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REPORT

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Efficacy of Water Treatment with the AquaHort®-System against *Agrobacterium tumefaciens*

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Client:
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Introduction

Aqua-Hort® is an utility for a controlled electrolytic supply of copper and an electromagnetic water treatment to irrigation water. It shows an approved efficacy against zoospores of oomycetic pathogens such as *Pythium* spp. and *Phytophthora* spp. First experiments have shown, that phytopathogenic bacteria, such as *Xanthomonas hortorum* pv. *pelargonii*, are quite more resistant. A minimum Cu-concentration of 2 ppm and exposure times of at least 4 hrs have been necessary to eliminate this pathogen. Based on these first results the susceptibility of other phytopathogenic bacteria should be examined.

Objectives

To test the efficacy of the Aqua-Hort®-System against *Agrobacterium tumefaciens* (syn.: *Rhizobium radiobacter*) in a range of 0 to 4 ppm Cu at various exposure times (60 min to 24 hrs).

Material and Methods

Aqua-Hort® Danmark ApS installed a Aqua-Hort®-unit at the experimental greenhouse of the department of phytomedicine. The unit has been put into operation and tested for its functional capability by Mr. De Lasson. He trained the personal involved into the project to handle and maintain the Aqua-Hort®-equipment.

From a 1000 L reservoir a nutrient solution contaminated with *Agrobacterium tumefaciens* was pumped with about 1 m^3/h through the Aqua-Hort®-unit. After passage through the unit the solution was dumped.

800 L nutrient solution were prepared by 0.5 g/L of the complete fertiliser FERTY® 3 MEGA (ingredients see table 1). The solution ready for use had an electric conductivity of about 1 mS/cm and the pH was adjusted to about 6.5 by adding sulphuric acid. Preparation took place one day in advance to achieve a adaptation to the ambient temperature of about 19 °C.

Immediately before the first treatment the nutrient solution was contaminated with *Agrobacterium tumefaciens* (strain B049Gshm). From a 48 h plate culture (YDC agar) a bacterial suspension (Ringer solution) was prepared (OD 53 %) and an aliquot added to the 800 L nutrient solution to achieve a density of about 10^3 to 10^4 cfu/ml. The nominal Cu-concentrations (displayed on the unit) were 1, 2 und 4 ppm Cu. Table 2 shows the realised (photometrically determined) Cu-concentrations of the various treatments.

Samples were taken 1 min after adjusting the respective concentration at the display (pump continuously working). The samples were immediately transferred to the lab and there stored at room temperature. To realise the various exposure times three sub-samples each were plated onto semiselective agar plates (Medium 1A acc. Schaad et al., 2001) 1, 2, 4 and 24 hrs after the samples were taken (spiral plater; Meintrup DWS Laborgeräte GMBH, Lähden – Holte, Germany). At start and end of each treatment beside the Cu-concentration (Kupfer-Test Aquaquant®; range 0.3 – 5.0 mg/l; Merck KGaA, Darmstadt), the electric conductivity, pH and temperature of the nutrient solution were determined.

Each treatment was repeated four times. The bacterial counts (cfu/ml) were statistically analysed by ANOVA and significant differences to the control were determined by the Dunnett-test (STATISTICA for Windows version 7.1)

table 1: nutrient contents of the complete fertiliser FERTY® 3 MEGA (Planta Düngemittel GmbH, Regenstauf, Germany)

Plant Nutrients	**Content (%)**
nitrogen	18
potassium	12
phosphorus	18
calcium	2
boron	0.02
copper	0.04
iron *)	0.10
manganese	0.05
molybdenum	0.01
zinc	0.01
*) partially as chelate (EDDHA)	

table 2: Cu-concentrations (ppm) of the treated fertiliser solution at the various repetitions (rpt.1 – rpt. 4)

set value (displayed)	**rpt. 1**	**rpt. 2**	**rpt. 3**	**rpt. 4**
0.0	0.0	0.0	0.0	0.0
1.0	0.3	0.6	0.3-0.6	0.6
2.0	1.5-2.0	no value	1.5-2.0	1.5-2.0
4.0	4.0	4.0	4.0	4.0

Results and Discussion

The AquaHort® treatment with the lowest Cu-concentration of 1 ppm showed significant reductions of the bacterial counts only at the longer exposure times of 4 and 24 hrs, but produced no complete elimination. Corresponding efficiency rates were 51 and 35 % respectively. With 2 ppm Cu a significant reduction was produced already after 2 hrs (48 %) and after 4 hrs (76 %). Complete elimination (efficiency rate = 100 %) was achieved after a exposure time of 24 hrs. The 4 ppm Cu-treatment produced a significant reduction (54 %) already after 1 hr. Efficiency rates of the 4 ppm Cu-treatment were 89 and 99.8 % after 2 and 4 hrs reaction time. Finally, the longest exposure time of 24 hrs resulted in a complete elimination of the pathogen.

table 3: Means of bacterial counts (cfu/ml) of *Agrobacterium tumefaciens*) in a fertiliser solution after treatment at different Cu-concentrations and exposure times

treatment	**exposure time**			
	1h	**2h**	**4h**	**24h**
control	2.886	2.608	2.852	63.013
1 ppm	2.473	1.931	*1.406*	*40.754*
2 ppm	2.832	*1.369*	*681*	*0*
4 ppm	*1.336*	*280*	*7*	*0*
values in italics are significantly ($p<0.05$) different from the control				

The results confirmed the potential of the AquaHort®-system to eliminate phytopathogenic bacteria under practical conditions. For a complete elimination of *Agrobacterium tumefaciens* at least 2 ppm at a 24 hr exposure time have been necessary. With the highest concentration of 4 ppm an efficiency rate close to 100 % (99.8 %) could be obtained already after 4hrs.

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