Symptom of ozone injured leaves in 3 kinds of birch species in Hokkaido

Graduate School of Agriculture, Hokkaido University, MAO Qiaozhi
Research Faculty of Agriculture, Hokkaido University, HOSHIKA Yasutomo・WATANABE Makoto・KOIKE Takayoshi

Introduction

Ozone (O_3) is one of the most abundant troposphere oxidant and an essential component of photochemical pollution (*1*). Tropospheric O_3 is formed by volatile organic compounds and the oxides of nitrogen through photochemical reactions. Elevated concentration of near-surface O_3 that principally occurs has been shown to be damaging vegetation (*1,14*). Unfortunately, near-surface O_3 level has reported to be continuously increasing (*13*).

Most studies reported on the harmful effects of long-term low concentration O_3 on the yield or growth to crops and trees at around 40-80 ppb (*7,9,14*). However, near surface ozone does not keep in a constant concentration, the peak sometimes found to be more than 150 ppb in the atmosphere (*7,12,13*).

Birches (*Betula* sp.) are representative deciduous broadleaf species that widely distributed in Hokkaido (*5,8*). Which kinds of visible injury of birches will be caused by short-term high concentration O_3? In our on-going research (*6*), plants are sometimes suffered from very high O_3 near the matrix instrument. We should know the visible symptoms in leaves by the high O_3 for protection of plants.

For attaining these raised questions, three kinds of birch, mountain birch, Monarch birch and white birch were exposed to high concentration ozone. The treated leaves were detected by fluorescent emission method. Moreover, the possible effects of high O_3 on birches will be discussed.

Materials and methods

1. Plants materials

Three-year-old mountain birch (*Betula ermanii*, Be), 5-year-old Monarch birch (*B. maximowicziana*, Bm) and 5-year-old white birch (*B. platyphylla* var. *japonica*, Bp) planted in 7 liter pots filled with brown forest soil were exposed to ozone in an open air. Leaf-age of samples was about 40-50 days old positioned at first or second part of a leader shoot of individual plants.

The diameter (standard deviation: SD) and height (SD) were following: mountain birch 5.2 (1.5), 34.7 (11.4) cm, Monarch birch; 9.5 (0.7) cm, 85.3 (11.9) cm and white birch; 12.5 (2.0) cm, 110.6 (19.9) cm.

2. Light environmental conditions

The plants were transplanted to pots in 2010 and were set in Sapporo Forest Experiment Station of Hokkaido University (43°04'N, 141°20'E, 15m a.s.l., recent annual mean temperature: about 11.5˚C, total precipitation: about 1200 mm). The light condition during ozone fumigation period was monitored by HOBO data loggers (Pendant Temperature/Light Data Logger, 64K-UA-002-64, Onset, USA.).

3. Ozone fumigation

Ozone was generated and fumigated at fluctuate high concentration, hourly mean 400 ppb in the day time for 11 hours during late September to early October, 2011.

4. Assessment of O_3 visible injury

Ozone visible injury was identified by the same two observers with the help of ICP-Forest manual (*4*). The brown stippling visible injury of the 3 birches appeared between leaf veins on adaxial leaf surface, and disappeared in shaded leaves and apparently more severe in older than in younger leaves.

5. Fluorescence measurement

Fluorescence of chlorophyll *a* was measured after the 0 hr, 48 hrs and 72 hrs by a pulse amplitude modulation fluorometer (PAM, EM-FluorCam-800MF, Photon system Inst., Drasov, Czech Republic).

Chlorophyll *a* plays an important role in photosynthetic system II (PSII) for generating electrons. The leaves were adapted in dark condition for 40 min by wrapped with aluminum foil before the PAM measurement. Minimal fluorescence (F_0) was measured using the low measuring modulated light which was too sufficiently to induce any significant variable fluorescence. Maximum fluorescence (F_m) was determined by applying a saturated light pulse. The light reaction centers were opened during F_0 determining progress and were closed during F_m measurements. The variation of F_m and F_0 is defined as F_v ($F_v = F_m-F_0$) (*11*). The efficiency of excitation energy transfer to open PSII traps was estimated by F_v/F_m ($=\Phi_{exc}$). In healthy leaves show F_v/Fm=0.80~ 0.83 (*1*).

Results and Discussion

1. Light condition

In the daytime from 6:00 am to 5:00 pm, the Photosynthetic Photon Flux (PPF) during 11th Oct. to 14th Oct. was monitored in sunny day and cloudy day (1835.21μmol quanta m^{-2} s^{-}). PPF of each day from 11th to 14th Oct. was 969.47, 294.78 (cloudy), 893.30 and 970.07μmol quanta m^{-2} s^{-1} respectively.

