Fluoranthene fumigation and exogenous scavenging of reactive oxygen intermediates (ROI) in evergreen Japanese red pine seedlings (*Pinus densiflora* Sieb. et. Zucc.).

Scavenging reactive oxygen intermediates in stressed Japanese red pine seedlings.

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Abstract

Generation of reactive oxygen intermediates (ROI) such as O_2^-, H_2O_2, and $^{\cdot}OH$ is known to be a major mechanism of damage in biological systems. This study investigated and compared effectiveness of scavenging ROI generated in fluoranthene (FLU) pre-fumigated Japanese red pine seedlings. Three kinds of eco-physiological assessments were used to express the impact of the different fumigants used inside the green house. Gas exchange measurements showed negative changes induced by 10 μM FLU on Japanese pine seedlings during a 10 d exposure period whilst no negative change was found during a 5 d exposure period. Moreover, during a 14 d FLU exposure incorporating ROI scavengers, results revealed that chlorophyll fluorescence, needle chemical contents and needle dry mass per unit area of the seedlings were affected. The negative effects of FLU on the conifer were dependent on both the dose and period of FLU fumigation. Peroxidase (PERO), superoxide dismutase (SOD) and mannitol (MANN) were all effective scavengers of ROI. MANN scavenged $^{\cdot}OH$, the most lethal of the ROI. For practicable use, MANN is more economical, and may be the best ROI scavenger among the three considered. It can be concluded that efficient scavenging of ROI in biological systems is important to mitigate the negative effects of FLU on Japanese red pine trees.

Keywords: Fluoranthene, reactive oxygen intermediates, pine needles, chlorophyll fluorescence, eco-physiology, enzymes.

1. Introduction

Fluoranthene (FLU) belongs to a group of organic compounds known as polycyclic aromatic hydrocarbons (PAHs) that comprise at least two fused condensed aromatic rings. It is also one of the 17 US Environmental Protection Authority (USEPA) priority PAHs. These compounds are generally formed by pyrolysis and incomplete combustion processes at temperatures of approximately 700 °C (ATSDR, 2006). Anthropogenic sources of PAHs include combustion of fuels, refining, coke production, bush burning, automobile exhausts and cigarette smoke. Plants' needles and leaves are important sinks for atmospheric PAHs. The structures of these plant organs are complex, and the route by which PAHs move through them, and become stored or processed by them dictates their environmental fate and plays a role in their annual cycling (Wild et al., 2005; Wild et al., 2006). A common mechanism of contaminants' toxic action is inhibition of biological pathways such as photosynthesis and mitochondrial electron transport (Babu et al., 2001). Photosynthesis is a very sensitive indicator of plant disorders. Its measurement enables the detection of early reversible changes in plant metabolism that are difficult to detect otherwise (Black and Unsworth, 1980). Therefore, measuring inhibition of photosynthesis was found to be useful in assessing the potential toxic effects of xenobiotic contaminants (including PAHs) on plants (Huang et al., 1997). Studies have shown that plant responses to PAHs depend on the intensity and duration of fumigation, the developmental stage of the plant, and the concentration of fumigating solutions (Wieczorek and Wieczorek, 2007). Lipophilicity (in terms of log K_{ow}) and availability are two factors found to control the bioavailability of PAHs in inner needle compartments, and thus, that influence the uptake efficiency of PAHs into plants (Wenzel et al., 1997).

Photo-induced toxicity of PAHs could be based in the formation of intracellular singlet oxygen and other reactive oxygen intermediates (ROI) that lead to biological damage

72 (Eisenberg and Cunningham, 1985; El-Alawi et al., 2002). Phytotoxicities appear to vary,
73 depending on the particular PAH and plant species (Hwang et al., 2003). The production of
74 reduced and excited species of ROI in chloroplasts has been reviewed (Asada, 2006). The
75 reaction centers of photosystem I (PSI) and photosystem II (PSII) in chloroplast thylakoids
76 are the major sites of ROI generation. Superoxide dismutase (SOD) constitutes the first line
77 of defense against ROI within a cell (Alscher et al., 2002). SODs are among the fastest
78 enzymes known (V_{max} of 2×10^9 M^{-1} s^{-1}; Karlson, 2003). Peroxidases (PERO, hydrogen
79 peroxide oxidoreductase) are widely found in plants, and oxidize a vast array of compounds
80 in the presence of hydrogen peroxide (H_2O_2) (Chen and Schopfer, 1999). Mannitol (MANN)
81 is produced in some plants and is recognized as a potent ROI quencher. It was shown to
82 scavenge hydroxyl radicals (·OH) generated by cell-free oxidant systems (Upham and Jahnke,
83 1986). The rate constant of MANN with ·OH at pH = 7 is $(1{\cdot}8 \pm 0{\cdot}4) \times 10^9$ M^{-1} s^{-1} (Goldstein
84 and Czapski, 1984).
85 FLU toxicity has been studied in higher plants (Huang et al., 1996; Kummerova and
86 Kmentova, 2004; Kummerova et al., 2006a). Previously, we investigated the fumigation
87 effects of FLU and phenanthrene over a long exposure period (~3 months) on the needles of
88 Japanese red pine (Oguntimehin et al., 2007) using open-top chambers. The negative effects
89 of FLU were more pronounced than those of phenanthrene. In the present study, our aims are
90 as follows: (a) to investigate the fumigation effects at two different molar concentrations of
91 FLU on Japanese red pine over 5 and 10 d exposure periods; (b) to relate the generation of
92 ROI induced by FLU with the resulting physiological or morphological changes in 2-year-old
93 Japanese red pine; and (c) to compare the efficiency of exogenous ROI scavengers in
94 reducing the negative eco-physiological effects of FLU on Japanese red pine needles.
95 Generally, three kinds of eco-physiological assessment criteria were considered: (i) gas
96 exchange parameters including net photosynthesis rate at saturated irradiance, A_{max}; stomata

