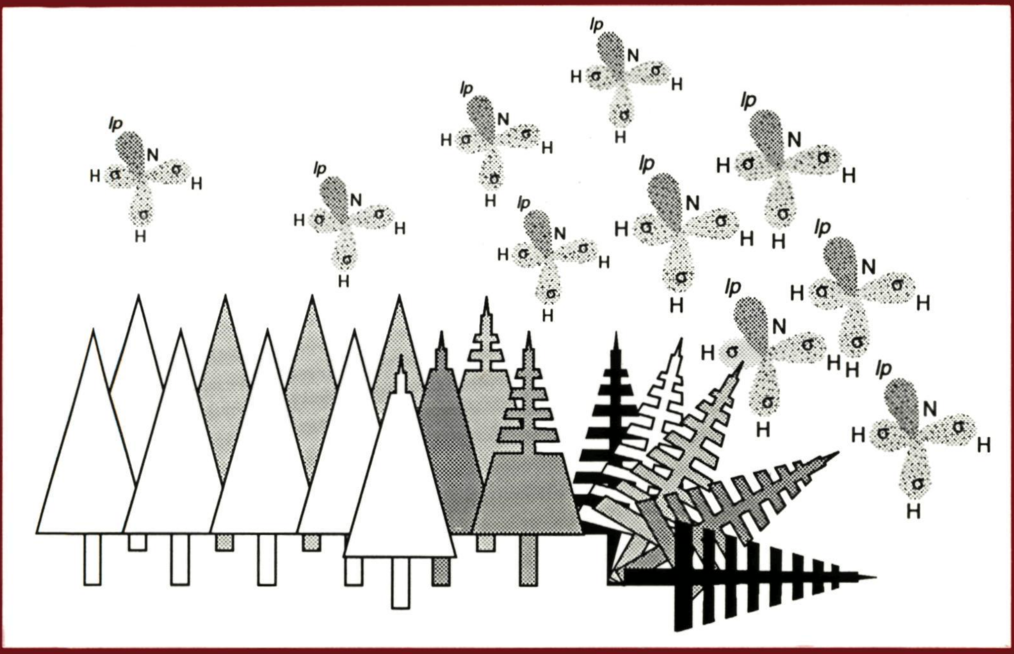
EXCESS NITROGEN DEPOSITION: A STRESS FACTOR IN DUTCH PLANTATION FORESTS



Harri F. .v

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Aan mijn vader, die er alles voor over had om mij de kansen te geven, die hij zelf nooit kreeg.

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EXCESS NITROGEN DEPOSITION:
A STRESS FACTOR IN DUTCH PLANTATION FORESTS

een wetenschappelijke proeve op het gebied
van de Natuurwetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Katholieke Universiteit te Nijmegen
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op dinsdag 11 mei 1993
des namiddags te 1.30 uur precies

door

HENRICUS FRANCISCUS GERARDUS VAN DIJK

geboren op 15 oktober 1957
te Venlo

Drukkerij Elinkwijk BV - Utrecht

Promotor: prof. dr. C. den Hartog

Co-promotor: dr. J.G.M. Roelofs

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CONTENTS

VOORWOORD

Het is nu ruim negen jaar geleden dat ik als vrijwilliger kwam werken op de afdeling Aquatische Oecologie van de Katholieke Universiteit te Nijmegen. Het mag enige bevreemding wekken dat ik mij daar ging bezighouden met de invloed van een overmatig stikstofaanbod op de vitaliteit van bomen, vooral naaldbomen, toch niet de meest uitgesproken voorbeelden van in het water levende organismen. De verklaring hiervoor schuilt in de ontwikkeling die het onderzoek van de afdeling doorgemaakt heeft en die sterke gelijkenis vertoont met de evolutionaire ontwikkeling van de gewervelde dieren, die vanuit het water geleidelijk het land op kropen. In opdracht van het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer keken medewerkers van de afdeling reeds naar de invloed van 'zure regen' op de flora en fauna van vennen in heidegebieden. Uit dit onderzoek bleek dat vooral de grote hoeveelheden stikstof, met name ammoniak, die vanuit de lucht aangevoerd werden, verantwoordelijk waren voor de aftakeling van deze ecosystemen. Een blik in de omgeving van de heidevennen maakte duidelijk dat ook de omringende heide en bossen niet gezond waren. Mede op aandringen van de heer Theo Janssen, toenmalig districtshoofd van Staatsbosbeheer in Noord-Limburg, werden oriënterende studies verricht naar de relatie tussen stikstofaanbod en bossterfte. Dit resulteerde in een tweede subsidie-aanvraag bij het Ministerie van VROM, die in oktober van 1984 werd gehonoreerd. Als prettige bijkomstigheid veranderde mijn status van officieel erkend vrijwilliger in die van betaald medewerker. Voor de duur van drie jaar mocht ik mij gaan bezighouden met 'het effect van landbouw-ammoniumdeposities op bos- en heidevegetaties'. Omdat deze opdracht wel erg breed was, beperkte ik mij noodgedwongen al snel tot de effecten op bossen. Meteen bij het begin van mijn aanstelling maakten zowel het afdelingshoofd, prof. dr. C. den Hartog, als mijn directe begeleider, Jan Roelofs, mij duidelijk, dat mijn onderzoek geacht werd te resulteren in een promotie. Een vervolg-project, dat vrijwel meteen aansloot op het voorgaande project en gericht was op de studie van herstelmogelijkheden van bossen na reductie van de stikstofaanvoer, maakte het mij lange tijd onmogelijk om de nog noodzakelijke, aanvullende metingen te verrichten en de talrijke gegevens tot een proefschrift samen te smeden. Pas toen we extra analytische ondersteuning kregen in de persoon van ing. Paul van der Ven, kon ik tijd vrijmaken voor de afronding van mijn proefschrift. Het bleek niet altijd even gemakkelijk te zijn om een goed evenwicht te vinden tussen de belangen van de opdrachtgever, in dit geval de overheid, die vooral geïnteresseerd is in snelle en duidelijke uitspraken waarop beleid gebaseerd kan worden, en de belangen van de wetenschapper, die overal het fijne van wil weten en vooral geïnteresseerd is in de achterliggende mechanismen. Ik hoop dat ik erin geslaagd ben de gulden middenweg te vinden. Het dilemma, dat ik als 'bomenman' op een aquatische afdeling rondliep, heeft zich inmiddels vanzelf opgelost door fusie van de diverse oecologische afdelingen tot één grote vakgroep Oecologie, bestaande uit verschillende werkgroepen, waaronder die van Milieubiologie, onder welke mijn werkzaamheden nu ressorteren.

Dit proefschrift zou nooit tot stand gekomen zijn als niet velen mij met raad en daad hadden bijgestaan. In de eerste plaats bedank ik mijn collega's Dries Boxman en Paul van der Ven, met wie ik werkzaam ben in het voornoemde project, waarin de herstelmogelijkheden van het bos onderzocht worden. Zij werden extra zwaar belast doordat ik een belangrijk deel van mijn tijd aan de vervaardiging van mijn proefschrift moest besteden. Ook dank ik hen, evenals Anneke Houdijk, voor de vele stimulerende en interessante discussies. Veel dank ben ik verschuldigd aan mijn directe begeleider en co-promotor Jan Roelofs. Zijn ideeën en inzichten vormen de grondslag van dit proefschrift. Ik heb veel van hem geleerd en hij heeft mij voortdurend gestimuleerd om dit proefschrift te schrijven. Dat geldt ook voor mijn promotor prof. dr. C. den Hartog. Mijn onderzoeksonderwerp ligt ver van zijn vakgebied, de aquatische oecologie, af; hij liet daarom de directe begeleiding aan Jan over. De afstand die hierdoor ontstond, maakte echter een frisse kijk op de problematiek mogelijk en hij dwong mij voortdurend tot helderheid. Zijn kritische kanttekeningen bij de manuscripten van mijn publicaties alsmede bij dit proefschrift, heb ik altijd zeer gewaardeerd.

Veel studenten hebben bijgedragen aan het verzamelen en interpreteren van gegevens. In chronologische volgorde noem ik: Jules van Lier, Wil Smeets, die helaas onlangs is overleden, Jeroen Rijniers, Johan Verburgh, Karel Herberigs, Willem Engelaar, Peter Verbeek, Kees Nuyten, René Krekels, Carlo van de Rijt, Anne ter Elst, Alexandra de Haan, Erik-Jan Kamerbeek, Raymond Creemers, Marian de Louw, Anita Vervenne, Hein van Lieverloo, Petra Perik, Brigit Schoenmakers, José Dobbelsteen, Kor Goutbeek en Patrick Weijers.

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Veel collega's van andere universiteiten en onderzoeksinstellingen hebben bijgedragen aan de totstandkoming van dit proefschrift, hetzij door stimulerende discussies en goede adviezen, hetzij door kritische kanttekeningen bij concept-versies van publicaties of het proefschrift. Met name noem ik: dr. H. F. Bienfait, prof. dr. C. W. P. M. Blom, prof. dr. N. van Breemen, ir. J. van den Burg, dr. L. J. M. van der Eerden, ing. M. de Kam , prof. dr. H. J. Laanbroek en prof. dr. J. M. Verstraten. Even onmisbaar waren de ruime veldervaring en scherpe blik van de heren Theo Janssen en Leon Hahn van Staatsbosbeheer in Noord-Limburg, alsmede de medewerking van talloze terreinbeheerders en opzichters, waarvan ik de heer P. van der Aa van het landgoed 'De Stippelberg' met name wil noemen.

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CHAPTER 1

GENERAL INTRODUCTION

Dutch forests

The Netherlands belong to the least afforested countries of Europe. Only 7% of their land area, *i. e.* 330,000 ha, is covered by forests, of which 281,000 ha is timber forest. In the nineteenth century this was even less. In the preceding period, most woods had been destroyed by litter removal or were felled to supply building materials and fuels. Most of the forests are situated in the southern, eastern and central parts of the country on nutrient-poor, acidic, sandy soils (predominantly podzol soils and inland dunes), that were too infertile for agricultural practices. These areas were formerly covered by extensive heathlands that were part of the agricultural system at that time. Sheep grazed on the heathlands and their excrements together with sods from the heathlands were used to fertilize the fields. However, at the beginning of the twentieth century artificial fertilizers became available and the heathlands lost their function. As mining industry came up in the southern part of the country, there was a growing demand for wood to make pit-props. Therefore, many heathlands were planted with pine trees. Nowadays, all Dutch forests are man-made plantations. The last pristine forest, the Beekbergerwoud, was logged in 1871.

Scots pine (*Pinus sylvestris* L.) is quantitatively the most important of the tree species, which constitute Dutch forests. This tree species was probably native but disappeared long ago (Heukels & Van der Meijden, 1983). It has a continental distribution and The Netherlands are situated at the western boundary of this distribution area. It is able to grow on very infertile soils (Carlisle & Brown, 1968). Therefore, it was planted extensively on the abandoned heathlands. Later, other, more productive, species were introduced, such as Corsican pine (*Pinus nigra* var. *maritima* (Aiton) Melville) from the Mediterranean, Austrian pine (*Pinus nigra* var. *nigra* Arnold) from the low mountain ranges of southeastern Europe and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) from the northwest coast of North America. Amongst the deciduous trees oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) are the most prominent species (Table 1).

In the past, sowing was common practice to create new forest stands, especially in case of Scots pine (Schütz & Van Tol, 1981). Today, however, forest stands are usually planted. Young plants of 2 to 4 years old are used. In the past, often seeds or young plants of unsuitable provenances, that could not thrive under the Dutch circumstances, were used. This resulted in poor growth and susceptibility to disease, drought and frost damage. However, nowadays only selected provenances are used. During afforestation mostly 2500 to 4500 trees per hectare are planted. Sometimes fertilizers containing N, P or K are applied to the soil prior to planting. But often this is not done, because most forest trees are not very demanding and grow well on infertile soils. At regular intervals during stand development the stand is thinned, *i. e.* poorly growing and deformed individuals are removed to give the other trees more space and light. Economical and management factors define the moment of felling.

Table 1. Forest tree species in The Netherlands and their part in the total afforested area (in ha and in %). (Source: Staatsbosbeheer, 1984)

tree species	area (ha)	(%)
Pinus sylvestris	113,400	40
Pinus nigra	15,860	6
Pseudotsuga menziesii	15,530	6
Picea abies	13,120	5
Quercus robur	43,850	16
Fagus sylvatica	8,550	3
Other coniferous species	24,030	9
Other deciduous species	47,010	17
All species together	281,350	100

This is usually long before the trees reach maximum age. Clear felling is common practise in silviculture, but sometimes some of the older trees are spared temporarily or felling takes place in zones to protect the newly planted young trees from frost damage and desiccation. As a consequence the forest stands are normally monospecific, with even-aged and straight stemmed trees. These characteristics may be favourable to the production of high-quality wood and thus increase the economical value of the forest, but they greatly reduce its natural and recreational merits. Particularly in coniferous forests the undergrowth is poorly developed if present at all. This is because tree density is often high, allowing for little light to reach the forest floor. Moreover, the soil is infertile and acid and the litter is degrading only very slowly. The small variability within the stands does not create many habitats for different plant species. However, many fungi occur naturally in the Dutch forests. Many of them produce fruiting bodies that are known as toadstools and mushrooms. Partly, this concerns mycorrhizal species which live in close association with the roots of the trees. They are essential for tree growth as they provide water and nutrients to the trees (Harley & Smith, 1983). In return they receive sugars which the trees make with their green aerial parts. Other fungi play an important role in degrading litter. The low soil pH favours fungal above bacterial degradation.

Forest dieback

During the nineteen seventies and the early eighties extensive tree damage and forest dieback were reported from Germany. Initially, only silver fir (*Abies alba* Miller) was affected, but later on also Norway spruce (*Picea abies* (L.) Karsten), Scots pine (*Pinus sylvestris*) and other tree species showed symptoms of decline. Damage occurred both in the middle and high mountains and in the lowlands (Schütt, 1984). Alarmed by the news from Germany the Dutch National Forest Service started a national survey to assess the vitality of the Dutch forests in 1983 (Staatsbosbeheer, 1983). It was concluded that its condition gave cause to great concern. The investigation was repeated more extensively in 1984 (Staatsbosbeheer, 1984). It revealed that almost 50% of the Dutch forests had a reduced vitality (vitality classes 2, 3 and 4, Table 2). The trees showed extensive loss of needles or leaves or severe discolouration of these. In 8% of the stands these symptoms were so pronounced that recovery was thought to be unlikely (class 3); 1.5% of the forests was so heavily affected that immediate felling was deemed necessary (class 4). The situation had slightly worsened since the previous year. Damage was most evident in the Peel area, central and eastern Noord-Brabant, the Utrechtse Heuvelrug, the western part of the Veluwe, the lateral moraines of Nijmegen and Overijssel and the Friese Wouden. Both coniferous and deciduous trees were affected, but the situation was worst for Scots pine and Douglas fir. Stands growing on podzolic soils and inland dune soils, *i.e.* soils susceptible to acidification, were less vital than stands growing on other soil types. Moreover, very young and very old trees proved to be less affected than middle-aged trees.

Table 2. The vitality of the Dutch forest in 1984 (Source: Staatsbosbeheer, 1984)

vitality class	area (ha)	(%)	description
1	143,000	50.5	vital
2	112,000	40.0	less vital
3	22,400	8.0	hardly vital
4	4,000	1.5	not vital

There has been much speculation on the cause of the sudden forest dieback (Poortinga, 1984; Schütt, 1984). Unfavourable climatic conditions, pathogens and wrong silvicultural practices have all been suggested to be most important in this respect. However, none of these can satisfactorily explain the massive occurrence of forest decline under so many different climatical and soil conditions, involving so many different tree species. Most scientists do agree that air pollution is the main primary cause of the observed forest dieback (Lichtenthaler, 1984; Schuck, 1984;

Schütt, 1984). Sulphur dioxide - released during the burning of sulphur containing fossil fuels - , nitrogen oxides - produced by combustion engines - , and ozone and peroxiacetylnitrate - arising in the atmosphere from nitrogen oxides and organic compounds under the influence of ultraviolet light - are blamed in this respect. As some of these compounds generate sulphuric and nitric acid upon dissolution in rainwater, the air pollution is often referred to as 'acid rain'. This description was used first by the English chemist Robert Smith in 1872, but it took almost a century before scientists used it again. Since 1982 the general public is familiar with it (Poortinga, 1984).

Origin, transport and deposition processes of these substances, as well as interactions with soil and vegetation are all studied intensively (Hutchinson & Havas, 1980; Drabløs & Tollan, 1980; Smith, 1981; Ulrich & Pankrath, 1983). The mechanisms, by which tree vitality is impaired, are still a matter of debate. Some investigators take the position that the influence of pollutants on trees is mainly indirect. Some of these substances cause soil acidification, resulting in raised aluminium concentrations in the soil solution, which affect the root systems of the trees (Ulrich, 1983a). Others are of the opinion that the aerial parts are directly affected by the pollutants, leading to reduced photosynthesis (Lichtenthaler, 1984; Schütt, 1984) or leaching of nutrients from the leaves or needles (Krause *et al.*, 1984).

Ammonia

The significance of ammonia as an important pollutant was underestimated for a long time. Only in 1982 Van der Eerden remarked that 'Although ammonia is not one of the major air pollutants, it is necessary to have some knowledge about its toxicity and about the concentration that will give no adverse effect.'. Damage to vegetation near poultry and pig farms and fields dressed with animal slurry caused by ammonia toxicity was noticed by several authors (Kühne, 1966; Garber & Schürmann, 1971; Hunger, 1978; Tesche & Schmidtchen, 1978). However, it was looked upon as a local problem. Nevertheless, Ewert (1978) estimated the total area of forest stands injured by emissions from livestock farms in the German Democratic Republic at 2,000-3,000 ha.

In 1982 Van Breemen *et al.* reported that ammonium and sulphate were the most important solutes in rainwater dripping from the leaves, needles, twigs and branches of trees in two Dutch forest stands. They found that the concentration of ammonium in this so-called canopy throughfall water was 4 to 5 times higher than in the incident rainwater and that the total nitrogen deposition in the forest stands under investigation amounted to more than 60 $kg.ha^{-1}.yr^{-1}$. They ascribed this to the filtering action of the tree crowns. During dry weather gaseous ammonia, sulphur dioxide and ammonium containing aerosols are deposited in the canopy. During the next shower these substances are washed down as ammonium sulphate. As the concentrations of ammonium and sulphate, which they found in the throughfall water, were approximately equal and sulphur dioxide and ammonia have opposite effects on the pH of rainwater, Van Breemen *et al.* (1982) suggested that one substance may stimulate the deposition of the other. They supposed that the ammonia originated mainly from liquid manure sprayed onto pastures in winter and early spring. The high ammonium sulphate input resulted in considerable soil acidification, as most of the ammonium was converted into nitrate by microorganisms living in the forest soil.

Soon it became evident that ammonia is quantitatively one of the most important pollutants in The Netherlands. Though ammonia is supposed to be transported only over short distances, due to reaction with acidic compounds in the atmosphere (Nihlgård, 1985), its sources are so numerous that the ammonia deposition proved to be high throughout the entire country. Buijsman *et al.* (1984) estimated the total ammonia emission in The Netherlands in 1980 at 130,500 tons. Approximately 90% of this amount originated from manure. Emissions were highest in sandy areas. According to Van Aalst (1984) the total deposition of acidifying substances in The Netherlands in the period 1978-1982 amounted to 6000 $eq.ha^{-1}.yr^{-1}$, 32% of which was accounted for by ammonia and ammonium. He estimated the average nitrogen deposition at 50 $kg.ha^{-1}.yr^{-1}$. Slightly over 50% was deposited in the ammonium form.

In 1982 Janssen suggested that there was a relation between the forest dieback, which he observed in the so-called Peel-area in the southeastern part of the country, and the vicinity of numerous, large ammonia emitting sources like stockbreeding farms and fields that were dressed regularly with animal slurry (Janssen, 1982). Roelofs and his co-workers started to investigate the relation between forest dieback and excess ammonia availability and presented their first results at the Acid Rain Conference in Den Bosch in 1983 (Roelofs *et al.*, 1984) and published them two years later (Roelofs *et al.*, 1985). They found that Corsican pine stands with a reduced vitality were characterized by nutrient imbalances in both the soil and the needles. Nitrogen - in the soil predominantly in the ammonium form - was present in excess whereas there were shortages in potassium and magnesium. They postulated that excess ammonium deposition resulted in ammonium accumulation in the soil and that this impaired uptake of base cations by the trees. They also gave experimental evidence that leaching of base cations from needles was enhanced by high ammonium sulphate concentrations in rainwater. Furthermore, they suggested a link between the epidemical spread of fungal diseases in pine stands in the Peel area and the large atmospheric nitrogen input. Increasing evidence that excess nitrogen nutrition played an important role in the observed forest dieback came also from other European countries (Nihlgård, 1985).

It may be clear from the foregoing that the term 'acid rain' is not very well chosen, as not all pollutants are acidic and do not necessarily need to be dissolved in rainwater to become harmful. However, the designation has become too current to abolish.

Outline of this thesis

In 1984 the Catholic University of Nijmegen obtained a grant from the Dutch Ministry of Housing, Physical Planning and Environment (project 621.046-01) to investigate the effects of excess nitrogen availability on forests. Some of the results of this study are presented in this thesis. In chapters 2 and 3 a greenhouse experiment is described in which young coniferous trees were planted in pots and treated with artificial rainwater containing different amounts of ammonium sulphate. Main objective of this experiment was to investigate whether ammonium sulphate is detrimental to coniferous trees, when applied in large amounts. Because ammonium deposition results in ammonium accumulation in some soils (Roelofs *et al.*, 1985), whereas it causes acidification due to nitrification in others (Van Breemen *et al.*, 1982), two types of soil, which could be expected to react differently in this respect, were used in this experiment. The experiment was carried out with three coniferous tree species, which are quantitatively most important in The Netherlands. The effects on the availability and balance of nutrients in the soil, the soil pH and the dissolution of detrimental substances like aluminium, are described in chapter 2. Chapter 3 focusses on the impact of the chemical changes in the soil on tree growth, development of mycorrhizae and nutritional status of the trees.

In chapters 4 and 5 a special type of needle discolouration in Scots pine is described. The current needles are yellowish and the discolouration is most pronounced at the needle base. The symptom bears resemblance to iron-deficiency chlorosis, as observed in plants growing on calcareous soils. However, the trees afflicted with the present disease grow on acidic soils. Both field measurements and experiments were conducted to gain insight in the cause of this chlorosis. Special attention was focussed on the possible role of excess nitrogen availability, as the phenomenon is commonly observed in areas with high nitrogen deposition.

In chapter 6 the possible role of nitrogen in the host-parasite relation between Corsican pine and the fungus *Sphaeropsis sapinea* is discussed. This fungus, that formerly hardly occurred in Dutch forests, was found in the Peel area in 1982. Two years later it had spread all over the country and caused great damage. Symptoms were not merely confined to shoot blight: branch dying and crown wilt were observed increasingly as well. Because of the large extent of the damage in the Peel area, where the nitrogen deposition is very high, it was investigated whether there is a relationship between nutrient availability, especially that of nitrogen, and the occurrence of the disease. Therefore, tree nutritional status and soil nutrient availability in infected and non-infected stands were assessed in a field study. The results are compared with those of infection experiments carried out simultaneously at the 'Dorschkamp', Research Institute for Forestry and Urban Ecology, in Wageningen.

In chapter 7 the main results of the previous chapters are summarized and discussed in relation to the findings of other investigators. The various ways in which excess nitrogen availability can affect the forests are shown and the general role of nitrogen in the forest dieback in The Netherlands is discussed.

CHAPTER 2

IMPACT OF ARTIFICIAL, AMMONIUM-ENRICHED RAINWATER ON SOILS AND YOUNG CONIFEROUS TREES IN A GREENHOUSE. I. EFFECTS ON THE SOILS

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ABSTRACT

A pot experiment was used to investigate whether ammonium sulphate, when applied in amounts comparable to those deposited in the Dutch forests, is detrimental to trees. Young coniferous trees were planted in pots and treated with artificial ammonium-enriched rainwater in a greenhouse. The deposition of ammonium resulted in a strong acidification of the soil solution, leaching of base cations and an increased solubility of aluminium, manganese and zinc. Ammonium accumulated in the soil solution, causing severe imbalances between plant nutrients. The effects of these chemical changes in the soil on tree performance are described in a second paper.

INTRODUCTION

Air pollutants are generally accepted to be a major cause of forest decline in Europe and North America. Toxic and acidifying substances like ozone, sulphur dioxide and nitrogen oxides are often mentioned in this context and their effects on plants are being studied intensively. However, during the last few years several investigators have pointed out that overloading of the forest stands with nitrogen may also play an important role (Nihlgård, 1985; Roelofs *et al.*, 1985; Encke, 1986 and Zedler *et al.*, 1986).

In The Netherlands nitrogen deposition is extremely high. Most of the nitrogen originates from ammonia that is released from intensive stockbreeding farms or evaporates from animal slurry dumped on arable land. In humid air, or in water films covering plant surfaces, the ammonia reacts with sulphur dioxide forming ammonium sulphate. Due to the filtering action of tree canopies large amounts of this substance are deposited in the forests. In the so-called 'Peel'-area, in the southeastern part of the country, nitrogen deposition in forests averages 60 to 75 $kg.ha^{-1}.yr^{-1}$, but locally, in wood edges and groves, values of up to 500 kg may be reached. In many other parts of the country nitrogen deposition amounts to 45 to 60 $kg.ha^{-1}.yr^{-1}$ (Staatsbosbeheer, 1986a). From other European countries peak values of 20 to 70 $kg.ha^{-1}.yr^{-1}$ are reported (Ellenberg, 1985; Andersen, 1986; Encke, 1986 and Grennfelt & Hultberg, 1986). According to Encke (1986) the forest stands need only 5 to 8 kg $N.ha^{-1}.yr^{-1}$ for the production of new biomass and Ågren (1983) states that pine and spruce forests become saturated with nitrogen within 25 to 50 years at a deposition rate of 25 kg $N.ha^{-1}.yr^{-1}$. So, in large parts of western Europe the forest stands receive much more nitrogen than is needed.

