

How sensitive is birch to ozone? Responses in structure and function

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ABSTRACT: Although *Betula pendula* and *B. pubescens*, the tree species of birch in Europe, cover large and ecologically diverse areas of distribution, evidence about limiting ozone (O_3) impact in terms of unambiguous cause-effect relationships is scarce, regarding growth under actual site conditions. Similar to other tree species, considerations about risks imposed by chronic exposure to elevated O_3 levels must largely rely on principles of O_3 action as known for young trees grown under controlled chamber conditions. Such principles are well documented for birch, concerning physiological processes and structural differentiation at the cell, organ and whole-plant level, and interactions are highlighted between O_3 impact and effects of different light, water and nutrient availabilities. Although the resulting O_3 responses can be scaled consistently across the internal levels of tree functioning, evidence is rather poor for upscaling to old and tall trees and their specific habitats. Taking into account the ecophysiology of birch, hypotheses about this latter kind of upscaling are proposed, being aware, however, of the demand for experimental validation in the field. A means for approaching this validation are experimental 'free-air' O_3 fumigations which have been conducted extensively in northern Europe on juvenile birch plants. Findings of this approach are summarized and compared with O_3 responses of birch plants at central-European or sub-Mediterranean sites. Overall, it is concluded that birch is sensitive to chronic O_3 stress, although the extent of stress response can strongly be governed by the genetical constitution and other environmental influences. 'Free-air' O_3 fumigations must be extended to natural habitats and tree age beyond the juvenile stage in order to achieve a process-based and quantitative risk assessment of ecological relevance for the long-term performance of birch trees and forests under chronic O_3 impact.

Keywords: *Betula pendula*; *Betula pubescens*; ozone; sensitivity; scaling; ecophysiology

THE TREE SPECIES OF BIRCH IN EUROPE AND THE RISK OF OZONE EXPOSURE

Birch is a species-rich genus with about 40 species being spread across the northern hemisphere (BARTELS 1993). Many of them displaying a shrub-like growth habit, *Betula pendula* and *B. pubescens* can develop into trees of more than 30 m in height, but rarely grow older than 120 years (SCHÜTT et al. 1992). Although these two species represent the only trees of the genus birch which are autochthonous to Europe, both of them cover large natural areas of distribution, expanding from France and Great Britain in the west to far beyond the Ural mountains in the east (MEUSEL et al. 1992), and from low to high elevations of about 2,000 m a.s.l. in the Alps. Both species reach northern Scandinavia, where *B. pubescens* can form the boreal timberline, however, it is *B. pendula* which

clearly extends into the Mediterranean region, namely Italy and the Balkans. The expansion to southern Europe may reflect the higher drought tolerance of *B. pendula*, although both birches are water-demanding, with *B. pubescens* – the so-called 'swamp birch' – typically occupying the rather wet sites (ELLENBERG 1996). Both species have a compact root system, i.e. they may be shallow-rooted but can extensively expand their horizontal roots underneath the soil surface. The soils are typically acidic and poor in nutrition, and the modest requirements for soil quality in combination with high demands for light characterize both birches as distinct pioneer species. This conclusion is supported by high growth rates as based on an indeterminate or 'free' growth pattern, i.e. the longitudinal shoot growth and leaf formation can continue throughout the growing season. Nevertheless, due to the high light demand both trees are weak competitors and,

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thus, restricted to poor sites which include cold habitats at high latitude and altitude.

Given the wide and ecologically diverse distribution of the two birch species across Europe, their areas of distribution have been affected almost in total, during the 20th century, by distinct increases in the tropospheric ozone (O_3) concentration to above pre-industrial levels (FOWLER et al. 1999). Since 1870, in Central Europe the mean O_3 exposure has increased in rural regions by a factor of 2 to about 6 at high elevations of the Alps, and peak concentrations can distinctly exceed 100 nl/l in urban areas (STOCKWELL et al. 1997; MATYSSEK et al. 1997). Upward trends in O_3 levels were reported to persist in the upper troposphere (STAEHELIN, SCHMID 1991). Continued increases have been found also in the British Islands (DOLLARD et al. 1995), and in northern Europe high O_3 episodes are observed as early as during spring (SKÄRBY, KARLSSON 1996). These primarily occur downwind of industrialized areas, perhaps being enriched by long-distance advection from the central and western parts of Europe or, also, by incursions of stratospheric ozone (DAVIES, SCHUEPBACH 1994). High O_3 concentrations were reported from eastern Europe (Poland, Ukraine) and discussed as causes of leaf injury in sensitive species (BYTNEROWICZ et al. 1993; BLUM et al. 1997). The situation is rather specific in southern Europe, where interactions between the orography of the Mediterranean Basin, macro-climatic gradients in atmospheric pressure between the Atlantic Ocean and the Asian continent, high exhausts of O_3 precursors and intense insolation can lead to local, meso-scale air circulation systems which accumulate ozone to rather high levels (MILLAN et al. 1997; SANZ, MILLAN 1998). The combination of Mediterranean climate and excessive air pollution along with the peculiarity of high summer precipitation as occurring at the southern edge of the Alps appears to be particularly conducive to O_3 injury in the local vegetation (SKELLY et al. 1999). Attempts of modeling the environmental constraint posed by ozone across Europe (HETTELINGH et al. 1996, 1997) suggest the entire geographic ranges of both birch species to be at risk by adverse O_3 effects (MATYSSEK, INNES 1999). One needs to be aware, however, that current threshold definitions of O_3 impairment are rather vague with respect to trees (e.g. 'Critical Level for Ozone' concept defined as AOT40 which is the 'accumulated exposure over a threshold of 40 nl/l'; cf. FOWLER et al. 1999; FUHRER, ACHERMANN 1999; VANDERHEYDEN et al. 2001).

