Luncheon Seminar on 2011.12.9

Ozone Stress Response from the Viewpoint of Organic Compound Emission from Leaves

By Korin Kawaguchi

First...

- VOCs (Volatile Organic Compounds) are important in atmospheric science.
  - Formation of photochemical oxidants and O_3 / degradation of OH or O_3
  - Formation of aerosols
  - Carbon cycle (奥村 2009)

- VOC can be divided into two types.
  - AVOCs: Anthropogenic VOCs from solvent and burning fossil fuel, etc.
  - BVOCs: Biogenic VOCs mainly from vegetation and ocean

- AVOCs and BVOCs are different in that;
  - Larger global emission of BVOCs (0.5~1.2PgC/yr) than AVOCs (0.1Pg) (Guenther 1995 J. Geophys. Res.)
  - High reactivity of BVOCs (terpenes) compared to AVOCs with OH or O_3 (Griffin et al. 1999 J. Geophys. Res.)

BVOCs from vegetation

- Isoprene (hemi-terpene)
  Acacia, *Eucalyptus*, *Populus*, *Salix*, *Quercus* are high emitters among tree species
- Mono-terpene, di-terpene, sesqui-terpene
- Methanol
- Ethylene (alkene)
- Alkanes, organic acids, aldehydes, alcohols…
- Methane
- Categorized as green leaf volatiles, herbivore-induced volatiles, allelochemicals
- There are also belowground VOCs (root exudates)
  (Kegge and Pierik 2010 Trends Plant Sci.).

Isoprene

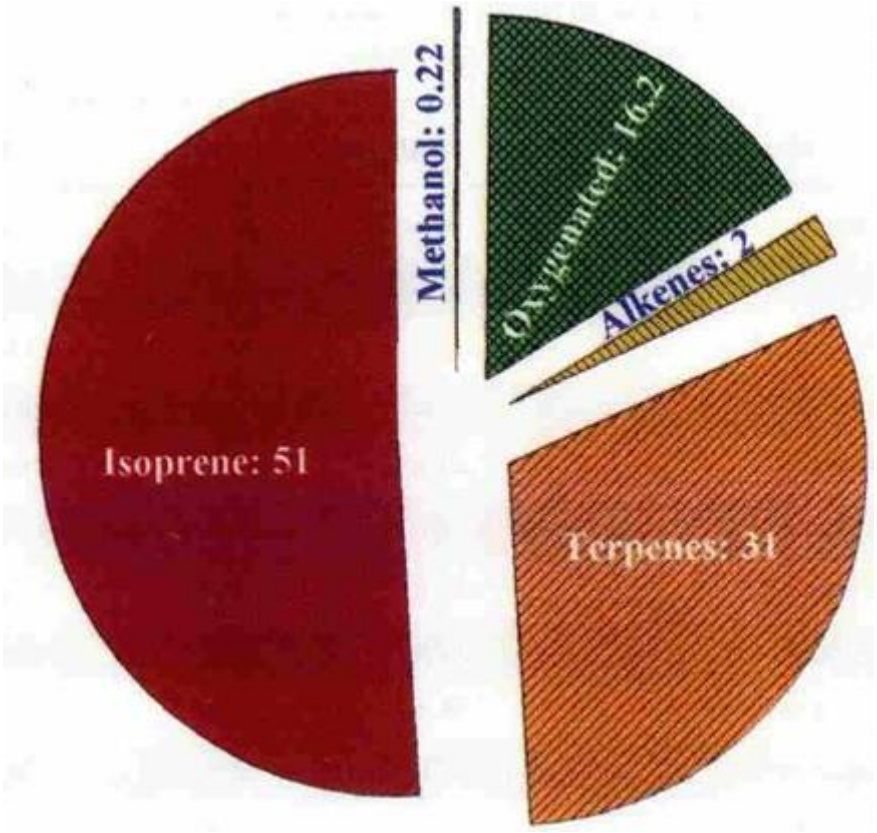
- Protection against heat
  → This may explain why many tropical plants emit isoprene.
  (Sharkey and Singsaas 1995 Nature)

- Protection against O_3
  (Loreto and Velikova 2001 Plant Physol.)

- 67% of the photosynthetically fixed carbon was reemitted as isoprene from Kudzu (*Pueraria lobata*) leaves
  (Sharkey and Loreto 1993 Oecologia).

