Phyton (Austria) Special issue: "Global change"	**Vol. 42**	Fasc. 3	(149)-(155)	1.10.2002

Photosynthetic Behavior of Two Italian Clones of European Beech (*Fagus sylvatica* L.) Exposed to Ozone

By

E. PAOLETTI[1)], C. NALI[2)] & G. LORENZINI[2)]

Key words: Carboxylation efficiency, chlorophyll a fluorescence, leaf greenness, electron transport rate, leaf gas exchange, oxidative stress.

Summary

PAOLETTI E., NALI C. & LORENZINI G. 2002. Photosynthetic behavior of two Italian clones of European beech (*Fagus sylvatica* L.) exposed to ozone. - Phyton (Horn, Austria) 42 (3): (149)-(155).

Two *Fagus sylvatica* clones, one from central and the other from southern Italy, were exposed to charcoal-filtered or ozone-enriched (150 ppb, 5 h d^{-1}) air for 14 days. Leaf gas exchange, chlorophyll a fluorescence, leaf greenness and maximum RuBP-saturated rate of carboxylation have been measured. Even though the two clones came from sites with different environmental features, their photosynthetic performances were similarly depressed by fumigation, apart from stomatal conductance. An uncoupling between stomatal conductance and carbon assimilation was assumed to take place. Mesophyllic limitations were likely to be the most limiting factor. Photosynthetic light reactions (i.e. apparent electron transport rate) responded to ozone earlier than dark reactions (i.e. maximum RuBP-saturated rate of carboxylation).

Introduction

Contrasting results have been reported concerning the effects of ozone (O_3) on European beech (*Fagus sylvatica* L.), one of the most important broad-leaved tree species in central and Western Europe (see SANDERMANN & al. 1997 for a review).

[1)] Istituto Protezione Piante, Consiglio Nazionale Ricerche, Piazzale Cascine 28 I-50144 Firenze, Italy.

[2)] Dipartimento "Giovanni Scaramuzzi", Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy.

European beech distribution area extends south in the Mediterranean basin. Southern populations show leaf sclerophylly and high tannin content (BUSSOTTI & FERRETTI 1998, GROSSONI & al. 1998), high potential for drougth acclimation (GARCÍA-PLAZAOLA & BECERRIL 2000) and low susceptibility to photoinhibitory damage (TOGNETTI & al. 1998). PALUDAN-MÜLLER & al. 1999 investigated long-term responses to low levels of O_3 of seedlings of 12 European beech provenances, and found that northwest provenances were more sensitive than southeast ones in terms of leaf gas exchange and growth parameters.

The purpose of this study was to analyze how two European beech clones from Central and Southern Italy respond to O_3 in terms of photosynthetic performance. A previous work (NALI & PAOLETTI 2002) reported that these two clones differed in the background levels of some physiological and biochemical parameters. These differences could be interpreted as a result of the adaptation of each clone to its environment.

Material and Methods

Seeds were collected from two beech forests with contrasting climatic conditions: Abetone (Tuscany, Central Italy; altitude 1,200 m a.s.l., annual rainfall 2,568 mm, annual average temperature 6.9°C) and Nebrodi mountains (Sicily, Southern Italy; altitude 1,250 m a.s.l., annual rainfall 1,467 mm, annual average temperature 10.8°C). Ramets from five-years-old seedlings were selfrooted in the greenhouse of the Niedersächsische Forstliche Versuchsanstalt (Staufenberg-Escherode, Germany). As after-rooting survival was low, two years later only one clone per provenance (clone C from Central Italy and clone S from Southern Italy) was available for the experiment. Twelve even-sized plants from each clone were moved to a growth chamber at 20 ± 1°C, 85 ± 5 % R.H. and 500 μmol m^{-2} s^{-1} photon flux density at plant height during a 12 h photoperiod. Plants were allowed to acclimatize for one week and then exposed to O_3.