Similar to other tree species, also in the case of birch considerations about risks that may arise from chronic exposure to elevated O_3 levels must largely rely on studies with young trees grown under controlled experimental conditions (e.g. in exposure chambers: MATYSSEK, INNES 1999; SKÄRBY et al. 1998; KOLB et al. 1997). This is due to a prevalent lack of rigorously demonstrated cause-effect relationships by ozone under actual site conditions, given the logistic limitations of ecophysiological field work on tall trees beyond the juvenile stage. The following ac-

count will first address, therefore, principles of O_3 action in birch as observed in studies on young trees under controlled exposure conditions. This account will be extended then to the issue on how the sensitivity of *B. pendula* and *B. pubescens* may be modified by physiological interactions between O_3 stress and effects of variable nutrient and water supply or light conditions. Findings will be integrated from studies which have documented O_3 responses in young birch trees as growing in the 'open air', i.e. under actual site conditions. Given this evidence, it will be hypothesized to which extent findings from young birch plants may be 'scaled' to mature trees in the field. A perspective will be proposed on how to overcome the deficits in knowledge about the O_3 tolerance of ageing trees as growing in their actual habitats. On the grounds of the available evidence, a conclusion will be drawn about the sensitivity of the two European tree species of birch to chronic O_3 stress.

PRINCIPLES OF OZONE ACTION IN BIRCH

There is evidence that ozone after passage through the stomata rapidly decomposes into secondary, oxidative derivatives which themselves can be injurious to the metabolism and structure of leaves so that the concentration of ozone approaches zero in the intercellular space of the leaf mesophyll (LAISK et al. 1989). The decay of ozone into reactive derivatives, unless having already been happened during the diffusive influx process, largely occurs in the mesophyll apoplast which contains antioxidants like ascorbate that form the 'front line' in oxidant defense (POLLE 1995). To the extent ozone or its derivatives reach the plasmalemma, they may link to receptors that can initiate 'oxidative burst' reactions (ELSTNER et al. 1996; PELLINEN et al. 1999) and 'programmed cell death' (KANGASJÄRVI et al. 1995), leading to local necroses as a means against spreading injury. Such latter defenses are mediated at the gene level through molecular signal chains including the oxidative O_3 derivatives, ethylene formation as well as salicylic or jasmonic acid (SANDERMANN 2000). As ozone hardly reaches the chloroplasts (URBACH et al. 1989), molecular signaling is also suggested to induce the decline of chloroplasts (loss of pigments and rubisco activity; SANDERMANN 1996; PÄÄKKÖNEN et al. 1998). The mechanistic nature of the receptors and signal chains has only partly been unraveled to date, however, it appears that primary defense against ozone is rather similar, at the cellular level, to responses elicited by biotic agents (SANDERMANN et al. 1997; LANGEBARTELS et al. 1997; SANDERMANN 2000). It can be concluded that the primary response mechanisms to ozone in birch are consistent with findings in other woody and herbaceous plants (PELLINEN et al. 1999).

In leaves of *Betula pendula*, chronic O_3 impact in fact increased the level of apoplastic ascorbate, and together with the pool of dehydroascorbate, the overall defense capacity appeared to be raised (POLLE et al. 2000). Other constituents involved in oxidant scavenging like glu-

tathione, glutathione reductase and peroxidase tended to be activated as well in the apoplast. Also in the symplast, levels of ascorbate and glutathione were enhanced, although this response depended on nutrition (see below). Effects of the primary O_3 impact on the mesophyll were also reflected in the cell walls facing the intercellular space by droplet-like protrusions (at the outer cell wall surface) which appeared as 'unetchable exudates' under low-temperature scanning electron microscopy (= LTSEM: MATYSSEK et al. 1991; GÜNTHARDT-GOERG et al. 1993). Combinations of different microscopic techniques have revealed these protrusions to mainly consist of calcium pectate and to be distinguishable, by chemical composition and appearance, from structural changes as induced by other kinds of stress (GÜNTHARDT-GOERG et al. 1997). As oxidative stress may increase the cytosolic calcium level (LOCK, PRICE 1994), 'trapping' of calcium in cell walls by incorporation into pectate may function as a kind of detoxifying mechanism and apparently spreads from the sub-stomatal cavities, the sites of O_3 uptake, into the intercellular space. Given such primary effects of ozone, other changes in the plant can be regarded as secondary responses to O_3 stress. For example, the overall width of cell walls tended to be increased under O_3 stress, being accompanied by increases in the leaf mass/area ratio (LMA) and stomatal density, but reduced leaf size (GÜNTHARDT-GOERG et al. 1993). The cellular extension growth appears to be inhibited by ozone which may render membranes leaky to water loss and lower the turgor during the process of leaf differentiation (HEATH, TAYLOR 1997; cf. MATYSSEK et al. 1988). Loss of cell fluid also seems to be the cause of advancing O_3 injury, when shrinkage in volume leads to cell collapse, by this enhancing the intercellular space as long as the epidermal cell layer stays intact.

The structural changes affected the gas exchange of the birch leaves: The photosynthetic performance declined as a consequence of the collapsing mesophyll cells (MATYSSEK et al. 1991). This was preceded by a reduced rubisco activity (SAURER et al. 1995) and paralleled by a lowered, effective quantum yield of photosystem II (F_v/F_m of PS II) but increased non-photochemical quenching (NPQ, SHAVNIN et al. 1999), whereas the optimal quantum yield of PS II, F_v/F_m , tended to be rather stable (MAURER et al. 1997). Both the photosynthetic capacity of CO_2 fixation and carboxylation efficiency declined dramatically so that the CO_2 concentration in the intercellular space of the mesophyll increased. This latter change may have been the stimulus for some degree of stomatal closure which overrode the effect of the increased stomatal density on leaf transpiration, and the stomatal narrowing was microscopically confirmed by LTSEM (MATYSSEK et al. 1991). The partial stomatal closure could not prevent, however, a decline in the water-use efficiency of photosynthesis (WUE). Macroscopically, the leaf-internal decline was accompanied by discoloration, progressing from chlorotic stippling through a stage of 'bronzing' of the entire leaf lamina into the formation of large brownish necroses (MATYSSEK et al. 1990).