Mono-terpene

- Non-stomatal O_3 flux
  (Goldstein et al. 2004 Geophys. Res. Lett.)

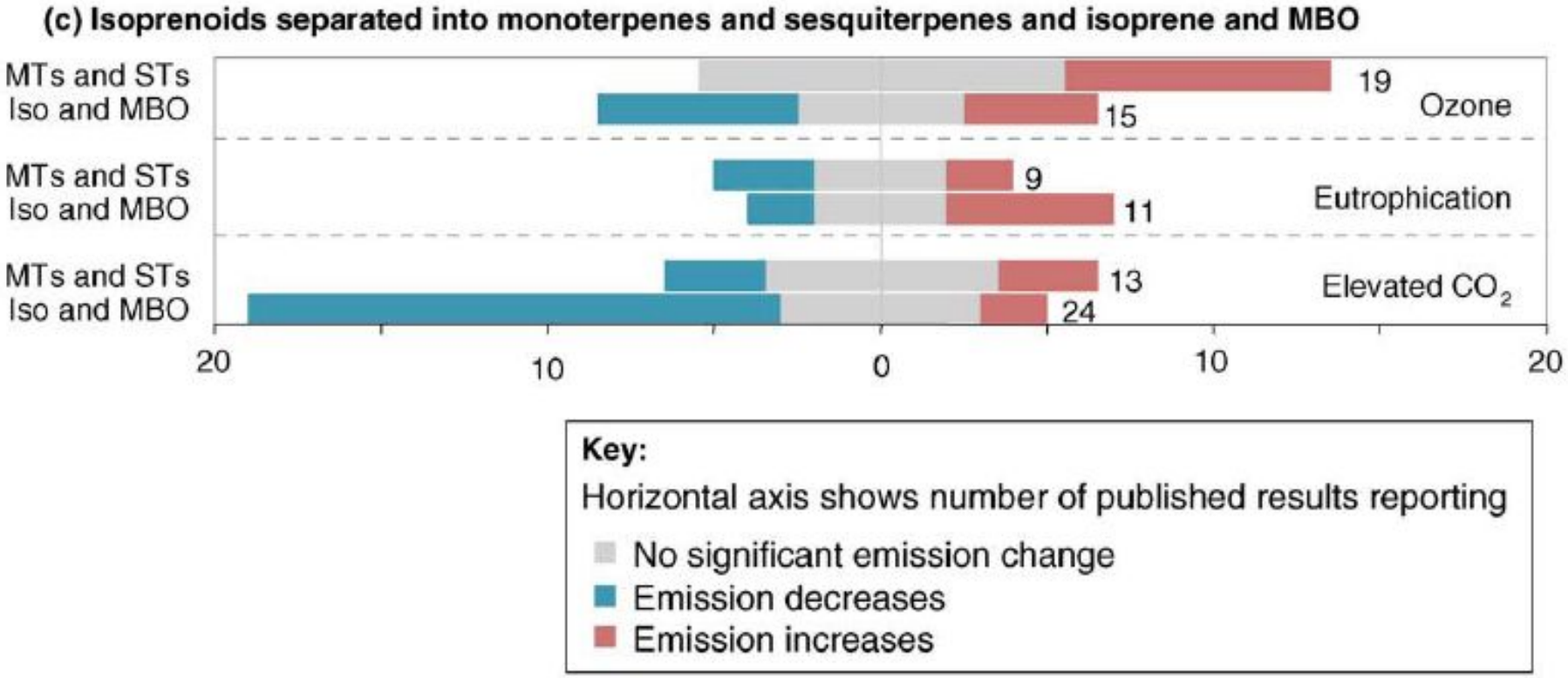


(Fuentes et al. 2000 Bull.Am.Meteol.Soc.)

