REFEREED MANUSCRIPT

Proc. Fla. State Hort. Soc. 124:281–284. 2011.

Efficacy of Copper Sanitizers in Subirrigation Tanks

GAVIN S. MOHAMMAD-POUR, DALE W. HASKELL, JINSHENG HUANG, AND PAUL R. FISHER*

University of Florida, Environmental Horticulture Department, P.O. Box 110670, Gainesville FL 32611-0670

ADDITIONAL INDEX WORDS. copper ionization, copper salt, algae, chlorophyll, yeast and mold, aerobic bacteria, Fe-EDDHA, Fe-EDTA

Algae growth in irrigation systems can increase equipment clogging and insect pest problems, increases the required application rate for sanitizing agents for controlling waterborne plant pathogens, decreases aesthetic quality on growing media surfaces of ornamental plants, and can be a worker safety hazard on floors. The objective was to quantify the efficacy of copper for inhibiting growth of algae-inoculated nutrient solutions. Transparent vessels (500 mL) contained a blend of subirrigation solution used in irrigation at a Florida nursery, 100 mg·L^{-1} N from a water soluble fertilizer (17N–1.8P–14.1K), eight Cu concentrations from 0 to 8.0 mg·L^{-1} from either Cu ionization via electrolysis or $Cu(NO_3)_2$, Fe at 1 mg·L^{-1} from either FeEDTA or FeEDDHA, and deionized water, with pH correction to 6.0. After 16 days of incubation in a greenhouse environment, vessels containing 0 to 0.25 mg·L^{-1} Cu from $Cu(NO_3)_2$ showed similar increases in chlorophyll and biomass, after which algae content decreased to near zero at 4 mg·L^{-1} Cu. Yeast and mold density followed similar trends with a 99.9% drop at 2 mg·L^{-1} and a near zero colony count at 4 mg·L^{-1}. Cu ionization was more effective at a given mg·L^{-1} Cu than $Cu(NO_3)_2$ at controlling algae and fungi. Algae content was higher with FeEDDHA than FeEDTA at concentrations of Cu below 2 mg·L^{-1}. Aerobic bacteria count, which is an indicator of potential equipment clogging from biofilm, was resistant to all Cu levels and was above recommended levels for irrigation water, averaging 3.56 × 10^6 colony forming units/mL. This study establishes a protocol for quantifying algal content that can be applied to test the effectiveness of other algaecides.

Cu(II) ions are widely used as algaecides and fungicides in irrigation systems and other applications such as swimming pools as an alternative to chlorination. The mode of action for copper ions may result from activity at the cell or capsid protein surface or on the nucleic acid of cells or viruses (Thurman and Gerba, 1989). Historically, the more common form of copper delivery is as a salt (Chase and Conover, 1993), paired with an anion such as sulfate, which is low cost and widely available. However, more recently there has been an increasing trend towards copper applied by way of electrolysis of elemental copper (Wohanka and Fehres, 2007). Copper bars or plates are exposed to a solution containing sufficient ionic content to facilitate an electric exchange. Through a redox reaction, the neutral copper is stripped of two valence electrons (oxidized) and dissolves into the solution. The electrons reduce water molecules, creating two hydroxide ions and releasing hydrogen gas. Copper dosage rates have a linear relationship with electrical conductivity at a given electrical charge.

Manufacturers of copper ionization units claim that copper from electrolysis provides more effective control of algae than an equivalent concentration of copper salts. However, scientific data comparing copper forms are limited.

Other considerations regarding copper as an algaecide are its interactions with various macro and micro nutrients, most notably the chelates used to keep Fe nutrients dissolved in solution (Toppe and Thinggaard, 1998). Because of the tendency of Fe at higher pH levels to form oxides and precipitate out of solution, chelating agents such as EDTA and EDDHA are used to create metal complexes that keep the Fe dissolved over a wider range of concentrations and pH levels (Bugter and Reichwein, 2007). A potential issue is that Cu(II) has a slightly higher affinity for both EDTA and EDDHA than that of Fe(II). If a substitution reaction occurs, Cu(II) may remain in solution as a copper chelate, but may potentially become less active as an algaecide.

The purpose of this research was to test the efficacy of copper salt and copper ionization for control of algae, yeast, mold and aerobic bacteria based on measurements of chlorophyll, biomass and microbial density, in the presence of two different iron chelate types (Fe-EDTA and Fe-EDDHA).

