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# The use of Sunpatiens (*Impatiens* spp.) as a bioindicator of some simulated air pollutants – Using an ornamental plant as bioindicator

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ABSTRACT

Sunpatiens were exposed separately or combined to ozone gas (130 ppb), fluoranthene (10 μM) and sulphuric acid mists (pH 3) sprayed as simulated pollutants in chamber conditions for 21 d. The treatments negatively affected the gas ($CO_2$ and moisture) exchange, leaf chlorophyll fluorescence, and the leaf-quality expressed in chlorophyll value (SPAD). Fluoranthene and the acid individual negative effects on the measured eco-physiological variables were nearly the same on Sunpatiens; their effects became aggravated on combining the duo. The foliar symptom assessments of chlorosis, necrosis and stippling revealed severe damages in ozone containing treatments compared with other treatments. The presence of fluoranthene exuberate ozone negative effects on some of the plant eco-physiological status. Where mannitol (1 mM) additions were contained in treatments, mitigation effects of the negative impact of pollutants resulted. These findings indicated that Sunpatiens can be used as an active bioindicator of singular and multiple pollutants in field conditions.



## 1. Introduction

Over the past few decades, there has been a progressive global industrialization and increased population which have led the environment into severe pollution problems. Acid rain and ground level ozone pollution caused by the interaction of heat, sunlight, emissions from automobiles, industries, and coal fired power plants are threats to our public health and to the plants and animals that share our habitat (Kohut, 2005).

Many terminologies such as biosensors, biomarkers and bioindicators are in use freely, to express different interactions of stressors with the biosphere. Abiotic plant stress inducers include water, light, salt, high and low temperatures, heavy metals, nutrients and environmental pollutants. The stress responses of plants can be divided into three levels: growth, physiology, and molecular biology. A major pathway to stress tolerance in plants involves stress recognition, signal transduction, gene induction, gene products, protection, repair, and stress tolerance. Therefore, to grow plants which can tolerate in the changing global environment, we need joint efforts within the fields of physiology and molecular biology, plant breeders working with genetic engineers (Rao et al., 2006).

Bioindicating is an anthropogenically-induced response involving biomolecular, biochemical, or physiological parameters in plants or animals that has been causally linked to biological effects in organisms, populations, communities, and ecosystems (Arndt et al., 1987; McCarty and Munkittrick, 1996). Bioindicators are used in the direct determination of the biological effects of a single pollutant, the determination of the synergetic and antagonistic effects of multiple pollutants on an organism, and in the early recognition of pollutant damage to plants as well as toxic dangers to humans. Bioindicator tests are often operated at relatively low cost compared to technical measuring methods (Posthumus, 1982).

Several types of air-pollution effects on plants have been described (Manning and Godzik, 2004; Heath, 2008). They range from the acute effects of exposures to high concentrations over short periods, to the chronic effects of exposures of low concentrations over long periods. Examples of acute effects are clearly visible in the chlorosis and necrosis of leaf tissues, leaf, flower or fruit abscissions, and the epinastic curvatures of leaves and leaf stems. Chronic effects may appear as retardation or disturbance of normal growth and development (resulting in reduction of yield or quality of agricultural, horticultural, and forestry crop plants), or slow discoloration (chlorosis), leaf-tip necrosis, and ultimately total dieback of plant organs. In some cases the symptoms of acute and chronic effects may be fairly specific for a particular air pollutant or for a combination of different pollutants (Krol et al., 1982).

When natural vegetation and the cultivated plants present in the studied area are used to monitor the biological effects of a pollutant (passive bio-monitoring), differences in soil, water or even climatic conditions may influence the effects or diminish the comparability of results from different sites. It is therefore better to use selected indicator and accumulator plants, cultivated and exposed in conditions of soil and watering that are standardized as far as

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possible (active bio-monitoring). Indeed, the potential of bioindicators for environmental monitoring is often confronted with some criticisms, e.g. those related to the effects of environmental load that cannot always be clearly differentiated from natural stress factors, or the lack of practical experience with certain bioindicators that sometimes makes clear interpretation of findings more difficult, most especially if no comparable pollutant measurements are available. Nevertheless, numerous plant bioindicators have satisfied the requirements of convenience, standardization, cost and evaluative capability. Examples include pine tree needles, lichens, rye grass, green kale, tobacco, and beans (Manning et al., 2002).

Sunpatiens (*Impatiens* spp.) is a horticultural plant that originated in Japan. The variety 'Spreading Salmon with variegated leaves' was chosen in the present study because of its profuse growth characteristics and superior environmental cleanup capability as compared with conventional horticultural plants (Sakata, 2008). By monitoring some eco-physiological traits of Sunpatiens, we aimed at investigating the suitability of the native portable plant as a bioindicator of some simulated air pollutants. In addition, using a reactive oxygen species scavenging solution (mannitol), we may be able to acquire some new knowledge concerning plant protection against the simulated atmospheric pollutants.

The concentrations of pollutants employed in the present study on Sunpatiens have been reported in past studies involving *P. densiflora*. The ozone daily average concentration of between 120 and 150 ppb employed in this study (Oguntimehin and Sakugawa, 2009), was comparable to the measured daily average concentration measured in some forest mountains in Japan, where declination of different tree species were observed. Examples are Konara oak (*Quercus serrata*) and Mizunara oak (*Quercus mongolica*) at the seaside areas of Ishikawa, Tottori and Shimane. In addition, some other species such as Japanese fir (*Abies firma* Siebold and Zucc) in Fukuoka prefecture; Siebold's beech (*Fagus crenata* Blume) at Mt. Tanzawa in Kanagawa prefecture; Erman's birch (*Betula ermanii*) in the Oku-Nikko area of Tochigi prefecture were affected (Shimizu and Feng, 2007) by same concentration of ozone. A 10 μM fluoranthene concentration employed has been reported in Oguntimehin et al. (2008), the concentration was argued to be comparable to the minimal concentrations that were used elsewhere (Huang et al., 1996; Kummerova and Kmentova, 2004). Acid solution of pH = 3 used in the present work is comparable to those used elsewhere (Reich et al., 1986; Takemoto et al., 1988; Miwa et al., 1993), also, another report had showed the lowest pHs of ambient acid rain to be close to pH 2.3 (Cape, 1993).

