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project: Wo0602

REPORT

(04 Jan 2007)

Efficacy of Water Treatment with the AquaHort®-System against *Clavibacter michiganensis* ssp. *michiganensis*

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Client:
Aqua-Hort® Danmark ApS, Aksel de Lasson
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Objective

To test the efficacy of the Aqua-Hort®-System against *Clavibacter michiganensis* ssp. *michiganensis* in a range of 0 to 4 ppm Cu at various exposure times (1 to 24 hrs).

Material and Methods

Aqua-Hort® Danmark ApS installed an 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 *Clavibacter michiganensis* 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 6.0 by adding sulphuric acid. The temperature of the nutrient solution was about 14 °C.

Immediately before the first treatment the nutrient solution was contaminated with *Clavibacter michiganensis* ssp. *michiganensis* (strain B011Gshm). From a 48 h plate culture (YDC agar) a bacterial suspension (Ringer solution) was prepared (OD 51 % T) and an aliquot added to the 800 L nutrient solution to achieve a density of about 10^3 cfu/ml. The nominal Cu-concentrations (displayed on the unit) were 2 and 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
2.0	2.0	2.0	2.0	2.0
4.0	3.5	4.0	3.8	4.0

Results and Discussion

At the shortest exposure time of 1 hour only the high concentration of 4 ppm Cu reduced the survival of bacteria significantly (efficiency rate: 65 %). At an exposure time of 2 hrs both concentrations significant effects could be observed (efficiency rates 91 and 94 % resp.). At longer exposure times of 4 and 24 hrs in particular the efficiency rate of both concentrations was about 98 %. After an exposure time of 24 hrs the concentration of 2 ppm resulted in an elimination of 99.7 %.

Table 3: Means of bacterial counts (cfu/ml) of *Clavibacter michiganensis* ssp. *michiganensis* in a fertiliser solution after treatment at different Cu-concentrations and exposure times

treatment	**exposure time**			
	1h	**2h**	**4h**	**24h**
control	582	550	342	545
2 ppm	270	*52*	*7*	*2*
4 ppm	*203*	*33*	*8*	*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 *Clavibacter michiganensis* ssp. *michiganensis* at least 4 ppm at a 24 hr exposure time have been necessary. However, already after an exposure time of only 2 h the efficiency rates were about 90 % and after 4 hrs about 98 %.

Geisenheim, 04 January 2007

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