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Effects of living crown reduction on needle element status of Scots pine

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Academic dissertation

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ABSTRACT

A wide range of biotic and abiotic factors, operating over different time perspectives and intensities, cause defoliation and a rapid decrease in the crown size of trees. Scleroderris canker disease [*Gremmeniella abietina* (Lagerb.) Morelet] has caused widespread crown reduction and tree mortality in Scots pine in forests in Scandinavia during the last three decades. In the 1980's, attempts were made to show, on the basis of the higher foliar N and S concentrations of affected pines in the diseased area, that sulphur and nitrogen deposition predispose trees to *G. abietina*. Unfortunately, in many studies on defoliated trees, exceptionally high or low needle mineral nutrient concentrations are still often interpreted as one of the causes of tree injury and not, conversely, as the result. In this thesis, three different field experiments, with foliar analysis as the main study method, were conducted in order to asses the possible long-term effects of living crown reduction on the needle nutrient concentrations of Scots pine trees in southern Finland. The crown ratio and length of the living crown were used to estimate the amount of defoliation in the reduced canopies. The material for the partial studies was collected and a total of 968 foliar samples were analysed individually (15-17 elements/sample) on a total of 488 sample trees (140 diseased, 116 pruned and 232 control trees) during the years 1987-1996 in 13 Scots pine stands.

All the three experiments of this thesis provided significant evidence that severe disease-induced defoliation or artificial pruning of the living branches can induce long-lasting nutritional changes in the foliage of the recovering trees under the typical growing conditions for Scots pine.The foliar concentrations of all the 17 mineral nutrients/elements analysed were affected, to a varying degree, by artificial pruning during the following three years. Although Scots pine, as an evergreen conifer, is considered to have low induced chemical responses to defoliation, this study proved experimentally under natural forest conditions that severe artificial pruning or disease-induced defoliation of Scots pine trees may induce biologically significant changes in the concentrations of most of the important macro- and micronutrients, as well as of carbon, in refoliated needles.

Keywords: Foliar analysis, defoliation, needle loss, pruning, nutrients, *Pinus sylvestris*, *Gremmeniella abietina*

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Helsinki, April 2008

Heikki Nuorteva

LIST OF ORIGINAL ARTICLES

The thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I Nuorteva, H. & Kurkela, T. 1993. Effects of crown reduction on needle nutrient status of scleroderris-canker-diseased and green-pruned Scots pine. Canadian Journal of Forest Research 23: 1169-1178.

II Nuorteva, H., Kurkela, T. & Lehto, A. 1998. Rapid living crown reduction caused by *Gremmeniella abietina* affects foliar nutrient concentrations of Scots pine. European Journal of Forest Pathology 28: 349-360.

III Nuorteva, H. 2002. Increased boron concentrations of Scots pine foliage induced by green pruning. Canadian Journal of Forest Research 32: 1434-1440.

IV Nuorteva, H. Artificial pruning induces long-term changes in element concentrations of Scots pine needles. Manuscript.

AUTHOR'S CONTRIBUTION IN ARTICLES I AND II

I Heikki Nuorteva planned the experiment together with the co-author. H. Nuorteva performed the experimental and laboratory work and was responsible for the data analysis and preparing the first draft of the manuscript.

II Heikki Nuorteva planned the experiment together with the co-authors. H. Nuorteva performed the experimental and laboratory work, and was responsible for the data analysis and preparing the first draft of the manuscript.

CONTENTS

1 INTRODUCTION

A living healthy crown is the basis for the whole photosynthetic energy production of trees. Without the carbon assimilation ability of the chlorophyll in the foliage of the crown or in the other areas of the trees, e.g. chlorophyll underneath the bark (see e.g. Pfanz et al. 2002, Berveiller et al. 2007), there would be no energy for binding carbon in the growth processes and to keep the tree alive. Different tree species have a wide range of canopy structures. One parameter used in ecological modelling to estimate the crown size of evergreen conifers is the living crown ratio (see e.g. Hynynen 1995, Vanninen and Mäkelä 2000, Medhurst and Beadle 2001, Grazer et al. 2004, Lehtonen 2005, Mäkelä and Valentine 2006). The crown ratio, expressed as the percentual proportion of the living green crown in relation to the total height of a tree, is easy to measure. In small seedlings and saplings the photosynthetic foliage starts right from the tree base, and the living crown length might be up to 100% of the tree length. In the case of many conifer genera, e.g. *Picea*, *Abies*, *Thuja*, *Tsuga* and *Juniperus* the crown ratio of the trees can easily be over 90%, even in mature trees. This is also valid for the species of *Pinus* spp, especially in the case of open-grown trees.

In the 1980's the media and the scientific world were very concerned about the state and health of global forests. The effects of air pollution, i.e. forest death, defoliation/needle loss of trees, the impact of acidic deposition on the soil, water and vegetation, and the "new-type" of forest decline, were widely mentioned both in the news headlines and in the titles of scientific papers. Extensive monitoring of forest condition and major environmental projects were started in many European countries in the middle of the 1980's, including Finland (Mathy 1988, Roberts et al. 1989, Hanisch and Kilz 1990, Kauppi et al. 1990, Merilä et al. 2007). In the early 1980's in Sweden and Finland, a fungal disease, Scleroderris canker [*Gremmeniella abietina* (Lagerb.) Morelet], spread rapidly and caused a considerable amount of living crown reduction and tree mortality in Scandinavian Scots pine (*Pinus sylvestris* L.) and lodgepole pine (*Pinus* contorta Dougl. Ex Loud.) forests (Kurkela 1981, Karlman 1984, Karlman et al. 1994, Kaitera and Jalkanen 1995, Hansson 1996). The disese epidemic was so large and severe in Finland that tree defoliation and mortality was clearly visible in the countrywide 8th National Forest Inventory data and vitality surveys of Scots pine (Nevalainen and Yli-Kojola 1990, Jukola-Sulonen et al. 1990). The brown colour of the diseased pines was even visible in the satellite images taken two decades ago (Häme 1991).

An extensive area of pine forest affected by *Gremmeniella abietina* was reported in eastern Lapland at the end of the 1980’s (Kaitera and Jalkanen 1994). The epidemic was explained in some other quarters as nutritional disturbances caused by air pollution (especially sulphur emissions) from copper-nickel smelters on the Kola Peninsula, NW Russia (e.g. Tikkanen 1991). Attempts were made to show, on the basis of the higher foliar N and S concentrations of affected pines in the diseased area, that sulphur and nitrogen deposition predispose trees to G. abietina. Even though no correlation was found between acidic deposition or other pollutants and the occurrence of *Gremmeniella abietina* in Europe or in USA (e.g. Donaubauer 1984, Godbold and Hüttermann 1994), the Finnish media frequently claimed that the connection between air pollution and the incidence of the *Gremmeniella* disease was "an established fact" in the late 1980's and early 1990's. Later on the 1990's, the scientific and media debate about the hypothesised connection gradually settled down, as the disease epidemic in Scandinavia slowly abated. In many studies

concentrating on *Gremmeniella abietina* (e.g. the following dissertations alone: Karlman 1984, Barklund 1989, Ylimartimo 1993, Hellgren 1995, Ranta 1995, Hansson 1996, Petäistö 1996, Kaitera 1997), no clear evidence was found to link pollution effects with the susceptibility or disease incidence of pine forests. The disease itself, however, has not disappeared from the forests of Scandinavia. In Sweden, for example, the most serious outbreak of *Gremmeniella abietina* so far, has been estimated to have caused damage over an area of 484 000 ha of pine forests during the years 2001-2003 (Wulff et al. 2006). In Finland, symptoms of *G. abietina* were found in the years 2004-2005 in 215 400 ha of forest, of which 205 100 ha were dominated by Scots pine (Korhonen and Nevalainen 2007). All the above studies clearly demonstrate that, even when the threat of air pollution effects on forest health has strongly diminished, the number of Scots pine trees with severely reduced living crowns due to *G. abietina* has not decreased.

