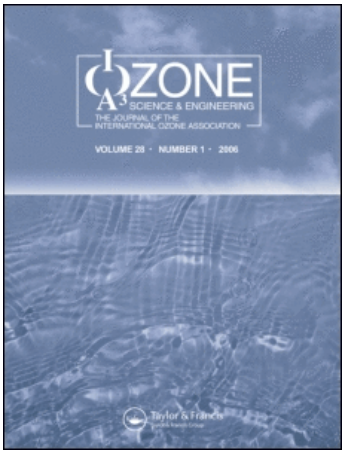

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Spraying Ozonated Water under Well-Ventilated Conditions Does Not Cause Any Visible Injury to Fruit-Vegetable Seedlings

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RESEARCH NOTE

Spraying Ozonated Water under Well-Ventilated Conditions Does Not Cause Any Visible Injury to Fruit-Vegetable Seedlings

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To determine whether horticultural plants are visibly injured by being sprayed with ozonated water (OW) for airborne-disease control, we sprayed OW with a dissolved ozone concentration (DOC) of 4.0 or 8.0 mg L^{-1} or distilled water (DW) on melon (Cucumis melo L. 'Andes'), tomato (Lycopersicon esculentum M. 'Momotaro-York'), watermelon (Citrullus lanatus M. 'Fujikou'), cucumber (Cucumis sativus L. 'Sharp'), green pepper (Capsicum annuum L. 'Kyounami'), and eggplant (Solanum melongena L. 'Senryo-nigou') seedlings under well-ventilated conditions. The OW and DW were sprayed on the seedlings at 10 a.m. and 2 p.m. on 3 successive days. No yellowing, chlorosis, necrosis, or malformation was observed for any of the seedlings during this period and also 1 day after the last OW spraying. These results indicate that intensive spraying of OW with a high DOC, for airborne-disease control, does not cause any visible injury to the fruit-vegetable seedlings used as long as the spraying is carried out under well-ventilated conditions.

Keywords Ozone, Agricultural Applications, Dissolved Ozone Concentration, Greenhouse Cultivation

INTRODUCTION

The reduction in agricultural chemical usage has become an important issue for various reasons; therefore, we require practical and effective treatments using substances that reduce agricultural chemical usage and are less harmful to humans and the environment and also less persistent (Fujiwara and Fujii, 2002). Ozonated water (OW) can be used as an alternative to agricultural chemicals for controlling powdery mildew on cucumber (Fujiwara and Fujii, 2002; Fujiwara et al., 2009).

OW with a dissolved ozone concentration (DOC) of 4.0 mg L^{-1} (Fujiwara and Fujii, 2002) or 8.0 mg L^{-1} (Fujiwara et al., 2009) was intermittently sprayed 4 times for 2 weeks for controlling powdery mildew infection; no visible injury, i.e., yellowing, chlorosis, necrosis, or malformation, was observed on the cucumber leaves at later growth stages. However, spraying OW daily or even more frequently in an attempt to increase the efficacy of disease control may cause visible injury to horticultural plants, especially in the early growth stages. Plants are more susceptible to ozone gas injury in the early stages of growth than in the later stages (Timonen et al. 2004); therefore, they are expected to be more susceptible to intensive OW spraying in the early growth stages than in the later growth stages.

Above a certain concentration, ozone gas has been reported to exert deleterious effects not only on plant growth and productivity (Schreiber, 1978; Health, 1980; Cooley and Manning, 1987) but also on leaf appearance (Gentile et al., 1971; Karlsson et al., 1995; Bergmann et al., 1999; Khan and Khan, 1999). However, little is known regarding the effects of spraying OW on horticultural plants with visible injury caused by factors other than ozone gas.

Therefore, we performed an experiment to determine whether spraying OW can cause visible injury to horticultural plants if the effects of ozone gas released from sprayed OW droplets are eliminated. To minimize visible injuries caused by ozone gas, we sprayed OW on the horticultural plants after moving them outside the greenhouse in order to provide good ventilation. Several fruit-vegetable seedlings in the early growth stages were used as horticultural plants.

