Ecotoxicology and Environmental Safety 73 (2010) 1028–1033

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Simultaneous ozone fumigation and fluoranthene sprayed as mists negatively affected cherry tomato (*Lycopersicon esculentum* Mill)

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ARTICLE INFO

Article history:
Received 16 January 2010
Received in revised form
29 March 2010
Accepted 4 April 2010

Keywords:
Ozone fumigation
Fluoranthene
Mannitol
Pigment content
Tomato
Additive effect

ABSTRACT

Ozone (O_3) fumigated at 120 μg L^{-1} for 12 h d^{-1} was combined with 10 μM fluoranthene, and other treatments, including Mannitol solution to investigate the interaction of the two pollutants on tomato plant (*Lycopersicon esculentum* Mill). Using ten treatments including Mannitol solution and a control, exposure experiment was conducted for 34 d inside six growth chambers used for monitoring the resulted ecophysiological changes. Visible foliar injury, chlorophyll *a* fluorescence, leaf pigment contents, CO_2 uptake and water vapor exchange were monitored in tomato. Ozone or fluoranthene independently affected some ecophysiological traits of the tomato. In addition, simultaneous treatments with the duo had increased (additive) negative effects on the photosynthesis rate (A_{max}), stomatal conductance (g_s), chlorophyll pigment contents (Chl *a*, Chl *b* and $Chl_{(a+b)}$) and visible foliar symptoms. Contrarily, alleviation of the negative effects of O_3 on the leaf chlorophyll *a* fluorescence variables by fluoranthene occurred. Mannitol solution, which functioned as a reactive oxygen species scavenger was able to mitigate some negative effects of the two pollutants on the tomato plants.

1. Introduction

In the last two decades, research focus in the field of environmental science has changed rapidly from forest declination that mainly dealt with single factor experiments to multiple-stressors effects. The change has been attributed to the much evidence on antagonistic and synergistic factorial pathways that have accumulated from the studies in the past 30 years (Paoletti et al., 2010). Tropospheric ozone (O_3), a phytotoxic pollutant and an agent of climate change (Andrady et al., 2006; Wilson et al., 2006) is a by-product of the photochemical processes associated with air pollution, or, chemical reactions involving nitrogenous oxides (NO_x) and volatile organic compounds (VOCs) in the presence of sunlight (Simpson et al., 1995). VOCs are produced mainly by incomplete combustion processes, in similar way to polycyclic aromatic hydrocarbons (PAHs). PAHs emitted into the atmosphere are from both natural and anthropogenic sources (Simonich and Hites, 1995). The natural sources may include forest fires and volcanic eruptions (Nikolaou et al., 1984). However, the major sources of PAHs in the atmosphere are anthropogenic, which includes, incomplete combustion emissions from petrol and diesel engines, residential heating, wood fires, industrial processes, cigarettes, cooking and biomass burning (Finlayson-Pitts and Pitts, 1997; Li et al., 2003).

PAHs have received much attention, as they are emitted worldwide, and are known to have carcinogenic and/or mutagenic properties (Menzie et al., 1992). PAHs may have significant ecological impacts on higher plants, and thus leading to economic consequences as well (Lee et al., 2001). They react with oxidants such as •OH radicals, ozone and NO_3 radicals in the troposphere, often yielding degradation products, which may be more toxic than the parent PAH (Pitts et al., 1978). Fluoranthene (FLU), a four ring PAH, is among the most widespread PAHs in the environment, often indicating the presence of other PAHs (Wetzel et al., 1994).

Uptake of O_3 into the internal leaf through stomata results in the production of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals, all of which impair metabolic processes (Mehlhorn et al., 1990; Wohlgemuth et al., 2002). Similarly, FLU's mechanism of action is similar to that of other PAHs, disturbing thylakoid membranes and reversibly inactivating photosystem II (Huang et al., 1997; Mallakin et al., 2002). The involvement of ROS in the mechanisms by which PAHs inflict their phytotoxic effects on plants has been discussed (Oguntimehin and Sakugawa, 2008).

The need to alleviate the negative effects of O_3 and/or other pollutants on plants prompted the search for chemical compounds that counteract pollutant-induced phytotoxicity. Mannitol is produced in some plants, and is recognized as a

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doi:10.1016/j.ecoenv.2010.04.003

potent ROS quencher. Mannitol can scavenge •OH radicals generated by cell-free oxidant systems (Upham and Jahnke, 1986). Previous study compared several exogenous ROS scavengers (peroxidase, superoxide dismutase and mannitol), and found that mannitol was the most effective ROS scavenger. Mannitol effectively alleviated some of the negative ecophysiological effects of FLU on Japanese red pine needles (Oguntimehin and Sakugawa, 2008). In addition, the singular or combined effects of O_3 and FLU on Japanese red pine, an evergreen coniferous tree (Oguntimehin et al., 2008; Oguntimehin and Sakugawa, 2009) had been reported, also, the significance of using tomato, a world recognized agriculturally important crop was reported (Oguntimehin et al., 2010) from a different study. Tomato being a major vegetable crop has achieved tremendous popularity over the last century. In the present study, we wish to examine the interactive effects of ozone and fluoranthene treatments on the ecophysiological status of tomato plants.

