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Academic Dissertation in Forest Entomology

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Abstract

Concern about forest health has fluctuated over the years and the aspects and opinions about the different threats have varied. However, the importance of sustaining vital forest ecosystems has been generally accepted. Nutrient availability and cycling is one of the key elements in a healthy, functioning forest ecosystem. Human activities pose a threat to balanced nutrition, for example through air pollution and the acidification of forest soils. Pest insects are a natural part of the forest ecosystem, and in Finland they seldom cause extensive damage in otherwise healthy stands. However, abiotic factors can reduce the pest resistance of trees, thus accelerating the development of insect outbreaks into serious epidemics leading to forest decline. At best this only leads to economic losses through a temporary reduction in productivity. The situation is more serious if the regeneration of trees is endangered by the same factors, e.g. imbalanced nutrition, that predisposed the previous tree generation to pests and diseases. One way of alleviating acidification and restoring the nutrient pool on sites with an imbalanced nutrition is site-specific compensatory fertilization.

The literature review on the effects of fertilization on insect performance revealed that most of the studies have concentrated on nitrogen, which is usually the nutrient limiting growth. In most cases nitrogen fertilization benefited herbivores when measured directly at the individual insect level, which was consistent with the hypotheses predicting that a fertilizer-induced growth increase leads to reduced defence. In such cases more carbon is allocated to growth at the expense of defence. At the population level the effects were less positive or even negative from the insect point of view. This was probably due to indirect effects at the ecosystem level, especially the proliferation of predators and parasitoids in the fertilized stands. Damage occurrence was probably also reduced due to a dilution effect, because defoliation causes less relative damage in fertilized trees with lush foliage.

Traditionally the objective of forest fertilization has been to increase wood production through the addition of nitrogen, but the main aim of compensatory fertilization is to restore the nutrient balance and improve tree vitality. The effects of different nutrient combinations on nutrient cycling, soil properties, tree growth and pest resistance were monitored and measured in a series of field trials in Scots pine and Norway spruce in different parts of Finland. The measured resistance traits were mainly studied from the constitutive defence point of view, but some aspects of induced defence were also included. Besides the several compensatory fertilization experiments, an additional study was conducted in a pine stand where trees of different defoliation intensities were fertilized in order to accelerate their recovery. Physiological and morphological traits connected with resin exudation and various defence compounds in the phloem or needles were measured as indicators of defence readiness. Radial stem growth was measured and a vigour index calculated from the growth data. Special emphasis was laid on the predicted and observed relationship between growth and different defence traits. While some indications of a negative relationship between growth and defence were found, no consistent effects of fertilization on defence were found even though the treatments including nitrogen markedly enhanced growth. In some cases the different defence traits showed opposite responses, and usually did not correlate with each other. The results indicated that neither the constitutive nor the inducible resistance level could be markedly increased by fertilization. However, compensatory fertilization did not reduce the defence capacity of the trees, and can therefore be used to increase the vitality or to accelerate the recovery of conifers from e.g. defoliation.

List of publications

This thesis is based on the following journal articles, which are referred to in the text by their Roman numerals:

I Kytö, M., Niemelä, P. & Larsson, S. 1996. Insects on trees: Population and individual response to fertilization. *Oikos 75:148-159.*

II Kytö, M., Niemelä, P. & Annila, E. 1996. Vitality and bark-beetle resistance of fertilized Norway spruce. *Forest Ecology and Management 84:149-157.*

III Kytö, M., Niemelä, P. & Annila, E. 1998. Effects of vitality fertilization on the resin flow and vigour of Scots pine in Finland. *Forest Ecology and Management 102:121-130.*

IV Björkman, C., Kytö, M., Larsson, S. & Niemelä, P. 1998. Different responses of two carbon-based defences in Scots pine needles to nitrogen fertilization. *Écoscience 5:502-507.*

V Kytö, M., Niemelä, P., Annila, E. & Varama, M. 1999. Effects of forest fertilization on the radial growth and resin exudation of insect defoliated Scots pines. *Journal of Applied Ecology 36* (in press).

Maarit Kytö has participated in the planning of studies I-V, collected and screened the literature in I, participated in the collection and processing of the field samples and data of II-V, performed the statistical analyses for II, III, V, and in part IV, written the first draft versions for papers I-III, V and participated in the preparation of manuscript IV.

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two of the papers in this thesis would have ended up in the recycling-bin, and one paper would probably not have been started at all. Erkki Annila and Pekka Niemelä have also participated in and supported me through all the stages of the work, from planning to writing. Discussions with them and numerous other colleagues both at Metla and abroad have stimulated and helped me to avoid learning quite everything the hard way by trial and error. In good company it has also been easier to deal with the occasional mistakes.

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1. Introduction

1.1 Growth, vigour and resistance

The review by Stark (1965) has had a strong impact on subsequent thinking because the data indicated that the resistance of conifers to folivorous insects increases following fertilization. Since then, many more fertilization experiments have been carried out and progress has also been made in understanding fertilization-induced biochemical changes in tree tissues and their influence on insect feeding. Plant resistance against various kinds of harmful organism can be based on both structural (e.g. bark thickness, tissue toughness) and functional (e.g. hormonal activity, hypersensitivity) traits. Compared to fast-growing annual or perennial plants, constitutive defence in conifers is relatively important. In addition, induced defences are of crucial importance, especially in protecting the conducting tissues in the stem phloem and sapwood. Nitrogen-based alkaloids are important means of defence in many plants, but are of little importance in conifers that are adapted to sites with limited nitrogen availability. Moderate nutrient deficiency leads to an imbalance between nutrient uptake and the rate of photosynthesis, which is thought to explain why conifers and many other plants almost exclusively employ defensive compounds based on carbon (Ericsson 1979, Coley *et al.* 1985). The amount of excess carbon is believed to determine the concentration of carbon-based defensive compounds, such as phenolics and terpenoids (Waring 1985). Although the concentrations of plant defensive compounds are to a large extent genetically determined, their concentrations can also be influenced by environmental conditions (Gershenzon 1984, 1994, Larsson *et al.* 1986, Björkman *et al.* 1991, Rousi *et al.* 1993).

In their defensive role, the carbon-based secondary chemicals act as toxins or repellents against harmful insects and fungi. Especially resin flow from the stem is a crucial form of defence against bark beetle attack and air-borne microbes, and the constitutive and attack-induced secondary metabolites are essential in stopping invasion by the pathogenic fungal associates of the beetles (Raffa & Berryman 1983, Berryman 1986, Christiansen *et al.* 1987, Solheim 1991, Paine *et al.* 1997). The response of plants to changes in the environment may affect their susceptibility to attack and damage by herbivores and pathogens. For example, the risk of attack can increase in response to a decrease in the concentration of defensive compounds, and hence in resistance (Rhoades 1979). Decreased host resistance has been suggested as one explanation for increased insect densities in plant stands treated with fertilizers (Waring & Cobb 1992) or exposed to abiotic stress such as drought or pollutants (Larsson & Tenow 1984, White 1984, Larsson 1989, Waring & Cobb 1992). Insect-defoliated conifers may not be able to replace carbon as rapidly as mineral nutrients because of their inherently low rates of photosynthesis, which can lead to a relative carbon deficiency (Herms & Mattson 1992). This is predicted to result in a decreased C/N balance, decreased concentrations of carbon-based secondary metabolites, and the absence of delayed induced resistance (Tuomi *et al.* 1988, Herms & Mattson 1992).

The concept of tree vitality or vigour is difficult to define unambiguously. Good growth is usually considered to be a sign of good vitality, and reduced growth correspondingly a sign of decreased vitality or stress (Price 1991). It is more difficult to incorporate pest resistance (or tolerance) in the concept of vitality, especially as resistance against all kinds of attacking organism requires a variety of different defence mechanisms and characteristics that can be mutually exclusive. In the current theoretical frameworks on pest resistance, growth and defence are considered as two competing sinks because they both depend on the availability of assimilates for building bricks and for energy. Several hypotheses have been proposed to predict how the assimilated carbon is allocated within the plant into growth or defence (Herms & Mattson 1992, Tuomi 1992). When the allocation problem is discussed it is usually treated as a physiological trade-off between two alternative sinks, of which growth is the strong sink as long as it is not limited by e. g. low nutrient availability, and defence receives the carbon that is left over. As Mole (1994) points out, it is also of importance to take into account possible "third party traits" when predicting allocation. When a growth-limiting nutrient is added to the soil, the tree usually responds by enhanced growth. If a trade-off between growth and defence exists, the defence level should consequently diminish. In such a case, whether the vitality of the tree can be considered to have improved or declined would then depend on how much relative value is given to growth and defence, and how large the relative and actual changes in growth and defence would be. In some cases fertilization does not enhance growth, but can still affect the physiology of the tree by stimulating photosynthesis and thereby possibly improving defence (Reichardt *et al.* 1991, Bryant *et al.* 1993).

The growth/differentiation balance hypothesis, as well as the carbon-nutrient balance hypothesis, predict that, under conditions of moderate nutrient deficiency, fertilization will lead to decreased concentrations of carbon-based secondary metabolites (Herms & Mattson 1992). It has been hypothesized that when growth is limited by low nutrient availability, the "excess" carbon is used for producing carbon-based secondary metabolites (Bryant *et al.* 1983, Tuomi *et al.* 1988). After fertilization, when nitrogen is no longer limiting, carbon should be incorporated into new tissues, resulting in lower levels of carbon-based secondary metabolites. Several empirical studies support these predictions (Tuomi *et al.* 1984, Bryant *et al.* 1993).

1.2 Objectives of the study

As background to the empirical studies we first reviewed the existing knowledge on the effects of forest tree fertilization on the insects feeding on them (I). In particular, we were interested in looking for explanations to the seemingly contradictory results on herbivore performance in relation to tree nutrition, reported in various studies. An additional objective was to evaluate whether the results support the hypotheses on the relationship between growth and defence.

The empirical part of the study was mainly conducted in a number of field experiments as part of the research project “Forest vitality fertilization” of the Finnish Forest Research Institute. The series of field trials was established in different parts of Finland in order to study the possibility of using compensatory fertilization for improving the vitality and pest resistance of conifers, alleviating soil acidification, improving soil biological activity, and maintaining balanced nutrient cycling. Papers II-IV report the results from these studies, focusing on how different fertilizer treatments affected tree vigour, various resistance traits, and the relationship between growth and defence. In Norway spruce the study dealt with the defence traits involved in resistance against bark beetles and their associate blue-stain fungus (II). In Scots pine the resistance traits connected with defoliators (IV) and bark-beetle-fungus associations (III) were studied. In the compensatory fertilization experiments the studies on pine were mainly focused on constitutive resistance, and in spruce on both constitutive and induced resistance.

A special case study related to compensatory fertilization, more correctly called “recovery fertilization”, was conducted in an insect-defoliated pine stand (V). The main aim was to study whether the recovery of Scots pines suffering from different defoliation intensities could be accelerated, the survival secured, and growth losses decreased by fertilization.

The growth and vigour of the trees was monitored in connection with resistance traits. In the light of current hypotheses on the relationship between growth and defence (see Herms & Mattson 1992), there may be problems in finding measures that simultaneously increase growth (a sign of improved vitality) and improve resistance. If growth and defence are alternative competing sinks for assimilates, there is a risk that if fertilization increases growth it will lead to reduced resistance.

The studies were conducted to find answers to the following questions:
- Can selective (site-adapted) fertilization increase resistance?
- Is it possible to simultaneously enhance growth and resistance, or does an increase in growth lead to reduced resistance?
- Does fertilization affect defence traits if growth is unaffected?
- Are the effects on constitutive and induced defence the same?
- Are different defence traits affected in a similar fashion?

2. Materials and Methods

2.1 Literature review (I)

We reviewed publications dealing with the effects of fertilization on forest tree-feeding insects and mites. The search for papers was performed using the forestry literature databases TREECD (CAB International), AGRIS (FAO) and AGRICOLA (National Agricultural Library) and by checking the reference lists of the articles found. Most of the papers dealt with nitrogen fertilization, which is why we focused on nitrogen when reporting the effects of fertilization, even though in several cases it was not possible to separate the effects of nitrogen from those of the other nutrients applied. We divided the effects of fertilization on the performance (size, survival, growth rate, abundance, or food consumption) of the insects into positive, negative and non-significant from the insect point of view. We classified the studies into three groups according to the feeding mode of the insect species: leaf-chewing, sap-sucking, and other insects. The third group included a heterogeneous sample of studies on insect species with a wide range of feeding modes that were treated together because the number of studies was too low to form more appropriate groups of e.g. wood-boring or gall-forming insects. In the group of leaf-chewing insects, we further divided the effects into the individual level, i.e. directly on the performance of individual insects, and the population level in which the experiments were conducted in uncontrolled natural surroundings.

2.2 Field experiments (II-V)

2.2.1 Study sites and experimental design

2.2.1.1 Compensatory fertilization experiments (II-IV)

A series of experiments was established by the Finnish Forest Research Institute in 1990-1992 to study the possibilities of using slow-release fertilizers (so called vitality fertilizers) to prevent or alleviate forest decline in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst.) stands subjected to air pollution, soil acidification, nitrogen deposition and/or biotic factors. Papers II-IV deal with the studies on tree vigour and pest resistance that were conducted in some of the field experiments belonging to this series. Other aspects studied in the project include nutrient cycling, deposition and soil properties, soil microbiology, tree growth and yield, root dynamics, forest diseases and ground vegetation (Mälkönen 1998, Mälkönen *et al.* 1999). The resistance studies were conducted in two spruce experiments and eleven pine experiments (Fig. 1). The experiment on each site consisted of 3-4 replicates of different fertilizer treatments on randomized plots. The size of each plot was 30 × 30 m, and the plots were located 10 meters apart. The number of replications and treatments varied between the sites according to the specific characteristics of the test site and the available area. All the experiments had an unfertilized control and 3-5 different fertilizer treatments with and without nitrogen.

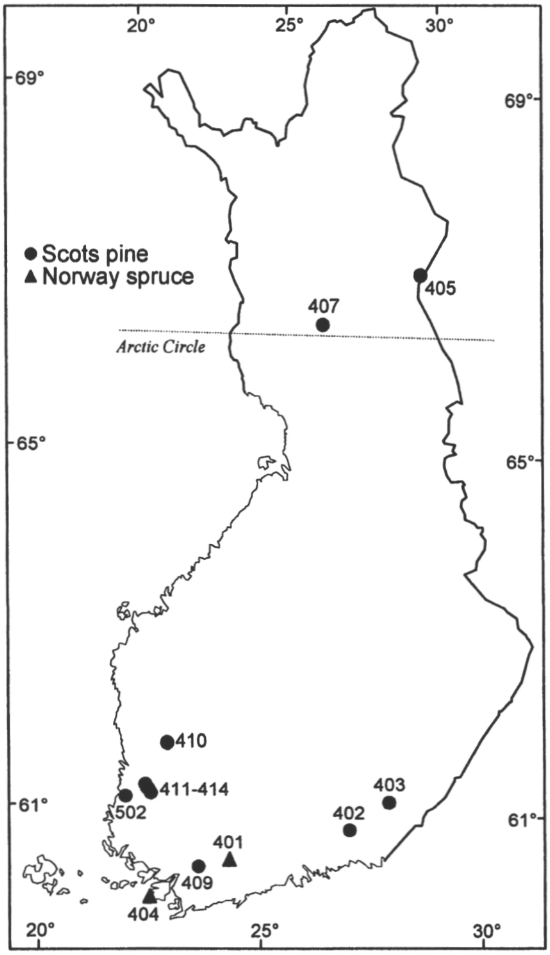

Figure 1. *Location of the experimental sites in the compensatory fertilization series. Circles indicate pine, triangles spruce experiments.*

2.2.1.2 Recovery fertilization experiment (V)

The fertilization experiment on defoliated pines was carried out in a stand located halfway between experiments 412 and 413 in Harjavalta, SW Finland. In 1989 there was an outbreak of the pine sawfly (*Diprion pini* L.) and some of the trees were heavily defoliated, while others within the same stand were practically untouched. The insect population collapsed the following year, and tree mortality in the stand remained negligible. In the spring following the defoliation year, 70 groups of three trees were chosen for the fertilization experiment. Each group consisted of one visibly undefoliated tree, one tree that had lost about half of its needle biomass, and one almost totally defoliated tree. The groups were randomly assigned to treatments (control and six different fertilization treatments) so as to give 10 undefoliated trees, 10 half-defoliated trees, and 10 heavily defoliated trees in each treatment.

2.2.2 Constitutive and induced resistance

2.2.2.1 Resin exudation and resin ducts (II,III,V)

The resin flow of Norway spruce was measured at two sites, and of Scots pine at ten sites in different parts of Finland. The resin sampling at most of the pine sites was performed at the end of the second growing season after fertilization. Spruce resin was sampled in the beginning of the fourth growing season. On each plot three trees growing closest to the plot border, altogether 12 trees per treatment, were included in the study. The resin flow on Norway spruce was measured by inserting, at seven points on each stem, thin plastic tubes through the bark into the cambium (Christiansen & Horntvedt 1983) (II). The amount of resin exudation was determined by measuring the length of the resin column in the tube after 24 h. In experiment 401 (Karkkila, Loppi) previously wounded and unwounded sample trees were used for sampling in order to compare possible effects of fertilization on both constitutive and induced resin flow. In experiment 404 (Dragsfjärd) all the study trees were previously unwounded ones. Pine resin was sampled according to the method of Lorio & Sommers (1986) and Lorio *et al.* (1990) on previously unwounded trees (III). Resin was tapped at two points on each stem by punching a round hole through the bark into the cambium and attaching an empty pre-weighed test tube under the wound to collect the exuding resin for 24 hours. At two sites the collected resin volume was also determined ocularly after 4 hours to compare the amount of resin collected after 4 and 24 hours. The resin tubes were removed after 24 hours, sealed and taken to the laboratory for weighing. The number of vertical resin ducts in the outermost sapwood of spruce were counted on the increment cores collected for growth measurements (II). After the measurements the cores were cut longitudinally and the number of resin ducts in the cross-sections of the 1991-1993 annual rings was counted under a stereomicroscope.

In the "recovery fertilization" experiment half of the original 70 tree groups were assigned for resin flow measurements in 1992 (V). At that time all the trees had recovered rather well from defoliation, and had needles from the current and two previous years 1990-1992. Resin was sampled using the same method as in the other pine experiments described above. The amount of resin collected was determined after the first two hours, and after 24 hours the tubes were removed from the trees. Increment cores from 61 undefoliated or totally defoliated trees, sampled in November 1992, were used to study the effect of defoliation and nitrogen fertilization on the formation of vertical resin ducts.

2.2.2.2 Phloem phenolics and biotests with a fungus (II,III)

The concentration of total phenolics in the phloem tissue was determined using the bark plugs removed from the resin sampling wounds of one spruce experiment and several pine experiments (II, III). The sample trees of the spruce experiments were artificially inoculated at four points around each stem with *Ceratocystis polonica* (Siem.) C. Moreau, a pathogenic blue-stain fungus vectored by the bark beetle *Ips typographus* L. (II). The fungus induces defence reactions (resin exudation and a characteristic lesion in the phloem) around the infected wounds on spruce. Five weeks after inoculation the length of the resin stripe below the inoculation wound was measured. The outer bark was removed and the length and width of the lesion in the phloem around each inoculation point measured.

2.2.2.3 Resistance traits of the needles (IV)

In three of the pine experiments needles were collected from control and nitrogen-fertilized (treatment F2) trees in winter 1992/93 for chemical and anatomical analyses (IV). Three needle age-classes, formed before (1990) and after (1991, 1992) fertilization, were sampled. The sampling was performed during the dormant season when needle chemistry is known to be relatively stable (Helmisaari 1990, Linder 1995), thus enabling comparisons among sites as well as among treatments. The concentrations of carbon, nitrogen, total phenolics and resin acids were determined. The length and cross-sectional area of the needles and the number and size of resin ducts were measured.

2.2.3 Growth and vigour of the trees (II,III,V)

Two increment cores were taken from each tree at 1.3 m height for radial growth measurements (II, III). The annual growth rings and the width of the sapwood and heartwood were measured on the cores. A tree vigour index was calculated as BA_1/SA, where BA_1 is the cross-sectional area of the annual ring of a specific year (year of resin measurement) and SA is the sapwood basal area (Waring *et al.* 1980).

In the recovery experiment two increment cores were also taken from each stem at 1.3 m height in November 1992 (V). The mean of the two increment cores was used to calculate the basal area of sapwood and heartwood for each tree for calculating the vigour index. In November 1995 one more increment core was taken from all the trees for diameter growth measurements. When the radial growth data were examined it was obvious that annual rings were missing from some of the trees. The growth series of all the trees were therefore cross-dated, and the vigour index for 1992 (the resin sampling year) calculated using the corrected ring width data as described above.

2.3 Chemical analyses

Most of the chemical analyses were performed in the Central Laboratory of the Finnish Forest Research Institute. The resin acid analyses were conducted at the Swedish University of Agricultural Sciences. Nitrogen and carbon concentrations were measured with a CHN elemental analyzer (LECO CHN-600) (II-IV). Phenolics were analysed with a UV-spectrophotometer using Folin-Ciocalteus phenol reagent, after extraction in 80% methanol for 30 minutes at room temperature (II-IV). Tannic acid was used as the standard (Julkunen-Tiitto 1985). Resin acid concentrations were analysed with a gas chromatograph (Varian 3700) after the resin acids had been extracted and treated as described in Gref & Ericsson (1985) (IV).

2.4 Data analysis

In the compensatory fertilization experiments analysis of variance with plots (mean value of the three sample trees) as replicates was used to test the differences in resin flow, phenolic content, and tree vigour between treatments and sites. When necessary, transformations were used to normalize the distributions in the data before testing. When significant differences between sites were found in the analysis of variance, Tukey's test was used for pairwise multiple comparisons. The effects of the fertilizer treatments were tested with Dunnett's test. The within-tree variation in resin flow was tested using the matched t-test. The mean value of the two resin flow and increment core measurements of each tree was used for computing the bivariate correlations with the Pearson correlation test. The tests were conducted with the programs included in the VAX/VMS 1990 version of the BMDP statistical package.

In the needle trait study (IV) the effects of treatment, site and needle age-class on resin acid, phenolic and nitrogen concentrations, C/N ratio, number of resin ducts, resin duct diameter, needle length and needle cross-sectional area were assessed using repeated measures multivariate analysis of variance. Site and treatment were treated as fixed factors in the analyses. Data in the form of proportions were arcsine-transformed before analysis. Imbalance in the data set due to missing values was dealt with using the GLM procedure in SAS. Regression analyses were performed to estimate the degrees to which morphological and chemical properties of the needles were related to the concentrations of resin acids and phenolics.

In the defoliation study the VAX/VMS 1990 version of the BMDP statistical package was used. Differences in the tree age, diameter, and heartwood and sapwood basal areas between treatments and defoliation classes were tested using analysis of variance. Differences in the resin flow, the resin duct number and density, the vigour index and the annual radial increments between defoliation classes and treatments within the defoliation classes were tested with the non-parametric Kruskal-Wallis analysis of variance. When significant differences were indicated, the multiple comparisons were computed with the nonparametric test included in the Kruskal-Wallis analysis of the BMDP program 3S. Bivariate linear correlations were tested using Pearson's product-moment correlation test.

3. Results and Discussion

3.1 Literature review (I)

Fertilization can affect herbivores through changes in tree physiology causing altered nutritional quality of the plant tissue (individual level effect), and through effects on population-regulating processes like parasitism, predation and competition within and between species (population level effects). When leaf-chewing insects were affected by fertilization, the effect was usually positive when measured directly on individual insects. However, when the studies were conducted at the population level the effects on leaf-chewers were usually negative or non-significant. Sucking insects, mainly aphids and adelgids, usually benefited from nitrogen fertilization. The mutualistic relationship with ants may protect the populations of some aphid species from the increased predation or parasitism due to changes in the ecosystem. Also, the effects of a fertilization-induced increase in certain defense chemicals would be less pronounced in sap-feeders than chewing insects. However, an increase in the total nitrogen concentration did not automatically benefit sap-feeders: fertilizers comprising different forms of nitrogen had different effects on the amino acid composition of the sap and on the performance of the sap-feeding aphids (Carrow & Betts 1973). Investigations on insects other than leaf-chewing or sucking ones usually dealt with occurrence of damage on the trees, mainly reflecting population level effects. The small number of studies may not give sufficient grounds for generalization, but fertilization seemed to decrease to some extent the damage caused by bark beetles and shoot-feeding insects. The decrease in damage occurrence could partly depend on the fact that the more vigorous shoots on fertilized trees were less vulnerable to feeding damage compared to the more slender shoots of unfertilized trees. In defoliation surveys fertilization may cause a "dilution" effect because the foliage mass usually increases, which is why the abundance of insects may seem to decrease in cases where the population actually remained constant or even increased (Wickman *et al.* 1992).