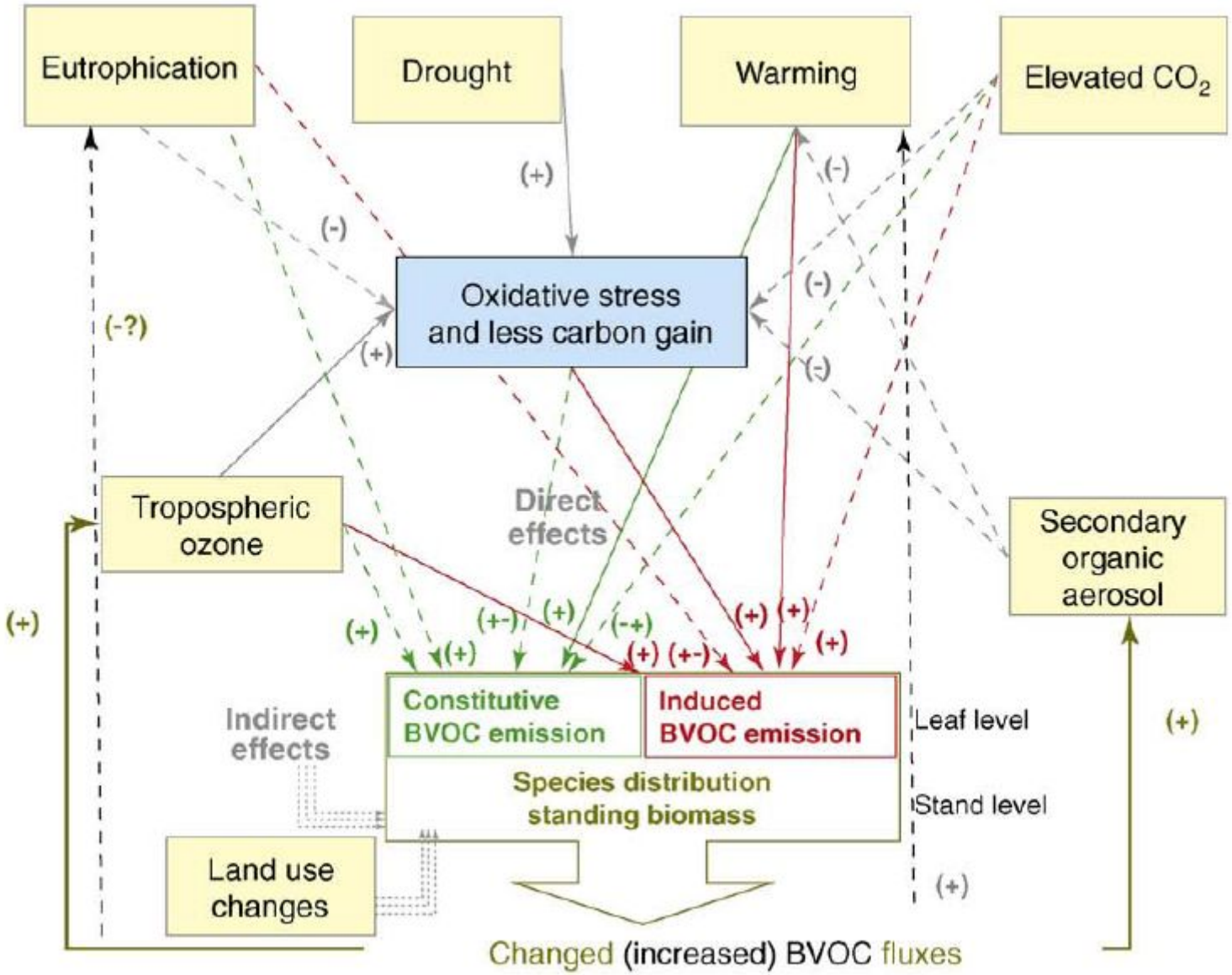
BVOCs and climate change

- It can be estimated that climate warming could have increase global BVOCs emissions by 10%.

- Generally elevated CO_2 decreases and elevated O_3 increases isoprene emission



(Peñuelas and Staudt 2010 Trends Plant Sci.)



(Peñuelas and Staudt 2010 Trends Plant Sci.)

References

- 奥村智憲 (2009) 森林植生からの揮発性有機化合物放出量の推定に関する研究. 京都大学エネルギー科学研究科博士論文.

- 2010. Trends in Plant Science, "*Special Issue: Induced volatile organic compounds from plants*" vol. 15 no.3

For more recent reviews,

- Laothawornkitkul J, Taylor JE, Paul ND, Hewitt CN. (2009) Biogenic volatile organic compounds in the earth system. New Phytologist, 183:27-51.

