Ozone and ozone injury on plants in and around Beijing, China

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ABSTRACT
Ozone (O3) levels were assessed for the first time with passive samplers at 10 sites in and around Beijing in summer 2012. Average O3 concentrations were higher at locations around Beijing than in the city center. Levels varied with site locations and ranged from 22.5 to 48.1 ppb and were highest at three locations. Hourly O3 concentrations exceeded 40 ppb for 128 h and 80 ppb for 17 h from 2 to 9 in August at one site, where it had a real-time O3 analyzer. Extensive foliar O3 injury was found on 19 species of native and cultivated trees, shrubs, and herbs at 6 of the 10 study sites and the other 2 sites without passive sampler. This is the first report of O3 foliar injury in and around Beijing. Our results warrant an extensive program of O3 monitoring and foliar O3 injury assessment in and around Beijing.

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1. Introduction
Episodes of high concentrations of particulate and gaseous pollutants, referred to as smog, occur frequently in Beijing, raising concerns about effects on human health. Ozone (O3) is a major part of this complex urban pollution mixture. Ozone levels increased at a rate of 1.1 ± 0.5 ppb/year from 2001 to 2006 (Tang et al., 2009). Ozone levels have been increasing significantly from 2002 to 2010 in China (Wang et al., 2011; Wang et al., 2012a,b,c; Xu and Zhang, 2006). The new air quality standards for O3 for China established in 2012 says that an 8 h average of 80 ppb and an hourly average of 50 ppb are allowed for the First Grade Standard for nature reserves and scenic areas, and 100 ppb and 80 ppb for the Second Grade Standard for most areas, separately (GB 3095-2012). The higher value is permitted in the new standard than in the old one (GB 3095-1996). It reflected the incidence of high levels of ambient O3 in China.

The effects of O3 on crop plants and some tree seedlings have been reported from controlled field experiments with Open-Top Chambers and Free-Air Exposure Systems in China (Wang et al., 2011; Wang et al., 2012a,b,c; Xu and Zhang, 2006). The new air quality standards for O3 for China established in 2012 says that an 8 h average of 80 ppb and an hourly average of 50 ppb are allowed for the First Grade Standard for nature reserves and scenic areas, and 100 ppb and 80 ppb for the Second Grade Standard for most areas, separately (GB 3095-2012). The higher value is permitted in the new standard than in the old one (GB 3095-1996). It reflected the incidence of high levels of ambient O3 in China.

The effects of O3 on crop plants and some tree seedlings have been reported from controlled field experiments with Open-Top Chambers and Free-Air Exposure Systems in China (Wang et al., 2011; Tang et al., 2011). Little is known about the effects of ambient levels of O3 on plants in and around Beijing or anywhere else in China.

Older leaves of ozone-sensitive plants exhibit characteristic injury symptoms when O3 levels are high enough and exposure is long enough and environmental conditions are appropriate for gas exchange. Symptoms of ambient O3 injury on a wide variety of native plants have been observed in the field and then verified as being caused by O3 (Skelly et al., 1999; Innes et al., 2001; Porter, 2003; Manning and Godzik, 2004; Kohut, 2005; Davis, 2007; ICP Forests, 2010; Paolletti et al., 2009; Sanz and Catalayud, 2011). Using these references and pictorial data bases as a guide, symptom expression is a widely accepted and reliable method of assessing plant response to O3 in the field and providing biological significance for O3 monitoring data (Manning, 2003).

Coulson et al. (2003) rated tree species in mid-Atlantic States using O3 injury symptoms. The Carpathian Mountain Range was surveyed for O3 injury on plants over a 10-year period (Manning and Godzik, 2004). Davis (2007) and Davis and Orendovici (2006) used foliar O3 injury symptoms on plants as evidence of O3 injury to plants in Maine and Pennsylvania. Native bioindicator plants for O3 were extensively used in forest health monitoring programs over a 16-year period in the Eastern USA (Smith et al., 2003, 2008; Smith, 2012). Ozone injury on ornamental plants has also been investigated in the field. Ozone symptom expression on Hibiscus
syriacus was detected in the field and verified by treatment of plants to EDU (ethylenediurea) and exposure of plants to O₃ in open-top chambers (Paoletti et al., 2009).

