond-water and examined for *Pythium* sporangia after 3 days.

Xanthomonas leaf spot study

One hundred and ninety two *Hedera helix* plants were potted from 9 cm liners into two litre pots (30/05/07). For each plot, 12 plants were grouped around a plant infected with *Xanthomonas hortum hedera* leaf spot and stood out on a gravel standing area. Treatments are given in Table 3.

Table 3. Treatment list for experiment on *Xanthomonas* leaf spot control.

1.	Untreated water at each watering
2.	E Cu water at each watering, target 2 ppm Cu.
3.	Composted green waste (Gardenscape) mulch 2.5cm depth applied to pot surface after potting. Untreated water at each watering
4.	Composted green waste (Gardenscape) mulch 2.5cm depth applied to pot surface after potting. E Cu water at each watering, target 2 ppm Cu.

Irrigation treatments were maintained for 21 weeks from 11/6/07.

Assessments

Plants were visually assessed for brown/black leaf spots, the foliar symptoms of *Xanthomonas* infection, on 27/06/07, 25/07/07, 22/08/07, 02/09/07 and 09/11/07. The total number of leaf spots per 12 plant plot was recorded on each occasion.

Additionally, on 26/7/07, 4/9/07 and 15/11/07, bulk samples of 20 symptomatic leaves per treatment were collected and the total number of aerobic bacteria determined (Eurofins method EUMM3.14). On 12/11/07, 20 asymptomatic leaves per plot were collected and then tested for the numbers of Xanthomonas bacteria associated with them (by isolation onto a semi-selective medium); species were identified by Fatty Acid Profiling (FAP).

Copper ionizer operation and nutrient level monitoring

A 500 mL growing medium sample was taken at the start of the experiment (11/06/07) and at the end of the experiment (09/11/07) from a representative selection of plants and analysed for water soluble macro and micro nutrients determined by extraction of 1/15th density in 400-mL deionised water to BS 4156 1990.

Mains water, copper feed and E Cu water samples were taken weekly and analysed for dissolved copper (mg/L). Because the water analysis for copper generally showed a level of less than the target of 2 ppm various measures were taken to try to increase the output of the Aqua Hort ioniser:

1. The water flow rate was adjusted to ensure it was not too fast for the capacity of the equipment
2. The equipment was always run for 10 minutes before sampling and application

3. In order to raise the EC of the water supply, a liquid feed of Scotts Agrolution 3:1:6, 13-5-28, (EC 1.1 mS/m^3 for 1 g/L) was applied at 0.5 g/L from 30 July to all treatments to raise the EC of the water from 520 uS/m^3 to approximately 1000 uS/m^3
4. The setting of the equipment was raised from 2 ppm to 4 ppm
5. The copper electrodes were cleaned.

Results and discussion

Phytophthora *root rot study*

In spite of the use of infector plants and watering to encourage the spread of infection, very few plants showed signs of foliage dulling and plant death. An average of only 0.6 plants per plot died during the course of the experiment with no difference in number of dead plants between treatments (Table 4).

Similarly, although root growth was not extensive and some root death was recorded and confirmed as *Phytophthora* (Appendix 3), there was no significant difference between treatments (Table 4).

Table 4. Plant death, root death and root pot fill scores for *Chamaecyparis* 'Chilworth Silver' – November 2007

Treatment	*Mean No. dead plants (of 15)*	*Mean root death score**	*Mean pot fill*
1. Untreated water	0.25	1.7	36.5
2. E Cu treated water	0.75	2.0	24.6
3. *Trichoderma* incorporation, untreated water	1.25	1.7	32.5
4. *Trichoderma* incorporation, E Cu treated water	0.25	1.7	48.4
F pr.	0.411	0.568	0.292
Df	9	9	9
s.e.d	0.656	0.24	11.62

*Root death score: 1 = 0-10%, 2= 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100%

It was concluded that the *Phytophthora* infection failed to establish sufficiently for any treatment effects to become apparent.

Microbiological analysis of the growing media (Appendix 2) indicated that the *Trichoderma* incorporation had established well and was unaffected by the E Cu copper treatment (Table 5).

