Photosynthesis of *Hedera canariensis* var. *azorica* variegated leaves as affected by ozone

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**Abstract**

A differential response to long term ozone exposures (50 and 100 mm3 m-3) was observed in the green and white areas of variegated leaves of *Hedera canariensis* var. *azorica* L. In green tissue the photosynthetic activity was depressed via a stomatal mechanism, and in white regions no effect was observed. Chlorophyll fluorescence parameters remained unchanged in green portions, whereas in the white ones $F_m$ and $F_v/F_m$ significantly diminished following ozone fumigation.

*Additional key words:* chlorophyll; fluorescence; ivy; net photosynthetic rate; stomatal conductance; transpiration.

**Introduction**

Ozone ($O_3$) is the main constituent of photochemical smog, and its importance is increasing. This air pollutant determines macroscopic responses in sensitive plants, but also long subliminal ("hidden") effects, such as metabolic disorders which cause reductions in growth or yield even in the absence of any visible marking, have been detected. Phytotoxicity mechanisms of $O_3$ are complex and still a matter of debate, especially as far as photosynthesis is concerned (Heath 1994).

Variegated leaves are an interesting material to investigate in the same organ the influence of pigment on the photosynthetic response to an environmental stress (Lafray et al. 1991, Beerling and Woodward 1995). The aim of this work was to

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*Abbreviations* (Chl: chlorophyll; ChloF: chlorophyll a fluorescence; DMF: N,N-dimethylformamide; $E$: transpiration rate; $F_m$: maximal fluorescence; $F_v$: variable fluorescence; $F_o$: ground fluorescence; $g_s$: stomatal conductance; $F_N$: net photosynthetic rate; PS: photosystem.

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247
investigate the gas exchange and chlorophyll a fluorescence (ChlaF) parameters in green and white leaf portions of variegated plant, Heder a canariensis var. azorica L., subjected to realistic O\textsubscript{3} levels in long-term exposures.

**Materials and methods**

**Plants**: Rooted cuttings of ivy deriving from a single mother plant were raised in plastic pots containing a steam-sterilized soil:peat:perlite mix, and grown in open air. Uniform plants were selected when six leaves were fully expanded, and pre-adapted to greenhouse conditions a week before the treatment.

**Ozone fumigation** was performed during the summer of 1996 in a set of Perspex chambers, each measuring 0.90×0.90×0.65 m, that were continuously ventilated with charcoal-filtered air (two complete air changes in 1 min). O\textsubscript{3} was produced by electric discharge via an air-cooled generator (Fischer 500, Zürich, Switzerland), supplied with pure oxygen, and it was mixed with the inlet air when entering the fumigation chambers. O\textsubscript{3} concentrations at plant height were continuously monitored with a photometric ML8810 analyzer (Monitor Labs, San Diego, CA, USA); for details see Lorenzini et al. (1994). The target doses were 50 and 100 mm\textsuperscript{3} m\textsuperscript{-3} for 28 d (5 h d\textsuperscript{-1}, from 09:00 to 14:00 h, solar time). The control plants were exposed to charcoal-filtered air only.

**Measurements and observations**: Epidermal impressions from both sides of interveinal portions (proximal, medial, and distal) of leaves were made onto acetate sheets (Beersling and Chaloner 1992). Stomatal density was determined using a Leitz microscope at 250×. Three fields per leaf were counted, and four leaves from each plant were analyzed.

Gas exchange was measured using a CIRAS-1 infrared gas analyzer (PP Systems, Stotfold, UK) at ambient CO\textsubscript{2} concentration, 80% relative humidity, and 25 °C. Saturating irradiance (800 μmol m\textsuperscript{-2} s\textsuperscript{-1}) was obtained with a halogen lamp. Measurements were taken at the end of the treatment on five plants (three recently mature leaves per plant, one green and one white area per leaf).

*In vivo* ChlaF excited by modulated red radiation (centered at 655 nm) was measured at room temperature and wavelengths longer than 700 nm with a PAM-2000 fluorometer (H. Walz, Effeltrich, Germany) as previously described (Guidi et al. 1997). After 40 min dark-adaptation, the maximum quantum yield of photosystem 2 (PS2) photochemistry was assessed as (F\textsubscript{m} - F\textsubscript{0})/F\textsubscript{m} = F\textsubscript{v}/F\textsubscript{m}, where F\textsubscript{0} is the initial level of ChlaF, and F\textsubscript{m} is the maximum fluorescence induced by an 800 ms flash of saturating "white light" (Schreiber and Bilger 1993). Measurements were taken at the end of the treatment on five plants (one recently mature leaf per plant, one green and one white area per leaf). The Chl content per unit leaf area was finally measured spectrophotometrically in N\textsubscript{2}N-dimethylformamide extracts according to Moran (1982).
Results and discussion

Characterization of variegated leaves: *H. canariensis* var. *azorica* leaves are hypostomatous, and in the adaxial surface only rare stomata are present. No significant differences exist for stomatal density between different comparable areas (proximal, medial, and distal) in white and green regions (379±10.3 vs. 350±18.7 stomata per mm², respectively, p>0.05, Student's *t*-test). This is in accordance with results of Aphalo and Sánchez (1986) in variegated *H. helix* leaves, but may be an exception because white portions of variegated leaves usually have a smaller stomatal density in comparison with the green ones (Downton and Grant 1994, Beerling and Woodward 1995).