conductance, g_s; and intercellular CO_2 concentration, C_i; (ii) chlorophyll fluorescence parameters including initial chlorophyll fluorescence, F_o; and maximal photochemical efficiency of PSII, F_v/F_m; and (iii) physiological parameters of the needles, including contents of chlorophyll a, (Chl a); chlorophyll b, (Chl b); and total chlorophyll, (Chl $_{a+b}$); and needle dry mass per unit area (NMA). In considering (b) and (c) above, we assumed that the activities of the antioxidant enzymes would provide an eco-physiological measure of Japanese red pine's relative resistance to FLU toxicity. Previous studies related to enzyme activities have used biological and biochemical assays. In our study, however, we fumigated MANN, PERO, SOD, and an equal-units mixture of PERO and SOD directly onto needle surfaces of Japanese red pine over a 14 d exposure period with 10 μM FLU. This method is similar to horticultural and agricultural foliar feeding practices, and is modified for the application of non-toxic formulations of organic enzymes (Gorton and Sollinger, 2005).

2. Materials and Methods

2.1. Greenhouse growth conditions

Experiments were carried out from May to June 2007 in the green house (metal-framed shelters) built inside the Hiroshima University campus (34°24'N, 132°44'E). Shelters were constructed in a way to prevent rain and dew from falling on the seedlings. The shelters were semi-cylindrically shaped on the horizontal axis, well ventilated, and covered an area of about 8 m × 12 m on the ground. The upper halves of the framed structures (excluding the two ends) were covered with a 0.06 mm thick Tefzel® film. The film is transparent to visible light and UV-A (DuPont, Wilmington, DE, USA). The mean photosynthetic photon flux density (PPFD), (LI-190SA Quantum Sensor, Licor, USA*)* incident on the green house between May and June, 2007 in the first, second and third batches of the experiments were

536 (0 – 1665), 533 (0 – 1707) and 536 (0 – 1719) μmolm^{-2}s^{-1} (respectively. The mean air temperature and mean relative air humidity (Thermo recorder TR-72S, T&D Corp., Japan) measured in ambient environment of the green house from May to June, 2007 in the first, second and third batches of the experiments were 14.7 (1-27) ^{o}C, 14.8 (1-30) ^{o}C, 14.8 (3-31) ^{o}C and 69 (13-99)%, 68 (13-99)%, 68 (12-96)% respectively. Although we have not measured these parameters inside the greenhouse, but we are of the opinion that the differences between the ambient conditions (outside) and the inside conditions of the greenhouse during the exposure period were negligible based on the design of the green house. Also, the difference in values of these parameters during the three different batches of the experiments can be considered negligibly small to effects any variations in the results of the experiments.

2.2. Plant and soil materials

Two-year-old Japanese red pine seedlings grown in a nursery in Fukuoka prefecture were purchased and transplanted into 0.35 m × 0.3 m deep pots (1 seedling per pot) on March 4, 2007. The pots were filled with 21 L soil, which was a mixture of yellow sandy soil (weathered granite), perlite (white loam 4–20 mm, Toho-Leo Co.), isolite (CG2, Isolite Insulating Products Co.) and humus soil (Midori-Sangyo Inc.) at 11:2:2:4 volumetric ratios, respectively. Neat litters composed of healthy pine woodland from the university campus were collected and spread over the soil surface in each pot (approximately 50 g pot^{-1}). Inside the green house, the pots were watered with de-ionized water once daily by 7:00 AM using an auto-irrigation system.

145

146 2.2. Fumigation systems

147 A stock solution was prepared by dissolving FLU (Sigma-Aldrich, USA) in 50% acetone
148 (ACT, Wako pure chem. Ind., Japan) and MilliQ (MQ) water (Millipore Co., Japan) adjusted
149 to 1 mM (202 mg L^{-1}). The stock was diluted as appropriate to final concentrations of 5 and
150 10 μM with MilliQ water. This made the final concentration of ACT in solutions 0.5%. The
151 highest concentration of FLU used is comparable to minimal concentrations used elsewhere
152 (Huang et al., 1996; Kummerova and Kmentova, 2004). 1 mM MANN (Nacalai, Kyoto,
153 Japan) was prepared and used as an ·OH scavenger. The fumigation system in the first
154 exposure experiment consisted of MQ, ACT, MANN, FLU and FLU+MANN. The solutions
155 were applied to the foliage of pine seedlings using an electronic spray machine with a nozzle
156 (BS-4000, Fujiwara Sangyo, Miki, Japan) twice daily (6:00–7:00 AM and 6:00–7:00 PM),
157 over a 5 and 10 d period. On the average each seedling was fumigated with 50 mL of the
158 fumigant per one spraying period. The soil surface was covered with a waterproof sheet
159 during fumigant application to prevent solutions from entering the roots of the seedlings. The
160 coverings were removed about 2 hrs after the fumigation. Three experiments were conducted
161 in batches. In the first experiment (5 d fumigation), MQ, ACT, and 5 or 10 μM FLU were
162 used. Secondly, the 10 d experiment involved all the five components of the fumigation
163 system mentioned above with FLU concentrations at10 μM. In addition, mannitol was
164 introduced as part of the fumigation system.