To investigate whether ammonium sulphate is detrimental to coniferous trees when applied in amounts comparable to those deposited in Dutch forests, a pot experiment was designed. In a greenhouse young coniferous trees were planted in pots filled with soil and treated with artificial rainwater enriched with different amounts of ammonium sulphate. Because in some soils deposition of ammonium results in ammonium accumulation (Roelofs *et al.*, 1985), whereas in others it causes acidification due to nitrification (Van Breemen *et al.*, 1982), two types of soil were used in this experiment, which could be expected to react differently in this respect. Both tree performance and processes in the soil were studied simultaneously. Here, the results of soil analysis are presented. The effects on tree performance are described in a second paper (Van Dijk *et al.*, 1990).

MATERIALS AND METHODS

Two types of soil were derived from the field: the mixed Ah and Ae horizons of an acidic podzol soil from the edge of a young Scots pine stand, displaying no symptoms of decline, and the mineral top layer of a less acidic riverdune soil without a well developed profile.

Polypropylene pots (capacity 12 l, diameter 25 cm) with three holes in the bottom were filled with 14 kg of either of the two soil types. Two-year-old Scots pine (*Pinus sylvestris* L., var. Grubbenvorst), Corsican pine (*Pinus nigra* var. *maritima* (Aiton) Melville, var. Texel-OL) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco, var. Darrington) were obtained from a commercial nursery. They were planted in the pots in March 1985. Though the trees already had mycorrhizae a handful of humus from a mixed oak pine forest was added into the planting holes to stimulate further mycorrhizal formation. The trees were placed in a greenhouse and were allowed to recover and to adapt to the new circumstances for approximately two months. During this period the trees received demineralized water containing 5 $mg.l^{-1}$ seasalt, the amount naturally present in rainwater at a distance of 100 km from the shore (Leeflang, 1938). The chemical composition of this artificial 'clean' rainwater is given in Table 1. After this acclimatization period 10 trees of each species and from each soil type were randomly chosen and both trees and soils were analyzed to define the starting situation.

The remaining trees were randomly divided into three groups. One of these groups continued to receive clean rainwater, the other two received clean rainwater enriched with either 500 or 5000 μM ammonium, which was added as ammonium sulphate. The former can be regarded as a minimal value for the ammonium concentration in the throughfall in many Dutch coniferous forests, the second as an maximal value, that is only found continuously in the direct vicinity of ammonia emitting sources (Boxman, Houdijk, Roelofs & Van Dijk, unpublished data). Hereafter, the treatments are referred to as 0 N, 500 N and 5000 N, respectively.

Table 1. Chemical composition of a solution containing 5 mg seasalt.l^{-1}, *i.e.* artificial, 'clean' rainwater, in μmol.l^{-1}. Seasalt used: 'Marinemix + Bio-elements' of Wiegandt GmbH & Co KG, Krefeld, FRG.

Na	66	Br	0.118
K	1.42	I	0.0005
Mg	7.52	P	0.0009
Ca	1.48	SO4	4
Sr	0.021	HCO3	0.34
F	0.010	H3BO3	0.06
Cl	77.5		

+ other micro-elements < 0.0005

The ammonium application started on 1 June, 1985. From December 1985 onwards 25 μmol.l^{-1} ammonium nitrate were added to all types of rainwater, because the seasalt did not contain any nitrogen and the control trees showed symptoms of nitrogen deficiency. The rainwater was supplied with a watering-can in portions of 0.5 l, once to three times a week, according to the needs of the trees. All the trees got the same amount of water. This resulted in a water addition of 690 mm per year and a nitrogen deposition of 0, 48 and 480 kg.ha^{-1}.yr^{-1} for the different treatments. For the supply of other nutrients see Table 2. To investigate the importance of needle uptake, the rainwater was poured over the tree canopies in the case of half of the trees, allowing the water to reach the soil, while the other ones received the water directly on the soil. However, there were small differences in relative air humidity in the greenhouse, resulting in a slightly higher air humidity for the first group. For all treatments 10 replicates were used. Thus, the complete experiment included: 3 tree species x 2 soil types x 3 ammonium concentrations x 2 watering methods x 10 replicates = 360 trees.

Black polyethylene bottles (capacity 1 l) with funnels (diameter 16 cm) were placed beneath 6 of every 10 pots of one treatment to catch the excess water draining from the pots. The bottles contained 1 ml of a fixing agent ($HgCl_2$, 200 mg.l^{-1}) to prevent bacterial conversions. The drainage water of one month was pooled per treatment prior to analysis.

To estimate the impact of the trees on soil processes 18 pots were filled only with soil. Three replicates were used. They were treated in a similar way as those with trees.

Table 2 Amounts of nutrients added to the pots via the rainwater during ten months of treatment (μmol pot^{-1}). The underlined numbers indicate the ammonium concentration in the rainwater of the different treatments.

	0	500	5000
Na	2013	2013	2013
K	43	43	43
Mg	229	229	229
Ca	45	45	45
Cl	2364	2364	2364
P	0.026	0.026	0.026
NO3	50	50	50
NH4	50	15300	152550
SO4	122	7747	76372

Seven months after the beginning of the ammonium application two trees of every treatment were harvested. The soil was analyzed by extraction procedures. These were carried out as described earlier (Van Dijk & Roelofs, 1988). The climatic conditions prevailing in the greenhouse are shown in Fig. 1.

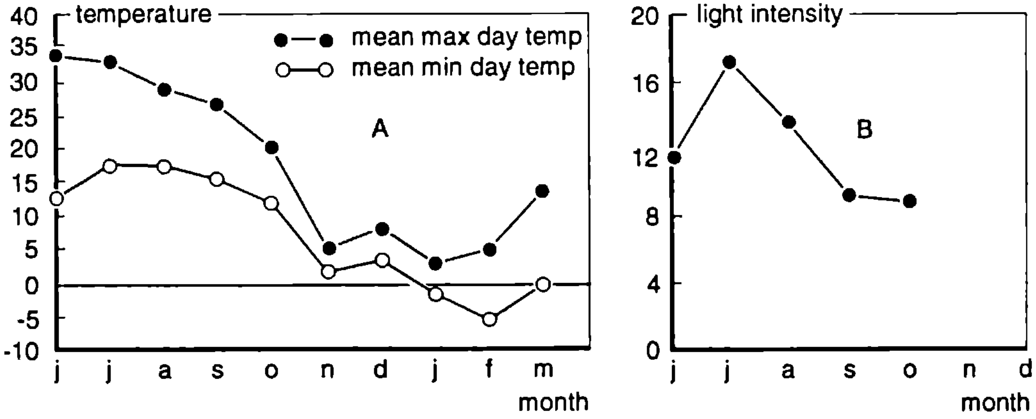

Figure 1. Climatic conditions prevailing in the greenhouse during ten months of treatment. A: temperature in °C, B: light intensity in Einstein.m^{-2}.day^{-1}.

Average nutrient concentrations in the drainage water were calculated by dividing the total amounts of nutrients leached from the pots by the total amounts of water that flowed from the pots. Prior to statistical analysis these values and all concentrations of nutrients in the soil extracts were log-transformed to make the variance independent of the mean (Sokal & Rohlf, 1981). For presentation all data were transformed back and presented as geometric means.

The influence of the experimental factors on the nutrient measurements was analyzed with four-way analysis of variance models including all first-order interactions, except those that were not significant for neither of the measured nutrients. The dependent variables were the nutrient concentrations in the drainage water and the soil extracts (K, Ca, Mg, Al, Fe, Mn, Zn, NH_4, NO_3, SO_4), the pH and the amount of drainage water. The independent variables were ammonium concentration in rainwater (3 levels), soil type (2 levels), tree species (3 levels) and watering method (2 levels).

In addition Scheffé's multiple-comparison procedure was performed on all main-effects. These statistical analyses were performed with the General Linear Models (GLM) procedure available in the Statistical Analysis System (SAS) software package (Sas Institute Inc., 1985).

RESULTS

Drainage water

Analysis of variance clearly demonstrates that the ammonium concentration in the rainwater, the soil type and the tree species significantly affected the concentrations of nutrients in the drainage water (Tab. 3). There were significant interactions between ammonium concentration and soil type, ammonium concentration and tree species, soil type and tree species, as well as tree species and watering method. These are specified below.

The results of Scheffé's multiple-comparison procedure are presented in Table 4. It is shown that an enhanced ammonium concentration in the rainwater resulted in significantly increased concentrations of nutrients in the drainage water and thus in increased leaching from the pots. Iron was the only element that did not show a clear rise in concentration. As the ammonium concentration increased more than the concentrations of the other ions, the ratios of ammonium to potassium, calcium, magnesium and nitrate in the drainage water rose. So did the ratio of aluminium to calcium. The pH of the drainage water decreased.

However, the two types of soil reacted differently to the application of increasing amounts of ammonium via the rainwater, resulting in significant interaction terms

Table 3. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on nutrient concentrations in the drainage water.

independent variables	dependent variables H2O	pH	K	Ca	Mg	Al	Zn	Mn	Fe	SO4	NH4	NO3	NH4 /K	NH4 /Ca	NH4 /Mg	NH4 NO3	Al /Ca
ammonium	-	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
soil type	***	***	-	***	***	***	***	**	***	-	***	***	***	***	***	***	***
tree species	*	**	*	***	***	***	***	-	-	***	-	***	-	***	***	***	***
watering m	-	-	*	-	-	**	-	-	-	-	-	-	-	-	-	-	*
interactions																	
ammonium*soil	-	***	-	***	***	***	***	***	**	-	**	***	***	***	***	***	***
ammonium*tree	-	-	-	-	*	*	-	-	-	-	-	***	-	*	-	*	*
soil*tree	***	**	-	-	*	**	-	-	-	-	-	*	-	-	-	*	**
tree*water	*	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	*

P-values of the analysis of variance: *** P≤0.001, ** 0.001<P≤0.01, * 0.01<P≤0.05, - not significant. Abbreviations: ammonium: ammonium concentration in rain, watering m: watering method, water: watering method.

between soil type and ammonium concentration (Tab. 3): the leaching of calcium, magnesium, manganese and zinc from the riverdune soil was stimulated more by the elevated nitrogen deposition than was leaching from the podzol soil (Tab. 5). The same applies for nitrate. The rise of the ammonium concentration was most pronounced in the drainage water of the podzol soil and very high ammonium to potassium, calcium, magnesium and nitrate ratios occurred in the drainage water of the 5000 N treatment. Also the aluminium to calcium ratio increased strongly. No such elevated ratios were observed in the drainage water of the riverdune soil. Most of them even decreased slightly when the ammonium concentration in the rainwater was raised from 0 to 500 μM. The decrease in pH of the drainage water was also most pronounced in the case of the riverdune soil.

Soil type greatly affected the chemical composition of the drainage water (Tab. 4). Calcium, magnesium and nitrate reached significantly higher concentrations in the drainage water of the riverdune soil. The same goes for manganese in the 5000 N treatment. Aluminium, iron and ammonium ions were more abundant in the drainage water of the podzol soil. This was also true for zinc, but only in the 0 N and 500 N treatments. The pH value of the drainage water of the riverdune soil was significantly higher than that of the other soil type. As much more water percolated through the

Table 4. Average nutrient concentrations in the drainage water for three ammonium concentrations, two soil types, three tree species and two watering methods.

	ammonium in rain			soil type		tree species			watering m	
	0 n=12	500 n=12	5000 n=12	podz n=18	river n=18	dfir n=12	cpine n=12	spine n=12	crown n=18	soil n=18
H2O	1.75a	1.69a	1.77a	2.39a	1.27b	1.89a	1.92a	1.45b	1.66a	1.82a
pH	4.44a	4.29b	3.71c	3.51b	4.78a	4.18a	4.20a	4.06b	4.14a	4.15a
K	7b	11b	42a	15a	15a	18a	15ab	12b	17a	13b
Ca	166c	387b	1638a	177b	1257a	412b	614a	415b	475a	469a
Mg	48c	108b	467a	69b	259a	118b	195a	104b	139a	129a
Al	49c	59b	1044a	396a	52b	118b	122b	207a	134b	154a
Zn	4c	8b	56a	13a	11b	10b	14a	12a	12a	12a
Mn	2c	5b	47a	7b	10a	7a	8a	10a	8a	8a
Fe	7a	3b	6a	16a	2b	5a	5a	5a	5a	5a
SO4	145c	691b	7630a	937a	893a	651b	1002a	1175a	898a	933a
NH4	22c	32b	2915a	192a	84b	141a	116a	124a	132a	121a
NO3	61c	154b	1036a	73b	620a	227b	549a	78c	233a	196a
NH4/K	4b	4b	99a	17a	7b	11a	10a	13a	10a	12a
NH4/Ca	0.15b	0.09c	1.97a	1.14a	0.08b	0.38a	0.21b	0.34a	0.31a	0.29a
NH4/Mg	0.6b	0.3c	6.8a	2.9a	0.4b	1.3a	0.7b	1.3a	1.1a	1.0a
NH4/NO3	3.2a	0.8b	4.4a	9.6a	0.5b	1.7b	1.3b	4.8a	2.3a	2.1a
Al/Ca	0.34b	0.15c	0.61a	2.25a	0.04b	0.29b	0.22c	0.51a	0.30b	0.34a

Nutrients in µmol.l^{-1}, water in l.pot^{-1}, geometric means. Different letters within each row indicate statistical difference at the 5% level according to Scheffé's multiple-comparison procedure. Abbreviations: watering m: watering method, podz: podzol soil, river: riverdune soil, dfir: Douglas fir, cpine: Corsican pine, spine: Scots pine.

podzol soil, this soil type lost more potassium and sulphate, though concentrations in the drainage water did not differ. The ratios of ammonium to potassium, calcium and magnesium as well as the ratio of aluminium to calcium were significantly higher in the drainage water of the podzol soil.

Tree species also exerted an influence on the chemical composition of the drainage water, though its impact was less pronounced than that of the ammonium concentration in rain or the soil type (Tab. 4). Concentrations of nutrients were generally highest in the drainage water of pots planted with Corsican pine and the ratios of ammonium to calcium and magnesium and the aluminium to calcium ratio were significantly lower here. Drainage water from pots with Scots pine contained the largest concentration of aluminium and the pH was significantly lower, particularly in the case of the riverdune soil. The nitrate concentration was also lower in the water flowing from pots planted with Scots pine. On average, pots with this tree species lost

Table 5. Average nutrient concentrations in the drainage water for each ammonium concentration per soil type.

	podzol soil ammonium in rain			riverdune soil ammonium in rain		
	0 n=6	500 n=6	5000 n=6	0 n=6	500 n=6	5000 n=6
H2O	2.50	2.26	2.40	1.22	1.27	1.31
pH	3.70	3.53	3.31	5.18	5.04	4.12
K	8	12	38	7	10	46
Ca	86	157	409	318	951	6562
Mg	35	58	165	65	201	1326
Al	117	254	2080	20	13	524
Mn	4	5	16	1	5	134
Fe	15	13	21	3	1	2
Zn	6	12	36	2	6	88
SO4	155	659	8055	136	725	7223
NO3	44	47	192	84	510	5569
NH4	25	51	5620	19	20	1512
NH4/K	4	5	248	3	3	39
NH4/Ca	0.29	0.35	15	0.08	0.02	0.27
NH4/Mg	0.74	0.86	37	0.41	0.11	1.25
NH4/NO3	4	4	52	3	0.1	0.4
Al/Ca	1.4	1.6	4.9	0.08	0.01	0.08

Nutrients in µmol.l^{-1}, water in l.pot^{-1}, geometric means.

the smallest amount of water. However, there was a significant interaction with the soil type (Tab. 3): pots containing the podzol soil lost larger amounts of water when planted with Douglas fir than with either of the two pine species. However, when the riverdune soil was used, pots with Douglas fir lost the smallest quantities of water. The total amounts of nutrients lost via the drainage water varied accordingly.

The way of watering the trees had no effect on the chemical composition of the drainage water, except for small but significant effects on the potassium and aluminium concentrations (Tab. 3). There was a significant interaction between tree species and watering method for the amount of drainage water: watering over the crown resulted in significantly smaller amounts of drainage water in the case of Scots pine and Douglas fir, whereas the smallest quantities of water flowed from the pots with Corsican pine when water was applied directly to the soil surface. Although this hardly influenced the concentrations of nutrients, it did affect the total amounts of ions that leached from the pots.

The pots without trees lost larger quantities of water and nutrients than did the pots with trees. The only clear exception was aluminium, that leached in smaller amounts from the soil in the 5000 N treatment in the absence of trees, particularly in the case of the podzol soil. The decrease in pH was smaller when trees were absent (data not shown).

Soil extracts

Very similar results were obtained from the water extracts made after 7 months of treatment (Tab. 6). All nutrients, except iron, showed a significant increase in availability when the ammonium deposition was enhanced (Tab. 7). However, for potassium, calcium, magnesium and nitrate this ammonium effect was much stronger on the riverdune soil than on the podzol soil (Tab. 8).

Table 6. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the extractability of nutrients with water

independent variables	dependent variables pH	K	Ca	Mg	Al	Zn	Mn	Fe	SO4	NH4	NO3	NH4 /K	NH4 /Ca	NH4 /Mg	NH4 NO3	Al /Ca
ammonium	***	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***
soil type	***	-	***	***	***	-	***	***	-	-	***	-	***	***	***	***
tree species	*	***	-	-	-	-	*	-	*	-	**	***	-	-	*	-
watering m	-	-	-	*	-	-	-	-	-	-	-	-	-	*	-	-
interactions																
ammonium*soil	***	***	*	*	-	-	-	-	-	*	***	**	**	**	***	***
ammonium*tree	-	*	-	-	*	-	-	-	-	-	**	-	-	-	**	**
ammonium*water	-	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
soil*tree	-	-	*	*	-	-	-	-	-	**	-	**	-	**	*	*
tree*water	-	-	-	-	-	-	-	-	-	-	*	-	-	-	*	-

For the explanation of significance symbols see Tab. 3. Abbreviations: ammonium: ammonium concentration in rain, watering m watering method, water watering method

Compared to the starting situation (data not shown), the extractability of potassium, magnesium, ammonium and nitrate in both soil types, and of calcium, aluminium and sulphur in the podzol soil had decreased in the 0 N treatment. The

Table 7. Extractability of nutrients in the soil as determined by water extraction for three ammonium concentrations, two soil types, three tree species and two watering methods.

	ammonium in rain			soil type		tree species			watering m	
	0 n=12	500 n=12	5000 n=12	podz n=18	river n=18	dfir n=12	cpine n=12	spine n=12	crown n=18	soil n=18
pH	4.48a	4.23b	3.65c	3.65b	4.59a	4.10a	4.17a	4.09a	4.11a	4.13a
K	372c	621b	1005a	604a	626a	680a	842a	406b	612a	618a
Ca	952c	2407b	7601a	1140b	5896a	2506a	2312a	3009a	2291a	2934a
Mg	229c	439b	1401a	304b	890a	479a	515a	572a	460b	589a
Al	633c	882b	2392a	1421a	852b	967a	1057a	1305a	1030a	1177a
Zn	37b	241a	421a	128a	187a	145a	186a	138a	159a	150a
Mn	15c	41b	225a	26b	100a	39b	42ab	81a	40a	65a
Fe	176a	153ab	102b	203a	97b	169a	129a	126a	146a	135a
SO4	1103c	7274b	28226a	4837a	7685a	4110b	6277ab	8779a	6105a	6086a
NH4	361b	348b	24785a	1605a	1330a	1252a	1606a	1549a	1542a	1383a
NO3	126b	225b	1676a	148b	889a	272b	612a	285b	394a	332a
NH4/K	0.97b	0.56c	24.6a	2.66a	2.12a	1.84b	1.91b	3.81a	2.52a	2.24a
NH4/Ca	0.38b	0.14c	3.3a	1.41a	0.23b	0.50a	0.69a	0.51a	0.67a	0.47a
NH4/Mg	1.58b	0.79c	17.7a	5.27a	1.49b	2.61a	3.12a	2.71a	3.36a	2.35b
NH4/NO3	2.9b	1.5b	14.8a	10.9a	1.5b	4.6ab	2.6b	5.4a	3.9a	4.2a
Al/Ca	0.66a	0.37b	0.31b	1.25a	0.14b	0.39a	0.46a	0.43a	0.45a	0.40a

Nutrients in µmol.pot^{-1}, geometric means. For the explanation of significance symbols and abbreviations see Tab. 4.

same applies for ammonium and nitrate in the 500 N treatment of both soil types and for nitrate in the 5000 N treatment of the podzol soil. The pH value of the water extract increased in the 0 N treatment, but decreased significantly when more ammonium was added. This decrease was most pronounced in the riverdune soil.

The ratios of ammonium to potassium, magnesium, calcium and nitrate decreased slightly when the ammonium concentration in the rainwater was raised from 0 to 500 µM, but increased when more ammonium was added (Tab. 7). This increase, however, was strongest in the case of the podzol soil (Tab. 8), resulting in significant interactions between soil type and ammonium concentration in rainwater for these ratios (Tab. 6). In the podzol soil the ratios of ammonium to potassium, magnesium, calcium and nitrate reached values of 39, 48, 13 and 111, respectively. In the other soil type these values were 16, 6, 0.8 and 2 (Tab. 8).

The aluminium to calcium ratio varied from 0.9 to 1.5 in the extracts of the podzol soil and was lowest in the 500 N treatment. In the riverdune soil this ratio remained far below 1 and decreased significantly when more ammonium was added.

Table 8. Extractability of nutrients in the soil as determined by water extraction for each ammonium concentration per soil type.

	podzol soil ammonium in rain			riverdune soil ammonium in rain		
	0 n=6	500 n=6	5000 n=6	0 n=6	500 n=6	5000 n=6
pH	3.90	3.68	3.36	5.05	4.77	3.95
K	522	489	863	266	788	1170
Ca	517	1086	2639	1753	5340	21895
Mg	162	252	692	324	766	2838
Al	781	1001	3670	512	777	1558
Zn	55	167	232	25	349	763
Mn	9	24	79	23	68	645
Fe	222	210	181	140	112	57
SO4	926	4447	27474	1313	11897	28999
NH4	403	308	33323	324	394	18416
NO3	115	93	301	138	546	9330
NH4/K	0.8	0.6	38.6	1.2	0.5	15.7
NH4/Ca	0.8	0.3	12.6	0.2	0.1	0.8
NH4/Mg	2.5	1.2	48.1	1.0	0.5	6.5
NH4/NO3	3.5	3.3	110.7	2.4	0.7	2.0
Al/Ca	1.5	0.9	1.4	0.3	0.2	0.1

Nutrients in µmol pot^{-1}, geometric means.

When the two soil types are compared, it is seen that the podzol soil contained more extractable aluminium and iron, whereas the riverdune soil had larger amounts of extractable calcium, magnesium, manganese and nitrate. The ratios of ammonium to other cations as well as the aluminium to calcium ratio were significantly higher in the water extracts of the podzol soil. The pH was significantly lower there (Tab. 7).

For most nutrients tree species had no influence on the extractability (Tab. 7): however, that of potassium was significantly lower when the pots were planted with Scots pine instead of one of the other tree species and that of nitrate was highest in the case of Corsican pine. The ratios of ammonium to potassium and ammonium to nitrate differed accordingly. Pots with Douglas fir and Scots pine differed also regarding the extractability of sulphate.

The watering method had no effect on the extractability of nutrients, except for a small effect on that of magnesium and its ratio with ammonium (Tab. 7).

Analysis of variance shows, that treatment with different amounts of ammonium had no significant effect on the extractability of potassium, magnesium and iron with a salt solution (Tab. 9). However, amounts of extractable aluminium and ammonium rose when the ammonium concentration in rainwater was raised to 5000 µM (Tab. 10). For aluminium this rise was strongest in the riverdune soil, but for ammonium it was clearest in the podzol soil (Tab. 11). Th extractability of zinc and manganese ncreased only in the riverdune soil. The pH of the salt extract dropped significantly when the ammonium concentration in the rainwater rose. Again this was most clearly seen on the riverdune soil.

Compared to the starting situation (data not shown) the extractability of most nutrients increased, irrespective of the ammonium treatment. However, the extractability of magnesium from both soil types and calcium from the riverdune soil decreased. In the case of the riverdune soil this decline was positively related to the ammonium deposition. The ammonium extractability decreased in the 0 N and 500 N reatments of both soil types.

Table 9. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the extractability of nutrients with a salt solution.

independent variables	pH	K	Ca	Mg	Al	Zn	Mn	Fe	NH4
	dependent variables								
ammonium	***	-	*	-	***	**	*	-	***
soil type	***	***	***	***	***	***	***	-	-
tree species	-	***	***	***	**	**	*	-	***
watering m	-	-	-	-	-	-	-	-	-
interactions									
ammonium*soil	***	-	-	-	***	*	**	-	***
ammonium*tree	-	-	-	-	-	-	-	-	*
soil*tree	-	-	-	*	*	**	-	-	*

For the explanation of significance symbols see Tab. 3. Abbreviations: ammonium: ammonium concentration in rain, watering m: watering method.

Table 10 Extractability of nutrients in the soil as determined by salt extraction for three ammonium concentrations, two soil types, three tree species and two watering methods

	ammonium in rain			soil type		tree species			watering m	
	0 n=12	500 n=12	5000 n=12	podz n=18	river n=18	dfir n=12	cpine n=12	spine n=12	crown n=18	soil n=18
pH	3.82a	3.83a	3.49b	3.33b	4.09a	3.71a	3.71a	3.71a	3.71a	3.71a
K	2752a	2714a	2697a	2065b	3583a	3121a	3362a	1920b	2804a	2640a
Ca	16681a	12772b	14501ab	4789b	44267a	18503a	14843a	11241b	15184a	13964a
Mg	2641a	2090a	2317a	903b	6057a	2788a	2591a	1771b	2416a	2264a
Al	8039b	9228b	20599a	24835a	5340b	12422a	12446a	9874b	11673a	11358a
Zn	582ab	443b	694a	431b	737a	659a	643a	422b	593a	536a
Mn	189ab	183b	229a	128b	356a	210ab	141b	250a	194a	199a
Fe	132a	184a	180a	206a	133a	171a	196a	135a	160a	169a
NH4	585b	630b	25438a	2173a	2043a	2173a	2471a	1744b	2211a	2009a

Nutrients in µmol pot ^{1}, geometric means For the explanation of significance symbols and abbreviations see Tab 4

Table 11 Extractability of nutrients in the soil as determined by salt extraction for each ammonium concentration per soil type

	podzol soil ammonium in rain			riverdune soil ammonium in rain		
	0 n=6	500 n=6	5000 n=6	0 n=6	500 n=6	5000 n=6
pH	3.33	3.42	3.23	4.31	4.23	3.74
K	2189	1953	2063	3460	3774	3523
Ca	5902	3663	5085	47146	44489	41357
Mg	1041	714	990	6701	6118	5426
Al	21960	22765	30669	2942	3741	13822
Zn	551	319	457	616	615	1054
Mn	149	100	139	240	377	1010
Fe	171	233	212	107	145	153
NH4	582	497	35525	588	797	18215

Nutrients in µmol pot ^{1}, geometric means

Most nutrients could be extracted in larger amounts from the riverdune soil, but aluminium was present in larger quantities in the podzol soil. This type of soil had also a lower pH value (Tab. 10).