Structural changes probably also affected the translocation of assimilates in the birch leaves, given the disruption of symplastic pathways due to the progressive cell collapse. Also phloem loading appeared to be disturbed as indicated by starch accumulation along leaf veins (MATYSSEK et al. 1992; GÜNTHARDT-GOERG et al. 1993). This accumulation tended to be paralleled by increased levels of sucrose, glucose and fructose, while reduction of sucrose phosphate synthase activity and stimulation of sucrose synthase and invertase reflected favored degradation of sucrose rather than synthesis (LANDOLT et al. 1997; EINIG et al. 1997). The metabolic status resembled an end-product inhibition of photosynthesis in the leaves, induced by reduced assimilate export, with the carbon flow being switched from sucrose synthesis to glycolytic and anaplerotic processes feeding the detoxification and repair of O_3 injury. This view is supported by enhanced respiratory activity and stimulation of the CO_2 -binding PEP-carboxylase enzyme (PEPC, MAURER et al. 1997; LANDOLT et al. 1997), enhancing the carbon pool of the citric cycle (cf. WISKICH, DRY 1985). It was the low ^{13}C discrimination of PEPC rather than a limited CO_2 uptake through the stomata that caused the increase of ^{13}C in the biomass of O_3 -exposed birch trees (Fig. 1(A), SAURER et al. 1995; cf. FARQUHAR et al. 1989), given the decline in

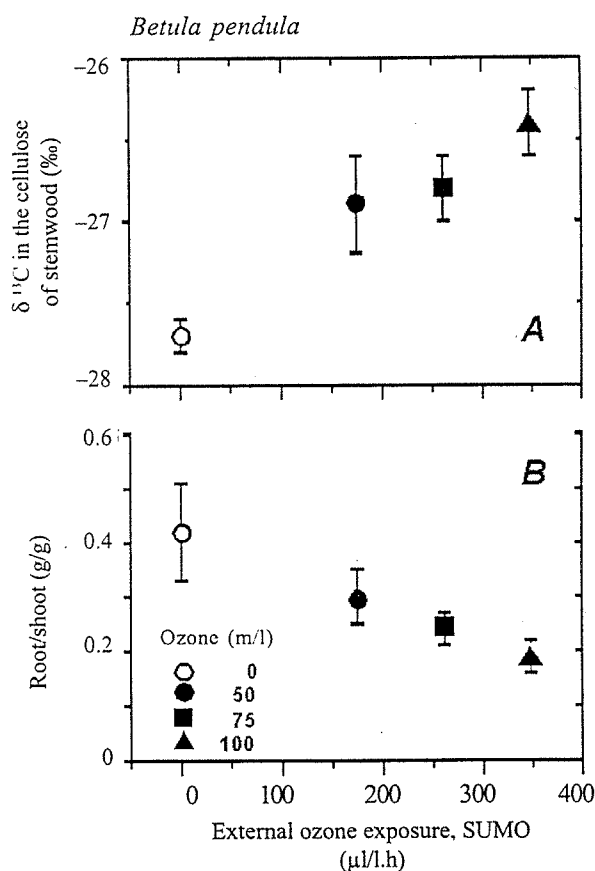


Fig. 1. ^{13}C in the cellulose of stem wood (A), and root/shoot biomass ratio (B) of *Betula pendula* as related to the external O_3 exposure (i.e. the SUMO dose) experienced throughout the growing season (means \pm standard deviation); after data from MATYSSEK et al. (1992) and SAURER et al. (1995)

WUE despite partial stomatal closure (MATYSSEK et al. 1992). The linear relationship between ^{13}C and external O_3 exposure indicates a constant ratio of the latter to the physiologically effective O_3 influx into the leaves and a proportional metabolic response to the accumulating O_3 impact on the mesophyll.

Given the increased demand for detoxification and repair processes and the inhibited assimilate translocation from the leaves, the development of the non-green organs must be expected to be limited under O_3 stress. The organ limited most is the root system so that the root/shoot biomass ratio (R/S) declined – again, in a linear relation to the external O_3 exposure (Fig. 1B). Increment in stem length was less limited than radial stem growth, and stems developed a similar number of nodes and leaves along their axes irrespective of O_3 stress (MAURER 1995) so that changes in biomass partitioning by ozone do not appear to merely reflect retarded ontogeny (MATYSSEK et al. 1998; cf. WALTERS et al. 1993). Limitation in radial stem growth was accompanied by structural decline in the cortex and phloem tissue (GÜNTHARDT-GOERG et al. 1993; POLLE et al. 2000). Ozone exposure also suppressed lateral branching so that this effect on the annual whole-plant biomass production can be quantified along with those of reductions in leaf size and photosynthesis, and of premature leaf loss at the lower stem parts (Fig. 2). These oldest leaves on axes which result from indeterminate growth experience the highest O_3 exposure (i.e. external O_3 dose) and are shed first, and their loss may limit the increments of the lower stem and the root (DICKSON,

ISEBRANDS 1991) in addition to the inhibited translocation of assimilates. The efficiency of the whole-plant production as based on the foliage area formed during the growing season may be reduced by up to 60% (MATYSSEK et al. 1992), and the biomass production again tends to decline in a linear way with the increasing O_3 exposure.