- Pinto DM, Blande JD, Souza SR, Nerg AM. Holopainen JK. (2010) Plant volatile organic compounds (VOCs) in ozone (O3) polluted atmospheres: the ecological effects. Journal of Chemical Ecology, 36:22-34.

2011.12.9 @ Luncheon seminar at Hokkaido U.

Physiologia Plantarum 128: 456–465. 2006

Impact of high ozone on isoprene emission, photosynthesis and histology of developing *Populus alba* leaves directly or indirectly exposed to the pollutant

Silvano Fares (a), Csengele Barta (a), Federico Brilli (a), Mauro Centritto (b), Luisa Ederli (c), Francesco Ferranti (c), Stefania Pasqualini (c), Lara Reale (c), Domenico Tricoli (a) and Francesco Loreto (a)*

(a) Consiglio Nazionale delle Ricerche, Istituto di Biologia Agroambientale e Forestale, Via Salaria Km 29,300, 00016 Monterotondo Scalo, Rome, Italy
(b) Consiglio Nazionale delle Ricerche, Istituto sull'Inquinamento Atmosferico, Via Salaria Km 29,300, 00016 Monterotondo Scalo, Rome, Italy
(c) Universita'degli Studi di Perugia, Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali e Zootecniche, Borgo XX Giugno, 74-06121 Perugia, Italy

doi: 10.1111/j.1399-3054.2006.00750.x

Introduction

- Recent studies have shown interactions between biogenic volatile organic compounds (BVOCs) and atmospheric ozone (O_3).

  1. contribution of isoprene (BVOCs) to O_3 formation (Chameides et al. 1988)
  2. O_3 stimulates isoprenoid emission by leaves (Loreto et al. 2004)
  3. isoprenoids reduce O_3 damage, (Loreto et al. 2001, 2004) and
  4. the mechanism may be that isoprene reduces intercellular NO and H_2O_2 formation (Velikova et al. 2005)

- Present and predicted climate change is expected to increase the formation of these gases.

  1. 50%-increase of atmospheric O_3 by 2100 (Fowler et al. 1999)
  2. climate warming is also expected to increase isoprenoid emissions by plants because of the strong temperature-dependence of isoprenoid biosynthesis (Loreto and Sharkey 1990)

- The objective of this study was to know
  whether O_3 only affects the physiology and anatomy of leaves directly exposed to high levels of O_3,
  or if it also affects new leaves not directly exposed to O_3.

Material and Methods

– Plant material & The ozone fumigation system-

- 3-year-old plants of *Populus alba* were used.
  5 plants referred to as treatment, and other 5 as controls.

- Branch cuvettes (3 l of volume) were installed at the bottom of the stem including the basal (3 to 4) leaves.
  The top part of the cuvettes was tied to the stem to avoid leaks.
  The stems existing on the top part of the cuvettes were cut to allow the sprouting of 1 bud only per stem during the O_3 treatment. New buds appeared after 1 week and new shoots grew within 3 weeks after cutting.

- O_3 treatment (150 ppb) was done for 11 h / day (7:00-18:00) and for 1 month.
  In control, O_3 concentration was 0 ppb at night but 40 ppb during the central hours of the day.
  The buds developing on the stem out of the cuvettes grew at ambient O_3 concentration (similar to control).

Material and Methods

– Photosynthesis and isoprene measurements

- Photosynthesis, stomatal conductance, intercellular CO_2 concentration (Ci) were calculated after measuring gas-exchange with Li-6400.

- Chlorophyll fluorescence was measured simultaneously.

  In particular, the quantum yield of photosystem 2 in darkened leaves (Fv/Fm) and illuminated leaves (⊿F/Fm') were measured.

- Isoprene emission from leaves was detected simultaneously to gas-exchange measurements, connecting the outflow of the Li-6400 to a proton-transfer-reaction mass-spectrometer.

Material and Methods
– Histological observations & RNA analysis

- At day 30 of the experiment, leaf tissue pieces were excised from 4 portions of each leaf on 5 replicates per treatment.

- Leaf sections were observed under a light microscope or a transmission electron microscope.

- Total RNA was extracted from frozen, homogenized leaf tissue.

- A given amount of total RNA was reverse transcribed.

