Forest decay in a continental-wide polluted environment: control by silvicultural measures

J.-Ph. Schütz

Institute for Forest and Wood Research of the Swiss Federal Institute of Technology, Chair of Silviculture, CH-8092 Zürich (Switzerland)

Key words. Forest decay; silviculture; air pollution.

Introduction

Present rates of pollution in forest ecosystems of Central Europe show an increase of between 10 and 100 times or even more compared to the situation in forests untouched by civilization[55]. This does not only lead to temporary disturbance but causes permanent modification of the environment of these forest ecosystems and, consequently, seriously upsets regulation mechanisms. The problem of forest decline should therefore be looked at from its ecosystemic[50] and cybernetic aspects[44]. Not only the immediate reaction of trees to air pollution is involved, but also the adaptive ability of trees[34] as well as that of the ecosystem to which they belong. In an analysis of silvicultural measures careful attention should therefore be paid in particular to site conditions, existing species, and types of canopy[34].

The ability of forest ecosystems to overcome environmental stress has, admittedly, been overestimated[9]. In fact ecosystems such as forests, which are still comparatively natural, have turned out to be particularly vulnerable ecologically, and they are also vulnerable because of their long life, which gives them little elasticity[53].

In fact, it is easier to adapt technical systems, by the use of appropriate technologies, to meet the needs of particularly sensitive biological systems than the other way round. It is absolutely essential that we should now try to find and define the exact equilibrium between industrial development and sensitivity of ecosystems. Industry should no longer be allowed to continue development, as in the past 100 years, at the expense of nature[9].

On the other hand, concrete silvicultural measures cannot be formulated without first having as much and as precise information as possible on the etiology of the decline and its mechanisms[27]. But our present knowledge is still very fragmentary and, above all, limited to individually evaluated facts. What we lack most is an appreciation of the overall situation and of the various interactions.

Based on the knowledge acquired in the course of more than 100 years of damage by noxious gases in industrial regions[45] as well as on results from experimental research on plant reaction to different pollutants, for instance in fumigation chambers[10,16], but also based on our fundamental knowledge of forest ecosystems, we can already now define general silvicultural strategies and outline certain particular measures. In view of the gaps in our knowledge and the complexity of interaction, the silvicultural strategies and measures proposed today can only provide a general framework, to be improved upon as our knowledge increases.

In any case, when proposing any measures today one should avoid the danger of presenting remedies for particular phenomena, however plausible and well-defined these measures may be, without first verifying, at least theoretically, their effect on the system as a whole. There is a risk in wanting to find an answer to each one of the manifestations of forest decline which might turn out to be negative in other respects, and might not finally strike at the root of the evil.

Definition of immission standards compatible with silvicultural objectives

The formulation of silvicultural measures starts with an analysis of the tolerance limits situation for forests. On the one hand, we may be faced with a pathological pollution situation for forests, i.e. a context of fatal or near-fatal pollution as we are experiencing at present and which has nothing to do with silviculture. On the other hand, we are concerned with the production of timber as raw material (in other words, a silvicultural activity) which can only be contemplated provided the level of pollution does not exceed a certain critical threshold[44].

It might sometimes be possible to consider different forms of timber production for different levels of pollution[33]. However, since Odén[28] showed the enormous distances over which these pollutants may be carried (something like 50 km for gas, 100 km for aerosols and as much as 1000 km for wet depositions) any regional differentiation of pollution seems to be highly illusory. The definition of compulsory immission standards is therefore, for silviculture, the first question to be solved and generally agreed upon. There are, however, many difficulties in the way of reaching an objective definition of such standards.

Different forest ecosystems show very different reactions to pollution. There are some comparatively stable ecosystems; others are rather more unstable because certain important site factors create marginal conditions. This may be the case with mountain forests because of the limiting effects of temperature, or that of forests on soils particularly sensitive to acidification or to deficiencies of certain nutrients, namely Mg and Ca (for instance on mottled sandstone, certain granites, metamorphic schists)[6,62]. Such marginal ecosystems are obviously more vulnerable to pollution[57]. Schütz[44] suggests that precisely those marginal ecosystems which provide essential production or protection, or have other important functions, should be used as standards for the definition of maximum immission.

