The impact of elevated ozone on the ornamental features of two flowering plants (Tagetes erecta Linn. and Petunia hybrida Vilm.)

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Abstract: There has been little investigation into the effects of ozone (O₃) on flowering plants around settlements, even though such plants are known to be threatened by elevated O₃. In this study, we used open-top chambers to assess the ornamental value of marigolds (Tagetes erecta Linn.) and four petunia (Petunia hybrida Vilm.) varieties in terms of their growth and physiological responses to elevated O₃. The aboveground biomass decreased by 7.4% in marigolds and by 19.4–23.6% in four varieties of petunia in response to
elevated O₃. The underground biomass decreased by 22.0% in marigolds and by 30.8–53.8% in four petunia varieties treated with elevated O₃. Flower biomass and diameter were markedly reduced by elevated O₃ in petunias, but not in marigolds. O₃ also accelerated leaf yellowing in different plant species and varieties, owing to a higher degree of degradation of chlorophyll than carotenoids, as well as an increase in flavonoid contents. Thus, O₃ stress responses should be considered when choosing flowering plants for their ornamental value.

**Keywords:** biomass; carotenoids; chlorophyll; elevated ozone; environmental pollution; flavonoids; flowering plants; marigold; open-top chamber; ornamental value; petunia.


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1 **Introduction**

Tropospheric ozone (O₃) is recognised as an important phytotoxic air pollutant with detrimental effects on plant growth and human health. O₃ originates naturally from the stratosphere and is produced in the troposphere by photochemical reactions involving
sunlight and O₃ precursor gases, including nitrogen oxide (NOx) and volatile organic compounds (VOCs) (Chou et al., 2011; Cooper et al., 2014; Han et al., 2011). With the rapid economic development and urbanisation in China, motor vehicle ownership is predicted to increase four-fold between 2000 and 2030, which might contribute to a large increase in NOx emissions (Shen, 2006). Moreover, projections indicate that a 34% rise in the emissions of the VOC isoprene will occur in China between 2000 and 2050 owing to the higher temperatures and solar radiation resulting from climate change (Wang et al., 2012, 2013).

An increase in both NOx and VOCs causes irradience-induced formation of O₃, especially in densely populated regions (Shao et al., 2006). Beijing is one of the largest mega-cities in China and is experiencing serious O₃ pollution. Feng et al. (2014) reported that the daily mean O₃ concentrations in urban and ex-urban areas of Beijing were 46 and 67 parts per billion (ppb), respectively, from May to September 2010, with hourly peak O₃ concentrations even reaching 181 and 209 ppb, respectively. These levels are expected to have detrimental effects on plant growth (Musselman et al., 2006), contributing to a loss of visual enjoyment and ecological benefit.

Flowering plants are planted in abundance along the sides of roads, in residential districts and in business zones and parks to decorate cities and enrich the environment, especially during important festivals. The ornamental value of flowering plants has frequently been visually assessed based on plant growth and signs of leaf injury (Cassaniti et al., 2009), the degree of leaf discoloration, defoliation and flowering (Bañón et al., 2011) and changes in flower colour (Bradley et al., 1999). However, increasing O₃ pollution is expected to interfere with the above indices, which would have detrimental effects on the ornamental features of flowering plants (Rämö et al., 2007; Findley et al., 1997).

Elevated O₃ concentrations induce visible foliar injury (Mills et al., 2011) and leaf senescence (Musselman et al., 2006) and inhibit plant growth (Franzaring et al., 2000; Hayes et al., 2012) mainly due to

1. less opportunity for carbon gain owing to reduced leaf area and longevity (Ainsworth et al., 2012)
2. a higher carbon demand for increased protein turnover and repair, as well as defence processes (Mäenpää et al., 2011).

The definition of adverse effects has been extended to flowers in which retarded flowering (Rämö et al., 2007) and reduced floral budding, flower blossoming and biomass (Findley et al., 1997; Rämö et al., 2007) are detected. However, the results of such studies are not always consistent and may have been influenced by O₃ concentration and exposure duration (Leisner and Ainsworth, 2012; Franzaring et al., 2000).

