JARQ 42 (4), 291 – 297 (2008) http://www.jircas.affrc.go.jp

# Effects of Ozonated Water-fog Cooling on Ozone Gas Concentrations and Population Densities of Airborne Bacteria and Fungi on Plant Leaves in a Naturally Ventilated Greenhouse

**Jong-Seok PARK[1], Masahisa ISHII[2], Limi OKUSHIMA[2]*, Sadanori SASE[2] and Kazuhiro FUJIWARA[1]**

[1] Graduate School of Agricultural and Life Sciences, The University of Tokyo (Bunkyo, Tokyo 113–8657, Japan)

[2] Department of Agricultural Environment Engineering, National Institute for Rural Engineering, National Agriculture and Food Research Organization (Tsukuba, Ibaraki 305–8609, Japan)

## Abstract

Ozone gas concentrations (OGC) and population densities of airborne bacteria and fungi on plant leaves were investigated in a naturally ventilated greenhouse cooled by a fogging system with ozonated water [OW; dissolved ozone concentration (DOC) of 10 mg/L at a generator outlet] or tap water (TW; DOC of 0 mg/L). The fog cooling occurred for 30 min with a combination of 20 s on and 100, 80, 60, 40, or 20 s off using 5 μm (catalog-specified fog size) nozzles and of 16 s on and 44 or 74 s off using 50 μm (catalog-specified fog size) nozzles. The population densities of airborne bacteria and fungi attached on the leaves of poinsettias (*Euphorbia pulcherrima*) and pothos (*Epipremnum aureum*) were determined using an agar stamp method before and after the fog cooling. There was no large difference in the greenhouse cooling efficiency between the TW and OW treatments. The highest OGC in the greenhouse never exceeded 0.04 mg/L with all treatments, even with a combination of fogging 20 s on and 20 s off. This result indicates that the OGC in the greenhouse cannot accumulate to harmful levels (over 0.05 mg/L during 8 ordinary working hours) because of the natural ventilation required for fog cooling. Although population densities of airborne bacteria and fungi on the plant leaves showed a slight decrease during OW-fogging, these levels were not significantly different from population densities during TW-fogging. However, it is still unknown whether repetitive OW-fog cooling for more than 30 min and/or several days of OW-fog cooling will reduce the population densities of airborne bacteria and fungi on plant leaves.

**Discipline:** Horticulture
**Additional key words:** evaporative-fog cooling, poinsettia, pothos

## Introduction

Ozone is a strong oxidant, which has been used to disinfect many industrial items, including food surfaces[13], food-processing equipment[8], drinking water, and industrial wastewater[12]. However, there is little information on the use of ozonated water (OW) in plant production. Fujiwara and Fujii[2] reported that powdery mildew was controlled by spraying OW directly onto cucumber plants. Ozone gas aeration has been used to disinfect several common root-infecting pathogens in salt solutions or hydroponic culture solutions[10,16,17]. Ozone concentrations for indoor working environments have been suggested from 0.05 to 0.3 mg/L or 0.1 to 0.6 mg/m$^3$ during 8 ordinary working hours by 18 countries including Japan[15]. Ozone has been added to irrigation waters with benefits that included increased water infiltration, increased disease resistance and a reduced need for fertilizers[11]. Significant reductions in the population density of *Fusarium oxysporum* in infested quartz sand but not in Kuroboku soil were observed with OW at dissolved ozone concentrations of 6 and 12 mg/L[4]. Kim et al.[7] reported that 0.05 and 0.1 mg/L ozone gas significantly reduced the levels of indolic compounds in swine building air.