Tree species affected the extractability of nutrients significantly: for most ions it was lowest in the pots planted with Scots pine. For magnesium, aluminium, zinc and ammonium this was most pronounced in the riverdune soil. The watering method had no influence on the extractability of nutrients (Tab. 10).

The extraction with a 0.2 N NaCl solution resulted in a higher yield of all nutrients than did the extraction with water. However, differences were large in the 0 N treatments and relatively small in the 5000 N treatments (compare Tab. 8 and 11). Aluminium in the riverdune soil was the only exception to this rule.

DISCUSSION

Deposition of ammonium sulphate at the rates used here appeared to have a strong impact on soil chemistry. Though neutral by itself, it caused severe acidification of both the soil and the soil solution. In the riverdune soil ammonium sulphate was rapidly converted into nitric and sulphuric acid by nitrifying bacteria. Only in the 5000 N treatment were appreciable amounts of ammonium found in the drainage water. So, possibly the conversion rate was too low or the retention time of the water in the soil too short to deal with all the ammonium supplied. Alternatively, the chemical reaction was impaired by too high concentrations of ammonium.

In the podzol soil the formation of nitrate was of minor importance. Apparently, the nitrification process was largely inhibited. This might be due to the low pH value or the infertile character of the soil. Furthermore, phenolic or other toxic substances could be present as many of the afforested soils were formerly covered by heathlands (Kriebitzsch, 1978; Kinzel, 1982). Here, acidification might be primarily caused by plant uptake of ammonium. It is well-known that ammonium uptake by plants can result in a considerable acidification (Runge, 1983).

As the protons generated were neutralized by exchange, mineralization and weathering processes, the concentrations of many cations rose in the soil solution and consequently in the drainage water. Thus, the acid neutralizing capacity (ANC) of the soil decreased (Van Breemen *et al.*, 1984).

In the riverdune soil, which was in the cation exchange buffer range (Ulrich, 1983b), the buffering action was mainly accounted for by calcium and magnesium compounds. The podzol soil was in a more advanced state of acidification, *i.e.* in the aluminium buffer range (Ulrich, 1983b) and predominantly aluminium ions were released by the reactions consuming the protons. However, in both soil types the buffer rate was insufficient to prevent a pH decline in the drainage water.

The loss of cations was most pronounced in the 5000 N treatments and clearly exceeded the supply from the seasalt in the rainwater. In the other two treatments however, nutrient leaching was fully compensated by the input, at least for potassium and magnesium. Nevertheless, as the advantage of nutrient supply from the atmosphere was cancelled by an approximately equivalent loss via the drainage water, the plants were completely dependent on the nutrient stock in the soil, which was gradually depleted by plant uptake processes.

As leaching was most severe in the 500 N and 5000 N treatments, one would expect nutrient availability to decrease here first. However, it can be concluded from the soil extractions and the drainage water analyses that the increasing losses of cations did not result in a decreased nutrient availability. On the contrary, it was enhanced by the ammonium addition, compared to the availability in the 0 N treatment. Apparently, rates of weathering and cation exchange were accelerated by the ammonium deposition to such an extent, that the enhanced losses were more than balanced. However, it is obvious that this will ultimately deplete the cation stock and impoverish the soil. In the 0 N treatment these processes were proceeding too slowly to compensate for leaching and plant uptake.

As acidification was progressing, toxic metals like aluminium, manganese and zinc started to participate in buffering and the concentrations in the soil solution and the drainage water rose. This was most clearly seen in the riverdune soil, where apart from the aluminium concentration those of manganese and zinc showed a clear rise. But calcium was still prevalent. In the podzol soil manganese and zinc were probably already largely depleted, as their concentrations in the drainage water did not respond so strongly to acidification. The same may hold true to a lesser extent also for calcium, because its concentration did not increase as fast as that of aluminium. Consequently the aluminium to calcium ratio was enhanced. In the 5000 N treatment the ratio rose to 5, a value that is regarded most critical for tree roots. Even the lower values of 1 to 2 in the other treatments are disadvantageous (Ulrich, 1983b). However, an unknown part of the free aluminium ions may be detoxified by chelating organic substances (Hüttermann, 1985).

The low nitrification rate in the podzol soil resulted in an accumulation of ammonium in the soil solution, when ammonium was applied in the highest concentration. As the liberation of other cations could not keep pace with the ammonium increase, the ratios of ammonium to potassium, magnesium and calcium rose steeply. In the water extracts of the podzol soil values were measured of 39, 48 and 13, respectively. Roelofs *et al.* (1985) and Roelofs (1986), who used the same extraction procedure, reported values for these ratios of approximately 5 in healthy and of far above 5 in moderately and severely damaged stands of Corsican pine and Douglas fir. Hydroculture experiments with young coniferous trees indicate that ammonium may impair potassium and magnesium uptake even at low concentrations

(Rygiewicz *et al.*, 1984; Bledsoe & Rygiewicz, 1986; Boxman & Roelofs, 1988; Boxman & Van Dijk, 1988). The presence of mycorrhizae may temper the adverse effect of ammonium on cation nutrition (Bledsoe & Rygiewicz, 1986; Boxman & Roelofs, 1988). Trees take up ammonium at a fast rate in the field. So, under optimal conditions they may be able to lower the ammonium concentration near the root surface to such an extent, that substantial uptake of other cations is allowed. However, if the ammonium concentration in the soil solution is too high, due to excessive nitrogen deposition, cation uptake may be seriously hampered. Lysimeter studies in the 'Peel'-area revealed ammonium concentrations in the soil solution beneath a pine forest of up to 2000 μM (Van Dijk, unpublished data).

No elevated ratios were observed in the 500 N treatment, as all the ammonium was used by the young trees. Nevertheless, it seems feasible, that this will change in a field situation in the course of time. As the trees grow older, their need for nitrogen may diminish and the uptake be impaired by shortages of other nutrients.

Accumulation of ammonium was less pronounced in the riverdune soil, where most of it was converted into nitrate and cation levels were relatively high. In the 5000 N nitrogen treatment the ammonium to potassium ratio was elevated due to the low potassium content of this soil type. Prolonged deposition of ammonium sulphate, however, will ultimately move the riverdune soil towards the aluminium buffer range and nitrification may be inhibited, resulting in ammonium accumulation.

Tree species had a smaller impact on soil chemistry than the ammonium concentration in the rainwater or the soil type. Its influence was probably largely the result of differences in tree size: concentrations of nutrients were highest in the drainage water of the pots with the smallest tree species, *i. e.* Corsican pine, and the largest tree species, Scots pine, depleted the soil most severely, as was demonstrated by the results of the salt extraction procedure. The total amounts of nutrients lost via the drainage water were also smaller for this tree species due to the relatively small amounts of drainage water.

As could be expected the way of watering the trees had little influence on soil chemistry. Small differences in nutrient concentrations could be the result of alterations in the waterfluxes, for the amount of drainage water was affected by a combination of tree species (tree size) and way of watering: in the case of the big Scots pine trees applying water to the crown resulted in considerably smaller amounts of drainage water than watering directly on the soil. To a lesser extent the same went for Douglas fir. This was probably caused by both evaporation of intercepted water and spilling of water. As the Corsican pine trees were much smaller these factors were of minor importance and the somewhat higher amounts of drainage water of the trees watered on the crown might be due to the slightly higher relative air humidity.

Finally, it can be concluded that

1. the ammonium concentration in the rainwater and the soil type had the strongest impact on soil and soil water chemistry and that both tree species and way of watering were of minor importance.
2. deposition of ammonium sulphate caused soil acidification due to nitrification and ammonium uptake by plants.
3. this acidification involved both a decline in pH of the soil solution and a decrease in the acid neutralizing capacity (ANC).
4. ammonium accumulated in the soil solution when the deposition rate exceeded plant uptake and nitrification rates.
5. important plant nutrients like potassium, magnesium and calcium leached from the soil.
6. toxic metals like aluminium, manganese and zinc showed an increased solubility.
7. imbalances between several plant nutrients arose, particularly in the podzol soil.

The effects of these changes in soil chemistry on tree performance are discussed in a second paper (Van Dijk *et al.*, 1990).

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CHAPTER 3

IMPACT OF ARTIFICIAL, AMMONIUM-ENRICHED RAINWATER ON SOILS AND YOUNG CONIFEROUS TREES IN A GREENHOUSE. II. EFFECTS ON THE TREES

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ABSTRACT

To obtain an insight into the effects of a high ammonium deposition on trees, young, coniferous trees were planted in pots in a greenhouse and treated with artificial ammonium-enriched rainwater. Application of 480 kg ammonium-N.ha^{-1}.yr^{-1} resulted in an increase of the shoot/root ratio. The biomass of fine roots strongly declined, as did the numbers of mycorrhizae. The fructification of the mycorrhizal fungi was totally inhibited. The nitrogen content of the needles was enhanced, but the levels of potassium, magnesium and calcium decreased sharply. The phosphorus content remained almost unaffected. Much of the nitrogen was stored as arginine. The levels of leaf pigments also increased. Within one year of treatment many of the trees died. The trees that were treated with 48 kg ammonium-N.ha^{-1}.yr^{-1} did not show any signs of deterioration.

INTRODUCTION

In The Netherlands and in other countries in Western Europe nitrogen deposition is extremely high and forest stands may receive much more nitrogen than is needed for the maintenance of biomass production. Several investigators have suggested that this excessive nitrogen nutrition may be a major cause of forest dieback (Meyer, 1985; Nihlgård, 1985; Roelofs *et al.*, 1985; Encke, 1986; Zedler *et al.*, 1986 and De Temmerman *et al.*, 1988).

To investigate the effects of an ample ammonium supply on trees and soils a pot experiment was carried out, in which young, coniferous trees were treated with artificial, ammonium-enriched rainwater. The effects on the soil were described in a previous paper (Van Dijk *et al.*, 1989): the ammonium deposition caused a considerable decrease in the pH of the soil solution, leaching of base cations and the mobilization of aluminium. Ammonium accumulated in the soil solution, resulting in a disturbed nutrient balance.

This paper describes how tree performance was affected by these chemical changes in the soil.

MATERIALS AND METHODS

A detailed description of the experimental setup and the climatic conditions prevailing in the greenhouse is given by Van Dijk *et al.* (1989).

Two trees of every treatment were harvested seven months after the beginning of the ammonium application. Fresh and dry weights (70 °C, 24 h drying) of the different tree parts were determined. The degree of mycorrhizal infection of the pine roots was estimated by cutting three subsamples out of the root systems by means of a sharpened metal cylinder (diameter 5 cm) and counting the typical bifurcate mycorrhizal roots. No distinction was made between dead and living mycorrhizae. Mycorrhizal infection was not assessed in the root systems of the Douglas firs. Fruiting bodies growing on the soil surfaces were identified in all pots.

Needle samples were used for elemental analysis (seven and nineteen-months-old needles) and measurements of leaf pigments and free amino acids (seven-months-old needles). Elemental analysis was carried out as described earlier (Van Dijk & Roelofs, 1988). To extract leaf pigments 4 ml 96% ethanol was added to 400 mg fresh needle tissue, previously ground in liquid nitrogen. The suspension was incubated in the dark at -20 °C for 24 h and centrifuged (5000 rpm, 5 min.). 125 μl of the supernatant was diluted to 1 ml with 96% ethanol and the leaf pigments were assessed spectrofotometrically according to Wellburn & Lichtenthaler (1984). The rest of the supernatant was used for an exploratory examination of the free amino acid pool. The corresponding samples of the two watering methods were pooled and dried at 35 °C. The residues were resuspended in 1 ml lithium citrate buffer (pH 2.6, 0.2 M Li) and the concentrations of the amino acids were measured according to Van Dijk & Roelofs (1988). Norleucine served as an internal standard.

All data were log-transformed prior to statistical analysis. The influence of the experimental factors on the tree parameters was analyzed with three-way and four-way analysis of variance models, including all first-order interactions that were significant for one or more of the measured parameters. The dependent variables were tree biomass parameters, numbers of mycorrhizae, element contents of the needles, levels of leaf pigments and arginine content. The independent variables were ammonium concentration in rainwater (3 levels), soil type (2 levels), tree species (3 levels) and watering method (2 levels). In the models used for the analysis of biomass and arginine data the watering method was omitted as independent variable. In the model analyzing the mycorrhizal data the tree species had only two levels (Douglas fir was omitted).

In addition Scheffé's multiple-comparison procedure was performed on all main-effects. These statistical analyses were performed with the General Linear Models (GLM) procedure available in the Statistical Analysis System (SAS) software package (SAS Institute Inc., 1985).

RESULTS

Biomass

The ammonium concentration in rainwater, the soil type and the tree species significantly affected most of the biomass parameters. The influence of the soil type on the biomass parameters, except for the biomass of the fine roots, depended on the tree species (*vice versa*) as is specified below (Tab. 1).

Table 1. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type and tree species on the biomass parameters of the trees.

	dependent variables							
independent variables	total biomass	7month needles	19month needles	stem+ branch	coarse roots	fine roots	s/r ratio	fr/cr ratio
ammonium	-	***	*	***	-	***	***	***
soil type	***	***	*	***	*	*	-	-
tree species	***	***	***	***	***	***	***	***
interactions								
soil*tree	***	***	***	***	***	-	**	***

P-values of the analysis of variance: *** P≤0.001, ** 0.001<P≤0.01, * 0.01<P≤0.05, - not significant. Abbreviations: ammonium: ammonium concentration in rainwater, s/r: shoot to root ratio, fr/cr: fine roots to coarse roots ratio.

No differences in biomass of the whole trees or the separate plant parts were observed between the two lower N treatments as was shown by Scheffé's multiple-comparison procedure (Tab. 2). When the ammonium concentration in rainwater was raised to 5000 µM total biomass still remained unaffected, but the dry weights of the seven-months-old needles and the stem and branches increased significantly, while that of the fine roots (diameter < 2 mm) decreased. The weights of the nineteen-month-old needles increased also, but only compared to the 500 N treatment. The weight of the coarse roots (diameter > 2mm) remained unchanged. Consequently, the shoot to root ratio increased and the fine to coarse root ratio decreased in the 5000 N treatment.

Table 2. Biomass of the trees and the various tree parts after seven months of treatment for three ammonium concentrations, two soil types and three tree species.

	ammonium in rain			soil type		tree species		
	0 n=24	500 n=24	5000 n=24	podz n=36	river n=36	dfir n=24	cpine n=24	spine n=24
total biomass	36.3a	38.9a	38.8a	34.3b	42.1a	34.8b	24.3c	64.8a
7m needles	8.1b	8.8b	10.3a	8.0b	10.1a	6.0c	8.0b	15.2a
19m needles	2.1ab	1.9b	2.7a	2.5a	1.9b	1.5b	2.6a	2.7a
stem+branches	8.0b	8.3b	10.4a	8.0b	9.8a	11.4a	4.7b	12.8a
coarse roots	3.8a	4.0a	4.7a	3.7b	4.6a	4.2b	2.4c	6.8a
fine roots	12.6a	13.8a	8.9b	10.4b	12.9a	10.6b	5.9c	24.6a
s/r-ratio	1.15b	1.10b	1.77a	1.34a	1.28a	1.26b	1.81a	0.98c
fr/cr-ratio	3.39a	3.48a	1.91b	2.78a	2.88a	2.49b	2.44b	3.71a

Dry weights in g, geometric means. Different letters within each row indicate statistical difference at the 5% level according to Scheffé's multiple-comparison procedure. Abbreviations: podz: podzol soil, river: riverdune soil, dfir: Douglas fir, cpine: Corsican pine, spine: Scots pine, 7m needles: seven-months-old needles, 19m needles: nineteen-months-old needles, s/r-ratio: shoot to root ratio, fr/cr-ratio: fine roots to coarse roots ratio.

The total biomass of the trees grown on the riverdune soil was significantly higher than that of the trees raised on the podzol soil, this being shown by all component parts except the nineteen-months-old needles which had a greater weight on the trees grown on the podzol (Tab. 2). However, not all tree species reacted in the same way to soil type, resulting in significant interactions between tree species and soil type (Tab. 1): in the case of Douglas fir the weights of the total plant and the separate plant parts, except for the nineteen-months-old needles, almost doubled when the trees were grown on the riverdune soil instead of on the podzol soil (Tab. 3). For the pine species these differences were small. Scots pine had even a slightly smaller biomass when grown on the riverdune soil. Generally, the shoot to root ratio and the fine to coarse root ratio were unaffected by soil type (Tab. 2), but Douglas fir had the highest shoot to root ratio on the riverdune soil, whereas the pine species had the highest ratio on the podzol soil. Scots pine had the highest fine to coarse root ratio on the riverdune, the other tree species on the podzol soil (Tab. 3).

Large differences in biomass were seen between the tree species (Tab. 2). Generally, Scots pine had the highest weights for all plant parts, whereas Corsican pine had the lowest. Douglas fir reached intermediate values, except for the biomass of the needles, which was always smaller than that of the pines. However, on the

Table 3. Biomass of the trees and the various tree parts after seven months of treatment for each tree species per soil type.

	podzol soil tree species			riverdune soil tree species		
	dfir n=12	cpine n=12	spine n=12	dfir n=12	cpine n=12	spine n=12
total biomass	25.6	23.4	67.2	47.2	25.3	62.4
7m needles	4.3	7.5	16.1	8.4	8.5	14.4
19m needles	1.4	2.7	4.4	1.7	2.6	1.6
stem+branches	7.6	4.7	14.3	17.2	4.7	11.4
coarse roots	3.0	2.1	8.1	6.0	2.8	5.7
fine roots	8.8	5.6	22.6	12.7	6.3	26.9
s/r-ratio	1.11	1.90	1.13	1.43	1.73	0.84
fr/cr-ratio	2.95	2.59	2.80	2.10	2.29	4.92

Dry weights in g, geometric means. For abbreviations see Tab. 2.

podzol soil the biomass of Douglas fir hardly exceeded that of Corsican pine (Tab. 3), resulting in the above mentioned interaction between soil type and tree species (Tab. 1).

Mycorrhizae

The ammonium concentration in rainwater, the soil type, the pine species and the watering method all affected the degree of mycorrhizal infection significantly (Tab. 4). The ammonium concentration had the strongest impact: the numbers of mycorrhizae were 5 times lower in the 5000 N treatment than in both other treatments (Tab. 5). Trees grown on the riverdune soil had more mycorrhizae than trees from the podzol soil. Scots pine had larger numbers of mycorrhizae than Corsican pine. However, this difference was most pronounced in the podzol soil (13 and 33 mycorrhizae g^{-1} DW in the podzol soil and 37 and 47 in the riverdune soil for Corsican and Scots pine, respectively). Trees given water over the crown had smaller numbers of mycorrhizae than trees that received water directly to the soil surface.

A similar picture was presented by the fruiting bodies of mycorrhizal fungi: *Telephora terrestris* Ehrh. ex Fr. was found only on the podzol soil. There were fruiting bodies found in 6 pots of the 0 N and in 4 pots of the 500 N treatment. However, none were observed in the 5000 N treatment.

Table 4. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the degree of mycorrhizal infection.

independent variables	dependent variable number of mycorrhizae
ammonium	***
soil type	***
tree species	**
watering m.	*
interactions	
soil*tree	*

For the explanation of significance symbols see Tab. 1. Abbreviations: ammonium: ammonium concentration in rain, watering m: watering method.

Table 5. Degree of mycorrhizal infection of the pine trees after seven months of treatment for three ammonium concentrations, two soil types, two tree species and two watering methods.

	ammonium in rain			soil type		tree species		watering m	
	0 n=16	500 n=16	5000 n=16	podz n=24	river n=24	cpine n=24	spine n=24	crown n=24	soil n=24
mycorr	59a	41a	10b	21b	42a	22b	40a	23b	37a

Number of mycorrhizae per g root DW, geometric means. For the explanation of significance symbols see Tab. 2. Abbreviations: see Tab. 2, mycorr: mycorrhizae, watering m: watering method.

Chemical composition of the needles

The ammonium concentration in rainwater, the soil type and the tree species proved to have a strong impact on the chemical composition of the seven-months-old needles

(ANOVA, Tab.6).

Table 6. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the nutrient levels in the seven-months-old needles.

independent variables	dependent variables K	Ca	Mg	Al	Mn	Fe	Zn	N	P	B	relative to N K	Mg	P
ammoniumain	***	*	***	-	-	-	-	***	***	***	***	***	***
soil type	***	***	***	***	***	-	***	-	-	***	***	**	-
tree species	***	***	***	-	-	***	-	***	***	***	***	***	**
watering m.	-	-	-	*	-	-	-	-	-	-	-	-	-
interactions													
ammonium*soil	-	-	-	-	***	-	-	-	-	*	-	-	*
ammonium*tree	-	-	-	-	-	-	-	*	-	***	*	-	*
soil*tree	-	-	-	-	-	-	-	-	*	***	-	-	-
soil*water.	-	-	-	-	*	-	-	-	*	-	-	-	-

For the explanation of significance symbols see Tab. 1. Abbreviations: ammonium ammonium concentration in rain, watering m. watering method, water. watering method

The levels of potassium, phosphorus and boron were significantly lower in the 5000 N treatment than in both other treatments (Tab 7). The contents of calcium and magnesium were also lower in the 5000 N than in the 500 N treatment, but did not differ significantly from those in the 0 N treatment. Most nutrients reached the highest levels in the 500 N treatment, but boron and nitrogen were the only elements for which this treatment differed significantly from the 0 N treatment. The nitrogen content of the needles increased when more ammonium was applied. The ratios of potassium, magnesium and phosphorus to nitrogen decreased accordingly.

The levels of potassium, calcium, magnesium, manganese, zinc and boron were significantly lower and that of aluminium higher in the needles of the trees grown on the podzol rather than the riverdune soil (Tab. 7). The soil type did not affect the iron, nitrogen and phosphorus contents of the needles. The ratios of potassium and magnesium to nitrogen were lowest in the needles of the trees from the podzol soil.

Table 7. Chemical composition of the seven-months-old needles for three ammonium concentrations, two soil types, three tree species and two watering methods, respectively.

	ammonium in rain			soil type		tree species			watering m.	
	0 n=24	500 n=24	5000 n=24	podz n=36	river n=36	dfir n=24	cpine n=24	spine n=24	crown n=36	soil n=36
K	0.321a	0.331a	0.240b	0.256b	0.339a	0.238b	0.325a	0.330a	0.292a	0.297a
Ca	0.354ab	0.392a	0.295b	0.252b	0.471a	0.439a	0.287b	0.325b	0.357a	0.333a
Mg	0.116ab	0.129a	0.100b	0.100b	0.131a	0.162a	0.084c	0.110b	0.115a	0.114a
Al	0.097a	0.102a	0.098a	0.104a	0.094b	0.096a	0.099a	0.102a	0.096b	0.102a
Mn	0.035a	0.040a	0.032a	0.030b	0.041a	0.038a	0.034a	0.035a	0.036a	0.035a
Fe	0.009a	0.011a	0.011a	0.011a	0.010a	0.012a	0.017a	0.006b	0.010a	0.010a
Zn	0.012a	0.013a	0.011a	0.010b	0.015a	0.014a	0.010a	0.012a	0.011a	0.013a
N	1.031c	1.210b	1.736a	1.256a	1.333a	1.442a	1.071b	1.403a	1.294a	1.294a
P	0.125a	0.126a	0.117b	0.124a	0.121a	0.127a	0.116b	0.125a	0.122a	0.123a
B	0.0018b	0.0023a	0.0016c	0.0016b	0.0022a	0.0024a	0.0016b	0.0017b	0.0019a	0.0019a
relative to N (N=100)										
K	31a	27a	14b	20b	25a	16c	30a	24b	23a	23a
Mg	11a	11a	6b	8b	10a	11a	8b	8b	9a	9a
P	12a	10a	7b	10a	9a	9b	11a	9b	9a	10a

Nutrients in % of DW, geometric means. For the explanation of significance symbols see Tab. 2. Abbreviations: see Tab. 2, watering m watering method.

Tree species differed markedly in the chemical composition of the seven-months-old needles (Tab. 7). Douglas fir had the lowest potassium content in the needles, but the highest levels of calcium, magnesium and boron. Corsican pine contained the smallest amounts of magnesium, nitrogen and phosphorus in its needles and Scots pine had the lowest levels of iron. The ratio of potassium to nitrogen was lowest in the needles of Douglas fir, but the ratio of magnesium to nitrogen was highest there. Corsican pine exhibited the highest potassium and phosphorus to nitrogen ratios.

The watering method had almost no effect on the chemical composition of the seven-months-old needles. Aluminium was the only element that reached slightly higher levels in the needles of the trees that were watered directly on the ground (Tab. 7).

The ammonium concentration in the rainwater had no effect on the cation levels in the nineteen-months-old needles (Tab. 8). However, the phosphorus and boron contents were significantly lower in the needles of the 5000 N treatment than in the needles of the other treatments (Tab. 9). The nitrogen content increased when more ammonium was added and consequently the ratios of potassium, magnesium and phosphorus to nitrogen decreased. The impact of soil type and tree species on the chemical composition of the old needles was very similar to that described previously for the young needles. The watering method had no effect at all.

Table 8. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the nutrient levels in the nineteen-months-old needles.

independent variables	dependent variables K	Ca	Mg	Al	Mn	Fe	Zn	N	P	B	relative to N K	Mg	P
ammonium	-	-	-	-	-	-	-	***	***	**	***	***	***
soil type	***	***	***	-	***	-	***	-	-	***	***	***	-
tree species	***	***	***	-	-	***	-	***	***	***	***	***	***
watering m.	-	-	-	-	-	-	-	-	-	-	-	-	-
interactions													
ammonium*soil	-	-	-	-	-	-	-	*	-	-	-	-	**
ammonium*tree	-	-	-	-	-	-	-	*	-	***	-	-	*
soil*tree	*	**	-	-	***	-	-	-	-	***	-	-	-

For the explanation of significance symbols see Tab. 1. Abbreviations: ammonium: ammonium concentration in rain, watering m: watering method.

