AUTUMNAL MOTH (*EPIRRITA AUTUMNATA*) INDUCED VOLATILE ORGANIC COMPOUNDS OF NORDIC MOUNTAIN BIRCH (*BETULA PUBESCENS* SPP. *CZEREPANOVII*)

Panu Piirtola
Master of Science Thesis
Department of Environmental Science
Faculty of Science and Forestry
University of Eastern Finland
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University of Eastern Finland
Faculty of Science and Forestry
Department of Environmental Science

Panu Piirtola: Autumnal moth (*Epirrita autumnata*) induced volatile organic compounds of nordic mountain birch (*Betula pubescens* spp. *Czerepanovii*)

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Supervisors: James Blande (PhD) and Jarmo Holopainen (Prof.)

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ABSTRACT

The aim of this study was to define the quantity and quality of volatile organic compounds emitted by the nordic mountain birch (*Betula pubescens* spp. *Czerepanovii*) for the first time, and to find out if there were any induction of volatile organic compounds when defoliated by the autumnal moth (*Epirrita autumnata*). The experimental part was conducted near by the subarctic research station of University of Turku, in Kevo, Utsjoki. Experiments were done with 15 pairs of mountain birch trees, where one of the pair was a control tree and the other one was defoliated by an autumnal moth. Volatile organic compound emissions were collected and measured from branches of these trees. 14 different compounds of volatile organic compounds were found and three of them were induced with statistical significance, linalool, (E)-DMNT and cis-3-hexenyl butyrate. The presented results show the composition and quantity of volatile organic compounds emitted by the mountain birch in normal and stress related conditions. There is a clear indication that defoliation of the mountain birch by the autumnal moth causes an induction of volatile organic compound emissions.

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The purpose of this study was to determinate the quantity and the quality of volatile organic compound emissions from the nordic mountain birch in normal conditions and under defoliation of herbivores. The experiments were done with Jarmo Holopainen's research group of chemical ecology. This project was funded by Academy of Finland.

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Jyväskylä, 26th June, 2011 Panu Piirtola

1. INTRODUCTION

Volatile organic compounds (VOCs) are a diverse group of hydrocarbons that are produced and emitted from anthropogenic and biogenic sources. Biological VOCs in plants are produced in the plant's leaves, flowers, fruits and roots, and are emitted as gas-like formations to atmosphere and from the roots also to soil. This group of compounds (VOCs) consists of isoprenoids, alkanes, alkenes, carbonyls, alcohols, esters, ethers and acids. The most prominent group for plants is isoprenoids (also referred as terpenoids, further in the text referred as terpenes that is most used name for this group) that is followed by alcohols and carbonyls (Kesselmeier and Staudt 1999, Hakola 2001, Tholl et al. 2006).

For plants, VOCs have been proven to work as the indirect defence against herbivores. Many plant species increase VOC emissions, in quantity and in quality, as a response to herbivores. This response attracts herbivore predators ("crying for help"), arthropods, on the site (Turlings et al. 1991, Turlings and Fritzsche 1999, Holopainen 2001, Kost and Heil 2006). There are also some experiments made that show the possibility of increased VOCs having significance on attracting birds (Mäntylä et al. 2008, Mäntylä et al. 2004), pollinators (Hakola 2001, Dudareva et al.2006) and there is also data that shows interaction with neighbouring plants (Kost and Heil 2006, Himanen et al. 2010).

The mountain birch (*Betula pubescens* ssp. *Czerepanovii* (Orlova) Hämet-Ahti) forms the upper and the northern tree line of sub-arctic and arctic regions of Fennoscandia and covers almost 600 000 ha (Lempa et al. 2004, Haapanala et al. 2009). It forms a dominant shrub/tree vegetation and plays a major ecological role in these regions as a source of food, shelter, nesting sites, soil formation, etc. (Ruuhola et al. 2007). The most common pests of mountain birch are the larvae of the autumnal moth (*Epirrita autumnata*) and the winter moth (*Operophthera brumata*). These two, mainly the autumnal moth larvae, can cause a massive defoliation (several square kilometres) during their outbreaks that take place on 10-year intervals (Tammaru et al. 2001, Karlsson et al. 2005).

In earlier studies *Betula pubescens* and *Betula pendula* have shown emissions of monoterpenes, homoterpenes, sesquiterpenes and green leaves volatiles (GLVs) (Vuorinen et al. 2007, Lindfors et al. 2000, Hakola 2001, Vuorinen et al. 2005). In stress conditions

(pathogen infection, herbivory, drought, etc.) *Betula pendula* VOC emissions have shown variations in quantity and in the composition of compounds present (Vuorinen et al. 2007).

The purpose of this study is to determinate VOC emissions of mountain birch growing in a natural forest stand and to investigate how the quantity of VOCs and the variation of compounds are affected by foliage damage caused by the larvae of autumnal moth.

Research hypothesis:

> Induction caused by the herbivory of autumnal moth larvae will increase the VOC emissions of mountain birch in quantity and in quality.

2. MOUNTAIN BIRCH (*BETULA PUBESCENS* EHRH. SPP. *CZEREPANOVII* (ORLOVA) HÄMET-AHTI)

2.1 TAXONOMY AND PHYSIOLOGY

The birches belong to the order *Fagales* and to the family *Betulaceae*. The exact taxonomy definition of the mountain birch has varied during time. It has been treated as a separate species and also with various names as a subspecies of the downy birch (*Betula pubescens*). At the moment it is treated as a subspecies of *Betula pubescens* Ehrh. called ssp. *czerepanovii* (Orlova) Hämet-Ahti (Wielgolaski 2005, Klemola 2009, Rikkinen 2010). The transition from the downy birch to the nordic mountain birch (when moved south to north) is continuous without a distinctive turning point: this makes the taxonomy of the Nordic mountain birch even further unclear as it shows a strong introgressive feature (Väre 2001, Klemola 2009).