In greenhouse cultivation, countermeasures against

*Corresponding author: e-mail limi@affrc.go.jp
Received 27 July 2007; accepted 8 January 2008.

a high-temperature phase are required for plant production since plant productivity decreases with physiological disorders and disease injuries. Evaporative-fog cooling is one method among many techniques that decreases greenhouse temperatures. However, the effects of OW instead of tap water (TW) or ground water for greenhouse evaporative-fog cooling have not been investigated. A disinfecting method using OW for recycling of inorganic salts solutions can be a promising method in large scale plant production facilities, because this application minimizes the risk of disease, reduces organic loading that is a microbial energy source and toxin source, and has no harmful by-products. OW can be used in greenhouse cultivation for disinfecting inorganic salt solutions in place of TW or ground water spray for evaporative-fog cooling, washing a harvest to preserve its postharvest quality, disinfecting seeds, and cleaning facilities. Depending on dissolved ozone concentrations (DOC) and fogging time, evaporative-fog cooling with OW during a high-temperature phase may reduce population densities of airborne bacteria and fungi in the air and on plant leaves that can cause plant diseases. Fujiwara and Fujii[3] reported that disease control with OW spraying was improved by minimizing the spray distance using a nozzle that produces a larger droplet size. However, previous studies have not investigated the effect of varying ozone gas concentrations (OGC) in a greenhouse on population densities of airborne bacteria and fungi in the air and on plant leaves during OW-fogging. The use of OW in greenhouses may serve as an alternative to agricultural chemicals that are harmful to humans and the environment.

The objectives of the present study were to investigate i) fog cooling efficiency, ii) changes in OGCs in a greenhouse when OW-fogging using 5 or 50 µm nozzles was carried out as evaporative fog cooling in a greenhouse, and iii) population densities of airborne bacteria and fungi both in the greenhouse air and those on the leaves of poinsettias (*Euphorbia pulcherrima*) and pothos (*Epipremnum aureum*) during OW-fogging using 50 µm nozzles. To measure population densities of airborne bacteria and fungi in the air and on leaves, two types of commercial agar strips and contact slides were used. The OGCs in the greenhouse cooled by OW-fogging were monitored with an ozone gas analyzer.

## Materials and methods

### 1. Greenhouse configuration and evaporative-fog cooling system

Two naturally ventilated single-span greenhouses (5.25 m in length, 3.4 m in width and 3.2 m in height), which were covered by glass with side and roof vents, were located at the National Institute for Rural Engineering in Tsukuba, Ibaraki, Japan. Two kinds of nozzles, with fog droplet size of 5 or 50 µm as described by the catalog, were used to investigate the effects of fog size on ozone gas change in the greenhouse. OW-fogging was conducted with a combination of 20 s on and 100, 80, 60, 40, or 20 s off using 5 µm (catalog-specified fog size with 7 MPa of discharging pressure) nozzles (0.01 Orifice, Mee Industries Inc., CA, USA) for 30 min on 27 Oct. 2006 (Fig. 1). A combination of 16 s on and 44 or 74 s off was executed for TW/OW-fogging using 50 µm (catalog-specified fog size with 2 MPa of discharging pressure) nozzles (KB-1/4 MKB 6016S303, Matsuzaka Engineering, Tokyo, Japan) for 30 min on 29 Nov. 2006 (Fig. 1). Water quantities for the evaporative-fog cooling were 45 ml per 20 s and 16 s with a 5 µm nozzle and 50 µm nozzle, respectively, during all treatments. Roof vents were opened 15 cm and side vents were closed during TW/OW-fogging to reduce the number of air exchanges in the greenhouse. After TW/OW-fogging treatments, side vents and an entrance door were fully opened for 30 min to restore the initial greenhouse conditions. Temperatures of two dry and wet bulbs in the greenhouse were measured with thermocouples located 1.5 m from the floor using a data collecting system (CR-10, Campbell, Scientific, Inc., UT, USA) and a PC.

The number of air exchanges in the greenhouse was continuously measured using the tracer gas method with a 1312 Photoacoustic Multi-gas Monitor (Innova AirTech

