Evaluation of the Performance of an Ozonization System for the Disinfection of the Nutrient Solution of a Greenhouse Tomato Crop

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Abstract

An ozonization system (Ozomax OZO 6VTT, IGT limited, Ontario, Canada) was installed in a commercial plastic-covered greenhouse in Danville, Quebec, Canada (www.savoura.com) to study the effects of the disinfection and recirculation of the nutrient solution in a tomato crop. The ozonization system was compared to a conventional growing system where the nutrient solution was not treated, neither recirculated. Samples of both treated and non-treated nutrient solutions were analysed for the presence of 38 potential pathogens using DNA Multiscan Technology. Detailed measurements of the presence of *Pythium* spp. and *Fusarium* spp. in the nutrient solutions, the growing media and the root system were achieved at various periods. We also determined the evolution of bacteria in the treated and non-treated nutrient solutions. A weekly complete mineral analysis, including pH and EC of both treated and non-treated nutrient solutions was done. For both treatments, plant growth and development were measured on a weekly basis while fruit yield and quality were determined for every harvest.

INTRODUCTION

Protection of the underground water and of the environment requires that nutrient solutions used for greenhouse tomato production are recuperated and recirculated. Various systems using heat or UV radiation are commonly employed mainly in northern Europe to treat nutrient solutions (Runia, 1994, 2001). These systems were shown to be very efficient to eliminate pathogens in the nutrient solutions, but relatively expensive to operate (0.15-0.25 Can$/m^3) (Le Quillec, 2002). Other systems, using strong oxidants such as ozone, peroxide or chloride to destroy pathogens were experimented (Rey, 2001; Poncet et al., 2001; Runia, 1994, Vanacher, 1988). The addition of chloride to the nutrient solutions caused symptoms of phytotoxicity to tomato plants when concentration exceeded 2-4 ppm (Le Quillec, 2002). A French company (Trailigaz, Garges-lès-Gonesse) developed an ozonization system to disinfect nutrient solutions using ozone and peroxide. In Canada, IGT Ltd developed and commercialized an ozonization system coupled with a bubbling system to increase oxygen concentration in the nutrient solution and potentially improve its efficiency. This project aims at measuring the efficiency and the agronomic performance of the latter system.

MATERIALS AND METHODS

The experiment was conducted in a 27 000 m^2 double polyethylene greenhouse equipped with a HPS supplemental lighting system supplying 120 μmol.m^{-2}.s^{-1}. Tomato plants Cv Trust were grown at a plant density of 2,4 plants/m^2 in rockwool slabs (Grodan master type) placed on gutters located 1 m. high. Standard cultural practices were used for

nutrition, irrigation and plant training. Biological control of insects was primarily achieved by introducing predators such as *Eretmocerus* spp. and *Dicyphus* spp. Before being reused, the nutrient solution from 50% of the growing area was recuperated and treated with the ozonization system (Ozomax OZO 6VTT, IGT limited, Ontario, Canada) initially able to produce 40 g and later upgraded to produce 60 g of O_3 per hour. The rate of nutrient solution treated was adjusted between 3 to 10 m^3 per hour. It was consequently possible to compare the effects of 3.0 , 5.3 , 7.7 and 14.1 g of ozone per m^3 of nutrient solution. The capacity of the ozonization system related to each treatment rate is described in Table 1. The nutrient solution of the other half of the growing area was neither recuperated, nor recirculated. In each section of the greenhouse, three bays of 337 m^2 were selected to measure growth, development, yields and fruit quality (Dorais, 2001). Samples of the nutrient solutions were taken before and after ozonization and analysed for their mineral contents.

Four additional experiments were conducted. First, we treated nutrient solutions for 30, 60, 90 or 120 minutes with ozone. Second, we sampled treated and non-treated nutrient solutions with ozone at 14.1 ppm and we later treated both of them with 0, 1, 2, 3, 4 or 5 ppm of peroxide. Thirdly, recirculated nutrient solutions were treated with both ozone (7.7 and 14.1 ppm) and peroxide at 5 ppm. Finally, nutrient solutions were treated with ozone at 7,7 ppm, peroxide at 5 ppm and chloride at 1, 2 or 5 ppm. For all four experiments, we counted bacteria on petri dishes as described further.