Compared to the starting situation (data not shown) the nineteen-months-old needles of Douglas fir and Corsican pine in the two lower N treatments contained less nitrogen. The potassium content strongly decreased in the needles of Douglas fir and to a smaller extent also in the needles of the pine trees growing on the podzol soil. The pine trees raised on the riverdune soil did not show decreased potassium levels.

Amino acids and leaf pigments

The most conspicuous result of the analysis of the free amino acid pool was the strong increase in free arginine, brought about by the addition of ammonium. In the 0 N and 500 N treatments the levels of arginine were 0.38 and 0.61 µmol.g^{-1} FW and did not differ at the 5% level. In the 5000 N treatment, however, this concentration rose to 26.2 µmol.g^{-1} FW, which was significantly more than in the other treatments

Table 9. Chemical composition of the nineteen-months-old needles for three ammonium concentrations soil types, three tree species and two watering methods, respectively.

	ammonium in rain			soil type		tree species			watering	
	0 n=24	500 n=24	5000 n=24	podz n=36	river n=36	dfir n=24	cpine n=24	spine n=24	crown n=36	s n
K	0.259a	0.285a	0.261a	0.225b	0.320a	0.166b	0.316a	0.373a	0.270a	0.2
Ca	0.581a	0.588a	0.542a	0.472b	0.692a	0.623a	0.601a	0.490b	0.565a	0.5
Mg	0.101a	0.099a	0.093a	0.085b	0.111a	0.119a	0.069b	0.112a	0.100a	0.0
Al	0.101a	0.102a	0.103a	0.103a	0.101a	0.102a	0.104a	0.100a	0.101a	0.1
Mn	0.031a	0.033a	0.029a	0.025b	0.038a	0.030a	0.035a	0.028a	0.032a	0.0
Fe	0.025a	0.022a	0.025a	0.022a	0.025a	0.032a	0.023ab	0.018b	0.022a	0.0
Zn	0.017a	0.018a	0.016a	0.015b	0.019a	0.019a	0.017a	0.016a	0.018a	0.0
N	0.762c	0.873b	1.402a	0.974a	0.987a	0.909b	0.898b	1.163a	1.009a	0.9
P	0.121a	0.121a	0.114b	0.119a	0.118a	0.120a	0.114b	0.122a	0.119a	0.1
B	0.0018ab	0.0020a	0.0016b	0.0015b	0.0021a	0.0026a	0.0014b	0.0016b	0.0018a	0.0
relative to N (N=100)										
K	34a	33a	19b	23b	32a	18b	35a	32a	27a	
Mg	13a	11a	7b	9b	11a	13a	8c	10b	10a	
P	16a	14a	8b	12a	12a	13a	13a	10b	12a	

Nutrients in % of DW, geometric means. For the explanation of significance symbols see Tab. 2. Abbrevia see Tab. 2, watering m: watering method.

($P<0.001$). Trees growing on the riverdune soil had slightly, but significantly higher arginine levels than trees from the podzol soil: 2.2 and 1.1 μmol.g^{-1} FW, respectively ($P=4.9\%$). The tree species did not affect the levels of free arginine.

Analyses of the leaf pigments (Tab. 10 and 11) revealed that the chlorophylls a and b increased significantly when more ammonium was added. The carotenoid levels were highest in the 500 N treatment. The ratio of chlorophylls to carotenoids was strongly enhanced in the 5000 N treatment. The ratio of chlorophylls a and b however, was lowest there.

Trees growing on the riverdune soil contained larger amounts of chlorophylls a and b and carotenoids than trees from the podzol soil (Tab. 11). However, in the case of the chlorophylls this was only observed in the lower N treatments. In the 5000 N treatment the chlorophyll levels were highest in the trees planted on the podzol soil. The same applied for the ratio of chlorophylls to carotenoids. This resulted in significant interactions between the soil type and the ammonium concentration in rainwater (Tab. 10).

Douglas fir contained more chlorophyll a and carotenoids than the pine trees. The watering method had almost no effect on the levels of leaf pigments. Only the

Table 10. P-values of the ANOVA model for the influence of ammonium in rainwater, soil type, tree species and watering method on the levels of leaf pigments in the seven-months-old needles.

	dependent variables				
independent variables	chlorophyll a	chlorophyll b	carotenoids	chlor.a /chlor.b	chlor.a+b /caroten.
ammonium	***	***	**	**	***
soil type	***	**	*	-	*
tree species	***	-	***	-	-
watering m.	-	-	*	-	-
interactions					
ammonium*soil	*	*	-	-	**

For the explanation of significance symbols see Tab. 1. Abbreviatios: ammonium: ammonium concentration in rain, chlor.: chlorophyll, caroten.: carotenoids, watering m: watering method.

Table 11. Levels of leaf pigments in the seven-months-old needles for three ammonium concentrations, two soil types, three tree species and two watering methods, respectively.

	ammonium in rain			soil type		tree species			watering m.	
	0	500	5000	podz	river	dfir	cpine	spine	crown	soil
	n=12	n=12	n=12	n=18	n=18	n=12	n=12	n=12	n=18	n=18
chl.a	453c	585b	736a	524b	641a	676a	539b	535b	584a	576a
chl.b	114b	142b	224a	137b	172a	174a	138a	151a	149a	159a
car.	102b	120a	102b	103b	113a	123a	102b	100b	113a	103b
chl.a/b	4.0a	4.1a	3.3b	3.8a	3.7a	3.9a	3.9a	3.5a	3.9a	3.6a
chl./car.	5.6b	6.1b	9.4a	6.4b	7.2a	6.9a	6.7a	6.9a	6.5a	7.2a

Pigments in $\mu g.g^{-1}$ FW, geometric means. For the explanation of significance symbols see Tab. 2. Abbreviations: chl. chlorophyll, car. carotenoids, watering m. watering method.

carotenoid content was slightly, but significantly higher in the needles of the trees watered over the crown instead of on the soil surface (Tab. 11).

Mortality

In spring 1986 some of the trees did not show bud-break and died soon afterwards. Mortality was highest in the 5000 N treatment. No trees died in the other treatments, except for two Douglas fir trees on the riverdune soil, one in the 0 N and one in the 500 N treatment. In the 5000 N treatment Douglas fir showed the highest mortality: almost all trees died on the podzol soil and almost 50 % of the trees on the riverdune soil (Tab. 12). Corsican pine displayed almost complete mortality on the podzol soil, but only a few trees did not show bud-break on the other soil type. Scots pine exhibited the lowest mortality. About 20 % of the trees died on the podzol soil and none on the riverdune soil. Differences in mortality between the watering methods were small.

Table 12. Tree mortality in the 5000 N treatment after one year of treatment (n=8=100%).

tree species	soil type	watering method	mortality
douglas fir	podzol	soil	100
douglas fir	podzol	crown	88
douglas fir	riverdune	soil	63
douglas fir	riverdune	crown	25
corsican pine	podzol	soil	100
corsican pine	podzol	crown	75
corsican pine	riverdune	soil	13
corsican pine	riverdune	crown	0
scots pine	podzol	soil	25
scots pine	podzol	crown	13
scots pine	riverdune	soil	0
scots pine	riverdune	crown	0

DISCUSSION

Application of high amounts of ammonium caused an increase of the shoot to root ratio. This was also reported by Encke (1986) and Gezelius (1986b). The decline of the root system was largely accounted for by a loss of fine roots. Nadelhoffer *et al.* (1985) also found a reduction of the fine roots with increasing nitrogen availability, though the production was greater as a result of an increased turnover.

The degree of mycorrhizal infection was also adversely affected. Container experiments with Norway spruce (*Picea abies* (L.) Karsten) (Meyer, 1985), Douglas fir (Van der Eerden & Wit, 1987) and Scots pine (Termorshuizen *et al.*, 1988) confirm the negative impact of ammonium on mycorrhizal formation. According to Meyer (1985) high amounts of inorganic nitrogen are particularly detrimental in soils with a low organic matter content and a low microbial activity, *i. e.* in podzol soils.

Simultaneously, the numbers of fruiting bodies of *Telephora terrestris* decreased. Similar results were obtained in fertilization experiments in forest stands (Menge & Grand, 1978, Wästerlund, 1982). This fits in very well with the observations of Arnolds (1985) and Arnolds & Jansen (1987), that fruiting bodies of mycorrhizal fungi are disappearing from the Dutch forests and that this process is most pronounced in the southern part of the country, where nitrogen deposition is highest.

It is not yet clear by what mechanism the mycorrhizae are influenced. Probably the production and functioning of growth substances is affected (Tomaszewski & Wojciechowska, 1973; Meyer, 1985).

All these physical changes may have far-reaching consequences for the trees. A smaller and less mycorrhizal root system has to supply water and nutrients to a more demanding shoot. Thus, the risk of desiccation and nutrient deficiencies is enhanced. This is aggravated by raised ammonium to cation ratios in the soil (Van Dijk *et al.*, 1989), which additionally impair nutrient uptake.

Indeed, cation levels in the young needles of the conifers were often lowest in the 5000 N treatment. This applied to both soil types and was most clear for potassium. The decrease could not be explained solely by growth dilution. Similarly, Turner & Olson (1976) observed a negative correlation between the cation levels in the needles of Douglas fir and the amounts of N given to the trees. The cation contents were lower in the needles of the trees growing on the podzol soil than in the needles of the trees planted on the riverdune soil. This could partly be explained by differences in nutrient availabilities in the two soil types, but most likely, the form of the nitrogen played an important role too: nitrate was dominant in the riverdune soil, whereas ammonium prevailed in the podzol soil (Van Dijk *et al.*, 1989). It is well-known, that, compared to ammonium, nitrate stimulates cation uptake (Runge, 1983). In the 5000 N treatment of the riverdune soil not all the ammonium was converted into nitrate. If the trees took up part of their nitrogen as ammonium, this could explain the lower potassium, magnesium and calcium levels in the trees of this treatment.

In comparing the nutrient levels in the needles with those reported in the literature (Van den Burg, 1988), it seems that nitrogen was deficient or that the levels were insufficient in all trees of the 0 N and in most of the trees in the 500 N treatment. The calcium and magnesium contents were generally sufficient or optimal. Phosphorus levels were insufficient in almost all trees but above the deficiency level of 0.1 %. These low phosphorus values are very commonly observed in Dutch coniferous forest stands (Mohren *et al.*; 1986, Oterdoom *et al.*, 1987 and Van den Burg *et al.*, 1988). Potassium levels, however, were extremely low: potassium was deficient in all

Douglas fir trees as well as in the Scots pine trees raised on the podzol soil. In all other trees the levels were insufficient. The values were lower than commonly observed in the Dutch forests. This is probably due to the low potassium content of the soil types used and the removal of the litter layer.

As deficiency of potassium, magnesium and phosphorus may occurr at higher levels of these nutrients when more nitrogen is present, the ratios of potassium, magnesium and phosphorus were calculated. In fixing the nitrogen content of the needles at 100, the critical weight proportions of potassium, phosphorus and magnesium are 25, 5 and 5 for coniferous trees (Van den Burg, 1988). From this it is clear that the phosphorus and magnesium supplies were low in the 5000 N treatments. However, the potassium proportions were the most critical here. They were far below the critical value of 25, particularly in the needles of Douglas fir. This was most pronounced on the podzol soil. So, although the nutritional status of the trees was generally low, it was balanced in the 0 N and 500 N treatments, whereas it was not so in the 5000 N treatment. Low cation levels, particularly compared to the nitrogen levels, are often observed in forests with a reduced vitality (Roelofs *et al.*, 1985, De Temmerman *et al.*, 1988).

The nitrogen rich amino acid arginine accumulated in the needles of the trees of the 5000 N treatment, irrespective of the tree species or the soil type. Rabe & Lovatt (1986) pointed out that both shortages and toxicities of many elements can cause accumulation of free arginine. As an elevated nitrogen nutrition also stimulates arginine production (Mulder & Bakema, 1956; Durzan & Steward, 1967; Rabe & Lovatt, 1986), it seems that the arginine accumulation may be the result of an unbalanced nutrition. Raised levels of non-proteinaceous nitrogen compounds involve a strong growth decline (Krauß *et al.*, 1986). Very high arginine concentrations were also measured in needles of Scots pines trees in the southeastern part of The Netherlands, where ammonium deposition is extremely high (Van Dijk & Roelofs, 1988).

In agreement with the findings of Turner & Olson (1976), the chlorophyll levels increased with increasing ammonium addition. The levels of chlorophyll b increased more than those of chlorophyll a. As the production of nitrogen-containing chlorophylls was greatly enhanced in the 5000 N treatment, whereas the production of the nitrogen-free carotenoids was not, the ratio of chlorophylls to carotenoids was enhanced.

Application of rainwater containing 5000 µM ammonium caused a high mortality of the trees within a year. As shown, ammonium may affect tree vitality in many ways. It is difficult to single out the ultimate killing factor. The facts that the trees did die first on the podzol soil and that the mortality was highest on this soil type indicate that this factor must have reached different intensities in the two soil types. The most obvious differences between the two types of soil were the form of inorganic nitrogen and the availability of cations. The cation levels in the needles of the trees of the 5000 N treatment were very low, especially on the podzol soil. In particular, the potassium levels were low. This was aggravated by the rather high nitrogen contents of the needles. As the weight proportions of potassium relative to nitrogen were much lower than the critical value of 25, it seems possible that the trees died of a (relative) potassium deficiency. Mortality was highest in Douglas fir, the tree species with the lowest potassium levels in the needles. Rygiewicz & Bledsoe (1984) also found that Douglas fir has very low rates of potassium uptake. Aluminium toxicity seems not to play an important role, because many trees on the riverdune soil died too, although the aluminium to calcium ratio was low there.

Waterstress may have additionally weakened the trees in the 5000 N treatment as a result of the reduction in size of the root system and the low relative air humidity in the greenhouse in early summer.

Application of rainwater containing 500 μM of ammonium (48 kg $N.ha^{-1}.yr^{-1}$) did not adversely affect the trees within one year of treatment. This may be due to the great growth vigour and resilience of young trees (Meyer, 1984). In many respects the trees performed even better than the control trees: higher biomass, higher nutrient and chlorophyll levels. Probably, this was the result of the very low availability of nitrogen and other nutrients in the soil of the latter (Van Dijk *et al.*, 1989). Meyer (1985) also reported a decline in tree performance when the nitrogen availability was reduced too much by the addition of too much sawdust to the soil. However, in the 500 N treatment the soil was acidifying and losing more nutrients with the drainage water than in the control treatment. So, in a field situation a prolonged nitrogen deposition of 48 kg may deplete the soil and cause nutrient shortages. Moreover, the nitrogen consuming capacity of the trees may decrease when they grow older, especially when other nutrients become scarce, leading to ammonium accumulation and nutrient imbalances in the soil. According to Ågren (1983) a deposition of 25 kg $N.ha^{-1}.yr^{-1}$ will result in the saturation of pine or spruce forests within 25 to 50 years.

Although both throughfall measurements (Miller *et al.*, 1976; Verry & Timmons, 1977; Grennfelt & Hultberg, 1986) and laboratory experiments (Roelofs *et al.*, 1985) clearly demonstrate, that needles can take up ammonium, no major differences in the chemical composition of the needles were observed between the trees that were watered over the canopy and those that received water directly on the soil. Probably, as a result of the high evaporation rate in the greenhouse and the large size of the raindrops, the needles were wet for too short a time to allow for any substantial cation exchange at the needle surface. More refined experiments in the laboratory are needed to investigate the importance of needle uptake.

Finally, it can be concluded, that

1. a deposition of 480 kg N.ha^{-1}.yr^{-1} caused high levels of nitrogen in the young needles and low levels of potassium, magnesium, calcium and other cations.
2. the latter coincided with an increased shoot to root ratio, a decline of fine roots, an impaired rate of infection with mycorrhizal fungi and unfavourable nutrient proportions in the soil.
3. the levels of free arginine in the needles of the trees treated with the highest ammonium concentration increased strongly.
4. these trees showed a greatly enhanced mortality.
5. application of 48 kg N.ha^{-1}.yr^{-1} did not adversely affect young coniferous trees during the time of the experiment.
6. application of ammonium enriched rainwater to young coniferous trees evoked many symptoms similar to those observed in the field.
7. soil type strongly interfered with the effects of nitrogen deposition.
8. Douglas fir was most strongly affected by the elevated nitrogen deposition and that Scots pine was the least sensitive species.

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CHAPTER 4

EFFECTS OF EXCESSIVE AMMONIUM DEPOSITION ON THE NUTRITIONAL STATUS AND CONDITION OF PINE NEEDLES

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ABSTRACT

In the southeastern part of The Netherlands many Scots pine (*Pinus sylvestris* L.) trees show signs of yellowing. To investigate whether there is a relation between this phenomenon and the high ammonium deposition, needle and soil samples were analyzed. Soil samples from the discoloured forests contained more extractable nitrogen than samples from healthy stands. whereas differences in pH values were small. Needles from yellow trees had higher levels of total nitrogen than needles from green trees as well as severe imbalances of Mg, K and P relative to N. The amount of leaf pigments was substantially lower in needles of the diseased trees, but they contained much higher quantities of free arginine, which accounted for a major part of total nitrogen. This may be an indication of a severe nitrogen overload. The linkage between this excessive nitrogen nutrition and the observed process of yellowing is discussed.

INTRODUCTION

The condition of the Dutch forests is alarming. A recent investigation by the Dutch National Forest Service reveals that 50% of the forests (141 000 ha) show a decreased vitality (Staatsbosbeheer, 1985). The geographical pattern of the damage does not fit in very well with the occurrence of well-known pollutants such as sulphur dioxide, nitrogen oxides and ozone (Den Boer, 1986). The situation is most critical in the so-called Peel area in the southeastern part of the country. Here nitrogen deposition in forest stands is very high, about 10 - 20 times the natural supply of 5 - 10 kg N $ha^{-1}.yr^{-1}$. Locally, the deposition has been estimated to amount to more than 500 kg N $ha^{-1}.yr^{-1}$ (Staatsbosbeheer, 1986a). According to Encke (1986) only 5 - 8 kg N $ha^{-1}.yr^{-1}$ is needed to maintain biomass production.

Most of this nitrogen originates from ammonia which is emitted by the numerous stockbreeding farms or evaporates from animal slurry dumped on arable land. In waterdrops, fog or on wet surfaces this ammonia reacts with sulphur dioxide to form ammonium sulphate. Due to the filtering action of the tree canopies, deposition of gaseous ammonia, sulphur dioxide and ammonium sulphate is considerably higher in the forests than in the surrounding meadows (Van Breemen *et al.*, 1982; Nihlgård, 1985; Roelofs *et al.*, 1985). Most of the Dutch forest stands are planted on acidic, nutrient-poor soils formerly covered by heathland. Since nitrification is inhibited in these soils (Roelofs *et al.*, 1985), ammonium is accumulated, resulting in a disturbed nutrient balance and a decreased vitality of the trees (Nihlgård, 1985; Roelofs *et al.*, 1985).

In the Peel area, Scots pine (*Pinus sylvestris* L.) trees show a special form of discolouration, which is also found in other parts of the country, albeit less frequently - the young needles have a yellow or even whitish colour. The yellowing is strongest at the needle base. Older needles are green. This discolouration does not conform to the discolouration of pine needles in autumn and winter, which is normally observed in The Netherlands for boreal or continental provenances. That phenomenon is strongest at the needle tip (De Vries, 1977). Potassium and magnesium deficiencies are, similarly, also not likely to be responsible (Van Goor, 1967). Moreover, one would expect symptoms to develop first in the older needles due to the translocation of these elements to the younger needles.

In the present study we investigated whether there is a link between the high nitrogen deposition in the southeastern part of The Netherlands and the poor vitality of Scots pine in this area. For this purpose soil and needle samples were collected in the field and analyzed. Since a disturbance in nitrogen metabolism is probably reflected in the composition of the amino acid pool, free amino acids were measured. Tissue pH was also estimated.

Preliminary results of this work were presented at the workshop on 'Direct effects of dry and wet deposition on forest ecosystems - in particular canopy interactions', jointly organized by the Swedish Univ. of Agricultural Sciences, the Swedish Environmental Research Inst. and the Commission of the European Communities, 19-23 October 1986, Lökeberg, Kungälv, Sweden.

MATERIALS AND METHODS

Soil and needle samples were taken from 17 Scots pine (*Pinus sylvestris* L.) stands in the southeastern part of The Netherlands. In 9 of these stands a considerable part of the trees (at least 10%) showed signs of yellowing. In the other 8 stands none of the trees showed such symptoms. In every stand a single tree was chosen for sampling. In the yellowed stands this was always a tree displaying severe yellowing.

Soil extracts

Soil samples were collected in November 1985. At each site 4 subsamples were taken from the mineral soil (0-20 cm depth, 1 m from the stem) and mixed. Seventy g of fresh soil were put into a 500 ml polyethylene bottle together with 200 ml of either bi-distilled water or a 0.2 M NaCl solution and shaken for 1 h, after which pH was measured. After centrifugation for 15 min at 27 500 g, the supernatant was stored at -20°C until analysis.

Needle destruates

Needle samples were collected in April 1986, just before bud-break. Needles were taken from several first and second order branches from the middle of the crown. The needles were washed three times with distilled water. For elemental analysis 50 mg of oven-dried (48 h, 60°C) and ground needle tissue were dispersed in 5 ml concentrated H_2SO_4, incubated at room temperature for 24 h, heated to 150°C and digested by slowly adding 2 ml 30% H_2O_2. The destruates were diluted to 100 ml with bi-distilled water and stored at 5°C until analysis.

Chemical analyses

In both soil extracts and needle destruates, Ca, Mg, Al, Fe, Mn, Zn, S and P were measured with an Inductively Coupled Plasma spectrophotometer (ICP), type IL Plasma 200. K^+ concentrations were determined by flame photometry. NH_4^+ and NO_3^- were measured colorimetrically with a Technicon AAII system according to Kempers & Zweers (1986) and Technicon AutoAnalyzer Methodology (Technicon Corporation, 1969), respectively.

Leaf pigments

For extraction of leaf pigments, fresh needle tissue was ground in liquid nitrogen and 0.5 g was put into a 10 ml centrifuge tube with 10 ml 96% ethanol. Tubes were stored for 96 h in the dark at -20°C, during which time the extraction solution was replaced twice. The 3 supernatant fractions were recovered by centrifugation (10 min, 4 000 g) and combined. Leaf pigment concentrations were determined spectrophotometrically according to Wellburn & Lichtenthaler (1984).

Amino acids

Free amino acids were similarly extracted, using 70% ethanol containing 10 ml thiodiglycol and 700 mg citric acid l^{-1}. The combined fractions were washed with 40 ml chloroform, freeze-dried, dissolved in 2 ml lithium citrate (0.2 M Li, pH 2.6) and stored at -20°C until analysis. Norleucine (150 nmol) was added as an internal standard at the beginning of the extraction procedure. Amino acid concentrations were determined by High Performance Liquid Chromatography (HPLC) (Varian Liquid Chromatographe 5000) using a cation exchange column (LKB UP8) and mixtures of lithium citrate (pH 2.6) and lithium borate (pH 10.6) of increasing molarity and pH. For quantification, fluorescence (excitation wavelength 390 nm, emission wavelength 460 nm) was measured after postcolumn derivatization with *o*-phthalaldehyde. Secondary amino acids were converted into primary amines with hypochlorite according to Ishida *et al.* (1981) prior to the derivatization with *o*-phtalaldehyde.

Thin-Layer Chromatography (TLC) was used to confirm the major results of the HPLC analyses. Some of the amino acid samples were desalted with Dowex resin

(50W-X2, 200-400mesh, Fluka 44465), eluted with a 5 M ammonia solution and freeze-dried. The amino acids were redissolved in a 0.1 M HCl giving a concentration of ca 1% (w/v). Aliquots (0.4 µl) of this solution were applied to plates of silica gel (60 F-254, Merck) using phenol/water (75:25, w/w) as the mobile phase (Stahl, 1962). To visualize the amino acids the plates were sprayed with ninhydrin (Stahl, 1962) or Sakaguchi's reagent (J. W. F. M. van Nispen, 1974. Ph.D. Thesis, Catholic Univ., Nijmegen, The Netherlands).

Tissue pH

To determine the average pH value in leaf tissue, fresh needles were ground in liquid nitrogen and 0.5 g was suspended in 3 ml bi-distilled water in a test tube. The suspension was boiled for 5 min and, after cooling, pH was measured with a pH electrode.

RESULTS

The contents of leaf pigments in the needles are shown in Tab. 1. The young needles of the yellow trees contained much lower levels of both chlorophyll a and b than the young needles of the green trees, the reduction being on the average 70 and 72%, respectively. Although the older needles looked green, they still had reduced chlorophyll levels, but the reduction was smaller than in the young needles, being 47 and 59% for chlorophyll a and b, respectively. Carotenoid levels were reduced in young and old needles of the yellow trees with 52 and 35%, respectively. The chlorophyll a/b ratio was significantly higher in the needles of the yellow trees.

The chemical composition of the needles is given in Tab. 2. The total nitrogen content was much higher in both young and old needles of the yellowed trees. In agreement with this were the higher amounts of extractable ammonium and nitrate in the soil samples of the discoloured forests (Tab. 3). Ammonium was the dominant nitrogen form. Also the calcium and zinc levels were elevated in the needles of the yellow trees, but for zinc differences were significant only in the young needles. This is reflected by the higher amounts of salt-extractable calcium and zinc in the soil samples of the yellowed trees. Aluminium reached higher concentrations in the young needles of the green trees. Iron showed a similar trend. The extractable amounts of aluminium and iron in the soil were also higher in the green forests, in spite of the slightly higher pH values. These differences were highly significant for iron, but not for aluminium. The potassium content in the needles of the green trees was also higher, but this difference was significant only in the old needles. In the soil samples no differences were observed in this respect.

Table 1. Contents of leaf pigments in needles of Scots pine collected in April 1986. Medians with 25% and 75% quartiles in parentheses. Significance according to Wilcoxon 2-samples test (normal approximation): n.s., not significant; (*), P≤0.1; *, P≤0.05; **, P≤0.01. Abbreviations: chl., chlorophyll.