It must be emphasized that the choice of species on such marginal sites is usually very limited. An example of this are the spruce forests of the upper montane and subalpine level in the northern Alps, consisting frequently of spruce stands alone, sometimes interspersed with silver fir, both species which are particularly sensitive to air

pollution[44, 59]. In such forests with predominantly protective functions one cannot replace either spruce or silver fir with other species. Mayer[26] has underlined the important role played by silver fir as ecological stabilizer.
More recent findings have shown that increased frost sensitivity as a consequence of air pollution plays a decisive part in the initiation of the decline process[3, 6, 35], and would explain the concentration of damage above a certain altitude, namely around 1000 m. One must realize, of course, that dieback of trees and forests is in fact only the end of a long and complex pathological process which may have been initiated several years earlier. According to the Council of Environmental Experts of the German Federal Republic[9] there are numerous valid reasons for believing that this process is due to the combined effects of several factors acting jointly or in parallel. These effects are not only additive but presumably also synergetic, as laboratory tests of the combined action of air pollutants have shown, even if not always clearly[61]. The effect of synergism may be said to apply to the action of abiotic factors (e.g. dryness, frost), and even to biotic ones too.
All this complicates singularly any definition of immission standards. Although controlled fumigation tests allow us to work out standards for the different types of pollutants, such tests apply to very favorable conditions, since they are done on young vegetation and therefore on more tolerant material than mature forest trees would be[44]. IUFRO experts on air pollution (International Union of Forest Research Organizations) propose different standards for SO_2 according to whether forests are used for timber production or have predominantly protection or welfare functions[58]. They suggest an average limit of 50 $\mu g/m^3$ of air per annum for production forests and 25 $\mu g/m^3$ for protection forests.
To take into account all the unpredictable factors, and particularly synergetic effects, Bucher[8] suggests the reduction by a factor of 3 of immission standards obtained in fumigation tests, for practical application. Wentzel[59] proposes the following scale of risks for SO_2, based on observation of actual damage:

Plantation risk of spruce and Scots pine forests in regions of SO_2 pollutions based on forest observation

Mean annual concentration ($\mu g/m^3$)	
15	Safe for coniferous trees
20	Damage restricted to medium altitude in mountains Reduced frost resistance
30	Slight chronic damage to pine and spruce on poor sites. Silver fir dieback
40	Damage becoming manifest at higher altitudes and predisposition for secondary damage in the plains
50	Symptoms of damage on good sites in plains
60	Chronic damage becoming manifest on good sites Loss of increment

Characterization of silvicultural measures

A distinction should be made between those silvicultural measures taken in pathological situations or times of crisis such as those existing at present in many European regions, which are emergency measures for forest hygiene[27], and those silvicultural measures which are to be applied within a context of tolerable pollution limits and which have a permanent and prophylactic character.
Observation of the decline of silver fir and spruce stands in southern Germany (Baden-Württemberg) shows that if the development of the last two years continues, the complete disappearance of silver fir and spruce is to be expected in about 10 years' time[40]. Such a situation calls for immediate action to avoid collapse and opening-up of stands, and to cope with subsequent erosion problems and the appearance of secondary damage. These are all emergency measures which are not going to be discussed here in more detail.
In view of the belated appearance of symptoms of dieback, which become manifest long after ecosystem stability has become affected and after a long period of invisible or latent damage, it seems to be completely illusory to apply measures of a therapeutic character in sick stands[27]. Silvicultural measures can therefore only be essentially preventive in character, and aim on the one hand at the improvement of ecological stability of the forests as such, by creating the most favorable situation for stands so that they can best resist disturbance and, on the other hand, at distribution of risks.

Silvicultural measures to be taken when stands are established

When stands are created, choice of species and their mixture are among the most efficient silvicultural measures, at least on sites offering alternative possibilities. There are in fact considerable behavioral differences in the reaction of species to air pollution, but Reemtsma emphasizes that one should also take account of the toleration of species towards other factors, in particular soil degradation[34], which is evidently not the same.
Selection of species suited to the site should in the first place be made to improve ecological stability. A species perfectly suited to the site tolerates disturbance better[44]. By creating stands of many species, as closely mixed as possible according to tending criteria, we comply with the second criterion postulating distribution of risks. This means, however, comparatively intensive forest tending.
It is important in this connection to realize that any form of silviculture based on a mechanistic production concept of maximum yield in monocultures, particularly coniferous ones, should be avoided, as emphasized by Ulrich[55] since the inherent risks are too great, even if the selected species, such as spruce, offer quite remarkable characteristics and benefits. We know now that such monocultures may have negative consequences, for example through soil acidification[56]. Only differentiated silviculture, near to nature and based on principles which minimize risks, is appropriate[44]. This type of silviculture requires, however, intensive forest management and especially forest tending, for which most Forest Services are not or are no longer organized. One of the first measures consists of adequate reorganization, especially in an increase of field personnel in order to intensify forest management[9].
The most important problem involved in the selection of species in a polluted environment is that forest tree spe-