165 In the third exposure experiment, 6 units mL^{-1} SOD (S2515-30 KU; Bovine erythrocytes;
166 Sigma) and 6 units mL^{-1} PERO (P8250; Type II Horseradish; Sigma) were prepared from 30
167 KU mL^{-1} SOD and 30 KU mL^{-1} PERO stocks that were stored at –20 °C and used within 2
168 weeks. In all, the total of each enzyme fumigated was 1200 units. FLU solutions were applied

169 to the foliage of pine seedlings using an electronic spray machine with a nozzle (BS-4000,
170 Fujiwara Sangyo) from 6:00–8:00 AM and 5:30–7:30 PM 4 d per week. On average, each
171 seedling was fumigated with approximately 100 mL FLU daily. Also 100ml each of MANN
172 and enzymes were applied twice weekly, between 6:00–8:00 AM. Here, the fumigant system
173 consisted of FLU + MANN, FLU + PERO, FLU, FLU + SOD, and FLU + SOD + PERO. In
174 the case of each fumigant, the components were fumigated singly, one at a time. In most
175 cases, the FLU was fumigated first and after making sure that the wet needles of the pine
176 seedlings were almost dried, the next component (either mannitol or enzymes) was then
177 applied.

178 2.3. Photosynthesis and chlorophyll fluorescence measurements

179 Gas exchange measurements were conducted on one-year-old needles of pine seedlings.
180 Starting from 7:00–10:30 AM, net photosynthesis at near saturating irradiance (A_{max}),
181 stomata conductance (g_s) and intercellular CO_2 concentration (C_i) were measured for healthy
182 needles in each treatment. A_{max}, g_s, and C_i were measured at near-saturating irradiance of
183 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and at needle temperature of
184 25 ± 2 °C. 'Leaf to air vapour pressure deficit' (VpdL) was maintained between 0.8 and 1.3
185 kPa, 'air into leaf chamber' CO_2 concentration was kept at 370 μmol CO_2 mol^{-1} at a flow rate
186 of 500 μmol s^{-1} by an open-flow infrared gas analyzer with light and temperature control
187 systems (LI-6400, Li-cor Inc., Lincoln, NE, USA). After each measurement, the needles used
188 were harvested and their width and length measured with a digital caliper (CD-15, Mitutoyo
189 Co., Kanagawa, Japan). The cross-section of the needle was approximated as a semi-circle
190 having a diameter equal to the measured width; half of the leaf surface area of the needle was
191 used as the effective leaf area for A_{max}, g_s and needle dry mass per unit area (NMA)
192 determinations (Kume et al., 2001; Nakatani et al., 2007). In general, photosynthetic capacity

is influenced by area-based chlorophyll content in needles. Therefore, needle eco-physiological traits are expressed based on the effective leaf area.

Chlorophyll fluorescence was measured at night (7:00–8:00 PM) using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) with leaf-clip holder 2030B (Heinz Walz GmBH). The needles were arranged compactly in a parallel array and clamped with the holder, then minimal fluorescence values (F_o) and the maxima photochemical efficiency of PS II (F_v/F_m) were measured.

2.4. Needle chlorophyll content

Chl a and Chl b contents were determined by extracting 100 mg needles (the same needles used for A_{max} g_s and C_i) with N, N-dimethylformamide (DMF). Absorption of the extract was measured in the scanning mode (600–700 nm) at 663.8 and 646.8 nm wavelengths with a spectrophotometer (UV-2400, Shimadzu Co., Japan.). Concentrations of Chl a, Chl b, Chl $_{(a+b)}$ were calculated with equations given by Porra et al. (1989).

2.5. Statistical analysis

Statistical evaluation of results was carried out using SPSS 13 (SPSS, USA). Results are average determinations from five seedlings in each treatment group ± standard error of the mean (S.E). Significances of the differences in average values between each treatment were evaluated by one-way ANOVA and Tukey analysis ($p < 0.05$). Pearson's correlation coefficient (r, $p < 0.05$) was used to test correlations among the needle eco-physiological traits.

3. Results and Discussion

3.1. Fluoranthene concentration effects

We examined the effect of two FLU concentrations (5 and 10 μM) on the eco-physiological status of the seedlings. Fig. 1(A-C) shows the gas exchange ecophysicological traits of Japanese red pine after 5 d exposure. There were no effects of 5 or 10 μM FLU on A_{max}, g_s, and C_i, Compared with the MQ and ACT treatments' values, there were no statistically significant differences among all the traits examined. It is probable that after 5 d of exposure, the FLU dosages were insufficient to cause any significant eco-physiological changes in Japanese red pine. More likely, the concentration of FLU used on seedlings during the 5 d period could not produce any significant change. However, considering the total reduction in values of the traits over the 5 d exposure period, it is evident that 10 μM had a greater negative effect than 5 μM FLU. A_{max} values decreased by 12% in response to 10 μM FLU, and by 2% with 5 μM FLU (from initial values of 16.2 and 14.3 μmol CO_2 m^{-2} s^{-1}, respectively). These results suggest that a longer application period of 10 μM FLU solution on the Japanese red pine might produce a statistically significant change in the eco-physiological traits.