Foliar analysis is a widely used method for evaluating nutritional and environmental status of trees, although with some reservations (Morrison 1974, Ballard and Carter 1986, Rautio 2000, Brockley 2001, Luyssaert et al. 2004, Mertens et al. 2005). Analysis of the elemental composition of foliage can provide additional information as a biomonitoring method in special cases (Manning & Feder 1980, Oliva and Mingorance 2006). In a few studies, however, higher or lower needle nutrient concentrations have been reported in defoliated trees following the loss of needles through artificial or herbivory-induced defoliation (e.g. Oksbjerg 1962, Piene 1980, Långström et al. 1990). According to Piene and Percy (1984), increased nitrogen concentrations continued for three years in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) foliage recovering from serious defoliation by spruce budworm (*Choristoneura fumiferana* (Clem.)). In defoliation studies on trees, based on foliar analysis, the results have been very heterogeneous as regards general trends in the changes in mineral nutrient concentrations in the recovering canopies of the trees (Tuomi et al. 1988, Nuorteva 1995). On the whole, there is clearly insufficient information on this phenomenon and its causal effects, particularly when interpreting the results of foliar analyses or nutritional recovery abilities of severely defoliated Scots pine stands. As foliar element analysis is a widely used tool to describe, indicate and predict the growth, nutritional, environmental and health status (Luyssaert et al. 2002) of trees, including crown condition and defoliation monitoring (Lindgren et al. 2000, Merilä et al. 2007), I considered it important to investigate the possible interactions between rapid living crown reduction and the foliar element status of the trees.

In this thesis, three different field experiments, with foliar analysis as the main study method, were conducted in order to asses the possible long-term effects of living crown reduction on the needle nutrient concentrations of Scots pine trees in southern Finland. The crown ratio and length of the living crown were used to estimate the amount of defoliation in the reduced canopies.

2 RESEARCH AIMS

The overall objective of this study was to investigate the long-term consequences in the foliar element concentrations of Scots pine trees after living crown reduction due to disease or to artificial pruning.

The main objectives of the present study were, in chronological order, as follows:

1) The purpose of the first experiment (I) was to determine in forest conditions the long-term effects of green crown reduction on the nutrient status of Scots pine needles *a)* 5-10 years after foliage loss due to *Gremmeniella abietina*, and *b)* 1-2 years after a reduction of the living crown by green pruning. Pruned trees provided an alternative mechanical form of defoliation for comparative study with the diseased trees. In both cases the loss of foliage was rapidly estimated from the crown ratio. The main objective was to clarify whether the anomalous needle nutrient concentrations of diseased or pruned trees differs significantly from the foliar nutrient concentrations of the control trees and if so, are the possible differences large enough to be detectable by foliar analysis. In all the diseased and pruned stands, the dead needles and other litter/pruned branches had been left to decompose underneath the defoliated trees.

2) The aim of the second experiment (II) was to estimate in forest conditions, the short-term qualitative and quantitative changes in foliar nutrient concentrations in Scots pine, one growing season after *G. abietina*-induced living crown reduction. The dead needles were mainly still attached to dead branches in the trees.

3) The main aim of the third experiment (III and IV) was *a)* to prove experimentally that a rapid and sufficiently large living crown reduction of Scots pine is the primary cause of increased or decreased mineral nutrient concentrations in the new needles that develop after severe defoliation. Additional aims were *b)* to study the longevity (during 3 consecutive years after pruning) of the pruning-induced responses, *c)* in different sized trees, and *d)* to determine approximately the minimum reduction in the living crown, resulting from pruning, that would be sufficient to induce a significant increase or decrease in foliar mineral nutrient concentrations. All the pruned branches and needles were immediately removed from the study area.

3 MATERIAL AND METHODS

The material for the partial studies was collected and the foliar samples analysed individually (15-17 elements/sample) on a total of 488 sample trees (140 diseased, 116 pruned and 232 control trees) during the years 1987-1996 in 13 Scots pine stands as follows (a total of 968 foliar samples analysed):

3.1. Experimental stands and the living crown reduction of the sample trees

All the experimental stands were located in southern Finland. Six of the 8 Scots pine stands suffering from *Gremmeniella abietina* (three in I and another three in II) were growing on mineral soil sites, classified as *Vaccinium* forest site type (see Cajander 1926, 1949), and 2 diseased stands on drained peatlands (one in I and another in II), the original site type of which was classified as a Cotton grass pine mire (see Heikurainen and Pakarinen 1982). One of the pruned stands in (I) and all the three pruned stands in (III and IV) were also growing on sites of the *Vaccinium* type. All of the 12 sites were typical, relatively infertile, habitats for pine. The site of only one pruning stand (I) was classified as the *Myrtillus* type (see Cajander 1926, 1949), which is slightly more fertile.

The age of the diseased stands was 25-30 years in (I) and 13-22 years in (II), and the mean height 6-12 m in (I) and 3-6 m in (II). The age of the pruned stands in (I) was 15-20 years and mean height 7-8 m. In the third experiment (III and IV), the trees were 2-8 m in the beginning of the study and 3-10 m three years later at the time of the last needle sampling. The age of the stands in III and IV was 10, 15 and 20 years.

The material for experiment (I) was collected in winter 1986-1987 from four Scots pine stands, that had suffered annually from *Gremmeniella abietina* during the last 10 years. The worst epidemic, which also caused the greatest crown reduction, occurred 5 years before the collection of needle samples. Two healthy stands, in which part of the trees had been green pruned 1-2 years before, were chosen for comparison.

The material for the second experiment (II) was collected in winter 1988-1989 from four Scots pine stands in which *G. abietina* had caused severe living crown reduction during the previous spring and summer (1988). Only minor visual symptoms of *G. abietina* infection were observed in the stands before the exceptionally cold and rainy summer of 1987, when most of the trees became seriously infected. Severe needle and shoot dieback occurred in the diseased trees in the spring and summer of 1988. The affected trees were easily recognizable on the basis of their reddish-brown needles and abundant pycnidia of *G. abietina* on the dead shoots and unflushed buds. The presence of G. abietina was also checked by isolating the fungus from dead shoots. The majority of the needles killed by *G. abietina* during the previous summer were still attached on the branches at the time of needle sampling in March 1989.