The positive effect of fertilization on insects could be due to enhanced nutritional quality of the consumed tissues, to a reduction in the effectiveness of the host defense system, or to a combination of these factors. In most studies fertilization improved leaf nutritional quality by increasing the concentrations of N and soluble amino acids, sometimes also the concentrations of sugars, water, and crude fat. Fertilization can also affect the hardness and toughness of the foliage, which should influence the performance of chewing insects. Mobile insect instars have the option of choosing between many plant individuals or tissue types, while sessile ones must rely on chance or settle for the choice of the egg-laying parent. However, some gall-formers have developed the ability to regulate the nitrogen concentration in the gall tissue they feed on, thus preventing the nitrogen concentration from rising to an injuriously high level after fertilization (Hartley & Lawton 1992).

The effects at the population level can differ depending on the length of the study period. Fertilization abruptly disrupts the system, and the improved food quality can initially affect the insects positively. After a period of time, however, the effect may decline and even become negative. The delayed negative effect can be due to the induction of some resistance mechanism, the establishment of a new host/herbivore equilibrium (Hargrove *et al.* 1984), or to the proliferation of natural enemies (Strauss 1987). Less than half of the reviewed population level studies were experiments or surveys dealing with long-term trans-generational effects, the rest reporting on within-generation effects on e.g. larval survival. However, in most of the population level studies the effects of natural enemies were not excluded, as was the case in the studies on individual level effects, most of which also dealt with short-term, within-generation effects.

A summary of the results reported in the literature leads to the conclusion that there is no predictable general pattern concerning the effects of fertilization on the defense mechanisms of trees. Some empirical studies support the hypothesis that, after fertilization, when nitrogen is no longer limiting, carbon is incorporated into new tissues leading to lower levels of carbon-based secondary metabolites. In accordance with the carbon/nutrient balance and the growth/differentiation hypotheses, fertilization seems to improve the quality of deciduous host trees as food for herbivores. However, among conifers the effects on needle quality seems to be more complicated with different responses counteracting each other. While in some studies phenolics were reduced after nitrogen fertilization, other papers reported increased resin flow, resin acid and monoterpene concentrations in the needles.

Under natural conditions the variation in plant nitrogen concentrations is lower in conifers than in many cultivated plant species. Because nitrogen is usually a limiting nutrient in the soil, fertilization enhances the nitrogen concentration in the needles and other tissues. However, it is possible that the insects adapted to a low nitrogen concentration with modest variation lack the plasticity required to benefit efficiently from the enhanced nitrogen concentration. According to the concept presented by Prestidge and McNeill (1983), the species that are host specialists and nitrogen generalists are not as dependent on available nitrogen levels as the species that are polyphagous but specialists in terms of nitrogen requirements. Most of the insect species feeding on conifers in Finland are highly specialized on their host species, but this may be due to the fact that both Scots pine and Norway spruce are the only representatives of their genus occurring naturally in Finland. A few other *Pinus* and *Picea* species have been introduced as ornamental trees, but forest insects that could feed on several *Pinus* species have no other options than to feed on *Pinus sylvestris*.

3.2 Field experiments (II-V)

3.2.1 Constitutive and induced resistance

3.2.1.1 Resin exudation and vertical resin ducts (II,III,V)

The resin sampling on spruce was performed in late May or early June, and the effect of temperature on the viscosity of resin, and thereby on the flow rate, was considerable. Resin exudation was copious from sun-warmed parts of the stem, while no resin was exuded on the shaded parts of the same trunk. Resin sampling on pines was conducted in July or August when the temperature variation was moderate, and the considerable variation in resin exudation both within and between trees could not be explained by temperature differences between the measurement points. The linear correlation between resin amounts after 4 and 24 hours was strong, which indicates that the trees with an intense resin flow immediately after wounding were able to maintain a high flow rate throughout the 24-hour period. More than half of the total amount of resin collected during the 24-hour period was exuded within the first 4 hours. Strong resin flow starting immediately after bark beetle attack or other wounding can effectively stop the invasion of pathogens by flushing the fungal spores and bacteria out of the wound and forcing the engraving beetles to withdraw.

In spruce it was evident that wounding and the inoculation of pathogenic fungus induced resin flow. The previously inoculated and wounded spruces produced significantly longer resin columns than the previously unwounded ones. Likewise, while mechanical control wounds caused some resin flow, the resin stripes exuding from wounds inoculated with *Ceratocystis polonica* were considerably longer. The control wounds probably exuded constitutive resin that sufficiently cleaned and covered the wound, while the fungus growing around the inoculated wounds induced additional resin synthesis (Christiansen *et al.* 1999). In many trees fresh resin was still exuding from the wounds several weeks after inoculation, indicating that resin production continues until either the fungus has stopped growing and become encapsulated or the tree has succumbed. The fertilizer treatments had no significant effect on either the constitutive or induced resin exudation of spruce. As the length of the resin stripe caused by inoculation of the blue-stain fungus correlated positively with the average resin column length of the same tree, the measures supported each other as estimates of the resin production capacity of a certain tree. Like the resin columns, the resin stripe length was also not affected by fertilization.

In most of the pine experiments the resin flow was highest in the control treatment, but no particular fertilizer treatment consistently affected resin flow throughout the series of experiments. Apart from the Harjavalta pollution gradient, the mean resin flow varied very little between the different experiments, despite the differences in geographical location and genetical background of the stands (Fig. 2). In the four sites along the pollution gradient the resin flow was lowest close to the smelter, and increased along the gradient up to a distance of 4 km from the emission source where the mean resin flow of the control trees was higher than at any other site. In the pine experiments the resin exudation reflected the constitutive level. Only in the defoliated stand was an induced component also included in the resin measurements on the defoliated trees. The groups of different defoliation intensity differed significantly from each other, the resin flow being strongest in the heavily defoliated trees and weakest in the undefoliated trees. None of the fertilization treatments had a significant effect on the resin flow.

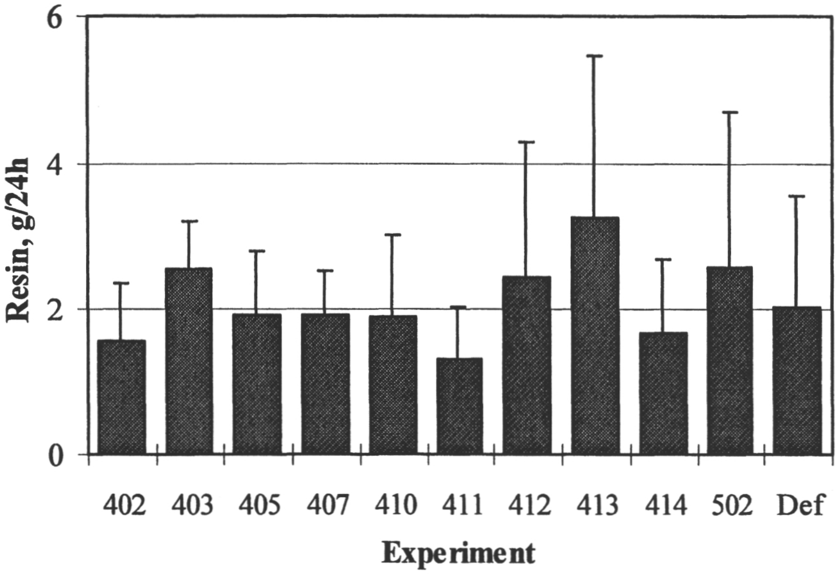

Figure 2. Resin exudation (mean + s.d.) of the control trees in the different pine experiments. See Fig. 1 for the location of the individual experiments, Def = undefoliated control trees in the defoliated stand.

Although the sites were poor rather than rich in nutrients, all the experiments were conducted on sites to which the tree species in question was well adapted. The variation in average resin flow between the different experiments was surprisingly low considering the geographical (site, climate) and genetic differences. This could indicate that the present level of constitutive resin is not far from the optimal, and therefore approximately the same result for adaptive evolution has been obtained in the range of climatic and edaphic conditions found in Finland. The moderate differences may also indicate that most of the stands had not been seriously traumatized in the immediate past. The strong resin flow in the defoliated trees was probably induced by the feeding sawflies. Furthermore, the exceptionally strong resin flow in the pollution gradient experiment 413 may have been induced by the *Diprion pini* outbreak that occurred in the area a couple of years prior to the resin measurements.

The density (number per mm^{-2}) of vertical resin ducts was much lower in spruce than in pine. In spruce, although the number of ducts was lowest in the control trees, the fertilizer effect was not statistically significant. In the defoliated pine stand the heavily defoliated trees had fewer vertical resin ducts in the annual rings formed after defoliation than the undefoliated trees. Nitrogen fertilization had increased the absolute number of ducts (per annual ring) in both defoliated and undefoliated trees. However, there were no significant differences in the resin duct density, although the density tended to be lower in the nitrogen-fertilized trees than in trees that had not received nitrogen. In both pine and spruce the annual ring width correlated positively with the number of resin ducts, but not with resin duct density. The number and size of the ducts affect not only the transportation capacity but also the potential resin production, as the ducts are lined with epithelial cells that synthesize the resin. These epithelial cells remain active for several years (Bannan 1936, Hillis 1987), which obviously also enabled the increase in resin synthesis in the stems of the defoliated trees in which no annual rings with new resin ducts were formed. However, the number or density of resin ducts did not correlate with the amount of resin flow in either spruce or pine.

3.2.1.2 Phloem phenolics and biotest with a fungus (II,III)

Phenolics can inhibit fungal growth and have a negative impact on insect performance (Berryman 1972, Johansson & Stenlid 1985, Nicholson & Hammerschmidt 1992). In our experiments, fertilization did not affect the phenolic concentration of the phloem in either spruce or pine, except at the severely polluted site (411) where soil amelioration had increased the phenolic concentration thereby probably increasing resistance. In the phloem of spruce the concentration of total phenolics was higher than that in pine, and there were also significant differences between the stands. No significant correlation was found between the phenolic concentration of the phloem and the resin flow in any of the experiments with either tree species.

The inoculated fungus grew in the phloem of spruce and induced the formation of a typical dark reaction zone extending upwards and downwards from the inoculation point along the grain (Solheim 1988). Growth to the sides, i.e. across the grain, was negligible. Apart from a narrow necrotic zone, no lesion was formed around the uninoculated control wounds. In addition to its constitutive defence, Norway spruce reacts to fungal attack by initiating the biosynthesis and accumulation of phenolics in the zone surrounding the pathogen (Stenlid & Johansson 1987, Brignolas *et al.* 1995a,b, Franceschi *et al.* 1998). Induced defence is very important in the inhibition of *Ceratocystis polonica* attack, because the fungus is more sensitive to the components of lesion resin than of preformed resin (Solheim 1991). The fact that the reaction to inoculation was similar in all treatments suggests that, along with the constitutive defence, the induced defence was also relatively unaffected by fertilization. The length of the lesion did not correlate with any of the measured resistance traits (the length of the resin stripe or the resin column, resin duct density, the phenolic concentration of the phloem). There was a significant difference in lesion length between the sub-experiments in the two different stands, indicating different levels of defence readiness. Of Norway spruce the resistance to bark-beetle attack has been empirically shown to have a treshold attack density that is related to host condition and site factors (Mulock & Christiansen 1986). In our experiments we were operating with a few single inoculations, far below the treshold even at the stressed sites. The treshold for successful *Ophiostoma/Ips* invasion in our experimental stands is estimated to be a few hundred beetle attacks or more than 100 artificial inoculations per tree (Christiansen 1985, Mulock & Christiansen 1986).

3.2.1.3 Resistance traits of the needles (IV)

Nitrogen fertilization increased the resin acid concentrations in the needles, but the effect was delayed and appeared first in the needles formed one year after fertilization. On the other hand, the total phenolic concentrations tended to be lower in the needles of fertilized than control trees. As expected, fertilization increased the needle length and nitrogen concentration, and decreased the C/N ratio. Although the number and size of resin ducts and the cross-sectional area of the needles tended to be bigger in the fertilized trees, the differences between the treatments were not statistically significant.

In the needles formed before fertilization none of the studied needle traits differed significantly between the fertilized and control trees. Only two traits, the number of resin ducts and the cross-sectional area of the needles, differed significantly among the experiments. All the studied traits changed significantly over time, probably reflecting the effects of weather and fertilization, but also the aging of the needles since all the needle age classes were sampled at the same time. Naturally the needles formed in the first two years after fertilization were more affected, but even the oldest needle age class that was fully formed at the time of fertilization was to some extent affected, i.e. as regards the nutrient content at least. Translocation of nutrients between plant parts can be very extensive and, during shoot elongation, older needles are an important source of carbon and nutrients for the growing shoot. The increase in the phenolic content of the needles over time could be due to age-related accumulation, or to the depletion of other substances from aging needles resulting in a relative increase of phenolics.

3.2.2 Growth and vigour of the trees (II,III,V)

As nitrogen addition increased growth in all the experiments, including the severely polluted and the defoliated ones, it is safe to assume that growth was limited primarily by nitrogen availability. In all the stands the vigour index closely reflected the absolute stem diameter growth, thus providing little or no additional information, apart from an index of growth, to the analyses. The calculation of the vigour index is based on the assumption that the sapwood basal area reflects the leaf area in the crown of the tree (Waring *et al.* 1980). However, even extensive defoliation had not affected the sapwood area of the studied trees. As the transformation of sapwood into heartwood is irreversible (Hillis 1987), a possible change in the proportion of heartwood should have been detectable even three years after the defoliation. Needle loss was probably not reflected in the conducting sapwood area because the branches remained alive in spite of total needle loss. Needle loss caused by internal physiological processes of the tree itself might be reflected in the sapwood area, but when the needle biomass is reduced by an external agent the sapwood basal area does not reflect the leaf area, and the vigour index is biased because of an overestimate of the leaf area.

Like growth, the vigour index was also always higher in the nitrogen-fertilized treatments than in the control and non-nitrogen fertilizer treatments. In the compensatory fertilization experiments there was no marked difference in the growth response between the treatments containing slow-release nitrogen and those containing only fast-release nitrogen. However, as the measurements were made only a few years after fertilization, it was too early to predict how long the growth-promoting effect lasts and how the vigour is affected in the long run by the different treatments. The vigour index differed substantially among the experiments. At the severely polluted site and defoliated site especially it was extremely low, the latter probably partly due to the biased sapwood-needle biomass ratio. Both defoliation and fertilization had a significant effect on the vigour index three years after defoliation and more than two years after fertilization. The index was always higher in the undefoliated than in the severely defoliated trees and, as a rule, the vigour index of the moderately defoliated trees fell between these two extremes.

In the defoliated stand the trees were divided into three classes according to the intensity of defoliation. Although the ovipositing sawflies may, instead of truly random selection, have actively chosen the trees on the basis of some characteristic, this trait was not reflected in the radial growth pattern of the trees before and during the outbreak. Starting from the year following the defoliation, the annual increments of the three defoliation classes differed significantly from each other, and the effect lasted for at least six years, throughout the study. The cross-dating of the increment data indicated that there were annual rings missing from about half of the trees. In the severely defoliated trees there were usually several rings missing, and some trees had little or no radial growth during the six-year period following defoliation. Compared to the undefoliated trees, the growth response to nitrogen application was delayed by one year in the moderately defoliated trees and by two years in the severely defoliated trees. This may be due to diminished assimilation and increased resource allocation into refoliation in the defoliated trees. In the undefoliated trees, the N and NPK treatments gave similar growth responses in spite of the fact that the NPK treatment contained only half the amount of nitrogen given in the N treatment (V). Two thirds of the nitrogen in the NVF (vitality fertilizer) treatment was in a slow-release form, which was reflected in the growth response. Nitrogen fertilization effectively counteracted the defoliation-induced growth reduction: compared to the undefoliated trees, the moderately defoliated trees had a 24 % growth reduction (1990-1995) in the nitrogen treatments, and a 51 % reduction in the treatments without nitrogen. In the severely defoliated trees the reduction was 52 % in the nitrogen treatments and 71 % in the non-nitrogen treatments.

3.2.3 Relationship between growth and defence (II-V)

In compliance with the growth/differentiation and carbon/nutrient balance theories (Loomis 1932, Bryant *et al.* 1983, Lorio 1986), some indications of a negative impact of enhanced growth on resin flow were detected, but the link between growth and defence was by no means clear or linear. In pines the most striking characteristic concerning the relationship between growth and defence was that, in spite of the large variation in the resin exudation rate, high resin flow and high vigour index never occurred together in any of the nearly 400 sampled pine trees (Fig. 3). While the resin exudation of low-vigour trees varied across the scale from very weak to very strong, the high-vigour trees never exhibited strong resin exudation. There were also indications of a similar negative relationship between the vigour index and the concentration of total phenolics in the phloem. Although growth and resin production cannot be completely independent, the results indicate that the relationship is unpredictable, apart from the non-existing combination of high resin flow and strong growth. This applies to constitutive resin at least. In our experiments the amount of resin probably represented the constitutive level in stands other than the defoliated one (and possibly also experiment 413). Whether the combination of strong growth and strong defence was unfeasible because of the resource-based trade-off, genetic constraints, lack of defence induction in vigorous trees, or some unknown reason, remained unresolved. It was impossible to estimate reliably the resource limitations, assimilation capacity and allocation patterns of the trees (Chapin *et al.* 1987, Mole 1994). A straightforward trade-off situation between growth and defence would only occur if carbon was scarce, which may not be the case. The defoliation experiment especially strongly implied that either there were considerable carbon stores in the pines, or that the normal assimilation rate is only a fraction of the maximal rate and, when necessary, the rate can be accelerated up to full capacity. Besides a physiological relationship, a trade-off could also be of genetical origin. In the evolution process, growth rate and defence rate may have been to some extent exclusive. However, the results seem to indicate that when it comes to constitutive defence there is a specific level of basic resistance that is maintained in all circumstances. It is unlikely that a pine would become totally defenceless even in the most favorable conditions with unlimited growth factors.

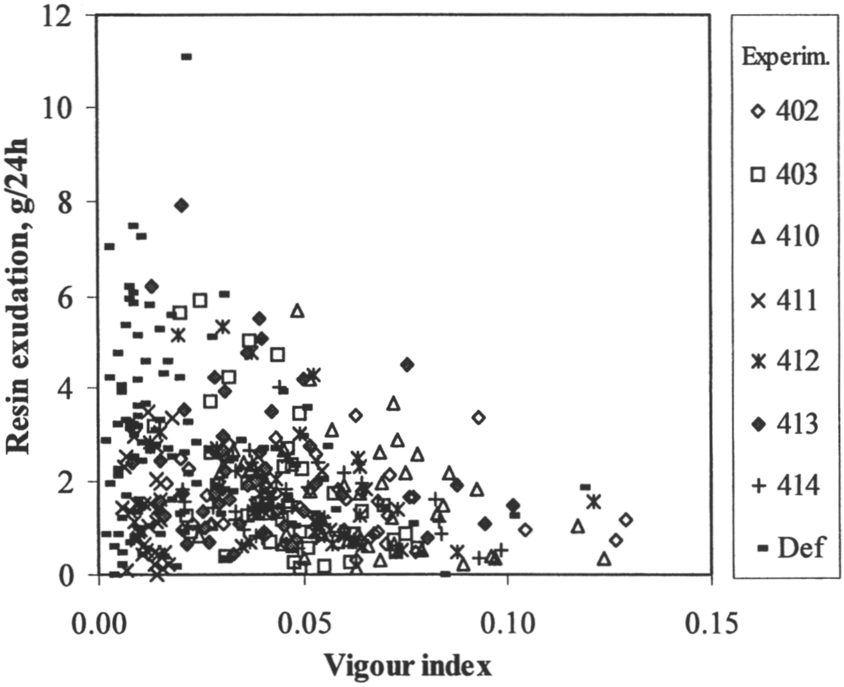

Figure 3. Correlation between the vigour index and resin exudation in the pines in the different experiments (see Fig. 1 for their location) and the defoliated stand (Def). Each point represents one tree. $R = -0.303$, $p < 0.01$, $n = 396$.

It would seem likely that the production of induced resin, being provoked by some traumatic incident not provided for in the regular physiological processes, would show a more straightforward relationship with growth if the availability of carbon was strictly limited. Artificial induction of a threefold resin concentration compared to the constitutive level in the trunk was reported to cause a 35 % growth reduction in pine (Kärkkäinen 1981). Increased assimilation efficiency may to some extent explain the high resin content of the severely defoliated trees, but it is likely that the trees had also utilized their carbon stores for foliage recovery and resin production (Ericsson *et al.* 1980, Hari *et al.* 1985). Lieutier *et al.* (1993) suggested that, especially in young pines, the resources for induced resin production originate from current photosynthesis, while in mature pines the mobilized energy might originate from tree reserves. This would mean that growth in mature pines is unaffected by moderate induced defence reactions, because the increased demand for carbon would be met from the energy reserves. Increased resin synthesis should thus cause growth reductions only in young pines. All the trees in our experiments were, even though relatively young compared to the natural life span of pines, already a few to several decades old and rather large. Therefore it seems reasonable to assume that they had sufficient carbon reserves to meet the increased carbon demands (due to enhanced growth) without cutting down their defence allocation. The slight reduction in resin exudation after fertilization in some of the experiments could, instead of reflecting a trade-off from defence to growth, merely indicate decreased production of induced resin due to the diminished physiological stress in trees with enhanced vitality.

In the defoliated trees the question of energy reserves and the relationship between growth and resin production is of a completely different order of magnitude. Extensive defoliation affects the source/sink balance of the tree in different ways depending on the pattern of the damage (Honkanen & Haukioja 1994). The defoliated trees must have depended heavily on their energy reserves because, in the worst cases, they had lost practically all their foliage. Within three years they had not only a recovered crown, but also exceptionally large resin reserves in the stem. According to Waring and Pitman (1985), new foliage and roots are favoured over stem diameter growth and the production of defence chemicals in the carbohydrate-allocation of a pine under stress conditions. In our study the resin flow measured three years after defoliation was more than 50 % higher in the defoliated trees than in the undefoliated trees. Assuming that the pre-defoliation resin content of the trees had not significantly differed between the defoliation classes, the results indicate that a considerable amount of resources had been allocated to induced defence in the defoliated trees at the same time as there was a marked reduction in growth compared to the undefoliated trees. This clearly indicates that induced resistance after a defoliation incident is a much stronger sink than diameter growth of the stem, unless the growth reduction was due to a lack of regulatory compounds rather than to a lack of available carbohydrates (Kozlowski 1969). However, while nitrogen fertilization clearly enhanced stem growth even in the heavily defoliated trees and the resin measurements coincided with growth peaks in the nitrogen treatments (Fig. 4), resin exudation was not significantly affected by fertilization and did not correlate with stem diameter growth. This means that a) the increase in resource allocation to growth was not taken from defence, at least as regards resin and phenolics, b) the trees were utilising nitrogen (but not carbon) at full available capacity, and c) the level of defence was regulated by induction rather than by resource availability.

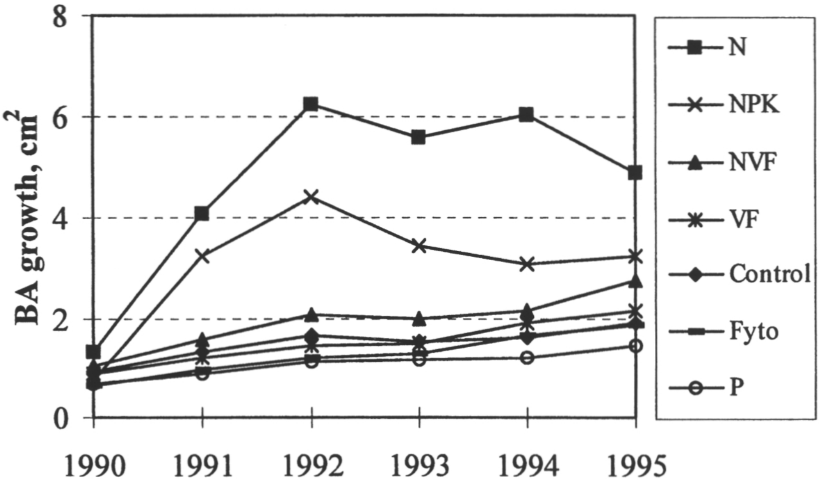

Figure 4. *Average basal area growth per tree in the different treatments of the defoliated stand. All defoliation classes pooled. Defoliation occurred in 1989, fertilizers were applied in 1990. Treatments N, NPK and NVF contained nitrogen 135, 69 and 71 kg/ha, respectively. In the NVF (vitality fertilizer) treatment 2/3 of the nitrogen was in slow-release form (Paper V).*

In the regression analysis, the 1991 annual ring width, the amount of resin flow, and fertilization together explained 69 % of the variation in the 1992 vigour index of the defoliation experiment. Growth and fertilization had positive, and resin flow negative regression coefficients. As the 1991 increment was affected by fertilization, these two variables were overlapping. When stepwise regression analysis was conducted without the annual increments after the treatments, and replaced by the average annual increment of the period 1985-1988 (growth rate indicator unaffected by the treatments), the average increment, defoliation, fertilization and resin flow together explained 40 % of the variation in the vigour index. High growth rate (determined by genetical and environmental factors) and fertilization had positive coefficients, while defoliation and resin exudation affected the vigour index negatively. This would indicate that, while growth and vigour are mainly regulated by intrinsic factors and site characteristics like nutrient availability, a high defence level to some extent reduces growth and the vigour index derived from it. Another question is, whether high resistance (at least upon attack, i.e. induced defence) should not be considered a sign of vigour even when it leads to growth reduction. No doubt a tree that has successfully rejected pathogenic organisms, even at the expense of growth, is more vigorous than a killed tree with an extremely high growth rate up until death. While the vigour index may be of use in questions connected with stand structure (density, inter-tree competition, etc.), it did not seem to provide a useful tool for silvicultural decisions in connection with pest problems on individual trees or in managed stands.

