

Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview

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Abstract

Tropospheric ozone exercises pressure on vegetation in combination with other oxidative factors such as strong sunlight, UV-B radiation, high temperatures and water shortage. The relationship established between these factors and the plants can be either antagonistic (cross-resistance) or synergistic. Response mechanisms are activated involving physiological, chemical and morphological features. Overall sensitivity (or resistance) is determined by the amount of total oxidative pressure in relation to the plant's physiological plasticity [i.e. its ability (within its own resilience potential) to alter its structure and functions in order to react to the oxidative pressure]. Within different populations of the same species, functional leaf traits (leaf area and thickness, leaf mass per area or specific leaf area, tissue thickness) may vary in relation to the extent that the plant succeeds in acclimating to ambient oxidative pressure. The behaviour of a tree in an oxidative environment can be interpreted by means of leaf structure analysis. Leaves presenting high tissue density (and thus low intercellular space content) display a high degree of acclimation to stressors, react little to environmental changes and are characterized by remarkable longevity. Leaves of this type also have a high photosynthesis capacity per surface unit (due to the N content per surface unit) and a high P_N/G_W (or water use efficiency, WUE). Thus, they are able to support detoxification processes. These morphological traits are to be found in adult plants in late successional stands and, above all, in ambients that have already been subjected to oxidative pressure. However, in early successional species and during the dynamic stages of growth (e.g. in the renewal occurring along forest edges), it is the opposite leaf traits that prevail: low leaf density, high photosynthesis capacity per dry weight unit, low WUE, low leaf longevity. These traits make plants far more reactive to environmental changes (e.g. they exploit the light from sun flecks much more effectively), but enable them to achieve only a low degree of acclimation and a poor ability to support detoxification processes. Whereas adult forests display a high level of ecological resilience and have a relatively good tolerance of ozone, the renewal stages are (at least potentially) more vulnerable. In these environments, ozone can alter the competition among genotypes and favour more resistant ones. Among the sectors most at risk, we must include communities growing at the edge of their ecological range, for whom even a slight increase in oxidative pressure can trigger substantial degenerative processes.

Keywords: acclimation, functional groups, functional leaf traits, oxidative stress, ozone, stress resilience

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Introduction

Plant communities have always been subjected to oxidative stresses, such as high sunlight radiation, extreme

temperatures, water and nutrient shortage, and/or parasite infestations. These factors have determined the differentiation of vegetation types, with different species adapting in different ways, according to the needs imposed by limiting environmental conditions. Acclimation to stress processes may differentiate similar (i.e. same species) plant communities in different

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ecological conditions, or even different parts of the same individual (e.g. sun leaves vs. shade leaves). Resistance derives from the very nature of the reactions triggered in the subcellular region by oxidative stresses, and especially by peroxidases, favouring the formation of compounds improving mechanical and defence performance, such as lignins and phenolic compounds (Passardi *et al.*, 2004, 2005). Plants react to stress with morphological changes ('stress induces morphogenic responses', SIMR; Potters *et al.*, 2007) that are part of a more general acclimation strategy. These responses are consequences of an altered phytohormone transport and/or metabolism.

Over the past decades, alongside oxidative stresses of natural origin, plants have also been affected by anthropogenic factors, such as increased tropospheric ozone (O₃) concentrations and increased UV-B radiations (Krupa, 2000). The impact of additional anthropogenic stress factors depends on the defences that the plants have already enacted to respond to prevailing environmental conditions. The overall effect of anthropogenic stresses may prevail over the effect of natural stressors, or it may be cancelled out within a complicated cross-resistance network (Mittler, 2006), or it may even be decisive in superceding the plant's resilience threshold, if the plant is already under extreme ecological conditions.

Today, in attempting to protect natural environments, the main problem researchers must address is evaluating an ecosystem's overall resilience to oxidative stresses within the ecological fluctuations of an environment that is constantly undergoing changes. For example, it is commonly stated that factors limiting stomatal aperture (i.e. increased drought) 'protect' plants from the effects of ozone (Bytnerowicz *et al.*, 2007). Yet, under these conditions, photosynthesis is reduced and plants are no longer capable of performing the metabolic functions necessary to fuel defence and detoxification processes. Thus, drought not only does not act as a protection factor, but it may actually increase the overall oxidative risk as well (Tausz *et al.*, 2007). However, some plant species living in stressful environments (as *Quercus ilex* L. in the Mediterranean region) are able to cope with oxidative pressure (high temperatures, drought, ozone and their interactive actions), thanks to foliar monoterpenes emissions, which act as antioxidant (Peñuelas & Llusià, 2002; Peñuelas *et al.*, 2005; Velikova *et al.*, 2005).

The purpose of this paper is to analyse the relationships between natural origin oxidative stresses and anthropogenic ones (primarily tropospheric ozone), as well as acclimation processes in plants. The thesis I uphold is that in mature forest ecosystems one should not expect to find very marked effects resulting from a moderate increase in oxidative stress. This intrinsic resilience is greater where environmental factors have

favoured the development of a well-adapted community (such as Mediterranean environments) or have stimulated adaptation responses in select populations.