One meta-analysis indicated that elevated O₃ concentrations have a relatively small effect on floral initiation, as well as flower number and weight (Leisner and Ainsworth, 2012). At lower O₃ levels (less than 40 ppb), flower number was significantly reduced (Leisner and Ainsworth, 2012), whereas a study involving similar O₃ concentrations (e.g., 25 ppb) revealed no differences in flowering or flower number compared to control conditions (Franzaring et al., 2000). One possible reason for this difference is that the plant species used by Franzaring et al. (2000) were perennials, which depend on vegetative reproduction. In addition, O₃ fumigation in this study ended before flowering and seed ripening. Any occurrence detrimental to flowering plants will contribute to the
weakening or loss of their ornamental value, thus reducing their aesthetic environmental
benefits.
Leaf yellowing, a morphological symptom of plant senescence induced by O3
exposure, may be attributed to changes in the types and amounts of pigments in the leaf
(Rowan et al., 2009). During any vigorous growth period, chlorophyll is the main
pigment that contributes to a plant’s green colour; as growth progresses, the leaves turn
yellow owing to preferential degradation of chlorophyll over carotenoids (Buchanan-
Wollaston, 1997). Flavonoids, which exist in almost all plant tissues, protect the plants
from stress damage owing to their antioxidant ability (Davies, 2004; Huang et al., 2013).
These widely known pigments absorb light at $\lambda_{350\text{nm}}$, contributing to leaf yellowing
(Schwinn and Davies, 2004). However, little research has focused on investigating
whether leaf yellowing is linked to flavonoid biosynthesis when the plant is exposed to
O3.

Marigold (Tagetes erecta Linn., Asteraceae) (Dasgupta et al., 2012), a widely-used
ornamental plant, bears bright yellow and orange flowers that are used as decorative
elements in grand festivals each year in China (Kiranmai et al., 2011). Petunia (Petunia
hybrid Vilm.), one of the most important members of the Solanaceae family planted for
ornamental purposes, is widely cultivated throughout the world owing to its broad range
of flower colours induced by transformation or loss of the anthocyanin gene (Meyer
et al., 1987; Quattrocchio et al., 1999; Stehmann et al., 2009). In the present study, we
exposed marigolds and four varieties of different coloured petunias to elevated O3 levels
to determine the effects of O3 on their ornamental features and to investigate whether the
changes were consistent among different plant species or varieties.

2 Materials and methods

2.1 Experimental site

The experiment was conducted in the village of Zhangtou (40°12’N, 116°8’E),
Changping District, Beijing, China. The average annual temperature and precipitation are
11.8°C and 550.3 mm (concentrated between June and August), respectively (Tong et al.,
2012).

2.2 Open top chambers

Open top chambers (OTCs) were established as in previous studies (Zheng et al., 2011,
2013) with minor modifications. The regular octagon OTC (2.8 m in height and 4.0 m in
diameter) comprised an aluminium alloy frame covered with stalinite. Ambient air or O3
was delivered by a centrifugal blower (2,800 r/min) through PVC pipes with a 110 mm
inside diameter and released from driven-rotating acrylic tubes with holes. The OTCs
were positioned 3 m apart. O3 was generated from pure oxygen via an O3 generator using
a high voltage discharge method (Zhang et al., 2014). The O3 concentration within the
OTCs was monitored and the data were measured using an ozone analyser (Model 49i,
Thermo Scientific, Waltham, MA, USA).
2.3 Plant management

Marigold (Tagetes erecta Linn.) seedlings bearing yellow flowers and seedlings of four petunia (Petunia hybrid Vilm.) varieties bearing pink (P), red (R), rose-red (Ro) and white (W) flowers, respectively, were purchased from Jindetai Landscape Engineering Co. Ltd. (Beijing, China). To ensure that the seedlings were exposed to O₃ throughout the entire flowering period, seedlings were transplanted to larger pots (17.0 cm in height and 22.0 cm in diameter) before the buds emerged when the average height of the marigold and petunia seedlings was approximately 12.8 and 7.0 cm, respectively. One individual seedling was planted for each pot, with 20 pots per treatment as 20 replications. The substrate was a mixture of turfy soil, vermiculite and perlite at a ratio of 100:1:2. Owing to high summer temperatures, the plants were adequately watered after 17:30 h every day when O₃ fumigation was stopped. To attenuate positional effects, the planted pots were re-positioned within the OTCs every seven days and re-distributed among three OTCs every 10–15 days (Feng et al., 2011).

2.4 Ozone treatment

Three treatments were applied, i.e., non-filtered air (NF), non-filtered air with the addition of 60 ppb O₃ (NF+60) and 120 ppb O₃ (NF+120). For each treatment, five plants were randomly sampled within each OTC plot for each replicate. The marigolds and petunias were exposed to O₃ from 15 August to 17 October in 2013, and from 24 March to 24 May in 2014, respectively, except during periods of rain and strong wind. The exposure began at 08:30 h and ended at 17:30 h.