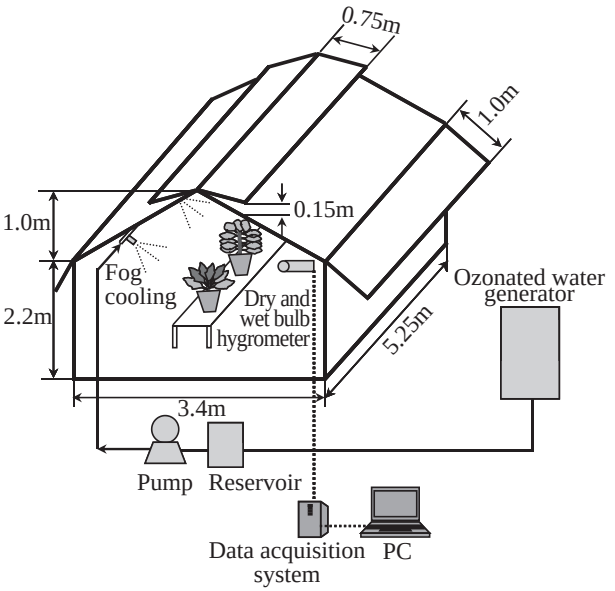


**Fig. 1. Schematic diagram of the experimental setup used for ozonated water-fog cooling**

Instruments A/S, Ballerup, Denmark), a 1303 Multipoint Sampler and Doser (Innova AirTech Instruments A/S, Ballerup, Denmark), a 7620 Application Software of Tracer-gas Monitoring System (Innova AirTech Instruments A/S, Ballerup, Denmark), and a PC. A tracer gas, sulfur hexaflouride ($SF_6$), which does not affect plant growth and is not harmful to humans was introduced at a corner and sampled in the center of the greenhouse during the experiment. The gas injection was regulated to keep the concentration constant at 5 µmol/mol. The number of air exchanges was calculated from the amount of gas supplied and recorded at approximately 120 s intervals, according to the method described by Ikeguchi et al.[6].

**2. Ozonated water preparation**

OW was generated with an electrolytically ozonated water generator (DO-30, Shinko Plant Engineering & Construction Co., Ltd., Hyogo, Japan). OW was generated by electrolysis with softened water which did not contain impurities such as Mg, Ca and Na, etc. OW generated with an electrolytically-ozonated water generator is characterized by less diffusion rate of ozone from the OW into the air and by producing high DOC of OW, compared with a gas dissolve-ozonated water generator. The OW with a DOC of 10.0 mg/L was sent to a stainless steel reservoir (volume: 23.0 L) through a 10 m flexible hose made of fluoric plastic material. Twenty ball-type fluoric buffers were in the reservoir to prevent a quick decrease in the DOC of OW in it (twenty buffers' volume: 12.9 L, OW volume in the reservoir: 10.1 L). The OW that overflowed from the reservoir was naturally released into a drain. The OW collected in the reservoir was sprayed out with a pump (FG-1, Matsuzaka Engineering Co., Ltd., Tokyo, Japan) which was connected to a 5 m hose that was linked to a 1.5 m stainless steel pipe with four nozzles (Fig. 1). The temperature of the OW in the reservoir was measured immediately before OW-fogging.

**3. Dissolved ozone concentration (DOC) and ozone gas concentration (OGC)**

The DOCs of the OW in the reservoir, at the pipe end, and at the nozzle outlet were measured using the indigo carmine method. To measure the DOC of the OW in the reservoir, 20 ml OW was withdrawn with a beaker and then immediately mixed with 100 ml TW to dilute the solution six-fold followed by an indigo carmine ampoule (detection range: 0–1.5 mg/L, Hach Co., Loveland, CO, USA). Absorbance was measured using a pocket colorimeter (DR-850, Hach Co., Loveland, CO, USA) calibrated against distilled water at zero DOC. The DOCs of the OW at the end of the pipe were measured with a three-fold diluted OW solution and an ampoule with a detection range of 0–1.5 mg/L. Those at the nozzle outlet were measured using directly collected OW and a 0–0.25 mg/L ampoule. Each DOC measurement was repeated three times. OGC in the greenhouse was continuously measured with an ozone gas analyzer (EG-5000, Ebara Jitsugyo Co., Ltd., Tokyo, Japan) during TW/OW-fogging using the ultraviolet absorption method at the center of the greenhouse.