Interactive oxidative stress, antagonistic (cross-resistance) and synergistic effects

Many oxidative stress factors occur simultaneously. For example, high temperatures and high sunlight radiations are self-correlated, and can induce water stress and the formation of tropospheric ozone. The molecular responses to different kinds of stresses are similar (Sandermann *et al.*, 1998), and defence mechanisms to a stress factor are usually capable of inducing resistance to other stressors as well. This process is usually referred to as 'cross-resistance' (see Alscher *et al.*, 1997; Pastori & Foyer, 2002; Mittler, 2006).

A typical example of a coordinated response to simultaneous stressors is the thermostability of photosystem II (PS II) under conditions of water stress (Havaux, 1992; Lu & Zhang, 1999). Heat treatment, which causes a complete and irreversible inhibition of PS II in well-watered leaves, resulted in a small and fully reversible reduction of the photochemical efficiency of PS II in drought-stressed leaves.

Cross-resistance can be the result of morphological changes. This kind of change includes sclerophylly (due to drought, nutritional shortages, etc.) that increases a plant's resistance (Mittler, 2006). There is also a physiological kind of cross-resistance (e.g. avoidance strategy, involving stomatal closure, enacted to react to water shortage which reduces the uptake of pollutants through the stomata, Emberson *et al.*, 2000, and thus reduces the damage those pollutants can cause, Schaub *et al.*, 2003); and biochemical cross-resistance (according to Selote & Khanna-Chopra, 2006, acclimation to drought endows corn seedlings with a tolerance to oxidative stress inducing antioxidant cellular and subcellular defence mechanisms). After a chronic ozone exposure of the ozone-tolerant Mediterranean shrub *Phyllirea latifolia* L., Paolacci *et al.* (2007) also detected the induction of some gene sequences induced by salt, drought and low temperature, as well as encoding pathogenesis-related proteins. This supports the hypothesis of convergent molecular response of plants to stress of different origins: plants growing in the Mediterranean area are exposed to recurrent stress factors, which may promote adaptive mechanisms of tolerance to oxidative stresses.

Examples of cross-resistance can be found in reaction to high sunlight radiations, UV-B and ozone. High sunlight exposure induces a high level of antioxidants in the leaves (García-Plazaola *et al.*, 2004) and guides morphogenetic processes leading to tissue thickening

and to the production of screening and antioxidant pigments (Neill & Gould, 2003): these are all factors that can affect overall behaviour (sensitivity/resistance) towards ozone. The increased sensitivity of shade leaves towards ozone (Wei *et al.*, 2004a,b) can be explained in terms of their morphological and functional traits, such as reduced leaf thickness (LT) and density, as well as wide intercellular spaces and a high quantity of antenna pigments (Walters, 2005), which are a target for ozone action. Schnitzler *et al.* (1999) have detected a mitigation of negative effects (visible symptoms and photosynthesis) in *Picea abies* (L.) Karst. and *Pinus sylvestris* L. subjected to ozone and UV-B, and the same observations were made by Hao *et al.* (2000) on *Lycopersicum esculentum* Mill.

Other studies have reported cases in which the combined action of oxidative stresses can exacerbate the overall effect, such as the combination of ozone and drought (see e.g. Alonso *et al.*, 2001, 2005; Grulke *et al.*, 2002; McLaughlin & Downing, 2002; Tausz *et al.*, 2007). Several explanations have been suggested for this behaviour, including an excessive accumulation of reactive oxygen species (ROS), due to the concomitant action of the two stressors (Tausz *et al.*, 2007), and an altered stomatal function, due to ozone. Maier-Maercker (1989) states that ozone is responsible for the lignification of stomatal guard cells, which, she suggests, are no longer capable of closing normally, thus favouring the outflow of water vapour. Pearson & Mansfield (1993), investigating *Fagus sylvatica* L., came to similar conclusions, highlighting the stomatal malfunction caused by ozone, which occurs under conditions of water stress. Paoletti (2005a) has taken this hypothesis further in investigations on the Mediterranean shrub *Arbutus unedo* L., suggesting that stomatal malfunction not only prevents the normal night-time water re-load, but also exposes plants to the risk of high nocturnal concentrations of ozone (Matyssek *et al.*, 1995; Grulke *et al.*, 2004).

Environmental stress and functional leaf traits

The adaptation that plants develop in relation to the environmental conditions of a site is expressed, among other things, through their leaf morphology (functional leaf traits). In environments characterized by high oxidative pressure (low rainfall and dry soil, strong sunlight, extreme temperatures, ozone pollution), one finds a prevalence of plants with reduced leaf surface, high LT and elevated mesophyll tissue density (Gutschick, 1999).

Usually, the degree of the plant's acclimation to its environment is expressed through the sclerophylly index LMA (leaf mass per area, dry weight per unit of leaf area, cf. Cowling & Campbell, 1983), where sclerophylly begins at an LMA value $>7.5 \text{ mg cm}^{-2}$. Its opposite

(SLA, specific leaf area) expresses the extent of leaf surface per unit of dry weight. High values of LMA (or, conversely, low values of SLA) can be a consequence of high density of mesophyll tissues (LD, leaf density; or DMC, dry matter concentration, expressed as dry weight per unit of leaf volume), or of elevated LT. This can lead to misinterpretations of the ecological significance of LMA, because LD and LT have different ecological meanings (Witowski & Lamont, 1994; Niinemets, 1999; Wilson *et al.*, 1999). In other words, LMA and SLA are not suitable parameters to use in comparing the ecological behaviour of different species, although within a single species LT and LD variations are often correlated (Bussotti *et al.*, 2005b). The findings referred to in this paper, however, are to be considered in relation to the parameters used by the authors of the papers mentioned.