Selection of the sample trees in both (I) and (II) was based on the crown ratio, using a pairwise sampling method. Twenty sample trees per stand in (I) and 40 sample trees in (II) were selected in pairs. In the diseased (II and II) or pruned (I) sample trees, the living crown reduction (>=50%) was concentrated in the lower part of the crown. A healthy looking, phenologically similar tree growing at a distance of less than 10 m from a diseased or pruned tree was chosen as a control tree in order to minimize the environmental variation between the paired trees. The diseased trees in (I) were slightly shorter than the control

trees but there was no significant difference between the height of the pruned and control trees in (I), or the height of the diseased and control trees in (II). All tree pairs per stand were chosen within a 0.5-3 ha area, and the average distance between the diseased and control tree was, on the average, 5 m (I and II), and 3 m between the pruned and control tree (I).

In the third experiment (III and IV) a total of 168 sample trees were carefully selected to be phenologically as similar as possible from three artificially regenerated Scots pine stands in southern Finland (48, 60 and 60 trees in Stands A, B and C, respectively) in winter 1992-1993. The sample trees were growing within a 1-2 ha area in each of the stands. All the selected trees were completely randomized into three different pruning treatment classes (on average 23%, 42% or 72% of the living crown length) and two control tree classes with untouched green living crowns: class I, in which trees were left totally unpruned and class II, in which the trees were dry pruned (only the dead branch whorls were removed). Half of the 72 control trees were unpruned, and the other half had the dead branch whorls removed. Removing the dead branch whorls had no effect on any of the physiological parameters measured. Reduction of the living crown of the sample trees in was carried out by manually pruning the lowest branch whorls. The trees were pruned with a pruning saw (large branches) and secateurs (small branches) between 29 March and 14 May 1993, before the height and diameter growth of Scots pine had begun. At the beginning of the experiment (before pruning), there were no statistically significant differences in the phenological characteristics of the trees between the pruning treatment classes and the control classes. For a more detailed description of the experimental stands, sample trees, reduction of the living crown by pruning and foliar sampling, see (III).

3.2. Foliar sampling, and chemical and data analyses

For mineral nutrient and element analyses of the needles, one or two green and visibly healthy, southerny orientated lateral top shoots of each sample tree were collected with a telescopic cutter. The foliar samples were collected during the late winter dormancy of the trees. In (I) March 1987 (23-31.3.1987), in (II) 1989 (13-17.3.1989), and in (III and IV) 1993-1996 (11-13.3.1993, 7-9.3.1994, 6-9.3.1995, 12-15.3.1996), the forest floor in the sampling areas was covered with snow and the monthly mean temperature was below 0°C (Finnish Meteorological Institute 1987-1996). Sampling for foliar analyses during the dormant period has for long been a common and recommended method in Finland and the rest of Scandinavia (Veijalainen 1977, Andersson et al. 1998, ICP Forest manual 2000). The foliar starch concentrations are also usually at their lowest level at this time (Adams et al. 1986, Fischer and Höll 1991, Anttonen and Kärenlampi 1995). The sample shoots, which contained only needles formed in the previous summer, were stored at -18°C prior to the analysis.

In (I) and (II) the sample shoots were dried for 48 h at 60 °C. The needles were then removed from the shoots, homogenised with a Wiley mill to pass a 2 mm screen (I) or milled to pass through a 20-mesh screen (II). The needle powder was stored in sealed plastic bags. Dry weight determination (105 °C) and ashing (550 °C) of the samples were carried out in special ovens (I) or in a LECO TGA-500 analyser (II, III and IV).The total concentrations of 15 (I and II) or 17 elements (III and IV) were determined on the unwashed needles of each individual sample tree. Nitrogen (N), carbon (C) and hydrogen (H) were analysed on a LECO CHN-600 (Leco Corp., St. Joseph, MI) analyser (I, II, IV) and sulphur (S) on a LECO SC-132 analyser (I, II) or on a TJA Iris Advantage ICP-atomic

emission spectrometer (ICP-AES) (IV). Phosphorus (P) was determined from a hydrochloric acid extract of the dry-ashed samples (Halonen et al. 1983) and boron (B) from a nitric acid extract of the ash by ICP-AES (ARL ICP 3580) (I and II).

In (III and IV) the frozen unwashed needles were separated from the shoots with tweezers, dried for 48 h at 60°C, and milled (Retsch 2M1) to pass through a screen with a mesh size of 0.75 mm. The B concentrations were determined spectrophotometrically (Shimadzu UV-240) by the azomethine H method (Halonen et al. 1983). P, K, Ca, Mg, Mn, Fe, Zn, Cu, Na, Al, nickel (Ni) and cadmium (Cd) were analysed following wet digestion in a microwave (0.5 gDW of milled needles digested in 50 ml HNO_3/H_2O_2), on a TJA Iris Advantage ICP-AES. The results in (I-IV) were calculated on the basis of the dry weight (+105° C) of the needles.

In (I) and (II) the significance of differences between diseased or pruned and healthy (control) trees in the tree pairs was tested by the Student's t-test for paired comparisons. The results were calculated *a)* within the individual stands, and *b)* by combining all the trees in the stands by crown classes (diseased, pruned or control) within the experiments (I and II). Relationships between foliar element concentrations and the crown ratio were tested using correlation analysis (Pearson). Only significant differences ($p<0.05$) are presented in the results. All the calculations were carried out using SAS statistical software (SAS Institute Inc. 1992, 1995).

In (III and IV) the mean values for the sample trees in different pruning classes and stands were compared using ANOVA and Tukey's comparisons. The relationships between foliar concentrations of B, LCRP (= living crown reduction by pruning, % of the initial crown ratio) and crown ratio were tested using Pearson's linear correlation analysis (III). For the final statistical analyses, i.e. in (IV), the pruning Classes I (=unpruned trees) and II (dry-pruned trees) were combined to form one large group of control trees. This decision was based on the statistical analyses of all the elements, pruning classes and stands, which showed that pruning of the dead branch whorls did not affect the concentrations of any of the foliar elements analysed in this study or in (III). All of the analyses in (III) were performed using a Systat 6.0 for Windows software package (SPSS Inc., Chicago, U.S.A.), and in (IV) using SPSS 15.0 for Windows.

4 RESULTS

The following is a summary of the main results in (I-IV):

(I) Needle element status of Scots pine trees 5-10 years after severe living crown reduction due to *Gremmeniella* or 1-2 years after green pruning

In the first experiment (I) the concentration of almost all the foliar elements (11 of the 15 elements analysed) was significantly affected ($p<0.05$) by living crown reduction (Figures 1 and 4). Most of the nutrient concentrations were higher in the diseased or pruned trees. This was evident especially when the results were calculated for all the sample trees of the stands by combined crown class (diseased, pruned or control). The B concentrations increased in the foliage of diseased or pruned trees in every single stand. Foliar B concentrations in the diseased trees were almost 60 % higher, and in the pruned trees more than 90 % higher, than in the adjacent control trees. The concentrations of foliar N, Ca, S were also higher, in both in the diseased and the pruned trees. In addition, foliar Mn increased in the diseased trees and Cu and Na in the pruned trees. In contrast, the foliar concentrations of Fe and Mg in the diseased trees were lower than those in the control trees, and C (and Mg in stand P1 on the *Vaccinium* site) in the pruned trees. Linear correlation was found between the crown ratio and the concentration of several elements (I).

