ABSTRACT

Introduction Humans are indirectly affecting forest trees by changing the Earth's atmosphere. Increasing concentrations of atmospheric CO_2 have a beneficial effect on tree growth whereas increase in tropospheric O_3 is a health risk for trees. Both gases also have effect on the metabolism of phenolic compounds, which are considered to be defences against herbivores. On the other hand, phenolics are also used as host recognition cues by herbivores. Taken together, atmospheric changes may affect the performance and preference of herbivorous insects and detritivorous soil animals by changing the quality of their host plant. The aims of this thesis were to study how atmospheric change affects phenolic metabolism in the leaves of silver birch over time and whether these changes have any effect on insects or soil macrofauna.
Materials and methods Seven years old saplings of two silver birch (*Betula pendula* Roth) clones were exposed to elevated concentrations of CO_2 and O_3, singly and in combination, for three years in an open-top chamber experiment. The effects of CO_2 and O_3 on the growth and phenolic accumulation of short- and long-shoot leaves, leaf litter and buds, and on the growth and food consumption and preference of insects and soil macrofauna were studied.
Results Elevated CO_2 increased the mass of short- and long-shoot leaves, irrespective of O_3, while the mass of leaf litter was increased in clone 4 only. Elevated O_3 decreased the mass and size of short-shoot leaves while long-shoot leaves were not affected by O_3. By contrast, both CO_2 and O_3 reduced the mass of buds, but the effects were prevented in the combined treatment. Elevated CO_2 increased the accumulation of intracellular phenolic compounds in leaves, while epicuticular compounds in leaves and buds were not affected. The effects of elevated O_3 on the accumulation of phenolic compounds were minor: fewer compounds were affected than by CO_2, and the effects were altered over time. The growth of larvae of two geometrid moth species was not affected by elevated CO_2, although their food consumption rate was increased. On the other hand, elevated O_3 reduced the growth of geometrid moth larvae fed with short-shoot leaves in spring. Food and oviposition preference was increased by elevated CO_2, but the effect was dependent on tree clone and insect species. Elevated O_3 decreased the growth of young earthworms fed with clone 80 litter, but otherwise the effects of CO_2 and O_3 on soil animal performance were minor. The measured plant quality related variables explained only up to half of the variation in CO_2- and O_3-mediated effects on insect performance, and the contribution of litter quality to soil animal performance was also low.
Conclusions The analysis of phenolic content values (i.e. the total amount per plant part) revealed that elevated CO_2 in general and elevated O_3 to some extent increase carbon allocation to certain phenolic compounds in birch leaves and that there are trade-offs among phenolic pathways between the studied tree clones. In silver birch, phenolic compounds may

serve as insect feeding stimulants, but the hypothesis of phenolics as silver birch defences against insect herbivores is questioned, since the growth of insects was not affected despite the observed CO_2 effects on phenolic compounds. It is obvious that O_3 is also detrimental to plant-feeding animals, but more research is needed to reveal the underlying factors. In addition, the effects of litter phenolics on soil animals may by nature be only indirect, and they should be investigated.

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I dedicate this thesis to my family. My parents took me to see the wonders of the woods, and dad taught me to hunt game and catch fish. I heartily thank Päivi for her support, encouragement and enthusiasm to my work and for sharing with me the spectacular sights of the Earth.

ABBREVIATIONS

CC	chamber control
CO_2	carbon dioxide
DHPPG	3,4'-dihydroxypropiophenone 3-β-D-glucoside
EC	elevated CO_2
EC+EO	elevated CO_2 and O_3 in combination
EO	elevated O_3
FACE	free-air CO_2 enrichment
HPLC	high-performance liquid chromatography
LMWP	low-molecular-weight phenolic compound
MS	mass spectrometry
O_3	ozone
OC	outside control
OTC	open-top chamber
PCA	principal component analysis
PGG	pentagalloylglucose
PLS	partial least squares
ppb	parts per billion, e.g. $nl\ l^{-1}$
ppm	parts per million, e.g. $\mu l\ l^{-1}$
RGR	relative growth rate
RCR	relative consumption rate
SI	simple interaction
SME	simple main effect

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by chapter numbers:

Chapter 2   Peltonen PA, Vapaavuori E, Julkunen-Tiitto R (2005) Accumulation of phenolic compounds in birch leaves is changed by elevated carbon dioxide and ozone. Global Change Biology 11: 1305-1324.

Chapter 3   Peltonen PA, Vapaavuori E, Heinonen J, Julkunen-Tiitto R, Holopainen JK. Food quality mediated interactive effects of CO_2 and O_3 concentrations on folivorous insects in birch (Betula pendula). Submitted for publication.

Chapter 4   Kasurinen A, Peltonen PA, Julkunen-Tiitto R, Vapaavuori E, Nuutinen V, Holopainen T, Holopainen JK. Effects of elevated CO_2 and O_3 on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. Submitted for publication.

Chapter 5   Peltonen PA, Julkunen-Tiitto R, Vapaavuori E, Holopainen JK (2006) Effects of elevated carbon dioxide and ozone on aphid oviposition preference and birch bud exudates phenolics. Global Change Biology 12: 1670-1679.

I participated in the planning of tests and collecting of plant samples, executed the tests, analysed the phenolic compounds from plant samples, carried out the statistical analyses and was the main author of Chapters 2, 3 and 5. In Chapter 4, I was responsible for the section that dealt with phenolic compounds and was co-author of this publication.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Atmospheric change affects forest trees

Human activities began to change the Earth's atmosphere dramatically during the final quarter of the last millennium. The burning of fossil fuels and changes in land use, particularly deforestation, have increased concentrations of the major greenhouse gas, carbon dioxide (CO_2) from 280 ppm in 1750 to 367 ppm in 1999 (IPCC 2001). It is estimated that the concentration of CO_2 could be 540 to 970 ppm by 2100. At the same time, the increased emissions of volatile organic compounds and reactive nitrogen species (i.e. NO_x) from anthropogenic sources have increased concentrations of photochemical air pollutant ozone (O_3) in the troposphere (Fowler et al. 1998, IPCC 2001, Vingarzan, 2004). Long-range (regional, continental or inter-continental) transport from distant sources, e.g. metropolitan areas, extends O_3 influence to remote, rural areas (Vingarzan, 2004). In fact, global background O_3 concentrations have increased from 10–15 ppb to the present-day level of 30–40 ppb within less than two centuries (Finlayson-Pitts & Pitts, 1997, Percy et al. 2003, Laurila et al. 2004). It is estimated that background O_3 concentrations will double by 2100, although there may be regional differences in this trend (Vingarzan, 2004). Consequently, it is estimated that by 2100, 60% of temperate and subpolar forests and almost half of all forests will be exposed to potentially phytotoxic O_3 concentrations (>60 ppb) (Fowler et al. 1999).

Numerous studies employing various enclosure techniques (growth chambers, open-top chambers, branch bags) and open field or free-air carbon dioxide exposure (FACE) facilities have shown that CO_2 enrichment has beneficial effects on forest trees at seedling stage. Elevated CO_2 increases carbon assimilation, and increases above-ground growth and litter production (Saxe et al. 1998, Norby et al. 1999, Pritchard et al. 1999, Karnosky et al. 2003, Ainsworth & Long 2005). By contrast, elevated O_3 decreases carbon assimilation and transport, increases carbon allocation to antioxidant defences and respiration, causes premature senescence of leaves and reduces growth cumulatively over time (Skärby et al. 1998, Wustman et al. 2001, Oksanen 2003). Because the concentration of both gases is increasing in the atmosphere, there has been discussion on whether CO_2 mitigates or ameliorates the deleterious effects of O_3 on trees. It has been found that elevated CO_2 may not protect trees from harmful O_3 effects (Karnosky et al. 2003), but may even exacerbate them (Wustman et al. 2001, Karnosky et al. 2005). However, making predictions on the responses of mature trees to CO_2 and O_3 on the basis of results obtained from seedling studies is not straightforward (Skärby et al. 1998, Norby et al. 1999, Kolb & Matyssek 2001), and therefore studies on older trees are needed.