LMA variations are linked to biomass allocation strategies (Wilson *et al.*, 1999) and to photosynthesis acclimation under different ecological conditions. Leaves with high LMA have a greater nitrogen content per surface unit; as a result, they have a greater photosynthesis potential (Niinemets, 1999). However, the content of nitrogen and photosynthesis capacity per dry weight unit is lowered (Reich *et al.*, 1998; Niinemets, 1999). LD correlates negatively to nitrogen concentration (Bussotti *et al.*, 2005b). This indicates that, in plants with dense leaves, the apoplastic fraction is increased. Conversely, leaves with low LMA react more readily to variations in environmental factors (Oguchi *et al.*, 2003). In the case of adaptation to water stress, a high LMA, combined with a reduced leaf surface, enables the plant to exploit more efficiently brief seasonal periods favouring photosynthesis activity, at the same time limiting the loss of water through transpiration (sclerophyllous species are more conservative in their water use than other functional groups, see Mediavilla *et al.*, 2001; Mediavilla & Escudero, 2003).

In the field, LMA variations according to ecological gradients have been observed and described in different species: Bauer *et al.* (1997) and Bussotti *et al.* (2005b) in *F. sylvatica*; Roderick *et al.* (2000) in *Eucalyptus* sp. pl.; Bruschi *et al.* (2003) in *Quercus petraea*; Gratani *et al.* (2003) in *Q. ilex*. In all cases, LMA increased in conditions of greatest water shortage, as well as under worsening environmental conditions. The differences in LMA persisted even when various ecotypes were cultivated in the same environment [*Q. ilex*: Gratani *et al.*, 2003; *Q. petraea* (Matt.) Liebl.: P. Bruschi, personal communication], thus suggesting that this trait is genetically established. At global level (Niinemets, 2001), LMA and LT values are higher in sites with the greatest R (daily annual mean global solar radiation) and mean temperature; these values correlate negatively (although

the correlation is weak) with rainfall. LD correlates negatively only with rainfall.

Enhanced UV-B radiations (Jansen, 2002; Gitz & Liu-Gitz, 2003) and tropospheric ozone are also important morphogenetic factors. Pääkkönen *et al.* (1997, 1998) have shown that *Betula pendula* Roth. leaves sprouted and grown in high ozone concentration ambients have a greater lamina thickness and a higher stomatal density than leaves grown in sites with filtered air. Ribas *et al.* (2005) have found, in *Ceratonia siliqua* L. exposed to an ozone-enriched atmosphere in experimental conditions in open-top chambers (OTC), an increase in LT and LMA. Bennett *et al.* (1992) and Ferdinand *et al.* (2000) have found that *Prunus serotina* Ehrh. and *Fraxinus pennsylvanica* Marsh. genotypes with elevated LT display a greater resistance to ozone than do the genotypes of the same species with thinner leaves. A strict correlation between ambient ozone concentrations and leaf morphology has been reported by Bussotti *et al.* (2005b) in native woodland communities of *F. sylvatica* in Italy: at increasing gradients of ozone concentrations there was a corresponding increase in LT, LMA and LD.

Sclerophylly is accompanied by increased water use efficiency (WUE; expressed as $P_N \times G_W^{-1}$ or $P_N \times \text{Trasp}^{-1}$) (Mediavilla *et al.*, 2001). WUE also expresses ozone resistance (Fredericksen *et al.*, 1996; Dizengremel *et al.*, 2008), because in condition of limited ozone uptake (drought stress), P_N declines less than G_W , and the detoxification pathways can be sustained more efficiently. Comparing some of the findings in the literature (Sala & Tenhunen, 1996; Zhang *et al.*, 2001; Bombelli & Gratani, 2003; Gratani *et al.*, 2003; Novak *et al.*, 2005; Warren *et al.*, 2007) with some of our unpublished findings, it appears that in *Populus* (ozone-sensitive clones) the P_N/G_W ratio ranges between 0.023 and 0.031; in *F. sylvatica* (intermediate) P_N/G_W ranges from 0.032 to 0.11; and in *Q. ilex* (resistant) P_N/G_W ranges from 0.08 to 0.15.

Enhanced WUE corresponds to the activation of the carbon-fixing PEP-carboxylase (PEPC) enzyme (Singh-Sangwan *et al.*, 1994). The result is a reduction in the discrimination carried out by the stable isotope of carbon ^{13}C , whose relative content (respect to the more abundant isotope ^{12}C) increases in plants subjected to water stress (Adams & Kolb, 2004). Ozone itself stimulates the production of PEPC, even without reduction of stomatal opening (Saurer *et al.*, 1995; Lütz *et al.*, 2000; Fontaine *et al.*, 2003; Inclan *et al.*, 2005), compensating the injury caused to Rubisco by this pollutant (Dann & Pell, 1989).