cies react differently to different types of pollution and their various ways of action. If we believe today[9] that the main mode of action is through the assimilation system of trees, this certainly does not exclude other modes of action such as leaching (removal of nutrient substances from leaves), reduced resistance to frost, and effects on the root system and biotic defense system of the tree.
Our knowledge of the resistance abilities of trees is essentially derived from laboratory fumigation tests mainly involving one way of action. Observation of actual damage does not always confirm these results. Although it was believed until recently that broadleaved trees were generally less vulnerable, we can see now that this is no longer the case[42]. Besides, as regards resistance to pollution, there is no such thing as actual immunity; some species and individuals tolerate disturbance better than others, but this only postpones the moment of decline for a while.

Selecting production goals and silvicultural treatment

Air pollution finally leads to premature ageing of forests, and final utilization is much earlier than the economically optimal production time. With the present pollution in Germany, Mülder[27] estimates that conifers will hardly survive for more than 30 to 40 years. Obviously, any additional disturbance would have ill-fated and probably synergetic effects in such a situation, whether these disturbance factors were endogenous (reaction to thinning) or exogenous (climatic extremes such as drought).
The type of silviculture to be practised should therefore promote the vigor of stands as well as attempt to produce maximum development of the dimensions of trees as these will be cut prematurely. As Tesar[47] suggests, this is only possible by very early and repeated interventions right from the earliest youth of stands, i.e. from the thicket stage onwards, even if the interventions as such cause financial loss. For it is at this period that trees react best to any changes in their environment. It is therefore important to ensure optimum development of crowns but at the same time avoid disruption of the canopy[31,47,57]. Although very active, this type of silviculture should avoid any brutal interventions. It is therefore necessary to have frequent and moderate interventions to achieve the best possible results in view of competition within the collectives.
Another reason for thinning is to profit from the possibilities of selection. This is an opportunity to rely on individual differences of reaction to disturbance appearing within a collective of trees, and to discover these differences early enough. The only useful criterion available at present is crown vitality[33,57]. Proper selection requires, however, a sufficiently large number of trees from which to select. It is therefore advisable to come back to higher densities when establishing new stands. Pollanschütz[33] suggests densities of not less than 3500–5000 stems per hectare.
Any other measures to ensure vigor and good health of stands, whether biotic ones (encouraging undergrowth for the improvement of biological soil activity) or technical ones (prevention of injuries; not using large machines, making the soil more compact, etc.) complete the range of silvicultural possibilities. One can see that they can be made use of only by means of comparatively intensified forest organization and management using 'soft' techniques and involving to a large extent highly skilled manual labor[44].
Such propositions may seem to contradict frequently-made observations in damaged areas of a negative reaction of stands to thinning intervention as well as to any opening of the canopy. It is obvious that any thinning intervention acts as a shock for the stand; a shock which is normally favorable because it reduces competition, but which may have unfavorable or even fatal effects if the stand in question is in a state of lability. Once again, any interventions have therefore to be made extremely early and at a time when the regeneration ability of the crowns is at its best, i.e. when height growth is most active, at the pole stage and timber stage. Naturally, in affected stands thinning cannot be a therapeutic measure, at the most a sanitary one. In such cases thinning should be limited to what is absolutely essential, precisely in order to avoid introducing additional stress factors.
Mention should also be made of the problem of stand stability with regard to storms. It has in fact been shown that the decline has unfavorably modified the form of trees[11,32] because annual tree rings get smaller from the top to the base of trees[1], thus decreasing tapering or increasing h:d coefficient and therefore sensitivity to wind break. Development of favorable tree forms is closely linked with crown education, namely encouraging the growth of long crowns. This can only be done by regulating density in young stands.

Forest regeneration methods

Selection of regeneration technique and the appropriate type of regeneration may present a certain dilemma. The danger of a sudden increase in soil acidification when soil is suddenly uncovered makes Ulrich[54,55] propose a technique of stand regeneration which avoids clear felling and works through progressive reduction of old stands. This would in fact imply the use of natural regeneration although frequently the acute problem of excessive game populations would have to be solved first.
On the other hand, it has been shown that the need for selection of species and provenances has often implied artificial regeneration. Moreover, Keller[16] has shown that comparatively weak doses of SO_2 caused perceptible reduction of pollen germination power, particularly of the European black pine, but also of other species, already after a few days of exposure[4]. Independently of any long-term effects regarding a possible waste of genetic material, these findings may explain increased field observation of more and more difficulties with natural regeneration, in particular with beech[12,27] although, of course, pollution cannot as yet be held entirely responsible.
Whatever the case may be, for reconciling these different aspects, more frequent use of underplanting is suggested[33]. Other possibilities are the use of pioneer crops and advance planting.