Table 5. Mean *Trichoderma* colony forming units/g at 25ºC

Treatment	*Cfu/g*	*Log Cfu/g*
1. Untreated water	0	0
2. E Cu treated water	1760	0.96
3. *Trichoderma* incorporation, untreated water	69500	4.35
4. *Trichoderma* incorporation, E Cu treated water	128012	4.23
F pr.		<0.001
Df		9
s.e.d		0.819

Pythium *root rot study*

Foliage dulling is a good indication of root death in growing crops of *Choisya*. Through July and August, some foliage dulling was recorded on all plots with no significant difference between treatments. However, by September some recovery had taken place particularly on the treatment plots, with only the untreated plots still showing signs of foliage dulling (Table 6). By October, further recovery had taken place on the untreated plots and there were no significant differences between treatments.

There were indications that root death was reduced by all treatments, however differences were small and not statistically significant.

Table 6. Foliage dulling and root death of *Choisya* 'Sundance' - 2007

Treatment	*Mean number of dull plants*				*Root death score* (Nov 7)*
	July 25	Aug 22	Sep 2	Oct 31	
1. Untreated water	3.25	6.75	1.50	0.50	2.7
2. E Cu treated water	2.00	8.75	0.00	0.50	2.5
3. *Bacillus spp.* drench, untreated water	3.50	8.00	0.00	0.50	2.1
4. *Bacillus spp.* drench, E Cu treated water	3.25	8.50	0.00	0.25	2.2
F pr.	0.701	0.436	0.259	0.972	0.783
Df	9	9	9	9	9
s.e.d	1.374	1.258	0.842	0.648	0.61

*Root death score: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

Thielaviopsis was prevalent in the Choisya samples taken during August, and *Phytophthora*, *Pythium* and *Thielaviopsis* were found in some samples taken during November following the conclusion of the experiment (Appendix 3).

Microbiological analysis of the growing media (Appendix 2) indicated that *Bacillus* was present in all samples including the untreated. Levels were higher where the *Bacillus* drench had been applied (treatments 3 and 4) but appeared to be reduced by the E Cu treatment, although there was some variability and the reduction was not statistically significant (Table 7).

Table 7. Mean *Bacillus* colony forming units/g (x1000)

Treatment	*Cfu/g (x1000)*
1. Untreated water	78
2. E Cu treated water	97
3. *Bacillus spp.* drench, untreated water	154
4. *Bacillus spp.* drench, E Cu treated water	104
F pr.	0.339
Df	9
s.e.d	40.2

Xanthomonas *leaf spot study*

Leaf spot levels were at a relatively low level (Table 8), reduced through August then increased by November, reflecting the prevailing weather conditions. The monthly variation was more marked than differences between treatments. By the end of the experiment the E Cu copper treatment (2) had the lowest level of leaf spots, however differences were relatively small and not statistically significant. There were some indications that the composted green waste treatment had increased the number of leaf spots at the June and July assessments but the effect was less marked by November.

Table 8. Leaf spot incidence in *Hedera helix*

Treatment	*Mean number of spots per plot (12 plants)*				
	June 27	July 25	Aug 31	Sep 2	Nov 9
1. Untreated water	46.5	28.0	7.8	12.8	81.5
2. E Cu treated water	44.0	39.8	7.5	10.2	64.8
3. Composted green waste mulch, untreated water	66.0	49.8	9.5	11.2	76.5
4. Composted green waste mulch, E Cu treated water	40.5	36.0	10.8	16.8	83.0
F pr.	0.119	0.382	0.768	0.658	0.677
Df	9	9	9	9	9
s.e.d	10.09	11.92	3.51	5.42	16.16

The leaf spot was confirmed as *Xanthomonas hortum hedera* (Appendix 2). Population numbers of *Xanthomonas* bacteria per leaf were very variable with no significant difference between treatments.

Growing media and water analyses

Analysis of the growing media (see Appendix 1) at the start and the conclusion of the experiment showed that water soluble copper levels increased from <0.06 mg/L to 0.11 mg/L for growing media with the E Cu treatment. Where plain water was given, the level finished at 0.06 mg/L.