OTHER FACTORS AS MODIFIERS OF THE OZONE RESPONSE IN BIRCH

Pursuing an ecophysiological perspective, the action of ozone must not be regarded as separated from other environmental impacts which altogether determine the sensitivity to stress (MOONEY et al. 1991; SKÄRBY et al. 1998). It is commonly agreed upon that high irradiance can increase the injurious potential of elevated O_3 levels (HEATH, TAYLOR 1997), as the antioxidative capacity which is inherently required to cope with the oxidant release during photosynthesis may be exceeded by additional oxidative stress. This kind of detoxification immanently depends, however, on the energy supply from photosynthesis (SCHUPP, RENNENBERG 1988; MENSER 1964) so that the question arises about the toxicity of ozone under light limitation. There is evidence that low-light conditions can raise the sensitivity to ozone (KOLB et al. 1997; MATYSSEK et al. 1995a), and such observations have been made also in birch (MATYSSEK et al. 1995b; GÜNTHARDT-GOERG et al. 1997). The same O_3 exposure at night, including dawn and dusk, not only fa-

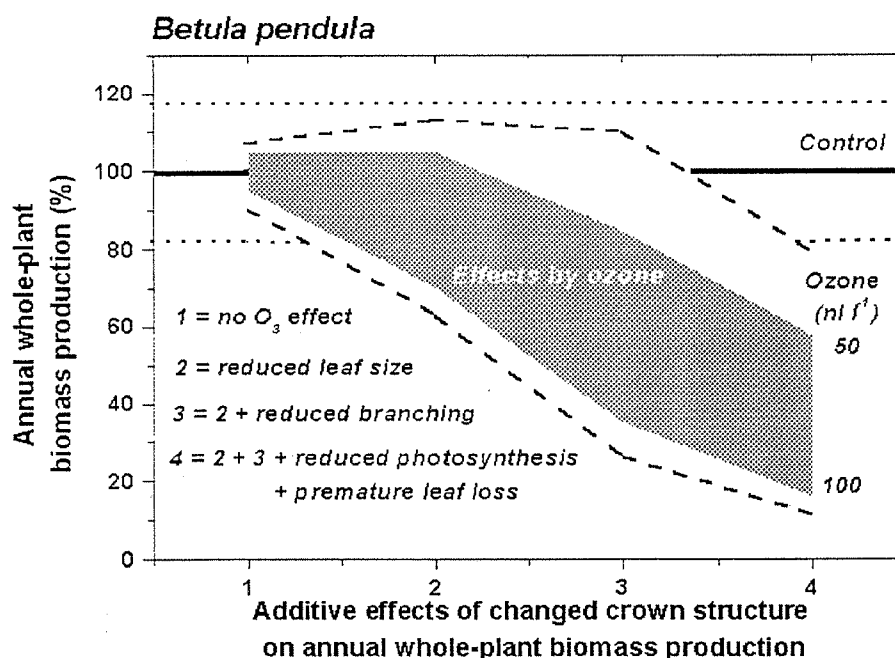


Fig. 2. Additive effects of O_3 -induced changes in crown structure on the annual whole-plant biomass production of *Betula pendula*: Influence of persisting O_3 levels between 50 and 100 nl l^{-1} (dark area, standard deviation given as dashed lines) relative to control plants in O_3 -free air (drawn bold line, standard deviation given as dotted lines; biomass production of the control set as 100%). Steps 1 through 4 represent the O_3 -caused consequences of reduced leaf size (2), plus inhibited formation of lateral branches (3), plus premature leaf loss in combination with photosynthetic decline (4) for the proportional decline in biomass production (redrawn from MATYSSEK et al. 1992)

vored leaf discoloration, but also led to more distinct declines in the whole-plant production and in the stem weight/length and root/shoot ratios (R/S) than during the daylight hours, and 24 h exposure resulted in additive effects of the declines under low and high-light conditions. Stomata were open at night, assuring O_3 influx, although the stomatal conductance was about 50 % only of the level under high irradiance. Given the lowered O_3 influx at night, plants were more sensitive to ozone in the absence of light (cf. KOLB, MATYSSEK 2001). Contrasting with the common view of stomatal closure in the dark, stomata have the potential of staying open at night (TOBIESSEN 1982), and there are a number of conditions (e.g. chronic O_3 impact itself) that may be conducive to such a stomatal behavior (MATYSSEK, INNES 1999). It is an open issue, though, if the light-demanding character of birch predisposes to high O_3 sensitivity under light limitation (KOLB, MATYSSEK 2001).

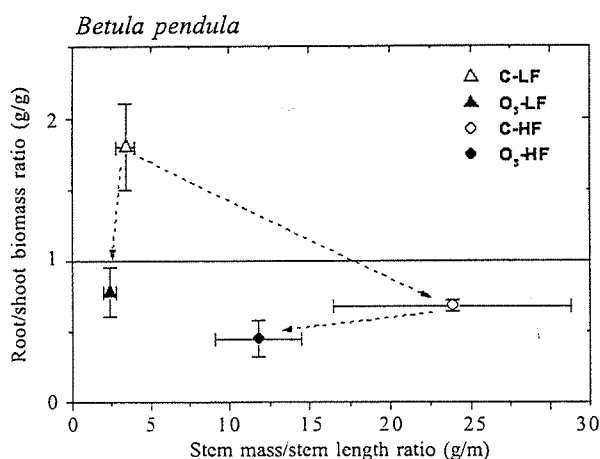


Fig. 3. Changes in whole-plant carbon allocation as expressed by the root/shoot biomass ratio in relation to the stem mass/length ratio of *Betula pendula* at the end of the growing season; Low and high-nutritional plants (triangles and circles, respectively) were exposed either to O_3 -free air (open symbols) or to an O_3 regime of 40 nl/l at night, and 90 nl/l during daytime (closed symbols; means \pm standard deviation). Abbreviations: LF = low-fertilized. HF = high-fertilized. C = O_3 -free air ('control'), O_3 = 40/90 nl/l night/day O_3 regime (after data from MAURER, MATYSSEK 1997)