1.2 Will climate change modify the fate of silver birch in the future?

It has been suggested that climate change, i.e. an increase in atmospheric CO_2 concentration and air temperature, may increase the competitive advantage of tree species with an indeterminate, continuous growth pattern, such as silver birch (*Betula pendula* Roth), over species with predetermined growth (e.g. conifers) (Kellomäki et al. 1996), and may thus increase the abundance of birches in Finland. However, the sensitivity of silver birch to ozone (Skärby et al. 1998, Oksanen & Rousi 2001, Oksanen 2003) confounds this prediction. Moreover, the fate of the birch may be complicated

even further because not only plants but also animals feeding on these plants will be affected by atmospheric change.

Besides its historical and economic importance for humans as firewood, building material, raw material for sawmills, the pulp and paper industry and even as a source of artistic inspiration, birch is an important food plant for several insect and mammalian herbivores. For example, silver birch and downy birch (*B. pubescens*) are hosts for approximately over 200, mostly Lepidopteran insect species in Finland (Saalas 1949, Annila 1987). Birch also improves soils by cycling nutrients (Perala & Alm 1990), and one important factor in this process is litter comminution by soil macrofauna (Swift et al. 1979), such as earthworms (Hendriksen 1990, Edwards & Bohlen 1996) and terrestrial isopods (woodlice) (Hassall et al. 1987).

To reduce herbivore damage, plants resort to mechanical and chemical traits that reduce herbivore performance (antibiosis) or preference (antixenosis) (Leimu & Koricheva 2006, Morris 2006). The nutritive quality of tissues plays a dominant role in the suitability of plants for plant-feeding animals and is generally determined by the concentration of nitrogen and secondary compounds (Scriber & Slansky 1981, Slansky 1992, Awmack & Leather 2002). Plants produce numerous secondary compounds that are harmful to herbivores or deter their feeding (Rosenthal & Berenbaum 1991). Of the non-nitrogenous secondary compounds, phenolic compounds occur universally in vascular plants (Harborne 1997). Although these compounds occur in all plant tissues, most studies have concentrated mainly on leaves and on high molecular weight oligomeric or polymeric compounds, such as condensed tannins, which occur in ferns, gymnosperms and woody angiosperms. It has been shown that hydrolysable and condensed tannins bind proteins and other substances, decreasing the palatability of food for herbivores, and affecting their food preference and growth (Hagerman & Butler 1991, Harborne 1997). In addition, phenolic compounds, including low molecular weight monomeric (LMWP) compounds, serve as chemical cues for feeding and oviposition, and are used in host selection by phytophagous insects (Bernays & Chapman 1994, Simmonds 2001, Städler 2002). In silver birch, condensed tannins and LMWP compounds seem to play a defensive role against insects (Mutikainen et al. 2000). However, in some cases herbivores have overcome the defences of birches and have defoliated vast areas of birch forests in different regions of Scandinavia and North America (Tenow 1972, Werner & Baker 1977, Annila 1979).

Food quality is also an important factor for litter feeding soil animals. Studies indicate that the concentration of minerals, proteins, sugars and phenolics in litter determine the suitability of food for earthworms (Edwards & Bohlen 1996). Food selection and consumption by earthworms seems to be influenced by phenolic compounds (Swift et al. 1979, Hendriksen 1990, Slapokas & Granhall 1991), which affect the palatability of litter at least in the early stage of decomposition (Edwards & Bohlen 1996, Schönholzer et al. 1998). Although earthworms are able to digest tannin-protein complexes (Kuiters 1990, Wardle & Lavelle 1997), this ability can be inhibited by the accumulation of other secondary metabolites in litter (Wardle & Lavelle 1997). Moreover, nitrogen concentration has been shown to affect earthworm growth and fecundity (Edwards & Bohlen 1996). These findings are supported by the observation that abundance of earthworms correlates negatively with litter C:N, lignin:N and

concentration of phenolic compounds (Edwards & Bohlen 1996).

Although some studies have shown that the consumption of terrestrial isopods is reduced by a high concentration of phenolic compounds, such as tannins (Cameron & LaPoint 1978, Zimmer 1999) and is increased by a high concentration of minerals (Ca, Mg, Na, K) in litter (Nicolai 1988), and that phenolics may affect the fecundity of isopods (Zimmer & Topp 2000), evidence of the effect of litter quality on them is controversial. For example, some studies show that litter with high concentrations of phenolic compounds are less preferred by isopods (Nicolai 1988), while others show absolutely no correlation between phenolics and isopod food preference (Neuhauser & Hartenstein 1978). Nevertheless, there is evidence that birch leaf litter with high tannin concentration is less preferred by isopods (Hassall & Rushton 1984, Hassall et al. 1987). Because phenolic compounds are abundant in birches (e.g. Bryant et al. 1993, Mutikainen et al. 1999, Ossipov et al. 2001), any change induced in the accumulation of these compounds in trees could thus affect not only leaf-feeding insects but also litter-feeding soil animals.

1.3 Atmospheric change affects plant quality and plant-herbivore interactions

1.3.1 Current knowledge on the effects of CO_2

Increasing atmospheric CO_2 concentrations may change plant-herbivore interactions by altering plant tissue quality (Bezemer & Jones 1998, Peñuelas & Estiarte 1998, Saxe et al. 1998, Norby et al. 1999). In fact, Körner (2000) has stated that "the rate by which the CO_2 concentration is doubling and the dietary change that it causes, are

unprecedented for the present biota". There has been intensive experimentation on this subject during recent decades, and information on the effects of elevated CO_2 on plant nutrients and secondary compounds has increased considerably. Studies have shown that CO_2 enrichment generally leads to a decrease in foliar nitrogen concentration (Bezemer & Jones 1998, Cotrufo et al. 1998a, Curtis & Wang 1998, Norby et al. 1999, Ainsworth & Long 2005), which may be caused by a dilution effect (Norby et al. 1999), because CO_2 increases leaf mass (Saxe et al. 1998), or by a decrease in the amount of RuBisCO (Ainsworth & Long 2005).

In contrast to nitrogen, elevated CO_2 usually increases the concentration of phenolic compounds (Bezemer & Jones 1998, Koricheva et al. 1998). Source-sink balance hypotheses, such as the carbon-nutrient balance hypothesis (Bryant et al. 1983) and the extended growth-differentiation balance hypothesis (Herms & Mattson 1992), have been formulated to explain carbon allocation to plant growth and secondary metabolism, but studies on the hypotheses have given variable results (Peñuelas & Estiarte 1998). One explanation for this variability could be unsuitability of the chosen variable. Because "analysis of ratios is based on the assumption of an isomeric relationship between denominator and numerator variables" (Raubenheimer & Simpson 1992), the use of concentration as a measure in carbon allocation studies may have led to misinterpretation of the results. In other words, ratio does not tell which variable has changed – the denominator or the numerator. To solve this problem, it has been suggested that the actual content or amount per plant or plant part be used instead (Koricheva 1999, Gianoli 2000).

Nevertheless, changes in food quality related variables measured as

concentrations appear to correlate with herbivore performance. CO_2 driven reductions in food quality have been shown to reduce the digestibility of food, and to reduce growth and increase consumption rates, development times and mortality of herbivores (Peñuelas & Estiarte 1998). However, the effects seem to be species-specific and should be examined in detail for each insect-plant system. So far, only relatively few species have been investigated (Coviella & Trumble 1999).

In accordance with the observed effect of elevated CO_2 on herbivores, litter quality is expected to influence the rate of litter decomposition by soil heterotrophs (Lambers 1993), especially during the initial stages of litter decomposition when the concentration of phenolic compounds is still high (Coûteaux & Bolger 2000). Studies have shown that CO_2 enrichment has indeed increased condensed tannins in the leaf litter of, for example, *Betula papyrifera* (Parsons et al. 2004), but more information is needed on the effects on soluble phenolics and tannins (Peñuelas & Estiarte 1998, Hättenschwiler & Vitousek 2000). In spite of evidence that the complexity of the soil fauna community is an important factor in the decomposition process (Coûteaux et al. 1991, Tian et al. 1997), especially macrofauna having a major effect during the early stages (Swift et al. 1979), the effects of CO_2 on soil macrofauna are underrepresented in the literature (Coûteaux & Bolger 2000). The few available studies have shown that elevated CO_2 can decrease preference (Cotrufo et al. 1998b) and increase consumption in isopods (Hättenschwiler et al. 1999, Hättenschwiler and Bretscher 2001), but contrary to observations in insects, nitrogen concentration does not appear to mediate isopod consumption (David et al. 2001, Hättenschwiler and Bretscher 2001). Moreover, the role of

phenolic compounds (lignin excluded) in this interaction is still unexplored, and there are no published reports on the effects of phenolic compound mediated responses of earthworms to elevated CO_2.