Measures for genetic improvement

There is general agreement that there are very marked individual differences within tree populations as regards resistance to air pollution. As we know since 1962[30], there are individual trees apparently showing good resistance to noxious air pollution in highly polluted and badly damaged regions. Experiments in fumigation chambers show that the same clones of different coniferous species appear to be resistant to the different types of pollutants (SO_2, NO_x, and O_3) and to their combined effects[61]. This suggests a common mechanism of resistance, and the possibility of using these characteristics for silvicultural prophylaxis. This correlation has, however, not been verified for all species, for example for the pedunculate oak *(Quercus robur)*[19].

Rohmeder and Schönborn[36] showed that spruce has very marked heritability of individual variability of resistance to air pollution. According to Tzschacksch[48] this heritability may be around 60% and be based on additive genic action.

Very considerable differences in resistance to decline are also found between populations (ecotypes or provenances) within the same species: A typical example of such a species is silver fir (*Abies alba* Mill.), the mimosa of forest trees[27]. Its inter- and intrapopulation variability has been known since Pavari[29] published his research results. Trials with old provenances, particularly those planted in Denmark in the thirties, showed considerable differences of production and vitality after 44 years of observation with regard to dieback. Provenances from the southern and eastern regions of the natural distribution range of silver fir, i.e. natives of regions with a Mediterranean maritime climate (Calabria, Carpathian Mountains, southern Serbia), offer the best resistance characteristics[21]. Such observations suggest that resistance to forest decline could be linked with drought resistance mechanisms[7,18]. In his research on silver fir seedlings Marcet[24] found very marked differences in reaction to water stress between ecotypes from regions with dry and humid climates, which confirms the interest that drought-reaction criterion can have for such studies. It would also be useful to know more about genetic variability within the natural distribution range, together with a knowledge of postdiluvian patterns of colonization and genetic differentiation mechanisms[26].

However, we should not overestimate the possibilities of genetic improvements. Tzschaksch[48] is right in pointing out that SO_2 acts mainly as an assimilation toxin and that we cannot expect complete immunity against this effect. At the very best can we improve intrinsic resistance characteristics. One must be aware that the selected trees are also going to decline, even if a little later than more sensitive individuals.

Moreover, in view of the long production time of trees and consequently the great number of biotic and abiotic risks and their interactions it does not seem to be advisable to aim at resistance to one single toxic agent or one single pest, if we do not want to risk that individuals resistant to one danger may turn out to be highly sensitive to others. A sufficient genetic basis should therefore be maintained to allow distribution of risks[44]. Maintaining genetic heterogeneity means, in fact, working with generatively reproduced populations, and this independently of the fact that the problems of vegetative reproduction of coniferous trees have not yet been solved, at least not for practical application. Inbreeding problems which may be caused by the use of clones should also be kept in mind.

In conclusion, the majority of writers agree on the long period of research needed to improve resistance, and that no rapid solution of these problems can be expected[19,38,44,48]. As a research method they suggest delimitation of resistant provenances, the creation of seed orchards composed of numerous resistant clones[48], and even intraspecific cross-breeding[19]. One of the basic methodological problems of such research is the definition of criteria for objective and early identification of resistance.

Liming as a silvicultural measure

No other method is so controversial as liming in forests. Its principle is based on Ulrich's ideas[52–55] about soil acidification caused by the intake of enormous quantities of weak acids into the soil system and subsequent modifications of its nutrient availability as well as toxic effects on the fine root systems of trees. This theory has frequently been queried[6,27] by writers who attribute the decline mainly to the leaching of Mg and Ca directly from the needles and less to nutrient deficiency of the soil.

Ulrich[55] points out that liming does not solve the fundamental problem of soil modification, and that only by drastically reducing pollution can the nutrient and filtering power of the soils be safeguarded and groundwater quality be maintained. According to him the situation existing at present in northern Europe justifies liming for the following reasons:

1) Retaining and neutralizing excess acids caused by pollution.

2) Keeping the soil system near its equilibrium condition and thus ensuring sufficient ecological elasticity in order to avoid unbalancing the system, with irreparable consequences for soil fertility which it would require several decades to remedy.

Liming could be accompanied by green fertilization, for instance by introducing soil-improving plants such as Lupinus[20]. In fact, liming should not be used on its own but be complemented by additions of P and Mg[13].