The toxic interactions among pollutant compounds need to be considered because of the potential for *antagonism*, *additivity*, and *synergism* in comparison to single components (Mauderly, 1993). The effects of pollutants on plant biochemistry and physiology depend on each individual pollutant and the combination of pollutants. Effects also vary according to the exposure regime, i.e. both the temporal variation in pollutant concentration and the cumulative dose (Weber et al., 1993). Plants' responses are affected by plant species and life stage (Momen et al., 1996), and environmental conditions, such as light and temperature (Anderson et al., 1997).

2. Materials and methods

2.1. Plant material and growth of plant

Seeds of tomato (*Lycopersicon esculentum* Mill. 'MiniCarol' from Sakata Seed Co., Ltd. Yokohama, Japan) were germinated in nursery cups containing commercial soil (Takii Co Ltd., Japan) on January 23, 2008. Seedlings were nurtured in a gas-heated glasshouse at Hiroshima University, Higashi Hiroshima, under a 12 h photoperiod (day, 20–28 °C; night, 15–20 °C; 55–70% mean relative humidity and 980 ± 110 μmol m^{-2} s^{-1} maximum photosynthetic photon flux density (PPFD) at plant height during day time).

When seedlings were 33 days old, they were transplanted into larger pots (10 L capacity), containing a commercial soil mixture (''Golden'' vermiculite; Iris Ohyama Co. Ltd., Sendai, Japan). Plants were watered as required during the growth period. Two months after tomato seedlings were transplanted into pots, nutrient solution (N/P/K=6:10:5; Hyponex, Murakami Bussan, Tokyo, Japan) was added at a rate of 1 mL concentrated nutrient solution per 500 mL MilliQ water per pot.

2.2. Open-top chamber (OTC) facility and ozone exposure

Plants were transferred into six open-top chambers (OTCs) on March 19, 2008.

The chambers were built inside the Hiroshima University campus (34°24′N, 132°44′E) were designed as described previously (Oguntimehin et al., 2010). The fan speed pumping air through the charcoal filters was reduced by 50% in the present study compared with the former. The chambers were covered with transparent ethylene–tetrafluoroethylene copolymer film (ETFE) (F-CLEAN®, Asahi Glass Green-Tech Co. Ltd., Japan), which allows maximum ultraviolet light transmission (over 95% sunshine transparency). The mean photosynthetic photon flux density (PPFD), (LI-190SA Quantum Sensor, Licor, USA) incident on the foliage of the tomato plants from May–July, 2008 was 1050 ± 23 μmol m^{-2} s^{-1}. The mean air temperature and relative air humidity in the chambers during that period were 23.5 ± 2 °C and 65 ± 4%, respectively. The mean temperature and mean relative humidity variations between the inside and outside of the chambers before and during the exposure period were negligible.

Charcoal filters installed in the chambers removed excess O_3 and maintained its concentration below 13.7 ± 3.4 μg L^{-1} (hourly mean ozone concentration for all chambers, measured on April 18, 2008). Two of the six chambers were used for the O_3 fumigation; therefore, they contained spent and ineffective charcoal filters, which were fitted to maintain the same air pressure similar to the ozone non-fumigated chambers.

2.3. Treatment system formulation

A stock solution (1 mM) of FLU (Sigma–Aldrich, USA) was prepared in 50% acetone (ACT) (Wako pure chem. Ind., Japan) and MilliQ water (Millipore Co., Japan). The stock was diluted to a final concentration of 10 μM with MilliQ water for each spraying period. This resulted in a final concentration of 0.5% ACT in solutions, which was the same as in the ACT treatment. 1 mM Mannitol solution (MANN; Nacalai, Kyoto, Japan) was prepared for use as an •OH radical scavenger. The treatment system comprised of 10 treatments namely: O_3, O_3+MANN, FLU, FLU+MANN, O_3+FLU, O_3+FLU+MANN, ACT, MANN, ACT+MANN and the Control (tomato fumigated with MQ water). Each treatment is made up of six tomato plants distributed carefully inside the chambers. Each chamber consisted of two different treatment types; the ozone fumigated chambers accommodated four treatments, while the remaining four filtered chambers accommodated the remaining six ozone non-containing treatments. Tomato seedlings were rotated bi-monthly in filtered (ozone non-fumigated) chambers to nullify any bias due to the relative positions of the chambers on the tomato seedlings.