Exposures to O_3 were carried out during the summer of 2000 in a controlled environment fumigation apparatus (LORENZINI & al. 1994), ventilated with charcoal-filtered air. Ozone was generated by a Fischer 500 (Zurich, Switzerland) supplied with pure O_2. Its concentration was continuously monitored using a PC-controlled photometric analyzer (Monitor Labs mod. 8810, San Diego, CA, USA). The exposure regime was 150 ppb (for O_3, 1 ppb = 1.96 μg m^{-3} at 20° C and 101.325 kPa) for 14 days (5 h d^{-1} from 09:00 to 14:00, solar time) in form of a square wave. The "Accumulated exposures Over the Threshold of 40 ppb" (the so-called "AOT40", sensu DE LEEUW & VAN ZANTVOORT 1997) was 7700 ppb.h. Control plants were maintained in charcoal-filtered air. Environmental conditions were the same as in the growth chamber. Analyses were performed after 14 days of exposure on a minimum of three well-watered plants per treatment (three mature leaves per plant).

Measurements of leaf gas exchange were carried out at noon, using O_3-free air, by an infra-red gas-analyzer (CIRAS-1 PP-Systems, Herts, UK) equipped with a Parkinson leaf chamber that controlled leaf temperature (25°C), relative humidity (80 %), light (800 μmol m^{-2} s^{-1} PAR) and CO_2 concentration (350 ppm). Stomatal conductance to water vapor (Gw) and apparent internal CO_2 concentration (Ci) were calculated according to VON CAEMMERER & FARQUHAR 1981 and related to one-sided leaf areas. For CO_2 response curves, photosynthetic rates were measured over a range of CO_2 concentrations (100-2000 ppm) under saturating PAR (1200 μmol m^{-2} s^{-1}). Parameter V_{cmax} (μmol m^{-2} s^{-1}), i.e.the maximum RuBP-saturated rate of carboxylation, was estimated according to FARQUHAR & al. 1980. V_{cmax} is positively correlated to carboxylation efficiency (CE), as calculated on the initial slope of the curve (FARQUHAR & VON CAEMMERER 1982).

Chlorophyll *a* fluorescence of photosystem II (PSII) was measured on the leaf adaxial surface of interveinal regions, using a fluorometer (FIM-1500, ADC, Herts, UK). Just before the measurement the leaves were dark-adapted for 40 min. In this state, initial fluorescence (F_0), when all PSII reaction centers were open, was measured under a low ambient background light. Maximum fluorescence (F_m), when all PSII reaction centers were closed, was measured by applying a saturating light pulse (5 s duration). Apparent electron transport rates through PSII (ETR) were estimated as $(F_m-F_0)/F_m$ * PPFD * a * f, assuming an absorptivity a of European beech leaves in photosynthetic active radiation of 0.84 and a light distribution factor between PSI and PSII, f, of 0.5 (KRALL & EDWARDS 1992).

Leaf greenness was measured using a SPAD-502 Chlorophyll Meter (Minolta, Osaka, Japan) (MANETAS & al. 1998). Three SPAD readings were averaged for each leaf to represent one observation.

Two-factor analysis of variance (ANOVAs) was used to assess which factors significantly influenced each variable (Tukey's HSD test). Two-factors ANOVA followed a collective multivariate analysis (MANOVA) for the not-correlated variables in Table 1 ($r^2 < 0.3$). A linear regression between ETR and Pnet was applied to the mean values per each plant.

Results and Discussion

After 14 days of treatment with O_3, no visible symptom of leaf injury was apparent. Both clones responded to O_3 exposure by decreasing net photosynthesis (Pnet) (-35.2 and -45.5 % in C and S, respectively, Table 1) and increasing Ci (+26.4 and +19.8 % in C and S, respectively, Table 2). Ci and Gw had higher values in clone S. In S, Gw was not affected by O_3, while a strong increase (+51.9 %) was recorded in C. LEONARDI & al. 1990 observed that European beech showed a transient increase in Gw and a persistent decrease in CO_2 assimilation in response to near-ambient O_3 levels. The "ozone x clone" interaction was significant also for Pnet and Ci.

Table 1. Two-factor ANOVA of ozone treatment and clonal variation on the not-correlated parameters of the two European beech clones (MANOVA-derived). Pnet = net photosynthesis (µmol CO_2 m^{-2} s^{-1}); Gw = stomatal conductance to water vapor (mmol H_2O m^{-2} s^{-1}); Chl = leaf greenness (arbitrary units). Values represent the mean (±SD). Asterisks indicate: * = P≤0.05, ** = P≤0.01, *** = P≤0.001; ns = P>0.05.