Pigment	Leaf pigments $\mu g.(g\ DW)^{-1}$ Green trees (n=8)	Yellow trees (n=10)	Significance
1984 needles			
chl. a	2390 (2250-2550)	1280 (890-1520)	**
chl. b	650 (610- 740)	265 (233- 339)	**
carotenoids	730 (680- 770)	480 (370- 540)	**
chl. a/chl. b	3.58 (3.41-3.87)	4.4 (3.78-4.9)	*
1985 needles			
chl. a	2120 (1940-2290)	650 (470- 820)	**
chl. b	470 (440- 580)	129 (102- 164)	**
carotenoids	630 (600- 660)	307 (266- 352)	**
chl. a/chl. b	4.4 (3.92-4.6)	5.1 (4.6 -5.4)	**

When the nutrient amounts in the needles are expressed as percentages of the total nitrogen content, the differences are even more pronounced (Tab. 2): the relative amounts of K, Mg, P, Fe and Al were significantly lower in both young and old needles of the yellow trees, whereas Mn levels were only decreased in the older needles.

Analysis of the free extractable amino acids is shown in Tab. 4. The most striking result is that the needles of yellow trees contained very large amounts of arginine, with levels above 140 μmol.(g DW)$^{-1}$. It was by far the most abundant amino acid and comprised 35 and 29% of the total nitrogen in the young and old needles, respectively. TLC confirmed the determination of this major constituent. It reacted positively with Sakaguchi's reagent, indicating the presence of a guanidino group.

Arginine was the most abundant amino acid also in the needles of the green trees, but the levels were much lower and it accounted for only 2 and 8% of the total nitrogen in young and old needles. Other nitrogen-rich amino acids such as histidine and lysine also reached significantly higher levels in the needles of the discoloured trees. The same was true for leucine and isoleucine. On the other hand glutamic acid and aspartic acid were more abundant in needles of the green trees. However, it should be noted that glutamic acid and asparagine were not separated. Other amino

Table 2. Chemical composition of needles of Scots pine collected in April 1986. In every second line the chemical composition relative to nitrogen is given. See text for further information. Medians with 25% and 75% quartiles in parentheses. See Tab. 1 for significance symbols.

Nutrient	Nutrients % of DW % of N Green trees (n=8)		Yellow trees (n=10)		Significance
1984 Needles					
N	1.94	(1.85 -2.22)	2.98	(2.26 -3.27)	**
	100		100		
K	0.48	(0.40 -0.52)	0.355	(0.288-0.43)	*
	23.6	(19.0-26.5)	12.2	(9.7-18.1)	**
Mg	0.048	(0.040-0.058)	0.050	(0.049-0.057)	n.s.
	2.4	(2.1- 2.7)	1.7	(1.6- 2.0)	*
Ca	0.340	(0.298-0.40)	0.46	(0.390-0.49)	*
	16.6	(15.8-20.9)	15.2	(13.8-17.1)	n.s.
P	0.104	(0.098-0.116)	0.097	(0.084-0.128)	n.s.
	5.1	(4.4- 6.3)	3.7	(3.2- 4.0)	**
Fe	0.022	(0.020-0.026)	0.023	(0.017-0.029)	n.s.
	1.15	(0.99-1.19)	0.88	(0.52-0.97)	*
Mn	0.029	(0.020-0.042)	0.023	(0.021-0.037)	n.s.
	1.25	(1.07-1.95)	0.82	(0.65-1.26)	*
Zn	0.015	(0.013-0.016)	0.018	(0.014-0.024)	n.s.
	0.71	(0.66-0.77)	0.68	(0.55-0.82)	n.s.
Al	0.023	(0.016-0.024)	0.017	(0.010-0.023)	n.s.
	0.97	(0.94-1.26)	0.60	(0.44-0.74)	**
1985 Needles					
N	1.94	(1.79 -2.15)	2.85	(2.28 -3.14)	**
	100		100		
K	0.57	(0.47 -0.59)	0.51	(0.42 -0.58)	n.s.
	28.1	(22.2-29.6)	19.3	(12.7-22.9)	*
Mg	0.056	(0.050-0.061)	0.060	(0.056-0.063)	n.s.
	2.9	(2.6- 3.3)	2.2	(1.8- 2.4)	**
Ca	0.210	(0.190-0.258)	0.270	(0.240-0.310)	*
	10.7	(9.4-13.4)	8.9	(7.9-12.0)	n.s.
P	0.121	(0.112-0.128)	0.116	(0.099-0.125)	n.s.
	5.9	(5.3- 6.7)	4.2	(3.7- 4.9)	**
Fe	0.015	(0.013-0.017)	0.012	(0.012-0.014)	(*)
	0.76	(0.70-0.80)	0.48	(0.38-0.57)	**
Mn	0.019	(0.015-0.027)	0.019	(0.016-0.027)	n.s.
	0.99	(0.71-1.31)	0.65	(0.51-0.96)	(*)
Zn	0.013	(0.011-0.014)	0.015	(0.014-0.016)	*
	0.62	(0.56-0.72)	0.53	(0.46-0.67)	n.s.
Al	0.020	(0.016-0.021)	0.013	(0.011-0.016)	**
	0.92	(0.86-1.15)	0.47	(0.39-0.61)	**

Table 3. Extractable nutrients in soils from Scots pine forests in November 1985. Medians with 25% and 75% quartiles in parentheses. See Tab. 1 for significance symbols.

Parameter	Extractable nutrients µmol.(100 g DW)$^{-1}$		
	Green trees (n=8)	Yellow trees (n=10)	Significance
Water extraction			
NH4+	18.5 (8.3 -29.6)	78 (42 -94)	**
NO3-	5.2 (2.2 - 7.6)	21.1 (18.9 -37.8)	**
K	7.0 (4.1 -10.4)	5.6 (3.6 -11.0)	n.s.
Mg	1.5 (1.3 - 4.0)	0.0 (0.0 - 1.5)	(*)
Ca	4.8 (0.6 - 8.9)	3.1 (0.9 - 4.7)	n.s.
Al	22.7 (18.2 -39.1)	10.1 (6.0 -23.8)	(*)
Fe	10.3 (3.6 -15.6)	1.8 (1.4 - 2.1)	**
Mn	0.07 (0.06- 0.12)	0.00 (0.00-0.02)	**
Zn	0.57 (0.32- 1.43)	0.63 (0.44- 0.83)	n.s.
P	1.2 (0.5 - 2.1)	3.4 (2.4 - 5.0)	**
S	46 (19.4 -57)	52 (30.3 -67)	n.s.
pH	4.1 (4.0 - 4.1)	4.0 (3.8 - 4.1)	n.s.
Salt extraction			
NH4+	26.0 (19.0 - 33.7)	146 (66 -188)	**
K	8.0 (4.1 - 13.8)	12.2 (5.9 - 17.5)	n.s.
Mg	14.8 (11.0 - 25.0)	27.8 (16.9 - 38.8)	(*)
Ca	53 (41 -104)	129 (93 -191)	**
Al	144 (117 -193)	81 (49 -173)	n.s.
Fe	5.1 (2.1 - 10.1)	0.7 (0.5 - 1.6)	**
Mn	0.8 (0.7 - 1.2)	1.7 (1.3 - 4.1)	*
Zn	4.7 (2.3 - 6.7)	8.3 (7.0 - 9.8)	*
pH	3.4 (3.1 - 3.5)	3.0 (2.9 - 3.0)	*

acids were found in too low quantities to allow accurate measurement.

No significant differences were found between the pH values of the needle-tissue suspensions of green and discoloured trees: In the young needles values of 5.06 and 5.24 were found for the green and the yellow trees, respectively. In the old needles these values were 5.26 and 5.13, respectively.

DISCUSSION

The needles of yellow trees had very low levels of leaf pigments (Tab. 1), as could be expected from their light colour. The higher contents in the older needles of yellow

Table 4 Free amino acids in needles of Scots pine collected in April 1986 Medians with 25% and 75% quartiles in parentheses See Tab 1 for significance symbols Glutamic acid includes asparagine Abbreviations γ-aminobut acid, γ-aminobutyric acid, glu, glutamic acid, gln, glutamine

Amino acid	Free amino acids μmol.(g DW)$^{-1}$				Significance
	Green trees (n=8)		Yellow trees (n=10)		
1984 Needles					
Alanine	1.31	(1.19-1.39)	1.36	(1.24-1.54)	n.s.
Leucine	0.048	(0.031-0.080)	0.101	(0.066-0.151)	*
Isoleucine	0.101	(0.053-0.122)	0.154	(0.126-0.206)	**
Serine	0.94	(0.84-1.40)	0.93	(0.83-1.27)	n.s.
Threonine	0.32	(0.27-0.42)	0.34	(0.21-0.40)	n.s.
γ-Aminobut. acid	0.31	(0.23-0.39)	0.33	(0.22-0.69)	n.s.
Proline	1.99	(1.31-5.7)	3.17	(2.14-9.7)	n.s.
Tryptophane	0.51	(0.34-0.57)	0.38	(0.30-0.46)	n.s.
Phenylalanine	0.132	(0.095-0.154)	0.144	(0.113-0.159)	n.s.
Aspartic acid	4.5	(4.3-5.3)	3.44	(2.65-4.3)	**
Glutamic acid	6.0	(5.5-6.3)	4.8	(3.97-5.3)	**
Glutamine	9.6	(6.2-12.6)	10.5	(7.1-19.9)	n.s.
Lysine	0.50	(0.25-0.77)	0.90	(0.72-1.11)	*
Histidine	0.48	(0.29-0.82)	0.94	(0.64-1.54)	*
Arginine	31.2	(4.6-51)	146	(92-234)	**
Glu/GlN-ratio	0.68	(0.47-0.97)	0.47	(0.26-0.79)	n.s.
1985 Needles					
Alanine	1.37	(1.21-1.67)	1.51	(1.00-1.63)	n.s.
Leucine	0.040	(0.034-0.060)	0.123	(0.073-0.141)	**
Isoleucine	0.071	(0.053-0.100)	0.178	(0.130-0.271)	**
Serine	1.08	(0.93-1.32)	1.63	(1.28-2.12)	*
Threonine	0.21	(0.18-0.30)	0.32	(0.23-0.34)	(*)
γ-Aminobut. acid	0.22	(0.15-0.35)	0.36	(0.23-0.43)	n.s.
Proline	1.91	(0.98-2.37)	4.2	(1.94-8.2)	(*)
Tryptophane	0.33	(0.14-0.42)	0.40	(0.28-0.46)	n.s.
Phenylalanine	0.147	(0.126-0.204)	0.187	(0.169-0.215)	n.s.
Aspartic acid	5.2	(4.6-6.3)	2.94	(2.53-3.95)	**
Glutamic acid	7.0	(5.9-7.2)	5.5	(4.1-6.7)	(*)
Glutamine	8.3	(6.2-9.6)	9.5	(6.9-22.1)	n.s.
Lysine	0.35	(0.17-0.51)	2.20	(1.65-3.54)	**
Histidine	0.38	(0.22-0.47)	1.63	(1.11-2.91)	**
Arginine	7.2	(2.3-39.6)	177	(106-293)	**
Glu/GlN-ratio	0.86	(0.65-1.17)	0.44	(0.27-0.89)	(*)

trees indicate that these low levels were not the result of a breakdown of pigments but rather a consequence of a partly inhibited synthesis. This view is confirmed by the observation that in May 1986, after bud-break, the new needles were yellowish. The fact that both chlorophyll and carotenoid levels were low suggests that this inhibitory process is rather unspecific. The two groups of pigments are, after all, completely different in chemical composition and have entirely different pathways of synthesis. As chlorophyll b is located in the grana, the higher chl. a/b-ratios in the needles of the yellow trees seem to indicate reduced thylakoid stacking in the chloroplasts of these trees.

Chemical analysis of the needle tissue (Tab. 2) seems to confirm that the yellowing of the trees was not simply the result of potassium or magnesium deficiencies. Although the levels of these elements were rather low, they were above deficiency concentrations (cf. Van Goor, 1967; Schütz & Van Tol, 1981). Moreover, there was no difference between green and yellow trees in this respect, except for potassium in the older needles. However, nutrient proportions may be more important than absolute levels. If the nitrogen content is set to 100, values of 25, 5 and 5 for potassium, phosphorus and magnesium, respectively, are supposed to be critical (J. van den Burg, personal communication). From this point of view, the yellow trees were suffering from severe potassium, magnesium and phosphorus shortages and the levels in green trees were also low (Tab. 2).

The total nitrogen content of the needles of the yellow trees was extremely high, higher than the 1.6 - 2.0% range considered to be optimal (Van Goor, 1967; Schütz & Van Tol, 1981; Aronsson, 1985; Krauß *et al.*, 1986) indicating that the trees must suffer from a severe nitrogen overload. Obviously, this must be the result of the high ammonium deposition leading to an accumulation of this nitrogen compound in the soil (Tab. 3). Most of the nitrogen was stored in the nitrogen-rich amino acids lysine, histidine and particularly arginine, the latter reaching pathological levels in the needles of yellowed trees (Tab. 4). In Sweden A. Aronsson (personal communication) found that only 0.5% of the total nitrogen was incorporated into free arginine in needles of Scots pine with a total nitrogen content of 2%. Durzan (1968) showed that just before bud-break, free arginine levels were low in needles of white spruce (*Picea glauca* (Moench) Voss). In Scots pine seedlings grown on a nutrient solution containing approximately optimal nutrient proportions, arginine was dominant in the free amino acid pool in the needles, but comprised <10% of total nitrogen in this pool (Gezelius, 1986a).

MacKenzie & Holme (1984) investigated the free amino acid pool of 10 North American conifer species in the dormant season. They concluded that the amounts of free amino acids varied between 3 and 7 μmol.g^{-1} of leaves, except for some spruces where 19 - 56 μmol.g^{-1} were found. The basic amino acids usually constituted less than 7% of total free amino acids and never exceeded 15%.

According to Chapin & Kedrowski (1983), more than 70% of the total N was always stored in proteins in both evergreen and deciduous taiga trees. This agrees well with the observations of Krauß *et al.* (1986) that under optimal conditions, soluble nitrogen comprised less than 30% of total N, but that under conditions of excessive nitrogen availability, due to fertilization or air pollution, higher values were reached. This always involved a strong growth decline. Zedler *et al.* (1986) reported elevated levels of amino acids in damaged spruce trees in polluted areas in south-western Germany and attributed this to increased ammonium and ammonia uptake. Several other investigators also found that ammonium nutrition raised free arginine levels in plant tissue (Durzan & Steward, 1967; Rabe & Lovatt, 1986). In a greenhouse experiment where coniferous trees were treated with ammonium-enriched rainwater, similar changes in amino acid concentrations were observed (H. F. G. van Dijk, unpublished data).

Other elements may also play a role in arginine accumulation. In particular, phosphorus or potassium deficiencies enhance arginine production (Mulder & Bakema, 1956; Krauß *et al.* 1986; Rabe & Lovatt, 1986). Aronsson (1985) found that fertilization of spruce stands with 90 - 180 kg N $ha^{-1}.yr^{-1}$ resulted in increased nitrogen and decreased phosphorus levels in the needles. At the same time the concentration of free arginine increased 100-fold to 46 $\mu mol.(g\ DW)^{-1}$ containing 14% of total N. He concluded that N to P ratios of 100 to 6-10 represented the lower limit beyond which irreversible damage appeared. These data fit in well with the results of the present investigation: the N to P ratio of the yellow needles clearly lay beyond this critical value, whereas that of the green trees was on the borderline (Tab. 2). Thus the low phosphorus content of the investigated needles may enhance the effects of the high nitrogen availability.

Krupa *et al.* (1973) and Krupa & Bränström (1974) reported high arginine levels (100 $\mu mol.(g\ DW)^{-1}$) in the roots of *Pinus nigra* and *P. sylvestris*. As arginine accumulation was more pronounced in non-mycorrhizal than in mycorrhizal roots, they suggested that nutrient (*e. g.* phosphorus or potassium) deficiencies may block the ornithine cycle. Many other nutrient deficiencies can cause elevated arginine levels in plants (see Rabe & Lovatt, 1986, and references therein).

Holley & Cain (1955) showed that many plants afflicted with iron-deficiency-type chlorosis accumulate large amounts of free arginine. Indeed, symptoms shown by the yellow trees were very similar to those of iron deficiency (Buchner, 1985). Moreover, in spite of the lower pH values, iron availability was much lower in soil samples around these trees (Tab. 3). In contrast to the iron levels in the soil, the iron contents of the needles from yellow and green trees did not differ significantly (Tab. 2). They were well above the minimal values of 0.004-0.005% of the dry weight (Van den Burg, 1985) and were much higher than those found by Buchner (1985) in chlorotic pine needles in the Lüneburger Heide, FRG. However, this paradox is also seen in lime-induced chlorosis (Kinzel, 1982) and little can be concluded from iron levels alone (Van den Burg, 1985) as iron may be rendered non-functional in the cell. Accumulation of arginine was not found to involve a rise in tissue pH, which might cause iron immobilization. However, the method used produces only an overall pH and it is possible, that elevated pH values do exist in some parts of the needles. In this connection it may be noted that iron chlorosis in conifers is often associated with nitrate nutrition (Nelson & Selby, 1974; Van den Driessche, 1978; Carter, 1980).

In a thorough review of lime-induced chlorosis, Kinzel (1982) suggested that the accumulation of amino acids and organic acids may not be the cause of iron deficiency but rather an effect. However, at least in the present case, the high concentration of free arginine was clearly the result of the excessive nitrogen nutrition, as can be seen from the high total nitrogen content in the needles (Tab. 2) and the increased availability in the soil (Tab. 3).

Some of the green trees appeared also to contain elevated levels of free arginine (Tab. 4), although the amounts were much smaller than in the yellow trees. Perhaps this is the first sign that these trees are also under (incipient) ammonium stress. It will be interesting to follow these trees in the course of time - maybe they will soon turn yellow. If so, arginine could function as a bioindicator; slightly raised levels may indicate a decreased vitality, at a time when visible symptoms have not yet developed.

It can be concluded that the diseased trees are suffering from a severe nitrogen overload. The consequent imbalance between nitrogen and other nutrients results in an accumulation of free arginine. The relation between this process and the observed chlorosis is not yet fully understood.

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CHAPTER 5

IRON-DEFICIENCY CHLOROSIS IN SCOTS PINE GROWING ON ACID SOILS

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Plant and Soil (submitted).

ABSTRACT

Scots pine (*Pinus sylvestris* L.) trees growing on acidic soils may show a special type of chlorosis. The current year needles are yellowish and the discolouration is most pronounced at the needle base. The disease bears resemblance to iron chlorosis in trees on calcareous soils.

In order to investigate the cause of the chlorosis, needle and soil samples were collected in 41 stands in the southern, central and eastern parts of The Netherlands. Needles of the chlorotic trees had low levels of chlorophylls and carotenoids and high ratios of carotenoids to chlorophylls and of chlorophyll a to chlorophyll b. These symptoms indicate iron deficiency. The current year needles of chlorotic trees contained on average 14 ppm Fe, whereas unaffected trees from the same stands contained 24 ppm. Healthy trees from stands without chlorotic trees had needle iron levels of 29 ppm. Spraying the needles of chlorotic trees with ferric-EDTA containing solutions resulted in partly regreening within a few weeks.

Chlorotic needles showed also high nitrogen and cadmium levels. Soils of chlorotic stands contained large amounts of extractable inorganic nitrogen and phosphorus. The possible causal relationships between the observed iron deficiency and the differences in elemental composition of needles and soils are discussed.

INTRODUCTION

In 1988 Van Dijk & Roelofs described a form of yellowing in Scots pine (*Pinus sylvestris* L.) trees growing on acid heathland soils. The current year needles were yellowish, whereas the older needles were green. The discolouration of the young needles was most pronounced at the needle base. Niehaus (1986) and Van den Burg (1991) described the same phenomenon. The first author estimated the number of Scots pine trees affected in this way in Lower Saxony in 1983 at approximately 2 %.

Discolouration of needles is commonly associated with nutrient deficiencies, *e.g.* of potassium and magnesium. Since these elements are relatively mobile and easily transported from older to younger needles, the discolouration normally starts in the older needles. This type of discolouration is often observed in areas where the soil has been impoverished by deposition of acidifying substances. Yellowing of young needles may be caused by iron or manganese deficiency. This kind of chlorosis is commonly observed in plants growing on calcareous soils and is then usually referred to as lime-induced chlorosis or calciose (Kinzel, 1982).

The discolouration of needles reported by Van Dijk & Roelofs (1988) strongly resembled the symptoms of lime-induced chlorosis, although this is characterized by a more even discolouration of the young needles (Zech, 1970; Schütt *et al.*, 1983). The resemblance is remarkable since Van Dijk and Roelofs's chlorosis occurred on strongly acidic soils (pH<4.5), whereas lime-induced chlorosis occurs on calcareous soils with pH values above 7 (Zech, 1970; Carter, 1980).

Chemical analysis of the tissue of the young needles by Van Dijk & Roelofs (1988) revealed differences in calcium, aluminium and zinc, but not in potassium and magnesium levels between discoloured and green trees. They did not find a significant difference in iron concentrations, though there was a trend towards slightly lower iron levels in the diseased trees. Van den Burg (1991) presented data, which suggested that iron deficiency might play a role, but again with low significance. However, even if no differences in the total iron content are found, this does not necessarily mean that iron is not involved. Iron may be immobilized within the plant and chlorotic plants may show normal levels of iron, or even higher levels than healthy plants (Oserkowsky, 1933; Iljin, 1952; Kinzel, 1982).

Van Dijk & Roelofs (1988) reported higher levels of nitrogen both in the needles and in the upper mineral soil layer of discoloured stands. Most of the additional nitrogen in the discoloured needles was stored in the free amino acid fraction, predominantly in arginine. The high nitrogen levels were probably due to the excessively high atmospheric nitrogen deposition in Dutch forests, which amounts to 80 $kg.ha^{-1}.yr^{-1}$ (Ivens, 1990; Houdijk & Roelofs, 1991). Main source of the nitrogen is ammonia that is emitted by livestock farms and volatilizes from animal slurry dumped on arable land.

In the present study the relation between the observed chlorosis and the iron metabolism was investigated more closely. Answers were sought to two questions: 1. Is the chlorosis caused by iron deficiency? 2. If so, which factor or factors caused trees to develop a 'lime induced'-like chlorosis on acid soils?

MATERIALS AND METHODS

Inventory field study

In November and December 1986 soil and needle samples were taken from 41 stands of Scots pine (*Pinus sylvestris* L.) in the southern, central and eastern parts of The Netherlands. In 23 of these stands several trees had yellowing young needles. In the other 18 stands no chlorosis was observed. In each stand, in which some of the trees showed chlorosis, samples were taken from both one yellowing and one green tree. In the stands without symptoms of yellowing one tree was sampled. Sampling, treatment of samples and measurements of leaf pigments, chemical composition of needle tissue and extractability of nutrients from the soil were performed as described by Van Dijk & Roelofs (1988), with a few alterations: a) to extract leaf pigments 20 mL 96% ethanol was added to 0.5 g freshly ground needle tissue. The suspension was shaken overnight at 5 °C. After centrifugation the supernatant was used to measure the leaf pigments colorimetrically after Wellburn & Lichtenthaler (1984). b) prior to chemical analysis of the needle tissue needles were washed 30 seconds with chloroform to remove iron adsorbed to the needle surface (Wyttenbach *et al.*, 1985). c) the cation exchange capacity of the soil and the amounts of cations adsorbed to the cation exchange complex were determined by suspending 5 g fresh soil in 40 ml 0.01 *M* silver thiourea (Ag-TU) and shaking for 4 hours (Chabra *et al.*, 1975). After centrifugation for 15 min at 27500 g the supernatant was stored at 4 °C until analysis. Ammonium was extracted from soil samples with 0.5 *M* KCl. solution.

Spraying of trees with iron-containing solution

In August 1990 two Scots pine trees were selected at the country estate 'De Stippelberg' in the Peel area. The trees were growing in a very open stand and had branches beginning just above ground level. They were at least 30 years old. The younger needles showed pronounced yellowing. In each tree 15 primary or secondary branches were labeled and sprayed with either 0.01 *M* Fe(III)NaEDTA or 0.01 *M* Na_4EDTA or remained untreated. Both spraying solutions contained 0.01% Triton X-100 as a wetting agent. Each treatment consisted of 10 replicates. During the next 2 weeks the treatments were repeated 8 times. Spraying was always performed between 7 and 10 p.m. to prevent the needles from drying too quickly. The branches were harvested on the 4th of September. Leaf pigments were determined separately in the top and the basic halves of the current year needles before and after the treatment.

Statistics

Needle and soil samples of the yellow and the green trees growing in the same, yellowing, stand were compared pairwise: for each diseased stand the value, adopted by a certain variable in the yellow tree, was divided by the value, adopted by the same variable in the companionate, green tree. After log-transformation these quotients fitted well to a normal distribution. A *t*-test in the UNIVARIATE procedure of the Statistical Analysis System software package (SAS Institute Inc., 1989) was used to determine whether these log-transformed quotients differed from 0 significantly.

A *t*-test in the TTEST procedure from SAS was used to compare the results of needle and soil analyses of yellowing and green stands. Tests were performed on log-transformed data as these fitted better to the conditions of normality and homoscedasticity. For presentation these data were backtransformed, so the mean values presented are geometric means.

The ANOVA procedure from SAS, including Scheffé's multiple comparison procedure, was used to evaluate the effects of the spraying treatments on the levels of leaf pigments in different needle parts. Values for pigment levels in each branch at the end of the experiment were divided by values for pigment levels in the same branch at the start of the experiment. These quotients, representing the change in pigment levels in each branch during the course of the experiment, were log-transformed prior to statistical analysis and backtransformed for presentation.

RESULTS

Inventory field study

Pigment and chemical analysis of needles

The chlorophyll a and b levels in needles of yellowish trees were approximately 50% and 40%, respectively, of those in needles of green trees growing in the same stands (Table 1). The average carotenoid content was also reduced, but not so strongly. Both the carotenoids to chlorophylls ratio and the chlorophyll a to chlorophyll b ratio were significantly higher in chlorotic needles. Pigment levels in green needles from healthy stands tended to be slightly higher than those in green needles from stands affected by chlorosis (Table 1); however, differences were only significant for the carotenoids.

Table 1 Pigment contents of 6-month-old needles of yellowing and healthy looking Scots pine trees.

	Yellowing stands		Green stands	Significance t-test#		
	Yellow trees	Green trees	Green trees	Paired 1-2	Unpaired 1-3	2-3
chlorophyll a	805	1767	1962	***	***	.
chlorophyll b	268	667	708	***	***	.
carotenoids	380	527	588	***	***	*
chlor.a/chlor.b	3.00	2.65	2.77	*	*	.
carot./chlor.a+b	0.35	0.22	0.22	***	***	.