- The complementary DNA was used for PCR with *actin* and isoprene synthase (*PaISPs*).

results

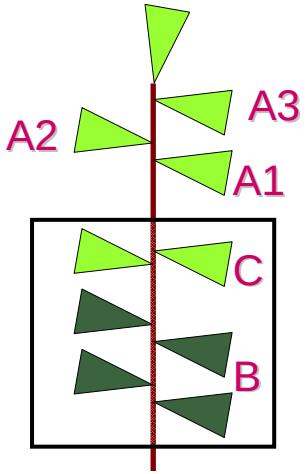
- Measurements were carried out following leaf material:

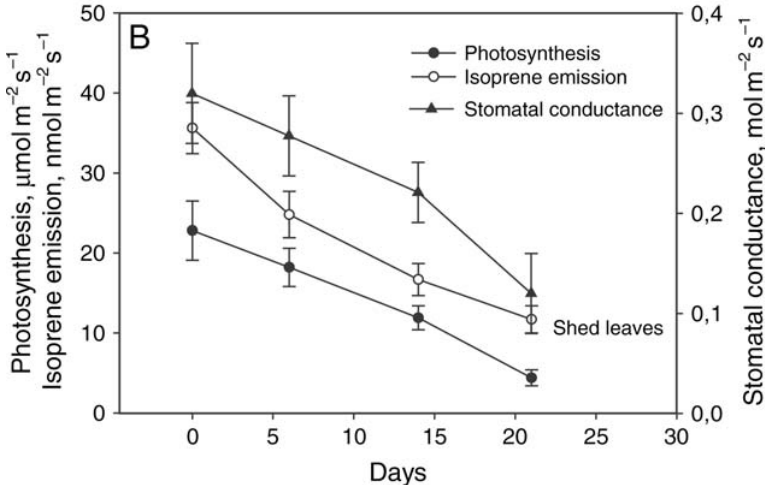
  1. the first three leaves expanding out of the cuvettes. These leaves were named A1 (first leaf), A2 (second leaf), A3 (third leaf).

  2. the leaves growing inside the cuvette since the beginning of the experiment. These leaves were named B.

  3. the leaves expanding from buds inside the cuvettes during the experiment. These leaves were named C.

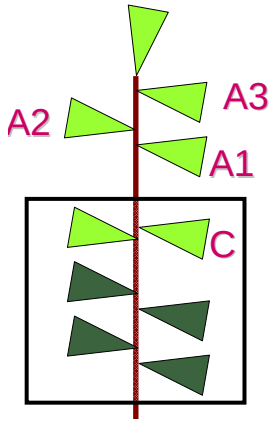
- In B leaves, O_3 damage were observed after 3 days of the treatment. Many of the leaves were shed during the experiment.





results

- A1 leaves within 1 week from beginning the O_3 treatment showed peculiar morphology and anatomy.
  The leaves were small and lost the typical shape of poplar leaves.
  The leaves were much thicker than other leaves.
  All components of the leaf contributed to make A1 leaves thicker and their mesophyll more packed.
- These features were lost in A2 and A3 leaves, which were similar to control leaves.
- New leaves developed inside the cuvettes (C) were resistant to O_3.
  C leaves developed with a time frame similar to A3 leaves, and were thinner in O_3-treated plants, because of a reduced height of the palisade parenchyma and reduced size of intercellular spaces in the spongy parenchyma.
  The mesophyll of O_3-treated C leaves was densely packed and palisade cells were smaller.