Leaf traits and sensitivity to oxidative stress: a meta-analysis of existing data

A homogeneous dataset was built with data coming from different surveys previously carried out by my

research group. Some of these data have already been published (Bussotti *et al.*, 1998, 2000, 2002, 2005b, 2007; Gerosa *et al.*, 2003; Gravano *et al.*, 2003). The dataset includes morphological features such as LT, LMA [= dry weight (LD)/leaf area (LA)] and LD. LD is expressed as:

$$\text{LD} = \text{DW}(\text{LA} \times \text{LT})^{-1}. \quad (1)$$

Each finding represents the mean value of leaves collected from at least three homogeneous (in terms of plot, height and age) trees. At least 25 sun leaves in adult trees, and five leaves in seedlings, were sampled from each individual plant, according to the experimental protocols. LA was measured by Li-Cor LI-3100 Area Meter (Lincoln, NE, USA); DW was determined after drying in an oven at 70 °C until constant weight. LT was measured on samples of at least four leaves from each tree. From the interveinal leaf areas, two 2-mm²-wide fragments of lamina were taken, and cross-sections were then obtained by Vibrotome 1000 Plus (St Louis, MO, USA). Four cross-sections from each leaf were measured with a light microscope Nikon Eclipse E400 (Japan) equipped with a calibrated micrometric grid. The analytical results are shown in Table 1.

Figure 1 is based on the factorial analysis performed using the homogeneous dataset. Factor 1 accounts for 77.59% of the variance and is determined mainly by LMA; Factor 2 accounts for 21.88% of the variance and is determined mainly by LT and LD. It is interesting to note that in Factor 2, unlike Factor 1, LT correlates negatively with LD and LMA. Figure 1 shows how, based on the considered morphological traits, groups with differing sensitivity to ozone can be clearly distinguished. The first such group includes the most sensitive plants [*Fraxinus excelsior* L., *Populus* (clones) and *Ailanthus altissima* (Mill.) Swingle] and is more influenced by Factor 2. Group 3 is clearly distinct from the other two: it includes only *Q. ilex*, and has a very low sensitivity. Group 2, the intermediate group, can further be subdivided into two subgroups: 2-A includes *F. sylvatica* and 2-B represents a combination of oak species (*Quercus robur* L. and *Quercus cerris* L.), as well as some individuals of *F. sylvatica* with more marked sclerophylly traits. These findings suggest that, among the species considered, ozone sensitivity is associated with high LT accompanied by low LD, rather than with reduced LMA. Within a single species (*F. sylvatica*), however, LMA variations may be considered sensitivity predictors.

Unlike the interpretations suggested by Pääkkönen *et al.* (1997) for *B. pendula*, we suggest that ozone tolerance is related to increase in LD rather than to LT (Barth & Conklin, 2003; Gerosa *et al.*, 2003; Bussotti *et al.*, 2007) (Fig. 2). Nevertheless, LMA can distinguish

Table 1 Morphological features in leaves of some tree species, according to the results of the present working group

Reference	Species	O ₃ sens	LT (μm)	LD (mg cm ⁻³)	LMA (mg cm ⁻²)
Gravano <i>et al.</i> (2003)	<i>Ailanthus altissima</i>	H	169	274	5.04
Gerosa <i>et al.</i> (2003)	<i>Fraxinus excelsior</i>	H	283	440	12.18
	<i>Fagus sylvatica</i>	ML	163	580	9.07
	<i>Quercus petraea</i>	ML	159–198		7–11
Bruschi <i>et al.</i> (2003)	<i>Quercus petraea</i>	ML	163		
Bruschi <i>et al.</i> (2000)	<i>Quercus pubescens</i>	L	184		
Bussotti <i>et al.</i> (2000)	<i>Fagus sylvatica</i>	ML	159	680	9.82
	<i>Quercus cerris</i>	ML	151	630	9.93
	<i>Quercus ilex</i>	L	230	790	17.96
Bussotti <i>et al.</i> (2007)	<i>Fagus sylvatica</i>	ML	125	568	7.13
	<i>Quercus robur</i>	ML	165	656	10.63
	<i>Populus nigra</i>	H	281	487	13.76
	<i>Fraxinus excelsior</i>	H	227	397	9
Bussotti <i>et al.</i> (1998)	<i>Fagus sylvatica</i>	ML	77–130	630–830	4.88–10.74
Bussotti <i>et al.</i> (1995)	<i>Fagus sylvatica</i>	ML	190–240	420–980	8–22
Bussotti <i>et al.</i> (1997)	<i>Fagus sylvatica</i>	ML	120–180	420–600	5–11
Bussotti <i>et al.</i> (2002)	<i>Quercus ilex</i>	L	233–296	710–920	16.8–23.1
F. Bussotti (unpublished)	<i>Quercus pubescens</i>	L			13.99
	<i>Quercus cerris</i>	ML			11.07

O₃ sens indicates the sensitivity to ozone of the considered species: H, high; M, medium; L, low. LT, leaf thickness; LD, leaf density; LMA, leaf mass per area.