3.2. Effect of increased application period

Japanese red pine seedlings were fumigated with 10 μM FLU for 10 d. After the 10 d fumigation, A_{max}, g_s and C_i were significantly reduced in the FLU-treated pine seedlings as compared with MQ, ACT and MANN treatments (Fig. 1D-F). However, F_o, $F_v/F_{m,}$ NMA and Chl a/ Chl b were not statistically different in all the treatments (F_o, $F_v/F_{m,}$ NMA and chlorophyll data not shown). The much decreased A_{max} in this study might have been caused by the limitation in stomatal conductance and the reduced internal CO_2 intake. Decreased

238 A_{max} of Japanese red pine needles by the FLU in this study is similar to that reported by
239 Kobayashi et al. (2002), using ·OH generating solutions as fumigants. This suggests that gas
240 exchange measurement is sensitive in detecting the negative changes inflicted on the
241 seedlings in 10 d. In addition, correlation coefficients of the eco-physiological traits
242 examined in this study indicated that only g_s and C_i showed positive correlations with A_{max} (*r,*
243 $p < 0.05$; = 0.85 and 0.63, respectively). There was no statistically significant difference in
244 the Chl a, Chl b and Chl $_{(a+b)}$ contents of the pine needles in the 10 d period. Also, Chl a/Chl b,
245 a characteristic stress indicator in plants (Shan et al., 1997), was unchanged in FLU-treated
246 seedlings. The decreased A_{max} by FLU in the present study might be due to the generation of
247 ROI such as O_2^- and ·OH (Krylov et al., 1997). This hypothesis is similar to that proposed by
248 Wang et al. (2005) on the photolysis of PAH in water. The wax layers in the needles of the
249 Japanese red pine may accumulate sufficient FLU for a photolysis reaction to take place
250 (Wang et al., 2005; Dolinova et al., 2004). In this study, MANN displayed a mitigating action
251 against the negative effects of FLU. MANN at 1 mM could be used as an ·OH scavenger,
252 even though some studies reported osmotic stress at concentrations above 90 mM (Lin and
253 Kao, 2002; Gill et al., 2002).

254

255 3.3. Scavenging of ROI in FLU pre-fumigated seedlings

256 Before fumigation, seedlings showed no significant differences among all determined eco-
257 physiological parameters. However, measurements made 2 weeks (14 d) after fumigation
258 indicated that A_{max} increased in all treatment groups except FLU-treated pine seedlings. Fig.
259 2A shows a lower A_{max} value in FLU-treated seedlings when compared with control 'MANN'
260 and the seedlings treated with enzymes. The decreased A_{max} in FLU-treated seedlings in this
261 study showed the inhibition of photosynthesis by FLU. However, the similar photosynthetic

rate found among MANN+FLU, SOD+FLU, PERO+FLU and SOD+PERO+FLU treatments implied that MANN, SOD and PERO mitigated the negative effect of ROI. Previous studies on the effects of sulphur dioxide (SO_2) on plants showed that high superoxide dismutase activity confers increased resistance to pollution (Tanaka and Sugahara, 1980). In addition, studies carried out using the green alga *Spermophilus armatus* cultured with anthracene provided evidence for strong oxidative stress under light, and in response, increased SOD activity (Aksmann and Tukaj, 2004). Stomatal conductance (g_s) is an important index for the transport of air pollutants into plants. Differential sensitivity in pine species may be related to the number and size of stomata. Unlike the area-based A_{max}, there was no significant difference after 14 d in FLU-treated seedlings (Fig. 2B). This seems inconsistent with our previous results in the 10 d. However, it is remarkable to note that FLU treated seedlings had the lowest value compared with other treatments. F_o values of the exposed pine seedlings slightly decreased in all treatments during 14 d fumigation. Values for PERO+FLU-treated pine seedlings were significantly lowered compared with FLU-treated seedlings (Fig. 2C). F_v/F_m was relatively stable for all treatments during fumigation except for FLU-treated seedlings, where the value was significantly different compared with SOD+FLU-treated seedlings (Fig. 2D). F_o and F_v/F_m are chlorophyll fluorescence parameters relating to PS II sites, and indicate light harvesting and utilization system efficiency. In this study we observed a little F_o increase and slightly decreased F_v/F_m in seedlings fumigated with FLU, this may imply a slightly lowered maximal photochemical efficiency in PS II. As previously reported in Oguntimehin et al. (2007), MANN mitigated the negative effect of FLU on Japanese red pine. Greater mitigating actions on the F_o increase and F_v/F_m depression were observed in the present study (Fig 2C, D). However, SOD+PERO did not appear to have any additive (synergistic) effect, but rather, showed similar effects to MANN. This might imply that H_2O_2 and O_2^- play similar roles in effecting chlorophyll fluorescence changes in the FLU-treated