In both pine and spruce the absolute number of resin ducts was much higher in the wide annual rings of vigorously growing trees than in the narrow rings of poorly growing trees. On the other hand, resin duct density (number per square millimeter) was much higher in the poorly growing pines than in the vigorously growing ones. Nitrogen fertilization increased the number of ducts, but decreased resin duct density (Fig. 5).

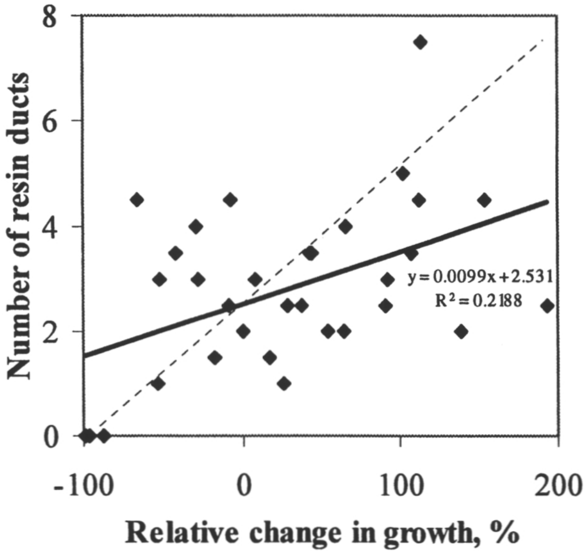

Figure 5. *The mean number of resin ducts per annual ring in 1991-92 plotted against relative change in growth (mean increment in 1991-92 compared to mean increment in 1988-89) in the fertilized trees (n = 32). The regression line shows that fertilization reduced resin duct density, because at constant density the number of ducts would double when growth doubles (indicated by the broken line).*

The concentration of resin acids in the pine needles depended on how large was the area of the needle cross section occupied by resin duct lumen. The more and/or the larger resin ducts there were in relation to the cross-sectional area, the higher the expected resin acid content. Fertilization increased the size of both the needles and resin ducts. The delayed increase in the number of resin ducts only in the second year after fertilization could partly explain why the concentration of resin acids in the fertilized trees increased in the second year, after being unaffected in the first growing season following fertilization when the increase in resin duct diameter was counteracted by the simultaneous increase in needle area. In contrast to resin acids, the concentration of phenolics was negatively correlated to the number of resin ducts. We hypothesize that the opposite responses were due to the differing modes of storage of these two groups of compounds. Terpenoids are primarily stored in the resin ducts, while phenolics are mainly stored in cell vacuoles. An increase in the number and size of the resin ducts would increase the space available for the storage of resin acids, and perhaps decrease the space available for cells containing phenolics. The size and location of the space containing plant defence compounds may affect the performance of insect herbivores. At least the species and instars that are capable of selective feeding can avoid or choose specific parts of the needles or phloem tissues (e.g. Tomlin & Borden 1994, Trier & Mattson 1997). Overall, increased nitrogen availability should make pines more susceptible to herbivores and pathogens directly after fertilization, especially as a result of the increase in nitrogen, but also due to the possible decrease in phenolics. The increase in resin acid concentrations one year after the treatment may, however, counteract this effect (Björkman *et al.* 1991). Another (or additional) explanation for the different responses of resin acids and phenolics could be the difference in their biosynthetic origin. Phenolics and proteins have a common precursor and are thus competitors, while terpenoid synthesis does not take place via the same pathway (Haukioja *et al.* 1998, Koricheva *et al.* 1998).

In spruce the radial growth and vigour index correlated positively with the length of the lesion caused by the pathogenic blue-stain fungus. All the trees were inoculated with the same fungal strain, so there should not have been any differences in the aggressiveness of the fungus. Therefore, a short lesion indicates high resistance because the tree has successfully prohibited the expansion of the fungus. While there was a relationship between growth and resistance, the lesion length correlated better with the annual increment before fertilization than with growth in the inoculation year. This suggests that the relationship was regulated by genetical constraints or some complex physiological processes rather than the availability of carbon or nutrients. This would also explain why the nitrogen-induced enhancement in growth did not lead to decreased resistance. It would appear that the carbon pool was not limited, either because of storage or because of flexibility in the assimilation capacity that allowed an increase in photosynthesis when fertilization enabled an enhancement of growth. The induced defensive reaction in the stems was quite evident in our study, but it was unaffected by the increase in growth following enhanced nutrient availability. However, it must be stressed that we were operating with a few single attacks per tree, far from an intensity comparable to a mass attack of bark beetles where efficient defence is a matter of life and death for the tree. Mass inoculation approaching the lethal dose could reveal differences in resistance between trees with different growth rates that did not appear in our study.

4. Conclusions

In the light of current theories, the main concern about compensatory fertilization (from the pest resistance point of view) is the question of whether there is a risk of a shift from defence to growth when a limiting nutrient is made available for the trees. Our results from field experiments did not indicate any such danger. Based on the results, the following answers to the questions posed in the introduction seem justified:

- Can selective (site-adapted) fertilization increase resistance?

In general, no. The effects of fertilization on a number of resistance traits were negligible in the stands that were not seriously suffering from pollution or heavily defoliated (i.e. subjected to stress that probably induces defence reactions). Nitrogen enhanced growth and may also have increased folivore tolerance, but non-nitrogenous fertilizers had significant positive effects on resistance only at the site damaged by heavy metal pollution.

- Is it possible to simultaneously enhance growth and resistance, or does an increase in growth lead to reduced resistance?

No. It is difficult to increase resistance with means that simultaneously enhance growth, but an increase in growth did not lead to reduced resistance either. The relationship between growth and resistance did not seem to be linear or even predictable, except for the fact that high growth and high resistance never occurred together. Still, this is not necessarily due to a trade-off caused by limited carbon availability, but may just as well be a sign of diminished stress leading to diminished induced defence.

- Do the fertilizers affect defence traits if growth is unaffected?

Generally no. Only at the severely polluted pine site the phenolic concentration of the phloem was significantly increased by liming and the non-nitrogenous compensatory fertilizer, although radial growth was unaffected. At the other sites the studied defence traits were mostly unaffected by the treatments that caused no growth response in the trees.

- Are the effects on constitutive and induced defence the same?

Yes, at low stress level. Both constitutive and induced resistance seemed to be relatively unaffected by the different fertilizer treatments. However, tests around or even above the treshold level (where trees start to be killed) might have given more decisive or even different results. Some treatments gave response only in trees experiencing severe stress (pollution, defoliation), meaning that the induced defence component was affected even though the constitutive defence wasn't.

- Are different defence traits affected in a similar fashion?

No. The results clearly indicated that different resistance traits cannot be expected to exhibit a consistent response to fertilization.

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Paper I

Kytö, M., Niemelä, P. & Larsson, S. 1996. Insects on trees: Population and individual response to fertilization. *Oikos 75:148-159.*

OIKOS 75: 148–159. Copenhagen 1996

MINI-REVIEW

Minireviews provides an opportunity to summarize existing knowledge of selected ecological areas, with special emphasis on current topics where rapid and significant advances are occurring. Reviews should be concise and not too wide-ranging. All key references should be cited. A summary is required.

Insects on trees: population and individual response to fertilization

Maarit Kytö, Pekka Niemelä and Stig Larsson

Kytö, M., Niemelä, P. and Larsson, S. 1996. Insects on trees: population and individual response to fertilization. – Oikos 75: 148–159.

We review the literature dealing with the effect of soil fertilization on phytophagous insects and mites on trees. Most published studies focus on nitrogen fertilization. The results of different experiments are often contradictory. In this review we attempt to facilitate the interpretation of the experimental results by distinguishing between direct effects on insect individuals and population level effects, and by grouping insects according to their feeding mode. By making these distinctions it is possible to explain at least some of the ostensibly contradictory results, such as where a fertilization-induced improvement in food quality from an individual point of view does not positively affect population growth. Enhanced nitrogen availability usually benefits individual herbivores by improving the nutritional quality of the host plant. However, except for aphids where a positive response to nitrogen fertilization is usually found, the effects of nitrogen fertilization on insects at the population level are often nonsignificant or even negative. The reason for this might be that fertilization also affects higher trophic levels in the ecosystem, i.e. predators and parasitoids, which control herbivore populations, and the effects mediated by community structure override the effects mediated by the improved quality of the host tree.

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Plant nitrogen concentration influences important interactions between herbivorous insects and plants. First, nitrogen is a vital component in the diet of all phytophagous insects. The nitrogen concentration in plant tissue is often much lower than that in the insect herbivore (Southwood 1973). Therefore, a scarcity of nitrogen-rich plant tissue has often been considered to limit resource utilization by phytophagous insects or their population density or both (White 1974, 1993, Mattson 1980). Plant nitrogen also influences phytophagous insects indirectly through effects on the production of plant secondary metabolites (Fig. 1). Such compounds often help protect plants by reducing insect growth and survival (Harborne 1993). Many secondary metabolites vary in concentrations depending on the nitrogen status of the plant. For example, compounds that contain nitrogen, such as alkaloids or cyanogenic glycosides, increase in concentration when nitrogen availability to plants increases (Bernays 1983). Conversely, concentrations of compounds such as tannins or terpenoids that contain no nitrogen, so-called carbon-based substances, often seem to decrease in plants

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as the amount of plant-available nitrogen increases (Bryant et al. 1983). Thus, a high plant nitrogen concentration may benefit the insect by increasing the nutritional value of host tissues or by lowering concentrations of deleterious carbon-based defensive compounds (Fig.1a). On the other hand, an increased nitrogen concentration can negatively affect insects if it leads to higher concentrations of nitrogen-containing secondary metabolites (Fig.1b).

It must be emphasized, however, that insect performance is not automatically influenced by nitrogen-related conditions such as those described above. Many factors contribute to a particular insect's responsiveness to a change in plant nitrogen concentration, including insect feeding habit (Larsson 1989), degree of host plant specialization (Scriber and Slansky 1981, Prestidge and McNeill 1983, Broadway and Duffey 1986), tissue specialization (White 1984), type of host plant (Mattson and Scriber 1987) and sensitivity to variable secondary metabolite concentration (Björkman et al. 1991, Larsson et al. 1992). For most insect species, detailed information concerning these relationships is still lacking. Consequently, it has proven difficult to develop mechanistic hypotheses relating insect performance to plant nitrogen concentration.

Much of our current knowledge about nitrogen effects on insect performance is based on experiments where host plant nitrogen status has been manipulated by various fertilization regimes. In general, such studies have demonstrated that fertilization of the host plant enhances insect performance and leads to increased population densities, although many exceptions have been observed (Stark 1965, Waring and Cobb 1992). The review by Stark (1965) has had a great impact on subsequent thinking because data indicated that the resistance of conifers to folivorous insects seems to increase following fertilization. During the last three decades, many more fertilization experiments have been carried out. Much progress has also been made in understanding fertilization-induced biochemical changes in tree foliage and their influence on insect feeding.

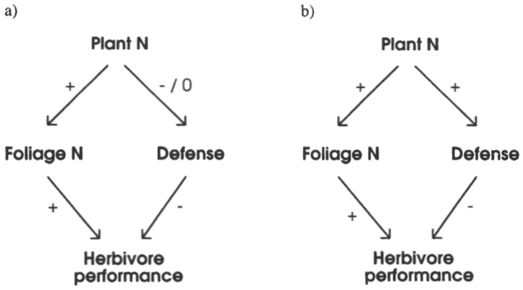

Fig. 1. Two scenarios describing the effects of the plant nitrogen pool on herbivore performance. In both cases the increased foliage nitrogen concentration increases herbivore survival and reproduction. However, in a) fertilization does not enhance host defense, resulting in a positive net effect on the herbivore, whereas in b) the net effect on the herbivore depends on the relative strength of the direct and indirect effects of plant nitrogen.

In the present review we attempt to update the literature on the nitrogen fertilization of forest trees and the responses of phytophagous insects. In particular, we seek to separate effects on insect individuals from effects on insect populations. This distinction is important when it comes to interpreting experimental results and developing hypotheses concerning the role of nitrogen in the population dynamics of forest insects. Past reviews have not fully considered this distinction, which could have contributed to the contradictory results.

Methods

We used TREECD (CAB International), AGRIS (FAO) and AGRICOLA (National Agricultural Library) databases for compiling publications dealing with the effect of fertilization on tree-feeding insects and mites. We focused on forest trees, excluding studies on species such as fruit trees that are efficiently bred, often grafted, and intensively cultivated. Of the 79 studies included in this review, 14 concern deciduous trees, and the remaining studies deal with coniferous species. There is great variation in the type of data, since they were obtained from studies ranging from general defoliation level inventories in forest stands to highly controlled greenhouse rearing experiments. Since many different insect taxa have been studied, we classified the studies according to the feeding mode of the studied insect species. The majority of the studies dealt with foliage-chewing insects, which constituted the first main group. This material was further divided into two subgroups: One group ("individual level" effects), included studies in which fertilization effects have been measured directly on the performance (growth, survival and reproduction) of individual insects, usually under conditions where natural enemies have been excluded. Another subgroup ("population level" effects) included studies where fertilization effects have been evaluated at the population level in uncontrolled field experiments. Sucking insects and mites were included in a second main group. The third main group was composed of chewing insects that feed on parts of the tree other than the foliage. Few studies have been performed with this insect type. Therefore, all studies on gall-forming, stem-, shoot-, root- and cone-feeding insects were grouped together, in spite of the heterogeneity of the feeding modes of the species included in this group. Most of the studies in the last two mentioned groups deal with population level effects.

In some experiments, nitrogen was the only fertilizer applied, but often it was combined with other nutrients.

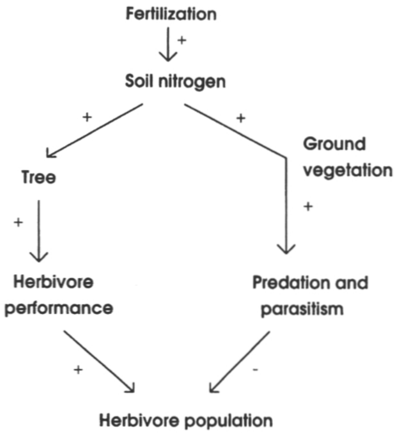

Fig. 2. Conceptual model describing the general effects that N-fertilization can have on herbivores living in trees.

For the sake of simplicity, when reporting the effects of fertilization we focused on nitrogen, even though in several cases it was not possible to separate nitrogen effects from those of the other nutrients applied. The separate effects of different nutrients are presented in the appendices in those cases where such effects were reported. In most cases the fertilizer was applied only once, but in a few studies the applications were repeated or fertilization was continuous. It was difficult to compare the results of different experiments because both fertilizer dosage and conditions at the experimental sites varied greatly from case to case. Also, the age of the studied trees varied considerably, and it seems likely that the response to fertilization will sometimes differ between seedlings and mature trees of the same species. The two experiments in which watering was included as a treatment (Young and Hall 1986, Mopper and Whitham 1992) indicate that in addition to soil nutrient status, the response to fertilization depends on soil water availability as well.

Effects of fertilization on herbivores with different feeding modes

The effect concept

Fertilization can affect herbivores in at least two ways (Fig. 2): First, tree physiology is changed which, in turn, influences the nutritional quality of plant tissue consumed by herbivores (individual effect). Second, fertilization may affect population-regulating processes like parasitism, predation and competition, by, for example, changing the composition and biomass of ground vegetation. Such ecosystem changes may, in turn, affect the growth of herbivore populations in the ground vegetation and through that also the generalist predators and parasitoids, which in turn would affect the tree-feeding herbivore populations (population level effects).

Leaf chewing insects

Effects on the individual level

Fertilization studies have focused on the application of nitrogen, either alone or in combination with phosphorus, potassium and/or calcium. In cases where a statistically significant effect of (nitrogen) fertilization on insect performance (body size, development time, survival) was recorded, it was nearly always beneficial for the insects (Table 1).

Effects on plant traits

The positive effect of fertilization on insects could be due to the enhanced nutritional quality of the consumed leaves/needles or to a reduction in the effectiveness of the host defense system, or to a combination of both these factors (Fig. 1a). In most studies fertilization improved leaf nutritional quality by increasing contents of N and soluble amino acids (Oldiges 1959, Schwenke 1960, Shaw and Little 1972, Mitchell and Paul 1974, Shaw et al. 1978, Hargrove et al. 1984, Ohmart et al. 1985, Young and Hall 1986, Bryant et al. 1987, Baylis and Pierce 1991, Björkman et al. 1991, McCullough and Kulman 1991, Mason et al. 1992, Mopper and Whitham 1992, Potter 1992, Bryant et al. 1993, Joseph et al. 1993). In some studies total sugar concentrations increased (Shaw and Little 1972) or were unaffected (Shaw et al. 1978, Mason et al. 1992). Further, there have been reports of increased water content (Schwenke 1960, Shaw and Little 1972, Baylis and Pierce 1991, McCullough and Kulman 1991, Joseph et al. 1993), increased crude fat content (Shaw and Little 1972, Shaw et al. 1978) and reduced (Shaw et al. 1978) or unaffected starch content (Shaw and Little 1972, Mason et al. 1992).

In a few studies fertilization changed the morphology of the leaves/needles. Potter (1992) reported a reduction in leaf moisture content although leaf thickness remained unaffected. Fertilization can affect the hardness and toughness of the foliage, which should influence the performance of chewing insects. According to Merker (1961) *Lymantria* larvae fed minced needles grew better and showed lower mortality than larvae raised on whole needles, although both food types had the same nutritional value.

Under natural conditions the variation in plant nitrogen content is lower in conifers than in many cultivated plant species. Because nitrogen usually is a limiting nutrient in the soil, fertilization enhances the nitrogen concentration in the needles and other tissues. However, it is possible that the insects adapted to a low nitrogen concentration with modest variation lack the

Table 1. Summary of the effects of nitrogen fertilization on different traits of individual leaf-chewing insects (from Appendix 1).

Trait	Effect of nitrogen fertilization		
	positive	non-significant	negative
Larval performance	5	9	–
Size and survival of pupae	8	7	3
Sex ratio and fecundity	3	8	–
Egg survival	–	3	–
Total	16	27	3

plasticity required to efficiently benefit from the enhanced nitrogen concentration. According to the concept presented by Prestidge and McNeill (1983), the species that are host specialists and nitrogen generalists (probably applies to most insects feeding on conifers) are not as dependent on available nitrogen levels as the species that are polyphagous but specialists in terms of nitrogen requirements. Also, differences in larval response to nitrogen concentrations between early and late instar larvae have been reported (Joseph et al. 1993).

The growth/differentiation balance hypothesis as well as the carbon-nutrient balance hypothesis predict that fertilization will lead to decreased concentrations of carbon-based secondary metabolites (Herms and Mattson 1992). It has been hypothesized that in cases of nutrient deficiency the "excess" carbon is used for producing carbon-based secondary metabolites rather than for growth (Bryant et al. 1983, Tuomi et al. 1988). After fertilization, when nitrogen is no longer limiting, carbon should be incorporated into new tissues, resulting in lower levels of carbon-based secondary metabolites. Several empirical studies support these predictions (Tuomi et al. 1984, Bryant et al. 1993). In deciduous trees fertilization reduced total phenolics and tannins (Bryant et al. 1987, 1993, Waring and Price 1988). In Douglas-fir phenolics were reduced (Joseph et al. 1993), whereas in grand fir tannins and lignins were unaffected (Mason et al. 1992). However, the fertilization of conifers seems to enhance their defensive capacity by increasing the resin flow (Oldiges 1959, Mopper and Whitham 1992), resin acid concentration (Björkman et al. 1991) and monoterpene concentration in needles (McCullough and Kulman 1991). These responses could be due to morphological changes, such as increases in the number and size of resin canals (Otto and Geyer 1970, Björkman et al. 1991). Obviously there is no predictable general pattern concerning the effects of fertilization on the defense mechanisms of the trees.

Effects on the population level

The effects of fertilization on insect populations and on the damage they cause are much more variable than the direct effects that fertilization has on individual insects (Table 2). In contrast to direct effects, the "overall" effect of fertilizers was negative more often than it was positive.

When analyzing the overall effects a wider perspective on the system is required. Fertilization affects not only the trees, but also the ground vegetation and properties of the soil. In most cases the biomass and species richness of ground vegetation increase dramatically. For example, in boreal forests the biomass of grasses increases sharply (Mälkönen et al. 1980). Changes at one trophic level may affect other levels. Thus, for instance, an increase in the growth of herbs and dwarf shrubs can benefit voles and shrews which are important predators on pupae of several herbivorous insects (Hanski and Parviainen 1985). Increases in the biomass and nutritional quality of the vegetation may enhance the overall abundance of herbivorous insects and, as a consequence, result in increased densities of general predators. Consequently, it is conceivable that in many cases effects mediated by community structure will override the effects mediated by the quality of the host tree (Fig. 2).

Baylis and Pierce (1991) reported that caterpillars of *Jalmenus evagoras* feeding on fertilized *Acacia* seedlings attracted a larger ant guard than caterpillars feeding on unfertilized seedlings. Fertilization has also been shown to result in greater numbers of phloem-feeding insects (aphids) and a concurrent increase in tending and patrolling by ants in *Artemisia ludoviciana* (Strauss 1987). However, the increased presence of these aggressive ants led to decreases in the numbers of beetles, weevils and leaftiers in fertilized plots. As a result, damage by leaf-chewing insects decreased significantly in fertilized plots. A similar mechanism may also operate in boreal forests where aphids and aphid-tending ants are very common (Rosengren and Sundström 1991). In addition, Zoebelein (1956) reported that parasitoids were attracted to the honeydew produced by the aphids and that the parasitoids feeding on honeydew laid an increased number of eggs. Such a parasitoid response would strengthen the negative effects mediated by community structure.

Population effects can also differ depending on the length of study period. Less than half of the population level studies (Appendix 2) were experiments or inventories dealing with long-term trans-generational effects, the rest reporting on within-generation effects on e.g. larval survival. However, in most of the population level studies the effects of natural enemies were not

Table 2. Summary of the effects of nitrogen fertilization on different traits of leaf-chewing insect populations (from Appendix 2).

Trait	Effect of nitrogen fertilization		
	positive	non-significant	negative
Performance, abundance	5	9	14
Consumption, feeding damage	4	2	9
Total	9	11	23

excluded, as was the case in the studies on individual level effects (Appendix 1), most of which also dealt with short term within-generation effects. Fertilization disrupts the system very suddenly, and the immediate effect on the defoliators can be positive, owing to the improved food quality. After a period of time, however, the overall effect on the defoliator population can become negative or non-significant. The delayed negative effect can be due to the induction of some resistance mechanism, the establishment of a new host/herbivore equilibrium (Hargrove et al. 1984), or to the proliferation of natural enemies. It should also be recognized that fertilization may have a "dilution" effect because foliage mass usually increases. As a consequence, the abundance of insects can wrongly be interpreted as having decreased in cases where they actually remained constant or even increased (Wickman et al. 1992).

Sucking insects and mites

Fertilization was usually beneficial for sucking insects and mites (Appendix 3, Table 3). By stimulating growth and increasing the amino acid concentration of the sap, fertilization obviously improves the nutritive quality of the host plant for sucking insects. This phenomenon is well documented among aphids feeding on cultivated plants (see Waring and Cobb 1992). However, Carrow and Betts (1973) showed that the effects of nitrogen fertilizers on the amino acid composition of the sap and on the performance of the sap-feeding balsam woolly aphids will vary depending on the form of the fertilizer; therefore, an increase in total nitrogen concentration need not necessarily benefit sap-feeders. In some studies nitrogen fertilization negatively affected the establishment of adelgid crawlers, whereas the performance of adelgid populations in other respects was promoted by fertilization (Carrow and Graham 1968, Carrow and Betts 1972, Mitchell and Paul 1974). The reduced establishment could have been due to physical changes in the tissue, such as enhanced tissue toughness, or to chemical changes resulting in reduced phagostimulation or nutritional value (Carrow and Graham 1968).