Pigment contents in the needles are expressed in µg per gram DW. The values presented are geometric means.

1-2 comparing yellowing with green trees in yellowing stands; 1-3: comparing yellowing trees in yellowing stands with green trees in green stands; 2-3. comparing green trees in yellowing stands with green trees in green stands For further explanation see Materials and Methods. Significance symbols: * $0.01<P<0.05$, ** $0.001<P<0.01$, *** $P<0.001$, . not significant. Yellow stands n=22, green stands n=18. Abbreviations chlor., chlorophyll; carot, carotenoids.

The needles of a yellow tree contained on an average only 59% of the amount of iron present in the needles of a green tree growing in the same stand (Table 2). Their nitrogen content was 16% higher and their cadmium content was 49% higher. Pairwise comparison showed that these differences were highly significant. These differences were even more pronounced when the diseased trees were compared with green trees from healthy stands. Aluminium levels were significantly higher in trees in healthy stands, calcium and zinc lower. For the other elements measured there were no significant differences between yellow and green trees growing in the same or in healthy stands.

Table 2. Chemical composition of 6-month-old needles of yellowing and healthy looking Scots pine trees.

	Yellowing stands		Green stands	Significance t-test#		
	Yellow trees	Green trees	Green trees	Paired 1-2	Unpaired 1-3	2-3
N	22200	19200	17700	**	***	.
P	1390	1320	1430	.	.	.
K	6020	5950	6280	.	.	.
Ca	2270	2300	1850	.	.	*
Mg	670	699	764	.	.	.
Fe	14	24	29	***	***	**
Mn	158	165	200	.	.	.
Zn	58	56	45	.	*	*
Al	102	112	164	.	***	***
Cd	0.353	0.237	0.251	**	*	.

Element contents in the needles are expressed in ppm of DW. The values presented are geometric means.
For explanation see Table 1. Yellow stands n=20, green stands n=17.

Chemical analysis of soils

Differences between soil samples from below yellowing and green trees in the same stand were small (Tables 3, 4 and 5). The latter contained significantly more water-extractable sulphur and Ag-TU-extractable sodium.

The differences between soils of yellowing and healthy stands were much more pronounced. Soils in yellowing stands showed higher amounts of water-extractable ammonium, nitrate, phosphorus and chloride than soils from healthy stands (Table 3).

Table 3. Water-extractable nutrients in the soils of yellowing and healthy looking Scots pine trees.

	Yellowing stands		Green stands	Significance t-test#		
	Yellow trees	Green trees	Green trees	Paired 1-2	Unpaired 1-3	2-3
Na	31	32	23	.	.	.
K	12	14	12	.	.	.
Ca	3.9	4.7	3.2	.	.	.
Mg	3.1	3.9	4.5	.	*	.
Al	35	41	39	.	.	.
Fe	4.8	6.6	10.8	.	*	.
Mn	0.07	0.09	0.12	.	**	.
Zn	0.24	0.24	0.20	.	.	.
NH4	36	35	15	.	***	***
NO3	15.3	12.3	6.0	.	**	*
S	26	34	24	**	.	*
P	6.1	7.1	2.5	.	***	**
Cl	183	164	57	.	***	***

Nutrients are expressed in µmol per 100 g DW. The values presented are geometric means.

For explanation see Table 1. Yellow stands n=23, green stands n=18.

Table 4. Silver thiourea-extractable nutrients in the soils of yellowing and healthy looking Scots pine trees.

	Yellowing stands		Green stands	Significance t-test#		
	Yellow trees	Green trees	Green trees	Paired 1-2	Unpaired 1-3	2-3
Na	27	34	23	*	.	.
K	28	31	26	.	.	.
Ca	103	105	48	.	**	**
Mg	24	24	17	.	.	.
Al	543	611	499	.	.	.
Fe	39	49	48	.	.	.
Mn	1.2	1.2	1.1	.	.	.
Zn	6.0	5.1	2.3	.	***	**
NH4	94	111	51	.	.	*
CEC	3685	3741	2500	.	*	*

Nutrients are expressed in µmol per 100 g DW, CEC in µeq. per 100 g DW. The values presented are geometric means.

For explanation see Table 1. Yellow stands n=23, green stands n=18.

Water-extractable amounts of iron, manganese and magnesium were highest in healthy stands, but these differences were only significant with regard to soil samples from yellowing trees. With Ag-TU significantly more ammonium, calcium and zinc could be extracted from soils in yellowing stands than from the other soils. The cation exchange capacity of the soil was also higher in diseased stands. The Ag-TU-extractable iron content did not differ significantly (Table 4). Soil pH was lower in yellow stands, organic matter and water content were higher than in healthy stands (Table 5).

Table 5. Organic matter and water contents and pH values of the soils beneath yellowing and healthy looking Scots pine trees.

	Yellowing stands		Green stands	Significance t-test#		
	Yellow trees	Green trees	Green trees	Paired	Unpaired	
				1-2	1-3	2-3
organic matter	5.40	6.16	3.46	.	**	**
water	15.0	15.7	9.3	.	***	**
pH-H2O	3.78	3.81	4.17	.	***	***
pH-NaCl	2.89	2.96	3.31	.	***	***

Organic matter content is expressed in % of DW, water content in % of FW. The values presented are arithmetic means.
For explanation see Table 1. Yellow stands n=23, green stands n=18.

Spraying experiment with iron-containing solution

During the experimental period (1 month) the levels of both chlorophylls and carotenoids in the needles increased in all treatments. Spraying with a Fe-EDTA solution had a significant additional effect on the pigment increase in the basic part of the needle, but not in the top (Table 6). Control sprays with Na-EDTA had no significant effect.

DISCUSSION

Chlorosis in Scots pine trees growing on acid soils was the result of reduced levels of chlorophylls a and b and carotenoids (Table 1). These low levels together with the

Table 6. Pigment levels in the top and basic parts of pine needles after treatment with different sprays, expressed in % of the levels before the start of the treatments.

	Top			Base		
	Control n=10	Na-EDTA n=10	Fe-EDTA n=10	Control n=10	Na-EDTA n=10	Fe-EDTA n=10
chlorophyll a	126a	127a	125a	132a	158a	339b
chlorophyll b	118a	155a	136a	117a	167a	429b
carotenoids	124a	121a	116a	120a	158a	261b

The values presented are geometric means. Different letters within one line and one needle part indicate significant differences at the 5% level according to Scheffé's multiple comparison procedure.

increased ratios of carotenoids to chlorophylls and of chlorophyll a to chlorophyll b are characteristic for iron deficient plants (Terry & Abadia, 1986).

The iron content of chlorotic needles (14 ppm) was about half that of green needles of trees in the same stands (24 ppm) or of trees in healthy stands (29 ppm) (Table 2). According to Zech (1970) iron deficiency in Scots pine is likely to occur below 20 ppm and is caused by an absolute shortage. Chlorosis in combination with levels above 20 ppm should then indicate a shortage due to inactivation of iron within the tissue. Therefore, the data presented here indicate an absolute shortage of iron in the needles of chlorotic trees. Figure 1 shows a good correlation between needle iron content and chlorosis and the apparent critical level of 22 ppm comes close to the value of Zech for trees in Germany. The observed shortage of iron in yellowing needles implies that either its uptake was insufficient or that its translocation from the root system to the needles was impaired.

Van Dijk & Roelofs (1988) reported iron levels in needles which were 5 to 9 times higher. Moreover, they found no significant differences in iron content between needles of chlorotic and green pine trees, though there was a trend towards lower values in discoloured trees. However, they used distilled water to wash the needles prior to determination of iron. Wyttenbach *et al.* (1985) showed that 81% of the total iron content of Norway spruce needles could be removed by washing the needles with chloroform. They concluded that this iron adhered to the needle surface as a result of atmospheric deposition. Krivan & Schaldach (1986) showed that washing with distilled water was ineffective in removing this fraction of iron. They postulated that a revision of previously published data on iron levels in needles, particularly in relation to deficiency levels, is necessary. When, in the light of the findings of Wyttenbach *et* *al.* and Krivan & Schaldach, the levels of Van Dijk & Roelofs are reduced by 80%, they fit within the same range as reported in the present study. Since the amount of iron deposited on the outer surface of the needles is not correlated with the internal amount of iron and, moreover, highly dependent on the place were the tree is growing within the stand (Wyttenbach *et al.*, 1989), it is understandable that Van Dijk & Roelofs (1988) did not find a significant difference between discoloured and green trees.

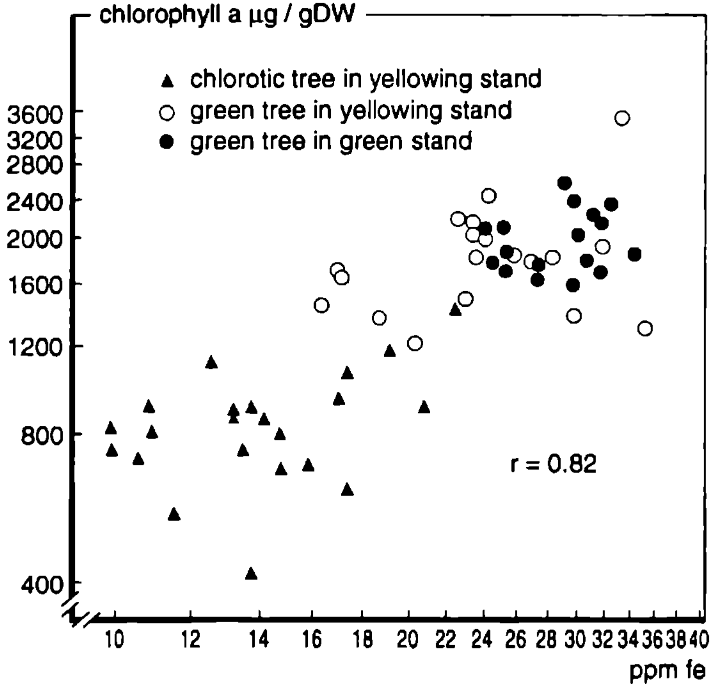

Figure 1. Correlation between chlorophyll a and total iron content in the 6-month-old needles of pine trees (double logarithmic scale).

Iron sprays caused a substantial greening of chlorotic needles (Table 6). This result is the most classic indication of iron deficiency (Gris, 1844). Chlorosis was most severe at the needle base and re-greening was most obvious there. Water and nutrient uptake by pine needles is highest at the basal part within the sheath (Leyton & Juniper, 1963) and this can explain the local effect of spraying.

From these data it must be concluded that the yellowing trees suffer from iron deficiency and that this deficiency causes the yellowing of needles. The needles of green trees in the discoloured stands contained significantly less iron than their counterparts in the healthy stands, indicating that the iron supply of the green trees in the affected stands was also critical.

The next question is, what causes the impairment in the iron metabolism of the trees in the diseased stands. Soil sample analysis provided evidence on the role which soil factors may play. Van Dijk & Roelofs (1988) found significantly less water- and salt-extractable iron in the soils in chlorotic stands. In the present study only a tendency was found to lower water-extractable iron levels in soils in the order: green stands - green trees in diseased stands - yellow trees in diseased stands (Table 3). Moreover, these soils showed no significant differences in the amount of iron adsorbed to the cation exchange complex (Table 4). Therefore, the iron deficiency was probably not caused by an absolute lack of iron in the soil. However, its availability to the trees may be reduced. This availability is known to depend on the form of iron, the properties of the soil and on the microbial flora in the rhizoplane (Mengel & Kirkby, 1987).

Soil samples from the discoloured stands contained more organic matter than those from healthy looking stands (Table 5). In agreement with this were the higher cation exchange capacity (Table 4) and the higher water content (Table 5). Chlorosis in Scots pine growing on wet, acid soils, rich in organic matter, was described by Anonymous (1985), Buchner (1985) and Niehaus (1986). Possibly, a high water and organic matter content of the soil stimulates the production of humic substances which chelate iron so strongly, that trees are not able to mobilize it. Houdijk (1993) found that Corsican pine trees took up less iron from a nutrient solution, when this solution contained humic acids.

Heavy metal toxicity is another possible cause of iron deficiency (Foy *et al.*, 1978; Woolhouse, 1983). Soil acidification, as in the diseased stands (Table 5), may increase heavy metal uptake. Lodenius (1990) showed that soil acidification caused by acid rain significantly increased cadmium levels in needles of conifers. Indeed, the chlorotic trees contained significantly more cadmium than the corresponding green trees (Table 2). However, cadmium levels were not very high (cf. Nuorteva *et al.*, 1986). Since the shortage of iron in the needles must be due to impaired uptake or transport, cadmium (and other heavy metals) contents in the roots are the more promising parameters to be investigated.

Inorganic nitrogen levels were higher in the soils of discoloured stands (Tables 3 and 4) and so were the levels in the needles of the trees growing there (Table 2). The same observations were made by Van Dijk & Roelofs (1988). The high concentrations of nitrogen in the soil and the needles are caused by high atmospheric nitrogen deposition due to intensive stockbreeding. High ammonium deposition can cause soil acidification both by nitrification and plant uptake and thus mobilize heavy metals. Excessive nitrogen availability can also hamper mycorrhizal development

(Meyer, 1985; Van Dijk *et al.*, 1990). Mycorrhizae probably play an important role in iron uptake by trees (Clement *et al.*, 1977; Meyer, 1984) and in the prevention of heavy metal toxicity (Bradley *et al.*, 1982; Brown & Wilkins, 1985).

The high soluble phosphorus content of the soil of diseased stands is remarkable (Table 3). Phosphorus can immobilize iron within the plant as a ferric-phosphate complex and thereby interfere with its transport to the needles.

Although there existed profound differences between yellowing and green trees growing in the same stand with respect to the chemical composition of the needles (Table 2), differences in those soil characteristics measured were negligible. So, it should be equally difficult for all the trees in the affected stands to meet their iron demand. Genetic variability within the population of trees, with regard to the iron metabolism, may then determine which individual trees are able to maintain a satisfactory iron status and remain green and which are not.

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CHAPTER 6

NUTRIENT AVAILABILITY IN CORSICAN PINE STANDS IN THE NETHERLANDS AND THE OCCURRENCE OF *SPHAEROPSIS SAPINEA*: A FIELD STUDY

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ABSTRACT

To investigate whether there is a relation between the availability of nitrogen and the incidence of damage caused by the parasitic fungus *Sphaeropsis sapinea* (Fr.) Dyko & Sutton in Corsican pine (*Pinus nigra* ssp. *laricio* (Poir.) Maire) stands, needle and soil samples were taken in 29 stands in spring and early summer of 1986. The infected stands proved to contain higher nitrogen levels in the needle tissue and concomitant higher ammonium levels in the soil. Phosphorus was also present in larger amounts in needle tissue and soil. Most of the additional nitrogen in the needles of the affected stands was stored in free amino acids. Particularly the levels of the nitrogen rich amino acids asparagine, glutamine, histidine, lysine and arginine strongly increased. Arginine was by far the most abundant free amino acid in both healthy and infected stands. Also proline showed a remarkable rise in concentration. These results are discussed with regard to other recent Dutch field and greenhouse studies concerning *Sphaeropsis sapinea*.

INTRODUCTION

Sphaeropsis sapinea (Fr.) Dyko & Sutton is a parasitic fungus belonging to the *Fungi Imperfecti*. It affects coniferous trees, predominantly belonging to the genus *Pinus*. In The Netherlands the infection can reveal itself as shoot blight or as branch or crown wilt (De Kam & Van Dam, 1987). In the case of shoot blight, shoots of the current year are affected. The needles do not grow out to full length, but remain short and turn brown. Often the shoots warp because the meristematic cells die earlier at the infected side of the shoot than at the other side. Crown and branch wilt are characterized by the die-off of the whole crown or parts of it, depending on the site of infection on the older branches or the main stem. They are the result of bark necrosis. This phenomenon is often accompanied by a blue stain of the wood.

The fungus, which occurs all over the world, was found in The Netherlands for the first time about 1866 and has sporadically been observed since then (De Kam, 1990). In 1982 it was discovered in the so-called Peel area in the southern part of the country (Van Dam & De Kam, 1984). Two years later it had spread all over the country and the disease turned epidemic. Symptoms were not merely confined to shoot blight: crown wilt and branch dying were observed increasingly as well (De Kam, 1985). In 1985, when the epidemic reached its peak (Staatsbosbeheer, 1986b; Van Dam, 1990), 35% of the Corsican pine (*Pinus nigra* ssp. *laricio* (Poir.) Maire) and Austrian pine (*Pinus nigra* Arnold ssp. *nigra*) stands were affected by the pathogen, of which 11% were heavily infected. Of the Scots pine (*Pinus sylvestris* L.) stands 32%, were affected, 6% of which were heavily infected (Staatsbosbeheer, 1985).

Because of the large extent of the damage in the Peel area, where the nitrogen deposition in the forests is approximately 100 $kg.ha^{-1}.yr^{-1}$, and because it is well-known that nutrient availability influences interactions of pathogens and their hosts (Huber, 1980), we investigated whether a relationship exists between the availability of nutrients, especially nitrogen, and the occurrence of the disease. For this purpose soil and needle samples were collected in Corsican pine stands, seriously or slightly attacked by *Sphaeropsis sapinea*, in the southern part of The Netherlands. Nutrient levels in the soil and in the needle tissue were measured. Levels of free amino acids were also determined, as these may better reflect the nitrogen status of the trees than the total nitrogen content of the needles does (Kim *et al.*, 1987).

MATERIALS AND METHODS

Sampling

Between May 28 and July 9, 1986 needle and soil samples were taken in 29 Corsican pine stands, varying in age from 15 to 45 years. A distinction was made between stands heavily affected by *Sphaeropsis* (13 stands) and forest stands slightly affected by the parasite (16 stands). To discriminate between these two types, the occurrence of shoot blight was used, as this is the most reliable feature to establish the presence of the fungus (De Kam & Van Dam, 1987). In addition to shoot blight, in many of the affected forests branch and crown wilt was observed.

In each stand one dominant tree was felled. In the heavily affected stands this was always a tree displaying severe shoot blight (year class 1985) with pycnidia of *Sphaeropsis sapinea* present on the infected needles. Most of these trees also showed dead branches and crown wilt. Several primary and secondary branches were taken from the middle of the tree crown and healthy looking needles were gathered and mixed per year class. One metre from the tree trunk four samples were taken from the top layer (0-20 cm) of the mineral soil, after removal of the litter layer. The four samples were put together in a polyethylene bag and mixed. Both soil and needle samples were stored at -28 °C until analysis.

Analysis of samples

The levels of nutrients in the soil samples were determined by means of extraction procedures with water and salt solutions. The latter were used to asses the cations adsorbed to the cation exchange complex. The chemical composition of the needle tissue was determined after destruction with sulphuric acid and hydrogen peroxide. The methods used have been described earlier by Van Dijk & Roelofs (1988).

Free amino acids were extracted by the method of Van Dijk & Roelofs (1988) with some slight modifications. Together with 20 mL of extraction solution, 0.5 g of freshly ground needle tissue was shaken for 24 h at 2 °C. The extract was washed with 40 mL of chloroform, freeze-dried and redissolved in 2 mL lithium citrate buffer prior to analysis with high performance liquid chromatography (HPLC). For quantification, fluorescence was measured after postcolumn derivatization with *o*-phtalaldehyde and acetylcysteine instead of *o*-phtalaldehyde and mercaptoethanol. All amino acid analyses (including extraction and measurement) were carried out in duplicate. For most amino acids the coefficient of variation (CV) of the duplicate measurements averaged 2 to 10%. Only glycine, isoleucine and leucine had CV values of 10 to 13%. The tryptophan values given may be too high as there are indications that this amino acid was not separated from an other, unknown substance.

Statistics

The TTEST procedure from the Statistical Analysis System software package (SAS Institute Inc., 1989) was used as a test of significance for differences between means. Bij means of the STEPDISC procedure in the same software package those sets of variables were selected, that discriminated best between infected and non-infected trees. The DISCRIM procedure was used to investigate how well these sets discriminated. Pearson correlation coefficients between needle and soil parameters were calculated with the CORR procedure in the SAS software package. Statistical operations were performed on log-transformed data, since these fitted better to the conditions of normality and homoscedasticity (Sokal & Rohlf, 1981; Slob, 1986). For presentation the data were back-transformed. Consequently, the mean values given are geometric means, which are the maximum likelihood estimators of the population medians. They are shown together with their 95% confidence limits.

RESULTS

Chemical composition of the needle tissue

Analysis of the needle tissue (Tables 1 - 3) showed that the infected trees contained significantly more nitrogen than the healthy ones. This applied to all year classes. Phosphorus was the only other element that also reached higher values in all year classes of the diseased trees. Aluminium was found in larger amounts in the first and second year class and zinc in the second and the third year class of the infected trees. Iron levels were significantly higher in the youngest needles of the healthy trees.

Discriminant analysis revealed that the combined measurements of nitrogen in the 2-year-old needles and phosphorus in the 1-year-old needles were best to discriminate between infected and non-infected trees. Even when assuming that only 11% of the Corsican pine trees in the field was severely infected by *Sphaeropsis sapinea* (Staatsbosbeheer, 1985), all the 29 investigated trees were classified into the correct

Table 1. Chemical composition of the 1-year-old needles of Corsican pine trees, infected or not infected by *Sphaeropsis sapinea*.

	non-infected (n=16)		infected (n=13)		
N	1.42	(1.31 -1.54)	2.23	(1.93 -2.56)	***
K	0.41	(0.38 -0.45)	0.43	(0.38 -0.49)	n.s.
Mg	0.042	(0.036-0.048)	0.050	(0.040-0.063)	n.s.
Ca	0.118	(0.092-0.150)	0.098	(0.065-0.149)	n.s.
P	0.062	(0.056-0.069)	0.103	(0.089-0.120)	***
Fe	0.019	(0.015-0.024)	0.013	(0.010-0.016)	*
Mn	0.022	(0.015-0.032)	0.016	(0.010-0.024)	n.s.
Zn	0.007	(0.006-0.009)	0.008	(0.006-0.010)	n.s.
Al	0.023	(0.019-0.028)	0.037	(0.026-0.051)	**

Values are given as a percentage of dry weight. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: n.s. not significant; (*) $0.05<P\leq0.10$; * $0.01<P\leq0.05$; ** $0.001<P\leq0.01$; *** $P\leq0.001$.

Table 2. Chemical composition of the 2-year-old needles of Corsican pine trees, infected or not infected by *Sphaeropsis sapinea*.

	non-infected (n=16)		infected (n=13)		
N	1.45	(1.33 -1.58)	2.12	(1.92 -2.34)	***
K	0.39	(0.35 -0.44)	0.39	(0.33 -0.46)	n.s.
Mg	0.033	(0.028-0.039)	0.039	(0.030-0.061)	n.s.
Ca	0.186	(0.146-0.239)	0.167	(0.119-0.234)	n.s.
P	0.054	(0.046-0.063)	0.093	(0.077-0.112)	***
Fe	0.024	(0.019-0.029)	0.023	(0.019-0.028)	n.s.
Mn	0.027	(0.017-0.041)	0.018	(0.012-0.027)	n.s.
Zn	0.002	(0.002-0.003)	0.005	(0.004-0.007)	***
Al	0.025	(0.020-0.031)	0.035	(0.029-0.042)	*

Values are given as a percentage of dry weight. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: see Tab. 1.

group according to these nitrogen and phosphorus levels, with posterior probabilities of membership of over 0.90. Nitrogen and phosphorus levels in other year classes

Table 3. Chemical composition of the 3-year-old needles of Corsican pine trees, infected or not infected by *Sphaeropsis sapinea.*

	non-infected (n=14)		infected (n=6)		
N	1.34	(1.20 -1.49)	1.90	(1.76 -2.04)	***
K	0.42	(0.38 -0.46)	0.36	(0.28 -0.48)	n.s.
Mg	0.034	(0.030-0.040)	0.042	(0.028-0.063)	n.s.
Ca	0.223	(0.171-0.291)	0.159	(0.087-0.290)	n.s.
P	0.056	(0.050-0.062)	0.095	(0.074-0.122)	***
Fe	0.021	(0.018-0.026)	0.024	(0.013-0.043)	n.s.
Mn	0.038	(0.025-0.060)	0.023	(0.009-0.057)	n.s.
Zn	0.005	(0.004-0.006)	0.007	(0.005-0.011)	(*)
Al	0.024	(0.019-0.029)	0.031	(0.020-0.048)	n.s.

Values are given as a percentage of dry weight. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: see Tab. 1.

were slightly less effective in discriminating between both groups of trees.

Free amino acids

The results of the analysis of the free amino acid spectrum of the one-year-old needles are presented in Tab. 4. Arginine was by far the most abundant amino acid in the needles of both healthy and infected trees. Also aspartic acid, glutamine, proline, alanine and γ-amino butyric acid reached relatively high concentrations. All amino acids but one showed higher levels in the diseased trees than in the healthy ones. For phenylalanine, however, this difference was not significant. Most amino acids reached levels 1.5 to 2.5 times higher in the diseased trees, but asparagine, glutamine, lysine, histidine, arginine and proline concentrations increased by a factor of 4 to 10. Glutamic acid was the only amino acid the levels of which were significantly lower in the infected than in the non-infected trees.

Combined measurements of glutamine and glutamic acid were most effective to distinguish between infected and non-infected trees as shown by discriminant analysis. When assuming that 11% of all Corsican pine trees were heavily infected, only one tree (from the infected group) was misclassified, based on these two amino acids, with posterior probabilities of correct membership of over 0.80 in 25 out of 28 cases.