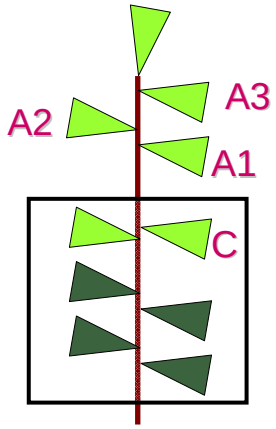


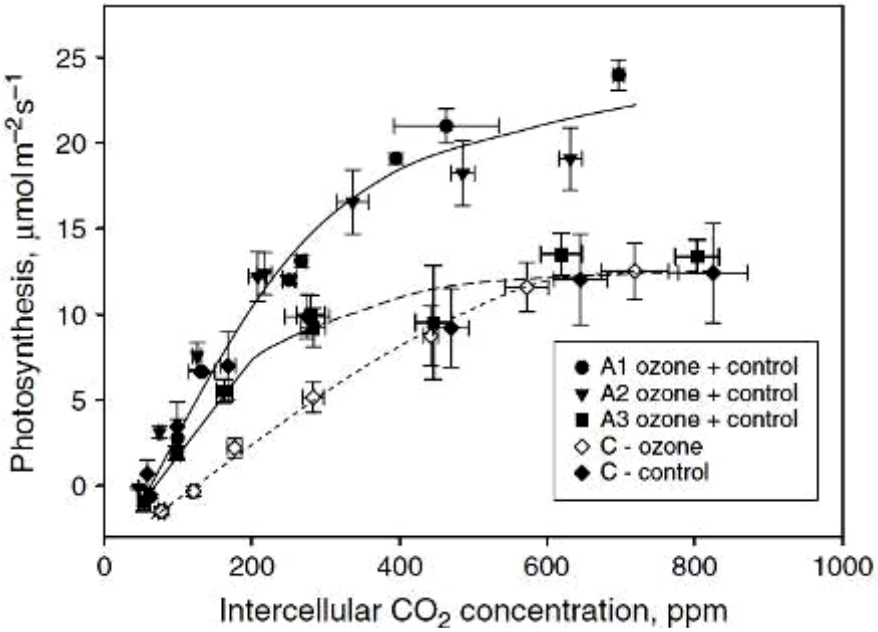
results

- A difference between C and A leaves in O_3-treated plants was indicated. Photosynthesis response to increasing Ci was less steep, indicating a lower Rubisco activity in C leaves.

- There were differences between A1 and A2 leaves of O_3-treated plants, which was observed at Ci higher than ambient.
  A1 and A2 leaves were affected by a strong developmental control on photosynthesis, rather than a direct effect of the O_3 treatment.

  For example, C leaves of control plants and A3 leaves of O_3-treated and control plants were ontogenetically similar and showed the same response of photosynthesis.

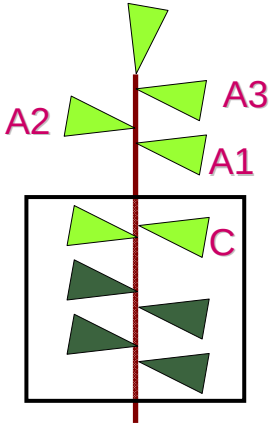




results

- No differences were found in the quantum yield of dark-adapted leaves measured by Fv/Fm.
  But in illuminated leaves the quantum yield of C leaves was affected by growth under high O_3 (or by developmental control).

- The ⊿F/Fm' was higher in control and O_3-treated A1 leaves.
  This confirmed the indications supplied by gas-exchange data, that photosynthesis of developing leaves was limited by the electron-transport-driven regeneration of RuBP, irrespective of O_3 treatment.