1.3.2 Interactive effects of CO_2 and O_3 seem to be complex and unpredictable

In field conditions, the effects of elevated CO_2 may not be so straightforward due to other interacting environmental factors, such as light, temperature, soil nitrogen availability and air pollution. Tropospheric O_3 may also influence plant-herbivore interactions by affecting plant quality, but interaction with elevated CO_2 may produce unpredictable results. For example, nitrogen concentration has been shown to decrease in leaves of deciduous trees under O_3 exposure (Lindroth et al. 2001, Oksanen and Rousi 2001, Holton et al. 2003, Yamaji et al. 2003), but the effect was enhanced, or only manifested, under simultaneous exposure to elevated CO_2 and O_3 (Kopper et al. 2001, Kopper and Lindroth 2003b, Agrell et al. 2005). Originally it was suspected that O_3 might hamper carbon assimilation and thus decreases the available precursors for the production of phenolic compounds in plants, but studies have shown that elevated O_3 actually increases the concentration of phenolic compounds (Koricheva et al. 1998, Yamaji et al. 2003). However, the interactive effects of CO_2 and O_3 on phenolics may be antagonistic (e.g. Percy et al. 2002) or synergistic (e.g. Kopper and Lindroth 2003a), a result that was not predictable from the results of the individual effects of gases.

In the literature, the number of papers dealing with the effects of O_3 on herbivores and detritivores is low in comparison to those describing the effects of CO_2. Nevertheless, it has been shown that O_3 reduces food palatability,

increases or decreases performance, and increases the food preference and consumption of herbivores (Docherty et al. 1997, Flückiger et al. 2001). However, recent studies with FACE facilities have shown that CO_2 and O_3 may have variable interactive effects on herbivores, even within the same plant-insect system (Percy et al. 2002, Holton et al. 2003, Kopper and Lindroth 2003b). On the other hand, not all plant-insect systems are affected by elevated CO_2 and O_3 applied either singly or in combination (Kopper et al. 2001).

It is reasonable to expect that O_3 induced alterations in leaf quality will be reflected subsequently in litter quality and decomposition. Indications of altered litter quality (Findlay et al. 1996, Liu et al. 2005) and reduced decomposition (Findlay et al. 1996, Kim et al. 1998) under O_3 enrichment has in fact been found, but contradictory results have also been reported (e.g. Boerner and Rebbeck 1995, Scherzer et al. 1998, Kainulainen et al. 2003, Parsons et al. 2004). Moreover, Loranger et al. (2004) have demonstrated that elevated CO_2 and O_3 as individual treatments can decrease the abundance of soil fauna (Acari, Collembola) in temperate forest ecosystems, although the effects are negated under the combined treatment. So far, the interactive, food quality mediated effects of CO_2 and O_3 on soil macrofauna have not been studied.

1.4 Aims of the study

In this thesis my aim was to analyse individual phenolic compounds in green leaves (Chapters 2 and 3), leaf litter (Chapter 4) and bud exudates (Chapter 5) of 7-year-old silver birch saplings, and to measure how atmospheric change affects carbon allocation to these compounds. Particular emphasis was placed on temporal changes in responses. I also wanted to find out how atmospheric

change affects the growth, food consumption and food or oviposition preference of herbivorous and detritivorous invertebrates, and to assess which phenolic compounds, if any, contribute to the observed effects.

References

Agrell J, Kopper BJ, McDonald EP & Lindroth RL (2005) CO_2 and O_3 effects on host plant preferences of the forest tent caterpillar (*Malacosoma disstria*). *Global Change Biology* 11: 588-599.

Ainsworth EA & Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165: 351-372.

Annila E (1979) Damage by *Phyllobius* weevils (Coleoptera: Curculionidae) in birch plantations (in Finnish with English summary). *Communicationes Instituti Forestalis fenniae* 97: 1-20.

Annila E (1987) Rauduskoivulla (*Betula pendula*) elävät hyönteiset. (in Finnish) *Sorbifolia* 18: 118-122.

Awmack C & Leather SR (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47: 817-844.

Bernays EA & Chapman RF (1994) *Host-plant selection by phytophagous insects.* Chapman & Hall, London.

Bezemer TM & Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. *Oikos* 82: 212-222.

Boerner REJ & Rebbeck J (1995) Decomposition and nitrogen release from leaves of three hardwood species grown under elevated O_3 and/or CO_2. *Plant and Soil* 170: 149-157.

Bryant JP, Chapin FS & Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40: 357-368.

Bryant JP, Reichardt PB, Clausen TP & Werner RA (1993) Effects of mineral nutrition on delayed inducible resistance in Alaska. *Ecology* 74: 2072-2084.

Cameron GN & LaPoint TW (1978) Effects of tannins on the decomposition of Chinese Tallow leaves by terrestrial and aquatic invertebrates. *Oecologia* 32: 349-366.

Cotrufo MF, Ineson P & Scott A (1998a) Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4: 43-54.

Cotrufo MF, Briones MJI & Ineson P (1998b) Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. *Soil Biology & Biochemistry* 30: 1565-1571.

Coûteaux M-M, Mousseau M, Célérier M-L & Bottner P (1991) Increased atmospheric CO_2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. *Oikos* 61: 54-64.

Coûteaux M-M & Bolger T (2000) Interactions between atmospheric CO_2 enrichment and soil fauna. *Plant and Soil* 224: 123-143.

Coviella CE & Trumble JT (1999) Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conservation Biology* 13: 700-712.

Curtis PS & Wang X (1998) A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. *Oecologia* 113: 299-313.

David J-F, Malet N, Coûteaux M-M & Roy J (2001) Feeding rates of the woodlouse *Armadillidium vulgare* on herb litters produced at two levels of atmospheric CO_2. *Oecologia* 127: 343-349.

Docherty M, Salt DT & Holopainen JK (1997) The impacts of climate change and pollution on forest pests. In: *Forests and Insects* (eds Watt AD, Stork NE, Hunter MD), pp. 229-247, Chapman & Hall, London.

Edwards CA & Bohlen PJ (1996) *Biology and Ecology of Earthworms.* Chapman & Hall, London.

Findlay S, Carreiro M, Krischnik V & Jones CG (1996) Effects of damage to living plants of leaf litter quality. *Ecological Applications* 6: 269-275.

Finlayson-Pitts BJ & Pitts Jr. JN (1997) Tropospheric air pollution: Ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles. *Science* 276: 1045-1052.

Flückiger W, Braun S & Hiltbrunner E (2001) Effects of air pollutants on biotic stress. In: *Air Pollution and Plant Life* (eds Bell JNB, Treshow M), pp. 379-406. John Wiley & Sons Ltd, Chichester.

Fowler D, Flechard C, Skiba U, Coyle M & Cape JN (1998) The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition. *New Phytologist* 139: 11-23.

Fowler D, Cape JN, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C & Stevenson D (1999) The global exposure of forests to air pollutants. *Water, Air and Soil Pollution* 116: 5-32.

Gianoli E, Ríos JM & Niemeyer HM (1999) Within-plant allocation of a chemical defense in *Secale cereale*. Is concentration the appropriate currency of allocation? *Chemoecology* 9: 113-117.

Hagerman AE & Butler LG (1991) Tannins and Lignins. In: *Herbivores. Their Interactions with Secondary Plant Metabolites.* Vol. 1 (eds Rosenthal GA, Berenbaum MR), pp. 355-388. Academic Press, San Diego, CA.

Harborne JB (1997) Role of Phenolic Secondary Compounds Metabolites in Plants and their Degradation in Nature. In: *Driven by Nature.* (eds Cadish G, Giller KE), pp. 67-74. University Press, Cambridge.

Hassall M & Rushton SP (1984) Feeding behaviour of terrestrial isopods in relation to plant defences and microbial activity. *Symposia of the Zoological Society of London* 53: 487-505.