Nutrition appears to be another appreciable modifier of the O_3 response of birch (POLLE et al. 2000), being less reflected by proportional O_3 -caused declines of whole-plant production rather than changed carbon allocation (MAURER, MATYSSEK 1997). The increase in R/S, as typically observed in low-nutritional plants (STITT, SCHULZE 1994), was prevented by O_3 impact which rather lowered this ratio to levels similar to those of high-fertilized plants (Fig. 3), although differential effects of nutrition on O_3 -induced declines in the stem weight/length ratio were less pronounced. High nutrition had minor effects only on the O_3 response of R/S. The change in whole-plant allocation apparently related to the indeterminate growth pattern of the birch shoots which promoted, at high nutrition, extension growth and leaf formation throughout almost the

entire growing season, whereas low nutrition made these increments cease early in the season. By this, low-nutritional plants were required to maintain their lower number of leaves, even if O_3 -injured, whereas the oldest and, therefore, O_3 -injured leaves at the basal stem parts of high-nutritional plants tended to be prematurely shed. As a consequence, the proportion of injured leaves in the foliage area along stems stayed below 50% by early September (Fig. 4), and this percentage was even lowered, when taking into account the largely intact foliage of the extensive branch formation at high nutrition. Foliage injury approached 100% only by early October. At low nutrition (and in the absence of branching), O_3 -caused injury affected more than 50% of the foliage throughout most of the growing season and reached 100 % already during early September (Fig. 4). The maintenance of a persistently high proportion of injured foliage apparently represented both an enhanced demand for assimilates as reflected by high respiratory carbon loss in the whole-tree carbon balance (MAURER, MATYSSEK 1997) and an aggravating impact of disrupted assimilate translocation on the below-ground carbon allocation (cf. RENNENBERG et al. 1996). Given these constraints, the WUE of the whole-plant biomass production was low in low-nutritional plants under chronic O_3 exposure, even though partial stomatal closure occurred in response to ozone under low nutrient supply (MAURER et al. 1997).

It were the O_3 -exposed leaves of the low-nutritional plants that showed, in most distinct ways, the changes in carbon metabolism (starch, sugars) mentioned above, including increases in ^{13}C and defense capacity (SAURER et al. 1995; LANDOLT et al. 1997; EINIG et al. 1997; POLLE et al. 2000). Apparently, nutrition offers two 'strategies' to birch for coping with O_3 stress (MATYSSEK et al. 1997b): Low nutrition 'forces' the plants to rely on the photosynthesis of O_3 -injured leaves and, thus, to maintain them – with drastic consequences for the whole-plant carbon allocation. High nutrition enables a high leaf formation

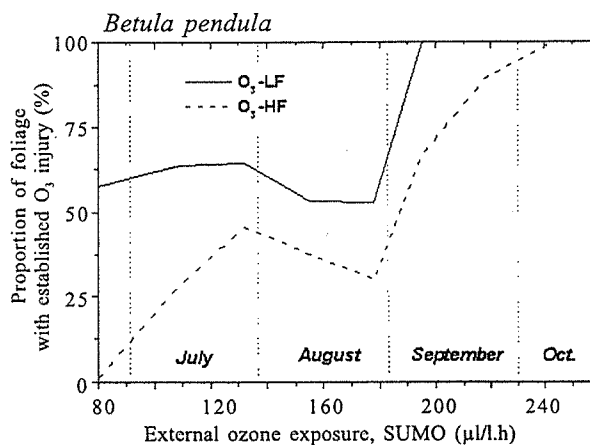


Fig. 4. Proportion of foliage with established O_3 injury in the whole-plant foliage area of low or high-nutritional birch plants (*Betula pendula*) during the seasonal course (and in parallel to the accumulating external O_3 exposure, i.e. the SUMO dose); treatments and abbreviations as given in Fig. 3 (after data from MAURER, MATYSSEK 1997)

rate so that the need for maintaining O₃-injured leaves and their effects on the whole-plant carbon balance are diminished. The extent to which these 'strategies' substantiate strongly determines the sensitivity of specific plant parameters under consideration and may explain the high variability found in tree responses to ozone (POLLE et al. 2000; MATYSSEK et al. 1995a). Variable nutrient availability may be a common situation for pioneers like the two birch species in question which can occupy edaphically disturbed sites of high nutrient availability, but which do also tolerate poor soils so that resulting O₃ responses in the field may be rather inconsistent. In another study by PÄÄKKÖNEN et al. (1995), high nutrition tended to decrease, in *Betula pendula*, O₃ injury and premature loss of leaves, although the nutritional status was mainly determined by nitrogen supply. It needs to be examined, to what extent the quality of nutrition (nitrogen vs. balanced nutrient supply) may as well modify the responsiveness to ozone (MATYSSEK et al. 1995a).

Also soil water supply can be variable in the habitats of the two birch species, and in particular the sandy and drier sites of *Betula pendula* may tend to develop moisture deficits, although this tree species is water-demanding. Findings disagree, however, as to whether drought may necessarily prevent O₃ injury through stomatal closure, or if chronic O₃ exposure may render stomatal regulation imprecise through mechanical or biochemical changes in the stomatal apparatus (WIESER, HAVRANEK 1993; GÖTZ 1996; HEATH, TAYLOR 1997; MAIER-MAERCKER 1998), by this increasing risks of impairment by both O₃ uptake and poorly controlled water loss. In *Betula pendula*, effects of drought and O₃ stress appeared to be additive in reducing leaf number, foliage area and starch formation in the mesophyll cells, but increasing epidermal cell wall width and tannin-like depositions in the vacuoles, both being regarded as defense reactions (PÄÄKKÖNEN et al. 1998a). Drought counteracted O₃ effects on leaf differentiation by decreasing the stomatal density, however, both stresses lowered the stomatal widths. Nevertheless, drought was beneficial only in slightly reducing the O₃-caused leaf discoloration, although the latter was related to the gene induction of stress proteins (PÄÄKKÖNEN et al. 1998b). Only severe drought appeared to protect from O₃ impact, whereas mild soil moisture deficit rather was conducive to O₃ injury (PÄÄKKÖNEN et al. 1998c). It was concluded that drought/ozone interactions were rather complex in birch so that generalizations were not readily achievable.