All writers agree, however, that liming means a brutal modification of the soil system and may cause considerable ecological damage[9,13,55], in particular to soil fertility. Numerous earlier lime fertilization experiments carried out in Germany showed some 15–25 years later that, once a short period of recovery of vitality and production has passed, the measure had on the whole not been beneficial[13,17]. In no instance has lime fertilization cured lack of stand vitality[13]. On soils with raw humus lime has a negative effect, leading to rapid mineralization and leaching of nitrogen[13], in some cases liberating as much as 75% of the easily mineralizable nitrogen[9]. The more removed a soil is from its normal equilibrium (optimum condition), the less liming can reestablish the equilibrium situation. Effects on microbial fauna and flora are still very little known[9], particularly effects on mycorrhiza, which are extremely sensitive to any changes in ecological soil conditions[43,60] and seem to play an essential part in the biotic defense system of trees. Systematic studies of fine root systems of affected trees show a very pronounced lack of fine roots and their insufficient mycorrhization[5], which

explains the entry of pathological fungi and their secondary role in the decline process.
Liming can therefore only solve the problem caused by the massive entrance of acids into the soil system by retaining and neutralizing them – but the solution will be merely temporary. Even then, the disadvantages for the groundwater and the ecological equilibrium are so big that its use cannot be recommended[9]. In view of all this, and based on the advice of the main nutrient specialists[13], the Council of Environmental Experts of the Federal Republic of Germany[9] does not recommend general use of liming. However, in some cases moderate liming fertilization with additions of K, P, Mg, applied on successive occasions and intermixed with the topsoil, may be recommended as prophylaxis for soils with nutrient deficiencies[13, 57]. The costs of such operations are about $ 500 per ha and have to be considered as very high. In cases where new stands are being established on poor soils, starting fertilization may also be considered[9].