Hassall M, Turner JG & Rands MRW (1987) Effects of terrestrial isopods on the decomposition of woodland leaf litter. *Oecologia* 72: 597-604.

Hättenschwiler S, Bühler S & Körner C (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO2. *Oikos* 85: 271-281.

Hättenschwiler S & Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution* 15: 238-243.

Hättenschwiler S & Bretsher D (2001) Isopod effects on decomposition of litter produced under elevated CO_2, N deposition and different soil types. *Global Change Biology* 7: 565-579.

Hendriksen NB (1990) Leaf litter selection by detritivore and geophagous earthworms. *Biology and Fertility of Soils* 10: 17-21.

Herms DA & Mattson WJ (1992) The dilemma of plants: to grow or to defend. *The Quarterly Review of Biology* 67: 283-335.

Holton MK, Lindroth RL & Nordheim EV (2003) Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. *Oecologia* 137: 233-244.

Intergovernmental Panel on Climate Change (2001) Climate Change 2001: *The Scientific Basis. Summary for Policymakers. Report of Working Group I of the Intergovernmental Panel on Climate Change.* http://www.ipcc.ch/pub/spm22-01.pdf

Kainulainen P, Holopainen T & Holopainen JK (2003) Decomposition of secondary compounds from needle litter of Scots pine grown under elevated CO_2 and O_3. *Global Change Biology* 9: 295-304.

Karnosky DF, Zak DR, Pregitzer K, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ, Kruger EL, Kubiske ME, Lindroth RL, Mattson WJ, McDonald EP, Noormets A, Oksanen E,

Parsons WFJ, Percy KE, Podila GK, Riemenschneider DE, Sharma P, Thakur R, Sober A, Sober J, Jones WS, Anttonen S, Vapaavuori E, Mankovska B, Heilman W & Isebrands JG (2003) Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17: 289-304.

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M & Percy KE (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell and Environment* 28: 965-981.

Kellomäki S, Väisänen H & Strandman H (1996) Response of the boreal forest ecosystem to climatic change and its silvicultural implications: modelling. In: *The Finnish Research Programme on Climate Change. Final Report.* (ed Roos J), pp. 252-253. Publications of the Academy of Finland, Edita.

Kim JS, Chappelka AH & Miller-Goodman MS (1998) Decomposition of Blackberry and Broomsedge Bluestem as influenced by ozone. *Journal of Environmental Quality* 27: 953-960.

Kolb TE & Matyssek R (2001) Limitations and perspectives about scaling ozone impacts in trees. *Environmental Pollution* 115: 373-393.

Kopper BJ, Lindroth RL & Nordheim EV (2001) CO_2 and O_3 effects on paper birch (Betulaceae: *Betula papyrifera*) phytochemistry and whitemarked tussock moth (Lymantriidae: *Orgyia leucostigma*) performance. *Environmental Entomology* 30: 1119-1126.

Kopper BJ & Lindroth RL (2003a) Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* 134: 95-103.

Kopper BJ & Lindroth RL (2003b) Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO_2 and O_3. *Agricultural and Forest Entomology* 5: 17-26.

Koricheva J, Larsson S, Haukioja E & Keinänen M (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* 83: 212-226.

Koricheva J (1999) Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia* 119: 467-473.

Körner C (2000) Biosphere responses to CO_2 enrichment. *Ecological Applications* 10: 1590-1619.

Kuiters AT (1990) Role of phenolic substances from decomposing forest litter in plant-soil interactions. *Acta Botanica Neerlandica 39: 329-348.*

Lambers H (1993) Rising CO_2, secondary plant metabolism, plant-herbivore interactions and litter decomposition. *Vegetatio* 104/105: 263-271.

Laurila T, Tuovinen J-P, Tarvainen V & Simpson D (2004) Trends and scenarios of gound-level ozone concentrations in Finland. *Boreal Environmental Research* 9: 167-184.

Leimu R & Koricheva J (2006) A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos* 112: 1-9.

Lindroth RL, Kopper BJ, Parsons WFJ, Bockheim JG, Karnosky DF, Hendrey GR, Pregitzer KS, Isebrands JG & Sober J (2001) Consequenses of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution* 115: 395-404.

Liu L, King JS & Giardina CP (2005) Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on leaf litter production and chemistry in trembling aspen and birch communities. *Tree Physiology* 25: 1511-1522.

Loranger GI, Pregitzer K & King JS (2004) Elevated CO_2 and O_{3t} concentrations differentially affect selected groups of the fauna in temperate forest soils. *Soil Biology & Biochemistry* 36: 1521-1524.

Morris WF, Traw MB & Bergelson J (2006) On testing for a tradeoff between constitutive and induced resistance. *Oikos* 112: 102-110.

Mutikainen P, Walls M, Ovaska J, Keinänen M, Julkunen-Tiitto R & Vapaavuori E (2000) Herbivore resistance in Betula pendula: effect of fertilization, defoliation and plant genotype. *Ecology* 81: 49-65.

Neuhauser EF & Hartenstein R (1978) Phenolic content and palatability of leaves and wood to soil isopods and diplopods. *Pedobiologia* 18: 99-109.

Nicolai V (1988) Phenolic and mineral content of leaves influences decomposition in European forest ecosystems. *Oecologia* 75: 575-579.

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW & Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant, Cell and Environment* 22: 683-714.

Oksanen E & Rousi M (2001) Differences of *Betula* origins in ozone sensitivity based on open-field experiment over two growing seasons. *Canadian Journal of Forest Research* 31: 804-811.

Oksanen E (2003) Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. *Tree Physiology* 23: 603-614.

Ossipov V, Haukioja E, Ossipova S, Hanhimäki S & Pihlaja K (2001) Phenolic and phenolic-related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. *Biochemical Systematics and Ecology* 29: 223-240.

Parsons WFJ, Lindroth RL & Bockheim JG (2004) Decomposition of *Betula papyrifera* leaf litter under the independent and interactive effects of elevated CO_2 and O_3. *Global Change Biology* 10: 1-12.

Peñuelas J & Estiarte M (1998) Can elevated CO_2 affect secondary metabolism and ecosystem function? *Trends in Ecology & Evolution* 13: 20-24.

Perala DA & Alm AA (1990) Reproductive ecology of birch: a review. *Forest Ecology and Management* 32: 1-38.

Percy K, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG, Pregitzer KS, Hendrey GR, Dickson RE, Zak DR, Oksanen E, Sober J, Harrington R & Karnosky DF (2002) Altered performance of forest pests under atmospheres enriched by CO_2 and O_3. *Nature* 420: 403-407.

Percy KE, Legge AH & Krupa SV (2003) Tropospheric ozone: A continuing threat to global forests? In: *Air Pollution, Global Change and Forests in the New Millenium.* (eds Karnosky DF, Percy KE, Chappelka AH, Simpson C, Pikkarainen J), pp. 85-118. Elsevier, Amsterdam.

Pritchard SG, Rogers HH & Prior SA (1999) Elevated CO_2 and plant structure: a review. *Global Change Biology* 5: 807-837.

Raubenheimer D & Simpson SJ (1992) Analysis of covariance: an alternative to nutritional indices. *Entomologia Experimentalis et Applicata* 62: 221-231.

Rosenthal GA & Berenbaum MR (1991) *Herbivores. Their Interaction with Secondary Plant Metabolites.* Academic Press, San Diego, CA.

Saalas U (1949) *Suomen metsähyönteiset.* (Finnish forest insects, in Finnish) WSOY, Porvoo.

Saxe H, Ellsworth DS & Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist* 139: 395-436.

Scherzer AJ, Rebbeck J & Boerner REJ (1998) Foliar nitrogen dynamics and decomposition of yellow-poplar and eastern white pine during four seasons of exposure to elevated ozone and carbon dioxide. *Forest Ecology and Management* 109: 355-366.

Schönholzer F, Kohli L, Hahn D, Daniel O, Goez C & Zeyer J (1998) Effects of decomposition of leaves on bacterial biomass and on palatability to *Lumbricus terrestris* L. *Soil Biology & Biochemistry* 30: 1805-1813.

Scriber JM & Slansky Jr. F (1981) The nutritional ecology of immature insects. *Annual Review of Entomology* 26: 183-211.

Simmonds MSJ (2001) Importance of flavonoids in insect-plant interactions: feeding and oviposition. *Phytochemistry* 56: 245-252.