Part of the complexity in birch response appeared to result from a high variation in the O₃ sensitivity between genotypes, and this conclusion substantiated both for *Betula pendula* and *B. pubescens* (PÄÄKKÖNEN et al. 1997). O₃ tolerance was associated with compact leaf structure and, remarkably, increased stomatal density, whereas sensitive genotypes were characterized by ultra-structural decline of chloroplasts. Individuals with low capacity of leaf formation had high proportions of O₃-injured foliage, which resembled findings on the O₃ response of low-nutritional birch plants (MAURER, MA-

TYSSEK 1997). It seems that the indeterminate growth pattern and the degree of its expression, either mediated through the genetical constitution or environmental influences, pre-determines the responsiveness of birch to ozone. It is also evident that the genetical variability in concert with the environmental scenario can initiate a broad spectrum of gradually differing stress responses in the two birch species so that the resulting range in sensitivity can easily differ from presumptions on the responsiveness to ozone (VANDERHEYDEN et al. 2001; MATYSSEK, INNES 1999; SKÄRBY et al. 1998).

EVIDENCE OF OZONE RESPONSES IN BIRCH UNDER PREVAILING FIELD CONDITIONS

Most findings, addressed so far, on responses of birch to ozone were based on investigations conducted in exposure chambers (which represents a situation similar to the state of knowledge about other tree species in Europe; MATYSSEK, INNES 1999). However, some of the addressed data, namely most of those provided by PÄÄKKÖNEN and co-workers, have in fact been elaborated by using the 'free-air' fumigation approach in the field. This methodology offers the advantage of preventing plant enclosure in containments and, by this, avoiding potential artifacts by micro-climatic bias (MUSSELMAN, HALE 1997). Hence, the ecological significance of such O₃ exposure studies is increased, and the prevailing ambient O₃ regime can serve as a 'control' of experimentally enhanced O₃ treatments (HÄBERLE et al. 1999). Although being more 'site-relevant', basic findings obtained from experimental 'free-air' exposure of birch were rather consistent with those from chamber studies (PÄÄKKÖNEN et al. 1993, 1997): Elevated O₃ levels reduced, in sensitive genotypes of birch, the height growth and foliage mass, promoted leaf discoloration and induced partial stomatal closure, but increased the stomatal density of leaves differentiating under O₃ impact. Ultra-structural decline occurred in the chloroplasts, and as observed elsewhere (GÜNTHARDT-GOERG et al. 1993; MATYSSEK et al. 1998), leaves formed by indeterminate growth in mid-summer were more sensitive to ozone than those which had flushed during spring. By comparing birch clones from different parts of Finland for O₃ sensitivity in response to 'free-air' fumigation, it was concluded that risks by O₃ impact may be highest in the southern, coastal region of the country (PÄÄKKÖNEN et al. 1997). 'Carry-over' effects were studied in birch during years succeeding experimental 'free-air' O₃ exposures, revealing persisting reductions in shoot and root biomass along with inhibited bud development, leaf growth and branching as well as decreases in photosynthesis and stomatal conductance (PÄÄKKÖNEN et al. 1999; OKSANEN, SALEEM 1999). Based on these experiments and silvicultural data of *Betula pendula* plantations, modeling attempts were made to estimate yield loss in timber production as caused by chronic O₃ exposure during rotation periods (KÄREN-LAMPI 1999). Although the model was parametrized, in part, with data from young birch plants, minor declines in

photosynthesis turned out to lower timber yield by about 10 to 20 % by the end of the rotation, whereas assumptions of a favored carbon allocation into the O₃-exposed leaves tended to counteract loss in production (cf. MOONEY, WINNER 1991).

Also in Central Europe, O₃ injury of birch leaves was identified in the open field, when plants grown under the ambient O₃ regime prevailing outside were compared with individuals exposed to controlled O₃ treatments inside exposure chambers (GÜNTHARDT-GOERG et al. 1998). The same kind of stippling occurred, when leaves of both sets of plants had experienced similar O₃ exposure. The southern edge of the Alps mediates between Central Europe and the Mediterranean region in displaying typically high precipitation of the insubric climate during the summer months, but being characterized also by high insolation and elevated O₃ regimes which are advected from the industrialized Milan area in northern Italy (WUNDERLI, GEHRIG 1990; STAFFELBACH et al. 1997; MATYSSEK, INNES 1999). The resulting high water availability during periods of high irradiance and O₃ impact may be rather conducive to an O₃-induced impairment of the vegetation in this sub-Mediterranean region. In fact, a survey conducted in this region, in the canton of Ticino (southern Switzerland), revealed a number of autochthonous and introduced species, comprising both herbaceous and woody plants, that exhibited symptoms of leaf injury (stippling, discoloration; SKELLY et al. 1999) which had previously been described to be indicative of O₃ impact (SKELLY et al. 1987). *Betula pendula* belonged, in this survey, to the array of symptomatic plants. Offsprings of symptomatic plants were grown, in a second step, in exposure chambers and submitted to either ambient O₃ regimes or treatments of reduced O₃ levels (filtered air). In offsprings of sensitive plants, same leaf symptoms were reproduced under the ambient O₃ fumigation regimes as found in the open field (VANDERHEYDEN et al. 2001).

Symptoms were consistent with known responses to enhanced O₃ levels, and were delayed in filtered-air treatments of reduced O₃ levels (SKELLY et al. 1999). Also *Betula pendula* behaved in this way, with the leaf symptoms being consistent with those found in central and northern Europe (VANDERHEYDEN et al. 2001). In this approach, which fulfilled the *Koch's postulates* in linking, in a rigorous way, leaf symptoms occurring in the field to ozone as the causing agent, birch appeared to be less O₃-sensitive relative to other woody species, in particular to the introduced *Prunus serotina* (cf. MATYSSEK et al. 1997a). One needs to be aware, however, that ranking of O₃ sensitivity across species is questionable, given – as pointed out above – the strong influences of genotypic constitution and accompanying site factors on the O₃ responses of trees.