(II) Needle element status of Scots pine trees less than 1 year after needle loss

In the second experiment (II), diseased trees had different foliar concentrations than the adjacent control trees in case of 10 elements. Foliar B concentrations were increased in every single stand, as were the Mg concentrations, on the contrary, decresed in every stand. The foliar Mn and Na concentrations were also higher than in the control trees in the diseased trees, as were Al in the peatland stand S4 (Figures 2 and 4). On the other hand, most of the other foliar nutrients (N,K, Mg, S, Fe, Cu and Zn) were lower in the diseased trees, when the results were calculated by combining all the sample trees of the four stands together by crown classes (diseased or control).

(III) Needle boron status of Scots pine trees before and 3 consecutive years after the pruning treatments

Before the reduction of the living crown by pruning (LCRP), there were no significant differences in foliar B between the pruning classes in any of the stands. One year after pruning, the pruned trees of Class V (LCRP 72%) in every stand and Class IV (LCRP 42%) in Stand C had significantly higher foliar B concentrations than the unpruned trees and dry-pruned control trees (Classes I and II, Figure 3). The B concentrations increased after pruning in the smaller trees (stands A and B) and were on the average, 40-50% higher than in the control trees and in the larger trees (Stand C) even as much as almost 180% higher. Pruning of all the dead branch whorls (Class II) or from only the few lowest whorls (Class III), did not affect the boron nutrition of the trees.

The larger the LCRP and the sample trees, the greater was the increase in the foliar B concentrations. In the stand with the largest trees (Stand C, mean height ca. 8 m) a reduction of about 50% in the living crown by pruning was enough to increase the foliar B concentrations for at least the next three years. In the stands with smaller trees (Stands A and B, mean height ca. 2 and 4 m, respectively) more intense pruning was needed to induce significant increases in foliar B. In every experimental stand there was also a clear linear correlation between the crown ratio and the foliar B concentrations three years after pruning.

(IV) Needle element status of Scots pine trees before and in 3 consecutive years after the pruning treatments

At the beginning of the experiment (before pruning), there were no statistically significant differences between the trees in the pruning treatment classes and the control trees, and not in needle chemistry or in the phenological characteristics of the trees. Severe pruning of the living branchwhorls induced significant differences (+ or -) at the stand or pruning-class levels in all of the 16 foliar element concentrations analysed (Figures 3 and 4); N (+/-), P (+/-), K (+/-), Ca (+), Mg (-) S (+/-), Fe (-), Mn (+), Zn (+/-), Na (+), Ni (-), C (-), H (-). Al (+), Cu (-) and Cd (+) tended to increase or decrease. The increase or decrease was the strongest and the most long-lasting in those trees with a 72 % reduction of the living crown length; three years after pruning, the concentrations of 11 elements in the current-year needles were still statistically significantly (ANOVA and Tukey HSD, $p<0.05$) changed in case of 11 nutrients as compared to the foliar concentrations of the control trees. The 23% or 42% pruning treatments had only minor effects on a number of elements.

In Stand A, 75% pruning induced a significant increase in the concentrations of foliar N^{1} (=1 year after), P^{13} (=1 and 3 years after), K^{1}, Ca^{123} (=1, 2 and 3 years after), S^{1}, Zn^{1}, Mn^{123}, Na^{1}, Cd^{13}, and a decrease in foliar Mg_{3}, Fe_{23}, Zn_{2}, Ni_{3}, C_{1} and H_{1}. The 35% pruning treatment had no effect on any of the foliar elements analysed in this study.

In Stand B, 70% pruning induced a significant increase in the concentrations of foliar Ca^{1}, Mn^{12} , Zn^{1} and Na^{23}, and a decrease in N_{2}, $P_{2(3)}$, K_{23}, S_{2}, Fe_{12}, Zn_{2} and C_{1}. The 40% pruning treatment increased the foliar concentrations of Na^{23}, while the 18% pruning treatment had no effect on any of the foliar elements.

In Stand C, 72% pruning induced a significant increase in the concentrations of foliar P^{1}, Ca^{1} and Na^{1}, and a decrease in N_{23}, P_{23}, K_{3}, S_{23}, Fe_{23}, Zn_{3}, C_{1} and H_{1}. The 52% pruning treatment decreased the concentrations of N_{2}, S_{2} and C_{1} and the 27% pruning treatment of 27% decreased the foliar concentrations of C_{1} and H_{2}.

When the similar pruning treatment classes of the three experimental stands were combined, 72% pruning (A 75%, B 70%, C72%) induced a significant increase in the concentrations of foliar Ca^{123}, Mn^{123}, Zn^{1}, Na^{1} and (Cd^{13}) and a decrease in the foliar concentrations of N_{23}, P_{23}, K_{23}, S_{2}, Fe_{23}, (Cu_{1}), Zn_{23}, Ni_{1}, C_{1} and H_{1} (IV: Tables 1 and 2). The concentrations of Cu and Cd, however, did not always exceed the Limit of Quantitation of the ICP-AES analyser. In general, there were some elevated Cd concentrations, but not sufficiently large to be of scientific importance. The 42% pruning treatment increased the foliar concentrations of Al^{1}.

