Basic mechanisms of pigment bleaching and loss of structural resistance in spruce *(Picea abies)* needles: advances in phytomedical diagnostics

E. F. Elstner, W. Osswald and R. J. Youngman

Institut für Botanik und Mikrobiologie, Technische Universität München, Arcisstrasse 21, D-8000 München 2 (Federal Republic of Germany)

Summary. Preliminary phytochemical diagnostic data in addition to scanning electron microscopy in spruce *(Picea abies)* needles from 18 different locations in Upper Bavaria support the following hypothesis: both physiological changes in the photosynthesizing cells and structural changes on the needle surfaces (loss of integrity of the wax layer over the stomatal cavities) are the result of the interaction of biomolecules with photooxidants. Damaged needles lose their resistance towards fungal invaders which finally cause necrotisation and abscission of the needles.

Key words. Spruce decline; photooxidations; fungal infections; phytomedical diagnostics; structural resistance.

Introduction

The diseases and decay or collapse of forest ecosystems have been the focus of discussions for several years from both scientific and political platforms. Several hypotheses have been presented on the basic events of induction and propagation of these severe disease symptoms, which are particularly expressed in pine species. These may be divided into three classes:

1) Intoxication of the rhizosphere system by acid rain, depletion of minerals in the soil accompanied by Al^{3+} toxicity.
2) Intoxication and primary effects on leaves and needles by SO_2, NO_x, O_3, PAN (peroxyacetylnitrate) and other air pollutants.
3) Infection by as yet unknown parasites such as viroids, viruses, mycoplasmas or bacteria.

Most researchers in the field take into account the fact that more than one primary event may be responsible for the visible symptoms, which may thus represent stages in a 'complex disease'. Nevertheless, in Bavaria we might have to differentiate between two main areas of disease. In the northeastern region (Fichtelgebirge, Bayrischer Wald) both SO_2 emission and sulphur contents in pine needles are high; the main causes of disease here are the effects of acid rain on mineral depletion on the one hand, and toxic SO_2 effects on primary biochemical metabolic events on the other. On the other hand, in southern Bavaria, and in the northern parts of the alpine region, neither high SO_2 emissions nor high sulphur contents in needles have been observed. These so-called 'clean air' regions also have relatively low acid depositions and there are no significant mineral deficiencies (Mg^{2+}, Ca^{2+}, Mn^{2+}, K^+) on these mainly calcareous soil types. As initiating principles of chronic intoxication, fluctuations of high and low concentrations of photooxidants with ozone (O_3) as the indicator gas are under discussion. In contrast to S- or N-derivatives oxygen forms such as ozone and organic peroxides cannot be detected per se by measuring changes in their concentration, or via typical fixation products, in the living photosynthesizing cell. Therefore, other parameters indicative of oxidative or photooxidative events or 'stress' have to be applied. Our investigations, based on the analysis of certain biochemical changes and on scanning electron microscopy (SEM), support the following hypothesis for the chain of events leading to symptom expression and finally death:

1) Photooxidants cause pigment bleaching.
2) The wax layer on the cuticles, and particularly the wax plugs covering the stomata are damaged.
3) After partial loss of both structural and physiological resistance towards fungal parasites, infection by pathogens may occur.
4) Needles invaded by fungal pathogens abscise after necrotization. In the following, data are presented which are discussed in the light of the proposed sequence above, together with reports by other groups.

Recent observations

The number of diseased trees and the severity of the visible symptoms have been reported to increase with rates resembling geometrical series or exponential functions. The increase in spruce disease especially since 1980, in the 'clean air' regions of southern Bavaria is particularly evident. Comparing the undamaged, green needles with bleached, yellow needles of the same growth period (1982 or 1983 shoots), we observed that:

1) Mean needle weights and needle length were not significantly different.
2) Water contents were essentially the same.
3) Chlorophyll concentration (based on fresh weight) was reduced by 50–80% in bleached needles.
4) Bleached needles exhibited properties indicating photorespiratory 'oxygen stress'.

The last point will be dealt with in more detail in the following discussion of the relationship between activated oxygen and bleaching.

'External' diagnostics

The term 'external' diagnostics encompasses observations made without the need for homogenizing, extraction or cutting of the biological specimen. Such data can be obtained by visual observation and by headspace gas analysis.

A) Visual observations

Three batches of spruce needles were collected according to their visible symptoms on diseased spruce trees from 17 different locations, namely, green, bleached and partially necrotized needles (10–100% of the needle area). Under SEM at a magnification of 100- to 1500-fold, we observed that bleached needles exhibited more damage symptoms to the wax layer adjacent to the stomata (figs 1–3). Multiple fungal structures and spores were visible on all batches of needles; penetration of hyphae into the interior of the needles was only observed at the points or regions of visible damage (figs 4 and 5). In addition, necrotic needles showed masses of reproductive structures on their surfaces (figs 6 and 7).

B) Gas analysis

Gas chromatographic determination of ethylene and ethane has frequently been used as a marker for physiological irritation. Ethylene is an indicator for stress and decompartmentalization on the one hand, while ethane is a marker for tissue damage on the other[1–4].