Table 3. Summary of the effects of nitrogen fertilization on different traits of sucking insect and mite populations (from Appendix 3).

Trait	Effect of nitrogen fertilization		
	positive	non-significant	negative
Performance	9	5	3
Abundance	3	2	3
Total	12	7	6

Other insects

Surprisingly few studies have been conducted on the effects of fertilization on stem-, shoot-, root- and cone-feeding or gall-forming insects; thus, it is difficult to see any consistent patterns. Most investigations dealt with indirect effects or attack level and were conducted under uncontrolled field studies, which makes it difficult to compare studies. Bark beetle attack levels either decreased or were not significantly affected, following fertilization of host trees (Appendix 4). In general, fertilization increases tree growth and vigor. Vigorous trees produce thicker and longer shoots, a response assumed to be beneficial for shoot-feeding insects (Price 1991). However, in most studies this did not seem to be the case. On the contrary, damage levels decreased after fertilization. Such decreases could have partly been due to the fact that more vigorous shoots on fertilized trees are less vulnerable to feeding damage compared with the more slender shoots of unfertilized trees. Nitrogen fertilization benefited stem-feeding *Hylobius abietis* adults, whereas root-feeding *Hylobius rhizophagus* larvae responded negatively to nitrogen. Nitrogen fertilization enhanced cone production (Mälkönen 1971) and had a positive effect on the performance of cone-feeding *Laspeyresia strobilella* larvae (Bakke 1969). Some gall-formers were able to regulate the nitrogen concentration in the gall tissue they fed on, thus preventing the nitrogen concentration from becoming injuriously high after fertilization (Hartley and Lawton 1992). This ability is one strategy to deal with shifts in nitrogen concentrations, comparable to the ability of mobile insects to choose between many plant individuals or tissue types.

Conclusions

Among leaf- and needle-chewing insects there seems to be a fundamental difference between direct, individual-level effects and indirect, population-level effects. Fertilization seems to improve the quality of deciduous host trees as food for herbivores by increasing the nutritional quality and water content which, in turn, results in enhanced larval growth, pupal weight and survival. In some cases a decrease in carbon-based secondary compounds has also been reported. This response is in accordance with the carbon/nutrient balance and growth/differentiation hypotheses and improves insect performance (Fig. 1a).

Among conifers the effect of fertilization on needle quality is more complicated. Although fertilization seems to increase the nutrient content, it also increases the resin content (Fig. 1b). The fact that these responses tend to counteract each other in terms of their effects on insects could explain cases where fertilization has not had any significant effect on herbivore performance (Björkman et al. 1991).

The effects of fertilization were more variable at the insect population level than at the individual level. However, most effects were negative. A potential explanation for the negative population level effects is that fertilization affects the factors controlling herbivore population growth, i.e. predation and parasitism (Fig. 2). Strauss (1987) provides some good evidence for this.

Fertilization is most beneficial for sucking insects. Because of their mutualistic relationship with ants, populations of some aphid species are not easily affected by increased predation or parasitism due to ecosystem changes. Also, the effects of any fertilization-induced increase in the concentration of defense chemicals or in tissue hardness would be less pronounced in sap-feeders than chewing insects.

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Appendix 1. Effects of fertilization on leaf-chewing insects at the individual level.
Effect compared to control: – negative, + positive, n. s. no statistically significant effect, ( ) no statistical test provided.

Insect species/ Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Diprion pini/ *Pinus sylvestris*	NCaPK	Cocoon diam. (female)	–	Schwenke (1960)
Neodiprion sertifer/ *Pinus sylvestris*	N	Larval performance	n. s.	Björkman et al. (1991)
		Fecundity	n. s.	
		Egg survival	n. s.	
Neodiprion edulis/ *Pinus edulis*	NPK + watering	Cocoon mass	+	Mopper and Whitham (1992)
		Sex ratio	n. s.	
		Reproductive potential	+	
	NPK	Cocoon mass	n. s.	
		Sex ratio	n. s.	
		Reproductive potential	n. s.	
Choristoneura pinus pinus/ *Pinus banksiana*	N	Larval survival	n. s.	McCullough and Kulman (1991)
		Development rate	n. s.	
		Female adult weight	n. s.	
Choristoneura occidentalis/ *Pseudotsuga menziesii*	N	Larval survival	+	Brewer et al. (1985)
		Pupal survival	n. s.	
		Development time	+	
		Pupal weight	+	

Appendix 1 (contd.)

Insect species Host tree species	Fertilizer treatment	Studied trait	Effect	Source
		Fecundity	+	
		Eclosion	n. s.	
Choristoneura occidentalis/	N	Larval survival	+	Brewer et al. (1985)
Abies concolor		Pupal survival	+	
		Development time	+	
		Pupal weight	+	
		Fecundity	+	
		Eclosion	n. s.	
Choristoneura occidentalis/	N	Larval weight	+	Mason et al. (1992)
Abies grandis		Pupal weight	+	
Choristoneura fumiferana/	NPK	Larval development time	n. s.	Shaw and Little (1972)
Abies balsamea		Pupal weight (female)	+	
		Adult weight (female)	+	
Choristoneura fumiferana/	high N + PK, Ca	Pupal weight	+	Shaw et al. (1978)
Abies balsamea		Sex ratio	n. s.	
	high K + low NP	Pupal weight (female)	–	
		Sex ratio	n. s.	
Choristoneura conflictana/	N (+P)	Pupal weight	+	Bryant et al. (1987)
Populus tremuloides				
Pristiphora abietina/	N	Cocoon size	n. s.	Merker (1961)
Picea abies				
Bupalus piniarius/	NCaPK	Pupal weight	(–)	Oldiges (1959)
Pinus sylvestris		Sex ratio	n. s.	
Lymantria monacha/	NCaPK	Pupal weight	(–)	Oldiges (1959)
Pinus sylvestris		Sex ratio	n. s.	
Lymantria dispar/	N	Larval period	n. s.	Joseph et al. (1993)
Pseudotsuga menziesii		Pupal weight	+	
		Pupal period	n. s.	
Epirrita autumnata/	NPK	Pupal weight (female)	n. s.	Haukioja and Neuvonen (1985)
Betula pubescens		Developmental rate	n. s.	
Rheumaptera hastata/	N,P,NP	Larval survival	n. s.	Bryant et al. (1993)
Betula resinifera		Pupal weight	n. s.	
Xanthogaleruca luteola/	NPK + watering	Pupal weight (female)	+	Young and Hall (1986)
Ulmus hybrid		Developmental time	+	
		Preoviposition period	+	
		Fecundity	+	
	NPK	Pupal weight (female)	n. s.	
		Developmental time	n. s.	
		Preoviposition period	n. s.	
		Fecundity	n. s.	
Phytomyza ilicicola/	N	Pupal weight	n. s.	Potter (1992)
Ilex opaca				
Jalmenus evagoras/	NPK	Larval survival	n. s.	Baylis and Pierce (1991)
Acacia decurrens		Oviposition	+	
Paropsis atomaria/	N	Pupal weight	+	Ohmart et al. (1985)
Eucalyptus blakelyi		Developmental time	+	

Appendix 2. Effects of fertilization on leaf-chewing insect populations.
Effect compared to control: – negative, + positive, n. s. no statistically significant effect, ( ) no statistical test provided.

Insect species/ Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Diprion pini/	NCaPK	Larval survival	(–)	Oldiges (1960)
Pinus sylvestris				
Diprion pini/	NCaPK		–	Schwenke (1960)
Pinus sylvestris				
Diprion pini/	Ca,K,CaPK	Larval survival	n. s.	Nef (1966)
Pinus sylvestris	P	Larval survival	+	
Neodiprion sertifer/	N	Larval consumption	–	Larsson and Tenow (1984)
Pinus sylvestris		Cocoon abundance	–	
		Pupal survival	n. s.	

Appendix 2 (contd.)

Insect species/ Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Neodiprion lecontei/ *Pinus taeda*	NK	Attack rate	(−)	Posey and McCullough (1969)
Neodiprion swainei/ *Pinus banksiana*	N	Larval survival	−	Smirnoff and Bernier (1973)
		Frass production	−	
Choristoneura occidentalis/ *Abies grandis*	N	Density of eggs and larvae	n. s.	Mason et al. (1992)
		Total herbivore biomass	+	
		Parasitization	−	
		Overall survival	n. s.	
		Outbreak prevalence	n. s.	
Choristoneura occidentalis/ *Larix occidentalis*	N,NP,NK,NPK,PK	Larval feeding	(+)	Schmidt and Fellin (1983)
Choristoneura fumiferana/ *Abies balsamea*	NPK	Larval survival	+	Shaw and Little (1972)
		Pupal survival (fem.)	n. s.	
Choristoneura fumiferana/ *Abies balsamea*	high N + PK	Larval survival	n. s.	Shaw et al. (1978)
		Larval development time	n. s.	
	high N + CaPK	Larval survival	+	
		Larval development time	n. s.	
	high K + low NP	Larval survival	n. s.	
		Larval development time	n. s.	
Choristoneura fumiferana/ *Picea glauca*	N	Performance	n. s.	Fogal et al. (1993)
Choristoneura fumiferana/ *Picea mariana*	N	Feeding damage	−	Fogal et al. (1993)
Pristiphora abietina/ *Picea abies*	Ca,N,P	Larval survival	(−)	Büttner (1956)
	K,MnB	Larval survival	(+)	
	NPKCa + micr.	Larval survival	(−)	
	NPK + micr.	Larval survival	(+)	
Pristiphora abietina/ *Picea abies*	CaN	Larval feeding	−	Merker (1958)
		Cocoon abundance	(−)	
Pristiphora abietina/ *Picea abies*	N	Pupal survival	(−)	Merker (1961)
Pikonema alaskansis/ *Picea glauca*	N	Larval survival	+	Popp et al. (1986)
	high N	Larval survival	n. s.	
Panolis flammea/ *Pinus sylvestris*	NCaPK	Larval survival	(−)	Oldiges (1960)
Dendrolimus pini/ *Pinus sylvestris*	NCaPK	Larval survival	(−)	Oldiges (1959)
Bupalus piniarius/ *Pinus sylvestris*	NCaPK	Larval survival	(−)	Oldiges (1959)
Lymantria monacha/ *Pinus sylvestris*	NCaPK	Larval survival	−	Oldiges (1959)
Lymantria monacha/ *Picea abies*	Ca,N,P,NPK	Larval survival	(−)	Büttner (1956)
	K	Larval survival	(+)	
Lymantria dispar/ *Fagus* sp.	NPK,NCa	Larval survival	n. s.	Oldiges (1960)
Lymantria dispar/ *Quercus* sp.	NPK,NCa	Larval survival	n. s.	Oldiges (1960)
Brachyderes incanus/ *Pinus sylvestris*	N,P	Feeding damage	+	Heliövaara and Löyttyniemi (1989)
	K	Feeding damage	+, −	
Brachonyx pineti/ *Pinus sylvestris*	N	Feeding damage	+	Heliövaara and Löyttyniemi (1989)
	P, K	Feeding damage	−	
Calomicrus pinicola/ *Pinus sylvestris*	N, K	Feeding damage	−	Heliövaara and Löyttyniemi (1989)
	P	Feeding damage	n. s.	
Cryptocephalus pini/ *Pinus sylvestris*	N, K	Feeding damage	−	Heliövaara and Löyttyniemi (1989)
	P	Feeding damage	n. s.	
Phytomyza ilicicola/ *Ilex opaca*	N	Larval survival	−	Potter (1992)
		Developmental rate	n. s.	
		Mine abundance and area	n. s.	
Defoliators/ *Robinia pseudacaia*	NPKNa	Leaf consumption	+/−	Hargrove et al. (1984)
Defoliators/ *Quercus robur*	NPK	Leaf consumption	n. s.	Grimalskii and Lozinskii (1979)
Defoliators/ *Pinus sylvestris*	N	Leaf consumption	−	Grimalskii and Emelyanchik (1983)

Appendix 3. Effects of fertilization on sap-sucking insects and mites.
Effect compared to control: – negative, + positive, n. s. no statistically significant effect, ( ) no statistical test provided.

Insect species/ Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Adelges cooleyi/	N	Fecundity	+	Mitchell and Paul (1974)
Pseudotsuga menziesii		Winter survival	+	
		Establishment	–	
Adelges piceae/ *Abies amabilis*	N	Performance	–	Carrow and Graham (1968)
Adelges tsugae/ *Tsuga canadensis*	N	Performance	+	McClure (1991)
Adelges tsugae/ *Tsuga canadensis*	NPK + micr.	Survival	+	McClure (1992)
Elatobium abietinum/ *Picea sitchensis*	N, P, NP	Attack intensity	+	Seaby and Mowat (1993)
Elatobium abietinum/ *Picea sitchensis*	N, NPKMg	Attack intensity	n. s.	Thomas and Miller (1994)
Sacchiphantes abietis/	NPK	Performance	(+)	Thalenhorst (1972)
Picea abies	NK, NP	Performance	(n. s.)	
	PK	Performance	(–)	
Fiorinia externa/ *Tsuga canadensis*	N	Performance	+	McClure (1980)
Lachnidae/	NP, NPK	Performance	(+)	Thalenhorst (1972)
Picea abies	PK, NK	Performance	(n. s.)	
Cinara pinea/ *Pinus sylvestris*	N	Performance	+	Neuvonen et al. (1992)
Cinara sp./ *Pinus tabulaeformis*	N	Performance	–	Zhou et al. (1986)
Lygus rugulipennis/	NPK + micr.	Nymph performance	n. s.	Holopainen and Rikala (1991)
Pinus sylvestris		Oviposition	n. s.	
Aradus cinnamomeus/ *Pinus sylvestris*	N	Abundance	+	Heliövaara et al. (1983)
Aradus cinnamomeus/ *Pinus* sp.	N	Abundance	–	Król (1986)
Aradus cinnamomeus/ *Pinus* sp.	N	Tree damage	–	Fuflygin and Grigorev (1987)
Oligonychus berless/ *Pinus tabulaeformis*	N	Abundance	–	Zhou et al. (1986)
Oligonychus ununguis/	NP, NPK	Performance	(+)	Thalenhorst (1972)
Picea abies	NK, PK	Performance	(n. s.)	
Oligonychus ununguis/	N	Egg density	n. s.	Löyttyniemi and Heliövaara (1991)
Picea abies	NP	Egg density	+	

Appendix 4. Effects of fertilization on gall-forming, stem-, shoot-, root- and cone-feeding insects.
Effect compared to control: – negative, + positive, n. s. no statistically significant effect, ( ) no statistical test provided.

Insect species Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Dendroctonus ponderosae/ *Pinus contorta*	N	Attack level/tree mortality	–	Waring and Pitman (1985)
Dendroctonusfrontalis and *D. terebrans* / *Pinus taeda*	NPK	Attack level	n. s.	Moore and Layman (1978)
Pissodes strobus/	NPK, N	Damage occurrence	n. s.	Xydias and Leaf (1964)
Pinus strobus	K	Damage occurrence	+	
Phaenops cyanea/ *Pinus* sp.	NK	Attack level	–	Mrazek (1983)
Tomicus spp./	N	Shoot attack frequency	+	Löyttyniemi (1978)
Pinus sylvestris	PK	Shoot attack frequency	–	
Exoteleia dodecella/ *Pinus* sp.	N + micr.	Attack level	–	Kulesza and Piskorz (1971)
Dioryctria splendidella/ *Pinus tabulaeformis*	N	Damage occurrence	–	Zhou et al. (1986)
Rhyacionia buoliana/ *Pinus sylvestris*	NKCa	Attack level	–	Nef (1966)
Rhyacionia spp./ *Pinus elliottii*	NPK + micr.	Damage occurrence	–	Pritchett and Smith (1972)
Rhyacionia frustrana/ *Pinus taeda*	NP	Damage occurrence	–	Young et al. (1979)

Appendix 4 (contd.)

Insect species/ Host tree species	Fertilizer treatment	Studied trait	Effect	Source
Euura lasiolepis/	NPK	Attack level	n. s.	Waring and Price (1988)
Salix lasiolepis		Larval survival	+/−	
Neuroterus quercus-baccarum/	N	Gall density	n. s.	Hartley and Lawton (1992)
Quercus robur		Insect performance	n. s.	
Andricus lignicola/	N	Gall density	n. s.	Hartley and Lawton (1992)
Quercus robur				
Hylobius abietis/	NPK, N	Damage level	+	Selander and Immonen (1991)
Pinus sylvestris	PK	Damage level	n. s.	
Hylobius abietis/	NPK	Feeding preference	n. s.	Selander and Immonen (1992)
Pinus sylvestris		Damage level	n. s.	
	PK	Feeding preference	−	
		Damage level	−	
Hylobius rhizophagus/	N, NP	Feeding preference	−	Goyer and Benjamin (1972)
Pinus banksiana		Larval development	−	
		Larval survival	−	
	P	Feeding preference	n. s.	
		Larval development	n. s.	
		Larval survival	n. s.	
Laspeyresia strobilella/	NP	Larval density	+	Bakke (1969)
Picea abies		Larval weight	+	
Cone insects/	N	Cone damage	n. s.	Fogal et al. (1993)
Picea mariana				

Paper II

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Vitality and bark beetle resistance of fertilized Norway spruce

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Abstract

The effects of different so-called vitality fertilizers on the resistance of Norway spruce against bark beetles and their associated pathogenic fungi were studied in field experiments. Stem diameter growth, phloem phenolic concentration, resin flow, and reaction to inoculation with *Ceratocystis polonica* were measured on the trees. The fertilization treatments increased stem diameter growth, but did not affect resin flow, phloem phenolic concentration, or the lesion length caused by the blue-stain fungus. Thus, contrary to expectations based on current theories, we could demonstrate no functional relationship between growth and defence. However, the number of resin canals correlated positively with tree vigour, and there were indications of a potential increase in this constitutive defence feature after fertilization. Resin flow increased two-fold in the trees wounded 1 year earlier, indicating an induced component in resin production. This induced mechanism was unaffected by fertilization. Thus, although it does not seem possible to markedly increase either the constitutive or the inducible resistance level of Norway spruce through fertilization, vitality fertilization did not reduce the defences in our study and we hypothesize that it can be used without endangering resistance.

Keywords: Picea abies; *Ceratocystis polonica*; Resin; Resource allocation; Defence

1. Introduction

Plant resistance against various kinds of harmful organism can be based on both structural (e.g. bark thickness, tissue toughness) and functional (e.g. hormonal activity, hypersensitivity) traits. Norway spruce possesses both constitutive and inducible defences. A variety of carbon-based chemical compounds, so-called secondary metabolites, are of importance as they act as toxins or repellents against harmful insects and fungi. Especially resin flow from the stem is a crucial defence against bark beetle attacks, and the constitutive and attack-induced secondary metabolites are essential in stopping the invasion of the pathogenic fungal associates of the beetles (Christiansen et al., 1987; Solheim, 1991).

The concept of vitality is difficult, if not impossible, to define unambiguously. However, good growth is usually considered to be a sign of good vitality, and correspondingly reduced growth a sign of decreased vitality. It is more difficult to incorporate pest resistance or tolerance in the concept of vitality, especially as resistance against all kinds of attacking organisms requires a variety of different defence mechanisms and characteristics that can be mutually exclusive. Growth and defence can be seen as two competing sinks because they both depend on the

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availability of assimilates for building bricks and for energy. Several hypotheses have been proposed to predict how the assimilated carbon is allocated within the plant into growth or defence (Tuomi, 1992; Herms and Mattson, 1992). When the allocation problem is discussed it is usually treated as a physiological trade-off between two alternative sinks, of which growth is the strong one as long as it is not limited by e.g. low nutrient availability, and defence receives the carbon that is left over. As Mole (1994) points out, it is also of importance to take into account possible 'third party traits' when predicting allocation.

Whichever defence theory one chooses to rely on, the fact remains that fertilization affects the tree. When a growth-limiting nutrient is added to the soil, the tree usually responds by enhanced growth. When this is the case, and if a trade-off between growth and defence exists, the defence level should diminish because photosynthates are allocated to growth instead of defence. Whether the vitality of the tree can be considered to improve or decline would then depend on how much defence is set before growth, and how large the relative and actual changes in growth and defence would be. In some cases fertilization does not enhance growth, but can still affect the physiology of the tree by stimulating photosynthesis and thereby possibly improving the defence (Reichardt et al., 1991; Bryant et al., 1993). In Norway spruce a growth reduction is a possible consequence of liming (Derome et al., 1986) and perhaps also of imbalanced fertilization. Such an effect could lead to improved defence as long as assimilation is not reduced.

The aim of this work was to study the effects of different types of so-called vitality fertilizers on the defence of Norway spruce against bark beetles and one of their associate blue-stain fungi, and to look for possible signs of a trade-off between growth and defence in the trees.

2. Materials and methods

2.1. *Study sites and treatments*

The study was conducted in south-western Finland in two field experiments (Nos. 401 and 404) that belong to a large series of revitalization fertilization trials. The experiments consisted of 30 m × 30 m plots with 10-m buffer zones between the plots. There are 3–4 replicates of each treatment in most of the experiments. Due to the small area of suitable forest, Experiment 401 was established in two separate stands, 14 km apart from each other, with Replicates 1 and 2 in Sub-experiment 401a, and Replicates 3 and 4 in Sub-experiment 401b (Table 1). Prior to fertilization, needle samples were taken

Table 1
The study sites and measures carried out

	Experiment		
	401a	401b	404
Location	60°34′N, 24°15′E	60°42′N, 24°10′E	60°05′N, 22°23′E
Site index (H_{100})	33	31	28
Soil textural class	Very fine sand	Very fine sand	Fine sand
Humus type	Mor	Mor	Moder
Basal area (m^2 ha^{-1})	24.0	24.4	23.2
Tree age (a)	61	58	71
Tree diameter (cm)	25.3	22.0	23.4
Fertilization	5/6 1991	5/6 1991	23/5 1991
Resin flow measurements	2–3/8 1993	2–3/8 1993	
	9–10/6 1994	9–10/6 1994	28–29/5 1994
Ceratocystis biotest	2/8–5/9 1993	2/8–5/9 1993	28/5–29/6 1994

Table 2
Nutrient concentrations in the current-year needles prior to fertilization. The needles were sampled during winter

	Experiment		
	401a	401b	404
N (mg g^{-1})	12.53	11.03	11.41
P (mg g^{-1})	1.72	1.37	1.37
K (mg g^{-1})	4.33	4.23	4.38
Mg (mg g^{-1})	1.24	1.11	1.22
Ca (mg g^{-1})	4.23	4.03	6.61
B (μg g^{-1})	16.29	14.85	18.15

from the current year class for nutrient analysis during winter 1990/1991 from 40 trees in both experiments (Table 2). The concentration of nitrogen was determined with a CHN-elemental analyser (LECO CHN-600), the other nutrients by inductively couple plasma atomic emission spectrometry (ICP/AES) after dry ashing and extraction with hydrochloric acid. The state of health of the stands appeared to be satisfactory, and there were no signs of severe needle loss or needle discoloration in the trees.

Three different fertilizer treatments and an unfertilized control were used in the study (Table 3). Treatment F1 was a test fertilizer without nitrogen, treatment F2 a test fertilizer with nitrogen, and treatment F3 the same as F2 with additional liming. The fertilizers were spread by hand on the plots and a surrounding 5-m-wide buffer zone in May/June 1991. As the experiments are intended for use as long-term trials, the trees within the plots could not be used for destructive sampling. The study trees were therefore selected from the buffer zones. On each plot three trees growing closest to the plot border, altogether 12 trees per treatment, were included in the study.

2.2. Resin flow measurements

The first attempt to measure the resin flow was conducted in Experiment 401 in August 1993 using the method described by Lorio and Sommers (1986). The outermost layer of the bark was removed with a barking knife to smoothen the surface, and a round hole (15 mm diameter) was punched through the remaining bark and the phloem. The removed bark and phloem plugs were put in plastic bags and taken to the laboratory for phenolic analysis. An aluminium trough and a test tube were fastened below the hole to collect the resin extruding from the wound. Two such holes were bored on opposite sides of the stem at 1.3 m height on each study tree. The test tubes and troughs were removed after 24 h. However, although we had successfully used this method on Scots pine, it did not work at all on spruce. At the end of the sampling period there was no resin in any of the 96 test tubes; only tiny resin droplets had developed in the wounds and on the cut bark surface. Therefore, the resin flow was measured in May/June 1994 using another method described by Christiansen and Horntvedt (1983). Eighty-millimetre-long plastic tubes with an inner diameter of 4 mm were cut at an angle and fitted into holes cut into the cambium through the bark with a cork borer. The length of the resin column in the tubes was measured after 24 h. Seven tubes were inserted at different points around the stem (1.3–1.7 m height) on each tree. In Experiment 401 the original, previously wounded sample trees were used. In order to compare resin flow between unwounded and previously wounded trees, three undamaged trees in each treatment were also included in the study. In Experiment 404 all the study trees were previously unwounded ones.