Materials and Method

Recirculated irrigation water was obtained from a commercial greenhouse (Agristarts, Apopka, Fl.) subirrigation tank that contained irrigation return water applied previously to crops on greenhouse benches, to provide a source of algae and other microbes. The algae were grown further in an open tank in a greenhouse supplemented with water-soluble fertilizer (17N–1.8P–14.1K; Greencare 17–4–17, Kankakee, IL) with 100 mg·L^{-1} (ppm) until enough algae inoculum was available for the trial. The algae was identified and enumerated by GreenWater Laboratories (Palatka, FL). The sample was dominated by green algae (chlorophyta) along with very small population of microflagellates and nitzchia. Microorganism analysis performed by University of Massachusetts, Plant Diagnostic Laboratory (Amherst, MA) found no *Pythium, Phytophthora, Rhizoctonia*, or *Fusarium* species present.

The fertilizer/copper/ iron-chelate solution treatments consisted of 100 mg·L^{-1} N from a water-soluble fertilizer (17N–1.8P–14.1K), eight copper concentrations (0, 0.125, 0.25, 0.5, 1, 2, 4, and 8

We thank the industry partners of the Young Plant Research Center (floriculturealliance.org) for supporting this research. The use of trade names in this publication does not imply endorsement of the products named or criticism of similar ones not mentioned.

*Corresponding author; phone: (352) 273-4581; email: pfisher@ufl.edu

mg·L^{-1}), two copper forms (ionization and $CuNO_3$ salt), along with two iron chelates (1 ppm of either Fe-EDTA or Fe-EDDHA)with pH correction to 6.0 in de-ionized water (Table 1). Transparent polypropylene vessels (946 mL; Phytotechnology, Shawnee Mission, KS) contained a total of 500 mL (450 mL fertilizer/copper/iron-chelate solution and 50 mL algae inoculum). Polypropylene lids with a 1.5-cm hole that was covered with gas exchange tape were snapped on each vessel. A randomized complete-block design with three blocks was used. Each block started one week apart and terminated after 16 d for data collection. Each of three sequential weeks therefore represented a block, with one vessel per combination of copper form, copper concentration, and iron chelate form replicated on each week.

The experiment was conducted on a greenhouse bench from 7 Feb. to 9 Mar. 2011, at an average 21.1 ± 3.7 °C air temperature, 19.4 ± 2.7C solution temperature, and 14.0 ± 3.7 mol·m^{-2}·d^{-1} of *PAR* light (mean ± standard deviation). Free and total copper were measured at the beginning and end of the trial using a Thermo Scientific Orion AQUAfast IV® AQ4000 colorimeter with Hach® free copper (Cat. 21823-69) and total copper (Cat. 2118869) reagents. Solution pH and EC were measured using a Thermo Scientific Orion 5 Star Bench-top meter. At the end of experiment, the solutions were filtered for chlorophyll and biomass determinations. For each treatment, a 50-mL sample of solution was filtered with a Whatman® 24-mm diameter 934-AH 1.5-µm fiberglass filter to collect algae that was then placed in 10 mL of dimethyl sulfoxide and heated to 65 °C for 40 min so as to extract the chlorophyll. The total chlorophyll concentration of resulting solution was determined using a UV-Vis Spectrophotometer setting to 645 and 663 nm. An additional 50-mL sample was filtered with a Whatman® 47-mm-diameter 934-AH 1.5-µm fiberglass filter and the filtrate was then oven-dried at 105 °C for 60 min for total biomass determination. Additionally, for each treatment, a 5-mL sample was collected in a sterile glass tube for determination of aerobic bacteria, and yeasts and molds, using 3M Petrifilms (3M Center, St. Paul, MN) at dilutions of 100 to 106.

Data were subject to SAS ANOVA (SAS PROC GLM) with mean separation using Tukey's HSD at $\alpha = 0.05$ level, and factors including the block, and interaction effects of copper type, copper concentration, and iron chelate type.

Results

The measured initial total copper concentration matched closely with the target concentrations (Table 1). Except for the low copper concentration treatments, the final total copper concentrations

Table.1. Experiment consisted of 32 treatments with the concentrations of initial free copper, initial total copper, final free copper, final total copper, and final pH and EC (means ± standard error, n = 3). Initial pH was 6.0, and EC was 0.96 mS/cm.