The four types of eco-physiological assessments were used to express the response of the plant to the stress variations: (i) $CO_2$ uptake and water vapour exchange, (ii) leaf chlorophyll *a* fluorescence, (iii) leaf-quality, and (iv) leaf foliar damage assessments. Most of the eco-physiological variables examined are pertinent to photosynthesis – a central anabolic pathways in plants, which results in the production of energy-rich organic compounds necessary for growth and the day to day life cycle of plants; therefore, where there is presence of pollutants in the biosphere, in excess of the amount than the detoxifying mechanisms of plants can naturally cope with, detection of abnormality is reflected in measured parameters.

## 2. Materials and methods

### 2.1. Plant and soil materials, plant growth and conditions

Sunpatiens seedlings were purchased from the horticultural plant shop at NAFCO (Higashi-Hiroshima, Japan) on August 3, 2009. With extra addition of some weathered rocks (Masado), the seedlings were later transplanted into larger pots (12 cm × 12 cm) containing a commercial soil mixture, "Golden" vermiculite, purchased from Iris Ohyama Co. Ltd. (Sendai, Japan). The soil was enhanced by amending it with some organic material to make it nutrient-rich with enhanced drainage. After transplanting, a liquid nutrient solution (N/P/K; 1:2:1; Hyponex, Murakami Bussan, Tokyo, Japan) was once in monthly (0.5 mL concentrated nutrient solution in 100 mL MilliQ water per pot).

Potted plants were left outside after the transplant for a week before being transferred to growth chambers located on the Higashi-Hiroshima University campus. The growth chambers were as previously described (Oguntimehin et al., 2008). In addition, the speed of the fan driving clean air through the charcoal filters was estimated using the 'Drafemaster, model 6311' and the air flow rates into the chambers from the outside were calculated to be $57.6 \pm 1.8\ m^3\ min^{-1}$ at daytime and $24.0 \pm 0.7\ m^3\ min^{-1}$ at nighttime. The mean maximum photosynthetic photon flux density (PPFD) was measured with an Li-190SA Quantum Sensor (Li-cor, USA). The mean PPFD incident on the plant foliage was approximately $1250 \pm 44\ \mu mol\ m^{-2}\ s^{-1}$ at midday (13:00–15:30) on a sunny day (August 31, 2009). A charcoal filter removed excess ozone ($O_3$) and sulphur dioxide ($SO_2$) from two of the four chambers used, and maintained their concentrations below $12 \pm 2$ and $3.5 \pm 1.1$ ppb, respectively (October 2, 2009). The mean air temperature in the chambers was $24 \pm 2\ ^\circ C$, while the mean relative air humidity was $74 \pm 3.5\%$. Once in the growth chambers, the plants were irrigated manually by adding a measured volume of water (usually before 08:00 HR) into each pot thrice weekly. The differences in the meteorological conditions of the inside and outside the experimental chambers were negligible.

### 2.2. Treatments preparation and ozone exposure

Ozone gas was supplied to two of the four experimental chambers using an ED-OG-S5, 350 W × 530D × 600H (EcoDesign®, Hikigun, Saitama, Japan). The system, a silent discharge method type fed with oxygen (generated by an air enrichment system 'PSA S0-005B' Sanyo Electronic Ind. Co. Ltd., Okayama, Japan), works at a voltage of 100 V; 50/60 Hz. The generated ozone was let into the two ozone exposure chambers at a rate <1 L $min^{-1}$. The concentration of $HNO_3$ measured by a low volume air sampler (September 16–18, 2009) was <2 ppb. Therefore, it is unlikely that $NO_x/HNO_3$ generation from the ozone generator caused a bias in the actual dosage of ozone fumigated. The daily average concentration of ozone measured inside the experimental chambers ranged from 120 to 140 ppb, culminating to mean fumigated dosage of $AOT_{40}$ $24.6 \pm 1.4$ ppm h in each chamber.

We prepared 1 mM stock solution of fluoranthene (Sigma–Aldrich, USA) in 50% aqueous acetone (Wako Pure Chem. Ind., Japan) diluted with MilliQ water (Millipore Co., Japan) as reported previously (Oguntimehin et al., 2008). For a single spraying period, the stock solution was diluted to a final concentration of 10 μM fluoranthene with MilliQ water, thus making acetone up to a final concentration of 0.5% v/v. A preliminary test conducted on Sunpatiens using this final concentration of acetone in spraying solutions showed that it had no negative effects on any of the eco-physiological traits examined in this study. More so, using two higher plants *Pinus densiflora* and *Lycopersicon esculentum* earlier, it was shown that 0.5% acetone solution had no negative effects (Oguntimehin et al., 2008, 2010). The concentration of fluoranthene in the sprayed solution was determined chromatographically (USEPA, 1990). Mannitol at a concentration of 1 mM (Nacalai Tesque, Kyoto, Japan) was prepared and used as an OH radical scavenger (Oguntimehin and Sakugawa, 2009). A stock of 0.1 M $H_2SO_4$ (Katayama Chemical Inc., Japan) was prepared by diluting an accurately measured volume of the concentrated acid into MQ water. For each use, aliquots of the acid stock were diluted in MQ water