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MATERIALS AND METHODS

Plant Materials

Melon (*Cucumis melo* L. 'Andes'), tomato (*Lycopersicon esculentum* M. 'Momotaro-York'), watermelon (*Citrullus lanatus* M. 'Fujikou'), cucumber (*Cucumis sativus* L. 'Sharp 1'), green pepper (*Capsicum annuum* L. 'Kyounami'), and eggplant (*Solanum melongena* L. 'Senryo-nigou') seedlings were obtained from a commercial seedling supplier (Berg Earth Co. Ltd., Ehime, Japan) on October 13; each seedling was grown in an individual cell in a 128-cell tray. The seedlings were then replanted on the same day in paper pots (diameter, 8 cm) filled with a commercial organic soil mixture and cultivated for 5 d on a growth shelf in a small greenhouse (length, 5 m; width, 3 m; height, 3.5 m) located at the top of an 8-story building at the University of Tokyo, Bunkyo-ku, Tokyo. Of these, 9 similar-sized seedlings from each species were selected for use as horticultural plants, and each pot containing a seedling was placed on a tray. The trays were placed on a growth shelf in the greenhouse and subirrigated once a day for 1 h from October 13 till the day after the last spraying.

Ozonated Water Preparation

Softened tap water was ozonated by passing it through an electrolytically ozonated water generator (Do-0100BESTA; Shinko Plant Engineering & Construction Co. Ltd., Japan) equipped with an ultraviolet-absorption-method-based DOC analyzer. The DOC of the OW collected in the reservoir of a stainless steel accumulator-injection sprayer (MH9D; Maruyama MFG Co. Inc., Japan) was adjusted to 4.0 or 8.0 mg L^{-1} by adjusting the set DOC value of the generator.

Spraying Treatments and Inspection for Visible Injuries

OW with a DOC of 4.0 or 8.0 mg L^{-1} or distilled water (DW) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days (thus, it was sprayed for a total of 6 times) on the above mentioned seedlings. For each spraying treatment, 135 mL (15 mL per plant) of OW or DW was sprayed outside the greenhouse with the sprayer. The spray distance between the nozzle and the fully expanded leaf was approximately 30 cm. According to the catalog, the Sauter mean diameter for the nozzle was approximately 0.22 mm at a discharge pressure of 0.3 MPa at the spray nozzle, and the discharge pressure of the sprayer was approximately 0.9 MPa. To avoid visible injuries caused by ozone gas, all the seedlings were left outside the greenhouse for 5 min after spraying. The seedlings were then replaced on the shelf in the greenhouse.

Every leaf was carefully inspected for visible injuries (yellowing, chlorosis, necrosis, or malformation) to determine whether visible injury had occurred or developed after spraying. The seedlings were inspected just before spraying and approximately 17 h after the second, fourth, and last spraying treatment.

RESULTS AND DISCUSSION

Figure 1 shows all the seedlings before the start of the spraying treatment and after the last spraying of OW (DOC, 4.0 or 8.0 mg L^{-1}) or DW. Although small yellow leaves can be seen in Figure 1, these are the cotyledons that were already yellow before the first spraying. No visible injuries or physiological disorders were observed for the leaves of any of the seedlings during the 3 successive days on which they were sprayed and even 17 h after the last spraying. These results indicate that when spraying is carried out under well-ventilated conditions, even extremely intensive (6 times in 3 successive days) spraying of OW with a high DOC of 4.0 or 8.0 mg L^{-1} does not cause any visible injury to the 6 species of fruits-vegetables seedlings.

Spraying OW may cause injury to or physiological disorders in the plant leaves because of the infiltration of the leaves by (1) ozone gas released into the air at the nozzle of the sprayer, from OW droplets while they are airborne, or from OW droplets that reach areas other than the leaves and the pot surfaces; (2) ozone gas released into the air from OW drops/droplets that come into contact with the leaves and pot surfaces; and (3) ozone from the OW drops/droplets that come into direct contact with the leaves.

We designed this experiment such that the ozone gas released into the air from the sprayed OW had a considerably lesser chance of reaching the seedlings; the OW was sprayed outside the greenhouse, and all the seedlings were left outside for 5 min after spraying. In this experiment, infiltration of the ozone gas into the leaves by the released in cases (1) and (2), while the seedlings were placed outside, is not considered to be high enough to cause visible injury to the leaves. In contrast, it was thought that the ozone gas released in case (2), after the seedlings were moved back into the greenhouse, might cause visible injury to the leaves; however no such visible injury was observed. In the same manner, for case (3), it may be said that infiltration of the ozone into the leaves present in the OW drops/droplets that come into direct contact with the leaves did not visibly injure them.

Ozone gas concentration has been considered as a key factor inducing physiological disorders in plant leaves (Health, 1980). Although the ozone gas concentration around seedlings was not measured in the present experiment, its concentration appeared to be quite similar to its atmospheric concentration; this is because the seedlings were placed outside the greenhouse. Park et al. (2008) reported that the ozone gas concentration in even a poorly ventilated greenhouse never exceeded 0.04 mL L^{-1} when OW with a DOC of approximately 7 mg L^{-1} was sprayed for 30 min for evaporative fog cooling in the greenhouse.