The bark was separated from the phloem in the

Table 3
The amounts of nutrients applied in the different fertilizer treatments. One third of the nitrogen was ammonium nitrate and two thirds methylene urea

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	S (kg ha^{-1})	B (kg ha^{-1})	Cu (kg ha^{-1})	Zn (kg ha^{-1})
F1	–	31	56	98	61	37	1.3	0.8	0.8
F2	150	31	56	98	61	37	1.3	0.8	0.8
F3	150	31	56	418	111	37	1.3	0.8	0.8

bark plug samples from Experiment 401 and the two phloem discs from each tree were combined into one sample. The samples were dried (+40°C, 48 h), ground, and extracted in 80% methanol for 30 min. The concentration of total phenols was determined by UV-spectrophotometry using Folin-Ciocalteu's phenol reagent.

2.3. Biotests with the blue-stain fungus

To test the effects of fertilization on the resistance of the trees against bark beetles, the sample trees were artificially inoculated with *Ceratocystis polonica* (Siem.) C. Moreau, an *Ips typographus* L.-associated pathogenic blue-stain fungus. The fungus induces wound reactions (resin flow and a characteristic lesion in the phloem) on spruce (Solheim, 1988). The inoculation procedure described by Solheim (1988) was used, the fungal isolate originating from southern Finland. A bark plug was removed with a 5-mm cork borer, a piece of malt agar with actively growing mycelium was inserted into the hole, and the bark plug was replaced. An additional control inoculation was made on five trees with a piece of sterile malt agar. Four inoculations were made on each tree, two on the southern and two on the northern side of the stem at 1.3 m height. In Experiment 401 the inoculations were made simultaneously with the first unsuccessful attempt to measure the resin flow in August 1993. The wounds for resin measurements were made on the eastern and western sides of the stems. In Experiment 404 the inoculations were made, simultaneously with the fitting of the resin measurement tubes, on the southern and northern sides of the stems. After 5 weeks the length of the resin stripe below the inoculation wound was measured. The outer bark was removed and the length and width of the lesion around each inoculation were measured.

2.4. Tree growth and vigour

In Experiment 401 two increment cores were taken from each tree at 1.3 m height in June 1994. The border between the sapwood and heartwood, based on the differential translucence, was marked on the cores (Münster-Swendsen, 1987). The annual growth rings and the width of the sapwood and heartwood were measured on the cores. A tree vigour index was calculated as BA_1/SA, where BA_1 is the cross-sectional area of the 1993 annual ring and SA is the sapwood basal area (Waring et al., 1980). After the growth measurements the cores were cut longitudinally and the number of vertical resin canals in the cross-sections of the 1991, 1992, and 1993 annual rings counted under a stereo microscope. The cores were 5 mm in diameter, but the examined area was only 4 mm wide, owing to the fact that a 0.5-mm section on each side was excluded because the tissue was often disrupted. Five cores had to be rejected because they had been snapped, and resin canals were therefore counted in altogether 115 cores.

2.5. Statistical analyses

For testing the statistical significance of the differences between treatments and sub-experiments and of the bivariate and multiple linear correlations we used analysis of variance, the Tukey test, and the Pearson correlation test included in the BMDP programs.

3. Results

The fertilizer treatments had no significant effect on the resin flow. In Experiment 404 the resin flow

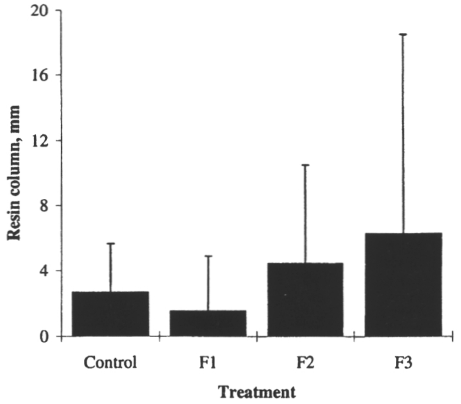

Fig. 1. Length of resin columns in Experiment 404, measured in May 1994. Twelve trees in each treatment, seven measurements per tree. See Table 2 for treatment explanations. The differences between treatments were not statistically significant ($F = 0.99$, df = 3, $P > 0.4$).

was quite weak, probably due to the low temperatures (max. +16°C, min. +5°C) during the sampling period, and the variation both within and between trees was considerable (Fig. 1). The resin column was usually long on parts of the stem exposed to sun, while on shaded parts there was often no resin flow at all. In Experiment 401 the sampling was done during a later and warmer (max. +24°C, min. +8°C) period, and empty resin tubes were less frequent. The resin flow in Sub-experiment 401b was stronger than that in 401a (Table 4). In Sub-experiment 401a the previously unwounded trees had significantly weaker resin flow than the previously wounded and inoculated trees (Fig. 2).

The fertilizer treatments had no effect on the phenolic concentration of the phloem, but the sub-experiments differed significantly from each other (Table 4). No significant correlation was found between the resin flow and the phenolic concentration of the phloem.

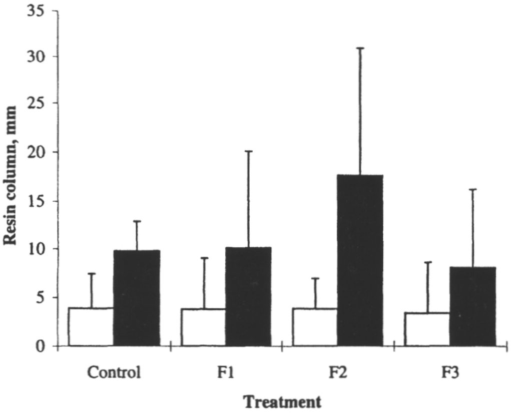

Fig. 2. Length of resin columns in one block of Experiment 401a in June 1994. Dark columns represent trees that had been wounded and inoculated with blue-stain fungus in 1993. Light columns represent previously unwounded trees. Each treatment includes three trees with seven measurements on each tree. See Table 2 for treatment explanations. There were no statistically significant differences between fertilizer treatments. The resin flow was significantly stronger in the previously wounded trees than in the unwounded ones ($F = 6.57$, df = 1, $P < 0.05$).

The length of the resin stripe caused by inoculation of the blue-stain fungus correlated positively with the resin column length in both experiments (Fig. 3). Fertilizer treatments did not affect the length of the resin stripe in Experiment 401 (Table 4). In Experiment 404 none of the treatments differed significantly from the control, but treatments F2 and F3 differed significantly from each other ($F = 3.12$, df $= 3$, $P < 0.05$). The difference, unless it was incidental, indicates that liming in addition to the nitrogen-containing fertilizer treatment enhanced resin flow from the inoculation wounds.

Table 4
Comparison between Sub-experiments 401a and 401b. Six trees in each treatment. F-values ($^{*} = P < 0.05$) from the analysis of variance at the bottom of the table

		Sapwood area 1993 (cm^2)	Phloem phenolics 1993 ($mg\ g^{-1}$)	Resin stripe 1993 (cm)	Lesion length 1993 (mm)	Vigour index 1993	Diameter growth 1993 (mm)	Diameter growth 1994 [a] (mm)	Resin column 1994 (mm)
401a	Control	294.3	34.07	9.0	40.9	0.032	1.2	0.2	7.4
	F1	243.7	39.05	17.4	33.8	0.039	1.5	0.2	11.0
	F2	284.6	39.18	11.9	33.3	0.052	1.9	0.1	9.7
	F3	259.9	40.16	10.9	33.0	0.039	1.4	0.1	9.4
401b	Control	219.2	45.14	16.6	40.2	0.043	1.6	0.3	13.0
	F1	299.4	45.63	16.9	75.2	0.060	2.3	0.4	19.0
	F2	321.4	45.00	17.9	55.8	0.062	2.3	0.6	12.7
	F3	280.2	41.61	18.2	53.1	0.083	2.9	0.5	14.9
Location (df = 1)		0.05	6.31 *	2.75	11.15 *	19.94 *	14.63 *	47.05 *	4.12 *
Treatment (df = 3)		0.24	0.26	0.34	0.97	4.76 *	3.06 *	1.25	0.59
Interaction (df = 3)		0.05	0.63	0.39	1.91	2.60	1.82	2.13	0.14

[a] Only the early growth, measured 16/6/1994.

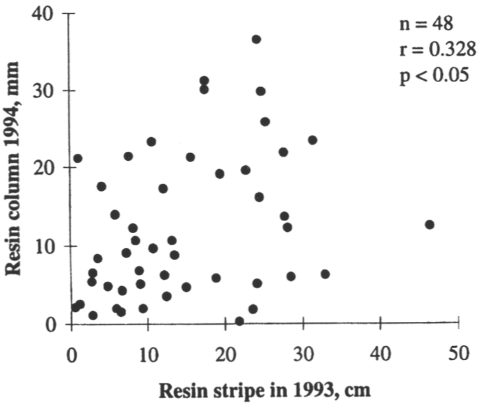

Fig. 3. The correlation between the length of the resin column, measured in June 1994, and the length of the resin stripe from the inoculation wound, measured in September 1993 in Experiment 401. In Experiment 404, where both measurements were made in May–June 1994, the statistics were $r = 0.658$, $P < 0.001$, $n = 48$.

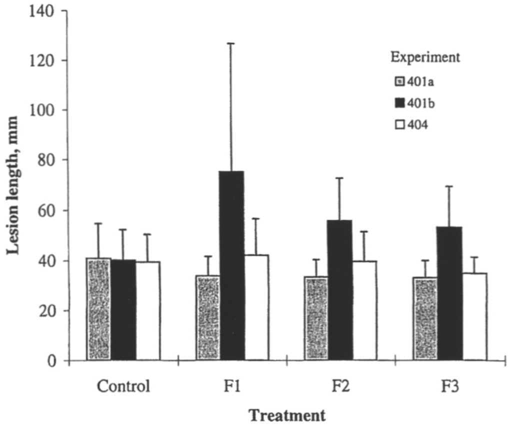

Fig. 4. Length of the lesion caused by the blue-stain fungus in the phloem 5 weeks after inoculation. The differences between treatments (see Table 2) were not statistically significant.

The inoculated fungus had spread in the phloem and produced a reaction zone (lesion) around all the inoculation points. The fungus had mainly grown both up and down the fibres at a similar rate. Growth to the sides had been very modest, only 2–3 mm. In the control wounds, where no fungus was inoculated, there was only a 2–3-mm-wide dark necrotic area around the wound, but no actual lesion. The resin flow from the control wounds was also weaker than that from the inoculation wounds. There was no correlation between the length of the resin stripe or the resin column and the length of the lesion caused by the fungus. Lesion length was not affected by the fertilizer treatments, but there was a significant difference between the sub-experiments in 401 (Fig. 4, Table 4). Lesion length and the phenolic concentration of the phloem did not correlate ($r = -0.107$, $P = 0.4$, $n = 48$).

Although Sub-experiments 401a and 401b did not differ as regards tree age, diameter or sapwood cross-sectional area, there was a significant difference in stem diameter growth between the sub-experiments (Table 4). Trees in 401b grew more and apparently also started growing earlier, because in June the 1994 annual ring width was already about 20% of the previous year's total growth, whereas in 401a the 1994 increment width was only about 11% of the 1993 increment. In the present material the vigour index more or less directly reflected the stem diameter growth ($r = 0.822$, $P < 0.001$, $n = 48$). The vigour index was affected by the treatments, in both sub-experiments the index was lowest in the control treatment (Table 4). There was also a significant difference in vigour index between the sub-experiments. Lesion length correlated positively with both diameter growth (Fig. 5) and vigour index ($r = 0.305$, $P < 0.05$, $n = 48$). Lesion length correlated better

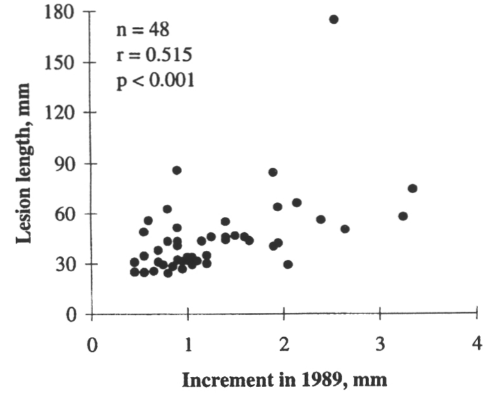

Fig. 5. The correlation between the length of the lesion caused by the blue-stain fungus in the phloem in 1993 and the diameter growth of the stem in 1989 (before fertilization) in Experiment 401. The corresponding correlation between lesion length and diameter increment of 1993 was $r = 0.374$ ($n = 48$, $P < 0.01$).

with the 1989 increment than with the 1993 increment (Fig. 5). Resin flow did not correlate with growth or vigour index.

Most of the vertical resin canals were located in the late-wood or in the latter part of the early-wood. The number of canals per annual ring (4-mm-wide section) examined varied between 0 and 5. The 1993 ring in particular lacked resin canals; 99 cores out of 115 had no vertical resin canals in the 1993 ring. The mean number of resin canals per ring section was thus only 0.22 (mode and median 0) in the 1993 ring, whereas in the 1992 ring the mean number was 1.54 (mode 0, median 2), and in the 1991 ring 1.26 (mode 0, median 1). To avoid having to deal with a large number of zero values in the computations, we used the sum of the canals in all three annual rings in both cores from each tree. In the whole material the average number of vertical resin canals per mm^2 cross-sectional area was 0.18. Although the number of canals was lowest in the control and highest in the F3 treatment in both sub-experiments, the difference was not statistically significant. The mean number of resin canals was higher in Sub-experiment 401b than 401a ($F = 4.22$, $P < 0.05$, df = 1). The number of resin canals in the 1991–93 annual rings correlated positively with diameter growth in 1991–93 ($r = 0.543$, $P < 0.001$, $n = 60$) and the vigour index of 1993 (Fig. 6), as well as with heartwood basal area ($r = 0.335$, $P < 0.01$, $n = 60$). There was no correlation between the number of resin canals and the resin flow, the phenolic concentration of the phloem, or the length of the lesion caused by the fungus.

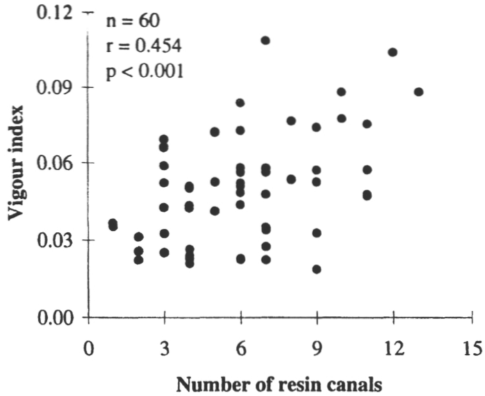

Fig. 6. The correlation between the vigour index of 1993 and the number of vertical resin canals in two 4-mm-wide sections of the 1991, 1992, and 1993 annual rings.

4. Discussion

Although the non-linearity of the suggested relationship between nutrient availability and the amount of carbon-based allelochemicals (Tuomi et al., 1988) impedes prediction of the effect of a growth-promoting fertilization treatment on defence, the theories lead us to expect a decrease in the defence level. In our experiments fertilization had, somewhat surprisingly, no clear effect on any of the measured resistance traits. As the growth response of the trees to fertilization was clear, we therefore conclude that there was no straightforward trade-off in resource allocation between growth and defence in this material. The only indication that enhanced growth might reduce the level of resistance was the modest positive correlation between lesion length and annual ring width. The fact that the resistance against the blue-stain fungus correlated negatively with stem diameter increment, and especially with the pre-fertilization increment, might be an indication of a genetical rather than a physiological trade-off between growth and defence. This might explain why there was no difference in resistance between the control and fertilized treatments in spite of the fertilizer-induced difference in growth. Furthermore, the different traits of resistance did not seem to be interdependent within a single tree. Our results did not indicate that a tree individual could be classified according to the level of overall resistance against all types of harmful organisms.

The fact that the sub-experiments (with trees of different stands) differed so much in their response to fertilization (in spite of the similar age, size and stand structure) suggests that in 'normal' conditions, as opposed to sites exposed to heavy stress factors, resource allocation to defence might be regulated genetically rather than by the availability of resources. Sub-experiments 401a and 401b had similar diameter growth histories because the age and average diameter were alike in both locations. During the short study period, however, the sub-experiments differed with respect to diameter growth and vigour index, as well as resin flow, reaction to the blue-stain fungus, and phenolic concentration of the phloem. The nutrient concentrations in the needles before fertilization were higher in 401a than 401b, and it would appear that the trees in Sub-experiment 401b had benefited more from the fertilization than the trees in 401a. This could either be due to differences in the ability (plasticity) of the trees to utilize the additional nutrients or to some stand characteristics that affect nutrient uptake, e.g. availability of nutrients or water in the soil. It seems that, despite the fact that we can usually at least partially predict the growth response to fertilization, it is far more difficult to predict the effects on defence mechanisms. The present experiments were established to test the effect of different fertilizers on the vitality of the stands. From that point of view the fact that there does not seem to be a physiological trade-off between growth and defence means that increased growth, a sign of improved vitality, does not inevitably lead to a reduced defence level and thus diminished vitality.

Phenolics inhibit fungal growth in the wood (Johansson and Stenlid, 1985). In our experiment the concentration of phenolics in the phloem was not affected by fertilization. In addition to its constitutive defence, Norway spruce reacts to attack by initiating the biosynthesis and accumulation of phenolics in the zone surrounding the fungal pathogen (Stenlid and Johansson, 1987). Induced defence is very important in the inhibition of *Ceratocystis polonica* attack, because the fungus is more sensitive to components in lesion resin than in preformed resin (Solheim, 1991). The fact that the reaction to inoculation with the fungus was similar in all treatments suggests that the induced defence against fungi was also more or less unaffected by fertilization.

Resin flow increased in the trees that had been wounded prior to the resin flow measurements. Whether this reaction was primarily induced by the mechanical wounding associated with the first unsuccessful resin flow measurements, or by the attack of the inoculated blue-stain fungus, remains unknown. However, in the light of earlier studies it seems likely that both of these factors were involved (Nylinder, 1951; Kärkkäinen, 1981; Christiansen and Horntvedt, 1983; Solheim, 1988). The fertilizer treatments did not reduce the production of induced resin. As was the case for lesion length, the frequency of vertical resin canals (both per annual ring and per mm^2) was also unaffected by the fertilizer treatments, although there was a positive correlation between growth and the number of resin canals. There was large variation in resin canal frequency between years. In agreement with Stephan's (Stephan, 1967) findings on Scots pine, we found more resin canals in wide annual rings than in narrow ones, but when counted per mm^2 the frequency was lower in wide rings. The increment cores on which the resin canals were counted were taken between the wounding points and therefore did not include traumatic resin ducts that are formed close to the wounded area in Norway spruce (Bannan, 1936; Nylinder, 1951). Although the occurrence of primary resin canals is probably of some importance in the success of a bark beetle attack, the frequency of vertical resin canals did not predict the amount of resin flow from the stem.

The readiness for defence varied within as well as between trees, and it did not seem to be very much affected by increased resource availability following fertilization. The induced defensive reaction in the stems was quite evident in our study. As no statistically significant effect of fertilization on induced resin production was detected, it would appear that an increase in growth due to enhanced nutrient availability neither diminished nor enhanced the readiness of the 'threatened' trees to allocate assimilates into defence. However, when it comes to revitalizing badly stressed trees, for example on severely polluted sites, the outcome might be quite different from that in the present experiments.

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Paper III

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Effects of vitality fertilization on the resin flow and vigour of Scots pine in Finland

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Abstract

The vitality and defence of Scots pine were measured in order to study how different fertilizer combinations affect these traits, and to determine whether an increase in growth leads to reduced defence as predicted by the theories based on the assumption of the existence of a trade-off between growth and carbon-based defence compounds. Stem diameter growth and vigour index were measured as indicators of vitality and stem resin flow and the concentration of phloem phenolics were used as indicators of defence level. Fertilizer treatments containing nitrogen had a growth-promoting effect and the vigour index was higher in the nitrogen-fertilized treatments than in the control and non-nitrogenous fertilizer treatments. Four of the ten experimental sites were situated along a heavy metal pollution gradient at 0.5, 2.5, 4 and 8 km distance from the emission source. The vigour index in the severely polluted site closest to the emission source was considerably lower than that in the other three sites along the gradient, but even there nitrogen gave a clear growth response. In spite of the large within- and between-tree variation in resin flow, the average resin exudation differed little between the experimental sites. In most sites the resin flow was highest in the control treatment. A weak negative correlation was found between resin flow and vigour index. In the pollution gradient the mean resin flow was lowest at the most polluted site and increased along the gradient up to 4 km from the emission source. The phenolic concentration of the phloem was not affected by fertilization in any of the experiments apart from the severely polluted one. Indications of a weak negative linear correlation between vigour index and phloem phenolics were found. There was no significant correlation between the phenolic concentration and the amount of resin flow in any of the different sites. Except for the liming treatment in the polluted experiments, we did not detect any response to non-nitrogenous fertilizer treatments.

Keywords: *Pinus sylvestris*; Phenolics; Defence; Resistance; Forest fertilization; Vigour index

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1. Introduction

The defence of pines against pest insects and microbes is believed to rely largely on carbon-based secondary compounds (Herms and Mattson, 1992). Resin flow from wounded stems protects trees against actively attacking xylophagous insects and airborne microbes that would otherwise infect a wound. If the insect or fungus succeeds in penetrating the bark, the invasion can be stopped by high concentrations of various carbon-based secondary metabolites like phenolics (Kosuge, 1969), that are inhibitory or toxic to the pest organisms. In addition to the constitutive

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defenses based on preformed and stored substances, the tree can further defend itself using induced resistance by synthesizing resin and defensive chemicals around the wounded area in response to attack (Berryman, 1986).

Theories dealing with carbon allocation to growth and defence predict that, under moderate nutrient limitation when growth is limited but photosynthesis is not, excess carbohydrates accumulate and are used in the synthesis of carbon-based secondary metabolites (Bryant et al., 1983). Consequently, increased growth results in decreased defence because less carbon is converted to carbon-based secondary metabolites. Fertilization with growth-limiting nutrients, usually nitrogen, increases tree growth while photosynthesis is not immediately affected. According to the carbon-nutrient balance (Bryant et al., 1983) and growth-differentiation hypotheses (Loomis, 1932; Lorio, 1986), this should lead to reduced concentrations of carbon-based secondary metabolites at sites with a moderate nutrient deficiency (Herms and Mattson, 1992, Tuomi, 1992).

Acidification and the deposition of nitrogen and air pollutants change nutrient availability and nutrient ratios in the forest soil and affects the whole forest ecosystem (Waring, 1985). Stress-induced changes in trees, as well as a range of pollutants, directly affect tree-feeding herbivores in a number of ways (Heliövaara and Väisänen, 1993). Human impact can severely disturb species composition and tree-herbivore relations, which in turn accelerates the decline of the ecosystem. As a balanced availability of nutrients is an important factor affecting tree vitality, amelioration measures can sometimes help to prevent or alleviate forest decline. Vitality fertilizers differ from traditional fertilizers in that the primary goal of the fertilization is, instead of increasing wood production, to improve tree resistance, to stabilize nutrient cycling, improve nutrient availability and decrease soil acidity for a long period of time (Huettl and Hunter, 1992; Aarnio et al., 1995). Traditionally, in order to obtain economic profit from fertilization, easily soluble nitrogen fertilizers are used because a quick growth response to the fertilizer investment is required. In the vitality fertilizers used in this study most of the nitrogen, if included at all, is in a slow release form in order to obtain an effect that is less intense but lasts for a considerably longer period of time than with traditional fertilizers (Martikainen et al., 1989).

An extensive series of field trials has been established in different parts of Finland to study the possibility of using fertilizers for improving the vitality and pest resistance of trees, alleviating soil acidification, improving soil biological activity, and maintaining balanced nutrient cycling. If growth and defence are alternative competing sinks for assimilates (Herms and Mattson, 1992), it is problematic to find measures that simultaneously increase growth (as a sign of improved vitality) and improve resistance.

The aim of this study was to survey how different kinds of fertilizer combinations aiming at enhancing the vitality of Scots pine (*Pinus sylvestris* L.) affect the constitutive defenses of the trees during the first few years after fertilization. We used stem resin flow and phloem phenolic concentration as indicators of the defence level, and tried to determine whether increased growth was reflected in these defence traits, as predicted by the theories based on the assumption of the existence of a trade-off between growth and carbon-based defence mechanisms.