Surveys for O₃ injury on native plants have been combined with and located near passive O₃ samplers or active real-time O₃ analyzers (Manning et al., 1996; Yuska et al., 2003; Bytnerowicz et al., 2004; Manning & Godzik, 2004; Sager et al., 2005). This makes it possible to relate O₃ injury symptoms on native plants to O₃ exposure.

Here we report the results from our survey of native and cultivated plants at 10 study sites with O₃ passive samplers and two additional sites without passive samplers in and around Beijing.
2. Methods

2.1. Site selections

The study was conducted in the urban region and peri-urban region of Beijing. The annual mean temperature is approximately 11–12 °C (State Statistical Bureau, 2011). Mean annual precipitation is about 585 mm, with more than 70% occurring from June to August (Beijing Water Authority, 2010). The annual mean concentrations of NO₂, SO₂, PM2.5 and O₃ were 21.5 ppb, 9.76 ppb, 71.8 μg/m³ and 22.1 ppb from Nov. 1, 2008 to Oct. 30, 2009 (Wang et al., 2011). The NO₂, SO₂, and PM2.5 concentrations were higher in winter than in summer because coal burning for heating was the major pollution sources, while O₃ concentration was higher in summer because of the good meteorological conditions for photochemical reactions to generate O₃, with higher temperature, higher ultraviolet radiation and lower wind speed (Wang et al., 2011; Xu et al., 2011). The peak value usually occurred at 15:00 to 17:00 o’clock in the afternoon (Wang et al., 2011). Higher O₃ concentrations during the growing period and the afternoon would cause greater uptake of O₃ by plants, resulting in more injury and growth effects. From the O₃ monitoring data in Beijing, Wang et al. (2012a,b,c) had estimated that the AOT₄₀ (accumulated exposure over a threshold of 40 ppb) during growing season was 20.12 ppm h, which far exceeded the critical level for ozone impact trees (UNECE, 2004).

In this study, 12 sites were selected according to vegetation types and prevailing wind patterns (Fig. 1). The Sites 1, 2, 3, 4, 10 and 12 are natural generated forests dominated mainly by 3 tree species: Ulmus pumila, Morus alba and Alianthus altissima. Around these sites, there were 3–5 shrub species, such as Grewia biloba var. parviflora, Prunus davidiana, and Cotinus coggyria var. pubescens and 15-20 herbaceous species. Other sites were park or roadside with cultivated plants and intensive managements with irrigation and fertilization, and were dominated by landscape plants, including trees (e.g., Populus tomentosa, Sophora japonica, Rhus typhina and Ginkgo biloba), shrubs (Amygdalus triloba, Kerria japonica var. plenifolia and Hibiscus syriacus) and many ornamental herbs. The location, elevation, land use and dominant species of these sites are showed in Table 1.

2.2. O₃ monitoring

One Ogawa passive sampler (Ogawa & Co. USA, Inc.) was installed at each of the study sites 1–10. Filters were replaced at 1-week intervals from 11 July to 9 August, resulting in 3 data samples by the time the vegetation survey was begun on 11 August. Mean ambient O₃ concentrations for each sampling location were statistically analyzed and used as an indicator of total O₃ exposure (SPSS 16.0). At one of the sites (Beijing Teaching Botanical Garden) O₃ was also continually monitored by a UV analyzer (Thermo Fisher 49i, Thermo Fisher Scientific Inc., USA). At site 2 (Tianchi), O₃ was also measured with a UV analyzer of the same model before the start of the survey.

2.3. Foliar O₃ injury assessment

Native and cultivated plants near the ten study sites with passive O₃ samplers and the two additional sites without passive samplers (site 11, Olympic Forest Park and site 12, Mutianyu) were surveyed for foliar O₃ injury from 11 to 15 August, 2012. Vegetation within 100 m from the passive samplers was surveyed for sites 1–10. Vegetation along roads was observed for sites 11 and 12.

The following criteria from the ICP Forests Manual part VIII, Assessment of Ozone Injury, were used to identify and characterize O₃ injury on the plants that we surveyed:

1. Visible symptoms are typically expressed as tiny purple-red, yellow or black spots (described as stipple) or sometimes as a general even discoloration, reddening or bronzing.

