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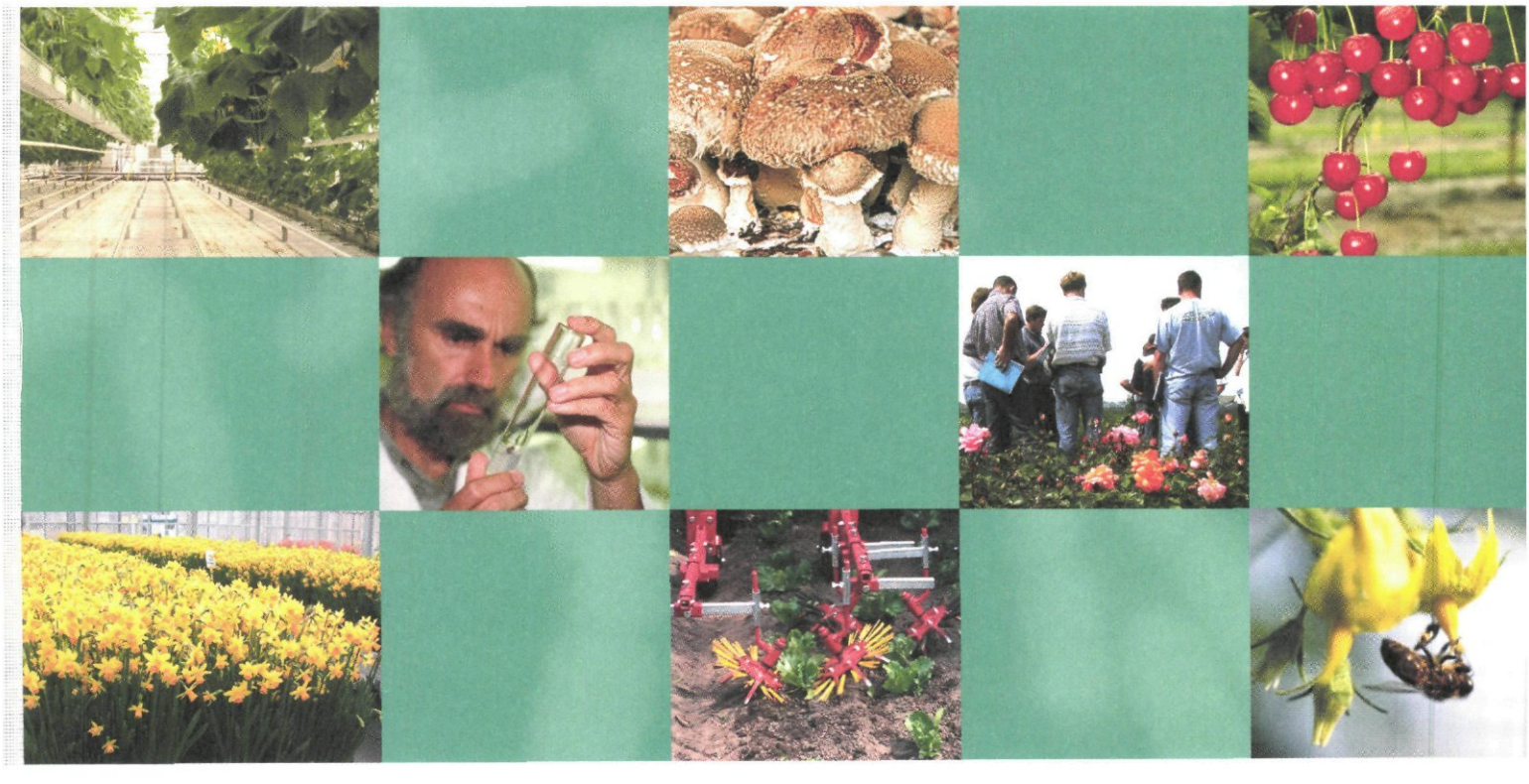

Study on the feasibility of Walenius AOT™ water purificationsystem from Senmatic A/S

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1 Introduction

In greenhouses in the Netherlands most vegetables, pot plants and some flowers (roses and gerberas) are grown in closed breeding systems on artificial substrates with re-use of the nutrient solution. This re-use means that there is a risk involving the introduction and spreading of pathogens in the system. Once the pathogens are present in the system, they can easily be spread through the nutrient solution (for example). The grower has to take measures to prevent the introduction of pathogens. In case that the pathogens are already present to prevent the spreading. The water can be disinfected in several ways; with ozone, heating or ultraviolet radiation. Recently a new water purification system was put on the market. This system, the Walenius AOT™ water purification system uses a new unique patented principle of **A**dvanced **O**xidation **T**echnology.
Applied Plant Research location Naaldwijk, has performed research on the feasibility of the system as a disinfective against a plant-pathogene fungus and virus.