Skärby L, Ro-Poulsen H, Wellburn FAM & Sheppard LJ (1998) Impacts of ozone on forests: a European perspective. *New Phytologist* 139: 109-122.

Slansky F, Jr (1992) Allelochemical-Nutrient Interactions in Herbivore Nutritional Ecology. In: *Herbivores. Their Interactions with Secondary Plant Metabolites.* Vol. 2 (eds Rosenthal GA, Berenbaum MR), pp. 135-175. Academic Press, San Diego, CA.

Slapokas T & Granhall U (1991) Decomposition of willow-leaf litter in a short-rotation forest in relation to fungal colonization and palatability for earthworms. *Biology and Fertility of Soils* 10: 241-248.

Städler E (2002) Plant chemical cues important for egg deposition by herbivorous insects. In: *Chemoecology of Insect Eggs and Egg Deposition* (eds Hilker M, Meiners T), pp. 171-204, Blackwell Publishing, Berlin.

Swift MJ, Heal OW & Anderson JM (1979) *Decomposition in terrestrial ecosystems.* Blackwell Scientific Publications, Oxford.

Tian G, Brussaard L, Kang BT & Swift MJ (1997) Soil Fauna-mediated Decomposition of Plant Residues under Constrained Environmental and Residue Quality Conditions. In: *Driven by Nature.* (eds Cadish G, Giller KE), pp. 125-134. University Press, Cambridge.

Tenow O (1972) The outbreaks of *Oporinia autumnata* Bkh. and *Operophthera* spp. (Lep., Geometridae) in the Scandinavian mountain chain and northern Finland 1862-1968. *Zoologiska Bidrag från Uppsala, Suppl. 2*, 1-107.

Vingarzan R (2004) A review of surface ozone background levels and trends. *Atmospheric Environment* 38: 3431-3442.

Wardle DA & Lavelle P (1997) Linkages between Soil Biota, Plant Litter Quality and Decomposition. In: *Driven by Nature.* (eds Cadish G, Giller KE), pp. 107-124. University Press, Cambridge.

Werner RA & Baker BH (1977) Spear-marked black moth. USDA, Forest Servive, Forest Insect & Disease Leaflet, 156, 8 pp.

Wustman BA, Oksanen E, Karnosky DF, Noormets A, Isebrands JG, Pregitzer KS, Hendrey GR, Sober J & Podila GK (2001) Effects of elevated CO_2 and O_3 on aspen clones varying in O_3 sensitivity: can CO_2 ameliorate the harmful effects of O_3? *Environmental Pollution* 115: 473-481.

Yamaji K, Julkunen-Tiitto R, Rousi M, Freiwald V & Oksanen E (2003) Ozone exposure over two growing seasons alters root-to-shoot ratio and chemical composition of birch (*Betula pendula* Roth). *Global Change Biology* 9: 1363-1377.

Zimmer M (1999) The fate and effects of ingested hydrolyzable tannins in *Porcellio scaber*. *Journal of Chemical Ecology* 25: 611-628.

Zimmer M & Topp W (2000) Species-specific utilization of food sources by sympatric woodlice (Isopoda: Oniscidea). *Journal of Animal Ecology*, 69, 1071-1082.

CHAPTER 6

GENERAL DISCUSSION

GENERAL DISCUSSION

6.1 Methodological considerations

Classic OTCs have advantages over other enclosure techniques (branch bags, growth chambers, glasshouses) in that they enable plant growth under natural light and soil conditions, but they do, however, suffer from altered microclimatic conditions (i.e. the "chamber effect") such as higher temperature and relative humidity, and lower wind speed and reduced light (Ceulemans & Mousseau 1994, Karnosky et al. 2005). Although OTCs are good for studying individual level effects, free-air exposure facilities, such as FACE, enable community level studies and are not hampered by the chamber effect (Karnosky et al. 2001). Nevertheless, OTCs are applicable, since for example interactions with temperature are difficult to study using free-air exposure facilities. Moreover, with regard to CO_2 effects on boreal forest trees, we have to rely on OTC studies because no FACE studies have yet been carried out on boreal forest ecosystems (Ainsworth & Long 2005). Furthermore, the results obtained from trees grown in OTCs and free-air fields have been similar for CO_2 (Ainsworth & Long 2005) and O_3 (Grantz et al. 2006, and references therein).

In our three-year long silver birch experiment, fumigations were successful and gas concentrations were close to target values most of the time (Vapaavuori et al. 2002, Chapter 2). However, the chamber effect was evident and affected the morphology, phytochemistry, physiology and growth of the trees (Eichelmann et al. 2004, Riikonen et al. 2004, Oksanen et al. 2005, Chapters 2 and 3). It appears that a chamber environment was less stressful than the ambient environment especially for clone 4 (Eichelmann et al. 2004, Riikonen et al. 2004) and may have diminished the effects of CO_2 and O_3 by reducing the stomatal conductance of trees (Riikonen et al. 2005). Hence, the results might have been higher under free-air conditions. On the other hand, the temperature inside the chambers is in accordance with that expected to prevail by the end of the century in Finland (Jylhä et al. 2004), and our OTCs may thus have given realistic results in this respect.

The sample drying and extraction methods and HPLC method that were used are good for analysing flavonoids in silver birch (Keinänen 1993, Keinänen & Julkunen-Tiitto 1996), but are less well suited for the analysis of hydrolysable tannins like ellagitannins (Salminen 2003). On the other hand, the accumulation of hydrolysable tannins is low in silver birch (Salminen et al. 2002). The accumulation of PGG, an ellagitannin precursor (Salminen et al. 2001), was not affected by elevated CO_2 or O_3 in silver birch (Chapters 2 and 3) indicating that ellagitannins were not affected either.

Plant-animal bioassays can be done either in the field or in the laboratory. In the field it is possible to study changes in free populations or individuals or groups of individuals confined in branch bags or clip cages etc. on their host plants. The OTC technology used did not allow population level studies, but provided plant material for laboratory bioassays. Although it was easier to study specific animal performance and preference, and food quality related hypotheses with bioassays in the laboratory, this methodology has some limitations. For instance, there are ecological factors that may modify animal responses to plant chemistry in the field (e.g. competitive ability, dispersal potential), but cannot be easily modeled in the laboratory (Berenbaum 1986). In addition, the controlled environment chambers and microcosms used in the bioassays of

insects and soil animals, respectively, are simplifications of their complex natural environments (Opp & Prokopy 1986), even though climatic conditions were set as close as possible to those prevailing in the field. On the other hand, complex climatic interactions, which were not the focus of the studies, such as increased temperature inside the chambers, did not confound the results obtained from laboratory tests.

The quality of test animals in laboratory-reared populations may change fast due to inbreeding, and genotypic alterations may influence animal behaviour (Kogan 1986, Opp & Prokopy 1986, Tingey 1986). The larvae of *E. autumnata* were reared under laboratory conditions for three years and may thus have suffered from some level of inbreeding, despite progeny control. Some of the larvae used in the experiments in 2001 were malformed prior to pupation and did not pupate normally. However, none of the larvae showed any such symptoms or abnormal behavior during the experiments. It is possible that subtle changes in food quality had caused this effect. Habituation or conditioning of larvae to food before the experiments (Berenbaum 1986) was avoided by keeping separate populations of moths for experiments and breeding.

Though laboratory-reared animals may have problems, animals collected from the field are not without problems either, because they can be infested with pathogens or parasites that change their behaviour (Tingey 1986). The larvae of *R. hastata* were obtained from eggs laid by captive wild adults under controlled conditions, whereas *O. antiqua* eggs were collected from the field. All eggs were stored under controlled conditions. Leaf weevil adults were collected from the field as well, but none of these wild individuals showed any indication of parasitism or disease during or after the experiments.

The great variation in the results of choice feeding tests (Chapter 3) shows that a higher number of replicates would have been needed (Opp & Prokopy 1986). In fact, low replication may have reduced the power of the statistical tests in all experiments (Quinn & Keough 2005), despite the fact that the range of statistical significance was broadened to 0.1 in order to minimize type II decision errors (Chapters 2-5). This meant, however, that inferences made from results within the 0.05-0.1 range were considered with caution, due to the increased probability of their being affected by chance.