Copper form	Fe chelate form (1 ppm Fe)	Copper concn (mg·L^{-1})	Inital free Cu (mg·L^{-1})	Initial total Cu (mg·L^{-1})	Final pH	Final EC (mS/cm)	Final free Cu (mg·L^{-1})	Final total Cu (mg·L^{-1})
Ionization	EDDHA	0	0.01 ± 0.01	0.05 ± 0.02	6.76 ± 0.49	0.91 ± 0.01	0.05 ± 0.04	0.42 ± 0.42
Ionization	EDDHA	0.125	0.07 ± 0.02	0.21 ± 0.01	6.00 ± 0.62	0.91 ± 0.01	0.11 ± 0.07	0.42 ± 0.42
Ionization	EDDHA	0.25	0.11 ± 0.03	0.38 ± 0.05	6.05 ± 0.75	0.92 ± 0.01	0.11 ± 0.07	0.30 ± 0.30
Ionization	EDDHA	0.5	0.32 ± 0.03	0.51 ± 0.06	5.49 ± 0.53	0.94 ± 0.02	0.20 ± 0.05	0.55 ± 0.42
Ionization	EDDHA	1	0.77 ± 0.12	1.03 ± 0.03	5.34 ± 0.24	0.94 ± 0.00	0.28 ± 0.03	0.85 ± 0.24
Ionization	EDDHA	2	1.56 ± 0.06	1.86 ± 0.09	6.41 ± 0.01	0.97 ± 0.01	0.72 ± 0.06	1.67 ± 0.18
Ionization	EDDHA	4	3.45 ± 0.08	3.80 ± 0.06	6.31 ± 0.00	0.98 ± 0	1.96 ± 0.39	3.25 ± 0.68
Ionization	EDDHA	8	7.63 ± 0.2	8.10 ± 0.26	6.12 ± 0.03	1.02 ± 0.01	6.34 ± 0.61	7.44 ± 0.66
Ionization	EDTA	0	0.02 ± 0.01	0.02 ± 0.01	6.55 ± 0.39	0.92 ± 0.01	0.07 ± 0.03	0.14 ± 0.09
Ionization	EDTA	0.125	0.17 ± 0.01	0.15 ± 0.03	5.41 ± 0.51	0.92 ± 0.02	0.12 ± 0.05	0.23 ± 0.13
Ionization	EDTA	0.25	0.26 ± 0.03	0.25 ± 0.02	5.19 ± 0.38	0.93 ± 0.02	0.24 ± 0.11	0.36 ± 0.13
Ionization	EDTA	0.5	0.43 ± 0.03	0.52 ± 0.03	5.92 ± 0.22	0.93 ± 0.02	0.46 ± 0.04	0.48 ± 0.03
Ionization	EDTA	1	0.82 ± 0.04	0.90 ± 0.02	5.94 ± 0.21	0.93 ± 0.02	0.63 ± 0.07	0.93 ± 0.16
Ionization	EDTA	2	1.66 ± 0.05	1.86 ± 0.01	6.32 ± 0.01	0.94 ± 0.02	0.82 ± 0.1	1.78 ± 0.16
Ionization	EDTA	4	3.24 ± 0.12	3.73 ± 0.03	6.25 ± 0.02	0.93 ± 0.02	2.04 ± 0.53	2.92 ± 0.49
Ionization	EDTA	8	7.53 ± 0.34	8.15 ± 0.32	6.06 ± 0.00	0.92 ± 0.03	5.54 ± 1.44	6.26 ± 1.36
$CuNO_3$	EDDHA	0	0.00 ± 0.00	0.04 ± 0.04	6.83 ± 0.08	0.91 ± 0.00	0.06 ± 0.06	0.37 ± 0.37
$CuNO_3$	EDDHA	0.125	0.06 ± 0.02	0.14 ± 0.03	6.74 ± 0.11	0.91 ± 0.00	0.12 ± 0.04	0.32 ± 0.28
$CuNO_3$	EDDHA	0.25	0.14 ± 0.02	0.38 ± 0.03	6.92 ± 0.16	0.91 ± 0.01	0.10 ± 0.03	0.53 ± 0.42
$CuNO_3$	EDDHA	0.5	0.33 ± 0.01	0.56 ± 0.02	7.41 ± 0.52	0.91 ± 0.01	0.20 ± 0.04	0.81 ± 0.35
$CuNO_3$	EDDHA	1	0.80 ± 0.04	1.20 ± 0.11	5.16 ± 0.16	0.93 ± 0.00	0.40 ± 0.06	1.44 ± 0.36
$CuNO_3$	EDDHA	2	1.71 ± 0.04	2.03 ± 0.04	5.94 ± 0.36	0.95 ± 0.00	0.72 ± 0.05	2.46 ± 0.58
$CuNO_3$	EDDHA	4	3.46 ± 0.08	3.77 ± 0.13	6.36 ± 0.01	0.97 ± 0.01	1.70 ± 0.75	3.16 ± 0.59
$CuNO_3$	EDDHA	8	7.36 ± 0.10	7.97 ± 0.11	6.27 ± 0.02	0.99 ± 0.01	5.64 ± 1.06	6.73 ± 1.28
$CuNO_3$	EDTA	0	0.02 ± 0.01	0.02 ± 0.01	6.50 ± 0.18	0.90 ± 0.00	0.12 ± 0.12	0.08 ± 0.08
$CuNO_3$	EDTA	0.125	0.12 ± 0.00	0.13 ± 0.03	6.61 ± 0.08	0.90 ± 0.00	0.08 ± 0.04	0.15 ± 0.1
$CuNO_3$	EDTA	0.25	0.24 ± 0.01	0.25 ± 0.00	6.75 ± 0.17	0.91 ± 0.01	0.14 ± 0.02	0.35 ± 0.13
$CuNO_3$	EDTA	0.5	0.47 ± 0.03	0.51 ± 0.03	6.01 ± 0.00	0.92 ± 0.01	0.28 ± 0.04	0.62 ± 0.11
$CuNO_3$	EDTA	1	0.96 ± 0.01	1.01 ± 0.01	5.66 ± 0.38	0.92 ± 0.01	0.57 ± 0.08	1.05 ± 0.08
$CuNO_3$	EDTA	2	1.83 ± 0.01	1.97 ± 0.03	6.10 ± 0.09	0.96 ± 0.01	0.84 ± 0.14	1.83 ± 0.27
$CuNO_3$	EDTA	4	3.57 ± 0.08	3.95 ± 0.05	6.30 ± 0.01	0.97 ± 0.01	2.28 ± 0.46	3.52 ± 0.57
$CuNO_3$	EDTA	8	7.38 ± 0.08	7.86 ± 0.20	6.19 ± 0.01	0.99 ± 0.01	5.96 ± 0.95	5.55 ± 1.02