Chl was not completely absent in white regions, but there was 45 times more of it in the green tissue (Table 1). Similar results have been reported for variegated ivy leaves (Aphalo and Sánchez 1986). Chl *a/b* ratio was three times larger in the green portions than in the white ones. The net photosynthetic rate (*P*ₙ) in green leaf tissues was about 4.5 μmol(CO₂) m⁻² s⁻¹, whereas the white leaf tissue did not show any net uptake of CO₂, and had lower values of both stomatal conductance (*g*ₘ) and transpiration rate (*E*) (∼85 %) than the green regions. Apparently, stomata in the white areas did not function properly. In variegated *H. helix*, *g*ₘ in the white areas was approximately half of that measured in the green ones (Aphalo and Sánchez 1986).

Table 1. Chlorophyll (Chl) content [mg m⁻²] and gas exchange parameters, i.e., net photosynthetic rate (*P*ₙ) [μmol(CO₂) m⁻² s⁻¹], stomatal conductance (*g*ₘ) and transpiration rate (*E*) [mmol(H₂O) m⁻² s⁻¹], of green and white portions of variegated leaves of *Hedera canariensis* var. *azorica* in pollutant-free air. Gas exchanges were measured at ca. 800 μmol(photon) m⁻² s⁻¹, 345 μmol(CO₂) mol⁻¹, and 21 % O₂. Values represent means of ten replicates for Chl content analysis, and five for gas exchange measurements. All difference were statistically significant according to the Student's *t*-test (p<0.001).

<table>
<thead>
<tr>
<th>Leaf portion</th>
<th>Chl <em>a</em></th>
<th>Chl <em>b</em></th>
<th>Chl <em>(a+b)</em></th>
<th>Chl <em>a/b</em></th>
<th><em>P</em>ₙ</th>
<th><em>g</em>ₘ</th>
<th><em>E</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>200.8</td>
<td>62.9</td>
<td>263.7</td>
<td>3.19</td>
<td>4.54</td>
<td>91</td>
<td>1.85</td>
</tr>
<tr>
<td>White</td>
<td>2.8</td>
<td>3.0</td>
<td>5.8</td>
<td>0.93</td>
<td>-0.80</td>
<td>13</td>
<td>0.26</td>
</tr>
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The Chl*ab* parameters were different in green and white portions (Table 2). In the white areas, *F₀* and *Fₘ* were very low (about -95 % in comparison with the green tissue), probably as a consequence of a very low Chl content. Usually, *F₀* and *Fₘ* depend on the unitary leaf Chl content, as the reabsorption of the emitted fluorescence is a function of the total Chl content (Lichtenthaler 1988). Surprisingly, in both the white and green areas the *F₀/Fₘ* ratio reached values considered normal for healthy plants (Björkman and Demmig 1987). This indicated that the electron transport efficiency around PSII was similar in the two kinds of tissue. The white portions of ivy leaves have apparently functional chloroplasts, as already reported by Aphalo and Sánchez (1986). This implies that NADPH and ATP can be formed, although at a rate much lower than in the green tissue. The question arises if NADPH and ATP can be utilized in areas where the CO₂ balance is negative (respiration...
prevails). Evidence indicates that a high dark CO₂ fixation via phosphoenolpyruvate carboxylase (PEPC) is induced in organs where photosynthesis is less efficient than respiration, i.e., at a net loss of CO₂ (Hedley and Rowland 1975, Soldatini et al 1982). Ploemann and Eschrich (1990) report that the white areas of variegated Coleus leaves fix CO₂ via PEPC.

Table 2. Chlorophyll a fluorescence parameters of green and white portions of variegated leaves of Hedera canariensis var., azorica in pollutant-free air. Measurements were carried out in leaves dark-adapted for 40 min. Values represent the means of five replicates. The last row indicates the significance of the differences (Student's t-test. ** = p<0.001; * = p<0.01; NS = p>0.05).

<table>
<thead>
<tr>
<th>Leaf portion</th>
<th>F₀</th>
<th>Fₘ</th>
<th>Fᵥ</th>
<th>Fᵥ/Fₘ</th>
<th>Fᵥ/F₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>125</td>
<td>511</td>
<td>386</td>
<td>0.754</td>
<td>3.88</td>
</tr>
<tr>
<td>White</td>
<td>9</td>
<td>26</td>
<td>17</td>
<td>0.657</td>
<td>1.88</td>
</tr>
</tbody>
</table>

Effect of O₃ fumigation: Plants subjected to a long-term O₃ fumigation did not show any visible foliar injury. So, H. canariensis var. azorica can be regarded as a good tolerant (resistant) plant to O₃ in terms of macroscopic effects. As confirmation, visible injury attributable to O₃ under natural conditions was never reported.