Ethylene formation in spruce needles is stimulated in the light with rates ranging from 50 to 200 pmoles/g fresh wt/h. Aminocyclopropane carboxylic acid, the immediate precursor of ethylene[5,6], stimulates ethylene formation in the dark (approx. 20-fold) as well as in the light (approx. 10-fold). Incubation of green or partially bleached (approx. 50% chlorophyll loss) needles for 20 h in 40 mM $NaHSO_3$ solution causes ethane production (approx. 0.5–1.0 nmoles/g fresh wt) which is not further enhanced by a subsequent light period (5 h, 30 klx). Control needles (incubation in H_2O), produce less than 10 pmoles ethane per g fresh wt during the same time period. Ethane, a saturated hydrocarbon gas, is rather unreactive. It can be taken as an indicator for damage or toxicity brought about by certain compounds or physiological situations or events. It reflects the availability of its precursor molecule, α-linolenic acid, in the plant cells if the tissue is mechanically damaged, for example by freezing in liquid nitrogen. Green spruce needles treated for 5 s with liquid nitrogen produce 3 nmoles ethane per g fresh wt. Bleached needles containing approximately 50% of the chlorophyll of the green needles evolve about half (1.5 nmoles) this amount after incubation for 1 h, following freezing. This is good evidence that bleaching is also accompanied by lipid peroxidation, i.e. degradation of α-linolenic acid, which is one of the important fatty acids of the thylakoid membranes. The question now arises as to whether bleaching of spruce needles, which is a slow process only observable in vivo over a period of

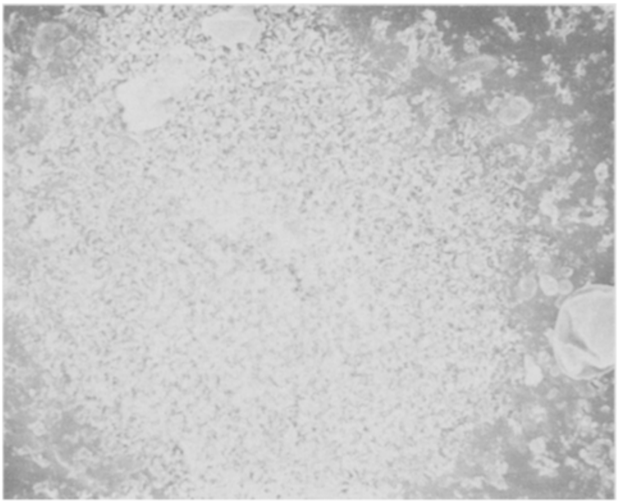

Figure 1. Intact structure of surface waxes covering the stomatal cavities of green needles. ×1400.

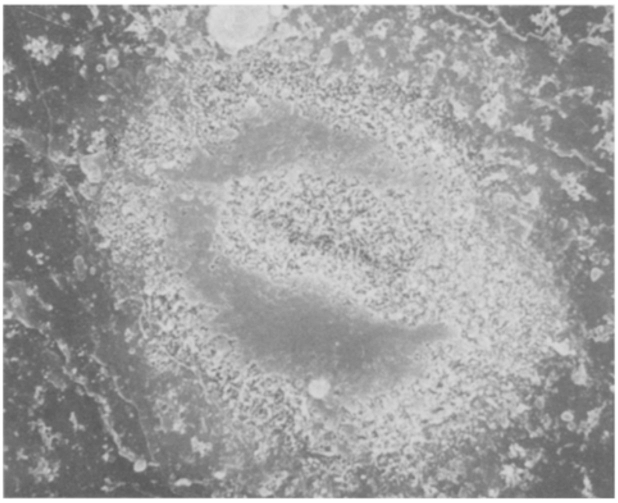

Figure 2. First signs of damage are visible as loss of fine structure of surface waxes similar to the formation of amorphous structures after 'melting'. ×1150.

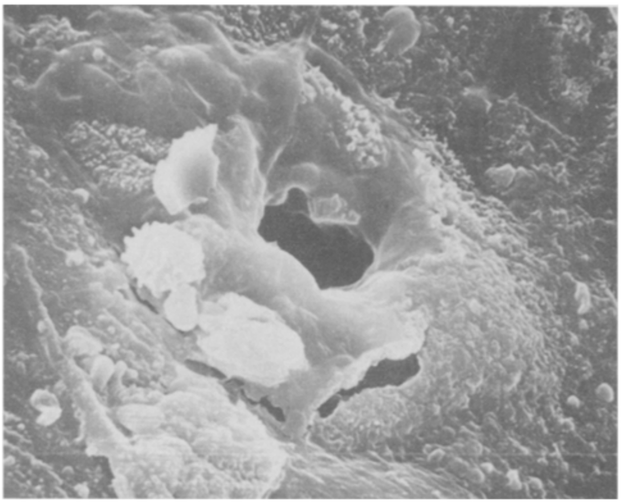

Figure 3. Strongly damaged needles show 'exposed' stomatal cavities after the destruction of wax network. ×1400.