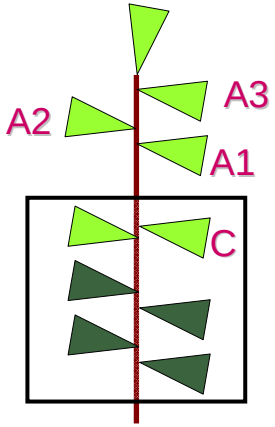


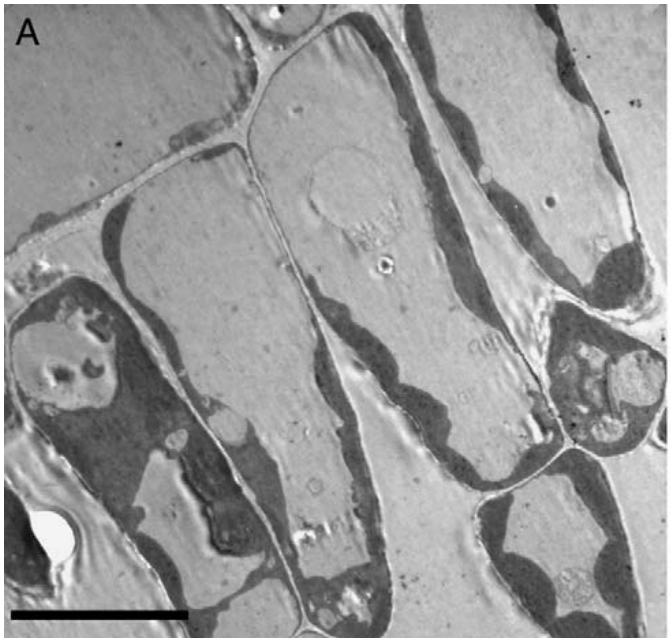
Leaf class	Fv/Fm	ΔF/Fm′
Controls		
C	0.79 ± 0.02a	0.23 ± 0.5b
A1	0.81 ± 0.04a	0.22 ± 0.4b
A2	0.77 ± 0.02a	0.16 ± 0.3a
A3	0.77 ± 0.04a	0.14 ± 0.3a
Ozone treated		
C	0.80 ± 0.02a	0.11 ± 0.5a
A1	0.77 ± 0.05a	0.21 ± 0.5ab
A2	0.79 ± 0.03a	0.14 ± 0.3a
A3	0.78 ± 0.01a	0.13 ± 0.4a

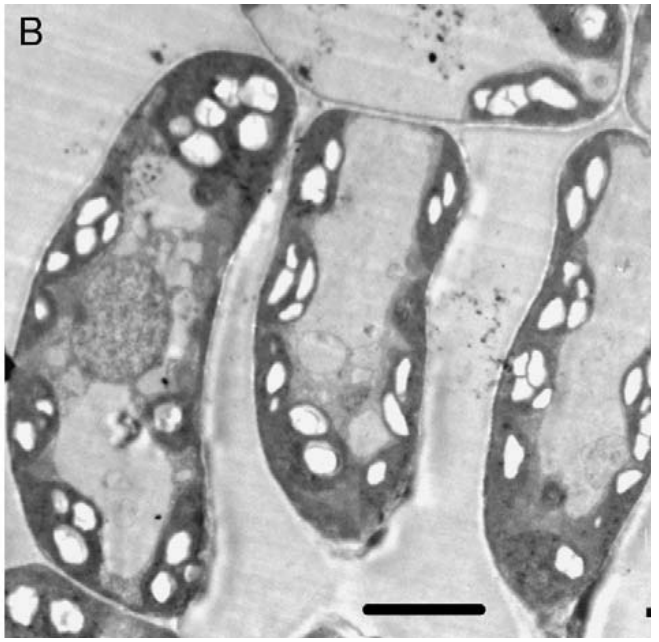
results

- Electron micrographs showed the accumulation of many starch granules in A leaves of control plants.
  No starch accumulation was observed in A3 leaves of O_3-treated plants.
  Starch accumulation in A2 leaves of O_3-treated plants was about 50% of that in controls, and that in A1 leaves was not different between treatments.

- Electron microphotograph of cells of O_3-treated (A: left) and control (B: right) A3 leaves.







results

- The O_3 treatment caused a stimulation of isoprene emission from leaves.

  This increase was significant in C and A1 leaves.

- In A1 leaves, isoprene emission was associated to a larger expression of isoprene synthase (*PaISPs*) mRNA.
  It's expression also matched the age-dependent reduction of isoprene emission in A leaves.

discussion - anatomy

- The anatomical changes of A1 leaves were dramatic and indicated that O_3 can strongly impact on their development.
  However, the impact of O_3 was rapidly lost in leaves developing after A1, suggesting that O_3 only affects the closest leaf to the O_3 source.

- The signalling system, which brings about these developmental changes, is unknown.
  It may be possible, alternatively, that O_3 sensitivity be mediated by the different developmental stage of the leaves, being stronger in leaves already developed (e.g. A1) than in those still developing (e.g. A3).

discussion - starch

- Similar to other studies (e.g. Oksanen 2003), O_3-induced starch depletion was observed.

  Interestingly, despite O_3 impact on leaf anatomy, A1 leaves revealed a well-preserved photosynthesis which are similar to those observed in control leaves of corresponding developmental stage.
  However, a very low accumulation of starch was noted in leaves developing after A1 in O_3-treated plants.

- These results suggest that the biosynthesis of starch is delayed, as this would explain why no differences were found in fully developed leaves (e.g. A1), whereas newly developing (A3) leaves of O_3-treated plants were unable to accumulate starch.
  However, it cannot be excluded that starch was in fact produced, but exported very rapidly from A3 leaves to stronger sinks of O_3-treated plants.