2. Look for ozone visible injury on fully developed, light-exposed leaves.

Fig. 2. Mean O₃ concentration of 3 passive sampler data with 1-week intervals from 11 July to 9 August at study sites 1 – 10 in 2012. The error bars are standard deviations. The location orders are the same as that in Fig. 1.
3. Symptoms are more severe on older middle-aged leaves than on younger leaves. Older leaves are the first ones to develop symptoms followed by accelerated senescence (age effect).

4. Shaded portions of two overlapping leaves do not show any visible injury (shade effect).

5. Ozone injury normally does not go through the leaf tissue. Both stippling and even discoloration occur only between veins (interveinal) and do not affect veins.

6. Towards the end of the growing season, foliar symptoms may progress to leaf yellowing or premature senescence, followed by leaf loss.

Based on the above information, the following questions should be answered when diagnosing possible ozone visible injury:

“Is there any stippling?”

“Is there any reddening and/or even discoloration?”

“Do the symptoms, as described above, occur on the upper leaf surface only (except during late season when visible injury becomes more severe and necrotic)?”

“Are the symptoms expressed between the veins only and are absent on the veins and veinlets (use hand lens and hold leaf against light)?”

“Are the symptoms evenly distributed?”

“Are the symptoms more developed on the older leaves (including leaflets, ‘age effect’)?”

If the above questions are answered affirmatively, the symptom can be considered as ozone visible injury.

Other handbooks, manuals and guides for identification of O3 injury symptoms on plants were also consulted (Innes et al., 2001; Porter, 2003; Kohut, 2005; Sanz and Calatayud, 2011; Smith, 2012).
3. Results

3.1. Ambient O$_3$ concentrations

Mean O$_3$ concentrations derived from passive samplers varied significantly from 22.5 to 48.1 ppb by sites during the month before the survey (Fig. 2). The highest concentrations occurred at sites 2, 3 and 4. From 11 July to 9 August, O$_3$ concentrations at site 9 (Beijing Teaching Botanical Garden) from the passive sampler and real-time analyzer were correlated (The coefficient of determination $R^2 = 0.56$). Results from site 2 (Tianchi) from the real-time analyzer (2–9 August) indicated a mean O$_3$ concentration of 54 ppb, with a maximum value of 135 ppb. During the period of 2–9 August, hourly O$_3$ concentrations exceeded 40 ppb for 128 h and 80 ppb for 17 h. The real-time analyzer at site 2 showed that higher O$_3$ concentrations occurred in May than August. The mean concentration was 74.4 ppb from 21 to 31 May and the peak value of 195.8 ppb occurred on 28 May.

3.2. Ozone injury on plants

Using the ICP Forests Criteria and questions during diagnoses, we found 19 species of native and cultivated trees, shrubs and herbs in and around Beijing that exhibited symptoms of foliar O$_3$ injury (Figs. 3–5). Upper surface stipple, ranging from white to yellow to brown to red to black was observed on 12 species (Table 2). “Classic stipple” was observed on leaves of Canavalia gladiata. Dark brown blotching was found on two species. Deep purple-brown older leaves were found on one species, interveinal chlorosis was observed on 3 species and general chlorosis on one species.

O$_3$ injury was observed on plants at 6 of the 10 study sites and 2 of the additional sites that lacked passive samplers (Table 1). Frequency for foliar injury symptoms on the life forms were 56.3% (18/32) for trees, 15.6% (5/32) for shrubs, 21.9% (7/32) for herbs, 6.3% (2/32) for crop plants. Ailanthus altissima and Populus tomentosa occurred at 4 of the 12 sites. And Sophora japonica occurred at 3 of the 12 sites.

3.3. Site differences for O$_3$ injury

There were significant differences in the number of species with O$_3$ injury symptoms by site locations (Table 1). Site 2 (Tianchi) and site 3 (Dazhuangke) had high O$_3$ levels and relatively higher occurrence of species with O$_3$ injury symptoms. At sites 1, 2, 3, 4 and 6, where were natural regenerated forest, the number of species with O$_3$ injury symptoms increased with measured O$_3$ concentrations. Site 5, a riverside city park rich in landscape plants had the second largest number of foliar injured species observed with a
middle level of mean O3 concentration. Site 11, the Olympic Forest Park, has extensive areas of cultivated ornamental plants. While O3 was not monitored there, the largest number of plant species with O3 injury was found there. Populus tomentosa, a widely planted native tree species, was the most sensitive plant. Extensive chlorosis and brown leaf blotching leading to defoliation was in progress. The Park is on the northern air flow gradient from central Beijing and should have high O3 concentrations given the large number of species affected and the intensity of the injury that occurred there.