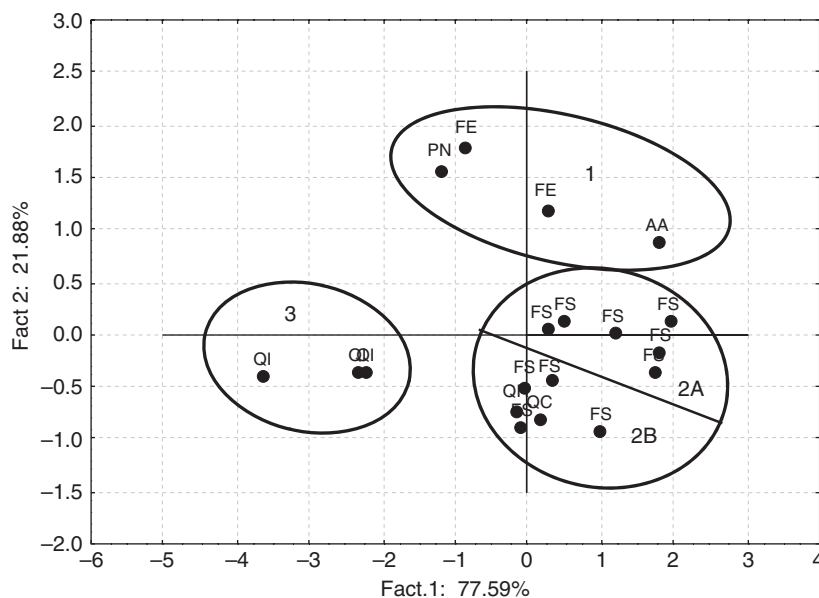


Fig. 1 Factorial analysis based on the leaf traits of several tree species with different ozone sensitivity. FS, *Fagus sylvatica*; QI, *Quercus ilex*; FE, *Fraxinus excelsior*; PN, *Populus nigra*; AA, *Ailanthus altissima*; QC, *Quercus cerris*; QR, *Quercus robur*. Three groups homogeneous for sensitivity to ozone were found – leaf mass per area: Factor 1, -0.994 ; Factor 2, -0.028 ; leaf density: Factor 1, -0.840 ; Factor 2, -0.537 ; leaf thickness: Factor 1, -0.794 ; Factor 2, 0.605 .

roughly between ozone-sensitive and ozone-tolerant species. Figure 3, based on LMA data provided by Cornelissen *et al.* (1996), shows a clear distinction between the two groups of species with different levels of ozone tolerance.

Functional traits, functional categories, vegetation types and stress resistance

The functional traits of leaves, and the stress resistance properties linked to these traits, are reflected in the

different functional categories (evergreen trees and shrubs, frugal deciduous trees and shrubs, demanding deciduous trees and shrubs, megaphorbia, herbaceous plants), and thus in the various types of terrestrial vegetation. The prevalence of species with specific functional leaf traits determines the overall behaviour of a plant community towards environmental stresses. Table 2 summarizes both the main traits associated with resistance and the ones associated with sensitivity in woody plants. Although they are not exactly the same, these traits are comparable with those described by Grime (2001) in his CSR strategy (competitive–stress tolerant–ruderal) in relation to competitive–stress-tolerant plants vs. competitive–ruderal plants.

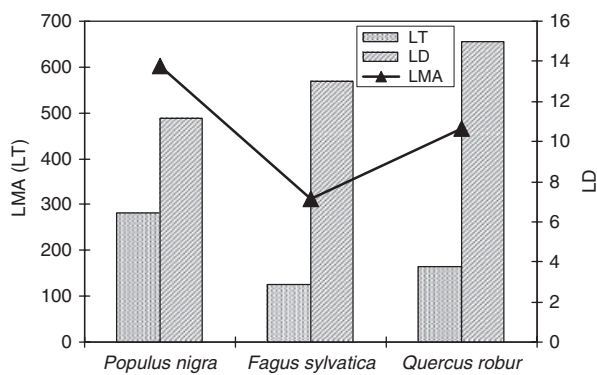


Fig. 2 Leaf traits of the tree species utilized in the Curno experiment (Bussotti *et al.*, 2007), arranged according to a scale of progressive sensitivity to ozone: *Populus nigra* > *Fagus sylvatica* > *Quercus robur*. LMA, leaf mass per area (mg cm^{-2}); LT, leaf thickness (μm); LD, leaf density (mg cm^{-3}).

In general, in environments characterized by low rainfall and low fertility such as the Mediterranean region, tree and shrub communities are usually made up of species belonging to functional groups with high LMA and high leaf lifespan (LL), and the opposite occurs in environments displaying the opposite characteristics (Reich *et al.*, 1998; Wright *et al.*, 2001; Lamont *et al.*, 2002; Wright & Westoby, 2002). The function of these traits is to improve the plants' nutrient use efficiency (NUE; Escudero *et al.*, 1992) and to protect leaves from drought and from parasite attacks (Gutschick, 1999).

Mediterranean vegetation has evolved under conditions of marked oxidative pressure and is considered tolerant towards anthropogenic stress factors as well, such as ozone (Bussotti & Ferretti, 1998; Bussotti & Gerosa, 2002; Paoletti, 2006) and UV-B (Nogués & Baker, 2000; Paoletti, 2005b). Authors have generally assumed that the ozone resistance of Mediterranean plants is due to their reduced stomatal conductance, occurring at the time of day and in periods when the water shortage is most acute (Bussotti & Gerosa, 2002: depressed stomatal conductance limits stomatal ozone flux). The above considerations, however, suggest that an important role is also played by the intrinsic features of Mediterranean species (functional traits). And, along the same lines, deciduous species from sub-Mediterranean and oligotrophic regions are also to be considered resistant.