Starch	A1	A2	A3
Control	-	-	-
O3	not different	50%	0%

- The observed isoprene stimulation in A1 leaves, developing above O_3-treated leaves, expands the observation that leaves recovering from O_3 stress emit more isoprene (Velikova et al. 2005) or monoterpenes (Loreto et al. 2004).

  As in the case of other physiological and anatomical features, also isoprene emission was not affected in leaves far from the O_3 treated leaves, although a residual stimulation was noted in A2 leaves.

- It should be considered that isoprene emission is under a strong developmental regulation, the low emission of young leaves being regulated at a transcriptional level because of the low amount of *PaISPs* mRNA and protein (Wiberley et al. 2005).

  Our finding confirms that *PaISPs* mRNA is very low in leaves that start to develop, and suggests that indirect O_3 treatment does not affect early stages of isoprene emission, at least on leaves far from the O_3 source.

  It remains to be tested, however, whether O_3 may induce earlier emission of isoprene in leaves near O_3 source (A1 leaves).

discussion – Isoprene 2

- Isoprene emission is often associated to the availability of photosynthetic intermediates.

  However, this is not the case in A leaves, which showed no photosynthesis stimulation with respect to A leaves of control plants.

- Isoprene stimulation in A1 leaves was associated with a greater expression of *PaISPs* gene, indicating that isoprene emission of leaves, developing above leaves being treated with O_3, can be regulated at the transcriptional level, as also observed in leaves exposed to light and heat stress (Sasaki et al. 2005).

discussion - O_3 resistance 1

- 1.
  New leaves (C leaves) developed inside the cuvettes under high O_3, while already developed leaves (B leaves) showed larger damage and were eventually shed. This observation reveals adjustments to the O_3 resistance.

- 2.
  As well as previous observation of O_3-induced reduction of leaf thickness and packed cell structure (Oksanen et al. 2005),
  C leaves exposed to O_3 was more packed than C leaves of controls and other leaf classes, suggesting that O_3 diffusion might be reduced and that this may be related to the observed resistance.

- 3.
  Probably, C leaves also have a more developed antioxidant system protecting them from O_3 (Diara et al. 2005, Pell et al. 1997).
- 3'.
  Isoprene was described to have an antioxidant action protecting leaves from O_3 (Loreto and Velikova 2001).
  We have detected a stimulation of isoprene emission in C leaves directly exposed to O_3.

discussion – O_3 resistance 2

- However, the emission of O_3-treated C leaves was lower than that in A1 leaves and in B leaves at the beginning of the treatment.

  Two aspects should be considered when analyzing this data set.
  First, C leaves were analyzed at a very young stage, comparable to those of A3 leaves. This is reflected in a yet low developmental capacity to produce isoprene as also indicated by the very low level of PaISPs mRNA detected in C leaves.
  Second, the photosynthesis of C leaves was perturbed by direct O_3 treatment and this may have further limited isoprene production.

- 4?
  A large limitation of photosynthesis was revealed in O_3-treated C leaves. The limitation was particularly evident at low CO_2 levels at which photosynthesis responds linearly to CO_2 and this is due to Rubisco activity (von Caemmerer and Farquhar 1981).
  Rubisco is known to be negatively affected by O_3, causing photosynthesis limitation in O_3-stressed leaves (Pell et al. 1997). It is suggested that Rubisco remains a parameter sensitive to O_3, even in leaves that acquire resistance to O_3.

summary

- Large changes in the anatomy of the leaves developing immediately after those treated by O_3 were detected.
  These leaves also showed a stimulation of isoprene emission not associated with higher photosynthesis levels, indicating that a larger fraction of the carbon was allocated to form isoprene as an indirect consequence of O_3 stress.

- O_3 also indirectly affected starch accumulation in developing leaves, probably delaying starch biosynthesis or causing a rapid translocation of starch to other plant parts.

- This study also revealed that direct treatment by O_3 may lead to the development of a thin class of leaves, with packed mesophyll and resistant to O_3.
  These leaves also showed isoprene stimulation, again revealing a larger carbon investment into the isoprenoid pathway, whereas photosynthetic rates were limited by Rubisco activity.