Water from all three irrigation treatments was monitored weekly for copper levels (see Appendix 1). It was soon apparent that the copper output from the ionizer was somewhat variable, being generally lower than the target 2 ppm. In spite of the various measures taken to improve the output from the ionizer, the recorded copper level averaged 1 ppm over the 4 months of the experiment. This was however, a 20 fold increase over the untreated water.

Conclusions

In the *Pythium* 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. *Thielaviopsis* was also detected in the root system and it is thought unlikely that the E Cu would have any effect on that disease.

Similarly, the *Phytophthora* infection in *Chamaecyparis* Chilworth Silver, although detectable, failed to develop sufficiently to cause a significant level of root or plant loss, unlike the results obtained in 2006. It may be that the cooler conditions of 2007 resulted in less stress on the plants, enabling the plants to overcome the infection.

Analysis of the *Chamaecyparis* growing media indicated that the *Trichoderma* incorporation had established well and was unaffected by the E Cu copper treatment. Analysis of the *Choisya* growing media indicated that *Bacillus* was present in all samples including the untreated, which had not received additional *Bacillus* – presumably a natural population from the *Choisya* liners. Levels were higher where the *Bacillus* drench had been applied but reduced back down to the natural population level by the E Cu treatment, although this reduction was variable and not statistically significant. These results suggest that the E Cu water treatment could be used in an integrated disease control system alongside biological growing media supplements such as *Trichoderma* but might be expected to reduce levels of *Bacillus.*

Xanthomonas leaf spot was present in the *Hedera* study 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. There was no significant effect from using the composted green waste mulch.

There were problems achieving the target level of 2 ppm Cu from the Aqua Hort equipment and the average over the experimental period was 1 ppm. This was surprising because a higher capacity machine was used in 2007 following similar problems in 2006. Although additional measures were taken to improve the output of the equipment – in particular, a nutrient feed was used to raise the conductivity of the water – it proved difficult to achieve a higher output. Further testing will be required before installation on a nursery site for the 2008 experiment.

Technology transfer

1. Presentation at EMR Water Centre day, 11 September (John Atwood).

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. Nutrient analyses.

Table 9. Effect of increased copper in irrigation water on growing media nutrient content (mg/L).

	Pre- treatment (11/6/07)	*Post-treatment (9/11/07)*	
		1. Mains water	2. E Cu water
pH	5.95	5.94	5.68
Chloride	38.1	126.8	128.7
Phosphorous	42.0	62.4	101.4
Potassium	147.9	484.3	759.9
Magnesium	164.0	91.6	170.6
Calcium	173.5	117.3	233.3
Sodium	45.5	198.0	194.8
Ammonia-N	6.4	13.5	9.0
Nitrate-N	263.2	199.5	414.0
Sulphate	288.6	448.8	545.1
Boron	0.18	0.33	0.34
Copper	**<0.06**	**0.06**	**0.11**
Manganese	0.58	0.19	0.25
Zinc	0.38	0.25	0.47
Iron	7.12	8.47	4.85
Conductivity uS/cm	459	547	877

Post-treatment samples were taken from the *Chamaecyapris lawsoniana* 'Chilworth Silver' *Phytophthora* study.

Table 10. Levels of copper (mg/L) achieved in irrigation water using an Aqua Hart Mini copper ioniser.during 2007

Date	*Mains water*	*E Cu water*
31/5/07	0	0.25
7/6/07	0.1	0.13
14/6/07	0.02	0.57
21/6/07	0	0.88
27/6/07	0	0.81
4/7/07	0.01	0.87
12/7/07*	0*	0*
19/7/07	0.02	0.82
25/7/07	0	0.19
1/8/07	0.11	1.76
8/8/07	0.04	2.35
16/8/07	0.28	4.8
22/8/07	0	0.58
30/8/07	0	0.8
5/9/07	0.07	0.78
13/9/07	0	0.56
18/9/07	0	1.08
26/9/07	0.03	1.22
4/10/07	0.1	0.84
11/10/07	0.09	0.68
18/10/07	0.08	0.66
Average	0.046	1.033

* possible sampling error

Appendix 2. Microbiological analyses

Table 11. Bacteria and fungal colony counts in growing media at the conclusion of the experiment – *Chamaecyparis*