plants. The mechanisms of ˙OH formation may comprise various pathways and combinations of compounds and pathways in these species. This may then necessitate the different cellular targets inside the plant (Halliwell, 2006). Needle dry mass per unit area generally reduced within the treatment period. NMA in FLU-treated seedlings is lowered compared with NMA in SOD+FLU-treated seedlings (Fig. 2E). Nakatani et al. (2007) found a strong positive correlation between A_{max} and NMA. Decreased NMA (approx. 10% decreases) at the end of the 14 d fumigation period in response to FLU treatment in the present study may agrees closely with the findings of Nakatani et al. (2007). Also, it supports the findings of a related study on *B. napus* and *Cucumis sativus* (Huang et al., 1996). In *C. sativus*, FLU had a reduction effect on the fresh weight of shoots and roots but in the present study, a reduction in NMA of FLU treated pine seedlings was recorded. Chlorophyll a/chlorophyll b ratio in Japanese red pine needles after 14 d fumigation is shown in Fig. 2F. FLU-treated pine seedlings had the lowest ratio, the highest being found equally in SOD+FLU and SOD+PERO+FLU treatments. The lower Chl a/Chl b ratio in mesophyll thylakoids of FLU-treated pine seedlings might be associated with lower photosystem II activity. The substantially decreased chlorophyll content in FLU-treated pine seedlings as compared with other treatments is consistent with previous findings on plant responses to FLU (Huang et al., 1996; Kummerova et al., 2006b). However, seedlings treated with SOD+FLU, PERO+FLU, SOD+PERO+FLU and MANN+FLU showed stable chlorophyll contents during the fumigation experiment.

3.4 Relationship between eco-physiological traits and fumigated FLU dosage per seedling

Total dosages of fumigated FLU per seedling during 5, 10 and 14 d exposure periods were calculated. The highest dosage of ≈10 nmol FLU per seedling was used for the 10 d exposure, followed by the 14 d exposure of ≈8 nmol FLU per seedling. The least was the 5 d exposure

of ≈5 nmol FLU per seedling. The relationships among A_{max}, g_s, C_i, F_o, F_v/F_m, and NMA and dosage of FLU applied per seedling are shown in Fig. 3. In this study, the high negative relationships (r values; 0.99, 0.97, 0.71 and 0.98) between A_{max}, g_s, C_i and NMA with FLU dosage respectively, suggests high dependence of these parameters on FLU dosage. This strongly indicated that the FLU dosage determined the extent of negative effects on these parameters (Fig. 3 A, B, C and F). However, there seems to be no direct relationship between F_o and F_v/F_m values in this present work (Fig. 3 D and E). These may be imply that PSII and other light reaction systems were not seriously damage, the primary decrease in g_s and the following physiological down regulation processes may likely be the cause of the decreased A_{max}.

5. Conclusion

FLU fumigation on the surface of the evergreen 'conifer' Japanese red pine produced significant negative effects in the needles in a short time. Both the dosage and period of fumigation were important factors in the extent of FLU-induced negative effects. We studied the scavenging mechanisms of ROI by fumigating FLU pre-fumigated seedlings with MANN, PERO, SOD, and an equal mixture of SOD and PERO. Scavengers of ROI used in this study mitigated the negative effects of FLU on the needles. MANN is more economical and has greater relative stability compared with the enzymes. This advantage might imply that MANN is the best ROI scavenger among the three considered. Also, that one of the negative effects of FLU on Japanese red pine is the production of $^{\cdot}OH$. A field-to-forest scale-up experiment may be used to establish additional results on the negative effects of FLU on Japanese red pine trees. Fluoranthene in the air, dew, rain or snow is a potential threat to the photosynthetic apparatus and tissues of Japanese red pine.

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LIST OF FIGURES

Fig. 1:

(A-C) Photosynthesis rate, stomatal conductance, and internal CO_2 concentration respectively, of Japanese red pine (*Pinus densiflora*) seedlings exposed to fumigation with 5 and 10 μM fluoranthene solutions for 5 d.

(D-E) Photosynthesis rate, stomatal conductance, and internal CO_2 concentration respectively, of Japanese red pine (*Pinus densiflora*) seedlings exposed to fumigation with 10 μM fluoranthene solutions for 10 d.