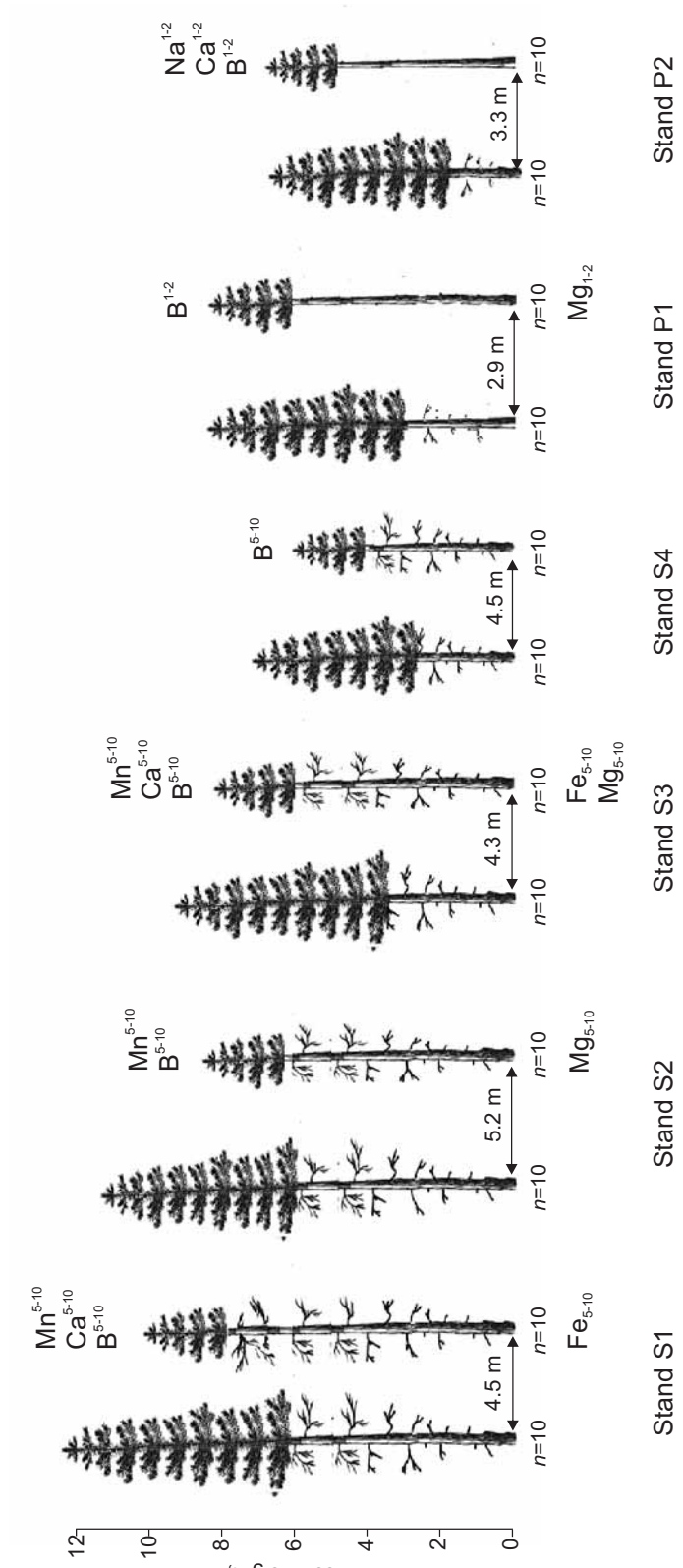

Figure 1. Increased (above the tree) or decreased (below the tree) foliar element concentrations of the diseased or pruned trees as compared to the concentrations of the adjacent healthy/unpruned control trees in Experiment 1 (I).
5-10 = five to ten years after the crown reduction due to disease (*Gremmeniella abietina*), 1-2 = one to two years after the pruning. Only significant differences are shown (t-test for paired comparisons, $p<0.05$).

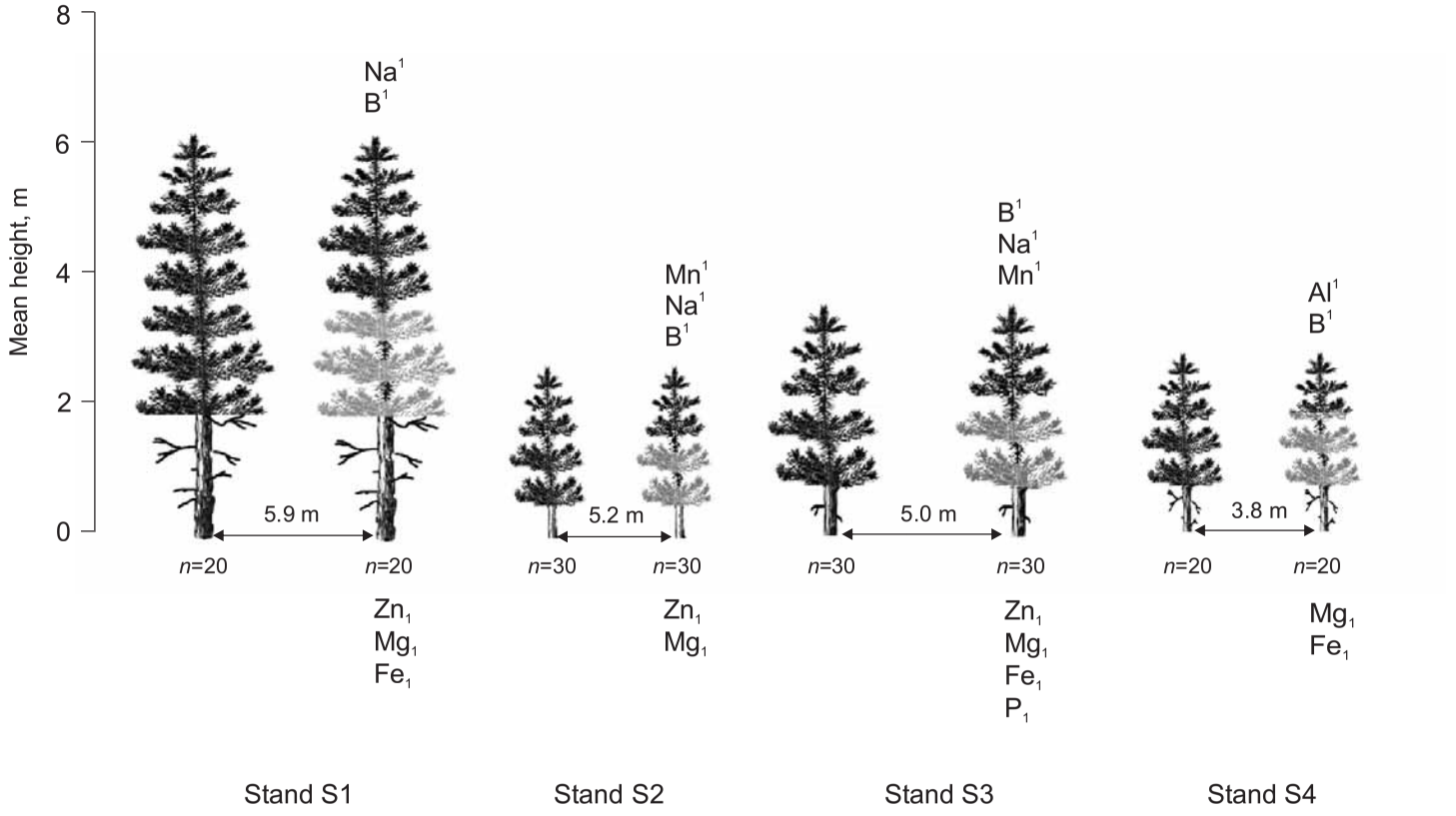

Figure 2. Increased (above the tree) or decreased (below the tree) foliar element concentrations of the diseased trees as compared to the concentrations of the adjacent healthy control trees in Experiment 2 (II).
1 = one year after the crown reduction due to disease (*Gremmeniella abietina*).
Only significant differences are shown (t-test for paired comparisons, $p<0.05$).