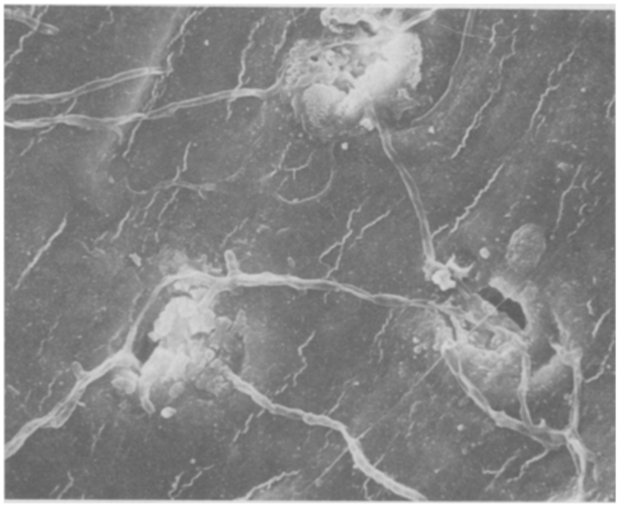

Figure 4. Fungal hyphae on the surface of spruce needles 'connecting' stomatal cavities after the loss of structural integrity. ×450.

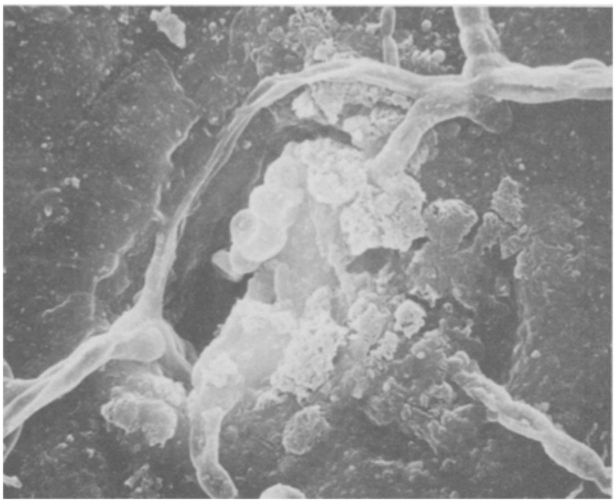

Figure 5. Penetration of hyphae into the interior of the needles after the loss of 'structural resistance'. ×1400.

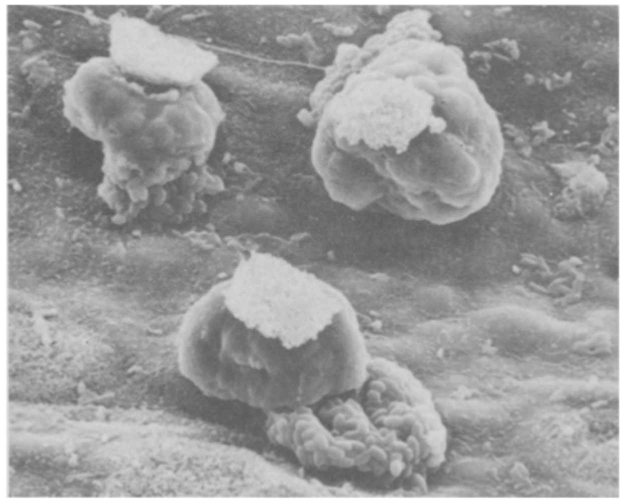

Figure 6. Reproductive fungal structures appear through the stomates carrying parts of the intact wax covers on their top. ×450.

weeks or months, implies a 'stress' situation for the needle tissue, which is accompanied by ethylene formation. Since we cannot measure ethylene in vivo for weeks or months, we have to make advantage of the 'historical' stress marker, malonyl-ACC[5,6].

'Internal' diagnostics

A) Ethylene precursors

In our first experiments in this respect, we found in extracts of spruce needles the following contents of ACC and malonyl-ACC (growth period, 1982).

Table 1. ACC and malonyl-ACC in spruce needles dependent upon the level of bleaching

Appearance	Chlorophyll content* (mg/g fresh wt)	ACC**	Malonyl-ACC***
Dark green	0.78	0	5
Yellow-green, partially necrotic	0.20	3	26
Bleached	0.15	9	170

*Determined according to Arnon[7]. **Determined fluorometrically after derivatization with phthalic dialdehyde and HPLC separation on a reversed phase column. ***Determined to ** after acid hydrolysis[5,6].

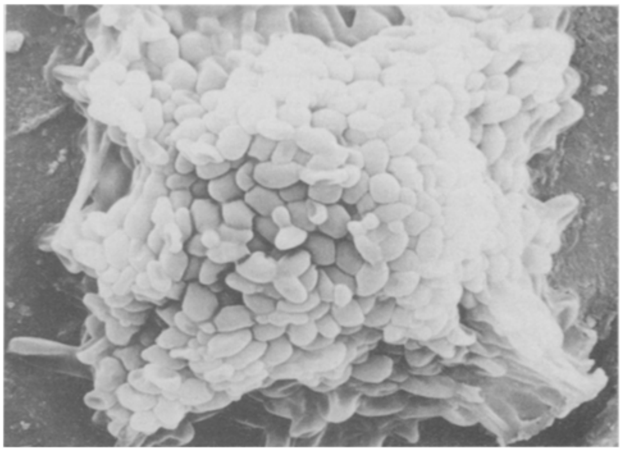

Figure 7. Masses of spores in 'pile formation' are visible on necrotic needles. ×1250.