Treatment	Rep	Bacteria aerobic colony count at 30°C (cfu/g)	Moulds at 37°C (%)						Yeasts at 37°C (cfu/g)	Yeasts at 25°C (cfu/g)	Moulds at 25°C (cfu/g)	Moulds at 25°C (%)						Moulds at 37°C (cfu/g)
			A. fumigatus	A. glaucus	Doratomyces	Mucor	Penicillium	Trichoderma				A. fumigatus	A. glaucus	Doratomyces	Mucor	Penicillium	Trichoderma	
1. Untreated water	1	113600000		71	29				120	700000	1500000			87		13		280
	2	31200000	40	60					<10	28000	22000			77		23		5000
	3	34800000	63		37				200	40000	56000			94	3	3		160
	4	15900000	96				4		200	200000	400000			95		5		240
2. ECu water	1	112000000	93		7				40	28000	31000			80		20		280
	2	19800000	91	9					30	38000	47000			90		10		350
	3	77600000	82			8		10	260	2000	16000			56			44	500
	4	18800000	91		9				190	1400000	300000	1		99				350
3.*Trichoderma* incorporated untreated water	1	168000000	81					19	90	14000	19000			74			26	420
	2	17200000	89					11	20	3000	8500000			51		47	2	650
	3	28800000	90			10			30	2000	18000			83			17	490
	4	10100000	63			20		17	100	12000	400000			17			25	240
4.*Trichoderma* incorporated ECu water	1	110400000	85					15	120	4000	15000			57			47	340
	2	16000000	75					25	2000	5000	20000			50	10		40	4000
	3	9900000	67					33	190	600000	1900000			74			26	360
	4	6700000	89			11			100	14000	15000			80			20	450

Table 12. Bacteria and fungal colony counts in growing media at the conclusion of the experiment – *Choisya*

Treatment	Rep	Bacteria aerobic colony count at 30°C (cfu/g)	Moulds at 37°C (%)						Yeasts at 37°C (cfu/g)	Yeasts at 25°C (cfu/g)	Moulds at 25°C (cfu/g)	Moulds at 25°C (%)						Moulds at 37°C (cfu/g)	Bacillus (cfu/g)
			A. fumigatus	A. glaucus	Doratomyces	Mucor	Penicillium	Trichoderma				A. fumigatus	A. glaucus	Doratomyces	Mucor	Penicillium	Trichoderma		
1. Untreated water	1	52600000	89		11				30	6000	21000		10	90				920	99000
	2	13200000	69		24			7	120	7000	46000			81		4	5	290	54000
	3	7200000	86		14				100	12000	18000			67		33		210	111000
	4	9600000	100						90	35000	12000			50			50	420	48000
2. ECu water	1	34100000		10	90				80	3000	17000		12	76		12		320	271000
	2	36000000	81		19				60	4000	32000			81		6	13	320	29000
	3	80000000	15	77	8				3000	46000	4000	25		50			25	13000	38000
	4	11700000	71					23	40	16000	20000			50			50	350	51000
3. *Bacillus* drench untreated water	1	6800000	68				32		<10	8000	41000	20		80				330	264000
	2	43200000	94			6			120	11000	25000			80	20			360	196000
	3	7900000	71		29				50	16000	22000			90		10		210	86000
	4	7100000	50		50				140	21000	4000			75			25	260	68000
4. *Bacillus* drench Ecu water	1	21100000	29		57	14			8000	10000	26000			90			10	7000	171000
	2	10600000	64		24			12	50	21000	16000			90			10	410	59000
	3	29700000	67				33		70	20000	30000			97		3		360	97000
	4	11900000	100						130	16000	18000			88	6	6		330	91000

Table 13. Aerobic bacteria colony counts @ 30° cfu/g on Hedera leaves

	Date		
Treatment	26/7/07	4/9/07	15/11/07
1. Untreated	68000	>300000	>300000
2. Copper	11900	>300000	>300000
3. Composted green waste mulch, untreated water	74000	>300000	>300000
4. Composted green waste mulch, copper ionizer water	156000	>300000	>300000

