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 $mm^3\ m^{-3}$) 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 (Laffray *et al.* 1991, Beerling and Woodward 1995). The aim of this work was to

Received 19 May 1997, *accepted* 23 July 1997.
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Abbreviations: Chl. chlorophyll; Chl*a*F. chlorophyll *a* fluorescence; DMF, *N,N*-dimethylformamide; *E*, transpiration rate; F_m, maximal fluorescence; F_v, variable fluorescence; F_0, ground fluorescence; g_w, stomatal conductance; P_N, net photosynthetic rate; PS, photosystem.
Acknowledgements: This work was supported by MURST (40 % funds) and by CNR (Special Project INGA), Rome (Italy). The authors are indebted to Dr. G. Ligasacchi for taking care of the plants in the fumigation chambers.

investigate the gas exchange and chlorophyll *a* fluorescence (Chl*a*F) parameters in green and white leaf portions of variegated plant, *Hedera canariensis* var. *azorica* L., subjected to realistic O_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_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_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 $mm^3\ m^{-3}$ for 28 d (5 h d^{-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 (Beerling 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_2 concentration, 80 % relative humidity, and 25 °C. Saturating irradiance (800 μmol $m^{-2}\ s^{-1}$) 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 Chl*a*F 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 $(F_m - F_0)/F_m = F_v/F_m$, where F_0 is the initial level of Chl*a*F, and F_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,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 mm^2, 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(CO_2) m^{-2} s^{-1}, whereas the white leaf tissue did not show any net uptake of CO_2, and had lower values of both stomatal conductance (g_w) and transpiration rate (E) (-85 %) than the green regions. Apparently, stomata in the white areas did not function properly. In variegated *H. helix*, g_w 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(CO_2) m^{-2} s^{-1}], stomatal conductance (g_w), and transpiration rate (E) [mmol(H_2O) 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(CO_2) mol^{-1}, and 21 % O_2. 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 ($p<0.001$).

Leaf portion	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*/*b*	P_N	g_w	*E*
Green	200.8	62.9	263.7	3.19	4.54	91	1.85
White	2.8	3.0	5.8	0.93	-0.80	13	0.26

The Chl*a*F parameters were different in green and white portions (Table 2). In the white areas, F_0 and F_m were very low (about -95 % in comparison with the green tissue), probably as a consequence of a very low Chl content. Usually, F_0 and F_m 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 F_v/F_m ratio reached values considered normal for healthy plants (Björkman and Demmig 1987). This indicated that the electron transport efficiency around PS2 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 CO_2 balance is negative (respiration

prevails). Evidence indicates that a high dark CO_2 fixation *via* phosphoenolpyruvate carboxylase (PEPC) is induced in organs where photosynthesis is less efficient than respiration, *i.e.*, at a net loss of CO_2 (Hedley and Rowland 1975, Soldatini *et al.* 1982). Plomann and Eschrich (1990) report that the white areas of variegated *Coleus* leaves fix CO_2 *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 = $p>0.05$).

Leaf portion	F_0	F_m	F_v	F_v/F_m	F_v/F_0
Green	125	511	386	0.754	3.88
White	9	26	17	0.657	1.88
p	**	***	***	NS	***

Effect of O_3 fumigation: Plants subjected to a long-term O_3 fumigation did not show any visible foliar injury. So, *H. canariensis* var. *azorica* can be regarded as a good tolerant (resistant) plant to O_3 in terms of macroscopic effects. As confirmation, visible injury attributable to O_3 under natural conditions was never reported.

Following the O_3 treatment, Chl *a* + *b* content in the green portions decreased significantly while the Chl *a/b* ratio was not changed (Fig. 1*A,B*). In the white leaf portions, Chl amounts and ratio were unaffected by O_3 (Fig. 1*A,B*).

Gas exchange responses to O_3 were very different in the two kinds of leaf. P_N was depressed in the green tissue only at a high O_3 concentration (Fig. 1*C*) which was related to a strong decrease in g_w and *E* (Fig. 1*D,E*). So, stomatal limitations were certainly involved in reduction of P_N induced by O_3 in the green portions. As confirmation, intercellular CO_2 concentrations were unaffected by O_3 concentration (225±7.3, 239±10.2, and 244±5.9 $mm^3\ m^{-3}$, respectively, in controls, at 50 and 100 $mm^3\ m^{-3}$ O_3: these differences were not significant according to the analysis of variance, $p > 0.05$). No effects of O_3 concentration on gas exchange were observed in the white regions. Here the P_N was negative (Fig. 1*C*) and the g_w and *E* were unaffected by O_3 (Fig. 1*D,E*). A greater sensitivity of the green portions of *Chlorophytum* variegated leaves to another oxidative air pollutant (*i.e.*, sulphur dioxide) has already been also reported by Miszalski (1994).