Microbial analysis were achieved by Relab den Haan in The Netherlands, the Laboratoire de diagnostic en phytoprotection of MAPAQ and by the Centre de recherche en horticulture (CRH) at université Laval. Relab den Haan uses DNA technology to detect for the presence of 38 pathogens in the nutrient solutions. Nutrients solutions and root samples were analysed for the presence of fungi by the Laboratoire de diagnostic en phytoprotection of MAPAQ. Root segments or 100 µL of nutrient solutions were spread on three different media: Synthetic nutrient agar with antibiotics, P_5ARP specific for *Pythium* and P_5ARPH specific for Phytophthora. Bacterial counts were made at Université Laval using 50 µL of nutrient solution spread in petri dishes containing a tryptic soy agar. Nutrient solutions were sampled before the ozonization treatment, immediately after and 18 hours after in the storage reservoir.

RESULTS AND DISCUSSION

Data measured by Relab Den Haan from DNA analysis (Table 2) indicate that the treatment of ozonization did not eliminate all pathogens from the nutrient solutions, even at the highest concentrations. However, ozonization reduced the level of infection in the nutrient solutions for many pathogens, especially *Pythium* spp., *Pythium dissocutum* and *Fusarium oxysporum*. In some cases, the level of infection was not reduced at all and in a few cases, it was even increased for unknown reasons. An experiment will be conducted shortly to verify if the sand filter located before the ozonization system was the source of sporadic contamination.

Analysis of fungi (data not presented) in the nutrient solutions and in roots by the Laboratoire de diagnostic en phytoprotection of MAPAQ indicated the presence of *Plectosporium* spp., *Fusarium oxysporum*, and *Cladosporium* spp. in similar amounts in both the treated and non-treated nutrient solutions. Fungi were also found in root segments.

The increase in the duration of ozonization from 30 to 120 minutes improved the efficiency of the disinfection treatment by reducing the number of bacteria present in the nutrient solutions (Table 3). These results may be explained by either a longer period of treatment and/or an increase in the oxydo-reduction potential caused by higher concentration of ozone in the nutrient solutions.

Data presented in table 4 clearly indicate the effectiveness of peroxide to reduce the number of bacteria in the nutrient solutions. The best results were obtained at the highest peroxide concentrations (4-5 ppm). Peroxide appears to be more efficient when added after the ozonization treatments (Table 5) when we sampled the treated nutrient

solutions in the storage tank. We however measured an increase in the bacterial counts 18H00 after storage. Our data support the strategy of Trailigaz to combine ozone and peroxide to disinfect nutrient solutions. Our results also lead to an experiment on the addition of chloride for a better disinfection.

Data presented in table 6 indicate that the addition of chloride in the nutrient solutions following disinfestation with ozone and peroxide was very effective not only at low concentrations (1-2 ppm), but also after a period of storage. The highest chloride concentration (5 ppm) cannot be recommended as it was shown to be toxic to various crops. This strategy seems to improve greatly the effects of ozone and peroxide, but also appears to offer an interesting alternative to inhibit bacterial development in the storage tank.

Ozonization did not affect the composition of the nutrient solutions as shown in table 7. In fact, EC stayed quite constant at around 4.0 mmhos/cm, while pH varied very slightly between 5.8 and 6.0. Major elements such as N, P, K, Ca and Mg were very constant following ozonization at either 5.3 or 14.1 ppm. Although Fe and Mn concentrations were shown to be reduced by ozonization (Vanachter et al., 1988), our data indicate that their concentrations in the nutrient solutions were not affected.

After 12 weeks of recirculation, ozonization did not affect growth and development (data not presented) of tomato as measured by stem diameter, leaf length, height of the first cluster or fruit number on the plants. Yields were neither affected by the recirculation and the ozonization of the nutrient solutions. Leaching of the nutrient solutions averaged 36 and 38 % for the recirculated and the not-recirculated treatments, respectively. The percentage of recirculation was satisfactory, being most of the time above 80% for the first 10 weeks of the experiments.

CONCLUSION

Our data agree with those of Runia (1994) and Rey et al. (2001) indicating that ozonization would reduce significantly the number of microorganisms in the nutrient solutions. However, it seems that the highest concentration of ozone alone was not sufficient to completely eliminate bacteria and fungi in the nutrient solutions. The addition of peroxide and/or the increase in the duration of the ozonization did improve the efficiency of the process, without obtaining complete disinfection. Complete disinfection was only achieved with the combination of ozone, peroxide and chloride. Further research will study the best combinations of ozone, peroxide and/or chloride to obtain complete and lasting disinfection at the lowest cost.