Applied Plant Research (PPO) is the leading Dutch organisation for applied research in arable farming, multifunctional agriculture and outdoor vegetable growing, bees, flower bulbs, nursery stock, fruit, greenhouse horticulture and mushrooms. PPO aims at co-innovations with partners from the different agricultural sectors, sciences, industry and government. PPO analyses together with clients questions regarding farm and crop management and translates these into applied research and development programmes.

2 Objective

The objective of this research was to examine the feasibility of the Walenius AOT™ water purification system as a disinfective against a plant-pathogene fungus and virus in water and nutrient solution for breeding the plants.

3 Materials and methods

3.1 The Walenius AOT™ water purification system

The Walenius AOT™ water purification system from Senmatic A/S was installed at Applied Plant Research location Naaldwijk, the Netherlands, where the tests were performed. The tests were performed with clean rain water (without addition of any nutrients) and a standard nutrient solution. The water came from the basin of the Applied Plant Research in Naaldwijk, the nutrient solution was prepared especially for the tests, by using the recipe shown in Appendix 1. The tests with water were performed at two different flow rates: 200 L/min and 300 L/min. The tests with nutrient solution were performed only at a flow rate of 200 L/min. Samples of the nutrient solution were taken for analysis of the nutrients, before and after the test. In all tests the water and nutrient solution with the pathogens were flushed through the system once.

3.2 The fungus

The tests were performed with *Fusarium oxysporum* f. sp. *lycopersici*. In the tests, conidiospores of the fungus were added to 1000 liter of water (for each flow rate), and 1000 liter of nutrient solution. The inoculated water was flushed through the system at the requested flow rates (see Appendix 2 for the settings of the system during the tests). During the tests, samples were taken from the 7 sample points in the system. The efficacy of the system was examined by plating the samples in duplicate in a range of dilutions on a *Fusarium*-specific agar. After five days the grown colonies of *Fusarium* were counted. The tests with *Fusarium* were performed on September 14, 2006.

3.3 The virus

The virus-test was performed with the tomato-mosaicvirus (ToMV). This virus belongs to the group of Tobamo-viruses. Also well-known viruses like Cucumber Green Mottle Mosaic Virus (CGMMV) and Pepper Mild Mottle Virus (PeMV) belong to this group. Viruses from the Tobamo-group are highly contagious and can, among other things, be spread by water. In the tests, the virus was added to 1000 liter of water (for each flow rate), and 1000 liter of nutrient solution. The inoculated water was flushed through the system at the requested flow rates (see Appendix 2 for the settings of the system during the tests). During the tests, samples were taken from the 7 sample points in the system. The efficacy of the system was examined in a bio-assay. Per sample 3 plants were used of which 2 leafs were inoculated. Three days after the inoculation of the plants the number of formed lesions on the leafs were counted. The virus-test was performed on October 16, 2006.

4 Results

4.1 Fungus

The results of the fungus-test, performed in water are shown in Table 1. The results of the fungus-test, performed in nutrient solution, are shown in Table 2. In these tables the average (two plates per sample point) amount of colony forming units (cfu) per treatment are shown. The efficacy of the system was compared to the inoculated, untreated water or inoculated, untreated nutrient solution (in the tables shown as "+ inoculum, untreated") and an untreated control (in the tables shown as "- inoculum, untreated).