Malonyl-ACC (M-ACC) may reflect 'historical' events in the needle since there is apparently no active metabolism of this compound. The bleached needles may have accumulated this substance after going through several or severe stress situations, when considerable amounts of ethylene must have been produced in addition to M-ACC. This ethylene production is in addition to the formation of several other typical unsaturated hydrocarbons or terpenes, produced under similar conditions by pine needles, which may have physiological and structural implications as outlined below.

B) Ascorbate

According to the 'Beck-Halliwell' cycle[8,9], ascorbic acid plays an important role in the balance of the redox situation and peroxide detoxication in the chloroplast, thus preventing oxygen toxicity[10,11] according to the following reactions:

$$\text{a) } H_2O_2 + AscH_2 \xrightarrow{\substack{\text{Ascorbate}\\ \text{peroxidase}}} Asc + 2H_2O$$

$$\text{b) } Asc + 2GSH \xrightarrow{\qquad} GSSG + AscH_2$$ (ascorbate reduction by glutathione)

$$\text{c) } NADPH_2 + GSSG \xrightarrow{\substack{\text{Glutathione}\\ \text{reductase}}} 2GSH + NADP$$

Since chloroplasts are devoid of the enzyme catalase, H_2O_2 detoxification at the expense of $NADPH_2$ via the ascorbate ($AscH_2$/Asc) and glutathione (2GSH/GSSG) redox pairs is of great importance. In this context, the determination of enzyme activities (ascorbate peroxidase, glutathione reductase) as well as the redox states of the cofactors ($AscH_2$, GSH) is of interest. Up until now, we only knew that the level of ascorbate is significantly increased in bleached spruce needles. Recent reports on the connection between ozone sensitivity and the above parameters in bean plants are in apparent contrast[12] to our present view[13].

C) Xanthoxin

During fatty acid peroxidation, carotenoids as well as chlorophyll have been shown to be cooxidized[13]. One cooxidation production of the carotenoid, violaxanthin is the growth-retarding plant hormone, xanthoxin, which induces stomatal closure, similarly to abscisic acid. In bleached plant tissue, xanthoxin concentrations appear to be significantly increased (Huber et al., personal communication).

In diseased (bleached) spruce needles, an increase in xanthoxin by a factor of up to 100 in comparison with green needles has been observed, whereas the abscisic acid concentration seemed to be decreased. Since certain problems arose concerning the reproducibility of the absolute values of xanthoxin concentration (determined by HPLC), more reliable data have to be obtained before any conclusions can be drawn from these results.

D) Metal ions

An analysis of K^+, Na^+, Ca^{++}, Mg^{++} and Mn^{++} ions in green and bleached needles from spruce trees on calcareous soils showed that only Mn^{++} content was significantly decreased in bleached needles. Sulphur, nitrogen, K^+ and Ca^{++} levels were almost identical in green and bleached needles, while Na^+ increased by about 5–10% and Mg^{++} decreased by approximately the same amount in bleached needles. Since neither the soil nor neighboring (healthy) trees showed any Mn^{++} deficiency, secondary Mn^{++} translocation in response to bleaching (degradation of photosystem II) may be assumed to have occurred.

E) Activity of key enzymes in oxygen metabolism

During biochemical oxygen activation in the aerobic cell, the following sequence of reactions are of importance:

$$\text{d) } O_2 + e \xrightarrow{E_0' = -160\text{ mV}} O_2^{\cdot -} \;;\; \longrightarrow$$

$$O_2^{\cdot -} + H^+ \longrightarrow HO_2^{\cdot} \; (pK_a = 4.8)$$

Monovalent oxygen reduction yields the formation of superoxide ($O_2^{\cdot -}$) and its corresponding acid, the hydroperoxyl radical ($HO_2^{\cdot}$).

$$\text{e) } O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{(SOD)} H_2O_2 + O_2$$

The dismutation of superoxide proceeds uncatalyzed at neutral pH with a rate constant of $2 \times 10^{-5}\ M^{-1}s^{-1}$. Under the catalysis of the metalloenzyme(s) superoxide dismutase (SOD), the above rate constant is increased by 10^4-fold, giving a value of $2 \times 10^9\ M^{-1}s^{-1}$.

$$\text{f) } H_2O_2 + H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

The degradation of hydrogen peroxide is catalyzed by the peroxisomal enzyme catalase (CAT) in a two-step reaction:

$$\text{f}_1\text{) } H_2O_2 + CAT \xrightarrow{k_a} \text{'compound I'}$$

$$\xrightarrow{k_b} \text{'compound II' (inactive)}$$

$$\text{f}_2\text{) compound I} + H_2O_2 \longrightarrow CAT + 2\,H_2O + O_2$$

Since there are several reports that SOD activities increase (induction of de novo enzyme synthesis?) under increased oxygen pressure or peroxidatic conditions (cf Elstner[11]), whereas catalase activity is decreased under the influence of certain bleaching herbicides, we examined these enzyme activities in green and bleached needles (table 2, Elstner and Osswald[13]).