Table 2. Statistical analysis results for algae chlorophyll, total biomass, yeast and mold, and aerobic bacteria.

	Chlorophyll		Biomass		Yeast and mold		Aerobic bacteria	
Source	*P*-value	Significance	*P*-value	Significance	*P*-value	Significance	*P*-value	Significance
Copper form	<0.0001	***	<0.0001	***	0.3763	NS	0.1656	NS
Chelate form	<0.0001	***	0.0219	*	0.6806	NS	0.1280	NS
Copper form × chelate form	0.9014	NS	0.0534	NS	0.6925	NS	0.2456	NS
Copper concn	<0.0001	***	<0.0001	***	<0.0001	***	0.4990	NS
Copper form × copper concn	0.0035	**	0.4934	NS	0.6711	NS	0.8102	NS
Chelate form × copper concn	0.0491	*	0.9632	NS	0.9987	NS	0.7919	NS
Copper form × chelate form × copper concn	0.2474	NS	0.2592	NS	0.5977	NS	0.1340	NS

NS, *, **, ***Nonsignificant or $P \leq 0.05$, 0.01, and 0.001, respectively.

at day 16 tended to be lower than the corresponding initial total copper concentration. Final copper concentration measurements for the low level of copper treatments were slightly higher than the corresponding initial concentration, which may have resulted from a lower solution volume. Overall the final EC values in the end were similar to the initial EC value (0.96 mS·cm^{-1}).