毛 巧芝（北海道大学大学院農学院、札幌 060-8589）・星加康智・渡辺 誠・小池孝良（北海道大学大学院農学研究院、札幌 060-8589）, 北海道に生育するカンバ類 3 種の個葉に見られるオゾン付加の影響の兆候

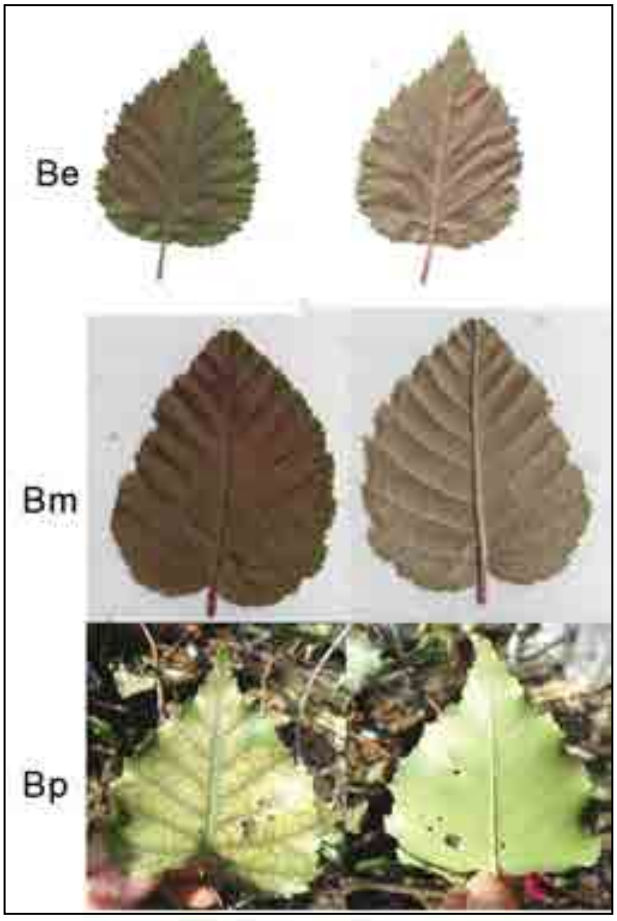

Figure 1. Typical visible injury in three birches. Left- and right-photo is adaxial and abaxial side of an example leaf, respectively. Be: mountain birch, Bm: Monarch birch, Bp: white birch.

2. Visible injuries

Visible injury was found in three kinds of birch species. Mountain birch showed dark brown stippling visible injury earlier than the other 2 birch species, and then the injured parts became dry within next 24 hours. Monarch birch was found the visible injury at late 48 hrs, and its inter-vein changed to reddish-brow stipple. Inter-vein light-brownish stippling was shown in white birch after 48 hrs exposure of O_3 (Fig. 1).

3. *Fluorescence measurement*

Detection of chlorophyll fluorescence has become one of the most powerful methods for assessment of environmental impacts on photosynthetic metabolism (*2,11*). The light absorbed by chlorophyll molecules has three alternative and competing fates: drive photosynthesis (photochemistry), dissipates as heat or re-emitted as light-chlorophyll fluorescence. The photochemical utilization of energy is to quench fluorescence: photosynthetic quenching (qP). Under light saturated condition, photosynthesis process cease and photochemical quenching become zero; and then there can be a large amount of quenching: non-photochemical quenching (qN). qN is induced as a mechanism for photo-protection of photosystem II (PS II). The fluorescence can be measured easily by a modulate equipment – Pulse Amplitude Modulation (PAM) method as shown in figure 2.

Fluorescence emission was measured after the visible injury shown in each species (Table 1, Fig. 2). Mountain birch showed fluorescence emission decreased around 10.25 %, Monarch birch showed fluorescence decreased around 37.67 % and white birch decreased 30.38 % in 72 hrs. Dark-brown stippling was shown quickly in mountain birch