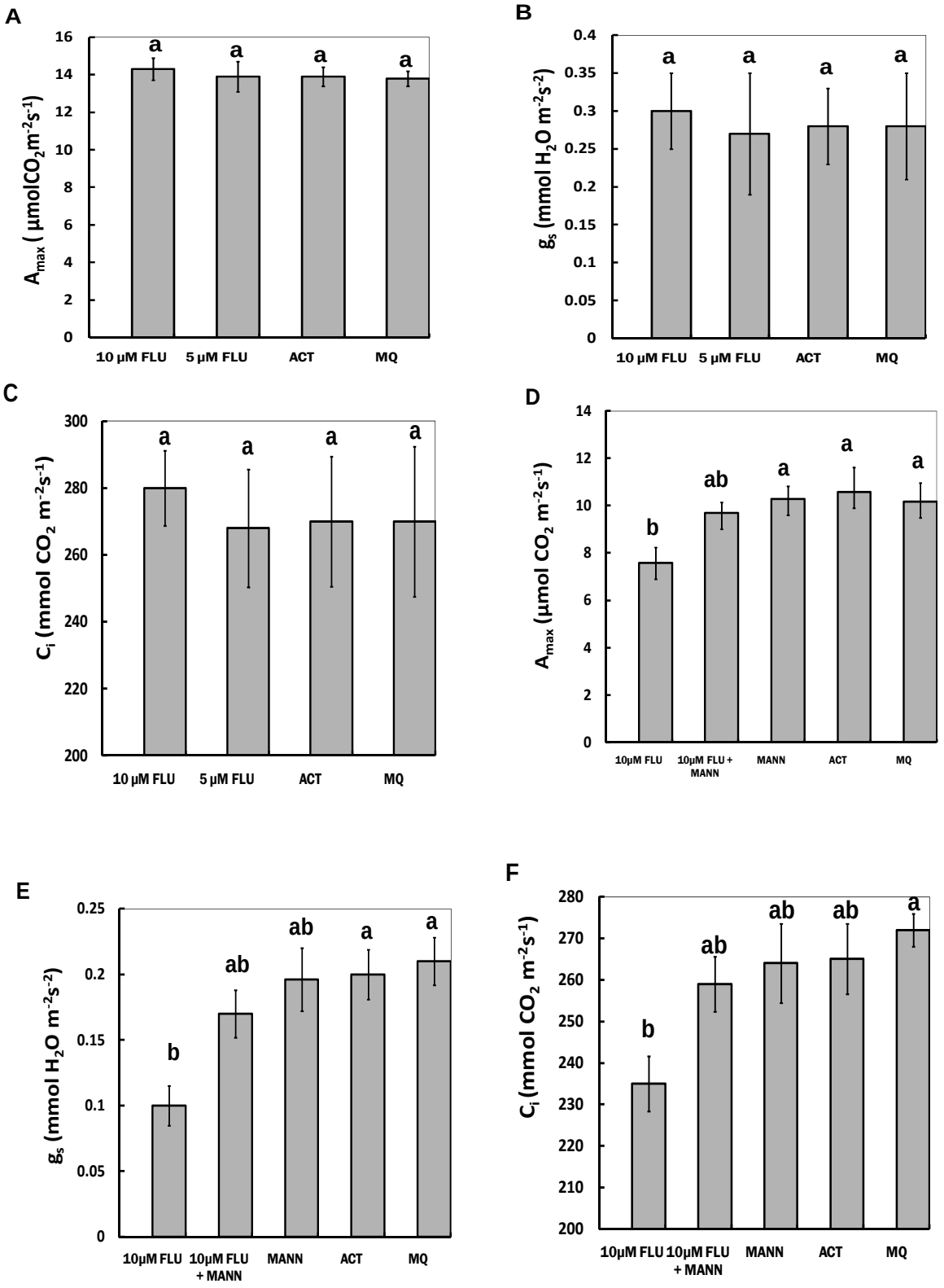
Data are means of determinations from five pine seedlings, error bars are ± standard error (S.E.). Identical superscript letters indicate the same homogenous groups; different letters indicate significant differences at $p < 0.05$ (Tukey analysis).

Fig. 2:

A) Photosynthesis rate measured at near-saturating irradiance (A_{max}). B) Stomatal conductance to water vapors (g_s). C) Minimal fluorescence value (F_o). D) Maximal photochemical efficiency of PSII (F_v/F_m). E) Needle dry mass per unit area (NMA). F) Chlorophyll content of pine seedlings measured after 14 d exposure with fluoranthene and ROI scavengers. Data are means of determinations from five pine seedlings, error bars are ± standard error (S.E.). Identical superscript letters indicate the same homogenous groups; different letters indicate significant difference at $p < 0.05$ (Tukey analysis).

Fig. 3:

Relationships between total dosages of FLU fumigated/seedling in the 5, 10 and 14 d (5, 10 and 8 nmol, respectively) fumigation periods with eco-physiological traits of Japanese red pine needles. Data are means of determinations from five pine seedlings, error bars are ± standard error (S.E.).


A
A_{max} (µmolCO_2 $m^{-2}s^{-1}$)
a a a a
10 µM FLU
5 µM FLU
ACT
MQ
B
g_s (mmol H_2O $m^{-2}s^{-2}$)
a a a a
10 µM FLU
5 µM FLU
ACT
MQ
C
C_i (mmol CO_2 $m^{-2}s^{-1}$)
a a a a
10 µM FLU
5 µM FLU
ACT
MQ
D
A_{max} (µmol CO_2 $m^{-2}s^{-1}$)
b ab a a a
10µM FLU
10µM FLU + MANN
MANN
ACT
MQ
E
g_s (mmol H_2O $m^{-2}s^{-2}$)
b ab ab a a
10µM FLU
10µM FLU + MANN
MANN
ACT
MQ
F
C_i (mmol CO_2 $m^{-2}s^{-1}$)
b ab ab ab a
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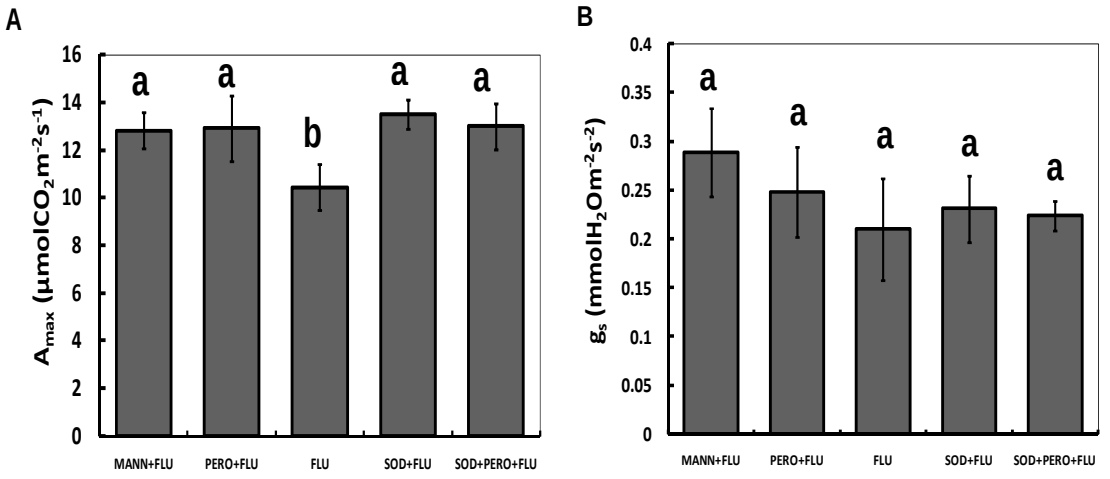
10µM FLU + MANN
MANN
ACT
MQ