Table 4 Amounts of free amino acids in the 1-year-old needles of Corsican pine trees, infected or not infected by *Sphaeropsis sapinea*

	non-infected (n=16)		infected (n=13)		
aspartic acid	1.878	(1.470-2.399)	2.773	(2.106-3.651)	*
threonine	0.185	(0.162-0.210)	0.304	(0.252-0.367)	***
serine	0.607	(0.496-0.742)	0.923	(0.763-1.116)	**
asparagine	0.099	(0.078-0.126)	0.543	(0.326-0.907)	***
glutamic acid	1.116	(0.958-1.301)	0.522	(0.307-0.887)	***
glutamine	0.787	(0.472-1.311)	7.316	(4.459-12.00)	***
proline	0.554	(0.454-0.677)	3.004	(1.781-5.068)	***
glycine	0.121	(0.103-0.143)	0.223	(0.163-0.305)	***
alanine	1.477	(1.280-1.704)	2.138	(1.893-2.416)	***
isoleucine	0.061	(0.048-0.076)	0.114	(0.086-0.152)	***
leucine	0.080	(0.068-0.095)	0.167	(0.119-0.234)	***
phenylalanine	0.170	(0.129-0.225)	0.225	(0.184-0.276)	n.s.
γ-amino but. ac.	3.896	(3.102-4.894)	9.025	(7.629-10.68)	***
tryptophan	0.395	(0.272-0.572)	0.638	(0.457-0.890)	*
histidine	0.330	(0.242-0.449)	1.405	(0.972-2.030)	***
ornithine	0.320	(0.213-0.480)	0.795	(0.417-1.514)	*
lysine	0.346	(0.222-0.542)	1.197	(0.696-2.059)	***
arginine	14.30	(7.185-28.45)	105.6	(70.11-159.2)	***

Free amino acid contents are given in µmol per g DW Geometric means are given with their 95% confidence limits in parentheses Significance according to the t-test see Tab 1 Abbreviations. γ-amino but ac , γ-amino butyric acid

The free amino acids comprised approximately 30% of the total nitrogen content of the needles in the infected trees (Tab. 5). In healthy trees this was only 8% Differences in the total nitrogen content were largely due to differences in free-amino-acid-bound nitrogen. Differences in non-extractable nitrogen were much smaller.

Soil analysis

Soil samples from infected and healthy stands displayed many significant differences in the extractability of nutrients. The soil in the diseased stands contained significantly more water-extractable NH_4 than the soil in unaffected forests (Tab. 6). There were no differences in NO_3. Also Na, Cl and P could be extracted in larger amounts from the soil samples of the infected stands. On the other hand, water-extractable K, Ca, Mg, Mn and S were present in larger quantities in the soil of healthy stands where the pH was slightly higher.

Table 5. Nitrogen fractions in the 1-year-old needles of Corsican pine trees, infected or not infected by *Sphaeropsis sapinea*.

	non-infected (n=16)		infected (n=13)		
N total	1.42	(1.31-1.54)	2.23	(1.94-2.56)	***
N in free amino acids	0.11	(0.07-0.18)	0.66	(0.45-0.98)	***
N in arginine	0.08	(0.04-0.16)	0.59	(0.39-0.89)	***
N not in free amino ac.	1.27	(1.20-1.35)	1.48	(1.39-1.58)	***

Values are given as a percentage of dry weight. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: see Tab. 1.

Table 6. Water-extractable nutrients in the soils of Corsican pine stands, infected or not infected by *Sphaeropsis sapinea*.

	non-infected (n=16)		infected (n=13)		
NH4	12.7	(7.0 -23.0)	28.8	(19.6 -42.4)	*
NO3	18.5	(14.8 -23.3)	20.3	(17.2 -24.0)	n.s.
K	12.9	(10.9 -15.3)	9.1	(6.8 -12.1)	*
Na	6.0	(3.7 - 9.6)	17.3	(7.7 -39.1)	*
Ca	4.3	(2.9 - 6.2)	2.4	(1.7 - 3.4)	*
Mg	2.6	(2.1 - 3.2)	1.1	(0.8 - 1.6)	***
Al	24.8	(17.9 -34.3)	20.9	(16.5 -26.4)	n.s.
Fe	7.5	(3.2 -17.6)	5.4	(4.0 - 7.2)	n.s.
Mn	0.24	(0.13- 0.43)	0.04	(0.02- 0.07)	***
Zn	0.17	(0.12- 0.23)	0.11	(0.08- 0.15)	(*)
Cl	18.7	(12.0 -29.2)	87.4	(63.8 -119.6)	***
S	19.7	(15.0 -35.6)	11.7	(9.2 -14.9)	**
P	2.0	(1.4 - 2.8)	6.7	(4.8 - 9.3)	***
pH	4.10	(3.97- 4.23)	3.95	(3.86- 4.04)	(*)

Nutrient content is given in µmol per 100 g DW. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: see Tab. 1.

Extraction with a salt solution gave similar results with respect to soil pH (Tab. 7). Most cations reached higher levels in the soil samples of the diseased stands. Exceptions to this rule were K and Mn, which did not differ significantly, and Fe, that was extracted in larger amounts from the soil samples of the healthy stands.

Table 7. Salt-extractable nutrients in the soils of Corsican pine stands, infected or not infected by *Sphaeropsis sapinea*.

	non-infected (n=16)		infected (n=13)		
NH4	25.5	(15.5 -42.0)	53.5	(30.6 -93.5)	*
K	29.7	(26.1 -33.7)	26.8	(19.4 -37.1)	n.s.
Ca	36.6	(23.7 -56.5)	76.7	(41.4 -142.2)	*
Mg	6.4	(4.8 - 8.5)	13.5	(8.5 -21.4)	**
Al	134.3	(106.9-167.0)	175.9	(134.6-230.0)	n.s.
Fe	1.20	(0.76- 1.89)	0.71	(0.48- 1.06)	(*)
Mn	1.35	(0.82- 2.22)	1.28	(0.78- 2.10)	n.s.
Zn	1.21	(0.84- 1.74)	3.22	(1.95- 5.32)	**
pH	3.54	(3.37- 3.71)	3.02	(2.91- 3.13)	***

Nutrient content is given in µmol per 100 g DW. Geometric means are given with their 95% confidence limits in parentheses. Significance according to the t-test: see Tab. 1.

DISCUSSION

The affected trees had significantly higher nitrogen levels in their needles than the healthy ones: in the 1-year-old needles the nitrogen content was 2.23 % in affected trees and 1.42 % in the healthy ones (Tab. 1). In 1983 Roelofs *et al.* (1985) investigated the relation between tree vitality and chemical composition of the needle tissue in Corsican pine. They also found that trees affected by *Sphaeropsis sapinea* or *Brunchorstia pinea* (Karst.) Höhnel contained more nitrogen in the 1-year-old needles and reported values of 1.90 % for the diseased trees and 1.34 % for the healthy ones. De Temmerman *et al.* (1988) and Van den Burg *et al.* (1988) obtained very similar results and Chou (1976a) reported that vigorous trees with plentiful nitrogen appeared to be much more susceptible to shoot blight than those that were nitrogen deficient. However, Smits (1986) did not find a correlation between ammonia emission data and the incidence of shoot blight in young plantations of Scots pine.

The extra amount of nitrogen in the diseased trees appeared to be largely stored in free amino acids (Tab. 4). The levels of most free amino acids increased, but this increase was most pronounced with the nitrogen-rich amino acids. Arginine was by far the most abundant amino acid in both infected and non-infected trees and accounted for 26% and 6% of total N, respectively (Tab. 5). Accumulation of arginine was also observed in Scots pine trees with a reduced vitality, growing in the same area (Van Dijk & Roelofs, 1988). In the diseased trees the free amino acids contained 30% of the total nitrogen content. According to Krauß *et al.* (1986) very high levels of soluble nitrogen compounds are detrimental to coniferous trees, but values up to 30% of total N are not accompanied by any reduction in growth in several coniferous species. However, this does not necessarily mean, that such levels do not influence the susceptibility to disease. Proline was the only not-nitrogen-rich amino acid that showed an increase comparable to the nitrogen-rich amino acids. This amino acid is known to accumulate under conditions of stress (Aspinall & Paleg, 1981).

Phosphorus levels were also remarkably higher in the needles of the trees that were infected by *Sphaeropsis sapinea*. Van den Burg *et al.* (1988) reported significantly higher phosphorus contents in the needles of infected trees as well, although the differences he found were much smaller. The higher phosphorus content in the needles of the infrected trees is rather remarkable, because in declining stands, particularly when the decline is caused by a high nitrogen availability, the phosphorus levels are often lower than in healthy stands as a result of growth dilution or impaired uptake owing to the disappearance of mycorrhizal fungi (Nihlgård, 1985; Mohren *et al.*, 1986; Aber *et al.*, 1989). However, Oren *et al.* (1988) also found considerably higher phosphorus levels in the needles of a declining Norway spruce (*Picea abies* (L.) Karst. stand than in the needles of a healthy stand. They ascribed this to an enhanced ammonium nutrition (Oren & Schulze, 1989).

The higher nitrogen and phosphorus levels in the affected trees were probably caused by edaphic factors and not (or at least not only) by the infection or a physiological disfunction of the needles. This was made plausible by the soil analyses that showed enhanced ammonium and phosphorus extractabilities from the soil samples of the diseased stands (Tables 6 and 7) and significant, positive correlations between nitrogen and phosphorus levels in the needles and extractable amounts of ammonium and phosphorus in the soils (Tab. 8). Nitrogen levels in the needles were less clearly correlated with extractable amounts of nitrate in the soil. Van den Burg *et al.* (1988) found a significant, positive correlation between nitrogen levels in the needles and the nitrogen deposition in Corsican pine stands. As nitrogen nutrition enhances the levels of amino acids in needle tissue (Krauß *et al.*, 1986; Kim *et al.*, 1987) and only healthy looking needles were sampled, the higher amino acid concentrations in the needles of infected trees were probably due to the higher nitrogen availability rather than protein breakdown.

Table 8. Pearson correlation coefficients between nitrogen and phosphorus levels in the needles and extractable ammonium and phosphorus in the soil.

	nutrient levels in needles of year class		
nutrient in soil	1	2	3
		nitrogen	
NH4 water-extr.	0.31	0.56	0.79
	(*)	**	***
	29	29	20
NH4 salt-extr.	0.24	0.47	0.83
	n.s.	**	***
	29	29	20
		phosphorus	
P water-extr.	0.80	0.64	0.76
	***	***	***
	27	27	18

Values are Pearson correlation coefficients with significance levels and numbers of observations. For explanation of the significance symbols see Tab. 1.

The nutritional status of all trees, regardless of infection, was poor. Potassium, magnesium and calcium levels were insufficient, when evaluated according to the criteria given by Van den Burg (1988).The non-infected trees were severely deficient in phosphorus. However, one should be careful in applying the above mentioned criteria, as they were developed for 0.5-year-old needles.

Though water-extraction showed higher levels of most cations in the soil samples of unaffected stands (Tab. 6), the amounts were higher in damaged forests when a salt solution was used for the extraction (Tab. 7). This suggests a higher cation exchange capacity of the soil in the forests that are affected by the fungus. The significantly higher amounts of Na and Cl in the soil of the diseased stands cannot yet be explained (Tab. 6).

The relation between the raised availability of nutrients, particularly nitrogen and phosphorus, and the occurrence of the fungal disease is not yet understood. One possible explanation is that the fungus is stimulated by the higher nutrient content of the young shoots. Indeed, Turner (1980) reported that parasitic fungi like *Sphaeropsis sapinea* use amino acids that do not contain sulphur as a nutrient source. However, according to M. De Kam (pers. communication), the young shoots are such a rich nutrient source that slightly raised levels of nitrogen and phosphorus do not matter.

An alternative possibility is that trees that receive too much N and P have a reduced physical and(or) chemical resistance against fungal attack owing to a reduced vitality. De Kam & Van Dam (1987), however, declared that there exists no relation between tree vitality and the occurrence of shoot blight, as undamaged, young shoots of vigorous trees are very susceptible to fungal attack as well. Chou (1976b) and Peterson (1977) found also that healthy, undamaged shoots could easily become infected. Apparently, *Sphaeropsis sapinea* acts like a primary parasite with respect to young, developing shoots. Indeed, infection experiments carried out at the Dorschkamp Research Institute for Forestry and Landscape Planning (Wageningen, The Netherlands) could not establish a relation between the nitrogen content of needles and the incidence of shoot blight (De Kam & Versteegen, 1990). However, these infection experiments should be interpreted with some restraint, because they were carried out under optimal temperatures and air humidity conditions for the fungus, with young trees with sufficiently high levels of P and K in the needles. Moreover, all trees were inoculated at the same time, though in the highest nitrogen fertilization treatment development of current shoots was retarded and poor. Probably, inoculation of current shoots should take place at the same developmental stage rather than at the same time. If no stress factors are involved in shoot blight, as is stated by De Kam & Versteegen (1990), it remains unclear why shoot blight rarely occurred before 1982. After all, it seems very unlikely that a mass occurrence of this phenomenon remained unnoticed for so long a time.

According to De Kam & Van Dam (1987) it is probably more useful to look for a relation between nitrogen availability and the occurence of necroses on branches and trunk, which cause branch and crown wilt. This view is based on the observations of De Winter (1986) that bark tissue older than one year becomes infected only after wounding and that the development of bark necrosis is stimulated by previously applied stress. So, with regard to bark necrosis the fungus apparently behaves like a weak pathogen. De Kam *et al.* (1990) found that ammonium fertilization stimulated the development of bark necrosis caused by *Sphaeropsis sapinea* in a pot experiment with 5-year-old Corsican pine trees. On the other hand, potassium fertilization retarded the development. De Kam *et al.* (1990) supposed that excessive ammonium nutrition reduced the tree's resistance by causing nutritional imbalances and a reduced water potential. The correlation between the incidence of shoot blight and the availability of nitrogen in the current field study, might be based on the coincidence of shoot blight and branch and crown wilt. The higher incidence of shoot blight in stands with higher nitrogen availability could then be explained by the presence of more inoculum due to bark necrosis. However, the fact that shoot blight had occurred massively before branch and crown wilt were observed does not support this idea.

In the past, several investigators have pointed out that trees suffering from drought are particularly susceptible to infection by *Sphaeropsis sapinea* (Chou, 1987). Nitrogen availability strongly influences the water balance of coniferous trees by affecting the shoot to root ratio (Van Dijk *et al.*, 1990), the mycorrhization of roots (Meyer, 1985) and the water potential of needles (De Kam *et al.*, 1990). Perhaps excessive nitrogen nutrition stimulates fungal infection by inducing drought stress. The remarkable accumulation of proline, which is often observed in drought-stressed plants (Aspinall & Paleg, 1981), may point in that direction. The fact that the *Sphaeropsis*-epidemic declined after 1985, while the nitrogen deposition remained high, indicates that at least other factors are also involved.

Finally, it can be concluded, that there is a clear and significant correlation between the availability of nitrogen and phosphorus in Corsican pine stands and the incidence of infection by the parasitic fungus *Sphaeropsis sapinea*. However, the nature of this relationship and the mechanisms involved remain to be elucidated.

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CHAPTER 7

GENERAL DISCUSSION

Ammonium and ammonia deposition in The Netherlands has increased by a factor of 3.7 during the last 110 years (Asman, 1987). Throughfall water analyses indicate that the nitrogen deposition in Dutch forests in 1986 and 1987 amounted to approximately 80 $kg.ha^{-1}.yr^{-1}$ (Ivens, 1990; Houdijk & Roelofs, 1991). About 80% was derived from ammonia and ammonium, 20% from nitrogen oxides. Reduced nitrogen compounds accounted for 44% of the deposition of potential acid (Houdijk & Roelofs, 1991). Calculations of deposition, based on pollutant concentrations in the air and deposition velocities yielded slightly lower values for ammonium-N and higher values for nitrate-N (Erisman & Heij, 1991; Erisman, 1992). The differences between the two methods are attributed to exchange processes in the canopy and the deposition of fog and coarse particles. There is considerable spatial (both within and between stands) and temporal variation in nitrogen deposition to the forest floor (Ivens, 1990; Houdijk & Roelofs, 1991).

The high atmospheric nitrogen input leads to an availability of inorganic nitrogen in excess of plant and microbial nutritional demand, a situation, which is referred to as nitrogen saturation (Aber *et al.*, 1989). However, this concept is still subject of much debate, as there is no scientific agreement on the exact definition (Skeffington & Wilson, 1988).

Under pristine conditions nitrogen is a growth limiting factor in forests on acidic soils (Durzan & Steward, 1983). Although the nitrogen deposition levels in forests, before man expanded his industrial and agricultural activities, are not known, measurements in remote, clean areas indicate a nitrogen deposition of less than 3 $kg.ha^{-1}.yr^{-1}$ (Aber *et al.*, 1989; Edfast *et al.*, 1990). Consequently, forest trees and plants are not well adapted to excess nitrogen availability, particularly, because the increase has taken place in such a short time span.

In this chapter the various ways in which excess nitrogen deposition, especially that of ammonium, interferes with tree health are shown by summarizing the results presented in the previous chapters and integrating them with those of other investigators. Finally, the role of nitrogen in the forest dieback in The Netherlands is discussed.

Effects on soil chemistry

High ammonium deposition may affect forest soil chemistry in various ways. In some soils little or none of the ammonium deposited is converted into nitrate (see chapter 2). Low pH values, low nutrient status or allelopathic inhibition may be the cause (Kriebitzsch, 1978; Killham, 1990). In these soils ammonium accumulates in the upper soil layers (chapter 2; Roelofs *et al.*, 1985; Harmer & Alexander, 1986; Schulze, 1989; Ferm *et al.*, 1990; Houdijk *et al.*, 1993). The ammonium ions replace base cations adsorbed to the cation exchange complex. This results in increased leaching of these cations to deeper soil layers together with sulphate. The ratio of ammonium to base cations in the soil solution increases (chapter 2). The presence of ammonium negatively affects the uptake of other cations by plant roots (Cox & Reisenauer, 1973; Scherer *et al.*, 1984; Boxman & Roelofs, 1988; Boxman *et al.*, 1991; Flaig & Mohr, 1991). For potassium this inhibition is probably by competition for a common carrier (Mengel & Kirkby, 1987).

In other soils - and their number may be increasing as a result of high ammonium deposition (Tamm, 1991) - most or all of the ammonium is nitrified (see chapter 2), even if the pH of the soil is very low (Kriebitzsch, 1978; Van Breemen & Van Dijk, 1988). Acid-adapted populations of chemolithotrophic bacteria (Tietema *et al.*, 1992; De Boer *et al.*, 1992) or heterotrophic micro-organisms, probably fungi (Killham, 1990), are regarded responsible for this conversion. The chemical reaction involves the production of two protons per nitrate molecule. Therefore, the high ammonium deposition constitutes an enormous acid load to these soils (chapter 2; Van Breemen *et al.*, 1982). The protons are neutralized by cation exchange processes and weathering of minerals in the soil. Prolonged ammonium sulphate deposition to these soils will thus exhaust successive buffering systems and lower the soil solution pH. Base cations will temporarily reach higher concentrations in the soil solution, but leach more and more to deeper soil layers together with nitrate and sulphate and become depleted (chapter 2). Aluminium and heavy metal containing compounds will start to participate in buffering when acidification progresses and free aluminium and heavy metal ions will enter the soil solution. These are toxic to plant roots (Foy, 1988; Godbold *et al.*, 1988) and hamper nutrient uptake (Boxman *et al.*, 1991). This may force the plants to restrict root growth to the organic top layer of the soil, were aluminium concentrations are lower and were aluminium may be detoxified by complexation with humic substances (Hue *et al.*,1986).

This organic top layer was left aside in the investigations described in the previous chapters. The main reason was, that sampling and extraction of nutrients are easier when the mineral soil is used. Moreover, nutrient availability in the mineral soil proved to correlate well with tree vitality (Roelofs *et al.*, 1985). The organic top layer, however, plays an important role in the nutrition of forest trees, because processes of mineralization and nitrification mainly take place in this layer and nutrient concentrations are high there. Therefore, it might have been better to include this layer in the investigations. Nevertheless, the conclusions would probably be exactly the same. Differences in availability of inorganic nitrogen in the mineral soil between forest stands are probably accompanied by similar or even larger differences in the organic top layer, because most of the inorganic nitrogen in the mineral soil has either been generated in the organic top layer by processes of mineralization and(or) nitrification, or it has entered the soil via it. Moreover, plant uptake, immobilization in microbial biomass and adsorption to the cation exchange complex will reduce the amounts of inorganic nitrogen leaching to deeper soil layers.

Effects on the root system

High levels of inorganic nitrogen in the soil have pronounced effects on the root system: it becomes smaller, largely because of a loss of fine roots (chapter 3; Boxman *et al.*, 1991). Nadelhoffer *et al.* (1985) reported the amount of fine roots to decrease when nitrogen availability increased, but the total production of fine roots was higher as a result of an increased turnover.

Also the degree of mycorrhizal infection of the roots of coniferous trees is negatively affected by an increased availability of nitrogen (chapter 3). Many other investigators found similar results with various tree species (Richards & Wilson, 1963; Björkman, 1970; Meyer, 1985; Termorshuizen & Ket, 1990; Boxman *et al.*, 1991; Van der Eerden *et al.*, 1992). Also fruiting body production by mycorrhizal fungi is negatively affected by nitrogen fertilization (see chapter 3; Menge & Grand, 1978; Wästerlund, 1982; Termorshuizen, 1990; Rühling & Tyler, 1991). This fits in very well with the observations of Arnolds (1985) and Arnolds & Jansen (1987) that the fruiting bodies of mycorrhizal fungi are disappearing from the Dutch forests and that the decline is most pronounced in the southern part of the country, where the nitrogen deposition is highest. According to Termorshuizen (1990) fungal carpophore production is more sensitive to nitrogen fertilization than mycorrhizal infection of the roots. A decline in number of fruiting bodies is preceding a decrease in mycorrhizal frequency.

Direct effects of gaseous air pollutants on mycorrhizae living in the soil are not very likely to occur. Soil and tree mediated effects seem to be of more importance (Jansen & Dighton, 1990; Arnolds, 1991). Deposition of nitrogen and sulphur compounds may cause soil acidification. The concomittant changes in soil solution chemistry (lower pH, higher concentrations of aluminium and heavy metals) may impair mycorrhizal development (Jongbloed & Borst-Pauwels, 1992). Moreover, high concentrations of inorganic nitrogen in the soil solution inhibit the production of phytohormones and phytohormone protecting polyphenols by the mycorrhizal fungi (Tomaszewski & Wojciechowska, 1973; Slankis, 1974; Cooke, 1977; Meyer, 1985). These hormones are necessary for the development of mycorrhizae. High ammonium concentrations in the soil solution may also affect nutrient uptake by the fungus (Boxman *et al.*, 1986). Tree mediated effects involve a reduction of the carbohydrate flow to the roots due to impaired photosynthesis or diversion of carbohydrates to processes of ammonia detoxification (Björkman, 1970).

Effects on above-ground plant parts

Initially, the biomass of the above-ground plant parts is stimulated by excess nitrogen nutrition (chapter 3; cf. Kenk & Fischer, 1988). Consequently, the shoot to root ratio increases (see chapter 3; cf. Ingestad, 1960; Meyer, 1985; Encke, 1986; Gezelius, 1986a; Boxman *et al.*, 1991; Flaig & Mohr, 1991). Probably, alterations in the production of growth substances play a role in this (Quispel & Stegwee, 1983). Consequently, a quantitatively and qualitatively degenerated root system has to supply water and nutrients to an ever more demanding shoot. The problem is aggravated by the fact that soil conditions become less favourable for nutrient uptake (see above). Nutrient deficiencies arise in the needles due to reduced uptake. Moreover, the balance between nutrients is disturbed. Trees require nutrients in more or less fixed proportions (Ingestad, 1979; Van den Burg, 1988). The elevated nitrogen availability stimulates growth. If other nutrients are not equally abundant, their concentrations in the plant tissue will decrease and ultimately limit growth. The nitrogen content of the tissue will rise as plants are not able to stop nitrogen uptake, even when the physiological need is satisfied (Hällgren & Näsholm, 1988; Skeffington & Wilson, 1988; Tamm, 1991). High levels of nitrogen and low levels of potassium, magnesium and phosphorus, in particular relative to nitrogen, are found in the needles of seedlings experimentally subjected to large amounts of ammonium in the rooting medium (chapter 3; cf. Boxman *et al.*, 1991; Flaig & Mohr, 1991). The same nutritional imbalance is commonly observed in forests exposed to high atmospheric nitrogen deposition (see chapters 4, 5 and 6; Nihlgård, 1985; Roelofs *et al.*, 1985; Mohren *et al.*, 1986; De Temmerman *et al.*, 1988; Oren *et al.*, 1988; Aber *et al.*, 1989; Schulze, 1989; Hüttl, 1990; Weissen *et al.*, 1990; Katzensteiner *et al.*, 1992; Houdijk & Roelofs, 1993). As potassium, magnesium and phosphorus are relatively mobile elements, deficiencies result in translocation of these nutrients from older to younger needles. The former needles start to show discolourations typical of nutrient deficiencies and may be shed prematurely. If the nutrient deficiencies become very severe even the current year needles can be affected.

Scots pine trees may also show iron deficiency symptoms in spite of low soil pH (chapters 4 and 5). As iron is not very mobile within plants, chlorosis develops characteristically in the current year needles whereas the older needles remain green. There is evidence that excessive nitrogen availability plays a role in this, but it is not clear whether it just acts by deteriorating the root system or that other mechanisms are involved. Based on discolouration and loss of needles or leaves 57% of the Dutch forests is regarded to have a reduced vitality (Smits, 1992).

High nitrogen availability may affect the appearance of the shoot also in other ways: it stimulates branch and diameter growth more than height growth (Will, 1971; Tamm, 1991). Moreover, it induces boron deficiency by growth dilution (see chapter 3; Miller & Miller, 1988; Ferm *et al.*, 1990) resulting in growth disturbances like thick-butted and crooked stems, multiple crowns and bushy growth. In The Netherlands malformed trees are very common throughout the country. In Finland they occur only near fur animal farms (Ferm *et al.*, 1990). High nitrogen availability in combination with boron deficiency also enhances frost sensitivity, either by inducing prolonged growth and retarding bud setting or by direct influence at cellular level (Aronsson, 1980; Hellergren, 1981; Skeffington & Wilson, 1988). It is evident that excess ammonium availability eventually leads to deterioration of the above-ground plant parts, in spite of its initially stimulating effect.

Although hardly any differences were observed between young trees in a greenhouse that received artificial rainwater on the soil either directly or after passage through the canopy (see chapters 2 and 3; cf. Thomas & Miller, 1992), there is ample evidence that trees are able to take up considerable amounts of nitrogen with their aerial parts (Van der Eerden, 1982; Lovett & Lindberg, 1984; Grennfelt & Hultberg, 1986; Lindberg *et al.*, 1986; Schulze, 1989; Eilers *et al.*, 1992). This does not only concern gaseous nitrogen compounds like ammonia and nitrogen oxides, but also aerosols and ammonium and nitrate that are dissolved in rainwater. The concentrations of these solutes are particularly high in fogwater (Fuhrer, 1986) and in the first rainwater dripping from needles or leaves after a dry period (Ivens, 1990). This facilitates uptake. The exact mechanism by which the solutes enter the trees is not known. Leyton & Juniper (1963) showed that pine needles may take up water via the needle base that is enveloped by the fascicular sheath. This uptake by the needles may be advantageous for plants growing in ecosystems where production is limited by nitrogen and consequently competition in the soil between plant roots and microorganisms for nitrogen is very heavy. However, in circumstances of excess nitrogen availability the problems of nutritional imbalances are exacerbated, especially when the trees compensate for ammonium uptake by enhanced leaching of potassium and magnesium from the needles and through the bark of twigs (Nihlgård, 1985; Roelofs *et al.*, 1985; Glatzel *et al.*, 1987; Leonardi & Flückiger, 1989; Schulze, 1989; Hogrebe, 1990).