**4. Population densities of airborne bacteria and fungi in the air and on plant leaves**

Greenhouse population densities of airborne bacteria and fungi in the air were determined by an air sampling and agar stamp method using soy-agar (SA) strips and SA-contact slides (TC, 40-AS0100, 40-CT0100, Biotest, Dreieich, Germany) for bacteria and rose bengal agar (RBA) strips and RBA-contact slides (YM, 40-AS0200, 40-CT0200, Biotest, Dreieich, Germany) for fungi. The air at the center of the greenhouse was sampled before and after TW/OW-fogging by an air sampler (RCA, Biotest, Dreieich, Germany) and SA/RBA strips were inserted into the sampler for one minute (equivalent to 40 L-air-contact).

Four potted poinsettias and pothos were used as model plants. They were purchased at a flower shop in Tokyo and then placed in the greenhouse maintained at 15 and 25°C (night and day) which were preset temperatures of a heater, respectively, for one week before the start of the experiment. Each poinsettia and pothos pot had an average of 140 and 60 leaves and was 48 cm and 41 cm in height from the bottom, respectively. The pots were placed on a 55 cm height shelf in the middle of the greenhouse during TW/OW-fogging (Fig. 1). Bacteria and fungi population densities were determined before and after TW/OW-fogging by imprinting the leaf surfaces with the SA/RBA contact slides (3.5 × 6.5 cm surface area). Each poinsettia and pothos was replaced for each TW/OW treatment during the experiment. Four leaves in a pot were imprinted before and after each fogging treatment for both bacteria and fungi. After imprinting bacteria and fungi in the air and on leaf surfaces with agar media, they were sealed in a portable clean bench (SS-MAC, Airtech Japan, Ltd., Tokyo, Japan) (0.24 $m^2$ × 0.7 m) which was set up for pre/post-treating the SA/RBA strips, contact slides, and the air sampler under sterile conditions and incubated at 32.5 ± 2.5°C for 24 h and 22.5 ± 2.5°C for 72 h, respectively, in two incubators (MIR 153, Sanyo Electric Co., Ltd., Tokyo, Japan). All pictures of agar media were taken with a digital camera (E10, Olympus, Corp., Tokyo, Japan), and visible colonies were counted to evaluate the population densities of airborne bacteria and fungi.

## Results and discussion

### 1. Dry and wet bulb temperatures, relative humidity (RH), and number of air exchanges

On 27 Oct. 2006 average dry bulb and wet bulb temperatures in the greenhouse were 18.4 ± 0.04 and 18.1 ± 0.04°C, respectively, during OW-fogging with 5 µm nozzles, and on 29 Nov. 2006, 20.5 ± 0.07 and 17.5 ± 0.11°C during TW-fogging with 50 µm nozzles, and 21.5 ± 0.20 and 17.9 ± 0.07°C during OW-fogging with 50 µm nozzles, respectively (Fig. 2). RH in the greenhouse increased from 80 to 100% and from 50 to 80% using 5 and 50 µm nozzles, respectively. Based on the dry and wet bulb temperatures on 29 Nov., there seems to be little difference in the greenhouse cooling efficiency between the TW and OW treatments. It is highly unlikely that the existence of ozone in water at concentrations of less than 0.17 mg/L (Fig. 3) exerts an influence on the greenhouse cooling efficiency. The number of air exchanges in the greenhouse with 15 cm opened roof vents was 15 to 30 per hour during TW/OW-fogging, but increased to 100 to 200 per hour when the side vents and entrance door were fully opened (data not shown). As described in the materials and methods, the greenhouse with 15 cm opened roof vents and closed side vents had a lower number of air exchanges than a greenhouse described by Handarto et al.[5] with fully opened side and roof vents during fogging. The number of air exchanges, an important factor in natural ventilation, affects the ozone gas concentration and the fog cooling efficiency when OW-fogging was carried out as evaporative-fog cooling in a greenhouse.