Fig. 1



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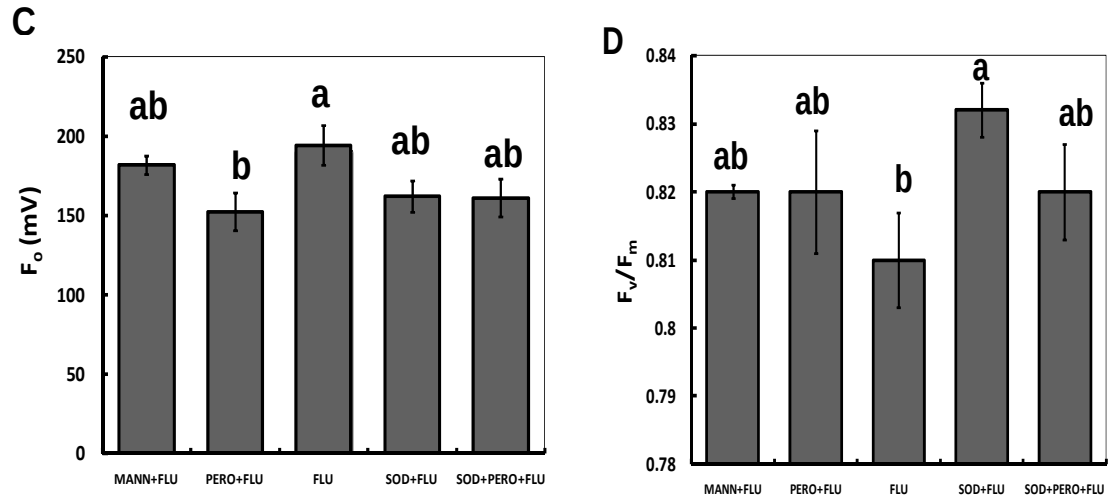

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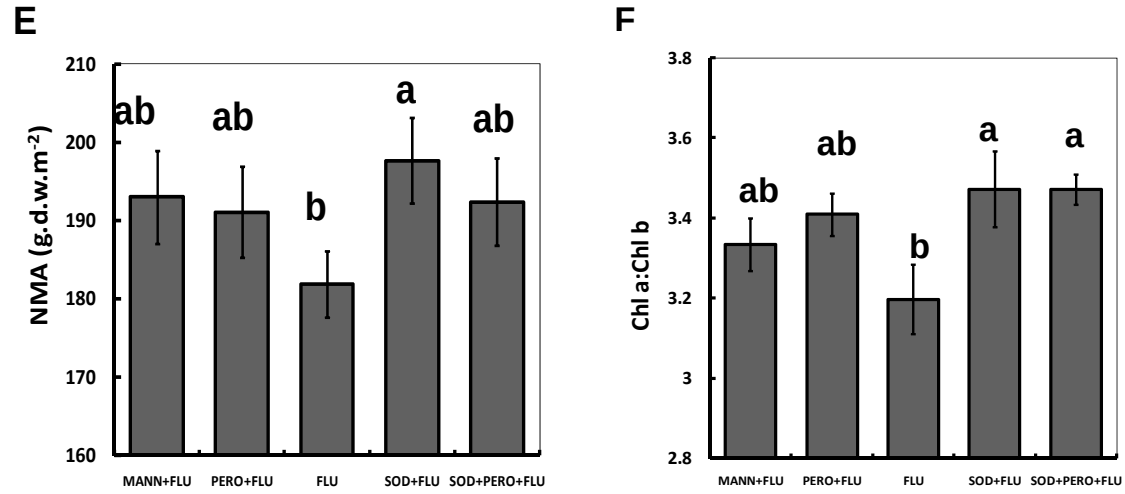