2. Materials and methods

2.1. Experimental design

The resin flow of Scots pine was measured in ten sites in different parts of Finland (Fig. 1, Table 1). The experimental sites belong to a larger trial series, the aim of which is to study how various so-called vitality fertilizers affect trees and stands. The primary reason for repeating the measurements in several sites instead of just one was to attain results that could be generalized and expected to apply also in other boreal pine stands. Site 502 is on a drained peatland and the other nine on mineral soil. Four of the experimental sites (411–414) were established along a heavy metal pollution gradient in the Harjavalta area close to a heavy metal smelter (Helmisaari et al., 1995). In site 411, located 0.5 km from the emission source, there was very little ground vegetation left, the trees were in an extremely poor condition, and hardly any living pine roots could be found in the upper 30 cm soil layer. In site 412, 2.5

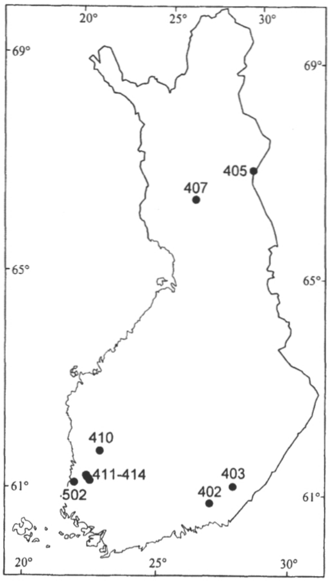

Fig. 1. The locations of the sites included in the experiment.

km from the industrial plant, the signs of pollution were not as clearly visible. The trees on this site seemed to suffer considerably less from the pollution than those in site 411, but the ground vegetation was sparse. In sites 413 and 414, 4 km and 8 km from the industrial plant respectively, the trees did not show visible signs of damage attributable to pollution.

The experiment on each site consisted of 3 or 4 replications of different fertilizer treatments on randomized plots in 40–80 year old pine stands. The size of each plot was 30 × 30 m, and the plots were 10 meters apart. The fertilizers were spread by hand on the plots and up to 3 m distance around the plots (a 4 m zone between plots remained unfertilized) in May/June 1991 or 1992. There were four different fertilizer treatments and an unfertilized control treatment in sites 402, 403, 405, 407 and 410 (Table 2). Treatments F1 (test fertilizer with no nitrogen), F2 (test fertilizer containing nitrogen, of which one third was ammonium nitrate and two thirds methylene urea) and F3 (fertilizer F2 combined with dolomitic limestone) were the same in all the above sites. Treatment F4 was designed specifically for each site, based on needle and soil nutrient analysis, to provide a balanced and adequate supply of macro- and micronutrients (Jukka, 1988). In the pollution gradient sites (411–414) there were three fertilizer treatments and an unfertilized control (Table 2). Treatments H1 (dolomitic limestone and boron) and H2 (test fertilizer with no nitrogen) were the same in all four sites. Treatment H3 was designed individually for each site to provide the nutrients that were deficient according to needle and soil analysis. In the peatland site (502) there were four fertilizer treatments and an unfertilized control (Table 2).

2.2. Sampling and measurements

Three trees standing closest to the edge of the plot proper were chosen for resin sampling. The trees within the plots could not be used for destructive sampling because it would have disrupted the 15-year

Table 1
The site characteristics. TGS = length of the thermal growing season. The values for normal precipitation and temperature sum (threshold +5°C) are means of the 1961–90 period

Site	TGS (day)	Temperature sum (d.d.)			Precipitation (mm)			Soil type	Site index
		normal	1992	1993	normal	1992	1993		H_{100}
402	166	1301	1372	1115	630	605	547	fine sand	18
403	163	1302	1368	1094	615	594	568	fine sand	20
405	122	701	736	585	532	812	541	loamy sand	17
407	129	855	877	717	526	785	524	fine sand	14
410	160	1163	1213	1015	598	705	602	coarse sand	17
411	165	1277	1322	1133	568	642	583	fine sand	20
412	165	1279	1324	1134	569	641	582	fine sand	20
413	165	1276	1321	1131	569	641	582	fine sand	19
414	165	1276	1323	1132	571	640	581	fine sand	23

Table 2

Fertilizer treatments, application dates, and resin sampling dates in the different sites. The 'F'-treatments were used in sites 402–410. Treatments F1, F2 and F3 were similar in all sites, treatment F4 was specially designed for each site based on needle and soil nutrient analysis. The 'H'-treatments were used in the pollution gradient sites 411–414, where treatments H1 and H2 were similar in all sites and treatment H3 specially designed for each site. The 'P'-treatments were applied in the peatland site 502

Treatment		N (kg/ha)	P (kg/ha)	K (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	S (kg/ha)	B (kg/ha)	Cu (kg/ha)	Zn (kg/ha)	Application date	Sampling date
F1		—	31	56	98	61	37	1.3	0.8	0.8		
F2		150	31	56	98	61	37	1.3	0.8	0.8		
F3		150	31	56	418	111	37	1.3	0.8	0.8		
F4	site 402	150	—	—	320	50	—	—	—	—	15/6 1991	10–11/8 1993
	site 403	120	40	—	320	90	—	—	—	2.0	20/6 1991	15–16/7 1992
	site 405	120	—	—	320	50	—	1.0	—	—	4/6 1991	4–5/8 1992
	site 407	120	—	—	—	—	—	2.0	—	—	27/5 1991	3–4/8 1992
	site 410	120	—	50	320	90	—	1.0	4.0	—	31/5 1991	22–23/7 1992
H1		—	—	—	640	100	—	2.0	—	—		
H2		—	31	56	98	61	37	1.3	0.8	0.8		
H3	site 411	150	—	—	480	125	—	—	—	—	26/5 1992	5—6/8 1993
	site 412	150	—	—	320	80	—	—	—	—	2/6 1992	5–6/8 1993
	site 413	150	30	—	320	80	—	—	—	—	8/6 1992	4–5/8 1993
	site 414	120	—	—	—	—	—	—	—	—	10/6 1992	12–13/8 1993
P1		—	—	81	4	19	—	1.0	—	1.0	7/5 1991	3–4/8 1993
P2		—	46	81	111	21	—	1.0	—	1.0		
P3		—	47	81	153	84	—	1.0	—	1.0		
P4		100	47	81	153	84	—	1.0	—	1.0		

growth and yield study. Since the fertilizers were also spread around the plots on a ca. 3 m belt, most of the root systems of the sample trees were affected by the fertilizer treatments.

The resin sampling in most of the sites was performed at the end of the second growing season after fertilization, and in sites 402 and 502 at the end of the third. Resin was sampled according to the method of Lorio and Sommers (1986) and Lorio et al., 1990. Part of the outer bark was removed to smoothen the sampling surface. A round wound (15 mm diameter) was then punched through the remaining bark and phloem on opposite sides of the stem at 1.3 m height. The bark and phloem were removed from the wound and placed in a plastic bag. An aluminium trough was pinned to the bark below the wound, and a plastic test tube attached to the stem below the trough with adhesive tape. Each tube was individually numbered and weighed beforehand. The wound and the tube opening were then covered with 6 cm wide adhesive tape to prevent rainfall from running into the tube. In sites 403 and 410 the resin volume was determined ocularly after 4 and 24 h. In all the sites the resin tubes were removed after 24 h, sealed and taken to the laboratory. The bark and phloem discs were frozen for later phenolic analysis.

In the laboratory each resin tube was weighed and the net weight of resin calculated. The bark was removed from the phloem discs. The phloem discs were dried (+40°C, 48 h) and milled, the two discs from each tree being combined into one sample. The samples were extracted in 80% methanol for 30 min at room temperature, and the total phenolic concentration in the phloem was determined by UV spectrophotometry using Folin–Ciocalteu's phenol reagent.

Two increment cores were taken at 1.3 m height from each sample tree in April 1994 for growth and vigour measurements. The border between the sapwood and heartwood was marked on the fresh cores with a pen. The annual increments and radius of the sapwood and heartwood were measured on the cores.

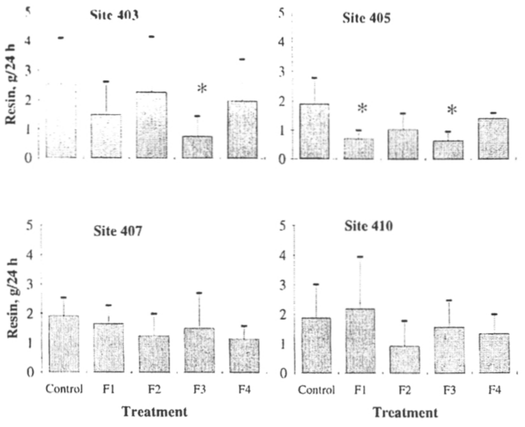

Fig. 2. Resin flow in different fertilizer treatments at the end of the second growing season after fertilization in sites 403, 405, 407 and 410. The asterisk indicates statistically significant ($p < 0.05$) difference from the control treatment according to Dunnett's test. See Table 2 for treatment explanations and Table 3 for ANOVA statistics.

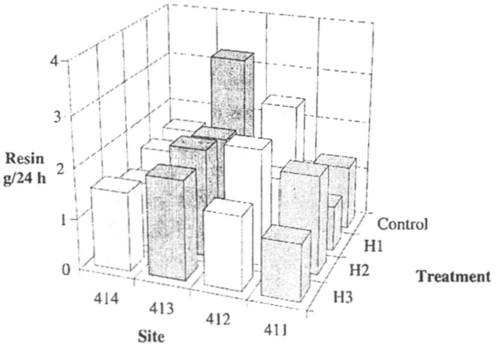

Fig. 3. Resin flow in different fertilizer treatments at the end of the second growing season after fertilization in the Harjavalta pollution gradient sites 411–414. The sites were at 0.5 km (411), 2.5 km (412), 4 km (413) and 8 km (414) distance from the emission source. See Table 2 for treatment explanations and Table 3 for ANOVA statistics.

Table 3
Analysis of variance statistics for the effect of site and fertilizer treatment on the resin flow in sites 403, 405, 407 and 410 (upper set) and 411–414 (lower set). See Fig. 2 and Fig. 3 for resin flow means

Source	F	df	p
Site	2.66	3	0.0610
Treatment	3.28	4	0.0205
$S*T$	1.47	12	0.1763
Site	5.59	3	0.0030
Treatment	2.75	3	0.0565
$S*T$	0.96	9	0.4918

Table 4
The phenolic concentration (mg/g) of the phloem in different sites and treatments. Standard deviations in parentheses. In site 502 the treatments are different from the other sites (P1–P4 instead of F1–F4). See Table 2 for treatment explanations

Site	Treatment					ANOVA		
	control	F1	F2	F3	F4	*F* value	df	*p*
402	26.8 (5.1)	28.1 (4.8)	30.4 (14.2)	25.8 (5.1)	23.6 (4.4)	0.98	4	0.43
403	20.3 (1.1)	20.1 (5.0)	17.5 (2.7)	18.5 (4.8)	19.3 (4.3)	0.7	4	0.59
410	14.9 (3.9)	15.1 (2.8)	14.4 (3.4)	16.6 (2.3)	13.8 (2.4)	1.15	4	0.35
502	29.2 (4.6)	30.5 (7.8)	24.8 (8.8)	36.1 (10.8)	28.4 (5.6)	2.36	4	0.07

The vigour index was calculated as BA_1/SA, where BA_1 is the cross-sectional area of the 1993 annual ring and SA is the sapwood basal area (Waring et al., 1980).

2.3. Statistical analyses

Analysis of variance with plots (mean value of the three sample trees) as replicates was used to test the differences in resin flow, phenolic content, and tree vigour between treatments and sites. $\text{Log}(x+1)$ transformations were used to normalize the distributions in the resin flow data before testing. When significant differences between sites were found in the analysis of variance, Tukey's test was used for pairwise multiple comparisons. Fertilizer treatment effects were tested with Dunnett's test. The within-tree variation in resin flow was tested using the matched *t*-test. The mean value of the two resin flow and increment core measurements of each tree was used for computing the bivariate correlations with the Pearson correlation test.

3. Results

3.1. Resin flow and phenolic concentration of the phloem

In most of the sites the resin flow was highest in the control treatment. The treatment effect was significant in the analysis of variance, but there was no indication of any particular fertilizer treatment consistently having had any especially strong effect on resin flow throughout the series of sites (Figs. 2 and 3, Table 3). Despite the different geographical locations and genetical backgrounds of the stands, there was no statistically significant difference in resin flow between the sites, apart from the pollution gradient, that were sampled at the end of the second growing season after fertilization (Table 3). In sites 402 and 502, which were sampled at the end of the third growing season after fertilization, there were no significant differences between control and fertilizer treatments.

In the four sites in the Harjavalta pollution gradient there was significant difference in mean resin flow between the sites (Table 3). The mean resin flow in site 411, which was the heavily polluted site closest to the smelter, was the lowest, and it increased along the gradient up to site 413 (4 km from the emission source) (Fig. 3). The resin flow in the control treatment of site 413 was higher than that in any of the other nine sites sampled in the series. The resin flow pattern along the Harjavalta gradient was similar to that in the other sites in the sense that, apart from site 411, the mean resin flow was highest in the control treatments (Fig. 3). According to the analysis of variance, however, the treatment effect only approached significance (Table 3). The resin flow was lowest in the liming treatment (H1). In

Table 5
The phenolic concentration (mg/g) of the phloem in different sites and treatments along the Harjavalta pollution gradient. Standard deviations in parentheses. See Table 2 for treatment explanations

Site	Treatment				ANOVA		
	Control	H1	H2	H3	*F* value	df	*p*
411	27.3 (4.8)	36.2 (9.8)	37.8 (5.6)	32.1 (6.3)	4.12	3	0.014
412	20.5 (6.1)	22.8 (3.8)	24.2 (11.6)	22.3 (4.2)	0.41	3	0.75
413	23.9 (6.5)	22.4 (4.9)	24.2 (4.5)	21.1 (6.6)	0.77	3	0.51
414	26.2 (5.7)	26.2 (7.9)	30.2 (17.5)	22.1 (5.3)	0.91	3	0.45

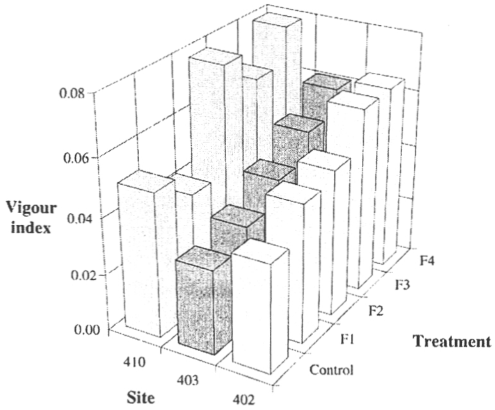

Fig. 4. The index of vigour in different treatments in sites 402, 403 and 410. See Table 2 for treatment explanations and Table 6 for ANOVA statistics.

experiment 414, the site least affected by pollution, the resin flow level was more or less the same in all treatments. There was no significant site/treatment interaction effect along the pollution gradient.

There was no statistically significant difference in the resin flow of the unfertilized trees between the different sites ($F = 1.50$, $\mathrm{df} = 9$, $p = 0.21$). The amount of resin flow varied greatly between and even within trees, but there was no significant difference in resin flow between the eastern and western side of the tree. The linear correlation between resin flow after 4 and 24 h was strong (in site 403 $r = 0.94$, $p < 0.001$, $n = 90$, and in site 410 $r = 0.98$, $p < 0.001$, $n = 88$), which indicates that the trees with intense resin flow immediately after wounding were able to maintain a high flow rate throughout the 24 h period. In site 403 nearly 50% and in site 410 approximately 75% of the total amount of resin collected during the 24 h period was exuded within the first 4 h.

The total phenolic concentration of the phloem differed significantly between sites 402, 403 and 410 ($F = 58.17$, $\mathrm{df} = 2$, $p < 0.001$). In the case of the pollution gradient, the phenolic concentration in site 411, closest to the plant, was significantly higher ($F = 16.02$, $\mathrm{df} = 3$, $p < 0.001$) than in the other three sites (412–414); these three sites did not differ from each other according to Tukey's test. The phenolic concentration of the phloem was not affected by fertilization in any of the sites except for the severely polluted site 411 (Tables 4 and 5). In site 411 the phenolic concentration in the fertilized treatments was higher than that in the control, but only treatments H1 and H2 differed significantly from the control according to Dunnett's test. There was no significant correlation between the phenolic concentration and the amount of resin flow in any of the sites.

3.2. Growth and vigour index

Nitrogen had a growth-promoting effect, and the vigour index was higher in the nitrogen-fertilized treatments than in the control and non-nitrogenous

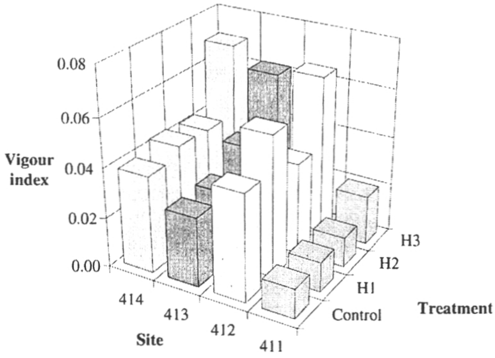

Fig. 5. The index of vigour in the different fertilizer treatments in the Harjavalta pollution gradient sites 411–414. See Table 2 for treatment explanations and Table 6 for ANOVA statistics.

Table 6

Analysis of variance statistics for the effect of site and fertilizer treatment on the vigour index in sites 402, 403 and 410 (upper set) and 411–414 (lower set). See Fig. 4 and Fig. 5 for vigour index means

Source	F	df	p
Site	10.39	2	< 0.001
Treatment	11.65	4	< 0.001
$S * T$	1.13	8	0.35
Site	44.56	3	< 0.001
Treatment	19.92	3	< 0.001
$S * T$	2.36	9	< 0.05

fertilizer treatments (Figs. 4 and 5). There was no marked difference in the growth response between treatments F2 and F3 (less soluble form of nitrogen) and treatment F4 (only fast-releasing nitrogen). As the measurements were made only 2–3 years after the fertilization it is still too early to determine how long the growth-promoting effect lasts and how the vigour is affected in the long run in the different treatments. There was a statistically significant difference in the vigour index between sites 402, 403, and 410 in the analysis of variance (Table 6). According to Tukey's test sites 403 and 410 differed from each other but not from site 402. In the case of the pollution gradient, the vigour index in the severely polluted site 411 was significantly lower than in the other three sites 412–414 (Fig. 5 and Table 6).

A negative linear correlation was found between resin flow and vigour index (Fig. 6). The correlation was always quite weak and in some sites not even statistically significant. However, the scattergram pattern for all sites was similar to the one shown in Fig. 6 in the sense that the upper right corner remained empty. The combination of high resin flow and high vigour index did not occur. Some indications of a weak negative linear correlation between the vigour index and phloem phenolics were also found, but the correlation was statistically significant only in experiment 412 ($r = -0.333$, $n = 36$, $p < 0.05$).

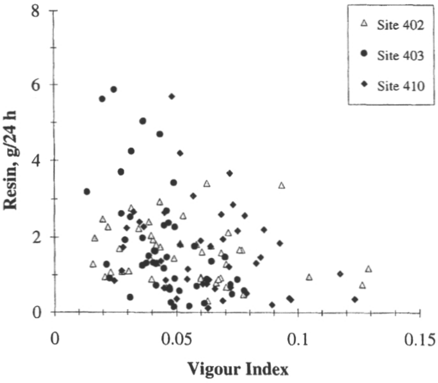

Fig. 6. The amount of resin flow plotted against the vigour index in experiments 402, 403 and 410. When the linear correlation was tested separately for each site, it was statistically significant only in site 403 ($r = -0.503$, $n = 45$, $p < 0.001$).

4. Discussion

Although the experiments were situated on relatively dry sites, the trees presumably did not suffer from a serious lack of water. Scots pines are well adapted to sandy soils and usually have deep root systems for water uptake. As nitrogen addition increased growth in all the experiments it can be assumed that growth was limited primarily by nitrogen availability. In compliance with the growth/differentiation and carbon/nutrient balance theories (Loomis, 1932; Bryant et al., 1983; Lorio, 1986), some indications of a negative impact of enhanced growth on resin flow were detected, but the link between growth and defence was by no means clear. It was also apparent that different defence traits cannot be treated as a whole, but instead should be considered as a series of loosely (if at all) connected characters. For example, the concentration of total phenolics seemed to be unaffected by changes in the growth rate of the trees and did not correlate with the resin flow. This is in accordance with the results for southern pines, where phloem tannin concentrations did not correlate with the growth rate or the site factors controlling it (Tiarks et al., 1989). In the study of Bridgen and Hanover (1982), the physiological traits associated with the oleoresin physiology of Scots pine were genetically independent of each other, and in most cases did not correlate with each other. A noteworthy feature in the results of this experiment was that high resin or phenolic amount did not seem to appear together with high vigour index, in spite of the large variation in resin flow and phenolic concentration and weak correlations with vigour index. Whether this combination was infeasible because of resource based trade-offs or genetic constraints is impossible to resolve without knowing the resource limitations, assimilation capacity and allocation patterns of the trees (Mole, 1994).

The trees in the experiments along the pollution gradient, especially the ones closest to the smelter, were visibly subjected to very heavy stresses and were suffering from an imbalanced nutrition and presumably also toxic concentrations of heavy metals in the soil (Fritze et al., 1996). Even in the most polluted experiment (411) nitrogen application increased growth and the vigour index substantially. Also liming seemed to have affected the trees in the most polluted area by enhancing the phenolic concentration of the phloem. In the treatment with both limestone and nitrogen application the phenolic concentration was not significantly higher than the control. However, whether this was because nitrogen somehow counteracted the lime-induced increase in phenolics, or because of increased growth in the nitrogen-fertilized treatment remained unsolved. The mechanisms through which the phenolic concentration could be affected by liming also remain open for speculation. It can be assumed that liming to some extent detoxified the soil by immobilizing heavy metals in the worst polluted surface layer. Furthermore, the higher pH and increased microbial activity (Fritze et al., 1996) probably had positive effects on nutrient mobilization and perhaps also on activities in and around the roots. However, growth was unaffected by liming when there was no nitrogen addition. Therefore if a relationship exists between growth and phenolic concentration, this would indicate a lime-induced increase in assimilation but not in growth, due to low nitrogen availability.

Except for the experiments suffering from heavy metal pollution, we did not get any response to non-nitrogenous fertilizer treatments. Of course the short time span between fertilization and the measurements should be kept in mind when interpreting the results. To register long-term effects of the different treatments we will have to wait another five or ten years. So far it is impossible to say with any certainty, for example, how long the effects of nitrogen application persist. In addition to short-term effects on the nutrient pool and other site qualities, the tree itself is also affected. For example, changes in foliage and root biomass after time affect the light interception within the crown and the uptake of water, which can lead to changes in the assimilation rate as well as in the allocation of the assimilates to various sinks.

The variation in average resin flow between the different experiments was surprisingly low in the light of the geographical (site, climate) and genetic differences. This could perhaps indicate that the present level of constitutive resin is close to the optimum and therefore more or less the same result of adaptive evolution has been acquired in the range of climatic and edaphic conditions found in Finland. Although growth and the amount of constitutive resin cannot be completely independent, our results indicate that the relationship is not very tight and predictable. However, in this study we did not examine the production of induced resin, which could have a more straightforward relationship to growth. The strong induced resin production of Scots pine has been well known and exploited for example in tar production in northern Europe during the last centuries. Kärkkäinen (1981) reported that inducing resin production to a threefold concentration compared to the constitutive level resulted in a 35% growth reduction. Lieutier et al. (1993) suggested that, especially in young pines, the resources for induced resin production originate from current photosynthesis, while in mature pines the mobilized energy might originate from tree reserves. The small differences in resin flow between our experiments lead us to believe that the stands had not been seriously traumatized in the immediate past. In another experiment, so far unpublished, we found that enhanced resin flow from the stem can also be induced by sawfly defoliation. It is possible that the strong resin flow in experiment 413 was partly induced by a *Diprion pini* outbreak in the area in 1991–1993. When induced resistance is included in the speculations, our results showing a reduction in resin production after fertilization could indicate a fertilizer-induced improvement of vitality in the trees. Instead of reflecting a growth-defence trade-off, the state could indicate enhanced vitality (increased growth) and therefore decreased production of induced resin due to diminished stress.