Following the O₃ treatment, Chl a + b content in the green portions decreased significantly while the Chl a/b ratio was not changed (Fig. 1A,B). In the white leaf portions, Chl amounts and ratio were unaffected by O₃ (Fig. 1A,B).

Gas exchange responses to O₃ were very different in the two kinds of leaf. P_N was depressed in the green tissue only at a high O₃ concentration (Fig. 1C) which was related to a strong decrease in gₙ and E (Fig. 1D,E). So, stomatal limitations were certainly involved in reduction of P_N induced by O₃ in the green portions. As confirmation, intercellular CO₂ concentrations were unaffected by O₃ concentration (225±7.3, 239±10.2, and 244±5.9 mm³ m⁻³, respectively, in controls, at 50 and 100 mm³ m⁻³ O₃; these differences were not significant according to the analysis of variance, p > 0.05). No effects of O₃ concentration on gas exchange were observed in the white regions. Here the P_N was negative (Fig. 1C) and the g₂ₙ and E were unaffected by O₃ (Fig. 1D,E). A greater sensitivity of the green portions of Chlorophyllum variegated leaves to another oxidative air pollutant (i.e., sulphur dioxide) has already been also reported by Miszalski (1994).

Neither F₀ nor Fₘ or the Fᵥ/Fₘ ratio changed in green portions following the O₃ exposure (Fig. 1F,G,I). In the white areas, Fᵥ/Fₘ significantly diminished following O₃ exposure as well as the Fᵥ/F₀ ratio (Fig. 1H).

Stomatal limitations were involved in the reduction of photosynthetic activity of green areas of the variegated leaves of H. canariensis var., azorica subjected to long-term exposure to realistic O₃ concentrations. The pollutant induced stomatal closure, and this in turn limited the CO₂ uptake, a phenomenon well-known in phytotoxicology (Unsworth and Black 1981). The observed changes in photosynthetic characteristics of fumigated leaves are consistent with O₃ having in these experimental conditions negligible direct effects on the photosynthetic
performance, as confirmed by the absence of alterations in ChlaF parameters.
Nevertheless, O$_3$ may induce not only direct damages at the stomatal level, but also induce the formation of reactive oxygen substances which may further affect cellular metabolism. This does not affect the leaves uniformly, but restricts itself to defined

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**Fig. 1.** Effects of ozone (50 and 100 mm$^3$ m$^{-3}$ for 28 d, 5 h per d) on chlorophyll (Chl) $a+b$ (A) content [mg m$^{-2}$], and the Chl $a/b$ (B), net photosynthetic rate, $P_N$ [C, mmol(CO$_2$) m$^{-2}$ s$^{-1}$], stomatal conductance, $g_s$ [D, mmol(H$_2$O) m$^{-2}$ s$^{-1}$], transpiration rate, $E$ [E, mmol(H$_2$O) m$^{-2}$ s$^{-1}$], and fluorescence parameters ($F_0$, $F_0$, $F_m$, $F_0/F_m$, $F_v/F_m$, $I$) in the green (closed circles) and the white (open circles) portions of Hedera canariensis var. acerifolia leaves. Vertical bars indicate fiducial limits ($p = 0.05$) (where they do not appear, their value is negligible).

areas where phenomena similar to those observed during senescence are provoked. Oxidation reactions reduce $P_N$ and possibly accelerate cell senescence (Dann and Pell 1989, Greitner et al. 1994). A reduction of photosynthetic activity without changes in the electron transport efficiency is typical of senescence, whereas maximum photosynthetic capacity decreases during senescence (Tichá et al. 1985). This would be expected as a consequence of the loss of components of the photosynthetic apparatus, especially ribulose-1,5-bisphosphate carboxylase/oxygenase (Pell et al. 1992). However, the quantum efficiency of PS2 photochemistry remains constant
during senescence (Jenkins et al. 1981), indicating that the efficiency with which individual PS2 complexes utilize photons for photochemistry does not change. The absence of any decrease in Fψ/Fm also indicates that no net photoinhibitory damage occurs to PS2 reaction centres. Similar results are reported by Nie et al. (1993) for wheat exposed to O3.

A different feature was shown by the white portions exposed to O3. Fψ and Fm did not change, but Fψ/Fm and Fψ/F0 significantly decreased. The value of this ratio was about 0.45, so much lower than the values of 0.80-0.85 considered normal in healthy plants (Björkman and Demmig 1987). Therefore, in the white portions, which emitted CO2, the efficiency of electron transport was affected by O3. The white portions had stomata not properly functioning, i.e., the principal path of O3 entrance should be strongly limited. The particular behaviour of the white portions as far as the Chla/ChlF parameters are concerned, and the limited knowledge about the CO2 fixation ability of non-green tissues do not permit explaining these parameters on the basis of biochemical events in the leaf.

References


PHOTOSYNTHESIS OF HEDERA CANARIENSIS VARIEGATED LEAVES


