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REPORT

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Efficacy of Water Treatment with the AquaHort®-System against *Ralstonia solanacearum* Race 3

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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 has 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 *Ralstonia solanacearum* race 3 in a range of 0 to 4 ppm Cu at various exposure times (<5 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 *Ralstonia solanacearum* race 3 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 a pH of 6.4. Preparation took place one day in advance to achieve an adaptation to the ambient temperature of about 19 °C.

Immediately before the first treatment the nutrient solution was contaminated with a rifamycin resistant *Ralstonia solanacearum* race 3 (strain B74Gshm). From a 48 h plate culture (YDC agar) a bacterial suspension (Ringer solution) was prepared (OD 60 %) and an aliquot added to the 800 L nutrient solution resulting in a density of 460 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 (YD agar containing 120 ppm rifamycin) immediately (<5 min), 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 transformed by $x'=\log10(1 + \text{cfu ml}^{-1})$ and statistically analysed by ANOVA and significant differences to the control were determined by the Dunnett-test at a p-level of <0.05 (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	0.5
2.0	1.6	1.6	1.6	1.6
4.0	4.0	4.0	4.0	4.0

Results

The AquaHort® treatment with the lower Cu-concentration of 2 ppm showed significant reductions of the bacterial counts at exposure times of 1 hr and more. Efficiency rates after 1 hr were 96.5 and 95.5 % for the low and high concentration. Immediately (<5min) after treatment no significant impact could be recorded. Exposures times of 4 and 24 hrs completely killed the bacteria even at the concentration of 2 ppm (1.6 ppm Cu determined). A 2 hr treatment eliminated the bacterium completely at the high concentration and reduced the contamination to almost zero at the lower concentration, producing an efficiency rate of 99.6 %.

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

treatment	**exposure time**				
	< 5min	**1h**	**2h**	**4h**	**24h**
control	478	522	450	482	3096
2 ppm	473	*18*	*2*	*0*	*0*
4 ppm	445	*23*	*0*	*0*	*0*
values in italics are significantly (p<0.05) different from the control (Dunnett-Test)					

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