Neither F_0 nor F_m or the F_v/F_m ratio changed in green portions following the O_3 exposure (Fig. 1*F,G,I*). In the white areas, F_v/F_m significantly diminished following O_3 exposure as well as the F_v/F_0 ratio (Fig. 1*H*).

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 O_3 concentrations. The pollutant induced stomatal closure, and this in turn limited the CO_2 uptake, a phenomenon well-known in phytotoxicology (Unsworth and Black 1981). The observed changes in photosynthetic characteristics of fumigated leaves are consistent with O_3 having in these experimental conditions negligible direct effects on the photosynthetic

performance, as confirmed by the absence of alterations in Chl*a*F 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

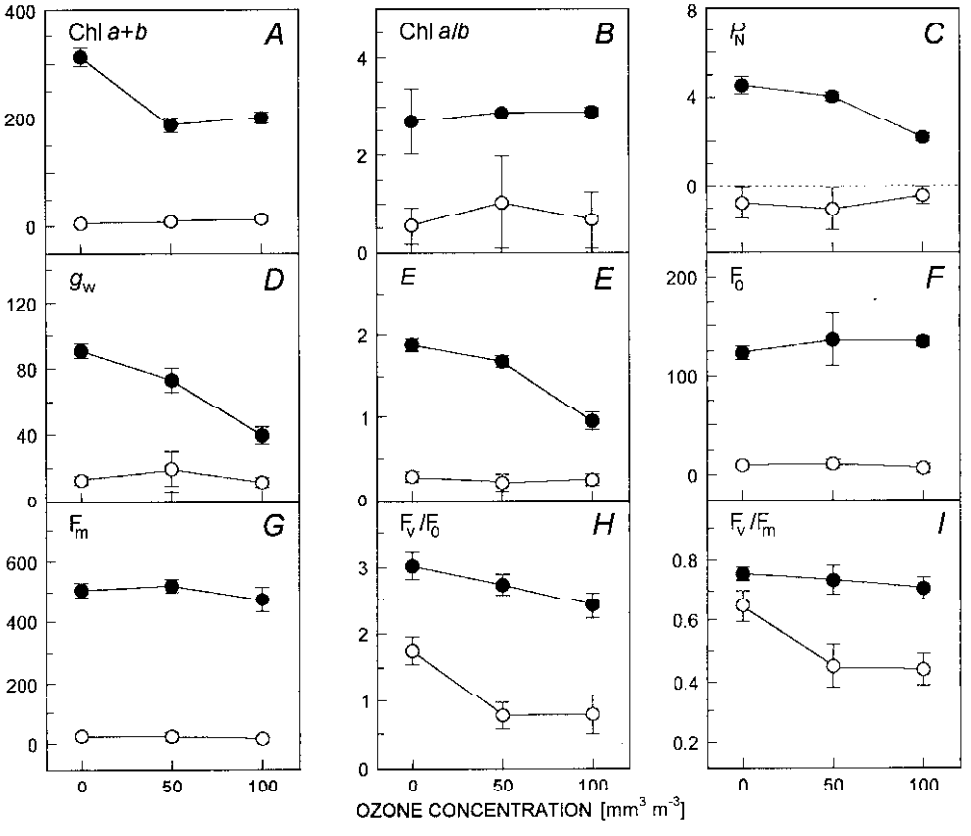

Fig. 1. Effects of ozone (50 and 100 mm^3 m^{-3} for 28 d, 5 h per d) on chlorophyll (Chl) *a* + *b* (*A*) content [mg m^{-2}], and the Chl *a*/*b* (*B*), net photosynthetic rate, P_N [*C*, μmol(CO_2) m^{-2} s^{-1}], stomatal conductance, g_w [*B*, mmol(H_2O) m^{-2} s^{-1}], transpiration rate, *E* [*E*, mmol(H_2O) m^{-2} s^{-1}], and fluorescence parameters (F_0, *F*; F_m, *G*; F_v/F_0, *H*; F_v/F_m, *I*) in the green (*closed circles*) and the white (*open circles*) portions of *Hedera canariensis* var. *azorica* 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 F_v/F_m 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 O_3.

A different feature was shown by the white portions exposed to O_3. F_0 and F_m did not change, but F_v/F_m and F_v/F_0 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 CO_2, the efficiency of electron transport was affected by O_3. The white portions had stomata not properly functioning, *i.e.*, the principal path of O_3 entrance should be strongly limited. The particular behaviour of the white portions as far as the Chl*a*F parameters are concerned, and the limited knowledge about the CO_2 fixation ability of non-green tissues do not permit explaining these parameters on the basis of biochemical events in the leaf.

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