Acknowledgements

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Paper IV

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Different responses of two carbon-based defences in Scots pine needles to nitrogen fertilization[1]

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Abstract: Concentrations of two groups of carbon-based defensive compounds, diterpenoid resin acids and phenolics in pine needles, responded differently to nitrogen fertilization. Resin acid concentrations were higher on fertilized Scots pine trees in needles formed one year after the treatment compared to the corresponding needle year-class on control trees. Phenolic concentrations, on the other hand, tended to be lower in fertilized trees than in control trees. These groups of defensive compounds represent two extremes in the continuum of modes of storage of plant defensive compounds; cell vacuoles (phenolics) and multicellular cavities (diterpenoids). This dichotomy may explain why the two groups differed in their response to fertilization. Regression analyses revealed a positive relationship between resin acid concentration and number and size of resin ducts in relation to needle area. There was a significant, but weak, negative relationship between phenolic concentration and number of resin ducts. These results raise the question whether, in addition to terpenoids, which are stored in complex compartments, phenolics, which are stored in cells in the space between resin ducts, to some extent may depend on the space available for their storage. The relevance of these findings for plant defence theories and for expected risks of increased damage by different types of insect herbivores are discussed.

Keywords: carbon/nutrient balance, phenolics, diterpenoids, resin ducts, *Pinus sylvestris*.

Résumé: Les contenus de deux groupes de métabolites secondaires à base de carbone, soit les acides diterpéniques contenus dans la résine et les phénols, ont répondu différemment à un traitement de fertilisation chez les aiguilles de pin. Les contenus en acides de la résine étaient plus élevés dans les aiguilles de pin sylvestre formées un an après le traitement de fertilisation comparé aux aiguilles de même classe d'âge des arbres témoins. Les contenus en phénols, d'autre part, tendaient à être plus faibles chez les arbres fertilisés que chez les arbres témoins. Ces groupes de métabolites secondaires représentent deux extrêmes du continuum de modes d'entreposage des métabolites secondaires, soit les vacuoles (phénols), d'une part, et les cavités multicellulaires (diterpènes), d'autre part. Cette dichotomie pourrait expliquer pourquoi les deux groupes diffèrent dans leur réponse à la fertilisation. Des analyses de régression ont révélé une relation positive entre le contenu en acides de la résine et le nombre ainsi que la taille des canaux résinifères en relation avec la surface des aiguilles. On a observé également une relation négative statistiquement significative, quoique faible, entre le contenu en phénols et le nombre de canaux résinifères. Ces résultats nous incitent à se demander si non seulement les terpènes qui sont accumulés dans des compartiments complexes, mais également les phénols qui sont accumulés dans les cellules situées entre les canaux résinifères, pourraient dépendre jusqu'à un certain point de l'espace disponible pour leur entreposage. La pertinence de ces résultats en regard des théories de défense des plantes et des risques attendus d'augmentation des dommages par différents types d'insectes herbivores est discuté.

Mot-clés: bilan carbone/nutriment, phénols, diterpènes, canaux résinifères, *Pinus sylvestris*.

Introduction

The responses of plants to changes in the environment may affect their risk of being attacked and damaged by herbivores and pathogens. For example, the risk of attack can increase in response to a decrease in the concentration of defensive compounds, and hence in resistance (Rhoades, 1979). Decreased host resistance has been suggested as one explanation for increased insect densities in plant stands treated with fertilizer (Waring & Cobb, 1992) or exposed to abiotic stress such as drought or pollutants (Larsson & Tenow, 1984; Waring & Cobb, 1992).

Plants growing on nutrient-poor soils have an excess of carbohydrates because nutrient uptake is not at parity with the rate of photosynthesis (Ericsson, 1979). This imbalance is thought to explain why conifers and many other plants almost exclusively employ secondary, or defensive, compounds based on carbon (Coley, Bryant & Chapin, 1985). The availability of excess carbon in the plant is believed to determine the concentration of carbon-based defensive compounds, such as phenolics and terpenoids (Waring *et al.*, 1985). Although concentrations of plant defensive compounds are to a large extent genetically determined, their concentrations can also be influenced by environmental conditions (Gershenzon, 1984; 1994a; Larsson *et al.*, 1986b; Björkman, Larsson & Gref, 1991).

The carbon/nutrient-balance hypothesis predicts that moderate exposure to nutrient limitation, *i.e.*, when growth, but not photosynthesis, is limited, leads to an accumulation of excess carbon that is used for producing carbon-based defensive compounds (Bryant, Chapin & Klein, 1983). As a consequence, it is predicted that increased nutrient availability leads to decreased resistance. Plants growing under more severe nutrient-limiting conditions (*i.e.*, when both

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[1] Rec. 1997-06-25; acc. 1998-03-09.

growth and photosynthesis are limited) could, on the other hand, become more resistant when supplied with nutrients (Bryant, Chapin & Klein, 1983).

The carbon/nutrient balance hypothesis does not make any differentiation with respect to how different groups of compounds respond to altered nutrient availability. Experimental tests, however, have provided mixed results concerning the responses of different groups of compounds. In general, concentrations of phenolic compounds decrease following nitrogen fertilization (Gershenzon, 1984; Bryant *et al.*, 1987; 1993; Waring & Price, 1988); whereas terpenoids increase in concentration or remain unaltered (Oldiges, 1958; Grimalskij, 1961; Björkman, Larsson & Gref, 1991; McCullough & Kulman, 1991; Mopper & Whitham, 1992; but see Bryant *et al.*, 1987; Mihaliak & Lincoln, 1985; Mihaliak, Couvet & Lincoln, 1989).

We earlier proposed that differences in the way that the two groups of compounds are stored may account for their opposite responses to fertilization (Björkman, Larsson & Gref, 1991). Phenolics in leaves are stored in simple compartments, such as vacuoles, whereas terpenoids are stored in multicellular cavities, such as resin ducts (McKey, 1979; Gershenzon, 1994a). Thus, the concentration of terpenoids may depend more on the size and/or number of ducts, which, in general, is limited by the same factors as tree growth, rather than by the availability of carbon.

The hypothesized link between the mode of storage and the response of carbon-based compounds to altered nitrogen availability has not been adequately tested. The responses of phenolics and diterpenoids to nutrient availability have, in almost all cases, been studied in separate experiments (but see Muzika, 1993). In the present study we compared the responses of these two groups of compounds in the needles of Scots pine to nitrogen fertilization. Specifically, we predicted that phenolics and diterpenoids would respond differently to nitrogen fertilization if their concentrations were determined by the mode of storage. If, on the other hand, their concentrations were determined by the availability of carbon, we then expected them to respond similarly to fertilization. In the latter case, both groups of compounds were expected to decrease in concentration.

Material and methods

STUDY SITES

The trees in this study were part of a national experimental series established by the Finnish Forest Research Institute in which a number of fertilizer regimes were evaluated in Scots pine (*Pinus sylvestris* L.) stands at several sites. The three sites used by us were all situated in southern Finland (60° N, 22-27° E) and were 150-250 km apart from each other. All sites were situated on sandy soils with low fertility. The climate was similar at all sites with an annual precipitation of approximately 600 mm and an annual temperature sum (above + 5°C) of 1200 degree days.

Tree age varied between 35 and 65 years. The stands had not been previously fertilized. Treatments were evaluated by measuring biomass production (estimated by weighing needles, branches, cones, etc. of cut sample trees), tree growth (estimated from data on the height growth and radial growth at several heights of cut sample trees) and chemical changes in the needles (see below).

FERTILIZER TREATMENT

The number of treatments varied among sites. We chose to use three sites (402, 409 and 410) where there were both unfertilized control plots and plots fertilized with 150 kg N/ha. The fertilizer (100 kg methylen urea/ha and 50 kg ammonium nitrate with lime/ha) was applied once, in late spring, 1991. There were three fertilized and three control plots at each site.

Experimental plots were 900 m^2 (30 m × 30 m) with a 10 m buffer zone between plots, reducing the risk for effects from adjacent plots. Trees grew several meters apart and trees sampled for needles did not grow adjacent to one another.

SAMPLING OF NEEDLES

Needles were sampled once during the winter of 1992/93. The sampling was performed during the dormant season when needle chemistry is stable, thus enabling comparisons among sites (Helmisaari, 1990; Linder, 1995). Needles formed in 1990, 1991 and 1992 were sampled from three trees in each of three blocks per treatment and site. Thus, in total, we collected 162 needle samples from this repeated measures, split-plot designed experiment (*i.e.*, 3 [years] × 3 [trees] × 3 [blocks] × 2 [treatments] × 3 [sites]).

The needle samples were collected from one upper crown (fifth whorl) branch on each tree. The branches were brought to the laboratory. From each branch, 30 needles were sampled from each of the three year classes. These needles were stored (-25°C) for analysis of resin acid concentrations. The remaining needles were dried for 48 hours at 40°C, ground and analyzed for nitrogen, carbon and phenolic concentrations.

CHEMICAL ANALYSES

Nitrogen and carbon concentrations were measured with a CHN-elemental analyzer (LECO CHN-600). The amount of dry matter analysed was 150 mg.

Phenolics were analysed with a UV-spectrophotometer using Folin-Ciocalteus phenol reagent, after extraction in 80% methanol for 30 minutes at room temperature. Tannic acid was used as the standard. See Julkunen-Tiitto (1985) for a detailed description of the method used. The amount of dry matter used in each analysis was 50 mg.

Resin acid concentrations were analysed with a gas chromatograph (Varian 3700) after the resin acids had been extracted and treated as described in Gref & Ericsson (1985). The chromatograph was equipped with a fused silica capillary column (15 mm × 0.25 mm ID) with a 0.25 μm film of DB-1 (J & W Scientific), a split injector and a flame ionization detector. The carrier gas was hydrogen used at a flow rate of 1.60 mL/minute. The chromatograph was operated isothermally at 210°C or temperature programmed to increase from 160° to 240°C at 4°/minute peak areas, relative to an internal standard, and retention times were measured with an electronic integrator.

MORPHOLOGICAL AND HISTOLOGICAL MEASUREMENTS

Thin sections from fresh needles were used to estimate the number and diameter of resin ducts. The outer and inner (*i.e.*, lumen) diameters of all the resin ducts of each needle were measured with a stereomicroscope (x40) equipped with an ocular micrometer. Resin duct areas were estimated assuming that ducts were circular. The needle cross-sectional area was measured with a digital area meter (delta-T devices) from contour-drawings made with the aid of a stereomicroscope (x25) equipped with a camera lucida. Needle length was measured prior to making the above measurements. One needle from each of the 162 sampling units (3 × 3 × 3 × 2 × 3; see sampling of needles) was used in these analyses.

STATISTICAL ANALYSES

The effects of treatment, site and time after treatment (*i.e.*, needle age-class) on resin acid, phenolic and nitrogen concentrations, C/N ratio, number of resin ducts, resin duct diameter, needle length and needle cross-sectional area were assessed using repeated measures multivariate analysis of variance. Site and treatment were treated as fixed factors in these analyses. Data in the form of proportions (*i.e.*, the concentrations and the C/N ratio) were arcsine-transformed before applying the analysis. Imbalance in the data set due to missing values was dealt with by using the GLM-procedure instead of the ANOVA-procedure in SAS.

To estimate the degrees to which morphological and chemical properties of needles were related to the concentrations of resin acids and phenolics, we performed regression analyses (Proc REG in SAS). Because of colinearity among independent variables (*i.e.*, needle length, needle cross-sectional area, nitrogen concentration, C/N ratio, number of resin ducts and resin duct outer and inner diameters) we tried to find the best single regression model based on one variable or a combination of variables.

Results

EFFECTS OF TREATMENT, SITE AND TIME

Nitrogen fertilization had a significant delayed effect on the concentration of needle resin acids: the resin acid concentration was higher in the needles of fertilized trees one year after fertilization (Figure 1a, Table I). Overall, the concentrations of phenolics tended to be lower in the needles of fertilized than control trees (Figure 1b, Table II).

Fertilization increased total nitrogen concentration (Figure 2a, Table III) and needle length (Figure 2c, Table III) but decreased C/N ratio (Figure 2b, Table III). The values for number of resin ducts (Figure 2d), resin duct inner diameter (Figure 2e) and needle cross-sectional area (Figure 2f) did not differ significantly between the treatments (Table III).

None of the studied traits differed significantly between fertilized and control trees when analyses were limited to needles formed in 1990, prior to the 1991 fertilization treatment. (Figure 1a-b, Figure 2a-f). Two traits, *i.e.*, number of resin ducts and needle cross-sectional area, differed significantly among sites (Table III). All needle traits changed significantly over time (Table I, II and III).

There was a significant interaction between time and treatment for resin acid concentration, as a consequence of the increase in the 1992 needles on fertilized trees. The significant time by treatment interactions found for nitrogen concentration and needle length was probably a consequence of the accentuated difference between treatments in the 1991 needles.

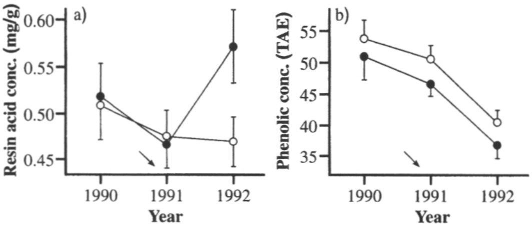

FIGURE 1. Response of mature Scots pine trees to nitrogen fertilization with respect to needle concentrations of (a) diterpenoid resin acids (mg/g) and (b) phenolics (Tannic Acid Equivalents). Each symbol indicates the mean of control (open) and fertilized (closed) trees from three sites ($n = 9$ per site and treatment). The bars indicate standard errors of the means. Treatment means (for a given year and trait) with both plus and minus standard error bars (within a year and trait) were significantly different ($p < 0.01$; LSD-tests), whereas means with only a plus or minus bar did not differ significantly ($p > 0.10$; LSD-tests). Arrows indicate when fertilizer was applied.

TABLE I. Effects of site, treatment and time after treatment (*i.e.*, needle age) and their interactions with resin acid concentration in the needles of unfertilized and nitrogen fertilized Scots pine trees as revealed by a repeated measures analysis of variance (a) between and (b) within subjects. Note the significant interaction between time and treatment, indicating that the two treatments differed in their response over time (see Figure 1a)

a) BETWEEN SUBJECTS

Source	df	MS	*F*	*p*
Site	2	0.0056	0.08	0.92
Error I (Block[Site])	6	0.0705		
Treatment	1	0.0355	0.58	0.47
Site × Treatment	2	0.0309	0.51	0.63
Error II (Block × Treatment[Site])	6	0.0610		

b) WITHIN SUBJECTS

Source	Wilks'lambda	*F*	Num[a] df	Den[b] df	*p*
Time	0.721	5.42	2	28	< 0.05
Time × Site	0.881	0.92	4	56	0.46
Time × Treatment	0.802	3.44	2	28	< 0.05
Time × Site × Treatment	0.965	0.25	4	56	0.91

[a] Numerator; [b] Denominator.

REGRESSION ANALYSES

The trait best describing resin acid concentration was the percent of the total needle area occupied by resin duct lumen, *i.e.*, a trait based on needle area, number of resin ducts and inner diameter of resin ducts ($r^2 = 0.241$, $p < 0.001$, $n = 154$, $y = 11.1\ x + 0.203$). No single trait or variable based on a combination of traits could describe concentration of phenolics as well. Two traits did, however, result in significant regressions: A negative relationship with number of resin ducts ($r^2 = 0.053$, $p = 0.008$, $n = 131$, $y = -1.36\ x + 60.0$) and a positive one with C/N ratio ($r^2 = 0.050$, $p = 0.01$, $n = 131$, $y = 0.539\ x + 25.4$). A similarly weak but significant relationship was found between resin acid concentration and C/N ratio ($r^2 = 0.034$, $p = 0.035$, $n = 131$, $y = 0.006\ x + 0.260$).

TABLE II. Effects of site, treatment and time after treatment (*i.e.*, needle age) and their interactions on phenolic concentration in the needles of unfertilized and nitrogen fertilized Scots pine trees as revealed by repeated measures multivariate analysis of variance (a) between and (b) within subjects

a) BETWEEN SUBJECTS

Source	df	MS	*F*	*p*
Site	2	407.2	0.91	0.45
Error I (Block[Site])	6	446.3		
Treatment	1	765.0	6.13	0.09
Site × Treatment	2	426.8	3.42	0.17
Error II (Block × Treatment[Site])	4	124.8		

b) WITHIN SUBJECTS

Source	Wilks'lambda	*F*	Num^a df	Den^b df	*p*
Time	0.068	48.22	2	7	< 0.001
Time × Site	0.357	2.36	4	16	0.10
Time × Treatment	0.295	2.45	2	7	0.09
Time × Site × Treatment	0.456	1.68	4	14	0.21

^{a}Numerator; ^{b}Denominator.

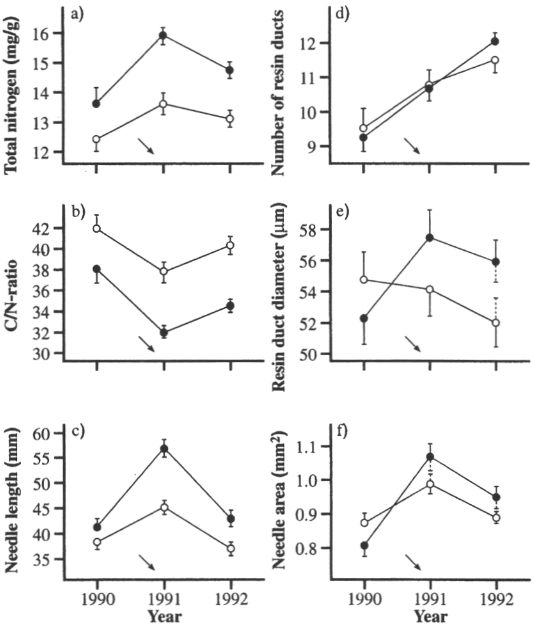

FIGURE 2. The response of mature Scots pine trees to nitrogen fertilization with respect to six needle characteristics: (a) total nitrogen concentration, (b) carbon/nitrogen ratio, (c) needle length, (d) number of resin ducts, (e) mean inner diameter of resin ducts and (f) needle cross-sectional area. The information given in Figure 1 is also valid here but, in addition, includes symbols with one of the error bars dashed which indicate that the means tended to be significantly different ($0.05 < p < 0.10$; LSD-tests). Arrows indicate when fertilizer was applied.

Discussion

Fertilization increased both the nitrogen content and length of needles. Conversely, fertilized trees exhibited a lower C/N ratio compared with controls. The lower C/N ratio in fertilized trees indicates that their needles may have contained less excess carbon than the needles of control trees. However, the lower C/N ratio may primarily be an effect of the increase in nitrogen. Thus, it is difficult to conclude that the tendency for phenolic concentrations to be lower in the needles of fertilized trees is an effect of lower availability of carbon, as predicted by the carbon/nutrient balance hypothesis (Bryant, Chapin & Klein, 1983). In contrast, the other group of defensive compounds, *i.e.*, the diterpenoid resin acids, responded differently to fertilization. The increase in resin acid concentration in the needles of fertilized trees was, however, delayed and only possible to detect in needles produced one year after treatment. This is a similar result to that found in a previous study (Björkman, Larsson & Gref, 1991).

A comparison of our results with those of the only previous study involving both terpenoids and phenolics suggests that both the length of the experiment and tree age can influence the results and hence the conclusions. Muzika (1993) found a significant decrease in phenolics but no change in terpenoids after fertilization of grand fir seedlings. The lack of any change in terpenoid concentrations can probably be explained by the fact that she did not measure concentrations in needles produced the year after treatment.

The fact that we sampled needles on one occasion rather than in the years when they were formed may partly explain why the nitrogen concentration tended to be higher in the 1990 needles, *i.e.*, those formed prior to fertilization. In fact, data from one of our sites showed that the difference in nitrogen concentration between fertilized and control trees was less than 4% in needles sampled and analyzed in 1990, but almost 10% for needles of this year-class sampled and analyzed in 1993. It has been shown that the transport of nitrogen between plant parts can be very extensive (Chapin, Johnson & McKendrick, 1980) and can affect the distribution of insect damage (Larsson, 1985). Even though there is no direct evidence that phenolics are transported within plants, translocation is a factor that, as in the case of nitrogen, could have tended to reduce the magnitude of difference in a specific year: Phenolic transportation could have resulted in a decreased difference between treatments in needles produced after treatment.

The regression analyses showed that the concentration of resin acids was positively related to the size and number of resin ducts in relation to needle area, *i.e.*, the percent of the total needle area occupied by resin duct lumen. In contrast, the concentration of phenolics was negatively correlated to the number of resin ducts.

The positive relationship for resin acid concentration is easy to understand because these diterpenoids, as well as other terpenoids, are primarily stored in the resin ducts. This result supports the hypothesis that the concentration of resin acids is affected by the relative growth and differentiation of the compartments that the terpenoid compounds are stored in, *i.e.*, the resin ducts.

The negative relationship between phenolic concentration and number of resin ducts is less easy to understand. It can, however, be understood if one takes into account the fact that phenolics do not occur at equal concentrations in all parts of a plant tissue (McKey, 1979; Solhaug, 1990). For example, in an elegant study, Solhaug (1990) showed

TABLE III. Indication of effects of site, treatment and time after treatment (*i.e.*, needle age) on six needle traits of unfertilized and nitrogen fertilized Scots pine trees as revealed by repeated measures multivariate analyses of variance. The between subject effects (a) were tested by mean square comparisons. The within subject effects (b) were tested with Wilks lambda's. The values in the table refer to the *p*-value of the test. Analysed as a split plot experiment; see Table I and II for error-terms used

	a) Between subjects			b) Within subjects			
Needle trait	Site	Treatment	Site × Treatment	Time	Time × Site	Time × Treatment	Time × Site × Treatment
Nitrogen concentration	0.09	< 0.01	0.16	< 0.001	0.89	< 0.05	0.42
C/N ratio	0.09	< 0.01	0.37	< 0.01	0.68	0.11	0.56
Number of resin ducts	< 0.05	0.05	0.15	< 0.001	0.07	0.24	0.76
Resin duct diameter (inner)	0.10	0.09	< 0.05	< 0.05	0.72	0.26	0.30
Needle length	0.33	< 0.01	0.95	< 0.001	< 0.01	< 0.01	0.45
Needle area	< 0.05	0.90	0.55	< 0.001	0.58	0.19	0.07

that one class of phenolic compounds, the stilbene glucosides, occurs almost exclusively in the center of *Picea abies* needles, thus being absent in the periphery where the resin ducts are found (Marco, 1939), as is also the case in Scots pine (Otto & Geyer, 1970). If phenolics in Scots pine needles also mainly occur in the center, this could explain why the phenolic concentration in our study was negatively correlated with the number of resin ducts. It could be hypothesized that even concentrations of stored (*e.g.*, in vacuoles) phenolic compounds may to some extent depend on the space available for their storage. In other words, an increase in resin duct number (and/or size) will decrease the space available for cells containing phenolics, *i.e.*, the number of cells with phenolics will decrease. The data presented here is far from sufficient for testing this hypothesis, but can merely be used to formulate it. More studies in which both chemical and morphological changes are studied simultaneously will be needed before it can be more critically tested. An interesting pattern in our data that may be taken as evidence in support of the hypothesis is that the steady decline in phenolic concentration, independent of treatments among the three years, is only matched by the steady increase in number of resin ducts. However, there are other possible explanations, not necessarily correlated to resin duct number, for the decline in phenolic concentration. For example, phenolics may accumulate with time and result in higher concentrations in older needles or phenolic synthesis may be higher in older than younger needles.

The size of the space between compartments containing plant defences may affect insect herbivores. For example, larvae of the needle-mining moth *Exoteleia pinifoliella* are able to avoid contact with terpenoids by feeding in between resin ducts, at least when needles are sufficiently large (Bennett, 1954). They cannot, however, avoid the phenolics. The situation is just the opposite for larvae of the externally feeding sawfly *Neodiprion sertifer.* Larvae avoid the middle part of needles in early instars (Schwenke, 1972). Late-instar larvae, however, prefer to feed on tissue rich in resin acids (Larsson, Björkman & Gref, 1986a; Björkman & Larsson, 1991). These two examples illustrate that any good hypothesis used for predicting the risk of plants sustaining damage in a changing environment may have to incorporate the feeding mode of the attacking organism (Larsson, 1989).

Increased nitrogen availability should make pines more susceptible to herbivores and pathogens directly after fertilization, especially as a result of the increase in nitrogen, but also due to the possible decrease in phenolics. The increase in resin acid concentrations one year after the treatment may, however, counteract this effect (Björkman, Larsson & Gref, 1991). Thus, predictions of how plant susceptibility to attacking organisms varies with the environment will depend on the time-scale as well as on the type of attacking organism. Whether or not changes in the magnitude observed here affect herbivores or not are, however, difficult to judge. Our findings that the concentration of resin acids, and possibly phenolics, depended on the space available for their storage, lend support to the growth/differentiation hypothesis (Loomis, 1932; Lorio, 1986; Herms & Mattson, 1992). The weak relationships between C/N ratio and phenolic as well as resin acid concentrations cannot be used as evidence for or against the carbon/nutrient balance hypothesis (Bryant, Chapin & Klein, 1983) because this ratio may not reflect availability of carbon. The study was not designed to test hypotheses of a more evolutionary nature, such as those dealing with trade-offs and costs involved in the development of defences in plants (Coley, Bryant & Chapin, 1985; Björkman & Anderson, 1990; Gershenzon, 1994a, b; Mole, 1994). It does, however, hint at mechanisms that need to be understood before a more comprehensive theory on plant defences can be developed (Tuomi, 1992; Mole, 1994).