6.2 Carbon allocation to growth and phenolic compounds in silver birch under elevated CO_2 and O_3 concentrations

6.2.1 Effects on growth

The results of studies conducted with these silver birch trees show unequivocally that CO_2 enrichment has beneficial effects on the growth of trees even after the seedling stage (Eichelmann et al. 2004, Kull et al. 2003, Riikonen et al. 2004, 2005), which is in accordance with recent studies (e.g. Ainsworth & Long 2005). At the plant part level, elevated CO_2 increased leaf dry weight markedly (Eichelmann et al. 2004, Chapters 2 and 4), whereas it had only a minor effect on bud dry weight (Chapter 5) and did not affect the size of these plant parts (Chapters 2 and 5). By contrast to CO_2, elevated O_3 reduced whole-tree growth (Kull et al. 2003, Riikonen et al. 2004) as was expected according to previous studies (e.g. Skärby et al. 1998), but the effects were less pronounced and often statistically insignificant at the plant part level

(Riikonen et al. 2004, Chapters 2, 4 and 5).

Contrary to findings in temperate deciduous forest tree species (Karnosky et al. 2003), the growth reductions observed in silver birch were ameliorated by elevated CO_2 (Kull et al. 2003, Riikonen et al. 2004, Chapters 2, 4 and 5). Differences in the elevation of CO_2 gas concentrations between the studies with temperate and boreal deciduous trees (+200 ppm vs. +360 ppm, respectively) may have affected the results, but they may also have been influenced by differences in other climatic factors. For example, it has been shown that springtime frost can predispose trees to ozone damage in northern boreal forest zone (Oksanen et al. 2006). Environmental conditions inside the OTCs may have been more benign and thus may have mitigated the effects of otherwise harsh northern conditions, especially in clone 4 (Eichelmann et al. 2004). On the other hand, high air temperatures in autumn may increase the deleterious effects of O_3 (Kubiske et al. 2006). Given the predictions of future climatic conditions in Finland, for example a 2–12°C increase in springtime temperatures and 2–7°C increase in autumn temperatures (Jylhä et al. 2004), the deleterious effects of O_3 on birch growth are difficult to predict. The wide genetic variation in the sensitivity of birches to O_3 (Pääkkönen et al. 1997, Yamaji et al. 2003) adds even more uncertainty to the predictions.

6.2.2 Effects on phenolics

The results demonstrate the applicability of graphical vector analysis and show that the content value is a good indicator of carbon allocation to phenolic compounds (Chapters 2, 4 and 5). The observed differences in phenolic composition between green and abscised leaves (Chapters 2, 3 and 4) indicate that some compounds are actively produced in green leaves, and may be subject to turnover, or that they are degraded or leached more rapidly during or after leaf abscission. Nevertheless, elevated CO_2 was found generally to increase carbon allocation to phenolic compounds in silver birch leaves and leaf litter, although not all individual compounds or compounds groups were affected. For example, the content of DHPPG and flavonoid aglycones was unresponsive to CO_2 enrichment, whereas the contents of phenolic acids, flavonol glycosides and condensed tannins were markedly increased (Chapters 2 and 4). On the other hand, the effects of O_3 on phenolics showed temporal variation in green and abscised leaves, indicating that O_3 was able to alter the carbon allocation patterns over time, but not to the same degree as did CO_2.

Interestingly, in parallel to leaf epicuticular compounds, the content of flavonoid aglycones found in bud exudates was also unresponsive to CO_2 (Chapter 5). These results imply that the accumulation of epicuticular compounds in leaf and bud glandular trichomes may be independent of the accumulation of intracellular compounds in leaves, as suggested by Cuadra et al. (1997) and Onyilagha and Grotewold (2004). Lavola and Julkunen-Tiitto (1994) and Lavola et al. (1994) have reported similar differences in responses to CO_2 enrichment among leaf and stem phenolics of birch. This indicates that phenolic pathways are also regulated independently in different plant parts.

6.3 Phenolic compounds as silver birch defences under changing atmospheric conditions

6.3.1 Implications for insect herbivores

Bioassays showed intriguing variation in the responses of herbivorous insects and

detritivorous soil macrofauna to CO_2- and O_3-mediated effects in birch quality. Despite clear changes in phenolic compounds under elevated CO_2 and O_3, the growth of juvenile insects was affected only by elevated O_3 (Chapter 3). The results contradict the hypothesis that phenolic compounds are defences against insect herbivores in silver birch. Similarly, information on the vast structural diversity of ubiquitous condensed tannins (Ayres et al. 1997) and their variable effects on insect herbivores (Ayres et al. 1997, Forkner et al. 2004) cast doubt on the importance of phenolics as defences against insects and thus on the relevance of current, herbivory-oriented plant defence hypotheses (reviewed by Stamp 2003).

It is clear that resistance is comprised of many interacting factors in plants (Kause et al. 1999, Ossipov et al. 2001, Haukioja et al. 2002, Haukioja 2003) and phenolics may play only a partial role. The raison d'être of plant phenolic production is thus an interesting question. Harborne (1991) has suggested that flavonoids have evolved with a primary, physiological function (e.g. protection from UV-radiation), while a secondary, ecological function as a cue for feeding and oviposition has evolved thereafter from feeding-inhibitory activity against herbivores. It is possible that one compound may have several functions (Harborne 1991), but it has also been suggested that plants may produce a variety of compounds with no actual purpose; a certain reservoir or "screen" of compounds waiting for an opportunity, such as altered environmental conditions, to enhance the plant's fitness (Firn & Jones 2003). However, more experiments using a wider range of species are needed before any firm inferences on the role and importance of phenolic compounds in plant resistance can be made.

Interestingly, Swihart and Bryant (2001) have shown that tree ontogeny (maturation) has an influence on tree resistance against mammalian herbivores in northern latitudes: juvenile-stage trees are less browsed than mature-stage trees. In other words, the resistance is higher in juvenile trees. Because the silver birch trees used in the experiments were 10 years old and were approaching maturity at the time when the bioassays were done (Riikonen et al. 2004), it is possible that the overall resistance of the trees against insect herbivores had declined. Moreover, although it is possible that phenolics serve merely as feeding and oviposition stimulants, i.e. as cues for host recognition, the food or oviposition preference was affected in birch by tree genotype and insect species (Chapters 3 and 5). Swihart and Bryant (2001) found that the density of herbivory diminishes with an increase in the latitudinal origin of conspecific plants. However, in insect herbivores, this correlation may be different (Swihart & Bryant 2001 and references therein), as was seen in the results for two tree genotypes in this thesis: southern clone 4 was preferred by birch aphids, whereas northern clone 80 was preferred by *P. argentatus* weevils and *R. hastata* moths.

Slansky (1992) suggested that allelochemicals (secondary compounds) may also be harmful to insect intestinal microbes or to the detoxication enzymes they produce, but the unresponsiveness of *E. autumnata* larvae to phenolics induced by CO_2 (Chapter 3) does not support this idea. The observed O_3 effect on insect growth may be better explained by O_3 induced foliar oxidative stress, which may be an important mechanism of plant resistance to insects (Appel 1993, Bi & Felton 1995). The observed increases in ascorbate (ASC) levels and ASC use rate by ascorbate oxidase (AO) in O_3-treated leaves of the same trees used in the experiments (Padu et al. 2005) may provide one explanation for the observed decline in *E. autumnata*

growth. Felton and Summers (1993) have proposed that AO, which is a ubiquitous plant enzyme, is able to reduce the bioavailability of ascorbic acid for insects that require this vitamin for growth, molting and reproduction. Although herbivores are generally able to detoxify pro-oxidant chemicals in their diet (Lindroth 1991) the detoxification capacity of the young *E. autumnata* larvae may have been overcome. Recently, Cornelissen et al. (2006) presented the novel idea of foliar pH as a determinant of leaf quality. These authors reported that an increase in pH, which correlates, for example, negatively with plant carbon and positively with plant nitrogen, increases digestion by ruminant herbivores. Provided this effect is same for folivorous insects, the observed increase of carbon in O_3-exposed birch leaves could have led to decreases in pH and digestibility, and consequently to a reduction in larval growth.