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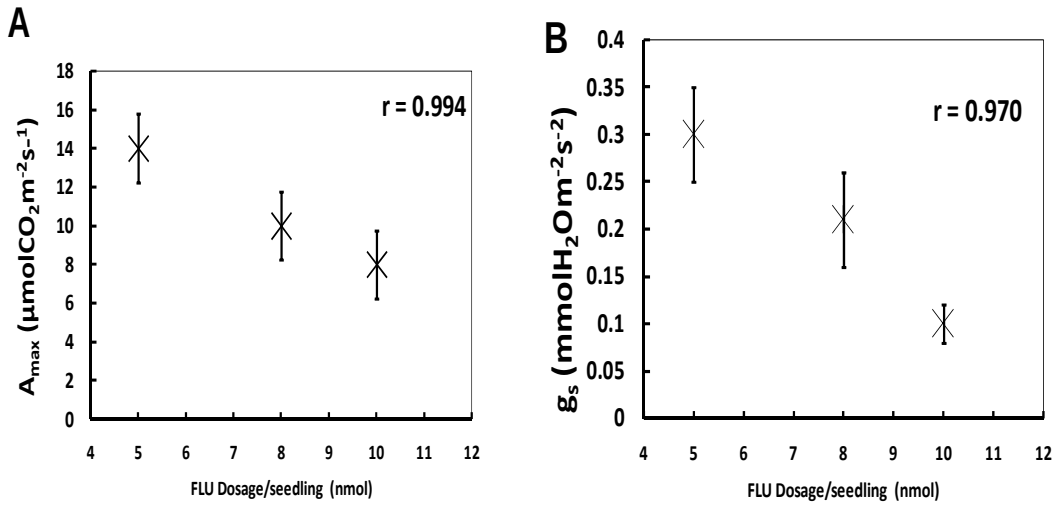
35 ig. 2



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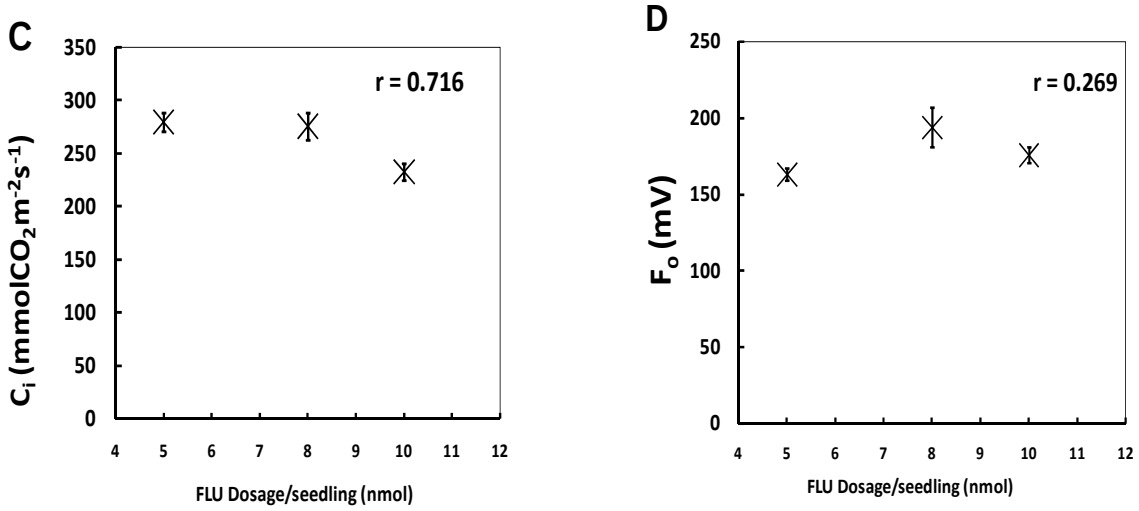

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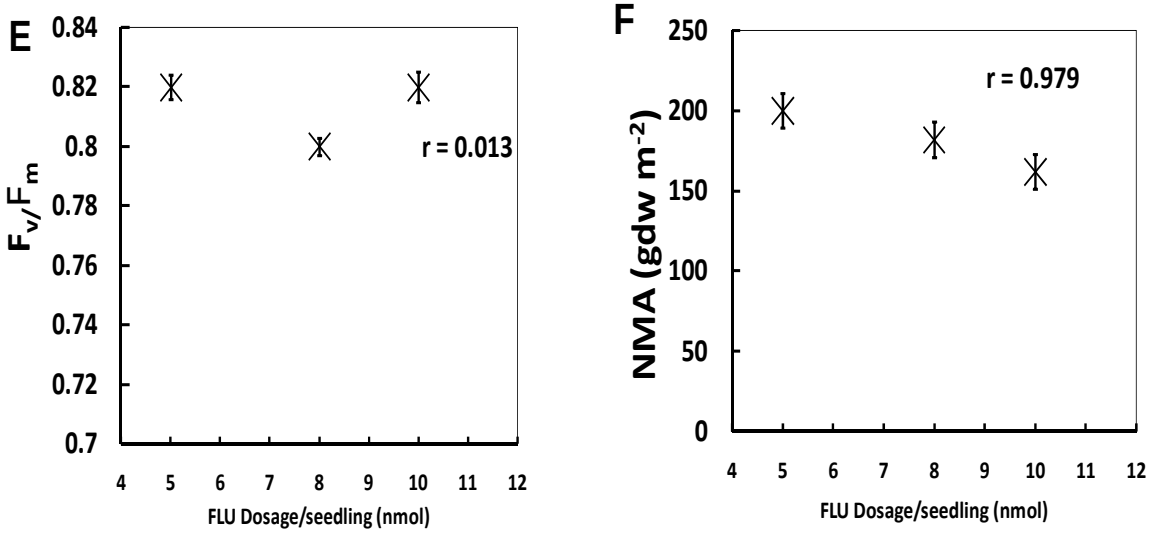

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39 Fig. 3