Table 2. Relative activities of catalase (CAT) and superoxide dismutase (SOD) in green and bleached spruce needles

Enzyme source*	Chlorophyll content and enzyme activities Chlorophyll (mg/g fresh wt)	SOD Units/mg protein	CAT Units/mg protein
Needles from *Picea abies*			
1981 growth period			
Green	1.58	120	6
Bleached	0.58	260	1
1982 growth period			
Green	1.11	125	10
Bleached	0.29	130	0.5
Needles from *Abies alba*			
1981 and 1982 growth periods			
Green	1.02	17	154
Bleached	0.6	40	not detectable

*Obtained by acetone precipitation and dialysis of the respective crude extracts.

As shown in table 2, catalase activities decreased significantly, while SOD tended to become increased in chlorophyll-deficient needles of two pine species *Abies alba* and *Picea abies* (cf reactions e and f).

Biochemical events initiating pigment bleaching

The processes involved in pigment degradation may be divided into two sequential categories of events:

1) induction reactions[14],
2) cooxidations during fatty acid peroxidation[15].

Reactions inducing bleaching may be generally described as occurring under conditions where photosynthesis is impaired. Such conditions comprise long dark periods, blocking of electron transport, uncoupling of electron transport, electron channelling to oxygen and blockage of transport systems. If we exclude long dark periods as being unphysiological, all other impairments may occur under physiological conditions, desirable or undesirable. As outlined recently[11, 13], the results of the above types of interference with the basic mechanism of photosynthetic carbon flow from CO_2 penetration through the stomata until translocation of photosynthetic products can lead to oxygen activation. The following distinctions can be made:

1) Photodynamic production of singlet oxygen $^1O_2$, according to lack of potential in charge separation after chlorophyll activation in the light;

$$\text{g) Chl} \longrightarrow \text{Chl*} \quad (\text{Chl* = activated chlorophyll})$$

$$\text{h) Chl*} \longrightarrow {}^T\text{Chl} \quad ({}^T\text{Chl = triplet state chlorophyll})$$

$$\text{i) } {}^T\text{Chl} + O_2 \longrightarrow \text{Chl} + {}^1O_2$$

Singlet oxygen may be quenched by cartenoids or α-tocopherol in the thylakoid membranes or directly react with unsaturated fatty acids such as linolenic acid (LH):

$$\text{j) } LH + {}^1O_2 \longrightarrow LOOH$$

2) Reductive oxygen activation by photosystem I (PS I) under conditions of electron channeling (paraquat-treatment or lack of $NADPH_2$ reoxidation (blocking of carbon flow):

$$\text{k) } 2\ PS\ I_{red} + 2O_2 \longrightarrow 2\ O_2^{\cdot -} + 2\ PS\ I_{OX}$$

where reactions identical to (d) and (e) occur.

As demonstrated earlier[16], H_2O_2 is further reduced by reduced ferredoxin (fd_{red}) yielding oxygen species with properties similar to the aggressive free $OH^{\cdot}$ radical, $OH^{\cdot}$:

$$\text{l) } fd_{red} + H_2O_2 \longrightarrow fd_{OX} + OH^- + (OH^{\cdot})$$

This species ($OH^{\cdot}$) may similarly to $HO_2^{\cdot}$ (cf reaction d) activate unsaturated fatty acids in the thylakoid membrane:

$$\text{m) } (OH^{\cdot})/HO_2^{\cdot} + LH \longrightarrow L^{\cdot} + H_2O/(H_2O_2)$$

The alkyl radical $L^{\cdot}$ may induce a chain reaction yielding hydroperoxides (as also described by reaction j):

$$\text{n) } L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$

$$\text{o) } LOO^{\cdot} + LH \longrightarrow L^{\cdot} + LOOH$$

Thus, the products of the induction reactions seem generally to be hydroperoxides of unsaturated fatty acids.

The following secondary events concern cooxidations in context with the degradation of the above hydroperoxides:

$$\text{p) } LOOH + M^{n+} \longrightarrow LO^{\cdot} + OH^- + M^{(n+1)+}$$

The metal-catalyzed production of alkoxy radicals is followed either by further chain reactions:

$$\text{q) } LO^{\cdot} + LH \longrightarrow L^{\cdot} + LOH$$

or, by β-scission of a neighboring C – C bond leading to the production of an alkyl radical fragment:

$$\text{r) } LO^{\cdot} \longrightarrow L_1 = O + L_2^{\cdot}$$

where $L_2^{\cdot}$ may be $CH_3CH_2^{\cdot}$ if the oxy-function was located at the ω-3 position of linolenic acid. The ethyl radical is reduced by an endogenous electron donor, to form the above-mentioned ethane molecule. $LO^{\cdot}$ on the other hand, in analogy to reaction (q) can cooxidize pigments (PH), initiating their oxidative bleaching.

$$\text{s) } LO^{\cdot} + PH \longrightarrow LOH + P^{\cdot}$$

$$\text{t) } P^{\cdot} \longrightarrow \text{decay, loss of color}$$

One of the consequences of these cooxidations is the visible loss of color as is possibly also the production of the inhibitory plant hormone, xanthoxin, when the cooxidized pigment is the carotenoid, violaxanthin.