Algae growth was estimated by the amount of chlorophyll and total dry biomass. Both chlorophyll content and biomass were significantly influenced by copper forms, copper concentration and iron chelate forms (Table 2). Both forms of copper showed inhibitive effects on algae growth as indicated by chlorophyll (Fig. 1A) and biomass (Fig. 1B). However, copper from the ionization was more effective in inhibiting algae growth than copper nitrate salt, showing complete algae control at 2 and 4 mg·L^{-1} for copper ionization and $CuNO_3$, respectively (Fig .1A). Averaged across copper concentrations, chlorophyll content for copper nitrate salt and copper ionization was 14.9 and 8.3 mg·L^{-1}, respectively. The average biomass was 76.3 and 54.1 mg·L^{-1} for copper nitrate salt and copper ionization across copper concentrations, respectively.

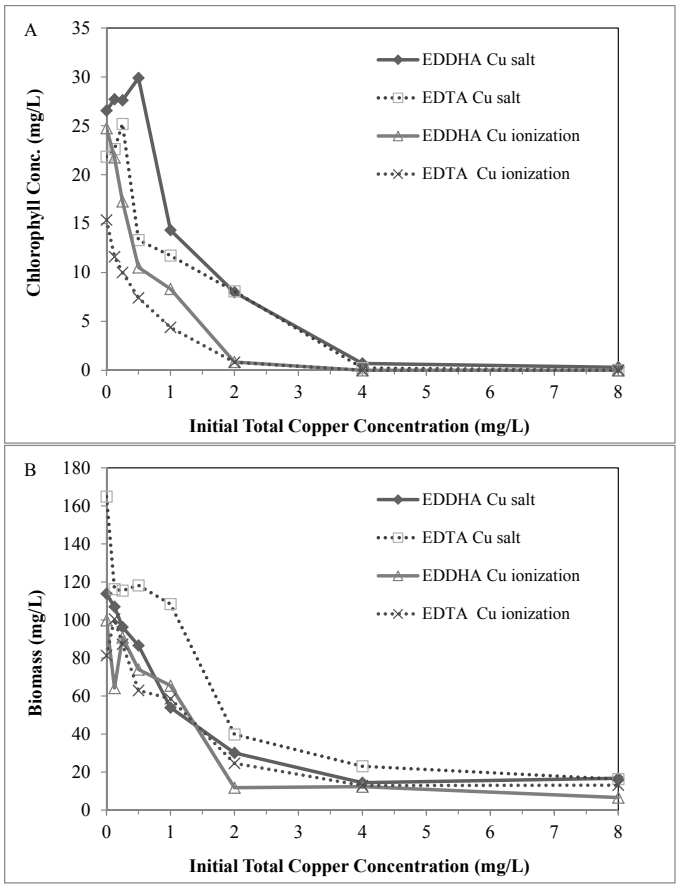

Fig. 1. Effect of copper form (ionization or nitrate salt) and concentration (0 to 8 mg/L) on (**A**) chlorophyll concentration and (**B**) total biomass with two different iron chelate forms (EDTA or EDDHA). Overall 95% CI for (**A**) was ±3.08 mg/L and (**B**) ±17.76 mg/L.

Chelate forms influenced the efficacy of copper sanitizers. The average chlorophyll concentration for Fe-EDDHA solutions was 13.7 mg·L^{-1}, compared with 9.5 mg·L^{-1} for Fe-EDTA solutions. For both forms of copper ionization and copper nitrate salt, vessels with Fe-EDDHA also showed a higher amount of chlorophyll compared with equivalent copper concentration with Fe-EDTA (Fig. 1A). The amount of biomass for each chelate type differed from the trend in chlorophyll, whereby Fe-EDTA solutions had higher biomass (71.5 mg/L) than Fe-EDDHA solutions (59 mg·L^{-1}).

Algae growth was inhibited as increasing copper concentration for both copper forms in either EDTA or EDDHA solutions (Fig.1A–B).The chlorophyll content was close to zero at 2 mg·L^{-1} total copper from copper ionization and at 4 mg·L^{-1} total copper from $CuNO_3$ salt, respectively (Fig.1A). Biomass estimates followed similar trends with a drop of approximately 85% at 2 mg·L^{-1} of total copper concentration (Fig.1B). With both chelate forms, solutions with the first two copper concentrations of 0.125 ppm and 0.250 ppm resulted in higher growth than the control for the samples dosed with copper nitrate, but resulted in an inhibition over the control for vessels dosed by copper ionization (Fig.1A).