1 Atharis, S., Jahrringausfall, ein meist unbeachtetes Problem bei Zuwachsuntersuchungen in rauchgeschädigten und gesunden Fichtenbeständen. Mitt. forstl. BundVersAnst. Wien *139* (1981) 7–27.
2 Bauer, F., Naturnahe Waldwirtschaft nach Leibundgut und waldbauliche und forstpolitische Folgerungen aus der Waldschadensentwicklung in der Bundesrepublik Deutschland. Allg. Forstz. *38* (1983) 19, 481–483.
3 Becker, A., Untersuchungen zur Verjüngungsfähigkeit der Buche in bodensauren Buchenwald-Ökosystemen. Forst- Holzwirt *38* (1983) 154–161.
4 Beda, H., Der Einfluss einer SO_2-Begasung auf Bildung und Keimkraft des Pollens von Weisstanne, *Abies alba* (Mill.). Mitt. eidg. Anst. forstl. Versuchswes. *58* (1982) 165–223.
5 Blaschke, H., Veränderungen bei der Feinwurzelentwicklung in Weisstannenbeständen. Forstwiss. Zentbl. *100* (1981) 190–195.
6 Bosch, C., Pfannkuch, E., and Baum, U., Über die Erkrankung der Fichte (*Picea abies* Karst.) in den Hochlagen des Bayerischen Waldes. Forstwiss. Zentbl. *102* (1983) 167–181.
7 Braun, G., Über Ursachen der Immissionsresistenz bei Fichte und Folgerungen für die Resistenzzüchtung. Forstwiss. Zentbl. *96* (1977) 62–67.
8 Bucher, J. B., Physiologische Veränderungen und ökotoxikologische Wirkmechanismen, Probleme der Differentialdignose, in: Waldschäden durch Immissionen? Ausmass bereits sichtbarer Schäden, erste Forschungsergebnisse, mögliche Massnahmen, pp. 91–109. Ed. Gottlieb Duttweiler-Institut, Rüschlikon 1983.
9 Der Rat von Sachverständigen für Umweltfragen, Waldschäden und Luftverunreinigungen. Sondergutachten März 1983, p. 172. W. Kohlhammer, Stuttgart, Mainz 1983.
10 Dässler, H.-G., Einfluss von Luftverunreinigungen auf die Vegetation, 2nd edn p. 211. G. Fischer, Jena 1981.
11 Franz, F., Auswirkungen der Walderkrankungen auf Struktur und Wuchsleistung von Fichtenbeständen. Forstwiss. Zentbl. *102* (1983) 186–200.
12 Gehrmann, J., and Ulrich, B., Der Einfluss des sauren Niederschlags auf die Naturverjüngung der Buche. Sonderh. Mitt. Landesanst. Ökol. 1982, 32–36.
13 Gussone, H. A., Die Praxis der Kalkung im Walde der Bundesrepublik Deutschland. Forst- Holzwirt *38* (1983) 63–71.
14 Gussone, H. A., Möglichkeiten und Grenzen der Bodenmelioration. Beih. Forstwiss. Zentbl. *38* (1983) 36–40.
15 Keller, Th., and Beda-Puta, H., Luftverunreinigungen und Schneedruck – eine Beobachtung an Buchen. Forstarchiv *52* (1981) 13–16.
16 Keller, Th., Zum Nachweis einer Umweltbelastung durch Luftverunreinigungen. Schweiz. Z. Forstwes. *133* (1982) 873–884.
17 Kenk, G., Zuwachsuntersuchungen in geschädigten Tannen-Beständen in Baden-Württemberg. Allg. Forstz. *38* (1983) 650–652.
18 Klein, B., Trockenresistenz und Immissionshärte bei Fichte, pp. 1–190. Diss. Ludw.-Maxim.-Univ. München, 1981.
19 Kleinschmit, J., Möglichkeiten der Züchtung resistenter Waldbäume für die immissionsbelasteten Flächen. Forst- Holzwirt *38* (1983) 196–199.
20 Kramer, W., Über den Anbau der Dauerlupine (*Lupinus polyphillus* Lindl.). Forst- Holzwirt *36* (1981) 173–177.
21 Larsen, J. B., *Abies alba*-Provenienzen in Dänemark, in: 3. Tannen-Symposium Wien 1980, pp. 78–91. Ed. H. Mayer, Wien 1980.
22 Leibundgut, H., Zum Problem des Tannensterbens. Schweiz. Z. Forstwes. *125* (1974) 476–484.
23 Leibundgut, H., Zur Rassenfrage und Provenienzfrage bei der Weisstanne. Schweiz. Z. Forstwes. *129* (1978) 687–690.
24 Marcet, E., Versuche zur Dürreresistenz alpiner 'Trockentannen' (*Abies alba* Mill.). Schweiz. Z. Forstwes. *122* (1971) 117–135 and *123* (1972) 763–766.
25 Materna, J., Bewirtschaftung des immissionsgeschädigten und -gefährdeten Fichtenwaldes. Beih. Forstwiss. Zentbl. *38* (1983) 44–46.
26 Mayer, H., Zur waldbaulichen Bedeutung der Tanne im mitteleuropäischen Bergwald. Forst- Holzwirt *34* (1979) 333–343.
27 Mülder, D., Möglichkeiten der Forstbetriebe, sich Immissionsbelastungen waldbaulich anzupassen bzw. deren Schadwirkungen zu mildern, in: Materialien zur Umweltforschung, H. 7, p. 124. N. Kohlhammer, Stuttgart, Mainz 1983.
28 Odèn, S., The acidification of air precipitation and its consequences in the natural environment. Ecol. Commit. Bull. *1*, Swedish Nat. Sci. Res. Council, Stockholm 1968.
29 Pavari, A., Esperienze e indagini su le provenienze e razze dell'Abete bianco (*Abies alba* Mill.). Pubbl. Staz. sper. selvicolt. Firenze *8* (1951) 96.
30 Pelz, E., Untersuchungen über das Auftreten individueller Rauchhärte und die Erkennbarkeit phänotypisch rauchharter Individuen in rauchgeschädigten Fichtenbeständen. Diss. TU Dresden (1962).
31 Pelz, E., and Materna, J., Beiträge zum Problem der individuellen Rauchhärte von Fichte. Archiv Forstwes. *13* (1964) 177–210.
32 Pollanschütz, J., Verfahren zur objektiven Abschätzung verminderter Zuwachsleistungen von Einzelbäumen und Beständen. Mitt. forstl. BundVersAnst. Wien *73* (1966) 129–191.
33 Pollanschütz, J., Möglichkeiten und Grenzen der Behandlung und Pflege immissionsbeeinflusster und -geschädigter Waldbestände. Beih. Forstwiss. Zentbl. *38* (1983) 47–57.
34 Reemtsma, J. B., Naturnaher oder konventioneller Waldbau unter Immissionsbelastung. Beih. Forstwiss. Zentbl. *38* (1983) 66–70.
35 Ranft, H., et al., Untersuchungen zum Zusammenwirken von Immissions- und Frosteinfluss im Fichtenrauchschadengebiet. Beitr. Forstwirtsch. *33* (1979) 160–165.
36 Rohmeder, E., and von Schönborn, A., Der Einfluss von Umwelt und Erbgut auf die Widerstandsfähigkeit der Waldbäume gegenüber Luftverunreinigungen durch Industrieabgase. Forstwiss. Zentbl. *84* (1965) 1–13.
37 Scholz, F., Timmann, T., and Krusche, D., Untersuchungen zur Variation der Resistenz gegen HF-Begasung bei *Picea abies*-Familien. Mitt. Inst. Forst- Holzwirtsch. Ljubljana 1979, 244–258.
38 Scholz, F., Kann Züchtung auf Immissionsresistenz zur Lösung des Problems 'Waldsterben' beitragen? Allg. Forstz. *38* (1983) 281–283.
39 Schrader, S., Greve, U., and Schönwald, H. R., Saure Niederschläge und Waldschäden. Bibliographie. Mitt. BundForschAnst. Forst-Holzwirtsch. Nr. 138, p. 108. Kommissionsverlag, Hamburg 1983.
40 Schröter, H., Krankheitsentwicklung von Tannen und Fichten auf Beobachtungsflächen der FVA in Baden-Württemberg. Allg. Forstz. *38* (1983) 648–649.
41 Schütt, P., Blaschke, H., and Hoque, W., Erste Ergebnisse einer botanischen Inventur des 'Fichtensterbens'. Forstwiss. Zentbl. *102* (1983) 158–166.
42 Schütt, P., and Summerer, H., Waldsterben-Symptome an Buche. Forstwiss. Zentbl. *102* (1983) 201–206.
43 Schütz, J.-Ph., Waldbauliche Bedeutung von immissionsbedingtem Waldsterben, in: Waldschäden durch Immissionen? Ausmass bereits sichtbarer Schäden, erste Forschungsergebnisse, mögliche Massnahmen, pp. 111–120. Ed. Gottlieb-Duttweiler-Institut, Rüschlikon 1983.
44 Schütz, J.-Ph., Hat der naturnahe Waldbau noch eine Bedeutung in der Immissionsfrage? Beih. Forstwiss. Zentbl. *38* (1983) 60–65.
45 Stöckhardt, J. A., Über die Einwirkung des Rauches von Silberhütten auf die benachbarte Vegetation. Polytech. Zentbl. *16* (1850) 257–278.
46 Tesar, V., Anfangswirkung des Schwefeldioxids auf Fichtenbestände mittleren Alters. Lesn. Čas. *12* (1966) 815–829.
47 Tesar, V., Immissionsschäden und Durchforstung. Allg. Forstz. *38* (1983) 491–492.