Demanding broadleaves display the opposite behaviour. In these leaves the plasmatic component is greater than the mechanical one. Rather than relying on a strategy based on protecting injured leaves, these species promote a rapid leaf turnover (Maurer *et al.*, 1997),

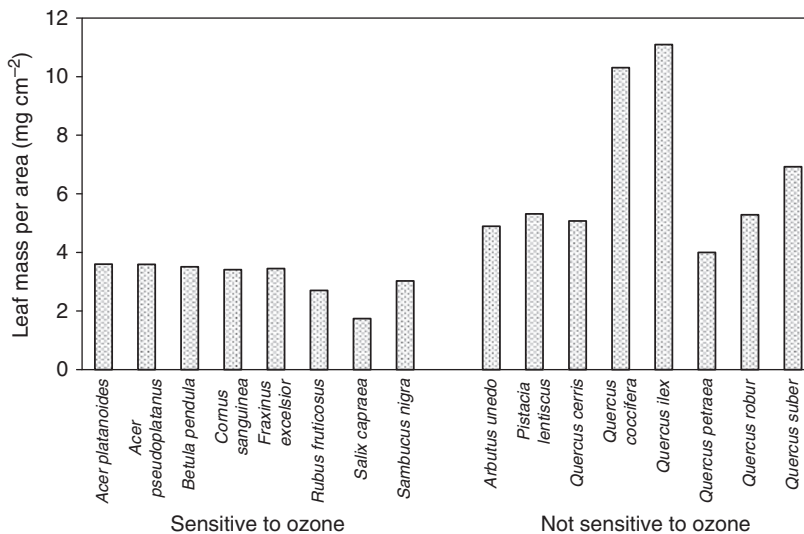


Fig. 3 Discrimination of ozone-sensitive and insensitive species (cf. Innes *et al.*, 2001) according to their leaf mass per area (data from Cornelissen *et al.*, 1996).

Table 2 Structural and functional features related to the high or low resilience to stress

High resilience to stress	Low resilience to stress
CS (competitive–stress resistant) strategy	CR (competitive–ruderal) strategy
Late successional	Early successional
Long-living leaves	Short-living leaves
High leaf density	Low leaf density
Low nitrogen concentration in leaves (mass per mass)	High nitrogen concentration in leaves (mass per mass)
High nitrogen/chlorophyll content in leaves (mass per surface unit)	Low nitrogen/chlorophyll content in leaves (mass per surface unit)
Abundance of mechanical structures in leaves	Scarcity of mechanical structures in leaves
High ability to produce defence compounds	Scarce ability to produce defence compounds
Low RGR (relative growth rate)	High RGR
High WUE	Low WUE
Low reaction to environmental changes	Fast reaction to environmental changes

exploiting environmental resources to reconstruct the photosynthesis apparatus. These are species and plant communities that have evolved under conditions of reduced oxidative stress, such as moderate sunlight and availability of water and nutrients. In these cases, plants display only minor morpho-functional adaptation, and additional stress factors can induce marked and clearly identifiable responses.

Another approach distinguishes early successional (ES) from late successional (LS) species. The former, which need to colonize a territory quickly, are characterized by high photosynthesis rates, low-density leaves and short LL (Matsuki & Koike, 2006). The latter, whose function is to ensure a stable cover of the territory, have opposite features (Matsuki & Koike, 2006). Poorter *et al.* (2004), studying the leaf traits of tropical plants, came to the conclusion that SLA, water content and nutrient concentration decrease in LS plants, while lignin concentration increases. Low LD makes ES species highly reactive to environmental stimuli through a rapid modulation of photosynthesis, although this increases their sensitivity to stress. Conversely, high LD in LS increases their resilience and ecological stability. Within a natural forest population, ES plants grow at the edges, in clearings, and in any case characterize the dynamic stages of growth; LS plants make up the stable forest populations, those that must last over time.

Bassin *et al.* (2007) reached similar conclusions investigating the behaviour of herbaceous communities. Ozone sensitivity correlates positively with productiv-

ity; therefore, species growing in more favourable habitats are more sensitive. Conversely, less productive habitats, with the presence of low-growth stress-tolerant species with low SLA, are less sensitive to ozone.

Leishman *et al.* (2007), in a comparative study of the leaf traits of native and exotic flora in Australia, observed that exotic and invasive flora, colonizing disturbed sites, display leaf traits that are very similar to those pertaining to ES, such as high SLA and elevated assimilation capacity; thus, these species are potentially ozone sensitive. This observation concurs with those reported on alien and invasive species in European flora, such as *A. altissima*, *P. serotina* and *Robinia pseudoacacia* L.