2.5 Sample collection

Five plants were selected randomly within each chamber. The fully developed leaves were sampled for pigment measurements as follows: for marigold, at the second branch from the top at pre-anthesis (4 September, 2013), anthesis (22 September, 2013) and wilting (18 October, 2013); and for the four petunia varieties, next to newly opened flowers (24 April, 2014). The sampled leaves were wrapped in aluminium foil, placed in liquid nitrogen and stored in a −80°C freezer prior to analysis.

Before harvest, the flower numbers of five randomly selected plants for each petunia variety within each treatment were counted, and flower diameters of both petunia (one flower per plant, 15 plants for each variety) and marigold (one flower per plant, 20 plants) were measured.

Five plants per chamber were randomly harvested on 18 October, 2013 for marigolds and on 24 May, 2014 for petunias and separated into aboveground parts (stem and leaves), flowers and roots. All parts were oven-dried at 75°C to achieve a constant weight for the biomass measurements.

2.6 Pigment determination

Chlorophyll (Chl: Ca and Cb) and carotenoids (Car) were extracted from 50 mg of fresh leaves or flowers using 15 ml of 95% ethanol until the tissue was colourless. Ca, Cb and
Car were quantified by measuring the absorbance at 665, 649 and 470 nm, respectively, using a UV/Vis spectrophotometer (Model V-530, Jasco, Japan). Concentrations were calculated using the following equations (Lichtenthaler and Wellburn, 1983):

\[
\begin{align*}
Ca &= 13.95 \times A_{665} - 6.88 \times A_{649} \\
Cb &= 24.96 \times A_{649} - 7.32 \times A_{665} \\
Car &= (1000 \times A_{470} - 2.05 \times Ca - 114.8 \times Cb)/245
\end{align*}
\]

The calculated results were converted to units of mg/g FW (fresh weight).

The flavonoid content was assayed according to the method of Sun (2011). Approximately 50 mg of marigold leaf or petal tissue that had been stored in the freezer at -80 °C was ground with a mortar in liquid nitrogen. Flavonoids were extracted with a 2 ml mixture of methanol (Fisher Scientific, Wilmington, DE, USA; chromatographic reagent), double-distilled H2O (ddH2O), methanoic acid (analytical reagent) and trifluoroacetic acid (analytical reagent) at a ratio of 70:27:2:1 (v/v/v/v) for 24 h in the dark at low temperature (4 °C); the extract was shaken manually every 8 h. Samples comprising 2 ml of extract were centrifuged using a microcentrifuge (Model 5424R, Eppendorf AG, Hamburg Germany) for 10 min at 2,000 × g and 4 °C. The supernatant was filtered through 0.22 μm PTFE syringe filters (13 mm in diameter; Jinteng, Tianjin, China) and placed in an autosampler vial to assay the flavonoids using a high-performance liquid chromatography (HPLC) system (Model 1100, Agilent Technologies, Santa Clara, CA, USA).

The HPLC system consisted of a Model 1100 pump, a PAD-100 photodiode array detector, an autosampler and an LC ChemStation B.01.01. The column used was a reverse-phase Waters C18 column (5 μm, pH 2–8, 250 mm × 4.6 mm internal dimensions). The injection volume was 10 μl, the flow rate was 1 ml min⁻¹ and the column temperature was 25 °C. A binary solvent system was utilised (mobile phase A: ddH2O: methanoic acid: trifluoroacetic acid = 97.9:2:0.1, v/v/v, B: ddH2O: acetonitrile [Fisher, chromatographic reagent]: methanoic acid: trifluoroacetic acid = 62.9:35:2:0.1, v/v/v/v). Separations were performed by altering the percentage of B: 0–20 min, 30–53%; 20–40 min, 53%; 40–45 min, 53–30% and 45–50 min, 30%. Total flavonoids were calculated based on the peak area, as measured at 350 nm against a standard curve prepared using rutin (Sinopharm Chemical Reagent, Beijing, China). The data were analysed using ChemStation B.01.01C (Agilent).