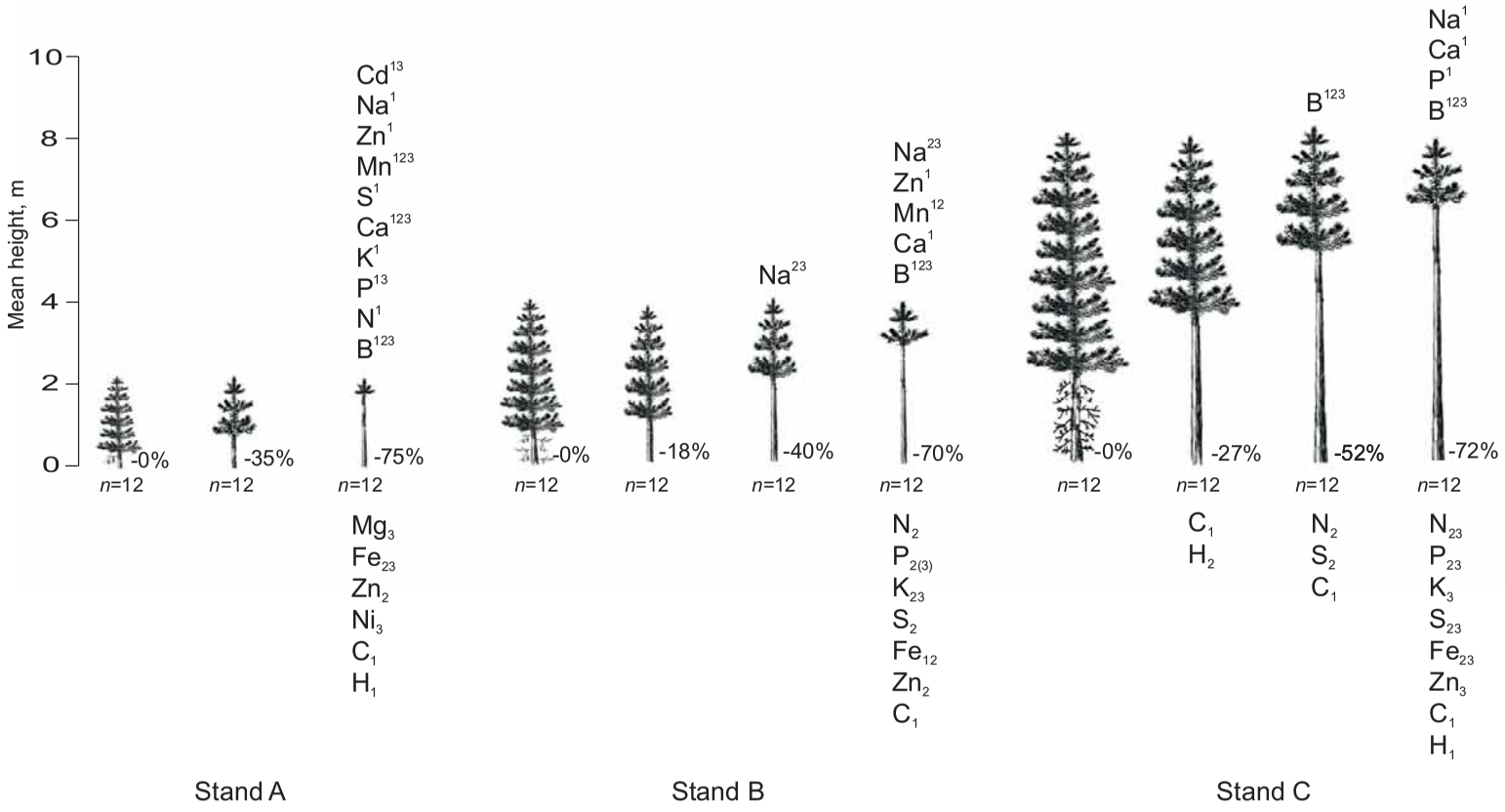

Figure 3. Increased (above the tree) or decreased (below the tree) foliar element concentrations of the pruned trees in different pruning treatment classes as compared to the concentrations of the control trees in Experiment 3 (III and IV). 1 = one year after the pruning, 12 = one and two years after the pruning,123 = one, two and three years after the pruning Only significant differences are shown (ANOVA and Tukey, $p<0.05$). See more details in text and (III and IV).

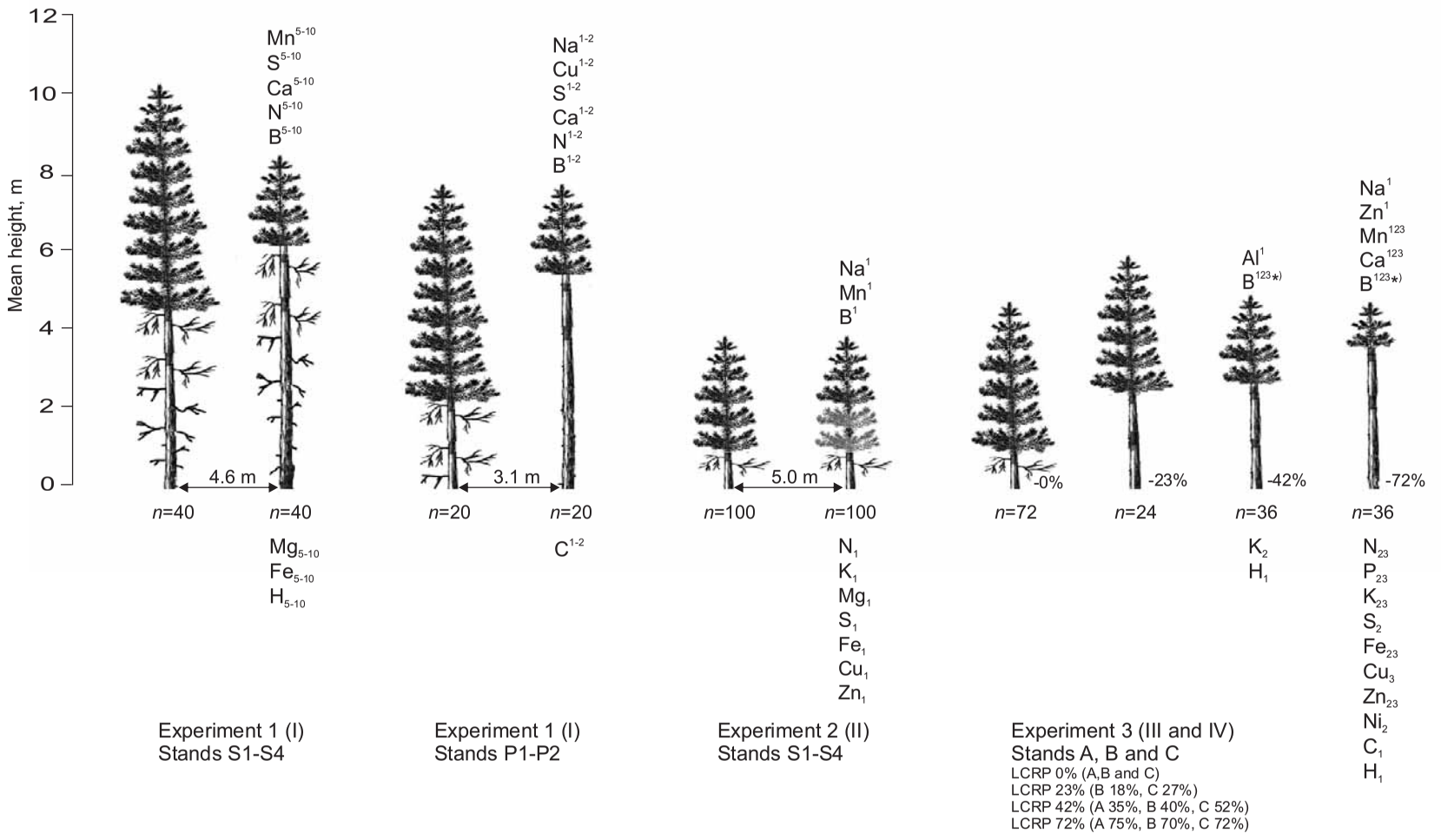

Figure 4. Increased (above the tree) or decreased (below the tree) foliar element concentrations of the diseased or pruned trees as compared to the healthy/unpruned control trees.The results are calculated for different crown reduction classes (diseased and pruned) within each experiment (1, 2 and 3) by pooling the data of individual stands in the given experiment. Stand specific data are presented in Figs. 1-3. LCRP=living crown reduction by pruning, % of the initial crown ratio.
*) = boron concentrations from (III) recalculated from the original data by combined pruning treatment classes