Possible mechanisms of damage involving air pollutants

According to the phytomedical diagnostic data described above, we have to assume that damage and finally death of spruce needles is brought about in three steps:

1) Disturbance of physiological reactions in the photosynthesizing cell.

2) Damage to the surface wax layer, especially close to stomatal apertures.

3) Colonization of the needles by pathogenic fungi after partial loss of physiological and structural resistance according to 1 and 2 followed by necrotization and abscission of the needles.

During the course of the above three phases involved in needle damage, we have to envisage at least three light-dependent processes both inside and outside the plant cells. The sequence of these processes may be outlined as follows:

I) Primary sources of major air pollutants (SO_2, NO, NO_2, unsaturated hydrocarbons)

These comprise power plants and combustion engines. SO_2 is not a major air pollutant and acid is not a major problem in calcareous habitats in the northern alpine region and southern Bavaria. However, in these clean air districts, NO_x produced by gasoline combustion (airplanes, cars) are probably the sources for the starting point of the deleterious reaction sequence. According the well-known conversions of NO_x into photooxidants, the photodynamic equilibrium

$$\text{u) } NO_2 + O_2 \xleftrightarrow{h \cdot \nu,\ UHC} NO + O_3$$

is dependent upon light ($h \cdot \nu$, $\lambda < 430$ nm). The equilibrium is shifted to the right by unsaturated hydrocarbons (UHC). The composition of these hydrocarbons determines the nature of the photooxidants, i.e. the alkyl moiety of the resulting peroxyacyl nitrates. Peroxyacetyl nitrate (PAN, $CH_3 \cdot CO \cdot OONO_2$) is one of the major photooxidants produced in industrial areas subjected to high quantum flux densities ('Los Angeles type smog'). Ozone is one of the markers for this type of photodynamic conversion. Since approximately 60–70% of worldwide unsaturated hydrocarbons are from biological sources, especially forests[17,18], the above equilibrium is strikingly governed by the availability of NO_x. Diesel and jet engines may introduce exceptionally large amounts of hydrocarbons, in addition to the natural sources.

II) Biological reactivity of photooxidants

Taking ozone (O_3) as the representative model compound and the visible symptoms of spruce needles (bleaching, loss of structural integrity of the wax layer), we have to consider the following possible chemical reactions and physiological consequences:

$$\text{v)}\ O_3 + C_2H_4 + H_2O \longrightarrow 2\ HCHO + H_2O_2$$
$$\text{(with extremely fast kinetics, } K \gg 10^8\text{)}$$

Ozone reacts with biogenic ethylene forming reactive compounds such as formaldehyde and hydroperoxides. Since we know that ethylene is a stress hormone, this reaction can be assumed to be of special significance for trees or branches under any kind of stress, for example drought, mechanical influences, flooding, mineral deficiency and others.

$$\text{w)}\ O_3 + H_2O + OH^- \xrightarrow{\text{aromatic compounds*}} H_2O_2 + O_2^{\cdot} + OH^{\cdot}$$

* for example from diesel exhaust.

There are several reports on the degradation of ozone in aqueous solution producing different active oxygen species such as the superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and the $OH^{\cdot}$ radical (Grimes et al.[19]). Very recently, the production of $OH^{\cdot}$ under physiological conditions (determined by ESR using 5,5-dimethyl-1-pyrroline N-oxide as a spin trap), has been reported to be catalyzed by aromatic compounds[19].

The very reactive $OH^{\cdot}$ radical with a redox potential of circa +2 V has an extremely short lifetime in biological systems. It has been shown to react almost randomly with all biomolecules. Ozone, on the other hand, has been reported not to damage protoplast integrity directly[19]; it seems, however, to damage specific sugar translocators in the plasma membrane (by oxidizing essential thiol groups?), resulting in a decrease in assimilate transport from the leaves to the rhizosphere[20]. This effect thus induces secondary symptoms similar to those of certain systemic viral infections which, by blocking phloem transport, lead to an increased starch accumulation and finally bleaching of the photosynthetic pigments. Older experiments by Dugger and Ting[21] support the hypothesis that blocks or limitations in sugar translocation increase sensitivity towards ozone on the one hand and, on the other hand, that ozone may be responsible for such blocks in the translocation.

Here we have to note certain cases where high sugar contents in the leaf cells may prevent later damage by acute doses of ozone but increase (reversibly) pigment bleaching.