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Tables

Table 1. Ozone concentration in the nutrient solutions according to different production capacity and treatment rates.

Ozone production* (g O_3 / hr)	Treatment rate (m^3 of water/hr) or ppm)	Estimated ozone concentration (g O_3/m^3)	Duration of O_3 injection* (min / m^3 of water)
32-40	9.1	3.5	7
40-60	9.1	5.3	7
40-60	6.2	7.7	10
40-60	3.4	14.1	18

* According to the manufacturer, the system produces 8-10 g of ozone per corona lamp. We assumed 8 g of ozone in our experiments * O_3 half-life is estimated to 30 minutes. After injection, O_3 continues to react until its breakdown.

Table 2. Influence of ozonization treatments on the detection of various fungi.

	3.5 ppm*		5.3 ppm		14.1 ppm	
Fungi	Non-Treated	Treated	Non-Treated	Treated	Non-Treated	Treated
Oomycetes	3**	1	2	3	2	3
Botrytis cinerea	0	2	0	1	3	1
Fusarium spp.	3	2	1	3	5	4
F. oxysporum	1	1	0	1	2	1
Pythium spp.	3	0	4	0	2	2
P. dissocutum	1	0	3	0	4	0

- *Calculated concentrations of ozone according to the power of the generator and the rates of the nutrient solution
- ** 0 = not infected; 1 = starting infection; 2 = light infection; 3 = moderate infection; 4 = infected; 5 = severely infected; 6 = very severely infected

Table 3. Influence of the duration of ozonization of the nutrient solution on the number of bacteria in petri dishes.

Duration of ozonization (min)	Total O_3 injected (g O_3 / m^3)	ORP* (mV)	Number of bacteria (CFU/ml)
Non-treated	---	---	4906
30	11.5	494	187
60	23.1	615	80
90	34.6	740	40
120	46.2	819	20

* Oxydo-reduction potential measured in the nutrient solutions in millivolts

Table 4. Influence of ozonization and peroxide concentration in the nutrient solutions on the number of bacteria in petri dishes.

Peroxide concentration (ppm)	Number of bacteria (CFU/ml) Treated*	Non-treated
0	3706	5653
1	800	2693
2	500	1467
3	566	1960
4	133	1413
5	80	1466

* Ozonization occurred at an estimated concentration of 14.1 ppm for a period of 18 min/m^3.

Table 5. Influence of ozonization followed by peroxide treatment (5 ppm) of the nutrient solutions on the number of bacteria and fungi (CFU/ml) in petri dishes.

Sampling sites*	Ozonization (ppm) 7.7 *		14.1	
	Bacteria	Fungi	Bacteria	Fungi
In the ozonization reservoir	1866	19	826	27
At the exit of the ozonization reservoir	113	13	266	13
Immediately in the storage tank	26	16	13	30
After 18H00 in the storage tank	260	24	N/A	N/A

*Counts of bacteria and fungi for the non-treated (no O_3, no peroxide) nutrient solution were 6296 and 56, respectively.

Table 6. Influence of chloride addition following ozone and peroxide treatments of the nutrient solutions on the number of bacteria (CFU/ml) in petri dishes.

Chloride concentrations (ppm)	Time of sampling After treatment	after 16H00 of storage
0	973	720
1	0	46
2	0	0
5	0	0

* Ozonization occurred at 7.7 ppm at a rate of 9,6 min/m^3 and 5 ppm of peroxide was added

Table 7. Influence of ozonization on the mineral concentration of the nutrient solutions.

Elements	Non-treated	Treated (7.7 ppm)	Non-treated	Treated (14.1 ppm)
EC*	4,02	4,15	3,89	3,90
pH	6,1	6,0	5,8	6,0
N	238	263	239	241
P	38	43	44	43
K	227	248	395	402
Ca	471	500	374	382
Mg	151	150	92	92
Sulphates	717	692	527	536
Na	25	25	17	17
Fe	1.68	1.88	116	1.23
Mn	0.93	1.84	0.86	1.25

* EC as electric conductivity in mmhos/cm