Forest regeneration, too, is a sector potentially sensitive to oxidative stresses. Seedlings and young plants have a greater SLA than adult plants of the same species, and have fewer reserves to use for detoxification (Kolb *et al.*, 1997; Kolb & Matyssek, 2001). Ozone can act as an additional selection factor in the competition for environmental resources: by reducing the above-ground growth in sensitive individuals, these individuals are placed at a disadvantage in relation to more resistant ones (competition for sunlight); furthermore, reduced root mass (Coleman *et al.*, 1996) in sensitive individuals makes them less competitive in their soil uptake of water and nutrients. Moreover, under the recruitment ecological conditions, seedlings are subject to continuous fluctuations of environmental parameters for example, when leaves normally growing in shade are exposed to sun flecks (i.e. sudden exposure to sunlight for a limited period of time). It is well known that, under these conditions, leaves increase their photosynthesis activity considerably, as compared with leaves growing in a steady and stable light condition (Küppers & Schneider, 1993; Naumburg & Ellsworth, 2000; Oguchi *et al.*, 2003). This favours a marked increase of hydrogen peroxide, which can temporarily exceed the leaf's detoxification capacity. Wei *et al.* (2004a,b) have detected a greater sensitivity to ozone in hybrid poplar leaves and in *Acer rubrum* L. in conditions of sun flecks.

In conditions of identical species composition and ecological characteristics, sensitivity or tolerance to oxidative stresses is an individual characteristic (Farage, 1996; Cascio *et al.*, 2007). Selection enacted by oxidative stresses will, thus affect only some of the genotypes of a given species. The result will be that tolerant individuals will establish a new forest cover, more tolerant than the pre-existing one (Berrang *et al.*, 1986, 1989, 1991). Thus, similar to other stressors, ozone acts as an evolution driver.

In general, it is possible to predict the sensitivity of ecosystems to ozone by means of an analysis of the overall ecological conditions and the leaf structure of

Table 3 Tree and shrub communities potentially sensitive (or insensitive) to ozone, and the main features related to their sensitivity

Tree and shrub communities potentially ozone sensitive
<i>Exotic-invasive vegetation (ruderal, disturbed areas)</i>
Low LMA and LD; high gas exchange rate; many species known as ozone sensitive, such as <i>Ailanthus altissima</i> , <i>Robinia pseudacacia</i> , <i>Prunus serotina</i> , <i>Rubus</i> sp. pl. (Innes <i>et al.</i> , 2001; Gravano <i>et al.</i> , 2003; Leishman <i>et al.</i> , 2007)
<i>Forest edges and fringes; forest gaps</i>
Low LMA and LD; high gas exchange rate; low leaf lifespan; sensitivity induced from sun flecks; many species known as ozone sensitive, such as <i>Cornus</i> sp. pl.; <i>Viburnum</i> sp. pl. (Fredericksen <i>et al.</i> , 1996; Innes <i>et al.</i> , 2001; Wei <i>et al.</i> , 2004a, b; Mills <i>et al.</i> , 2007)
<i>Forest recruitment and young plants</i>
Lower LMA and LD than mature trees; higher gas exchange rate; lower capacity of repair (Kolb <i>et al.</i> , 1997; Kolb & Matyssek, 2001)
<i>Early successional-pioneer forest communities</i>
Low LMA and LD; high gas exchange rate; low leaf lifespan; many species known as ozone sensitive (Innes <i>et al.</i> , 2001; Matsuki & Koike, 2006)
<i>Eutrophic-mesophytic forest communities</i>
Low LMA and LD; high gas exchange rate; low leaf lifespan; many species known as ozone sensitive, such as <i>Fraxinus excelsior</i> , <i>Acer pseudoplatanus</i> , <i>Populus</i> sp. pl., <i>Salix</i> sp. pl. (Innes <i>et al.</i> , 2001)
<i>Fast-growing tree plantations</i>
High growth rate; low foliar density and leaf defence (Bortier <i>et al.</i> , 2000; Karnosky <i>et al.</i> , 2005; Bussotti <i>et al.</i> , 2007)
Tree and shrub communities potentially insensitive to ozone
<i>Sclerophyllous evergreen Mediterranean formations</i>
High foliar density and detoxifying capacity; stomatal closure in the warmest hours of the summer days (Bussotti & Gerosa, 2002; Nali <i>et al.</i> , 2004; Paoletti, 2006)
<i>Oligotrophic-xeric forest communities</i>
High foliar density; synthesis of polyphenols and detoxifying compounds; high leaf lifespan (Gutschick, 1999; Matsuki & Koike, 2006)
<i>Late successional forest communities</i>
High resilience to stress; capacity of repair (Matsuki & Koike, 2006; Gielen <i>et al.</i> , 2007; Matyssek <i>et al.</i> , 2007)
<i>Mature forest trees</i>
Lower gas exchange and higher foliar density than seedlings; capacity of repair (Kolb <i>et al.</i> , 1997; Kolb & Matyssek, 2001; Gielen <i>et al.</i> , 2007; Matyssek <i>et al.</i> , 2007)

the main components of the vegetation. This statement can be confirmed empirically, by studies reporting a higher ozone sensitivity in species growing in moist environments (Power & Ashmore, 2002) and, conversely, a lower sensitivity in Mediterranean regions (Bussotti & Gerosa, 2002; Nali *et al.*, 2004; Paoletti, 2006). Forest edges and clearings are also considered ozone-sensitive ecosystems (Mills *et al.*, 2007). Table 3 shows the different

types of forest communities, trees and shrubs: some of the salient features, which may determine their sensitivity or resistance to ozone, are highlighted.