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Paper V

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Effects of forest fertilization on the radial growth and resin exudation of insect-defoliated Scots pines

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Summary

1. Scots pines that had been defoliated by the pine sawfly (*Diprion pini*) were fertilized with various nutrient combinations in the growing season following defoliation in order to study the possibility of accelerating tree recovery. Resin exudation, vertical resin duct density and vigour index of the trees were measured three years and radial growth six years after defoliation.
2. Defoliation reduced stem growth, and annual rings were missing especially in the severely defoliated trees. Nitrogen fertilization enhanced radial growth even in the severely defoliated trees.
3. Nitrogen fertilization increased the number of vertical resin ducts in both defoliated and undefoliated trees, but resin duct density tended to be lower in the nitrogen-fertilized trees than in trees that had not received nitrogen fertilizer. Resin duct density did not correlate with resin flow rate.
4. Resin exudation was strongest from trees that had been almost totally defoliated, intermediate from trees that had lost half of their foliage, and weakest from undefoliated trees, indicating that defoliation had induced resin production in the stem. Resin flow was not affected by fertilization.
5. There was a weak negative correlation between vigour index and resin flow. No trees with a high vigour index had strong resin exudation, while resin exudation varied from minimal to very strong in trees with a low vigour index.
6. The results indicate that nitrogen fertilization can be used to stimulate growth of defoliated trees without affecting resistance traits on infertile sites.

Key words:
Diprion pini, nutrient availability, *Pinus sylvestris*, resistance, vigour index

Introduction

Earlier studies have shown that defoliation has a long-term effect on Scots pine (*Pinus sylvestris* L.) growth (Austarå *et al.* 1987). Ericsson, Larsson & Tenow (1980a) found that the effects were most severe at a high degree of defoliation when the most recent needle year-classes were removed late in the growing season. On one hand, nutrients and carbon in defoliated trees are lost and the amount of assimilating leaf area is decreased. On the other hand, mineralisation of litter and frass increases nutrient availability in the soil. Defoliated conifers may not be able to replace carbon as rapidly as nutrients because of their inherently low rates of photosynthesis, which can lead to a relative carbon deficiency (Herms & Mattson 1992). This is predicted to result in a decreased C/N balance, decreased concentrations of carbon-based secondary metabolites, and no delayed induced resistance (Tuomi *et al.* 1988; Herms & Mattson 1992).

Based on the assumptions that the sapwood basal area is linearly related to leaf area, and that stemwood production has less allocation priority than root and shoot growth, Waring, Thies & Muscato (1980) proposed that the ratio of basal area growth to sapwood basal area is an index of tree growth efficiency. This vigour index may reflect the ability of trees to resist bark beetle attacks by producing defensive compounds, e.g. oleoresin. However, an increase in growth rate would decrease a tree's susceptibility to attack only then if photosynthesis is not limited (Waring & Pitman 1985). If carbohydrates are scarce, an increased allocation to growth would lead to a decreased defence (Bryant, Chapin & Klein 1983; Lorio 1986; Waring & Pitman 1985; Herms & Mattson 1992).

Fertilization is a means of enhancing tree growth on sites with limited nutrient availability. Depending on the photosynthesis capacity, a fertilizer-induced increase in the growth rate may lead to increased or decreased pest resistance. As long as the level of photosynthesis is enhanced along with growth, no trade-off between defence and growth should occur. Amelioration of nutrient availability should decrease nutrient stress and thereby speed up the recovery (refoliation and growth) of Scots pines, defoliated by the pine sawfly, *Diprion pini* L.. The aim of this study was to compare the effects of different fertilizers on the recovery of Scots pines suffering from different intensities of defoliation and to investigate the interaction between defoliation, growth and defence mechanisms against bark beetles.

Materials and methods

Study sites and experimental design

The experiment was carried out in western Finland (Harjavalta, 61°17'N, 22°09'E) in a 60-90 years old Scots pine stand growing on relatively infertile, dry sandy soil. Some of the trees were heavily defoliated by the pine sawfly in 1989. The insect population collapsed in the following year, and tree mortality in the stand remained negligible. A total of 70 groups with three trees in each group (one visibly defoliated tree, one tree with approximately 50% loss of needle biomass, and one almost totally defoliated tree with more than 90% loss of needle biomass) were selected for the fertilization experiments in the spring of 1990. The distances between trees within a group were small whereas the distances between groups were larger than 15 m. 10 groups of three trees were randomly assigned to each of seven different treatments (six fertilizer treatments and one control treatment (Table 1)). The mean stem diameter of all selected trees (n = 210) at 1.3 m of tree height (breast height) was 14.6 cm with no differences between the defoliation classes in 1990 at the beginning of the experiment.

The granular fertilizers were spread manually in a circular area around the individual trees (up to 6 m from the tree base) after budbreak in late May 1990. The nitrogen in the N and NPK treatments was in a soluble form (urea and ammonium nitrate, respectively), and in vitality fertilizer treatment NVF one third of the nitrogen was ammonium nitrate and two thirds slow-release methylene urea.

Table 1. Quantities of the major macronutrients applied in the different fertilizer treatments. NVF, Fyto and VF were test versions of commercial vitality fertilizers.

Treatment	**N**	**P**	**K**	**Mg**	**Ca**
			kg/ha		
N	135	-	-	-	-
NPK	69	27	58	3	64
NVF	71	21	39	35	67
Fyto	0.1	0.9	11	15	45
VF	-	28	49	57	92
P	-	46	-	-	-

Increment core sampling and analysis

In November 1992 two increment cores were taken from each tree at breast height for determination of heartwood and sapwood basal area. The heartwood-sapwood border was determined on the basis of differential translucence (Münster-Swendsen 1987). In November 1995 an additional increment core was taken from all the trees for diameter growth measurements. When the radial growth data were examined it was obvious that annual rings were missing from some of the trees. Therefore the growth series of all the trees were cross-dated, and the cross-dating was checked using the program COFECHA (Holmes 1994). Using the corrected ring width data, the vigour index for 1992 (the resin sampling year) was calculated for each tree as the ratio between the basal area of the 1992 annual ring (BA_1) and the sapwood basal area in 1992 (SA) (Waring *et al.* 1980).

Measurement of resin duct density and resin flow

Half of the trees in each treatment, 105 trees in all, were randomly selected for resin flow measurements on 11 August, 1992. All the trees had recovered rather well from the defoliation by this time, and had needles from the current and two previous years, 1990-1992. All trees were sampled on the same day. During the sampling period (24 h) the maximum temperature was 28 °C and minimum temperature was 13 °C. Resin was sampled according to the method described by Lorio & Sommers (1986) and Lorio et al. (1990). Part of the outer bark was removed to smoothen the sampling surface. Two round holes (15 mm diam.) were punched through the remaining bark up to the cambium on opposite sides of each stem at breast height. The bark was then removed, and an aluminium trough was pinned below the hole to conduct the resin into a plastic test tube attached to the stem below the trough. The test tubes were numbered and weighed beforehand. The amount of resin collected during the first two hours was marked on the tubes, and after 24 hours the tubes were removed from the trees, sealed, and taken to the laboratory. The amount of resin collected during the first two hours was calculated as a percentage of total amount collected.

Increment cores (5 mm diam.) from 61 trees were used to study the effects of defoliation and nitrogen fertilization on the formation of vertical resin ducts. Only those treatments were examined which represented the extremes in growth response; the 50% defoliation class was excluded. Cores that had been broken during the increment measurements were discarded. Therefore, the examined cores originated from 18 undefoliated and 14 heavily defoliated trees in the N and NPK treatments (showing clear fertilizer-induced growth enhancement), and 14 undefoliated and 15 heavily defoliated trees in the control and P treatments (no growth response). The cores were split longitudinally and the number of vertical resin ducts in the annual rings of 1988-1992 (annual rings formed both before and after defoliation and fertilization) was counted. The information from the cross-dating of increment data was used for correct dating of annual rings. A 4 mm-wide tangential segment of each annual ring was examined under a stereomicroscope, disregarding 0.5 mm at both edges because the tissue there was often disrupted. The area of the examined segment varied according to the width of each individual annual ring.

Analysis

Differences in the tree age, diameter, and heartwood and sapwood basal areas between different fertilization treatments and defoliation classes were tested using analysis of variance. Differences in resin flow, resin duct number and density, vigour index and annual radial increments between defoliation classes and between the fertilization treatments within the defoliation classes were tested with the Kruskal-Wallis analysis of variance. When significant differences were indicated, the multiple comparisons were computed with the nonparametric test included in the Kruskal-Wallis analysis of variance. Bivariate linear correlations were tested with Pearson's product-moment correlation test. All tests were performed with the VAX/VMS 1990 version of the BMDP statistical package (BMDP Inc 1990).

Results

Effects on growth

Although the ovipositing sawflies may have actively rather than randomly chosen the defoliated trees, there were no differences among defoliation classes in the radial growth pattern of the tree stems before the outbreak (Fig. 1). There were also no significant differences between the fertilization treatments or defoliation classes in tree age, stem diameter, sapwood basal area and heartwood basal area at breast height in 1992, two years after defoliation. The cross-dating indicated that there were one or more annual rings missing from four undefoliated trees, 41 trees with 50% defoliation and 58 trees with >90% defoliation. In the undefoliated and 50% defoliated trees only the 1990 ring was missing, but in the >90% defoliated trees there were usually several rings missing, and some trees had little or no radial growth after the defoliation event in 1989. The annual increments of the three defoliation classes differed significantly (ZSTAT > 2.39, df = 2, $P < 0.05$) from each other from 1990 until the end of the study in 1995, but not in the year of defoliation (1989) or before. The effect of defoliation was especially strong in 1990 and 1991, when practically no diameter growth occurred in most of the heavily defoliated trees (Fig. 1).

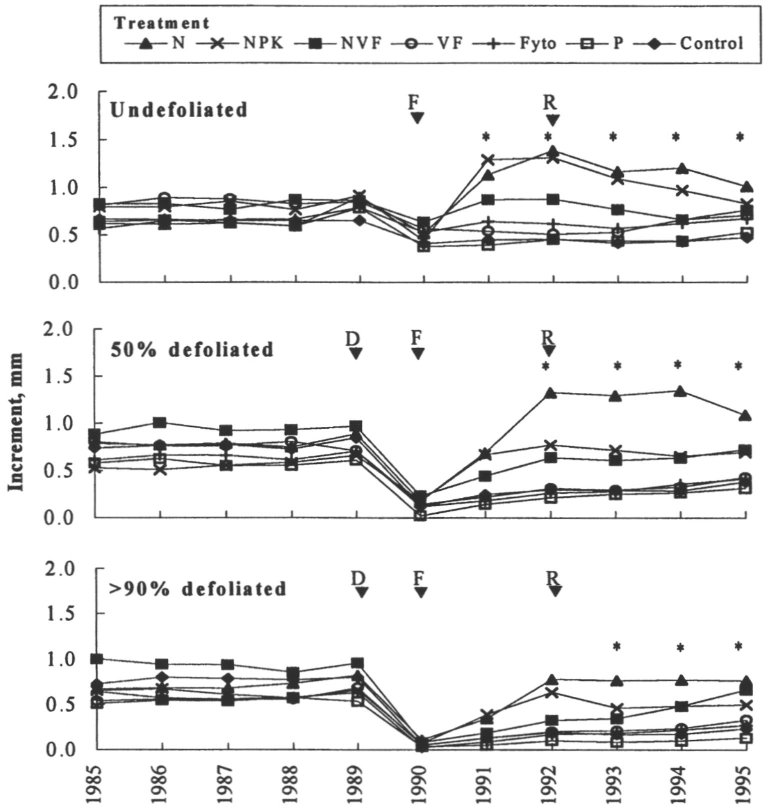

Figure 1. Average radial increment at breast height in trees of different treatments and defoliation classes. The occurrence of defoliation (D), fertilization (F) and resin flow measurement (R) are indicated in the figure. Asterisks mark significant (ZSTAT > 3.04, df = 6, $P < 0.05$) differences between control and one or more fertilizer treatments containing nitrogen. In each defoliation class n = 10 in each treatment. Fertilizer treatments are indicated in Table 1.

Although the fertilizers were applied in the spring of 1990, no significant effect of fertilization on radial growth was observed in 1990. In 1991 the effect of nitrogen fertilization was significant only in the undefoliated trees, while in the 50% defoliation class the fertilizer effect became significant first in 1992 and in the >90% class only in 1993 (Fig. 1). The trees receiving N and NPK treatments had greater radial growth than the unfertilized control and the trees receiving non-nitrogenous fertilizer treatments. Undefoliated trees in the N and NPK treatments showed similar growth responses although the NPK treatment contained only half the amount of nitrogen compared to the N treatment (Table 1, Fig. 1). Two thirds of the nitrogen in the NVF treatment were in slow-release form, and therefore the growth response was delayed (Fig. 1). However, the differences in growth between the three treatments containing nitrogen were not significant.

Radial growth was markedly reduced during 1990-1995 especially in the defoliated trees that did not receive any nitrogen fertilizer. In the 50 % defoliation class the growth reduction compared to undefoliated trees was 24 % in the nitrogen-fertilized treatments and 51 % in the treatments not receiving nitrogen. In the >90 % defoliation class, the reduction was 52 % in the nitrogen treatments and 71 % in the non-nitrogen treatments. Nitrogen fertilization had enhanced radial growth even in the severely defoliated trees; in 1993 their accumulative increment (since fertilization) already exceeded the growth of the undefoliated control trees.

Both defoliation and fertilization had a significant effect on the vigour index in 1992. All defoliation classes differed significantly from each other (Kruskal-Wallis test statistic = 48.81, df = 2, $P < 0.01$). The index was always higher in the undefoliated than in the >90% defoliated trees, and in most fertilizer treatments the 50% defoliation class lay between these two extremes. Since the vigour index is calculated from the radial growth, it was not surprising that in the treatments with growth-promoting nitrogen fertilizers the vigour index also increased, whereas there were no significant differences between the treatments not containing nitrogen and the control (Fig. 2).

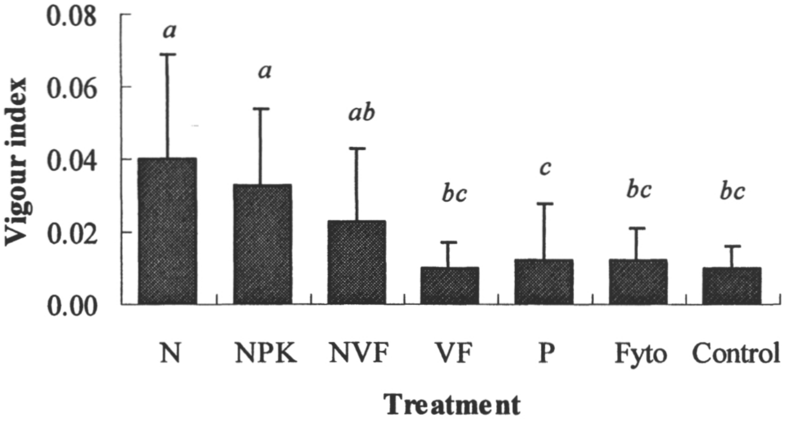

Figure 2. Vigour index (mean value and standard deviation) in 1992 in the different fertilizer treatments averaged across all defoliation classes. Means marked with the same letter do not significantly differ from each other. Fertilizer treatments are indicated in Table 1.

Effects on resin duct density and resin flow rate

The average number of resin ducts in 1988 and 1989 did not significantly differ between undefoliated and defoliated trees, nor between treatments receiving and not receiving nitrogen fertilizer. There were fewer vertical resin ducts in the annual rings 1990-1992 of the defoliated trees than of the undefoliated trees (Kruskal-Wallis test statistic = 22.92, df = 1, $P < 0.01$). Nitrogen fertilization increased the number of ducts in both defoliated and undefoliated trees (Kruskal-Wallis test statistic = 5.36, df = 1, $P < 0.05$). However, there were no significant differences in resin duct density, although the density tended to be lower in the nitrogen-fertilized trees (0.9 ± 0.1 ducts mm^{-2}) than in trees that had not received nitrogen fertilizer (1.4 ± 0.2 ducts mm^{-2}). The bivariate correlations between resin flow and the number or density of resin ducts were not statistically significant. The annual ring width correlated positively with the number of resin ducts ($r = 0.70$, $n = 61$, $P < 0.01$), but not with resin duct density ($r = -0.22$, $n = 61$, $P = 0.09$).

The between- and within-tree variation in resin flow was large. The difference between the two sampling points in the same tree was often considerable. The resin flow was strongest at the beginning of the sampling period; about one third of the total amount of resin accumulated during the first two hours. The correlation between the amount of resin after 2 hours and after 24 hours was very high ($r = 0.95$, $n = 105$, $P < 0.01$). None of the fertilization treatments had a significant effect on the resin flow, but the defoliation effect was significant (Kruskal-Wallis test statistic = 5.82, df = 2, $P = 0.05$). The resin flow was strongest in the heavily defoliated trees and weakest in the undefoliated trees (Fig. 3). Resin flow also correlated positively with heartwood basal area ($r = 0.43$, $n = 105$, $P < 0.01$), stem diameter ($r = 0.40$, $n = 105$, $P < 0.01$) and tree age ($r = 0.29$, $n = 105$, $P < 0.01$).

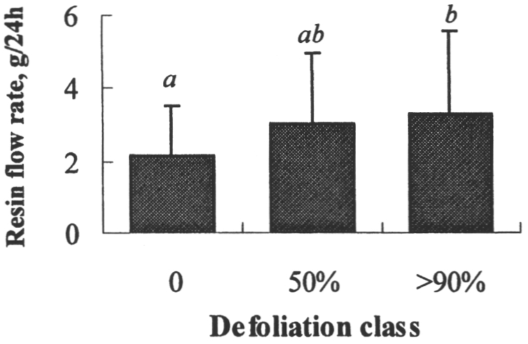

Figure 3. Resin exudation (mean value and standard deviation) in the different defoliation classes. Means marked with the same letter do not significantly differ from each other.

The width of the current-year growth ring did not correlate with the amount of resin flow. However, there was a weak negative linear correlation between vigour index and resin flow (Fig. 4). This relationship was in fact not truly linear; in trees with a low vigour index the resin flow was either weak or strong, while trees with relatively high vigour index never had a strong resin flow.

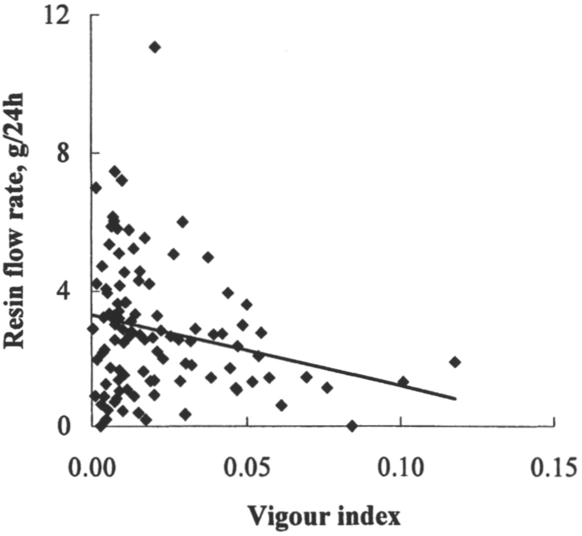

Figure 4. Relationship between resin exudation and vigour index. Regression line y = 3.2602 - 20.116x, n = 105, r = -0.227, $P = 0.02$.

Discussion

Although we were unable to find previous studies showing that crown defoliation induces resin production in the stem, the fact that the resin content of Scots pine can be increased considerably by mechanical wounding of the stem and certain chemical treatments has long been known (Kärkkäinen 1981; Hillis 1987). These results agree with our earlier observations in another defoliated pine forest where resin flow of severely defoliated trees was stronger than that of undefoliated trees (Annila *et al.* 1993). Assuming that the defoliation classes did not differ in their pre-defoliation resin content, the results indicate that trees allocated a considerable amount of resources to resin production following defoliation (over 50 % increase in resin flow), which was accompanied by a marked reduction in growth compared to the undefoliated trees. Waring & Pitman

(1985) proposed that carbohydrate-allocation in physiologically stressed pines will favour production of new foliage and roots over production of defence chemicals and stem diameter growth. In our study, supposing that carbon availability was limited, allocation to resin production (defence) seemed to have priority over stem diameter growth after defoliation. Kozlowski (1969) suggested that growth reduction after defoliation could be due to a lack of regulatory compounds rather than to a lack of available carbohydrates. This could explain the delay in growth response to fertilization in defoliated trees (Fig. 1). On the other hand, if the carbon pool was limited, the delay in growth response could be due to a carbon allocation pattern that favored refoliation and resin production.

The nitrogen-containing fertilizers promoted stem growth, indicating that nitrogen was a growth-limiting nutrient. In contrast, growth of trees receiving non-nitrogenous fertilizer treatments did not differ from the unfertilized control. As nitrogen fertilization clearly enhanced radial growth, but did not affect the amount of resin flow, no actual trade-off between growth and resin production or any indication of carbon shortage was detected. On extremely nutrient limited soils, fertilization is predicted by the carbon/nutrient balance and growth/differentiation balance hypotheses to increase growth and photosynthesis (Herms & Mattson 1992). The increased carbon pool could therefore increase both growth and secondary metabolism. In defoliated trees, transpiration and respiration are reduced and the need for water should therefore be smaller than that in undefoliated trees (Ericsson *et al.* 1980b). The remaining old needles and developing new needles in defoliated trees are less shaded than in undefoliated trees, which allows for more effective use of the trees' photosynthetic capacity because of increased light penetration (Hari *et al.* 1985). Increased assimilation efficiency may to some extent explain the high resin content of the heavily defoliated trees. It is also likely that the trees utilised their carbon stores for foliage recovery and resin production (Ericsson *et al.* 1980a,b). On nutrient rich sites, fertilization is not predicted to increase photosynthesis, so carbon would be diverted from secondary metabolism to growth and the trade-off is predicted to be manifested as a negative correlation between resin production and growth (Herms & Mattson 1992).

Although resin ducts have a defence function as a transport route and storage space for oleoresin, their formation is closely connected to growth. Wide annual rings have more vertical ducts than narrow ones. The number and size of the ducts affects not only the transportation capacity but also potential resin production, as the ducts are lined with epithelial cells that synthesise the resin. In pines these epithelial cells remain active for several years, thus enabling an increase in resin synthesis in stems where no annual rings with new resin ducts are formed (Bannan 1936; Hillis 1987).

The fact that trees with a high vigour index never had high resin exudation, while the resin exudation from low vigour trees ranged from very low to very high, has also been observed elsewhere in undefoliated trees on similar sites (Kytö, Niemelä & Annila 1998). As the vigour index reflects growth rate, this can be interpreted to be consistent with Herms & Mattson (1992), who predict that phenotypes with rapid growth coupled with high secondary metabolism are not possible because of the trade-off between growth and secondary metabolism.

The calculation of the vigour index is based on the assumption that the sapwood basal area reflects the leaf area in the crown of the tree (Waring *et al.* 1980). However, the results of this study show that defoliation did not affect the sapwood area in the studied trees. As the transformation of sapwood into heartwood is irreversible, a possible change in the proportion of heartwood should have been detectable even a few years after defoliation. Needle loss was probably not reflected in the conducting sapwood area because the branches remained alive in spite of total needle loss. Hence, when the needle biomass is temporarily reduced by an outside agent and not by physiological processes in the tree itself, the sapwood basal area will not reflect leaf area. In a case like this, calculating vigour indices based on the sapwood basal area of defoliated trees gives biased results, as the true leaf area is much smaller than the sapwood basal area indicates. By 1992 the observed trees had recovered from defoliation and held three age classes needles in the crown, which is normal on a site of this type. Therefore, the vigour index in 1992 should more or less accurately reflect the relationship between sapwood area and leaf area, although the sapwood basal area might still slightly overestimate the leaf area of defoliated trees, and thereby underestimate the vigour index.

In conclusion, we found that defoliation seemed to have induced resin production in the stem, and that resin production in the recovering trees was a stronger sink than stem growth. The amount of resin exudation did not correlate with annual increment. Nitrogen fertilization clearly enhanced stem growth even in the heavily defoliated trees, while resin flow was not affected by fertilization. We conclude that on sites with very limited nutrient availability, fertilization can be used to accelerate tree recovery from defoliation without deteriorating the pest resistance of the trees.

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