6.3.2 Implications for detritivorous soil animals

Both CO_2 and O_3 affected the growth of soil macrofauna, but the influence of phenolics on this interaction was uncertain (Chapter 4). Possibly the effect was indirect by nature. It has been shown that microorganism-mediated preprocessing of litter is important for earthworms (Wright 1972) and isopods (Zimmer and Topp 1997). In plants, O_3 induces mechanisms similar to the defence responses invoked by pathogens (Sandermann et al. 1998) including the synthesis of phenolic compounds, and at least some phenolics have been shown to possess both antimicrobial (Cowan 1999, Rauha et al. 2000) and antifungal (Alcerito et al. 2002) properties. Taken together, it is possible that soil macrofauna were affected via phenolic-mediated changes in microorganisms. There is in fact evidence that CO_2 and O_3

can alter the composition of microorganisms in soils (e.g. Coûteaux & Bolger 2000, Chung et al. 2006), but further investigations are needed to ascertain the validity of this hypothesis.

Elevated CO_2 has been shown to increase total leaf area per plant (Saxe et al. 1998, Norby et al. 1999, Pritchard et al. 1999), a result which was also observed in the trees used in this study (Riikonen et al. 2004). This means that the input of low quality litter will increase under CO_2 enrichment, which in turn may reduce the comminution and consumption of litter by soil macrofauna (Coûteaux & Bolger 2000). The effect that the increased input of litter phenolics into soils have on soil pH deserves attention in future studies, since soil pH is an important factor for earthworms (Edwards & Bohlen 1996).

6.4 A century is a long time to predict the effects of atmospheric change

To date, factorial experiments studying the effects of atmospheric change on forest trees have used only simple gradients, i.e. one elevated gas concentration level (e.g. +200 ppm or +360 ppm CO_2) that have been compared to control. However, environmental change is gradual by nature, and thus the current information gives only a glimpse of possible effects over time. Moreover, the modelled scenarios depend on emission abatement actions (IPCC 2001), but these agreements have not yet been undersigned by all countries.

The effects of atmospheric change on silver birch are also influenced by other abiotic factors that need to be taken into account in predictions. There are factors that may either mitigate the effects of elevated CO_2, such as increasing temperature (Zvereva & Kozlov 2006), or may exacerbate the effects of elevated O_3, such as an increase in various wavelengths of light (Ormrod et al. 1995,

Kubiske et al. 2006) and in temperature (Kubiske et al. 2006). In addition, large temporal variation in phenolic production in birch may be caused by interannual variability in climate (Kubiske et al. 2006) and by an ontogenic shift in trees (Boege & Marquis 2005). Morever, intraspecific variability in the phenolic compounds (Laitinen 2003) will increase the amount of variation within silver birch forests. Although these changes may not have an effect on common polyphagous insect species, there is a large number of untested species, including species that are insufficiently known or are rare, thereby making atmospheric change an issue affecting biodiversity. Multifactorial FACE experiments conducted under northern conditions and combining the above mentioned abiotic factors are therefore needed in order to gain a deeper understanding of the effects of atmospheric change on boreal forests.

6.5 Conclusions

Graphical vector analysis and the analysis of total contents are significant improvements in studies of carbon allocation in plants. Without this method, inferences on the responses of phenolic compounds to atmospheric change in silver birch could have been flawed, due to the interference of biomass change. My studies revealed how the accumulation of epicuticular phenolic compounds is different from that of other compounds. The relatively long duration of the fumigation experiment in comparison to most of the previous experiments on the subject showed that the effects of elevated CO_2 on phenolics are significant and stable over time, whereas the effects of elevated O_3 are more tissue- and compound-dependent and could accumulate over time. However, despite significant changes in phenolics under elevated CO_2, the growth

of two potential defoliator insect species was not affected, indicating the minor importance of phenolics as defensive compounds in silver birch. The growth responses of silver birch and early season insect larvae and detritivorous soil macrofauna feeding on birch leaves underline the threat represented by tropospheric O_3 to living organisms, although the exact reason for this effect in the studied animals remains to be clarified.

References

Ainsworth EA & Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165: 351-372.

Alcerito T, Barbo FE, Negri G, Santos DYAC, Meda CI, Young MCM, Chávez D & Blatt CTT (2002) Foliar epicuticular wax of *Arrabidaea brachypoda*: flavonoids and antifungal activity. *Biochemical Systematics and Ecology* 30: 677-683.

Appel HM (1993) Phenolics in ecological interactions: the importance of oxidation. *Journal of Chemical Ecology* 19:1521-1552.

Ayres MP, ClausenTP, MacLean Jr SF, Redman AM & Reichardt PB (1997) Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* 78: 1696-1712.

Berenbaum M (1986) Postingestive Effects of Phytochemicals on Insects: On Paracelsus and Plant Products. In: *Insect-Plant Interactions* (eds Miller JR, Miller TA), pp.121-154. Springer-Verlag, New York.

Bi JL & Felton GW (1995) Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *Journal of Chemical Ecology* 21: 1511-1530.

Boege K & Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* 20: 441-448.

Ceulemans R & Mousseau M (1994) Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist* 127: 425-446.

Chung H, Zak DR & Lilleskov EA (2006) Fungal community composition and metabolism under elevated CO_2 and O_3. *Oecologia* 147: 143-154.

Cornelissen JHC, Quested HM, van Logtestijn RSP, Pérez-Harguindeguy N, Gwynn-Jones D, Díaz

S, Callaghan TV, Press MC & Aerts R (2006) Foliar pH as a new plant trait: can it exlpain variation in foliar chemistry and carbon cycling processes among subarctic plant species and types? *Oecologia* 147: 315-326.

Coûteaux M-M & Bolger T (2000) Interactions between atmospheric CO_2 enrichment and soil fauna. *Plant and Soil* 224: 123-134.

Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12: 564-582.

Cuadra P, Harborne JB & Waterman PG (1997) Increase in surface flavonols and photosynthetic pigments in *Gnaphalium luteo-album* in response to UV-B radiation. *Phytochemistry* 45: 1377-1383.

Edwards CA & Bohlen PJ (1996) *Biology and Ecology of Earthworms*. Chapman & Hall, London.

Eichelmann H, Oja V, Rasulov B, Padu E, Bichele I, Pettai H, Möls T, Kasparova I, Vapaavuori E & Laisk A (2004) Photosynthetic parameters of birch (*Betula pendula* Roth) leaves growing in normal and in CO_2- and O_3-enriched atmospheres. *Plant, Cell and Environment* 27: 479-495.

Felton GW & Summers CB (1993) Potential role of ascorbate oxidase as a plant defence protein against insect herbivory. *Journal of Chemical Ecology* 19: 1553-1568.

Firn RD & Jones CG (2003) Natural products – a simple model to explain chemical diversity. *Natural Products Reports* 20: 382-391.

Forkner RE, Marquis RJ & Lill JT (2004) Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of Quercus. *Ecological Entomology* 29: 174-187.

Grantz DA, Gunn S & Vu H-B (2006) O_3 impact on plant development: a meta-analysis of root/shoot allocation and growth. *Plant, Cell and Environment* 29: 1193-1209.

Harborne JB (1991) Flavonoid pigments. In: *Herbivores. Their interactions with secondary plant metabolites. Volume I: The chemical participants* (eds Rosenthal GA, Berenbaum MR), pp. 389-429. Academic Press, San Diego.

Haukioja E (2003) Putting the insect into the birch-insect interaction. *Oecologia* 136: 161-168:

Haukioja E, Ossipov V & Lempa K (2002) Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata* 104: 125-136.

Intergovernmental Panel on Climate Change (2001) Climate Change 2001: *The Scientific Basis. Summary for Policymakers. Report of Working Group I of the Intergovernmental Panel on Climate Change.* http://www.ipcc.ch/pub/spm22-01.pdf

Jylhä K, Tuomenvirta H & Ruosteenoja K (2004) Climate change projections for Finland during the 21st century. *Boreal Environment Research* 9: 127-152.

Karnosky DF, Gielen B, Ceulemans R, Schlesinger WH, Norby RJ, Oksanen E, Matyssek R & Hendrey GR (2001) FACE Systems for Studying the Impacts of Greenhouse Gases on Forest Ecosystems. In: *The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems* (eds Karnosky DF, Ceulemans R, Scarascia-Mugnozza GE, Innes JL), pp. 297-324. CABI Publishing, Wallingford.