5 DISCUSSION

All the three experiments of this thesis provided significant evidence that severe disease-induced defoliation or artificial pruning of the living branches can induce long-lasting nutritional changes in the foliage of the recovering trees under the typical growing conditions for Scots pine.The foliar concentrations of all the 17 mineral nutrients/elements analysed were affected, to a varying degree, by artificial pruning during the following three years (III and IV). In the case of relatively small trees (height 2-3 m), most (10) of the nutrient/element concentrations appeared to increase rapidly in the first year after pruning, while only the C and H concentrations were at a lower level. During the second and third year after pruning, the Mg, Fe, Ni and Zn concentrations dropped, and only B, Ca, Mn and P had remained at an elevated level by the end of the study. A similar phenomenon, but at a different scale, apparently occurred in the larger pruned trees (height 4-8 m); after one year 7 different elements increased, while only Fe, C and H decreased. In the second and third year, only foliar B, Ca and Mn increased, and six N, S, P, K, Fe and Zn decreased. It was interesting to see that the foliar C concentration returned almost back its original level during the second and third year, despite the decreased concentrations of photosynthetically important nutrients like N and S.

In the pine stands in which *Gremmeniella abietina* caused a reduction in the living crown, 7 (N, S, K, Mg, Fe, Cu and Zn) nutrients decreased and 3 elements increased (B, Mn and Na) within one year (II). In the stands with symptoms of long-term severe infection (5-10 years) (I), the concentrations of only Mg and Fe decreased but that of and B, N, S, Ca and Mg increased. It is interesting to see that N and S remained at a higher level for so many years after living crown reduction. One possible explanation for this is the partial decomposition and N mineralization of the increased litterfall of dead needles from the the trees with severe living crown reduction over the years.

Coniferous trees store a significant amount of their nutrients in the foliage, and they retranslocate part of the nutrients from older needles to the new growing shoots depending on the nutrient status and demand of the tree (Fife and Nambiar 1982, Lim and Cousens 1986, Nambiar and Fife 1991, Helmisaari 1992, Finer 1989, Salif and Timmer 2001). Defoliation disturbs/inhibits this internal cycle but, normally, part of the nutrients gradually return from the soil to the nutrient pool in the tree through leaching and decomposition of the litterfall. In (I), the dead needles and other litter/pruned branches had become partly decomposed underneath the defoliated trees. This was not the case in the third experiment (III and IV), where all the pruned branches and needles were immediately transported away from the study area. In the second experiment (II), retranslocation was also probably disturbed, because *Gremmeniella abietina* primarily kills the phloem of the branches during the dormant period, before needle death in the spring. The inhibited or disturbed internal cycle of nutrients between the defoliated foliage in (II, III and IV) may partly explain some of the different changes in e.g. nitrogen and sulphur concentrations, compared to the results obtained in the first experiment (I).

The paired-tree comparison method used in (I)and (II) was successful in eliminating several disturbing factors affecting the tree nutrient status in the *ex-post* investigations, where the adjacent control trees represented the *ex-ante* situation. However, the differences in genetic properties of the trees and the species composition and diversity of the ectomycorrhizal associations may affect the foliar nutrition (Knight 1978, Schmidtling 1995, Baxter and Dighton 2001, Xu et al. 2003, Kennedy et al. 2007, Korkama-Rajala et al.

2008). The selection mechanisms employed by *Gremmeniella abietina* in achieving successful infection of the trees are also variable, and the original foliar nutrient status of the unaffected control trees may have also been partly altered, especially in (I), over the years. On the whole, despite the somewhat heterogeneous foliar element reactions of the trees in this thesis, many of the consequences of the changes in foliar chemistry were relatively similar (e.g. B, Mn and Ca), irrespective the form (disease- or pruning-induced) of living crown reduction of the Scots pine trees.

In other studies on the defoliation of conifers, the foliar N concentration for instance has increased (Piene 1980, Piene and Percy 1984, Ericsson et al. 1985, Långström et al. 1990, McMillin and Wagner 1997), decreased (Wagner 1986,1988, Raffa et al. 1998, Roitto et al. 2003, Handa et al. 2005, Asshoff & Hättenschwiler 2006), first increased and then decreased (Oksbjerg 1962, Piene 1980) or remained unchanged (Lyytikäinen 1993*a*, Reich et al. 1993). There are many possible reasons for the discrepancies between these studies. Physiological differences between tree species, different defoliation mechanisms and degree of needle loss, or the time difference between defoliation and the sampling of the regrown needles, for instance, provide possible explanations. On the other hand, even in the present study which employed the same species and approximately the same intensity of defoliation, the results were frequently relatively different in the case of trees of different age, and in how many years the trees took to recover after the treatment (see e.g. Figure 3).

The analysed and predicted physiological reactions of the trees and higher plants to minimize the effects of reduced foliage have been explained in several studies on the basis of e.g. compensatory growth, increased rate of photosynthesis in the remaining and refoliated foliage and increased mobilization of carbon reserves, including the carbon based defense mechanisms against the consequences of damage (see e.g. Bryant et al. 1983, Piene and Percy 1984, Tuomi et al. 1984, Wilson 1992, Honkanen et al. 1999, Mutikainen et al. 2000, Stamp 2003, Rooke and Bergström 2007). Trees may produce new foliage or leaves relatively quickly, and have compensatory growth, increased size of their leaves, produce secondary buds etc. (Piene 1980, Piene and Percy 1984, Søgaard et al 2007). Most of these theories and studies, however, take account of the nutrient status only at a general level, and primarily concentrate on the relationship between nitrogen and carbon.

One possible explanation for the increased mineral nutrient concentrations in the remaining and new foliage within one year after pruning is the altered root/shoot(crown) ratio, and a consequence of the temporary "overcapacity" of the roots after rapid and severe loss of foliage. In a pruning study on Norway spruce (*Picea abies* Karst.), the increased nutrient concentrations lasted for only a few months after crown reduction (Oksbjerg 1962). I assume, based on the results in (III and IV), that during the following years at the latest, after the root system has utilised the possible nutrient reserves, the uptake of mineral nutrients will probably decline as a result of decreased carbohydrate flow from the reduced canopy to the roots. In several studies, the root system has usually been depressed after defoliation. However, in a defoliation study on Scots pine trees (Kuikka et al. 2003), the fungal biomass in the roots and the mycorrhizal colonization percentage remained unchanged, even though the number of sporocarps was more than three times higher near the undefoliated control trees than the defoliated trees one year later There are also some indications of altered water uptake of the roots after a reduction in the canopy (Jackson et al. 2000, Snyder and Williams 2003).