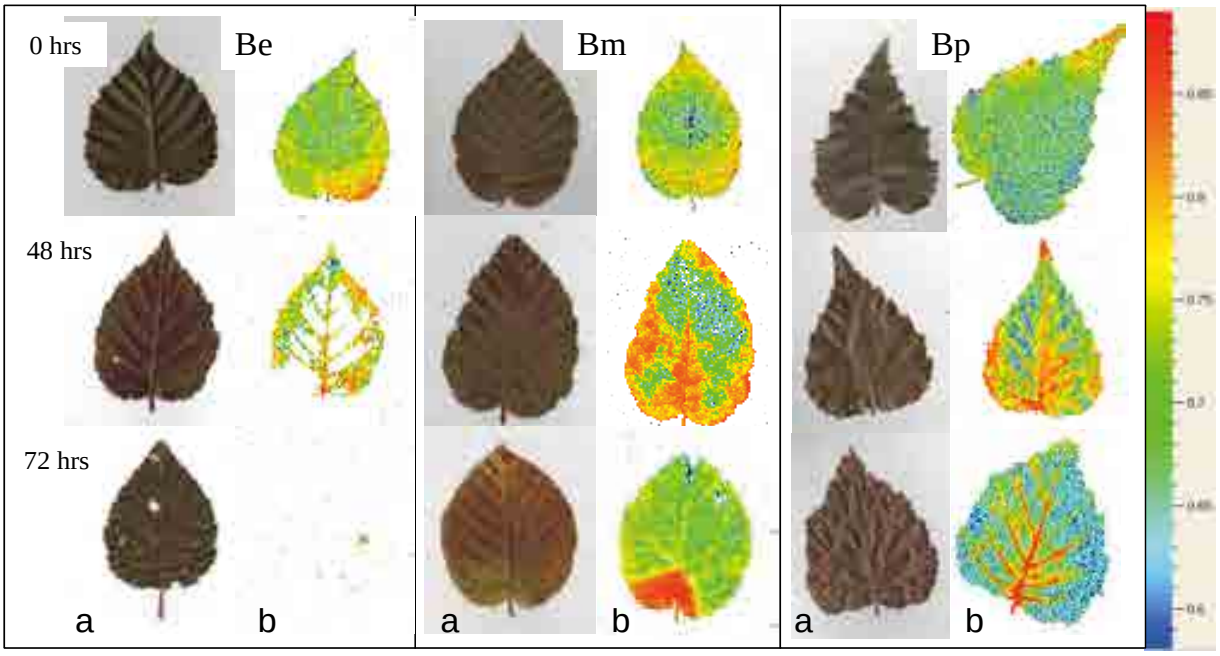

Figure 2. Fluorescence measurement images of three kinds of birch species at deferent period. Be: mountain birch, Bm: Monarch birch, Bp: white birch. a: a column is scanning image, b: b column is PAM image of excitation efficiency of energy transfer. Scale at right shows the value of F_v/F_m. 0 hr: initial images, 48 hrs: 48 hrs later images, 72 hrs: 72 hrs later images.

within 24 hrs by high concentration O_3 fumigation. This birch leaves showed partially dead (dead area ratio was 37.51 % and 78.16 % in the 48 hrs and 72 hrs, respectively) since the second day. Dead leaf is referred as the damaged part became dark brown or dry, further more in PAM measurement F_0 and F_m were zero. As a result the fluorescence value of active part in mountain birch leaves showed relatively high. In 72 hrs, Monarch birch leaves were partially dead (dead area ratio, 31.28 %) too. The chlorophyll in vein kept active within the experimental period for 72 hrs of Monarch birch. In contrast, no dead part of leaf was found in white birch even though leaf color showed pale-green. The dead leaf phenomonen suggests that growth decline of birch trees (*9*).

Table 1. Fluorescence (ϕ_{exc}) of three birches in active part of the whole leaves

	Φ_{exc}			source		
	0 hrs	48 hrs	72 hrs	time	species	time*species
Be	0.78 (.01)	0.64 (.11)	0.70 (.14)			
Bm	0.77 (.01)	0.43 (.09)	0.48 (.03)			
Bp	0.79 (.01)	0.53 (.06)	0.55 (.24)			
ANOVA				<0.01	<0.01	0.03

Data in the table is Fv/Fm value (standard deviation). Be: mountain birch, Bm: Monarch birch, Bp: white birch. 0h: n = 6, 48h and 72h day: n= 3. Two-way ANOVA was applied.

All of these results indicated that three birch species showed significantly specific sensitivity to O_3. Comparing the time cost of showing visible injure of the three birches, mountain birch was the earliest one. In the end of this experiment mountain birch leaf lost 78.16% active area, the most serious among these 3 birches. On the other hand, monarch birch showed visible injury 48 hrs later and 31.28% of injured leaf lost activity while white birch showed visible injury after 48 hrs and no leaf dead. Considering the response speed and damaged serious degree, obviously, mountain birch was the most sensitive one. While mountain birch was quite sensitive to short period of the high O_3 concentration, white birch was the most tolerance species among these 3 birches. Chlorophyll *a* plays an essential role in PSII for generating electrons. Lower photo-protective components in chlorophyll indicate the tolerance of plants decreased. Here, decreasing F_v/F_m indicated that chlorophyll *a* activity decreased by O_3. It is clear that electron yield will be decreased, the electron transport progress will not sufficient. Without the donator, electron, ATP will not be synthesized sufficiently. As a result the CO_2 assimilation rate will decrease relatively. That means photosynthetic capacity will decrease. Though the mechanism of plants response to O_3 oxidative stress has not clear yet. Previous studies report that some photo-protective components synthesized in chloroplast such as carotenoids and light-harvesting complexes antennas (*3,11*). However, what is caused specific different tolerance of these 3 birches to high concentration O_3 is still unclear. Future study needs to be done. Firstly, the leaf morphology may contribute to the specific difference. For example, mountain birch leaf is much thinner, smooth while Monarch birch leaf is much thicker and dasyphllous. Anatomy structure maybe can show us some clue. Secondly, the stomata behavior plays an important role for preventing O_3 uptake damage (10). In addition, chloroplast is an essential place for synthesizing photo-protective components, at least chlorophyll content and other secondary metabolism should be detected such as ethylenediurea (EDU).