Environmental oxidative pressure also performs a selection among the genotypes of a given species. Very interesting is the case of *F. sylvatica*, a species very widespread in Europe, along a broad North-South gradient (from Southern Scandinavia to Sicily). In this species, leaf morphology variations, occurring along geographical and ecological gradients, reflect acclimation to the environment. Based on these findings, one could assume that this species may behave as either sensitive or tolerant to stress according to its geographical and/or ecological provenance. In this respect, Paludan-Müller *et al.* (1999) highlight the much greater ozone-tolerance of southern populations of *F. sylvatica*. Yet, *F. sylvatica* trees growing at the edge of their ecological range are more sensitive to environmental changes and to increased oxidative pressure. In *F. sylvatica* woods at high altitudes in Southern Germany, a worsening of crown condition has been observed, as well as reduced radial increments over the past 30 years (Dittmar *et al.*, 2003). The authors explain this trend as being the result of the combined action of ozone and climate changes.

Conclusions

Among anthropogenic oxidative stressors, ozone is the one that has the greatest potential impact on forests. Several studies have highlighted a broad range of possible effects on forest trees (see review: Skärbi *et al.*, 1998; Matyssek & Innes, 1999), including growth reduction (Karlsson *et al.*, 2007). These studies observed mainly young trees in experimental conditions but, due to complex interactions within forest stands, evidence of similar growth losses and damage within mature tree canopies remains elusive (Karnosky *et al.*, 2007; Matyssek *et al.*, 2007), especially mature forests growing in a climax balance with their environment. In mature forests of natural origin – forests which are assumed to have struck a good balance with their natural environment – one would not predict significant effects from a moderate increase of oxidative stress. These forests are in a steady state, protected against any sudden variations in environmental parameters (deep root structures, sunlight and microclimate parameter regulators within the crown, availability of reserves, high-density leaves). Thus, they are capable of regulating their own metabolism to fuel acclimation, defence and repair processes, so as to address environmental stressors. When the pressure exerted by stress of natural origin is already very strong to begin with, then even a limited increase of oxidative pressure can have devastating

effects. A short growth season or a marked resource shortage (conditions occurring on the ecological edges of a distribution area, such as the altitude margin – timberline – or in semiarid Mediterranean ambients) can prevent the establishment of an adequate defence system, thus exposing plants to the greater dangers due to environmental fluctuations and changes (Tranquillini, 1979; Turunen & Latola, 2005). However, as a result of worsening of environmental conditions, it is possible that a forest may find itself at the far threshold of its resilience capacity. In such a case, even a moderate additional oxidative stress contribution may cause severe damage.

Nevertheless, the dynamics actually observed in forest ecosystems suggest that what we are recording is a positive growth trend in northern forests, probably due to a combination of factors including increased CO₂, nitrogen depositions and climate changes (Nabuurs *et al.*, 2003). In this context, ozone is considered a factor potentially capable of reducing these 'benefits' (Loats & Rebbeck, 1999; Isebrands *et al.*, 2001; Karnosky *et al.*, 2003; King *et al.*, 2005; Kubiske *et al.*, 2006). CO₂, in turn, is a morphogenic factor, capable of increasing LMA and LT (Radouglu & Jarvis, 1990; Hirose *et al.*, 2005), as well as overall photosynthesis efficiency (Drake & González-Meler, 1997; Ishizaki *et al.*, 2003) and, therefore, the plant's ability to respond to stress.

Several studies have reported cases of extensive damage caused by ozone in forests characterized by pioneer, invasive and aggressive species. In North America, this damage affects *Populus tremuloides* Michx., *P. serotina* and several species of pine (Karnosky *et al.*, 2005, 2007). In Europe, ozone stress symptoms have been reported in several woody and grass species, under natural and semicontrolled conditions (VanderHeyden *et al.*, 2001; Working Group on Air Quality, 2004; Bussotti *et al.*, 2005a), but no real cases of actual forest decline clearly attributable to ozone have been reported. Ozone is more often a co-factor interacting with other stressors. The role of ozone in reducing crown density and/or stem growth is usually less significant than that played by other factors, of natural origin (Ferretti *et al.*, 2003, 2007; Karlsson *et al.*, 2006). However, some clones of the genus *Populus* (Soldatini *et al.*, 1998; Ranieri *et al.*, 2000; Guidi *et al.*, 2001; Novak *et al.*, 2007) have shown sensitivity, and this suggests a possible risk for fast-growing woody cultures and short rotation forestry.

Continuous and integrated monitoring of forests is needed to enable us to identify early risk signals. Special attention should be focused on specific forest sectors and sensitive communities, where negative effects are more likely, such as renewal (alteration of competition and possible biodiversity reduction) and,

more in general, the dynamic stages of vegetation establishing itself on the edges of the forest and in clearings (Mills *et al.*, 2007). Finally, special attention must be given to industrial forestry which, due to its intrinsic characteristics, may be very vulnerable (rapid growth, water availability, intrinsically sensitive species, low genetic variability).

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