### 2. OGC and DOC of OW during OW-fogging

OGC slightly increased during OW-fogging in the greenhouse (Fig. 2). An increased amount of OW-fogging with frequent on/off pump intervals increased and maintained the OGC levels during OW-fogging using the 5 µm nozzles on 27 Oct. 2006 (Fig. 2). The highest OGC in the greenhouse never exceeded 0.04 mg/L with all treatments, even with a combination of fogging 20 s on and 20 s off which is an excessive fogging for greenhouse fog-cooling. During TW-fogging with the 50 µm nozzles, the OGC was nearly zero, not different from outdoor air, but approximately 0.02 and 0.04 mg/L during OW-fogging with a combination of 16 s on and 74 or 44 s off, respectively (Fig. 2). As Fujiwara reported that dissolved ozone concentration in OW at the spray target decreased with decreasing droplet size[3], the OGC using the 50 µm nozzles was higher than that using the 5 µm nozzles in the greenhouse despite a small spraying quantity of OW. The

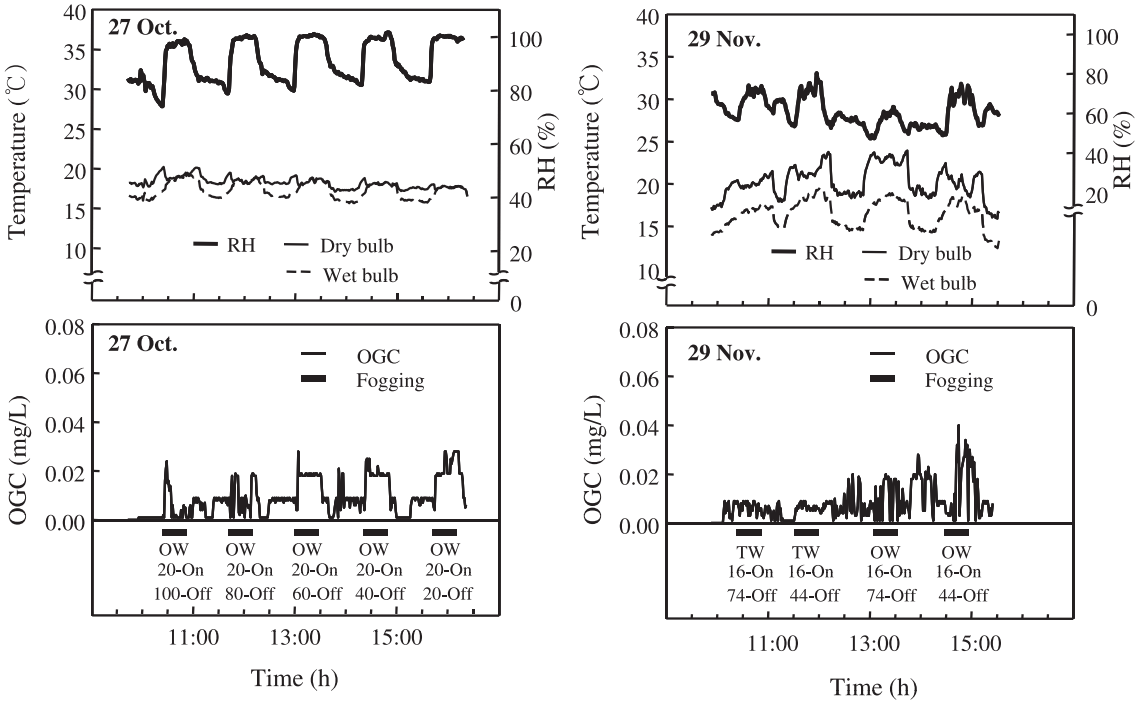


**Fig. 2. Change in relative humidity (RH), dry and wet bulb temperatures, and ozone gas concentrations (OGC) during ozonated water (OW)-fogging using 5 µm nozzles with a combination of 20 s on and 100, 80, 60, 40, or 20 s off on 27 Oct. 2006, and during tap water (TW)- and OW-fogging using 50 µm nozzles with a combination of 16 s on and 74 or 44 s off for 30 min on 29 Nov. 2006 in a greenhouse**

natural ventilation required for cooling the greenhouse contributed to a low OGC during OW-fogging. These results indicate that ozone gas during OW-fogging in the greenhouse cannot accumulate to harmful levels (0.05–0.3 mg/L or 0.1–0.6 mg/m$^3$ during 8 ordinary working hours suggested by 18 countries including Japan for an indoor working environment[15]) with the number of air exchanges of 15–30 times per hour.