2.7 Statistical analyses

The values are presented as the means of all measurements, and comparisons of means were analysed by one-way ANOVA with LSD tests. All data were analysed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was accepted at \( P \leq 0.05 \).
3 Results

3.1 O$_3$ exposure

O$_3$ exposure was interrupted several times during the plant growth periods owing to poor weather conditions (rain and strong winds). Consequently, the actual duration of fumigation was 39 and 53 days for marigolds and petunias, respectively. The accumulated O$_3$ exposure over a threshold of 40 ppb (AOT40) during the fumigation period was 1.6, 20.4 and 36.4 ppm·h under NF, NF+60 and NF+120 treatments for marigolds and 4.0, 25.0 and 47.6 ppm·h for petunias, respectively (Table 1).

3.2 Plant biomass

Under NF+120 treatment, the aboveground, underground and total biomass were reduced by 7.4%, 21.8% and 8.3% for marigolds and by 19.4–23.6%, 30.8–53.8% and 16.1–29.1% for the four petunia varieties, respectively. In addition, the flower biomass decreased significantly, with reductions of 20.0–40.4% for the four petunia varieties (Figure 1).

Figure 1 Effect of ozone (O$_3$) exposure on the biomass of (a) marigold and petunia plant parts [(b) petunia with pink flowers (c) petunia with red flowers (d) petunia with rose-red flowers (e) petunia with white flowers]

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O$_3$ treatments. AG indicates aboveground parts, including leaves and stems; UG indicates underground parts.

Under NF+60 treatment, the change in biomass depended on the plant organs and varieties investigated. The aboveground and total biomass decreased by 13.6% and 15.8% for P-petunias and by 18.2% and 20.2% for R-petunias, respectively. The underground biomass decreased significantly, with reductions of 23.1% and 26.6% for P- and Ro-petunias, respectively. Flower production for P- and W-petunias was reduced by 25.4% and 44.1%.

The shoot-to-root ratio for marigolds, P-, Ro- and W-petunias increased by 16.9%, 30.2%, 110.0% and 87.3%, respectively, under NF+120 treatment, while under NF+60 treatment, this ratio increased by 53.5% and 70.2% in P- and Ro-petunias, respectively (Figure 2).
Figure 2  Effect of ozone (O$_3$) exposure on the shoot-to-root ratio in marigold and petunia

![Figure 2](image)

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O$_3$ treatments. P-, R-, Ro- and W-petunia indicate petunia with pink, red, rose-red and white flowers, respectively.

Figure 3  Effect of ozone (O$_3$) exposure on (a) flower number and (b) flower diameter of petunia and marigold

![Figure 3](image)

Notes: Error bars indicate standard error, n(flower number) = 5, n (flower diameter of petunia) = 15, n (flower diameter of marigold) = 20. *indicates significant differences between non-filtered air (NF) and O$_3$ treatments. P, R, Ro and W indicate petunia with pink, red, rose-red and white flowers, respectively.
3.3 Flower number and diameter

In petunia, the flower number was not significantly influenced by O₃ stress [Figure 3(a)], while flower diameter was significantly reduced under high O₃ exposure, with reductions of 11.5%, 8.5%, 10.7% and 6.9% for P-, R-, Ro- and W-petunia, respectively [Figure 3(b)]. For W-petunia, flower diameter was also reduced under low O₃ exposure (by 7.3%). Unlike petunia, there was no difference in flower diameter between NF and O₃ treatments in marigold [Figure 3(b)].

3.4 Leaf chl and car contents

On 4 September, Chl and Car levels in marigold leaves were reduced by 50.8% and 46.5% under NF+60 treatment, respectively, and by 71.5% and 66.9% under NF+120 treatment, respectively [Figures 4(a), 5(a)]. On 22 September, Chl and Car contents were reduced by 30.5% and 25.2% under NF+60 treatment and by 45.6% and 39.0% under NF+120 treatment, respectively. At the end of the experiment, only NF+120 treatment caused a significant reduction in Chl and Car contents (48.2% and 37.2%, respectively).

**Figure 4** Effect of ozone (O₃) exposure on chlorophyll content in (a) marigold and (b) petunia leaves

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O₃ treatments. P, R, Ro and W indicate petunia with pink, red, rose-red and white flowers, respectively.

Under NF+60 treatment, leaf Chl levels were reduced by 48.4%, 31.9% and 33.2% for R-, Ro- and W-petunias, respectively; a downtrend occurred for P-petunia leaves (24.4%). There was no difference in the rates of reduction between the NF+60 and NF+120 treatments [Figure 4(b)]. Leaf Car levels decreased significantly in R-petunias under NF+60 and NF+120 treatments (by 37.0% and 28.9%, respectively) as well as in W-petunias under NF+120 treatment [by 36.7%; Figure 5(b)].
Figure 5  Effect of ozone (O₃) exposure on carotenoid content in (a) marigold and (b) petunia leaves

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O₃ treatments. P, R, Ro and W indicate petunia with pink, red, rose-red and white flowers, respectively.