HYPOTHESES ON THE OZONE RESPONSE OF AGEING BIRCH TREES AND PERSPECTIVES OF VALIDATION

The current knowledge about the impact of ozone on birch is mainly based on studies of juvenile plants, including the evidence from 'free-air' O₃ fumigations (see above). Concerning this early ontogenetic stage, principles in structural and functional response patterns to ozone have largely been understood, as *Betula pendula* is one of the plants that has been examined rather profoundly for its sensitivity to ozone. Basically, two pathways of O₃ action exist which both eventually result in changed carbon allocation (Fig. 5): One through injurious alterations, progressing from disruption of photosynthesis and assimilate translocation to premature leaf loss and productive decline, and the other one counteracting, through defense mechanisms, the risk of O₃ injury. This latter pathway tends to maintain production, relying on energy and substrate-demanding repair and detoxifica-

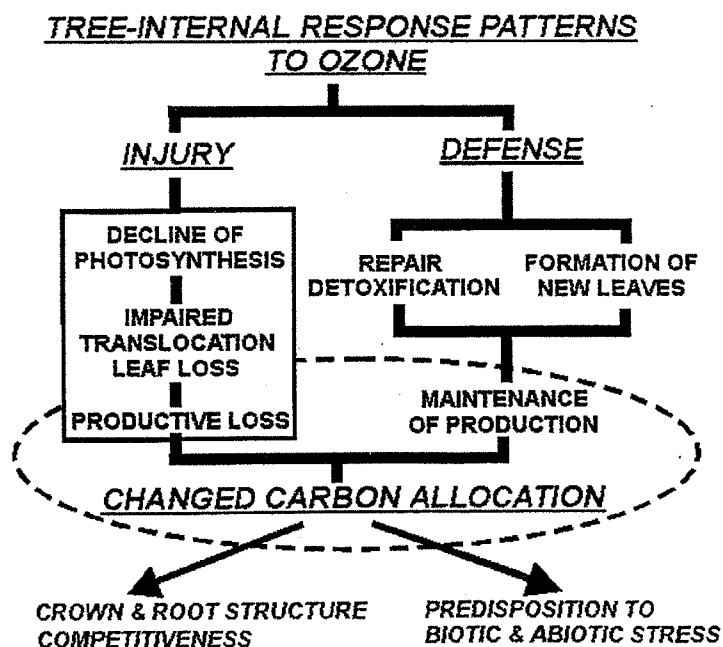


Fig. 5. Pathways of O₃ action in birch (see text for details)

tion processes and/or an enhanced capacity of growing new, initially intact leaves (WOLFENDEN, MANSFIELD 1991). The changed carbon allocation can be reflected in modifications of tissue morphology and above- and below-ground growth patterns, while associated changes in productivity and respiratory or transpiratory costs may lower the water-use efficiency of growth. The available evidence allows for 'scaling' processes involved in these pathways across the tree-internal levels of cells, organs and the whole plant (KOLB, MATYSSEK 2001).

What about extending, however, 'scaling' to tree age beyond the juvenile stage, or to growth under the actual site conditions of forests or plantations? Clearly, information on O_3 sensitivity is missing for such latter scenarios (MATYSSEK, INNES 1999; KOLB 2001), and birch is no exception in this respect. Hence, it may be concluded only that the principles of O_3 action known to date may be of some relevance also for ageing trees under given stand conditions, even though modifications are rather likely. The fact that O_3 -caused alterations in whole-plant allocation may change root and shoot architecture, e.g. reduce bud formation and branching, inherently has consequences for competitiveness (Fig. 5), considering the occupation of canopy or soil space and its exploitation for resources as the basic means of competitive interaction between plants (KÜPPERS 1994; BAZZAZ 1997). The competitiveness of light-demanding pioneers like birch trees may be vulnerable, in particular, through allometric changes in growth. Weakening through shading (also along crown-internal light gradients) may be exacerbated through chronic exposure to elevated O_3 levels, as light limitation appears to be conducive to O_3 injury in birch (see above). Gradients of O_3 regimes should be considered across the fairly open canopies of birch stands, given the observation that O_3 levels may be appreciably high even within rather closed canopies of shade-tolerant beech (BAUMGARTEN et al. 2000). Light limitation may be meaningful also at sites of persistently high O_3 impact throughout diurnal courses like in rural areas or at high elevation (WIESER, HAVRANEK 2001; MATYSSEK et al. 1997a; LEFOHN, JONES 1986). O_3 impact at night may be relevant, in particular, in the high-altitudinal habitats of *Betula pubescens*. It is uncertain, however, if high O_3 sensitivity under low-light conditions is inherently linked to high light demands of a tree species (KOLB 2001; TJOELKER et al. 1993).

High nutrient availability of disturbed sites can favor pioneers like birch, and it may be the indeterminate shoot growth that profits most from such conditions. A high rate of longitudinal shoot growth and leaf formation may sustain competitiveness and counterbalance productive loss by ozone, as the new leaves are initially intact in terms of photosynthesis and lose productivity only after having experienced prolonged O_3 exposure (cf. TJOELKER, LUXMOORE 1991). Nevertheless, the biomass production may be reduced, if the older injured leaves are prematurely shed. As trees grow in size, however, the proportion of leaves formed through indeterminate growth typically decreases relative to the older foliage of the

spring flush, and by this, the 'advantage' of stress compensation through new leaf formation is diminished (KOLB et al. 1997; KOLB 2001). Thus, ageing birch trees may become similar to individuals of low nutrition as the latter limits indeterminate shoot growth and represents a constraint for maintaining the existing foliage, even if O_3 -injured (KOLB, MATYSSEK 2001). Under such conditions, birch tends to resemble species with determinate shoot growth, and readjustment of whole-plant allocation for coping with O_3 stress may be crucial for survival (MAURER, MATYSSEK 1997). In this respect, responsiveness in allocation may be more meaningful in ecological terms than the maintenance of high productivity, although associated changes in the allometric relationships may distinctly affect competitiveness. Overall, it may be the low rather than high-nutritional sites which typically represent habitats of *Betula pendula* and *B. pubescens*, comprising poor sandy and wet bog soils. Chronic O_3 -caused limitation of root growth may weaken birch at such sites, losing below-ground competitiveness and becoming susceptible to nutritional deficiencies (cf. MATYSSEK et al. 1993).