The sprayed solutions (ACT, FLU) were applied to tomato foliage three times per week (06:00–7:00 HR) using an electronic spray machine with a nozzle (BS-4000, Fujiwara Sangyo, Miki, Japan) except for MANN solution that was applied twice weekly. On the average, each plant received about 50 mL of solution per spraying period, the solutions component were applied singly. In most cases, FLU was fumigated first, and the other component, e.g. Mannitol in FLU+MANN treatment was applied after the wet tomato leaves had dried (Oguntimehin et al., 2009). O_3 gas was generated through the electrical discharge of oxygen by an ozone generator (Super ceramic E-04 ozonizer) that has been pre-set to automatically let the generated ozone into the chambers at the rate of 14.4 L min^{-1} during the 00:80–20:00 HR daily. The hourly mean ozone concentration during the 34 d exposure period was 120.0 ± 25.0 μg L^{-1}. Inside the chambers, ozone concentration was monitored using a UV photometric O_3 analyzer (Model 49C Thermo Environmental Instrument Inc. Massachusetts, USA). The AOT_{40} value estimated for the total O_3 fumigated in 34 d period is about 1 mg L^{-1}, this value was lower than the ≈ 1.5 mg L^{-1} fumigated to Japanese red pine in the first 30 d of exposure (Oguntimehin and Sakugawa, 2009).

2.4. Photosynthesis rate, leaf pigment contents and chlorophyll fluorescence measurements

Carbon dioxide and water vapor exchange measurements were conducted on mature primary tomato leaves as previously reported (Oguntimehin et al., 2009). For each measurement, the leaf chamber area was fully covered with tomato leaf. From 7:00 to 10:30 HR, net photosynthesis at near-saturating irradiance of 1500 μmol m^{-2} s^{-1} (A_{max}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured in matured leaves in each treatment. Chamber leaf temperature was kept at 25 ± 2 °C while the leaf to air vapor pressure deficit (VpdL) was maintained between 0.8 and 1.3 kPa, and 'air into leaf chamber' CO_2 concentration was kept at 370 μmol CO_2 mol^{-1} at a flow rate of 500 μmol s^{-1} by an open-flow infrared gas analyzer with light and temperature control systems (LI-6400, Li-cor Inc., Lincoln, NE, USA).

Chlorophyll fluorescence was measured at night (19:30–21:00 HR) using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) with leaf-clip holder 2030B (Heinz Walz GmbH); and micro quantum sensor for selective PAR measurements (0–20,000 μmol m^{-2} s^{-1}). The leaves that were previously used for the leaf gas exchange measurements were dark adapted and arranged compactly in a parallel array and clamped with the holder, then the minimal fluorescence values (F_0) were obtained upon excitation of leaves with a beam from the light emitting diode; and maximum fluorescence (F_m) was measured following a 600 ms pulse of saturating white light. The yield of variable fluorescence (F_v) was taken as $F_m - F_0$. These measurements indicated maximal photochemical efficiency of PSII in the dark (F_v/F_m).

The concentrations of Chl *a*, Chl *b* and total carotenoids were determined by extracting 100 mg sliced tomato leaves with 10 ml 96% (v/v) ethanol. The same leaves used for A_{max}, g_s and C_i were collected 10 h after the end of exposure, pigment extraction analysis was performed immediately after sampling. Absorbance of the extract was measured in the scanning mode (400–700 nm) at 470, 663.8 and 646.8 nm with a spectrophotometer (UV-2400, Shimadzu Co., Japan.). Concentrations of Chl *a*, Chl *b* and total carotenoids were calculated using the equations given by Lichtenthaler and Wellburn (1983).

During the measurement periods discussed above, only the tomato leaves that were fully expanded were selected for clipping inside the chamber. When a single large leaf was obtained, it was ensured that the entire chamber area was covered with the leaf. However, for a small leaf area that is too small to fill the chamber space, the area suitable and used for clipping is estimated after measurements with a digital caliper (CD-15 Mitutoyo Co., Kanagawa, Japan). The value was later transferred and used in the calculation of A_{max} and g_s. Leaf age and position were put into consideration during measurements, even where the emergence of severe foliar symptoms on the plants made selection difficult, only leaves that have reached full expansion (between 24 and 30 days old) were considered suitable for clipping inside the chamber. Mostly the leaves were located at the top 1–8