Figure 6  Effect of ozone (O₃) exposure on the chlorophyll-to-carotenoid ratio in (a) marigold and (b) petunia leaves

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O₃ treatments. P, R, Ro and W indicate petunia with pink, red, rose-red and white flowers, respectively.

The ratio of Chl to Car in the marigold leaves decreased significantly under NF+60 and NF+120 treatments, with reductions of 7.2% and 10.7%, respectively on 22 September and 17.6% (under NF+120 treatment) on 17 October. These ratios decreased significantly in petunia in response to these treatments, with reductions of 20.4%, 17.8%, 21.7% and 9.5% for P-, R-, Ro- and W-petunias, respectively; no difference was found between NF+60 and NF+120 treatments (Figure 6).
3.5 Leaf flavonoid contents

Flavonoid contents in marigold leaves significantly increased under NF+60 and NF+120 treatments, with increases of 45.5% and 35.6%, respectively on 4 September. On 22 September and 17 October, the differences were not significant [Figure 7(a)]. For petunias, only NF+120 treatment caused a significant increase in leaf flavonoid content [81.4%, 216.1%, 24.0% and 84.3% for P-, R-, Ro- and W-petunias, respectively; Figure 7(b)].

Figure 7  Effect of ozone (O₃) exposure on flavonoid content in (a) marigold and (b) petunia leaves

Notes: Error bars indicate standard error, n = 5. *indicates significant differences between non-filtered air (NF) and O₃ treatments. P, R, Ro and W indicate petunia with pink, red, rose-red and white flowers, respectively.

4 Discussion

4.1 Elevated O₃ levels inhibit the productivity of flowering plants

In the current study, elevated O₃ obviously weakened the ornamental value of marigolds and petunias by reducing the aboveground and total biomass. This response may have been due to the significant reductions in photosynthetic pigment contents in leaves exposed to elevated O₃ (Figures 4, 5). A positive relationship between Chl and photosynthesis was detected in previous studies (Black et al., 2007; Rai et al., 2011; Yendrek et al., 2013), further corroborating the important role of Chl in CO₂ reduction. Elevated O₃ exposure not only affects total biomass accumulation, but it also alters biomass partitioning into the shoot and root (Hoshika et al., 2013). In the present study, less biomass was allocated to roots under O₃ stress, as shown by the increased shoot-to-root ratio (Figure 2). This finding is consistent with the results of Hoshika et al. (2013) and Nouchi et al. (1991) in crops and Köllner and Krause (2000) in trees. The influence of elevated O₃ on biomass allocation can be explained by the Optimal Partitioning Theory, which suggests that plants should allocate more biomass to the organ that requires the most limited resource (McCarthy and Enquist, 2007).
In this study, plant photosynthetic performance was weakened under O₃ stress, as indicated by the decline in leaf chlorophyll content (Figure 4), requiring more biomass to be allocated to the shoot in order to maintain high levels of photosynthesis. Additionally, O₃ stress obstructs phloem sieve elements by increasing the accumulation of callose, consequently hampering assimilate transport from shoot to root (Calatayud et al., 2011; Gao et al., 2016). Moreover, O₃ stress stimulates the root respiration rate, as suggested by Nouchi et al. (1991) and Thwe et al. (2013), which subsequently accelerates the consumption of root biomass. All of these effects might explain the increased shoot-to-root ratio observed under O₃ stress. R-petunia was the exception, as O₃ stress reduced the shoot-to-root ratio in this plant, which was also reported by Li et al. (2015). Perhaps leaf abscission in this plant was accelerated under O₃ treatment. Since roots play an important role in water and nutrient acquisition, poor root growth and development would have a negative effect on shoot growth.

Flowers are important ornamental organs for both marigolds and petunias. A change in their morphology or biomass would degrade the plant’s ornamental value. In this study, we found that under high O₃ stress, the flower numbers of the four petunia varieties were not reduced, but the flower diameter and biomass were significantly reduced. A similar result was also reported by Franzaring et al. (2000). The reduction in flower biomass under O₃ stress is attributed to a reduction in flower size (e.g., diameter). Zhang et al. (2014) found that in an undesirable environment, peony flower biomass and diameter decrease simultaneously. However, unlike petunia, no reduction in flower biomass or diameter was observed in marigold under O₃ exposure [Figure 3(b)]. This difference in flower responses between the two plant species may be ascribed to the fact that petunia experienced a higher O₃ exposure dose than marigold during reproductive growth. The durations and AOT40 of O₃ exposure during their reproductive growth were 52 days and 46.5 ppm·h for petunia and 27 days and 25.8 ppm·h for marigold (Table 1).