The OGC in the greenhouse with the full-open state of the side and roof vents, and the entrance door was around 0.02 mg/L without OW-fogging (Fig. 2). The ozonated water generator began to work after 30 min from the end of TW-fogging on 29 Nov. The OGC of around 0.02 mg/L without OW-fogging on 29 Nov. may be due to the ozone gas generated from the dumped OW which had overflowed from the reservoir. This overflowed OW was released into a drain at a distance 5 m apart from the greenhouse. So dumping of overflowed OW has to be done at a place far from the plants when OW is applied in plant production facilities. The main cause of the difference in the OGC between 27 Oct. and 29 Nov. without OW-fogging seemed to be the wind direction on those days.

The DOCs of the OW in the reservoir, at the pipe and at the outlet of the nozzles were measured by cracking indigo carmine ampoules in the collected OW during OW-fogging using the 5 and 50 µm nozzles (Fig. 3). The initial DOC of OW, 10 mg/L, generated with the electrolytically ozonated water generator markedly decreased as the distance between the generator and the measured points increased. This resulted from self-decomposition, dissolved ozone oxidation due to contact with transport materials and ozone diffusion from the droplets between release from the nozzle and collection in the beaker. The DOCs of the OW at the reservoir and at the pipe were 5.97 ± 0.219 and 2.79 ± 0.151 mg/L, respectively (Fig. 3). The DOCs of the OW collected in the beaker were 0.0 ± 0.00 and 0.17 ± 0.007 mg/L when released with the 5 and 50 µm nozzles, respectively (Fig. 3). The DOC of zero in the OW released with the 5 µm nozzle could be attributed to the small fog size and a 3.5 times higher discharge pressure (pressure applied to the nozzle) provided by the pump. Fujiwara and Fujii[3] reported that the DOC decreased with decreasing droplet diameter as the larger droplet surface area to volume ratios led to greater diffusion of dissolved ozone into the air. Although the nozzles specified with a 10–100 µm fog size are normally used for evaporative-fog cooling, these results indicate that a fog size smaller than that produced by the 50 µm nozzle would result in reduction of efficacy in decreasing bacteria and fungi population densities on the plant leaves.

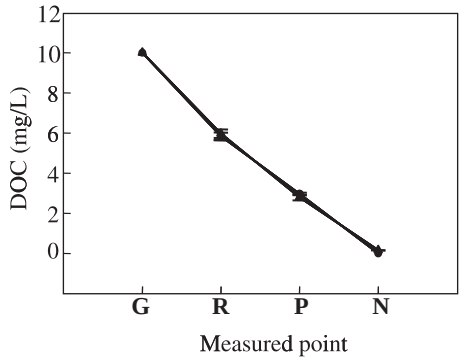


**Fig. 3. Dissolved ozone concentration (DOC) of OW at the outlet of the ozonated-water generator (G), in the reservoir (R), at the pipe end (P), and at the nozzle outlet (N) when released with the 5 and 50 µm nozzles**

Data are represented as the means ± standard error (n = 3). The flexible fluoric plastic hose between the generator and tank, and the tank and pipe end were 10 m and 5 m, respectively.
—●—: 5 µm nozzle, —▲—: 50 µm nozzle.

### 3. Population densities of airborne bacteria and fungi on plant leaves

Population densities of bacteria and fungi in the air were not significantly different before and after TW/OW-fogging (data not shown). This was expected, since the OGC in the greenhouse remained at low levels (less than 0.04 mg/L) during OW-fogging.

Disinfection effects of a sterilizer or its alternative on bacteria, fungi and mold within a range of 1–3 $\log_{10}$ colony-forming units per sample have been mentioned in many reports[1,9,13]. No significant differences in population densities of bacteria and fungi on poinsettia and pothos leaves before and after TW/OW-fogging were observed (Fig. 4). On the basis of simple quantitative comparisons between before and after TW/OW-fogging with a combination of same on/off time, the population densities were slightly reduced during OW-fogging compared with TW-fogging (Fig. 4), except the bacteria on pothos with the combination of 16 s on and 74 s off. This may be due to the disinfection effect of the dissolved ozone in the droplets deposited on the leaves. In addition, increased OW or TW fogging with the combination of 16 s on and 44 s off showed a slight decrease in bacteria population densities more than fogging with 16 s on and 74 s off. This can be attributed to a washing-off effect by the droplets; greater fogging amounts seemed to increase the chance of washing-off bacteria on the leaves. If decreased number of bacteria and fungi by TW-fogging between before and after treatment is regarded as the washing-off effect by the droplets, the difference in number of them