Yet another point has to be taken into account: resin acids with aromatic groups in pine needles such as 1-hydroxy dihydroabietic acid and [13]-hydroxypodocarpic acid both exhibit fungistatic effects and act as germination inhibitors for fungal spores[22]. It may thus not seem unlikely that the critical steps in the observed diseases of *Picea abies,* are represented by:

1) Destruction of these compounds in cuticular waxes by the above mechanism (O_3, $OH^{\cdot}$).
2) Stimulation of germination and growth of fungal germ tubes and hyphae by certain air pollutants, now acting as nutrients (S, N supply).
3) Leaching of minerals, substrates and vitamins from the leaf surface after damage, leading to the support of growth of the fungus.
4) Loss of structural resistance towards colonization.
5) Pigment bleaching by known reactions involving oxygen activation in response to physiological impairments[23–26].

Acknowledgments. The authors are grateful to Dr W. Mücke and Prof. H. Ziegler for discussions and to M. Freudenberg and G. Wagner for experimental support. The work was financed by the Bayer. Staatsministerium für Landesentwicklung und Umweltfragen.

1 Elstner, E.F., and Konze, J.R., Effect of point freezing on ethylene and ethane production by sugar beet leaf discs. Nature *263* (1976) 351–352.
2 Peiser, G.D., and Yang, S.F., Ethylene and ethane production from sulfur dioxide-injured plants. Pl. Physiol. *63* (1979) 142–154.
3 Bressan, R.A., LeCureux, L., Wilson, L.G., and Filner, P., Emission of ethylene and ethane by leaf tissue exposed to injurious concentrations of sulfur dioxide or bisulfite ion. Pl. Physiol. *63* (1979) 924–930.
4 Kimmerer, T.W., and Kozlowski, T.T., Ethylene, ethane, acetaldehyde and ethanol production by plants under stress. Pl. Physiol. *69* (1982) 840–847.
5 Amrhein, N., Schneebeck, D., Skorupka, H., Tophof, S., and Stöckigt, J., Identification of a major metabolite of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid in higher plants. Naturwissenschaften *68* (1981) 619–620.
6 Hoffman, N.E., Liu, Y., and Yang, S.F., Changes in 1-(malonylamino) cyclopropane-1-carboxylic acid content in wilted wheat leaves in relation to their ethylene production rates and 1-aminocyclopropane-1-carboxylic acid content. Planta *157* (1983) 518–523.
7 Arnon, D.I., Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Pl. Physiol. *24* (1949) 1–15.
8 Groden, D., and Beck, E., H_2O_2 destruction by ascorbate dependent systems from chloroplasts. Biochim. biophys. Acta *546* (1979) 426–435.
9 Foyer, C.H., and Halliwell, B., The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta *133* (1976) 21–25.
10 Elstner, E.F., Mechanisms and products of photosynthetic oxygen activation, in: Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase. Developments in Biochemistry, vol. 11b, p. 434. Eds J.V. Bannister and H.A.O. Hill. Elsevier/North Holland, Amsterdam 1980.
11 Elstner, E.F., Oxygen activation and oxygen toxicity. A. Rev. Pl. Physiol. *33* (1982) 73–96.
12 Guri, A., Variation in glutathione and ascorbic acid content among selected cultivars of *Phaseolus vulgaris* prior to and after exposure to ozone. Can. J. Pl. Sci. *63* (1983) 733–737.
13 Elstner, E.F., and Osswald, W., Fichtensterben in 'Reinluftgebieten': Strukturresistenzverlust. Naturw. Rdsch. *37*/2 (1984) 52–61.
14 Youngman, R.J., and Elstner, E.F., Primary photodynamic reactions occurring during the breakdown of photosynthetic pigments. Ber. dt. bot. Ges. (B) *96* (1983) 357–364.
15 Grosch, W., and Laskawy, G., Co-oxidation of carotenes requires one soybean lipoxygenase isoenzyme. Biochim. biophys. Acta *575* (1979) 439–445.
16 Elstner, E.F., Saran, M., Bors, W., and Lengfelder, E. Oxygen activation in isolated chloroplasts. Mechanism of ferredoxin-dependent ethylene formation from methionine. Eur. J. Biochem. *89* (1978) 61–66.
17 Bufalini, J.J., and Arnts, R.R. (eds), Atmospheric biogenic hydrocarbons, vols 1 and 2. Ann Arbor Sci. Publishers Inc., Ann Arbor, Michigan 1981.
18 Kohlmaier, G.H., Bröhl, H., and Siré, E.O., Über die mögliche lokale Wechselwirkung anthropogener Schadstoffe mit den Terpen-Emissionen von Waldökosystemen. Allg. Forst- Jagdztg *154* (1984) 170–174.
19 Grimes, H.D., Perkins, K.K., and Boss, W.F., Ozone degrades into hydroxyl radical under physiological conditions. A spin trapping study. Pl. Physiol. *72* (1983) 1016–1020.