Low-nutritional and shallow soils may be variable also in moisture so that limitation of the horizontal roots extending near the soil surface may impose another risk. Although the effects of water supply on the O_3 sensitivity of birch was rather ambiguous, there are indications that stomatal regulation, depending on water availability, may mediate the susceptibility to O_3 stress also in birch (PÄÄKKÖNEN et al. 1998). Water relations may determine, however, the O_3 sensitivity of ageing birch trees in an additional way that links, as found in other species, the stomatal conductance to the dimensions of the stem and crown: As the frictional resistance of the water flux increases with the length of the transport pathway through the tree, and gravitational constraints on the water flux gain importance with tree height, the stomatal pores tend to narrow (YODER et al. 1994; BAUERLE et al. 1999). Interrelationships of this kind represent the majority of findings on the water relations in ageing trees (KOLB 2001). Consequently, the O_3 influx into the leaves may be lowered as well so that old rather than young trees may be 'shielded' against O_3 impact. Also, lower metabolic activity may render old trees less susceptible to oxidative stress (LAURENCE et al. 1994). However, stomatal narrowing also limits the carbon gain (WARING, SILVESTER 1993; HUBBARD et al. 1999) so that the assimilate pool may be diminished in old trees for feeding repair and detoxification processes – and the O_3 sensitivity may be high. The situation may exacerbate in old trees, if raised O_3 -driven respiration costs lower the carbon pool required for maintaining the standing biomass (cf. WARING, SCHLESINGER 1985).

This latter issue again relates to O_3 -caused alterations in allocation which may create conflicts also with the carbon demands for other stress defenses (Fig. 5), namely, of biotic impacts by pathogens and phytophagous insects (HERMS, MATTSON 1992; LERDAU, GERSHENZON 1997). Such tradeoffs in resource partitioning mirror tree fitness

(BAZZAZ 1997) and appear to form the basis of O_3 -induced predispositions to biotic stress which are assumed (rather than the chronic O_3 exposure itself) to eventually lead to tree mortality and to diminish genotypic diversity (MILLER et al. 1997). In this context, interactions exist between O_3 impact, parasite resistance and mycorrhization, as limitation of the latter by O_3 exposure of the tree (ERICSSON et al. 1996) can lead to a lowered metabolic defense capacity and favor parasite attack (GEHRING et al. 1997; LANGEBAEDEL et al. 1997). Besides these peculiar relationships, attention needs to be directed, in general, to the aspect of mycorrhization and its effects on interactions between nutrition, water availability and the O_3 sensitivity of trees (cf. ANDERSEN, SCAGEL 1997). Potential effects of reduced assimilate pools under O_3 stress on the frost hardiness appear to deserve attention also in birch of high-altitude sites (cf. SKÄRBY et al. 1998).

The hypotheses posed above about the O_3 sensitivity of ageing trees under stand conditions need to be validated, in a rigorous way, at given field sites. The appropriate methodology for achieving this aim is the 'free-air' fumigation technique which allows exposure to elevated O_3 levels without micro-climatic bias, and the use of the unchanged O_3 regime at the site as a 'control' (MUSSELMAN, HALE 1997; HÄBERLE et al. 1999). Although this approach has been used in several investigations, it either has been operated with gases other than ozone, or if ozone was applied, then mainly juvenile trees or only discrete crown parts have been fumigated (KARNOSKY et al. 2001). This technique definitely needs to be extended to the experimental O_3 exposure of tall trees under given site conditions, and this postulate also holds true for trees of *Betula pendula* and *B. pubescens* in order to quantify the risks imposed by chronic O_3 stress in an ecologically meaningful context.

CONCLUSIONS

Viewing the evidence available to date, birch must be regarded as sensitive to O_3 impact as nowadays prevailing at many sites across Europe, and a few field studies did prove ozone as an agent that can cause injury to birch leaves. Findings are mainly based on extensive investigations of *Betula pendula*, but there is evidence of similar susceptibility to ozone in *B. pubescens*. Subtle responses in structural differentiation and metabolism have been unraveled in birch to experimental O_3 regimes of relevance for the chronic O_3 stress that nowadays prevails in the field, although most evidence is restricted to juvenile trees grown under controlled chamber conditions. As for other tree species, validation of response mechanisms is required in the field as the pre-requisite of a quantitative risk assessment of chronic O_3 stress, and risks may substantiate in tree fitness, via changes in resource allocation, growth pattern, competitiveness and capacity in stress defense, even in the absence of macroscopic injury. A means for achieving the required validation is the 'free-air' O_3 fumigation technique, in particular, if extended to use on ageing and tall trees under stand conditions.

One needs to keep in mind, though, that also in the two tree species of birch native to Europe, responsiveness to ozone may vary across wide ranges of genetic disposition and interactions with environmental influences. Assessments of O_3 risks in trees are gaining in relevance with respect to global change effects, as chronic O_3 impact will stay high (along with enhanced N deposition and increasing CO_2 concentration) during the decades to come (FOWLER et al. 1999) and may mitigate the carbon sink strength of forest ecosystems (SAXE et al. 1998; CEULEMANS et al. 1999). Thus, chronic O_3 regimes above the pre-industrial level as found in many forested areas have become and will remain one of the crucial components in global change scenarios.

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