Changes in the community, structure and biological activity of the mycorrhizal associations and thereby in active nutrient uptake, are also highly likely due to the smaller amount of photosynthates provided by a reduced crown. Kuikka et al. (2003) suggested that Scots pine continues to invest in the maintenance of the symbiosis despite the reduced

photosynthetic capacity resulting from defoliation. More recently, Saravesi et al. (2008) demonstrated that different timing of foliar defoliation induced different responses in the ectomycorrhizal fungal symbionts. Apparently, after severe defoliation, the amount of low-biomass ectomycorrhizae, which are assumed to require less carbon from the host tree, increased (Saravesi et al. 2008). Owing to the complex interactions between the photosynthetically active canopy and the root system processes in the soil, recent studies using natural abundances of nitrogen and carbon isotopes $\delta^{15}N$ and $\delta^{13}C$ (Hobbie et al. 1999, 2000, 2001) might give some new insights into the interactions between the foliage and mycorrhizal fungi. However, based on the above-mentioned studies and the widely held conviction about the important role of the mycorrhizas in the nutrient and water uptake of trees, I assume, that the active and energy-dependent nutrient uptake by fine roots and their mycorrhizal hyphae had, in the long-term, probably either decreased or changed in the pruned trees of this study and that the passive uptake of nutrients had relatively increased to some extent.

The nutritient status of trees has a considerable impact on the recovery processes after defoliation. If the trees are growing in poor nutritional conditions, their ability to recover decreases drastically. On the other hand, in the case of a good nutrition status, compensatory growth after defoliation has even increased the growth rate of the trees (Huttunen et al. 2007). Overcompensation after defoliation is a common phenomenon in many higher plants, especially in nutrient-rich, favourable growing conditions (e.g. Vanderklein and Reich 1999, Rautio et al. 2005, Ruiz et al. 2008). In the studies of this thesis, the increases in the concentrations of many of the nutrients may have had a temporarily beneficial growth effect in tree recovery. On the other hand, the decrease in the other nutrients is not likely to be a permanent process. It is possible that, after five or more years, the N and S concentrations in the defoliated trees for instance may return to normal or increase to slightly higher levels, partly as a result of decomposition and nutrient release from the defoliated needle litter. However, there are indications that, in some cases, those nutrients which increased in the present study after the pruning treatments, especially B, Ca and Mn may have growth-improving effects under certain conditions (e.g. Bergmann 1993, Marschner 1998, Saarsalmi and Tamminen 2005).

There might be connections between the tree nutrient/element status, defoliation-induced susceptibility and the resistance chemistry of trees, e.g. in the synthesis of carbon-based defence mechanisms against herbivores (Bryant et al. 1983, Wagner and Evans 1985, Lyytikäinen 1993*a*, 1994, Krause and Raffa 1995, 1996, McMillin and Wagner 1997, Raffa et al. 1998, Chen et al. 2002, Clancy et al. 2004, Cornelissen and Stiling 2006, Huttunen et al. 2008). Previously, Scots pine as an evergreen conifer is considered to have low induced chemical responses to defoliation (e.g. Niemelä et al. 1984, Tuomi et al. 1988, Lyytikäinen 1993*b*). Plant defence and growth hypotheses, however, are developing continuously (Honkanen 1995, Hamilton et al. 2001, Koricheva 2002, Stamp 2003). It is too speculative, on the basis of the results of this thesis, to draw direct conclusions about the relationship between the altered foliar element concentrations and the resistance against fungal diseases or herbivores. However, after severe pruning in the third experiment (III and IV), part of the heavily pruned trees were killed in subsequent years by moose (*Alces alces*) (a total of 7 trees in stands A and B) and by pine shoot beetle (*Tomicus piniperda*) (a total of 6 trees in stand C). Especially in case of *T. piniperda*, previous defoliation or severe pruning have increased the risk to beetle attacks of the pines (Långström et al. 1992, 2001, Cedervind 2003, Cedervind et al. 2003) . Apart from these cases of damage, all of the other pruned sample trees remained alive with only a slight growth reduction (see III for details). Nearly 15 years later, most of the pruned sample trees appear to be still alive and in a relatively

good condition (visual inspection of the experimental stands in the autumn of 2007, H. Nuorteva).

6 CONCLUSIONS

Based on the results of the studies (I-IV) the following conclusions can be drawn as a reply to the study aims, listed in chapter 2:

1) The foliar nutrient concentrations of both diseased and pruned trees differed significantly from the foliar nutrient concentrations of the adjacent control trees. Foliar B, N, S, Ca were significantly higher in both the diseased and pruned trees, while Mn was higher only in the diseased trees and Cu and Na only in the pruned trees. Foliar Mg and Fe concentrations were lower in the diseased trees than in the control trees. Most of the differences were at such a high level, that the large disparity should be noted in the interpretation of foliar analysis in recently defoliated conifers before any long-range conclusions.

2) One growing season after the *Gremmeniella abietina*-induced living crown reduction, the diseased trees had significantly higher foliar B, Mn and Na concentrations and lower Mg, Fe, Zn, Cu, K, N and S concentrations compared to the healthy trees. Because the reddish-brown dead needles were still attached on the dead branches in the trees, the results indicated that the foliar concentrations may change rapidly even without mineralization of the needle litter, and possible in conditions with minimal or any retranslocation from the dying needles (the phloem in the branches is killed by the fungus before the needle death).

3) The third study proved experimentally that a rapid and sufficiently large living crown reduction of Scots pine is the primary cause for the increased or decreased foliar element concentrations in the new needles that develop after severe defoliation. Especially after a rapid 72 % reduction in the live crown length, element concentrations in the current year/youngest needles of recovering Scots pine crown are significantly altered within one year. The concentrations of foliar B, and often also Mn and Ca probably increase for at least three or more years after defoliation. Several nutrients may become concentrated in the growing new needles during the first year, especially in trees smaller than 3 m, but during the second and third year the concentrations of e.g. N, S, Mg, Fe, Zn, P and K usually decrease.

This study proved experimentally in natural forest conditions that severe defoliation of Scots pine trees may induce biologically significant changes in the important macro- and micronutrients concentrations, as well as in the C concentrations, of refoliated needles.

In the field of biomonitoring, foliar analysis has been recently employed in several studies on forest and environmental health conditions. However, one should be carefull when comparing foliar nutrient results from injured and healthy conifers in order to explain the condition and vitality of trees. Most researchers take this into account, but it is also possible to use foliar analysis purpose oriented e.g. as an indicator of nitrogen and sulphur deposition, without taking into account the health of the trees. Especially during the heated public discussions in Scandinavia in the middle of the 80's about forest decline and *Gremmeniella abietina*, some researchers were tempted to overprove on the basis of foliar analysis the effects of environmental disturbances on forest health. Unfortunately,

exceptionally high or low needle nutrient concentrations are still occasionally interpreted as one of the explanations for tree injury, and not conversely, as a consequence.

Foliar analysis based methods are nowadays developing rapidly as a result of an increased ecological modelling capacity and new ideas. Considerable effort has been put in finding better solutions that would increase the diagnostic value of the foliar analysis of the trees (e.g. Braekke et al. 1998, Helmisaari 1998, Rautio 2000, Brockley 2001, Luyssaert et al. 2002). Scientific value and objective interpretation are important key questions in maintaining and improving the usability of foliar analysis in different fields of environmental monitoring. Concerning the studies in this thesis, I find them significant in providing new information about the long-term effects of rapid living crown reduction on the foliar nutrient and element status of Scots pine trees.

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