Clone	Treatment	Pnet	Gw	Chl
C	$-O_3$	7.1 ± 0.98	133 ± 21.6	30.7 ± 4.32
	$+O_3$	4.6 ± 1.43	202 ± 62.3	28.0 ± 4.35
S	$-O_3$	10.1 ± 1.94	186 ± 20.6	33.9 ± 3.98
	$+O_3$	5.5 ± 0.77	182 ± 40.4	34.5 ± 3.47
Ozone		***	***	ns
Clone		ns	*	**
Ozone x clone		*	***	ns

Both our clones did not suffer any significant alteration in leaf greenness after O_3 exposure, even if the clonal variation showed higher values in clone S (+10.4 %, Table 1). In both clones, O_3 did not significantly decrease V_{cmax} (Table

2), which represents the activity and/or amount of Rubisco during the photosynthetic dark reactions. This result contrasts with findings by BORTIER & al. 2000 and LIPPERT & al. 1996, who observed a significant reduction in V_{cmax} and CE caused by long-term exposure to O_3 of European beech. A decrease in CO_2 uptake was correlated to a decrease in CE for poplar as well (PELL & al. 1994), while FARAGE 1996 and FOSTER & al. 1990 did not find any effect on CE for *Quercus robur*. FARAGE & LONG 1999 reported that chronic O_3 fumigation did not affect the RuBP regeneration capacity of wheat and pea and the decreased CO_2 uptake was essentially due to a decrease in the amount of active Rubisco.

Table 2. Two-factor ANOVA of ozone treatment and clonal variation on apparent internal CO_2 concentration (Ci, ppm), maximum RuBP-saturated rate of carboxylation (V_{cmax}, µmol m^{-2} s^{-1}), maximal fluorescence of chlorophyll a (F_m, arbitrary units) and apparent electron transport rate through PSII (ETR, µmol m^{-2} s^{-1}) of the two European beech clones. Values represent the mean (±SD). Asterisks indicate: * = P≤0.05, ** = P≤0.01, *** = P≤0.001; ns = P>0.05.

Clone	Treatment	Ci	V_{cmax}	ETR	F_m
C	$-O_3$	220 ± 15.8	29.8 ± 0.98	273 ± 3.1	3740 ± 92
	$+O_3$	278 ± 15.2	26.0 ± 1.56	255 ± 11.9	3134 ± 252
S	$-O_3$	217 ± 15.1	29.3 ± 2.86	275 ± 2.5	3881 ± 124
	$+O_3$	260 ± 15.4	23.9 ± 8.41	263 ± 6.4	3453 ± 321
Ozone		***	ns	***	***
Clone		**	ns	*	ns
Ozone x clone		*	ns	ns	ns

CO_2 fixation acts as a major sink for the reducing equivalents and energy generated by the primary photochemical reactions (i.e. ATP and NADPH). The decreased CO_2 assimilation capacity observed in this study will cause ATP and NADPH demand to decrease in chloroplasts, and will lead to redox back pressure on PSII. In order to match the decreased ATP and NADPH demand by carbon metabolism in ozonated leaves, the primary photochemical reactions of PSII should be modified to down-regulate the linear photosynthetic electron transport. The results obtained in this experiment show a significant O_3-induced effect on ETR (Table 2), a photosynthetic light parameter, whose decrease may be due to photoinhibition. In fact, the decreased F_m (Table 2) is interpreted as a transformation of PSII reaction centers to fluorescence quenchers, i.e. to dissipators of excitation energy, without a decrease in trapping efficiency (SOMERSALO & KRAUSE 1990).