Yeast and mold were also inhibited as copper concentrations increased for both copper forms, and regardless of chelate solution (Fig. 2A). Yeast and mold content had a 99.9% drop at 2 mg·L^{-1} of total copper concentration and a near zero count at 4 mg·L^{-1} of total copper concentration. Copper sanitizers did not have any control of aerobic bacteria over the 0 to 8 mg·L^{-1} range (Table 2 and Fig. 2B). Aerobic bacteria count, which is an indicator of potential equipment clogging from biofilm, was very high, averaging 3.56 × 10^6 colony forming units (CFU)/mL.

Conclusions

Overall, the study indicated that copper ionization provided more effective control of algae and fungi at a given concentration than copper nitrate. However, neither copper form controlled aerobic bacteria. Copper concentrations of 2 to 4 mg·L^{-1} provided effective algae control. Further testing is required to ensure that this copper concentration is not phytotoxic to crop plants. This study establishes a protocol for quantifying algal content that can be applied to test the effectiveness of other algaecides.

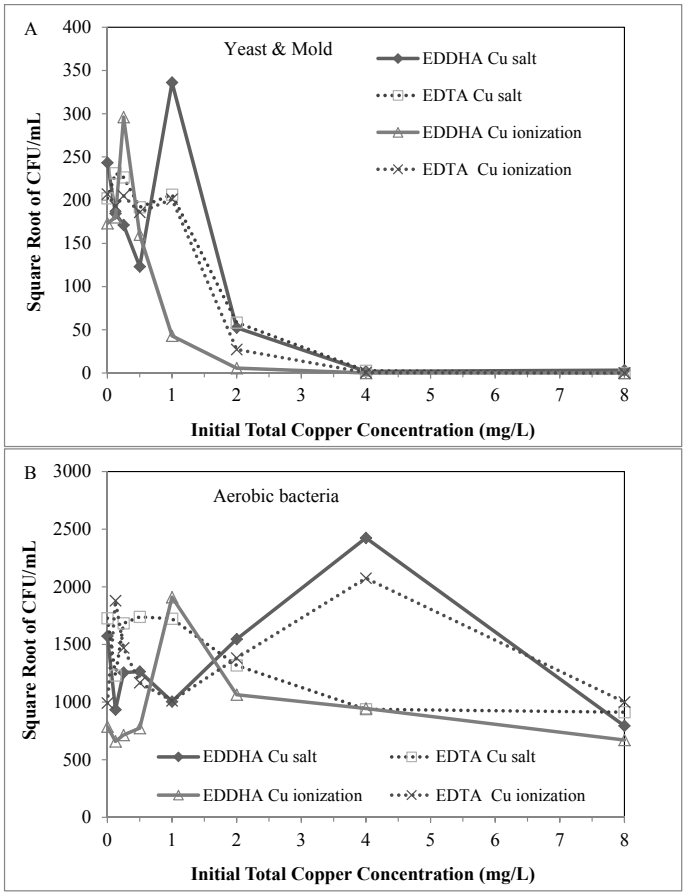

Fig. 2. Effect of copper form (ionization or nitrate salt) and concentration (0 to 8 mg/L) on density of (**A**) yeasts and molds and (**B**) aerobic bacteria with two different iron chelate forms (EDTA or EDDHA). Both yeast and mold count and aerobic bacteria count was in CFU (colony forming units) and the units were transformed to square root in the y-axis. Overall 95% CI for (**A**) was ±223.4 cfu/mL and (**B**) ±83.1 cfu/mL.

Literature Cited

Bugter, M.H.J. and A.M. Reichwein. 2007. pH stability of Fe-chelates in soilless culture. Acta Hort. 742:61–66.

Chase, A.R. and C.A. Conover. 1993. Algae control in an ebb and flow irrigation system. Proc. Fla. State Hort. Soc. 106:280–282.

Thurman, R.B. and C.P. Gerba. 1989. The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. Crit. Rev. Eviron. Control 18(4):295–315.

Toppe, B. and K. Thinggaard. 1998. Prevention of phytophthora root rot in gerbera by increasing copper ion concentration in the nutrient solution. European J. Plant Pathol. 104:359–366.

Wohanka, W. and H. Fehres. 2007. Efficacy of water treatment with the Aquahort® system against *Clavibacter michiganensis* ssp. *michiganensis*. Retrieved from Geisenheim Research Center, Dpt. Phytomedicine. 1 July 2011. <http://www.schetelig.com/file.php?855>.