Method Eurofins EUMM3.14

Table 14. Xanthomonas populations in 20 *Hedera* leaves

	Treatment	*Replicate*	*Number of Xanthomonas (X10^6)*	*FAP Identification*
1	Untreated	1	19	Xhh
		2	19.5	Xhh
		3	830	Xhh
		4	35	Xhh*
2	Copper iomser	1	8.5	Xhh
		2	130	Xhh
		3	>1000	Xhh
		4	2.3	Xhh
3	Mulch	1	435	Xhh*
		2	4.3	Xhh
		3	105	Xhh*
		4	420	Xhh
4	Mulch + copper ioniser	1	-	Not detected
		2	320	Xanthomonas sp
		3	45	Xhh*
		4	45	Xhh*

Xhh – probable *Xanthomonas hortum hedera*
Xhh* - possible *Xanthomonas hortum hedera*

Method: Isolation on semi-selective MKM medium; typical colonies of presumptive *Xanthomonas* were tested by Fatty Acid Profile (FAP) analysis.

Appendix 3. Fungal isolations

Study 1 – Phytophthora root rot in Chamaecyparis

Table 15. Occurrence of fungal pathogens-6 September 2007

Samples	*Agar*	*Diseased roots*	*Comments*
Rep 2 . ECu water	P5ARP	2 x phytophthora	
Rep 2 . ECu water	P5ARP	1 x phytophthora	
Rep 2 *Trichoderma* incorporated untreated water	P5ARP	3 x phytophthora	
Rep 2 *Trichoderma* incorporated untreated water	P5ARP	1 x phytophthora	
Rep 2 *Trichoderma* incorporated untreated water	PDA+S	2 x fusarium? 1 x pythium?	Floated
Rep 2 . ECu water	PDA+S	2 x fusarium? 1 x bacteria	

Table 16. Conifer root cultures: Number of roots infected – 28 November 2007

Rep	*Agar*	*Phytophthora*	*Pythium*	*Bacteria*	*None*
1	P5ARP	0	0	1	4
2	P5ARP	4	0	0	1
3	P5ARP	2	0	0	3
4	P5ARP	4	0	0	1

Table 17. Occurrence of fungi on roots by a float test-28 November 2007

Rep	*Phytophthora*	*Fusarium*	*Pythium*
1	N	Y	Y
2	N	N	N
3	N	Y	N
4	N	N	Y

Y-yes; N-no

Study 2 - Pythium root rot in Choisya

Table 18. Number of root pieces (of 5) resulting in: Choysia root isolation tests – 28 November 2007.

Rep	*Agar*	*Phytophthora*	*Pythium*	*Bacteria*	*Comments*
1	P5ARP	0	5	0	Nematodes
2	P5ARP	0	5	0	0
3	P5ARP	3	2	0	0
4	P5ARP	0	5	0	0

Table 19. Choysia root float tests – 28 November 2007.

Rep	*Phytophthora*	*Pythium*	*Thielaviopsis*	*Fusarium*
1	N	N	N	N
2	N	N	Y	N
3	Y	N	N	N
4	Y	N	N	N

Table 20. Choysia root floats – 3 August 2007.

Plant number/Sample id	*Pythium (y/n)*	*Comments*
1 (plot 2) – A	N	
2 (plot 2) – B	Y	Thielaviopsis also found
3 (plot 2) – C	N	Thielaviopsis present
4 (plot 3) – D	N	Thielaviopsis present
5 (plot 3) – E	N	Lots of myclelium but not identified

Table 21. Choysia agar floats - 7 August 2007 – testing possible pythium from plates .