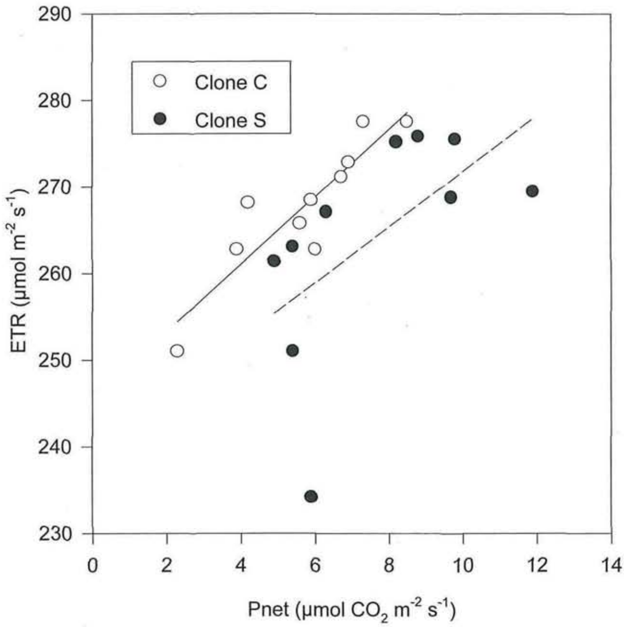

Fig. 1. Relationship between apparent electron transport rate through PSII (ETR) and net photosynthesis (Pnet) in the two clones of *Fagus sylvatica* after ozone exposure. Clone C: $y = 245.5 + 3.9x$, $r^2 = 0.80$; clone S: $y = 239.6 + 3.2x$, $r^2 = 0.38$.

The linear correlation between ETR and Ci was not significant, but showed positive and similar slopes in controls and negative slopes in the treated individuals (data not shown). A progressive lowering of slopes in European beech seedlings treated with O_3 has been described by GRAMS & MATYSSEK 1999. The significant correlation between ETR and Pnet in both clones (Fig. 1), suggests that the O_3-induced effects were already well established (GRAMS & MATYSSEK 1999).

Although the two clones investigated come from areas quite different in terms of environmental features, their photosynthetic performances did not show marked differential variations in response to a realistic O_3 fumigation, apart from an increase in Gw in clone C. In this case, it can be assumed that an uncoupling between Gw and Pnet took place; this phenomenon has been reported for other O_3-exposed woody species (TJOELKER & al. 1995). Ci increase and ETR decrease, lacking changes in the chlorophyll content (i.e. leaf greenness), were likely to be related to a mesophyllic limitation in CO_2 fixation under O_3 exposure (BORTIER & al. 2000). Photosynthetic light reactions (i.e. apparent electron transport rate) responded to O_3 earlier than dark reactions (i.e. maximum RuBP-saturated rate of carboxylation). The ecophysiological implications of these aspects are worthy of further investigations, which should also focus on different O_3 pollution scenarios.

Acknowledgments

The authors would like to thank Prof. J. KLEINSCHMIT (Niedersächsische Forstliche Versuchsanstalt, Staufenberg-Escherode, Germany) for providing the clones, Miss Katja LAAKSO (University of Oulu, Finland) for her assistance with sampling and measurements, and Dr. Filippo BUSSOTTI (University of Florence, Italy) for the loan of the fluorescence equipment.

References

BORTIER K., CEULEMANS R. & De TEMMERMAN L. 2000. Effects of ozone exposure on growth and photosynthesis of beech seedlings (*Fagus sylvatica*). - New Phytol. 146: 271 - 280.

BUSSOTTI F. & FERRETTI M. 1998. Air pollution, forest condition and forest decline in southern Europe: an overview. - Environ. Pollut. 101: 49 - 65.

DE LEEUW F. A. A. M & VAN ZANTVOORT E. D. G. 1997. Mapping of exceedances of ozone critical levels for crops and forest trees in the Netherlands: preliminary results. - Environ. Pollut. 96: 89 - 98.

FARAGE P. K. 1996. The effect of ozone fumigation over one season on photosynthetic processes of *Quercus robur* seedlings. - New. Phytol. 134: 279 - 285.

— & LONG S. P. 1999. The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: an in vivo analysis. - Photosynth. Res. 59: 1 - 7.

FARHQUAR G. D. & VON CAEMMERER S. 1982. Modelling of photosynthetic response to environmental conditions. - In: LANGE O. L., NOBEL P. S., OSMOND C. B. & ZIEGLER H. (Eds.), Encyclopedia of plant physiology, new Series. Vol. 12B Physiological plant ecology II, pp. 549 - 587. - Springer Verlag, Berlin.