Karnosky DF, Zak DR, Pregitzer K, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ, Kruger EL, Kubiske ME, Lindroth RL, Mattson WJ, McDonald EP, Noormets A, Oksanen E, Parsons WFJ, Percy KE, Podila GK, Riemenschneider DE, Sharma P, Thakur R, Sober A, Sober J, Jones WS, Anttonen S, Vapaavuori E, Mankovska B, Heilman W & Isebrands JG (2003) Troposheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17: 289-304

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M & Percy KE (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell and Environment* 28: 965-981.

Kause A, Ossipov V, Haukioja E, Lempa K, Hanhimäki S & Ossipova S (1999) Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia* 120: 102-112.

Keinänen M (1993) Comparison of methods for the extraction of flavonoids from birch leaves (*Betula pendula* Roth) carried out using high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 41: 1986-1990.

Keinänen M & Julkunen-Tiitto R (1996) Effect of sample preparation method on birch (*Betula pendula* Roth) leaf phenolics. *Journal of Agricultural and food chemistry* 44: 2724-2727.

Kogan M (1986) Bioassays for Measuring Quality of Insect Food. In: *Insect-Plant Interactions* (eds Miller JR, Miller TA), pp.155-190. Springer-Verlag, New York.

Kubiske ME, Quinn VS, Heilman WE, McDonald EP, Marquardt PE, Teclaw RN, Friend AL & Karnosky DF (2006) Interannual climatic variation mediates elevated CO_2 and O_3 effects on forest growth. *Global Change Biology* 12: 1054-1068.

Kull O, Tulva I & Vapaavuori E (2003) Influence of elevated CO_2 and O_3 on *Betula pendula* Roth crown structure. *Annals of Botany* 91: 559-569.

Laitinen M-L (2003) *Variation in secondary chemistry within a natural population of birch: Effects of genotype, environment and ontogeny*. Ph.D. thesis, University of Joensuu.

Lavola A & Julkunen-Tiitto R (1994) The effect of elevated carbon dioxide and fertilization on primary and secondary metabolites in birch, *Betula pendula* (Roth). *Oecologia* 99: 315-321.

Lavola A, Julkunen-Tiitto R & Pääkkönen E (1994) Does ozone stress change the primary or secondary metabolites of birch (*Betula pendula* Roth.)? *New Phytologist* 126: 637-642.

Lindroth RL (1991) Differential Toxicity of Plant Allelochemicals to Insects: Roles of Enzymatic Detoxication Systems. In: *Insect Plant Interactions. Vol III.* (ed Bernays E), pp. 1-34.CRC Press, Boca Raton, FA.

Norby RJ, Wullschleger SD & Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant, Cell and Environment* 22: 683-714.

Oksanen E, Riikonen J, Kaakinen S, Holopainen T & Vapaavuori E (2005) Structural characteristics and chemical composition of birch (*Betula pendula*) leaves are modified by increasing CO_2 and ozone. *Global Change Biology* 11: 732-748.

Oksanen E, Kontunen-Soppela S, Riikonen J, Peltonen P, Uddling J & Vapaavuori E (2006) The northern environment predisposes birch to ozone damage. Plant Biology, in press.

Onyilagha JC & Grotewold E (2004) The biology and structural distribution of surface flavonoids. *Recent Research Developments in Plant Sciences* 2: 1-19.

Opp SB & Prokopy RJ (1986) Approaches and Methods for Direct Behavioral Observation and Analysis of Plant-Insect Interactions. In: *Insect-Plant Interactions* (eds Miller JR, Miller TA), pp.1-22. Springer-Verlag, New York.

Ormrod DP, Landry LG & Conklin PL (1995) Short-term UV-B radiation and ozone exposure effects on aromatic secondary metabolite accumulation and shoot growth of flavonoid-deficient *Arabidopsis* mutants. *Physiologia Plantarum* 93: 602-610.

Ossipov V, Haukioja E, Ossipova S, Hanhimäki S & Pihlaja K (2001) Phenolic and phenolic-related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. *Biochemical Systematics and Ecology* 29: 223-240.

Pääkkönen E, Holopainen T & Kärenlampi L (1997) Variation in ozone sensitivity among clones of *Betula pendula* and *Betula pubescens. Environmental Pollution* 95: 37-44.

Padu E, Kollist H, Tulva I, Oksanen E & Moldau H (2005) Components of apoplastic ascorbate use in *Betula pendula* leaves exposed to CO_2 and O_3 enrichment. *New Phytologist* 165: 131-142.

Pritchard SG, Rogers HH & Prior SA (1999) Elevated CO_2 and plant structure: a review. *Global Change Biology* 5: 807-837.

Quinn GP & Keough MJ (2005) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Rauha J-P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H & Vuorela P (2000) Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology* 56: 3-12.

Riikonen J, Lindsberg M-M, Holopainen T, Oksanen E, Lappi J, Peltonen P & Vapaavuori E (2004) Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiology* 24: 1227-1237.

Riikonen J, Holopainen T, Oksanen E & Vapaavuori E (2005) Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO_2 and O_3 in the field. *Tree Physiology* 25: 621-632.

Salminen J-P (2003) Effects of sample drying and storage, and choice of extraction solvent and analysis method on the yield of birch leaf hydrolyzable tannins. *Journal of Chemical Ecology* 29: 1289-1305.

Salminen J-P, Ossipov V, Haukioja E & Pihlaja K (2001) Seasonal variation in the content of hydrolysable tannins in leaves of *Betula pubescens. Phytochemistry* 57: 15-22.

Salminen J-P, Ossipov V & Pihlaja K (2002) Distribution of hydrolysable tannins in the foliage of Finnish birch species. *Zeitschrift für Naturforschung* 57c: 248-256.

Sandermann H, Ernst D, Heller W & Langebartels C (1998) Ozone: an abiotic elicitor of plant defence reactions. *Trends in Plant Science* 3: 47-50.

Saxe H, Ellsworth DS & Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist* 139: 395-436.

Skärby L, Ro-Poulsen H, Wellburn FAM & Sheppard LJ (1998) Impacts of ozone on forests: a European perspective. *New Phytologist* 139: 109-122.

Slansky F Jr. (1992) Allelochemical-Nutrient Interactions in Herbivore Nutritional Ecology. In: *Herbivores. Their Interactions with Secondary Plant Metabolites.* Vol. 2 (eds Rosenthal GA, Berenbaum MR), pp. 135-175. Academic Press, San Diego, CA.

Stamp N (2003) Out of the quagmire of plant defense hypotheses. The Quarterly Review of Biology 78: 23-55.

Swihart RK & Bryant JP (2001) Importance of biogeography and ontogeny of woody plants in winter herbivory by mammals. *Journal of Mammalogy* 82: 1-21.

Tingey WM (1986) Techniques for Evaluating Plant Resistance to Insects. In: *Insect-Plant Interactions* (eds Miller JR, Miller TA), pp.251-284. Springer-Verlag, New York.

Vapaavuori E, Oksanen T, Holopainen JK, Holopainen T, Heiskanen J, Julkunen-Tiitto R, Kasurinen A, Oksanen E, Peltonen P, Poteri M, Repo T, Riikonen J & Syrjälä L (2002) *Technical report. Open-top chamber fumigation of cloned silver birch (Betula pendula Roth) trees to elevated CO_2 and ozone: description of the fumigation system and the experimental site*. The Finnish Forest Research Institute, Research Papers 838. http://www.metla.fi/julkaisut/mt/838

Wright MA (1972) Factors governing ingestion by the earthworm *Lumbricus terrestris* (L.), with special reference to apple leaves. *Annals of Applied Biology* 70: 175-188.

Yamaji K, Julkunen-Tiitto R, Rousi M, Freiwald V & Oksanen E (2003) Ozone exposure over two growing seasons alters root-to-shoot ratio and chemical composition of birch (*Betula pendula* Roth). *Global Change Biology* 9: 1363-1377.

Zimmer M & Topp W (1997) Does leaf litter quality influence population parameters of the common woodlouse, *Porcellio scaber* (Crustacea: Isopoda)? *Biology and Fertility of Soils* 24: 435-441.

Zvereva EL & Kozlov MV (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a metaanalysis. *Global Change Biology* 12: 27-41.