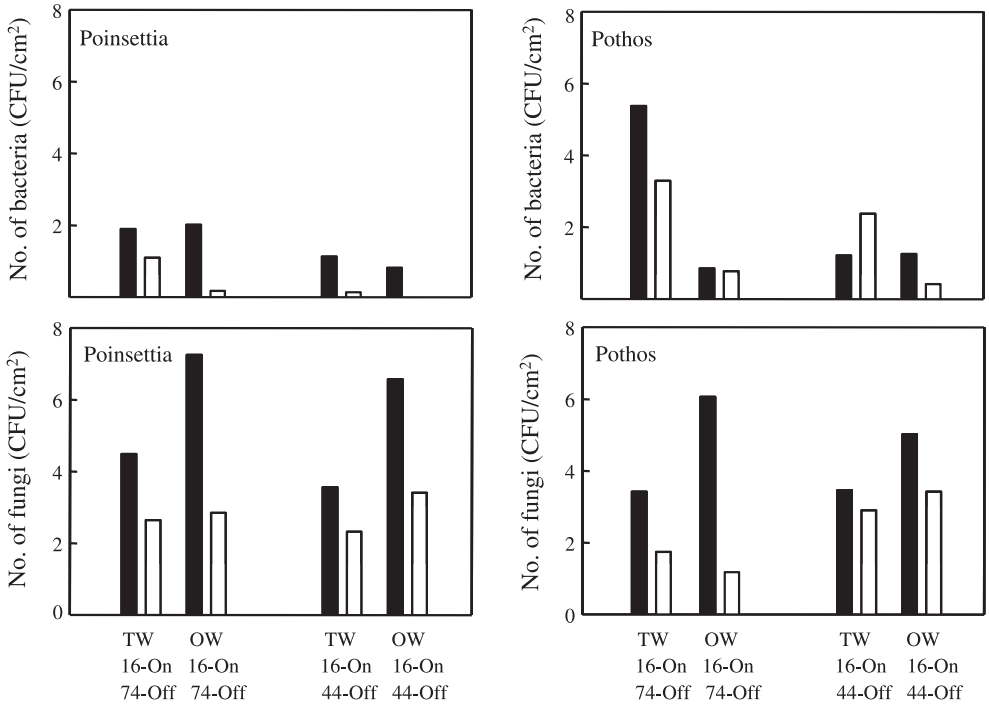


**Fig. 4. Number of airborne bacteria and fungi on poinsettia and pothos leaves before and after tap water (TW)- and ozonated water (OW)-fogging**

OW- and TW-fogging with 50 μm nozzles was conducted with a combination of 16 s on and 74 s or 44 s off for 30 min in the greenhouse on 29 Nov. 2006.

■ : Before, □ : After.

between TW/OW-fogging can be explained by disinfection of dissolved ozone in the droplets. To improve disease control, using a nozzle with a larger droplet diameter to a certain level appears to be an effective and appropriate strategy. Some reports suggest greater disease control efficacy with larger droplet size to the diameter of approximately 1 mm with pesticide treatment[13,14]. However, the decreases in deposition efficacy for droplet size greater than 1 mm were considered to be due to ricochet or shatter of droplets on contact with the leaf surface[12]. Although the DOC of the OW on the leaves after OW-fogging could not be measured in the present experiment, it would not be higher than the 0.17 mg/L DOC of the immediately released OW from the 50 μm nozzle (Fig. 3). These results indicate that repetitive OW-fogging or a fogging time greater than 30 min are needed to effectively decrease population densities of airborne bacteria and fungi and/or active disease control on plant leaves.