These results indicate that O₃ can diminish the ornamental performance of flowering plants by inhibiting the formation of biomass, including aboveground biomass, flowers and total biomass, although not all flowers were affected in both species.

### Table 1

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<th>Marigold</th>
<th>Petunia</th>
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<tr>
<td></td>
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<tr>
<td>[O₃]mean</td>
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<td>99.2</td>
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<tr>
<td>AOT40([Oct.17,2013])</td>
<td>1.6</td>
<td>20.4</td>
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<tr>
<td>AOT40([May24,2014])</td>
<td>–</td>
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<tr>
<td>AOT40(reproductive growth)</td>
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<td>14.8</td>
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Notes: AOT40(x) indicates accumulated O₃ exposure above a threshold of 40 ppb from the beginning of fumigation to the sampling date. NF = non-filtered air.

#### 4.2 Elevated O₃ levels accelerate leaf yellowing in flowering plants

In the current study, O₃ exposure decreased the ornamental value of marigolds and petunias by accelerating leaf yellowing, which is a natural morphological characteristic of leaf senescence due to changes in pigment types and relative levels (Mitchell et al.,
The impact of elevated ozone on the ornamental features

Chlorophyll is the most obvious and widespread plant pigment. This pigment is not only involved in initiating photosynthesis by capturing light energy, but it also gives leaves their green colour (Davies, 2004), especially during vigorous growth periods when the green colour masks other colours from, for example, carotenoids and anthocyanin. In the present study, O₃ accelerated leaf chlorophyll degradation in marigold and petunia, which is in line with the results of numerous studies (Hörtensteiner and Kräutler, 2011; Heath and Packer, 1968). Chlorophyll degradation resulted in the reduced expression of green colouration in leaves, which was confirmed in a previous study of Phaseolus vulgaris L., where high chlorophyll levels were retained in senescent leaves of a stay-green mutant, but not in the wild type (Fang et al., 1998). This indicates that chlorophyll degradation is an indirect cause of leaf yellowing by attenuating its masking effect on other pigments.

Carotenoids are another widely studied pigment in higher plants owing to their role in photoprotection (Bartley and Scolnik, 1995); these pigments result in a yellow colour owing to the presence of β-carotene and lutein (Hendry et al., 1987). In the present study, although the changes in carotenoid levels under O₃ stress varied with plant growth stage and plant species, we detected a reduced chlorophyll-to-carotenoid ratio, indicating a preferential degradation of chlorophyll over carotenoids. This effect may be one of the main reasons for leaf yellowing caused by O₃ stress, as revealed in the studies of Biswal (1995) and Burkart et al. (2013) on the yellowing of senescent leaves.

Previous studies have shown that flavonoid absorption at 350 nm is responsible for yellowing (Schwinn and Davies, 2004). However, whether leaf yellowing caused by O₃ is related to flavonoid accumulation has previously been unclear. In the present study, O₃ increased the flavonoid contents in marigold and petunia leaves, even when there was no significant reduction in the chlorophyll-to-carotenoid ratio [Figure 5(a)], suggesting that flavonoid biosynthesis may also be an important cause of leaf yellowing. Therefore, O₃-induced leaf yellowing could be attributed to the reduced ratio of chlorophyll to carotenoids and increased flavonoid levels; these effects may work alone or together.

5 Conclusions

Elevated O₃ levels significantly reduced the aboveground, underground and total biomass in marigolds and four petunia varieties. Under these conditions, root biomass decreased more than aboveground biomass. The flower biomass decreased significantly in all four petunia varieties, but not in marigolds. Chlorophyll levels in both marigold and petunia leaves decreased under O₃ exposure. The responses of carotenoids to O₃ varied with plant species. O₃ exposure also significantly increased leaf flavonoid contents, suggesting that leaf yellowing induced by O₃ could be attributed to increased flavonoid biosynthesis and chlorophyll degradation. In summary, elevated O₃ levels degraded the ornamental features of marigold and petunia, as represented by reduced biomass and accelerated leaf yellowing, the latter representing a morphological symptom of plant senescence.
References


The impact of elevated ozone on the ornamental features


The impact of elevated ozone on the ornamental features