Table 1 The average amount of colony forming units (cfu) per ml of water, at a flow rate of 200 L/min and 300 L/min

	200 L/min # cfu/ml (n=2)	efficacy (%)	300 L/min # cfu/ml (n=2)	efficacy (%)
- inoculum, untreated	0.00E+00		0.00E+00	
+ inoculum, untreated	3.65E+04		2.95E+04	
After unit 1, before unit 2	1.50E+04	58.9	1.24E+04	58.0
After unit 2, before unit 3	5.60E+03	84.7	8.40E+03	71.5
After unit 3, before unit 4	3.50E+02	99.0	2.70E+03	90.8
After unit 4, before unit 5	0.00E+00	100.0	1.50E+02	99.5
After unit 5, before unit 6	0.00E+00	100.0	0.00E+00	100.0
After unit 6	5.00E+00	100.0	2.00E+01	100.0

Table 2 The average amount of colony forming units (cfu) per ml of nutrient solution, at a flow rate of 200 L/min.

	200 L/min # cfu/ml (n=2)	efficacy (%)
- inoculum, untreated	0.00E+00	
+ inoculum, untreated	9.50E+03	
After unit 1, before unit 2	2.09E+03	78.0
After unit 2, before unit 3	2.18E+03	77.1
After unit 3, before unit 4	1.27E+03	86.7
After unit 4, before unit 5	4.70E+02	95.1
After unit 5, before unit 6	2.30E+02	97.6
After unit 6	9.50E+01	99.0

The results show that in the test with water at a flow rate of 200 L/min after unit 4 no *Fusarium* was found. In the test with water at a flow rate of 300 L/min no *Fusarium* was found after unit 5. In the test with nutrient solution at a flow rate of 200 L/min there still was *Fusarium* present after unit 6.

4.2 Virus

The results of the virus-test, performed in water at a flow rate of 200 L/min are shown in Table 3.
The results of the virus-test, performed in water at a flow rate of 300 L/min are shown in Table 4.
The results of the virus-test, performed in nutrient solution at a flow rate of 200 L/min are shown in Table 5.
In these tables the average of lesions per two leafs per plant are shown. The efficacy of the system was compared to the inoculated, untreated water or inoculated, untreated nutrient solution (in the tables shown as "+ inoculum, untreated") and an untreated control (in the tables shown as "- inoculum, untreated).

Table 3 The average amount of lesions per two leafs per plant, samples of water at a flow rate of 200 L/min,

	plant 1 (n=2)	plant 2 (n=2)	plant 3 (n=2)	total (n=3)	efficacy (%)
- inoculum, untreated	0.0	0.0	0.0	0.0	
+ inoculum, untreated	205.5	123.0	131.5	153.3	
After unit 1, before unit 2	11.0	8.0	13.0	10.7	93.0
After unit 2, before unit 3	19.0	15.5	10.5	15.0	90.2
After unit 3, before unit 4	2.5	2.5	0.0	1.7	98.9
After unit 4, before unit 5	0.5	1.0	0.0	0.5	99.7
After unit 5, before unit 6	1.0	1.0	0.0	0.7	99.6
After unit 6	0.0	0.5	0.0	0.2	99.9

Table 4 The average amount of lesions per two leafs per plant, samples of water at a flow rate of 300 L/min

	plant 1 (n=2)	plant 2 (n=2)	plant 3 (n=2)	total (n=3)	efficacy (%)
- inoculum, untreated	0.0	0.0	0.0	0.0	
+ inoculum, untreated	205.5	123.0	131.5	153.3	
After unit 1, before unit 2	47.5	44.0	42.0	44.5	71.0
After unit 2, before unit 3	39.5	36.0	52.5	42.7	72.2
After unit 3, before unit 4	23.0	18.0	25.5	22.2	85.5
After unit 4, before unit 5	11.0	11.5	7.5	10.0	93.5
After unit 5, before unit 6	11.0	4.0	5.5	6.8	95.5
After unit 6	0.0	0.0	0.5	0.2	99.9

Table 5 The average amount of lesions per two leafs per plant, samples of nutrient solution at a flow rate of 200 L/min

	plant 1 (n=2)	plant 2 (n=2)	plant 3 (n=2)	total (n=3)	efficacy (%)
- inoculum, untreated	0.0	0.0	0.0	0.0	
+ inoculum, untreated	52.0	43.0	56.0	50.3	
After unit 1, before unit 2	18.0	28.5	10.5	19.0	62.3
After unit 2, before unit 3	19.5	9.5	38.5	22.5	55.3
After unit 3, before unit 4	23.0	22.5	22.0	22.5	55.3
After unit 4, before unit 5	5.5	6.0	13.0	8.2	83.8
After unit 5, before unit 6	4.0	2.5	2.0	2.8	94.4
After unit 6	6.0	1.5	6.5	4.7	90.0

In all the tests the amount of virus was already decreased after the first unit. The amount of virus keeps decreasing but an efficacy of 100% was not reached in any of the tests. The amount of virus in the test with nutrient solution after the last unit was higher in comparison to the amount of virus in both tests with water.