— , — & BERRY J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. - Planta 149: 78 - 90.

FOSTER J. R., LOATS K. V. & JENSEN K. F. 1990. Influence of two growing seasons of experimental ozone fumigation on photosynthetic characteristics of white oak seedlings. - Environ. Pollut. 65: 371 - 380.

GARCÍA-PLAZAOLA J. I. & BECERRIL J. M. 2000. Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. - Trees 14: 485 - 490.

GRAMS T. E. E. & MATYSSEK R. 1999. Elevated CO_2 counteracts the limitation by chronic ozone exposure on photosynthesis in *Fagus sylvatica* L.: comparison between chlorophyll fluorescence and leaf gas exchange. - Phyton 39: 31 - 40.

GROSSONI P., BUSSOTTI F., TANI C., GRAVANO E., SANTARELLI S. & BOTTACCI A. 1998. Morpho-anatomical alterations in leaves of *Fagus sylvatica* L. and *Quercus ilex* L. in different environmental stress conditions. - Chemosphere 36: 919 - 924.

KRALL J. P. & EDWARDS G. E. 1992. Relationship between photosystem II activity and CO_2 fixation. - Physiol. Plant. 86: 180 - 187.

LEONARDI S., LANGEBARTELS C. & SANDERMANN H. 1990. Fall exposure of beech trees (*Fagus sylvatica* L.) to ozone and simulated acidic mist: immediate and post-treatment effects on whole plant physiology. - In: PAYER H. D., PFIRRMANN T. & MATHY P. (Eds.), Environmental research with plants in closed chambers, air pollution research reports, vol. 26, pp. 369 - 380. - CEC, Brussels.

LIPPERT M., STEINER K., PAYER H. D., SIMONS S., LANGEBARTELS C. & SANDERMANN H. 1996. Assessing the impact of ozone on photosynthesis of European beech (*Fagus sylvatica* L.) in environmental chambers. - Trees 10: 268 - 275.

LORENZINI G., MEDEGHINI BONATTI P., NALI C. & BARONI FORNASIERO R. 1994. The protective effect of rust infection against ozone, sulphur dioxide and paraquat toxicity symptoms in broad bean. - Physiol. Mol. Plant Path. 45: 263 - 279.

MANETAS Y., GRAMMATIKOPOULUS G. & KYPARISSIS A. 1998. The use of the portable, non-destructive, SPAD-502 (Minolta) chlorophyll meter with leaves of varying trichome density and anthocyanin content. - J. Plant Physiol. 153: 513 - 516.

NALI C. & PAOLETTI E. 2002. Risposta differenziale all'ozono in cloni di pioppo e faggio. - Inf. Fitopat. 52: 27 - 30.

PALUDAN-MÜLLER G., SAXE H. & LEVERENZ J. W. 1999. Responses to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry-matter partitioning. - New Phytol. 144: 261 - 273.

PELL E. J., ECKARDT N. A. & GLICK R. E. 1994. Biochemical and molecular basis for impairment of photosynthetic potential. - Photosynth. Res. 39: 453 - 462.

SANDERMANN H., WELLBURN A. R., & HEATH R. L. 1997. Forest decline and ozone. - Ecological Studies Vol. 127, pp. 400. - Springer, Berlin.

SOMERSALO S. & KRAUSE G.H. 1990. Reversible photoinhibition of unhardened and cold acclimated spinach leaves at chilling temperature. - Planta 180: 181 - 187.

TJOELKER M. G., VOLING J. C., OLEKSYN J. & REICH P. B. 1995. Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. - Plant Cell Environ. 18: 895 - 905.

TOGNETTI R., MINOTTA G., PINZAUTI S., MICHELOZZI M. & BORGHETTI M. 1998. Acclimation to changing light conditions of long-term shade-grown beech (*Fagus sylvatica* L.) seedlings of different geographic origins. - Trees 12: 326 - 333.

VON CAEMMERER S. & FARQUHAR G. H. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta 153: 376 - 387.