20 McCool, P.M., and Menge, J.A., Influence of ozone on carbon partitioning in tomato: potential role of carbon flow in regulation of the mycorrhizal symbiosis under conditions of stress. New Phytol. *94* (1983) 241–247.
21 Dugger, W.M., and Ting, I.P., Physiological and biochemical effect of air pollution oxidants on plants, in: Recent Advances in Phytochemistry, vol. 3, pp. 31–58. Eds C. Steelink and V.C. Runeckles. Appleton-Century-Crofts, New York 1970.
22 Franich, R.A., and Gadgil, P.D., Fungistatic effects of *Pinus radiata* needle epicuticular fatty and resin acids on *Dothistroma pini*. Physiol. Pl. Path. *23* (1983) 183–195.
23 Elstner, E.F., and Pils, I., Ethane formation and chlorophyll bleaching in DCMU-treated *Euglena gracilis* cells and isolated spinach chloroplast lamellae. Z. Naturf. *34c* (1979) 1040–1043.
24 Youngman, R.J., Dodge, A.D., Lengfelder, E., and Elstner, E.F., Inhibition of paraquat phytotoxicity by a novel copper chelate with superoxide dismutating activity. Experientia *35* (1979) 1295–1296.
25 Schobert, B., and Elstner, E.F., Production of hexanal and ethane by *Phaeodactylum tricornutum* and its correlation to fatty acid oxidation and bleaching of photosynthetic pigments. Pl. Physiol. *66* (1980) 215–219.
26 Youngman, R.J., Schieberle, P., Schnabl, H., Grosch, W., and Elstner, E.F., The photodynamic generation of singlet molecular oxygen by the fungal phytotoxin, cercosporin. Photobiochem. Photobiophys. *6* (1983) 109–119.

0014-4754/85/050591-07$1.50 + 0.20/0

Infectious diseases in forest trees caused by viruses, mycoplasma-like organisms and primitive bacteria

F. Nienhaus

Institut für Pflanzenkrankheiten, Abt. Virologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Nussallee 9, D-5300 Bonn (Federal Republic of Germany)

Key words. Viruses; mycoplasma-like organisms; Rickettsia-like bacteria; forest tree-diseases.

The spread of forest decline in Europe and other parts of the northern hemisphere in the last decade is associated with the complex interaction of a number of abiotic and biotic factors that lead to stress and to gradual or sudden degeneration of the trees. According to Manion[24] a decline syndrome may be caused by three or more sets of interchangeable factors of predisposing, inciting, and contributing categories interacting in a decline disease spiral (fig. 1).

Predisposing factors such as genetic potential, old age of the tree, and environmental conditions (climate change, wrong soil type or site conditions, poor fertility, air pollu-

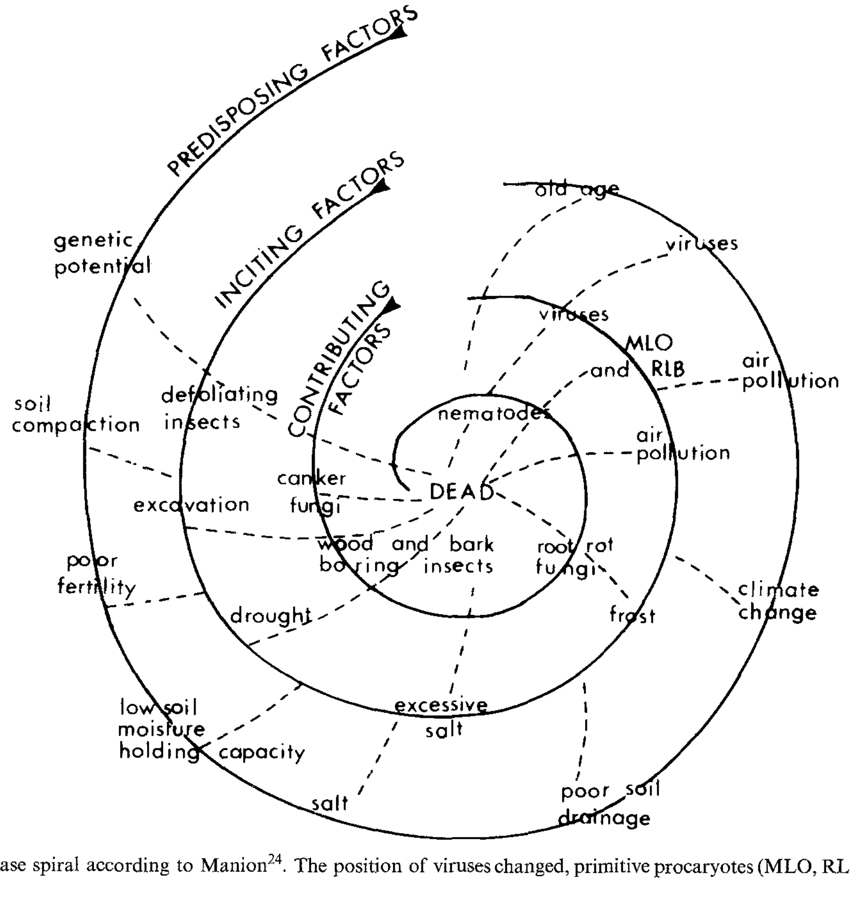

Figure 1. Decline disease spiral according to Manion[24]. The position of viruses changed, primitive procaryotes (MLO, RLB) added by the author.