4. Discussion

We were able to use passive O3 samplers to determine mean O3 concentrations at 10 study sites in and around Beijing in summer 2012. Passive samplers have been widely used by others in similar monitoring programs (Manning et al., 1996; Yuska et al., 2003; Bytnerowicz et al., 2004; Sager et al., 2005). Good agreement was also found between results from the real time O3 analyzer and the passive sampler at Beijing Teaching Botanical Garden, validating the method (Yuska et al., 2003).

We also found that the highest average O3 concentrations were at locations outside the center of the city (Figs. 1 and 2). These results are consistent of other reports that indicate that O3 levels are often higher downwind of city centers. Mean concentrations were in the range 22.5–48.1 ppb, with highest values at sites 2, 3, and 4. These values are only means and do not reflect high values that may have been episodically experienced. Data from real-time O3 analyzers showing the peak O3 concentration value of 198.5 ppb and mean value of 74.4 ppb which occurred in May were both higher than that of August. The time exceeded 40 ppb and 80 ppb were 128 h and 17 h separately during 2–9 in August on site 2. It revealed that plants in and around Beijing was frequently exposed to high ambient O3 concentration level.
Much has been written and reported about air pollution and its effects on humans in Beijing (Wan et al., 2012), but this is the first report of O₃ injury on plants in and around Beijing. This is evidence that not only are people at risk from air pollution in Beijing, but also is vegetation. Plants play an important part in ecosystem services in cities and anything that affects them will affect people. Finding O₃ injury on plants in Beijing means that air quality is also not appropriate for humans. O₃ injury on plants is a good bioindicator of relative O₃ pollution levels.

Following established criteria for identifying foliar O₃ injury in the field (Innes et al., 2001; Porter, 2003; Kohut, 2005; IPC Forests, 2010; Sanz and Calatayud, 2011, Smith, 2012), we identified 19 species of native and cultivated trees, shrubs, and herbs with O₃ injury. *Ailanthus altissima* is a verified bioindicator plant for ambient O₃ (Porter, 2003; Seiler, 2012) as is *Hibiscus syriacus* (Paoletti et al., 2009; Sanz and Calatayud, 2011). O₃ injury has not previously been described for 9 species (*Cassia tora*, *Phorbiitis purpurea*, *Canavalia gladiata*, *Amgdalus triloba*, *Kerria japonica var. plenifolia*, *Hyptis suaveolens*, *Vigna unguiculata*, *Sophora aureus* and *Juglans regina*). Ozone injury on these plants should be verified with controlled exposure experiments.

*Populus tomentosa* is widely planted in Beijing and surrounding areas. This is the first report of O₃ injury on leaves of *P. tomentosa*. Other species in the genus *Populus* are known to be O₃ sensitive as well (Innes et al., 2001; Kohut, 2005; IPC Forests, 2010). There is a very large grove of *P. tomentosa* in the Olympic Forest Park. At the time of our survey, the older leaves on the trees were chlorotic with no younger ones. The injury does not resemble O₃ injury. NO₂ injury only occurs on newer leaves and not older ones. The injury does not resemble O₃ injury. NO₂ injury also occurs on newer leaves and does not resemble O₃ injury. SO₂ injury might be possible, but levels of NO₂ have to be quite high to cause injury. Particulates could not cause the kinds of injury that we observed. Nutrient deficiency caused chlorosis always appeared on the whole leaf. Fungi caused injury include veins. Insects’ injuries were usually with traces of their biting or remnants, even the living insects. Basing on the criteria above, we could confirm at high confident that the foliar injury symptoms we documented were caused by elevated O₃ exposure.

### 5. Conclusions

O₃ was assessed for the first time with passive samplers and with real-time analyzers in and around Beijing. Results indicate O₃ levels of concern for plants and people. O₃ injury on 19 plant species was found at 8 of the 12 sites surveyed. This is the first report of O₃ injury to plants in this area. One of the sites, the Olympic Forest Park, is used extensively by residents of Beijing. Results from this short initial assessment provide justification for a much more extensive and long-term investigation of O₃ and its effects on vegetation in and around Beijing and elsewhere in China.

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