In this experiment chlorophyll fluorescence was detected. The chlorophyll fluorescence partially shows the activity of chloroplast in the leaf. The lower level chlorophyll fluorescence reflects lower anti-oxidation possibility. One of the main targets of O_3 damage is assessment of photosynthesis. The decrease of active leaf area and decrease of F_v/F_m indicate "photodamage" to PS II and suggests decline of photosynthetic capacity of the individual seedlings.

Conclusion

Short-term high concentration O_3 caused visible injury (inter-vein brownish) in 3 birches: i.e. mountain birch, Monarch birch and white birch. Sensitivities of these 3 birches showed significant specific difference. Among these 3 species mountain birch was the most sensitive species while the white birch was the most tolerance one. We presumed that photosynthetic activity decreased with damage of PS II caused by O_3

Acknowledgements

The first author (Mao Q.) thanks China Scholarship Council fund for her to study in Japan. We thank the financial support in part by the Environment Research and Technology Development Fund (B-1105) and Grant-in- Aid of JSPS (type B: 23380078).

References

(*1*) Barnes J., Zheng Y. and Lyons T. (2002) Plant resistance to ozone: the role of ascorbate. Air Pollution and Plant Biotechnolgoy - Prospects for Phytomonitoring and Phytoremediation. Edited by Omasa, K., Saji H, Youssefian S. and Kondo N. Springer Verlag, Tokyo, pp.235-252.

(*2*) Horton P., Ruban AV. and Walters RG.. (1994) Regulation of light harvesting in green plants, indication by nonphotochemical quenching of chlorophyll fluorescence. Plant Physiol. **106**: 415-420.

(*3*) Horton P., Ruban AV. and Walters RG.. (1996) Regulation of light harvesting in green plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. **47**: 655-682.

(*4*) ICP Forest (2004) United nations economic commission for Europe convention on Long-Range trans-boundary air pollution.

(*5*) Koike T., Kitao M., Quoreshi AM. and Matsuura Y. (2003) Growth characteristics of root-shoot relations of three birch seedlings raised under different water regimes. Plant and Soil **255**: 303-310.

(*6*) Koike T., Mao Q., Watanabe M., Inada N., Kawaguchi K.

and Hoshika Y. (2011) Introduction of ozone related research in forest of Hokkaido. Agr. Meteorol. Hokkaido **63**: 17-23 (in Japanese).

(*7*) Japan Meteorological Agency HP (2011). http://www.data.kishou.go.jp/obs-env/cdrom/report/html/2_6_2.html.

(*8*) Mao Q., Watanabe M. and Koike T. (2010) Growth characteristics of two promising tree species for afforestation, birch and larch in the northeastern part of Asia. Eurasian J. For. Res. **13**: 69-76.

(*9*) Matyssek R. and Innes JL. (1999) Ozone- a risk factor for trees and forests in Europe? Water Air Soil Pollut. **116**: 199-226.

(10) Paoleti E., Contran N., Manning WJ., Castagna A., Ranieri A. and Tagliaferro F. (2008) Protection of ash (*Fraxinus excelsior*) trees from ozone injury by ethylenediurea (EDU): roles of biochemical changes and decreased stomatal conductance in enhancement of growth. Environ. Pollut. **155**: 464-472.

(*11*) Pellegrini E, Carucci MG., Campanella A., Lorenzini G. and Nali C. (2011) Ozone stress in *Melissa officinalis* plants assessed by photosynthetic function. Environ. Exp. Bot. **73**: 94-101.

(12) Takigawa M., Niwano M., Akimoto H. and Takahashi M. (2007) Development of One-way nested global regional air quality forecasting model. SOLA **3**: 81-84.

(*13*) Vingarzan R. (2004) A review of surface O_3 background levels and trends. Atom. Environ. **38:** 3431-3442.

(*14*) Wang X. and Mauzerall DL. (2004) Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korean: 1990 and 2020. Atom. Environ. **38**: 4383-440.