Sample id	*Pythium (y/n)*	*Comments*
A	N	Thielaviopsis
D	N	Rhizopus
D	N	?
E	Y	Sporangia
D	N	Fusarium
Pythium plate – AR07/31B (from inoculation)	Y	Oospores only

Appendix 4. Trial plan.
XBM5511 – 2007 (Outside area)

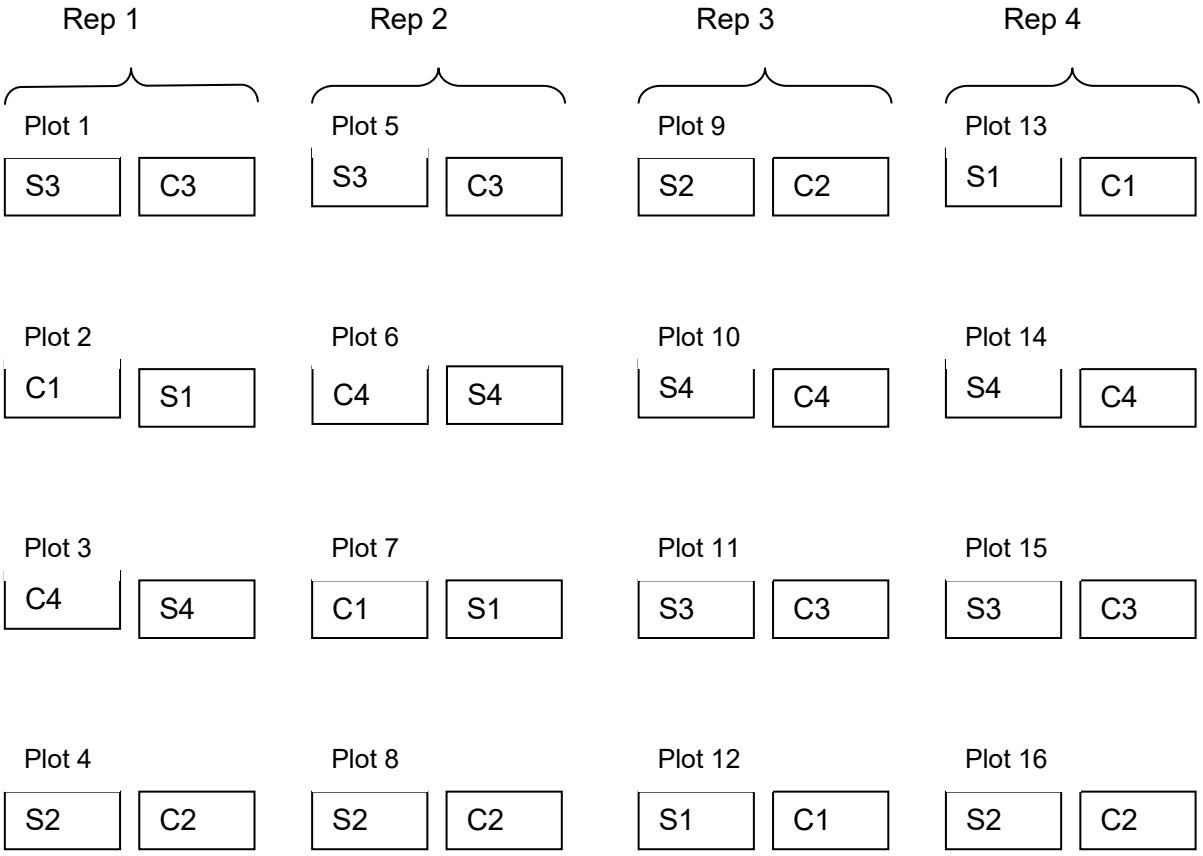
Rep 1	Rep 2	Rep 3	Rep 4
Plot 1	Plot 5	Plot 9	Plot 13
H3	H3	H2	H1
Plot 2	Plot 6	Plot 10	Plot 14
H1	H4	H4	H4
Plot 3	Plot 7	Plot 11	Plot 15
H4	H1	H3	H3
Plot 4	Plot 8	Plot 12	Plot 16
H2	H2	H1	H2

Treatments

Plants
H = Hedera helix

Watering
1 = Irrigation with untreated water
2 = Irrigation with E Cu treated water
3 = Industry standard biological control, irrigated with untreated water
4 = Industry standard biological control, irrigated with E Cu treated water

XBM5511 – 2007 (Tunnel area)



Treatments

Plants
C = Chamaecyparis
S = Choisya Sundance

Watering
1 = Irrigation with untreated water
2 = Irrigation with E Cu treated water
3 = Industry standard biological control, irrigated with untreated water
4 = Industry standard biological control, irrigated with E Cu treated water