## Conclusions

This study demonstrated that OGC during OW-fogging never accumulate to harmful levels when the number of air exchanges is over 15 per hour and that there is no large difference in the greenhouse cooling efficiency between the TW and OW treatments. Although population densities of airborne bacteria and fungi on the plant leaves showed a slight decrease during OW-fogging, these levels were too inconclusive to apply as a general antibacterial and antifungal technique. It is still unknown whether repetitive OW-fogging for more than 30 min and/or several days of OW-fog cooling will reduce the population densities of airborne bacteria and fungi on the plant leaves.

## Acknowledgments

The authors express appreciation to the Machinery and Engineering Company, Kobe Steel, Ltd., Japan for supplying the electrolytically ozonated water generator. This work was supported in part by the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (Grant No. 18052).

## References

1. Beltran, D. et al. (2005) Ozonated water extends the shelf life of fresh-cut lettuce. *J. Agric. Food Chem*., **53**, 5654–5663.
2. Fujiwara, K. & Fujii, T. (2002) Effects of spraying ozonat-

ed water on the severity of powdery mildew infection on cucumber leaves. *Ozone Sci. Eng.*, **24**, 463–469.
3. Fujiwara, K. & Fujii, T. (2004) Effects of ozonated water spray droplet size and distance on the dissolved ozone concentration at the spray target. *Ozone Sci. Eng.*, **26**, 511–516.
4. Fujiwara, K. et al. (2006) Effects of ozonated water application on the population density of *Fusarium oxysporum f. sp. Lycopersici* in soil columns. *Ozone Sci.Eng.*, **28**, 125–127.
5. Handarto, et al. (2005) Air and leaf temperatures and relative humidity in a naturally ventilated single-span greenhouse with a fogging system for cooling and its evaporative cooling efficiency. *Environ. Control Biol.*, **43**, 3–11.
6. Ikeguchi, A. et al. (2005) Efficient greenhouse cooling in semiarid climate (II) Natural ventilation measurement with gas tracer method in a single-span double-polyethylene. Proc. international conference on research highlights and vanguard technology on environmental engineering in agricultural systems, Kanazawa, Japan, September 12–15, 2005, 11–16.
7. Kim, Y. H. et al. (2005) Effect of ozonation on odor and the concentration of odorous organic compounds in air in a swine building facility. *Trans. ASAE*, **48**, 2297–2302.
8. Klingman, M. H. & Christy, A. D. (2000) Development of a continuous system for sanitizing whole apples with aqueous ozone. *Trans. ASAE*, **43**, 1989–1996.
9. Koseki, S. & Isobe, S. (2006) Effect of ozonated water treatment on microbial control and on browning of iceberg lettuce (*Lactuca sativa* L.). *J. Food Protect.*, **69**, 154–160.
10. Kusakari, S. (2000) Sterilization of root rot fungus of cucumber by ozone in hydroponics. *Shokubutsu Boeki (Plant Protec.* ), **54**, 11–16 [In Japanese with English summary].
11. Raub, L. C. et al. (2001) The effects of ozonated irrigation water on soil physical and chemical properties. *Ozone Sci. Eng.*, **23**, 65–76.
12. Rice, R. G. et al. (1981) Use of ozone in drinking water treatment. *J. Amer. Water Works Assoc.*, **73**, 44–57.
13. Smilanick, J. L. et al. (2002) Impact of ozonated water on the quality and shelf-life of fresh citrus fruit, stone fruit, and table grapes. *Ozone Sci. Eng.*, **24**, 343–356.
14. Smith, D. B. et al. (2000) Droplet size and leaf morphology effects on pesticide spray deposition. *Trans. ASAE*, **43**, 255–259.
15. Somiya, I. (ed.) (2004) Ozone handbook. Japan Ozone Association, Japan, 119–132.
16. Terazoe, H. (2001) Control of *Fusarium* wilt of spinach by using of ozone sterilizing system in NFT. Central Research Institute of Electric Power Industry Report No. U00020, JP, pp.20 [In Japanese with English summary].
17. Yamamoto, H. et al. (1990) Disinfectious effect of ozonation on water infested with several root-infecting pathogens. *Ann. Phytopath. Soc. Jpn.*, **56**, 250–251.