4.3 Analysis of nutrient solution

During each test, samples were taken from the nutrient solution before and after the system. The results of the analysis of the nutrient solution before and after the tests are shown in Appendix 3.
The amount of nutrients decreased after the nutrient solution was flushed through the purification system in the test of October 16, 2006.
The runs with nutrient solution were performed after the runs with water. Unfortunately, the nutrient solution could not be flushed through the system without the Walenius AOT™ units switched off. While flushing through the system, the nutrient solution was diluted by water still present in the system. This could explain the decrease in the amount of nutrients. The samples of the nutrient solution, used in the Fusarium test, were taken after the nutrient solution had already flushed through the system. This could explain that in this test there was no decrease in the amount of nutrients.

5 Conclusions and discussion

5.1 Fusarium

There was a decrease of the amount of *Fusarium* in all of the experiments (water at a flow rate of 200 L/min and 300 L/min, nutrient solution at 200 L/min). In the test with water at a flow rate of 200 L/min an efficacy of 100% was reached after the fourth unit. In the test with water at a flow rate of 300 L/min an efficacy of 100% was reached after the fifth unit. The pace in which the water flushes through the system has an effect on the efficacy against *Fusarium oxysporum* f. sp. *lycopersici*.
The test with nutrient solution shows a clear decrease of the amount of *Fusarium*. After the first unit already an efficacy of 78% was reached. After the last unit there was an efficacy of 99%.
The efficacy of the system was better when only water without nutrients was used.

5.2 Virus

The tests show a clear decrease in the amount of virus in all of the experiments (water at a flow rate of 200 L/min and 300 L/min, nutrient solution at a flow rate of 200 L/min). In both tests with water an efficacy of 99.9% was reached, while in the nutrient solution at a flow rate of 200 L/min an efficacy of 90% against ToMV (tomato mosaic-virus).
The pace in which the water flushes through the system has no effect on the efficacy. The efficacy of the system was better when only water without nutrients was used.

Appendix 1 Recipe of used nutrient solution

Fertilizers from Substrafeed liquid fertilizer (supplier Yara, formerly Hydro Agri)

Fertilizers are mixed into tank A and tank B. Dilution of these tanks with very pure rain water (without bicarbonate and other elements) until EC = 2.3 mS/cm (25°C) and pH = 5.5

Contents in A-tank, of 1 m^3 – 100 times concentrated

Calcium nitrate – liquid; CALSAL	– 96 L (= 144 kg)
Ammonium nitrate - liquid; AMNITRA	– 31 L (= 39 kg)
Magnesium nitrate – liquid; MAGNITRA	– 59 L (= 80 kg)
Iron DTPA (6 % Fe) – solid	– 2.75 kg
Manganese sulphate – solid	– 200 g
Zinc sulphate - solid	– 150 g
Borax	– 400 g
Copper sulphate (solid)	– 30 g
Sodium molybdate (solid)	– 10 g

Contents in B-tank, of 1 m^3 – 100 times concentrated

Potassium nitrate/nitric acid - liquid; NITRAKAL	– 27 L (= 36 kg)
Potassium sulphate/magnesium sulphate/sulphuric acid liquid; ZWAKAL	– 63 L (= 83 kg)
Potassium carbonate/potassium phosphate - liquid; BFK	– 37 L (= 54 kg)
Potassium carbonate - liquid; BASKAL	– 30 L (= 43 kg)

Recipe of used nutrient solution (in Dutch)

Voedingsoplossing Hydro Agri

Opkweek groenteplanten in steenwol

Zuur (H_3O) 0.0 mmol

Hoeveelheden per 0.10 m3

EC voedingsoplossing: 2.3
EC druppel: 2.0

Oplossing A

Kalksalpeter (CALSAL)	: 14.4 kg (9,6 L)
Ammoniumnitraat (AMNITRA)	: 3.9 kg (3.1 L)
Magnesiumnitraat (MAGNITRA)	: 8.0 kg (5.9 L)
IJzerchelaat DTPA 6%	: 275 g
Mangaansulfaat	: 20 g
Zinksulfaat	: 15 g
Borax	: 40 g
Kopersulfaat	: 3 g
Natriummolybdaat	: 1 g