48 Tzschacksch, O., Stand und Perspektiven der forstlichen Rauchresistenzzüchtung in der DDR. Beitr. Forstwirtsch. *15* (1981) 134–137.
49 Ulrich, B., Die Wälder in Mitteleuropa. Messergebnisse ihrer Umweltbelastung, Theorie ihrer Gefährdung, Prognose ihrer Entwicklung. Allg. Forstz. *35* (1980) 1198–1202.
50 Ulrich, B., Eine ökosystemare Hypothese über die Ursachen des Tannensterbens (*Abies alba* Mill.). Forstwiss. Zentbl. *100* (1981) 228–236.
51 Ulrich, B., Die Rolle des Waldes für die Wassergüte. Allg. Forstz. *36* (1981) 1107–1109.
52 Ulrich, B., Gefährdung von Waldökosystemen durch Akkumulation von Luftverunreinigungen, in: Stirbt der Wald? Alternative Konzepte 41, pp. 31–43. Cf. Müller, Karlsruhe 1982.
53 Ulrich, B., A concept of forest ecosystem stability and of acid deposition as driving force for destabilisation, pp. 1–29. Eds B. Ulrich and J. Pankrath. D. Reidel, Dordrecht, Boston, London 1983.
54 Ulrich, B., Auswirkungen der Immissionen auf die Bodenökologie des Waldes, in: Waldschäden durch Immissionen? Ausmass bereits sichtbarer Schäden, erste Forschungsergebnisse, mögliche Massnahmen, pp. 47–90. Ed. Gottlieb-Duttweiler-Institut, Rüschlikon 1983.
55 Ulrich, B., Waldbauliche Zielvorstellungen unter dem Gesichtspunkt der Stabilität und Elastizität der Waldökosysteme. Beih. Forstwiss. Zentbl. *38* (1983) 24–29.
56 Wentzel, K. F., Weisstanne = immissionsempfindliche einheimische Baumart. Allg. Forstz. *14* (1980) 313–314.
57 Wentzel, K. F., Tesar, V., and Seibt, G., and Materna, J., Waldbau in verunreinigter Luft. Forst- Holzwirt *36* (1981) 533–542.
58 Wentzel, K. F., Maximale Immissionswerte zum Schutze der Wälder, Überlegungen zur Resolution der IUFRO-Fachgruppe 2.09.00. Mitt. forstl. BundVersAnst. Wien *137* (1981) 175–180.
59 Wentzel, K. F., Ursachen des Waldsterbens in Mitteleuropa. Allg. Forstz. *45* (1982) 1365–1368.
60 Wodzinski, R.-S., Labeda, D. P., and Alexander, M., Effects of low concentrations of bisulfite and nitrite on microorganisms. Appl. envir. Microbiol. *35* (1978) 718–723.
61 Yang, Y.-S., Skelly, J. M., and Cherone, B. I., Clonal response of eastern white pine to low doses of O_3, SO_3 and NO_2 singly and in combinations. Can. J. Forest Res. *12* (1982) 803–808.
62 Zech, W., and Popp, E., Magnesiummangel, einer der Gründe für das Fichten- und Tannensterben in Nordostbayern, Forstwiss. Zentbl. *102* (1983) 50–55.