The nitrogen the trees are taking up is incorporated into amino acids. The binding of ammonium is absolutely necessary as free ammonium is toxic because of its interference with many processes in the cell (see *e.g.* Puritch & Barker, 1967; Wakiuchi *et al.*, 1970; Van der Eerden, 1982). If the rates of nitrogen uptake and subsequent amino acid synthesis exceed that of protein production, free amino acids accumulate. Upon changes in nitrogen supply these amino acids show manyfold greater changes in concentration than the total nitrogen content. Therefore, free amino acids are more informative parameters to evaluate the nitrogen status of a plant than the total nitrogen content (Van den Driessche, 1974; Kim *et al.*, 1987). In coniferous trees arginine is most important in this respect. As this amino acid holds as much as four nitrogen atoms and contains only six carbon atoms it is probably an ideal nitrogen storage compound requiring a minimum of carbohydrates to store a maximum of nitrogen. Under conditions of excess nitrogen availability it may be more abundant in needles of conifers than all other free amino acids together (see chapters 3, 4 and 6; Näsholm & Ericsson, 1990; Pietilä *et al.*, 1991) and Ferm *et al.*

(1990) regard it as a good indicator of detrimentally high ammonium deposition. In Dutch plantation forests free arginine may comprise 30% to 35% of the total nitrogen content of the needles (chapters 4 and 6). According to Krauß *et al.* (1986) this involves a clear reduction in growth.

Arginine accumulation is not only determined by nitrogen availability, but also by the availability of other nutrients. When these are in short supply, protein synthesis and growth are hampered and arginine accumulates. Thus, the primary role of arginine may be removal of excess ammonium rather than storage of nitrogen (Näsholm & Ericsson, 1990). Its accumulation seems not to be a response specific to shortage of a special mineral nutrient, as deficiencies of many macro- and micro-nutrients can cause arginine accumulation (Stewart & Lahrer, 1980 and Rabe & Lovatt, 1986 and references cited therein). Therefore, free arginine is probably a good indicator for nutritional imbalances in coniferous trees caused by excess nitrogen availability (cf. Edfast *et al.*, 1990; Tamm, 1991). The same role may be fulfilled by the amides glutamine and asparagine (see chapters 4 and 6; cf. Stewart & Lahrer, 1980; Flaig & Mohr, 1992), though they do not accumulate in needles of conifers to the same extent as arginine.

Not only the nutritional balance, but also the water balance is affected by high nitrogen availability. As a result of the increased shoot to root ratio the uptake of water is hampered, whereas transpiration is stimulated. This may explain the findings of De Kam *et al.* (1991), who reported that seedlings of Corsican pine, fertilized with ammonium sulphate, showed a reduced water potential in the needles. However, ammonium and ammonia seem also to interfere with stomatal control, possibly by reducing the potassium content of the guard cells (Gmur *et al.*, 1983; Van der Eerden & Pérez-Soba, 1992; Van Hove *et al.*, 1992). Evidence on the impact of ammonia and ammonium sulphate on the structure of the cuticular wax layer and on cuticular transpiration is conflicting. According to some investigators the wax layer is damaged by these compounds, resulting in increased water loss (Dueck *et al.*, 1990/91; Thijsse & Baas, 1990; Van der Eerden *et al.*, 1992). Others report no effect of ammonia or ammonium on the cuticular wax layer and cuticular water loss (Hogrebe, 1990; Bacic *et al.*, 1992; Van Hove *et al.*, 1992).

There are strong indications that nitrogen also interferes with tree - parasite interrelations (Hare, 1966). Corsican pine trees affected by the parasitical fungus *Sphaeropsis sapinea* have clearly higher nitrogen levels in their needles than unaffected trees (chapter 6, Roelofs *et al.*, 1985; Van den Burg *et al.*, 1988; De Temmerman *et al.*, 1988). Most of the additional nitrogen is bound in free arginine (chapter 6), indicating nutritional imbalances. Lambert (1986) found that seedlings and young trees of *Pinus radiata* are more readily infected by the parasitic fungi *Sphaeropsis sapinea* and *Dothistroma* upon induction of arginine accumulation by unbalanced nitrogen and sulphur nutrition. One possible explanation is, that young, developing shoots of nitrogen rich trees are a better nutritional source for the fungus, increasing its vigour. Another possibility is that the physical or chemical resistance of the trees is reduced as a result of the nutritional imbalances. High nitrogen availability may reduce the production of phenolic substances and thus lower resistance against attacks by parasites (Buchsbaum *et al.*, 1990; Muzika & Pregitzer, 1992; Balsberg Påhlsson, 1992).

Interactions between excess nitrogen availability and traditional stress factors

It may be clear from the foregoing that excess ammonium availability strongly interferes with traditional stress factors like soil infertility, drought, storm, frost and parasites. When the soil contains few plant available nutrients high ammonium deposition may more quickly result in soil acidification and unfavourable nutrient ratios. Moreover, a poorly developed root system may gather sufficient nutrients in a fertile soil, but it might not if nutrients are scarce. Likewise, a small and inefficient root system, large above-ground plant parts, malfunctioning stomata and a damaged wax layer may not be a too great disadvantage during periods of rainy weather or in case of a high groundwater table. However, during dry periods and on dry soils they may greatly reduce the chance of survival. A high shoot to root ratio is also a serious disadvantage during stormy weather, as the large shoot catches more wind whereas anchorage in the soil is less firm. Diminished frost hardiness will reveal itself only during severe cold. A tree with reduced chemical or physical defence will be more susceptible to infection by parasites. A single weak tree may still be able to escape from parasites when it is surrounded by healthy conspecifics, but it has little chance of survival when the whole stand is weakened. The traditional silvicultural practice to create monospecific stands of even-aged trees enlarges the risks considerably. In all these cases the deathblow is administered by a traditional stress factor. However, the underlying cause of death is the reduced resistance and resilience, brought about by air pollution.

The role of ammonia and ammonium in the forest dieback in The Netherlands

Of all pollutants that impair the vitality of the Dutch forests ammonium (including ammonia) seems to be the most important one. Indicative for this are the high nitrogen levels in the needles of and soils below diseased conifers. What is more, it does not matter whether the reduced vitality appears from needle loss (Roelofs *et al.*, 1985), needle discolourations (chapters 4 and 5) or fungal infections (chapter 6; Van den Burg *et al.*, 1988). Numerous laboratory, greenhouse and field experiments have demonstrated the profound, negative impact of excess ammonium availability on many processes in soil and plant, especially when other nutrients, water or light are not equally abundant (see *e. g.* chapters 2 and 3).

Nitrogen oxides seem to be less important in this respect, because nitrate nitrogen constitutes only 20% of the total nitrogen deposition in forests (Ivens, 1990; Houdijk & Roelofs, 1991). Its contribution to the total potential acid input is approximately 10% (Houdijk & Roelofs, 1991). Unlike ammonium, nitrate does not impair cation uptake (Runge, 1983).

The deposition of sulphur dioxide is approximately as large as that of ammonia and its role in soil acidification is equally important. However, its impact on the nutritional balance of the trees is much smaller. Plants need sulphur in relatively small amounts. It probably never was limiting plant production. Consequently plants have not evolved extremely efficient mechanisms to gather it and seem capable of excluding it.

Although ozone is known to reach periodically high concentrations that are harmful to plants, short-term effects are relatively unimportant. Insufficient data on long-term effects in The Netherlands are available at this moment, but the direct effects of gaseous pollutants on leaves and needles seem less important than first assumed (Heij & Schneider, 1991). Field measurements in Germany indicate that no long-term direct harmful effect from exposure to gaseous pollutants can be detected despite the measured high concentrations (Schulze, 1989). However, nitrogen oxides, sulphur dioxide and ozone constitute additional stress factors that exacerbate the effects of excess ammonium, drought, frost or parasites. Moreover, sulphur dioxide, nitrogen oxides and other acidic or acid-forming substances enhance the deposition of ammonia (Van Hove *et al.*, 1989).

Prospects

There are two possible ways to restore the nutrient balance in Dutch forests. The first option is to lime and fertilize the forest stands. In this way the consequences of acidification can be counteracted and the excess of nitrogen is compensated for by adding all the other essential nutrients in equally large amounts. Moreover, part of the nitrogen is got rid of by increased leaching of nitrate to the groundwater. A big advantage of this approach is, that it is relatively simple and cheap, but the disadvantages are numerous. First of all, not all problems are solved in this way. The shoot to root ratio for instance remains unfavourable, mycorrhizae may not develop and the cuticular wax layer continues to be damaged. Thus, the trees remain vulnerable to water stress and windthrow. The increased growth rate of the trees may have a negative impact on wood quality. Shifts in the species composition of the forest floor vegetation will occur. Slowly growing plant species that are adapted to nutrient poor soils will be replaced by those, which are able to use the ample nutrient availability for rapid biomass production. In that case the Dutch forests will loose even the appearance of a natural ecosystem. Moreover, liming and fertilization will not solve the problem of increased nitrate leaching to the groundwater, which is an important source for the production of drinking-water.

The other possibility is to reduce the atmospheric input of nitrogen into the forests by cutting down nitrogen emissions. This is the harder way, because it requires fundamental changes in agricultural management and involves great financial efforts. Measures should aim at a reduction of the ammonia production (for instance by using nitrogen-poor fodders or by livestock reduction) or at the reuse of ammonia containing materials. Just reducing the emission, by covering storage tanks and injecting animal slurry directly into the soil or ploughing the fields immediately after application, will be beneficial to the forests, it is true, but it will only move the problem to the ground-water, unless the amounts of slurry are reduced considerably. Injection of animal slurry directly into the soil might also have a severe negative impact on soil fauna.

A difficult question to answer is, how much nitrogen inputs into forests should be reduced. As nitrogen is often the factor that limits biomass production in forests on acid soils under pristine conditions (Durzan & Steward, 1983), every nitrogen input, regardless of its size, may be expected to change the ecosystem. Nature and size of these changes depend on input levels, soil and climatical conditions and ecosystem type. Defining a 'critical load', as is attempted by several authors (Nilsson & Grennfelt, 1988; Grennfelt & Thörnelöf, 1992) is only possible after it has been decided which of these ecosystem effects are regarded acceptable. However, if one does not want any changes at all, and that seems the safest way to ensure the survival of stable forest ecosystems with an optimal species diversity, nitrogen in- and outputs should be balanced (Hällgren & Näsholm, 1988; Ivens, 1990).

From measurements in remote areas it appears that the natural input of nitrogen in forests of the boreal and temperate zones amounts to less than 3 $kg.ha^{-1}.yr^{-1}$ (Aber *et al.*, 1989; Edfast *et al.*, 1990). According to Tamm (1991) most forests tend to accumulate nitrogen over the years under natural conditions, but these gains are compensated for by temporary losses due to storms, insect defoliation and, above all, fire. So, in the long run the nitrogen cycle is closed. However, nowadays incidence and scale of fires are checked by man. On the other hand, most present forests in the boreal and temperate zones are used by man for wood production and nitrogen is removed from the ecosystem by harvesting. In case of whole tree harvesting up to 11 kg $N.ha^{-1}.yr^{-1}$ is withdrawn from the system, in case of stemwood harvesting 6 kg (De Vries, 1988). Therefore, to prevent nitrogen accumulation in the forest ecosystem and/or leaching of nitrogen to the groundwater, atmospheric nitrogen inputs should be reduced to 6 to 11 kg $N.ha^{-1}.yr^{-1}$, depending on the method of harvesting. However, the loss of nutrients, other than nitrogen, caused by the harvesting should also be compensated for, either by atmospheric deposition, fertilization or in another way.

To investigate whether forests will recover once the ammonium input has been reduced, a roof was built in a Scots pine stand in the Peel area in The Netherlands which reduced the nitrogen and sulphur depositions onto the forest floor from over 50 to a few kg.ha^{-1}.yr^{-1} (Van Dijk *et al.*, 1992a). Preliminary results reveal that the ammonium and sulphate concentrations in the soil solution respond quickly to the reduced input levels. The nitrogen levels in the needles of the trees have not decreased sofar. This may be explained by the short duration of the treatment, canopy uptake, which was not prevented, or a large nitrogen stock within the trees and the soil. However, recent data indicate a slight, but significant improvement in the balance between nitrogen and other nutrients (Van Dijk *et al.*, 1992b). Nevertheless, it is very well possible that reduction of the nitrogen input alone will not be enough to restore the vitality of the trees. Additional measures may be necessary to undo acidification or to compensate for the loss of base cations caused by leaching or harvesting (cf. Den Hartog, 1993). Liming and or fertilization should, however, never increase nutrient availability beyond the levels that prevailed before air pollutants started to exert their detrimental effects and they should always be accompanied by efforts to reduce air pollution. In the long run, that is the only solution.

SAMENVATTING

Aan het einde van de jaren zeventig en in het begin van de jaren tachtig kwamen er berichten over massale bossterfte uit Duitsland. Daarop werd in Nederland een onderzoek ingesteld naar de gezondheidstoestand van het bos. Het bleek dat ook hier de vitaliteit van het bos sterk verminderd was. Hetzelfde gold voor andere landen in Europa en Noord-Amerika. Jaarlijkse inventarisaties van de gezondheidstoestand wijzen uit dat de situatie sindsdien niet verbeterd is. Over de oorzaken van de slechte vitaliteit wordt druk gespeculeerd. Hoewel ongunstige klimatologische omstandigheden, parasieten en verkeerd beheer door sommige deskundigen als schuldigen worden aangewezen, zijn de meeste onderzoekers het er tegenwoordig over eens dat luchtverontreinigende stoffen de belangrijkste boosdoeners zijn. Vooral aan de effecten van zwaveldioxyde, stikstofoxyden en ozon wordt veel aandacht besteed. De mogelijke rol van ammoniak in de hele bossterfteproblematiek is lange tijd onderschat. Daarin kwam verandering in 1982. Toen bleek namelijk dat ammonium en sulfaat tegenwoordig de meest voorkomende stoffen zijn in regenwater dat van boomkronen afdruipt. Het ammonium ontstaat uit ammoniakgas dat vrijkomt uit stallen en op het land uitgesproeide drijfmest. Het zwaveldioxyde wordt vooral door energiecentrales en de industrie geproduceerd. De stoffen zetten zich af op de boomkronen en spoelen met het regenwater naar beneden; dit leidt tot verzuring van de bosbodem. Enkele jaren later werd ontdekt dat de overmatige stikstofaanvoer ook een eutrofiëring van de bosbodem tot gevolg heeft, waardoor de mineralenbalans van de bomen verstoord wordt en de vitaliteit van het bos vermindert. Om meer informatie te krijgen omtrent de juistheid van deze theorie, werd het onderhavige onderzoek uitgevoerd.

Jonge grove dennen, Corsicaanse dennen en Douglas sparren werden in potten geplant en in een kas beregend met kunstmatig regenwater met daarin opgelost 0, 500 of 5000 µmol ammonium (als sulfaat) per liter (Hoofdstuk 2). Dit komt overeen met een depositie van resp. 0, 48 en 480 kg $N.ha^{-1}.j^{-1}$. De middelste waarde kan beschouwd worden als een minimale waarde voor de depositie in het Nederlandse bos. De laatste waarde wordt alleen aangetroffen in de onmiddellijke nabijheid van ammoniakbronnen. Bij het experiment werden twee bodemtypen gebruikt: de ene helft van de bomen werd geplant in een zure podzolbodem, de andere helft in een rivierduinvaaggrond. De gevolgen van de behandeling met ammoniumsulfaat-houdend regenwater voor de samenstelling van het bodemvocht bleken groot te zijn: de depositie van ammoniumsulfaat leidde tot een sterke verzuring van het bodemvocht door nitrificatie en ammoniumopname door de bomen. Ammonium hoopte zich op in de bodemoplossing, zodra de opname door de plant en de nitrificatie minder snel verliepen dan de ammoniumaanvoer. Dit was vooral het geval bij het hoogste depositieniveau en in de podzolbodem, waar de nitrificatie veel langzamer verliep dan in de rivierduinvaaggrond. Belangrijke voedingsstoffen als kalium, magnesium en calcium lekten uit de bodems weg en er ontstond een onbalans tussen ammonium en deze voedingsstoffen. Het is bekend dat dit hun opname door de plant bemoeilijkt. Toxische metalen, zoals aluminium, mangaan en zink, vertoonden een verhoogde oplosbaarheid.

De veranderingen in de bodemchemische samenstelling waren van grote invloed op de ontwikkeling van de boompjes (Hoofdstuk 3). Na zeven maanden beregenen bleken de wortelstelsels van de met de hoogste ammoniumconcentratie behandelde bomen kleiner te zijn dan die van de bomen in de andere behandelingen. Dit kwam voornamelijk door een verlies aan fijne wortels. Ook de vorming van mycorrhiza's was negatief beïnvloed, evenals het aantal vruchtlichamen dat de mycorrhiza-schimmels vormden. De scheutontwikkeling daarentegen bleek gestimuleerd te worden door het toedienen van ammoniumsulfaat. Dit betekende dat een kleiner en inefficiënter wortelstelsel een grotere, meer-eisende scheut van water en voedingsstoffen moest voorzien. In de vrije natuur vergroten zulke ontwikkelingen de kans op omwaaien, verdroging en een tekort aan voedingsstoffen. Inderdaad waren de gehalten aan kalium, calcium en magnesium veel lager in de naalden van de met 480 kg $N.ha^{-1}.j^{-1}$ behandelde boompjes dan in de naalden van de andere boompjes. Het stikstofgehalte was echter veel hoger. Veel van deze stikstof werd opgeslagen als vrij arginine. Binnen een jaar na het begin van de behandeling stierven de meeste van deze bomen, vooral op de podzolbodem. De bomen die met 48 kg $N.ha^{-1}.j^{-1}$ behandeld waren, vertoonden geen tekenen van een verminderde vitaliteit. Waarschijnlijk was dat het gevolg van de relatief grote veerkracht van jonge bomen en de korte duur van de behandeling.

Bij grove dennen in het veld werd enkele jaren geleden een massaal optreden van geelverkleuring van de jongste naalden vastgesteld (Hoofdstukken 4 en 5). Onderzoek wees uit dat deze geelverkleuring het gevolg was van lagere gehalten aan bladpigmenten. De verkleurde naalden bleken ook minder ijzer te bevatten. Besproeiing van de verkleurde naalden met een Fe(III)EDTA-oplossing resulteerde binnen enkele weken in het groener worden van de naalden, wat bewijst dat de geelverkleuring het gevolg was van ijzergebrek. De lagere gehalten in de naalden duiden erop, dat dit gebrek niet veroorzaakt werd door immobilisatie van ijzer in de naalden, maar door een geremde opname of een verstoord transport van de wortels naar de naalden. Wat de oorzaak van de verstoorde ijzerhuishouding was, blijft vooralsnog onduidelijk. Weliswaar was de extraheerbaarheid van ijzer wat kleiner in de bodem van aangetaste opstanden dan in de bodem van gezonde bossen, maar de verschillen waren niet altijd even duidelijk.

Opmerkelijk is de hoge stikstofbeschikbaarheid in de aangetaste opstanden. De naalden van de geelverkleurde bomen bleken meer stikstof te bevatten dan de naalden van hun groene soortgenoten. Veel van de extra stikstof bleek opgeslagen te worden in vrije aminozuren, waarvan arginine kwantitatief verreweg het belangrijkste was. In de bodem van de verkleurde opstanden kwam meer extraheerbaar ammonium voor. De stikstofbeschikbaarheid heeft grote invloed op de bodem-pH, de microörganismen in de rhizosfeer en het metabolisme van de bomen. Al deze factoren, alleen of in combinatie, kunnen ijzergebrek veroorzaken. Het is echter niet uitgesloten dat andere factoren, zoals de hoge fosforniveaus in de bodem van de zieke bossen of zware metalen, zoals cadmium, een rol speelden.

In 1985 heerste er een epidemie van de parasitaire schimmel *Sphaeropsis sapinea* in de Nederlandse dennenbossen. Om te onderzoeken of er een verband bestond tussen de overmatige beschikbaarheid van stikstof en het optreden van schade door de schimmel werden in het voorjaar van 1986 naald- en bodemmonsters verzameld in 29 opstanden van de Corsicaanse den en vervolgens geanalyseerd. De resultaten staan in Hoofdstuk 6. De aangetaste opstanden bleken hogere gehalten aan stikstof in de naalden en meer extraheerbaar ammonium in de minerale bodem te bevatten. Ook fosfor was in grotere hoeveelheden aanwezig in naalden en bodem. De extra stikstof, die aanwezig was in de naalden van geïnfecteerde bomen, bleek opgeslagen te worden in vrije aminozuren. Vooral de niveaus van de stikstofrijke aminozuren asparagine, glutamine, histidine, lysine en arginine waren sterk verhoogd. Van alle aminozuren bereikte arginine verreweg de hoogste concentratie, zowel in de zieke als in de gezonde bomen. Proline, een aminozuur dat wordt opgehoopt onder stress-omstandigheden, vooral bij watertekort, vertoonde ook een sterk verhoogde concentratie in zieke bomen. Wat het verband was tussen de hoge beschikbaarheid van stikstof en fosfor en de schimmelinfectie is vooralsnog onduidelijk. Mogelijk werd de schimmel gestimuleerd door het grotere voedselaanbod. Waarschijnlijker is echter dat de fysische en/of chemische weerstand van de bomen verminderd was door de onbalans in voedingsstoffen. Het is bekend dat een hoog stikstofaanbod de aanmaak van fenolische verbindingen door de plant vermindert. Deze verbindingen spelen een grote rol bij de afweer van parasieten. Infectieproeven onder gecontroleerde omstandigheden zullen het definitieve bewijs moeten leveren.

In de algemene discussie (Hoofdstuk 7) worden de nadelige gevolgen van een overmatige stikstoftoevoer samengevat en wordt gewezen op de mogelijke rol van arginine als indicator voor een door stikstof geïnduceerde onbalans in de minerale voeding van coniferen. De wisselwerking tussen overbelasting met stikstof en traditionele stressfactoren, zoals droogte, vorst, storm, parasieten en onvruchtbaarheid van de bodem, wordt benadrukt. Belasting met een overmaat aan stikstof wordt aangewezen als de belangrijkste oorzaak van de vitaliteitsvermindering van het Nederlandse bos. Andere luchtverPvuilende stoffen vormen een extra belasting. Er worden twee manieren aangegegeven, waarop de nutriëntenbalans van het bos hersteld kan worden: 1. in overmaat toedienen van de andere voedingsstoffen door middel van bemesting en bekalking; 2. verminderen van de stikstofdepositie in het bos op een zodanige manier, dat de aan- en afvoer weer in evenwicht met elkaar zijn. Hoewel de tweede manier de moeilijkste is, is het de enige manier om het voortbestaan van het bos als stabiel en soortenrijk ecosysteem op lange termijn te garanderen.

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CURRICULUM VITAE

Harrie van Dijk werd geboren op 15 oktober 1957 te Venlo. Na het behalen van het diploma Gymnasium β aan het St Thomascollege te Venlo, ging hij biologie studeren aan de Katholieke Universiteit te Nijmegen. In het kader van zijn hoofdvakstage was hij werkzaam op de afdeling Geobotanie van prof. dr. Westhoff, waar hij onderzoek verrichtte aan de rijke, maar bedreigde flora van de stroomdalgraslanden langs de Maas in Noord-Limburg. Dit werk leverde verschillende publicaties op in vakbladen. Daarnaast liep hij bijvakstages op de afdelingen Microbiologie (onderzoek aan methaanvergisting) en Bodemkunde (onderzoek aan graslandbodems) en haalde hij zijn onderwijsbevoegdheid.

Na zijn afstuderen (cum laude) in december van 1982 was hij enige maanden werkzaam als leraar biologie aan het Gertrudislyceum te Roosendaal, waar hij een tweetal zieke docenten verving.

In het najaar van 1983 ging hij als vrijwilliger werken bij de afdeling Aquatische Oecologie van de Katholieke Universiteit te Nijmegen, waar hij zich ging bezighouden met onderzoek naar de oorzaken van bossterfte. In deze tijd volgde hij tevens de cursussen "Popularisering van de Natuurwetenschappen" o.l.v. wetenschapsjournalist dr. J. Willems (K.U. Nijmegen) en "Onderzoeksmanagement" van het NIBI.

Sinds oktober 1984 is hij officieel in dienst van de Katholieke Universiteit te Nijmegen, en wel op project-basis. In opdracht van het ministerie van VROM bestudeert hij de effecten van de overmatige, atmosferische stikstofaanvoer (het betreft voornamelijk ammoniak) op bossen. De resultaten van dit onderzoek zijn voor een belangrijk deel verwerkt in het onderhavige proefschrift.

Vanaf 1988 richt zijn aandacht zich vooral op de herstelmogelijkheden van bossen onder invloed van een vermindering van de atmosferische stikstofaanvoer. Dit onderzoek, dat onder de naam NITREX wordt uitgevoerd in samenwerking met verschillende Nederlandse en buitenlandse onderzoeksinstellingen en dat mede door de EEG gefinancierd wordt, heeft veel aandacht getrokken van de pers en de auteur heeft dan ook talloze interviews gegeven voor dagbladen, radio en televisie. Door zijn werk aan de stikstofproblematiek komt hij veel in aanraking met gemeenten en landbouworganisaties. Op verzoek van diverse gemeenten, die bij het opstellen van bestemmingsplannen en milieubeleidsplannen in aanvaring kwamen met agrariërs, heeft hij lezingen gehouden om deze mensen bekend te maken met de gevolgen van een grote ammoniakuitstoot voor bossen. Tevens heeft hij meegewerkt aan de voorlichtingsvideoband 'Melkveehouderij en milieu in balans' van het Centrum Landbouw en Milieu.

Harrie is in 1990 getrouwd met Mia Wijers. Hun zoon Mark werd geboren in juni 1992.