Oplossing B

Kalisalpeterzuur (NITRAKAL)	: 3.6 kg (2.7 L)
Kalimagnesiumzwavelzuur (ZWAKAL)	: 8.3 kg (6.3 L)
Kalifosforcarbonaat (BFK)	: 5.4 kg (3.7 L)
Kalicarbonaat (BASKAL)	: 4.3 kg (3.0 L)

BASKAL nieuw (7 mol)
AMNITRA is 1 op 1 verdund met water

Belangrijk: Voor het bereiden van de voedingsoplossing eerst beide bakken gedeeltelijk vullen met water. Hierna de meststoffen volgens bovenstaande volgorde toevoegen.

Appendix 2 Settings of the system during the tests

14-09-06 test *Fusarium*

	flow (L/min)	flow meter	pressure before filter (bar)	pressure after filter (bar)
water	200	195 - 210	1.8	1.9
water	300	290 - 300	3.6	3.7
nutrient solution	200	191 - 205	1.7	1.8

16-10-06 test ToMV

	flow (L/min)	flow meter	pressure before filter (bar)	pressure after filter (bar)
water	200	198 - 210	1.6	1.65
water	300	295 - 304	3.6	3.7
nutrient solution	200	195 - 204	1.6	1.65

Appendix 3 Analysis nutrient solution

date of samples		*Fusarium* test 14-9-2006		ToMV test 16-10-2006	
		before the system	after the system	before the system	after the system
EC	mS/cm	2.2	2.2	2.1	1.8
pH		5.8	5.9	6.1	6.2
NH4	mmol/L	1.3	1.4	1.3	1.1
K	mmol/L	5.7	5.7	5.9	4.9
Na	mmol/L	0.6	0.6	0.5	0.6
Ca	mmol/L	4.1	4.2	3.7	3.2
Mg	mmol/L	2.2	2.3	2.1	1.8
NO3	mmol/L	14.6	14.8	13	11.2
Cl	mmol/L	0.3	0.3	0.3	0.3
SO4	mmol/L	1.7	1.7	2	1.6
HCO3	mmol/L	<0.1	<0.1	0.1	0.1
P	mmol/L	1.21	1.25	1.22	0.98
Si	mmol/L	<0.01	<0.01	0.01	0.01
Fe	µmol/L	24	23	20	15
Mn	µmol/L	13	13	9	8.3
Zn	µmol/L	7.9	8.4	7.6	8.1
B	µmol/L	33	33	33	27
Cu	µmol/L	1.5	1.6	1.4	1.1
Mo	µmol/L	0.8	0.8	0.6	0.5

Appendix 3 Analysis nutrient solution

date of samples		*Fusarium* test 14-9-2006		ToMV test 16-10-2006	
		before the system	after the system	before the system	after the system
EC	mS/cm	2.2	2.2	2.1	1.8
pH		5.8	5.9	6.1	6.2
NH4	mmol/L	1.3	1.4	1.3	1.1
K	mmol/L	5.7	5.7	5.9	4.9
Na	mmol/L	0.6	0.6	0.5	0.6
Ca	mmol/L	4.1	4.2	3.7	3.2
Mg	mmol/L	2.2	2.3	2.1	1.8
NO3	mmol/L	14.6	14.8	13	11.2
Cl	mmol/L	0.3	0.3	0.3	0.3
SO4	mmol/L	1.7	1.7	2	1.6
HCO3	mmol/L	<0.1	<0.1	0.1	0.1
P	mmol/L	1.21	1.25	1.22	0.98
Si	mmol/L	<0.01	<0.01	0.01	0.01
Fe	µmol/L	24	23	20	15
Mn	µmol/L	13	13	9	8.3
Zn	µmol/L	7.9	8.4	7.6	8.1
B	µmol/L	33	33	33	27
Cu	µmol/L	1.5	1.6	1.4	1.1
Mo	µmol/L	0.8	0.8	0.6	0.5