However, intensive and excessive OW spraying, similar to that performed in this experiment, can be used to control airborne plant-disease infection, considering that spraying OW twice a week has been reported to be effective in controlling powdery mildew (Fujiwara and Fujii, 2002). Thus, spraying OW for controlling airborne plant-disease infection

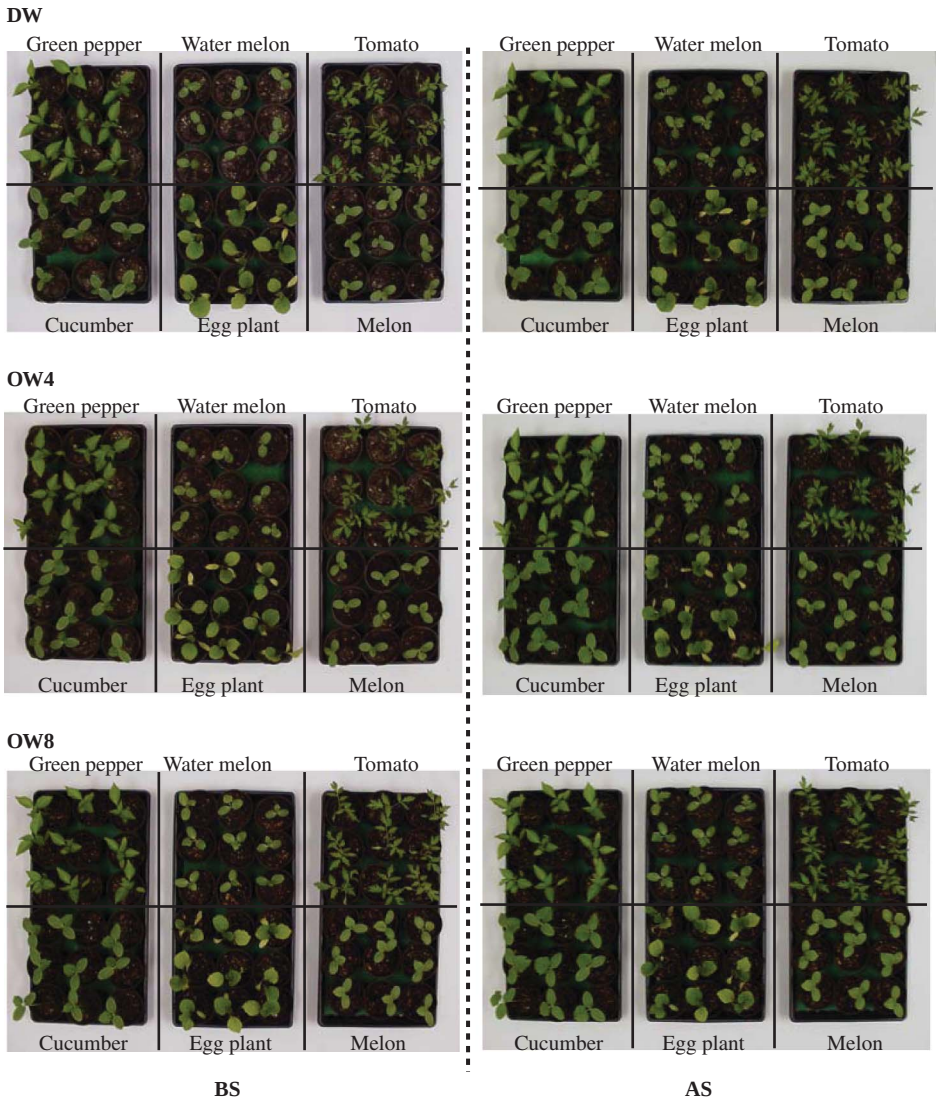

FIGURE 1. Seedlings of green pepper, watermelon, tomato, cucumber, eggplant, and melon before and after spraying (BS and AS) distilled water (DW) or ozonated water (OW) with a dissolved ozone concentration (DOC) of 4.0 mg L^{-1} (OW4) or 8.0 mg L^{-1} (OW8). In all cases, the water was sprayed twice everyday (10 a.m. and 2 p.m.) on 3 successive days (sprayed a total of 6 times in 3 d).

is unlikely to cause visible injury as long as spraying is carried out in a well-ventilated greenhouse. A greenhouse can easily be ventilated well by opening its side and ceiling windows or by using forced-ventilation fans. This suggests that for controlling airborne plant-disease infection more efficiently, we can spray OW with a DOC higher than 8 mg L^{-1} fairly frequently, e.g., once a day, without causing any visible injury.

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