0014-4754/85/030320-06$1.50 + 0.20/0

Full Papers

Cleavage of des-Arg^9-bradykinin by angiotensin I-converting enzyme from pig kidney cortex[1,2]

G. Oshima, Y. Hiraga, K. Shirono, S. Oh-ishi, S. Sakakibara and T. Kinoshita

School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane-5, Minato-ku, Tokyo 108 (Japan), and Peptide Institute, Protein Research Foundation, 476 Ina, Minoh-shi, Osaka 562 (Japan), 6 December 1983

Summary. Fast and very slow hydrolyses of des-Arg^9-bradykinin and angiotensin II by angiotensin I-converting enzyme were detected by high performance liquid chromatography. The Michaelis constants of the enzyme, K_m values, for des-Arg^9-bradykinin and bradykinin were found to be 0.24 mM and 4.4 µM, and the maximum velocities, V_{max} values (µmol·min^{-1}·mg protein^{-1}) for these compounds to be 3.24 and 0.34, respectively.
The enzyme also hydrolyzed Z-Gly-Pro-Gly-Gly-Pro-Ala to a tripeptide that was identified as dansyl-Gly-Pro-Ala by TLC on polyamide. These observations show that the enzyme hydrolyzes the peptides at the bond before the prolyl residue in the penultimate position.
Key words. Pig kidney cortex; des-Arg-bradykinin; angiotensin I-converting enzyme.

Introduction. The angiotensin I-converting enzyme (ACE; kininase II) is known to cleave a wide variety of peptides including angiotensin I and kinins[20], releasing a dipeptide from the C-terminus. This enzyme, therefore, is called peptidyl dipeptide hydrolase or dipeptidyl carboxypeptidase [EC 3.4.15.11][3]. The number of possible substrates of ACE is limited, because ACE cannot hydrolyze peptide bonds involving the imino group of proline[3,18,21]. Thus, angiotensin II is thought not to be hydrolyzed after its release from angiotensin I by ACE.
A HPLC method was developed in this laboratory for measuring the concentrations of metabolites of bradykinin and angiotensin I and II and characterizing them. After separation of peptides by single step, reversed-phase HPLC, the guanidino groups of arginyl residues in the peptides were detected fluorometrically by reaction with alkaline ninhydrin[9]. As reported previously, human plasma cleaved bradykinin to produce other peptides besides des-Arg^9-BK and des-[Phe^8-Arg^9]-BK, products of kininases I and II, respectively. Human plasma also hydrolyzed des-Arg^9-BK to form an unidentified peptide that differed from the heptapeptide des-[Phe^8-Arg^9]-BK. The formation of the unknown peptide from bradykinin seemed to be correlated with the disappearance of des-Arg^9-BK by human plasma. Since degradation of des-Arg^9-BK by human plasma was strongly inhibited by EDTA, an exopeptidase, probably a carboxypeptidase, was thought to be responsible for the hydrolysis[9].
We purified ACE to homogeneity from pig kidney cortex, and found that it converted angiotensin I to II, inactivated bradykinin and hydrolyzed several smaller synthetic peptide substrates[15,17]. Thus, it was a typical peptidyl dipeptide hydrolase. The purified preparation of ACE was essentially free from aminopeptidase, carboxypeptidase and other dipeptidases[16]. This enzyme preparation hydrolyzed des-Arg^9-BK at a rate comparable to that of bradykinin. This paper reports the hydrolyses by ACE from pig kidney cortex of the antepenultimate peptide bonds of Z-Gly-Pro-Gly-Gly-Pro-Ala to