The mountain birch shares genes with the dwarf birch (*Betula nana*) and has recognisable characteristics deriving from it (Holmåsen 1989, Wielgolaski 2005). Those characteristics can be seen visually as small leaves and bright autumn colours. Mountain birch is often polycormic (as seen in Picture 1) like the dwarf birch, while the downy birch is rather monocormic. The polycormous mountain birch can be formed up to 30 stems per genet (stems are connected below ground, appearance of several individual stems).

Picture 1. Polycormic mountain birch stand (Original picture: Panu Piirtola 2007).

It can grow as bushes or as two to six meters high trees (sometimes up to 10 meters). It has been suggested that different types of the mountain birch are adapted to different types of environments. (Starr et al. 1998, Wielgolaski 2005). The stem of the mountain birch is curved with white bark marked with dark horizontal lenticels. The branches are often almost horizontal. New buds and branches are normally brown and the length of buds is 3-4mm. The leaves' sizes vary between individuals, from tiny dwarf birch sized leaves to fairly average size downy birch leaves, 15-60mm in length and 15-45mm wide. The petiole length varies between 0.5cm to 2cm. The leaves are normally saw-edged like downy birch leaves. The flowers are wind-pollinated catkins that blossom in early spring (May-June) just after the new leaves sprout. As a monoecious tree, male and female flowers are in the same tree as separated catkins aside of shoots. Seed catkin is 6-12mm long and seed in the catkin is 1-2mm long with small wings on two sides (Kukkonen 1987, Rikkinen 2010).

2.2 DISTRIBUTION

The mountain birch is the most dominant tree species in the northern parts of Europe. It is a fairly old habitant in the Nordic countries, it migrated to the southern parts of Fennoscandia right after the last glacier period, about 12 000 B.P., and reached the northernmost parts 1000 years later (Wielgolaski 2005)

It grows from Iceland and Fennoscandia to the Central Kola Peninsula. It forms the tree line both in the north and at high elevations, except in the eastern Kola Peninsula, south eastern Norway and some isolated mountains of Sweden and Finland where the tree line is formed by conifers (Väre 2001, Wielgolaski 2005). Taxonomically very similar birch species grows also in Scotland and Greenland. The mountain birch forests cover the ecotone between the coniferous forests and the treeless areas in the Nordic countries, although it is hard to give exactly the limit of distribution because of the strong introgession towards the downy birch (Wielgolaski 2005). Inside the area of distribution, the percentage of the mountain birch of all birches can vary from the lowlands and southern areas 10% to higher and northern grounds 100% (Väre 2001).

2.3 ECOLOGICAL ROLE

The mountain birch forests play a significant role in the ecosystem within their distribution limits because it forms the majority of the plant biomass. In some regions it is estimated that it can consist 60-80% of the total live aboveground biomass. As a dominant tree species with relatively wide root networks, it also prevents soil erosion and acts as a base for soil formation (Wielgolaski 2005). The leaves of the mountain birch are used as a source of food by many animals, the majors being geometrid moths and semi-domestic reindeer. From the geometrid moths, the autumnal moth can cause defoliation of large areas and eventually the death of the trees. The mountain birch's epiphytes (for example foliose lichen (*Parmelia olivacea*)) are as well fairly important in the ecosystem as they are the sites for the overwintering eggs of the autumnal moth (Tammaru et al. 2001, Neuvonen et al. 2001).

3. AUTUMNAL MOTH (*EPIRRITA AUTUMNATA*)

3.1 GENERAL

The geometrid moths belong to class *Insecta*, order *Lepidoptera* and family *Geometridae*. The autumnal moth (*Epirrita autumnata* Borkhausen) is a pale grey moth with dark cross sectional stripes at the front wings. The wingspan for both the male and the female is 30-40 mm. The larva (Picture 2) of the autumnal moth is green with greenish yellow stripes on the sides. The length of the larva varies between 22-26 mm (Mikkola et al. 1985, Kankaanhuhta 2005).

The distribution area, considering all the subspecies, covers the north part of the northern hemisphere (Holarctic species). The southern limits in Europe are from France via Romania and the Caucasus to West Siberia. In Asia the southern distribution limits extends from Sajan Mountains to Japan via the Amur and the Sahel. In North America the geographical limits reaches from Newfoundland and Labrador to British Columbia and south Pennsylvania. The autumnal moth is very common in the whole of Finland, and it can be found in all kinds of environments where deciduous trees and bushes grow (Mikkola et al. 1985).

Picture 2. Larva of autumnal moth (Epirrita autumnata) (Original picture: http://www.vastavalo.fi/tuho-tuhoalue-ruija-norja-tunturimittari-17236.html 7th Oct. 2010.)

The reproduction cycle of the autumnal moth follows four stages. The flying moths group up and mate between the end of August and October, and the females lay the eggs on the branches of birch trees (Ossipov et al.2001, Kankaanhuhta 2005, Klemola et al. 2006). The female can lay 100-200 eggs, and the highest annual population increase recorded has been 6-10 fold (Neuvonen et al. 2005). The autumnal moth winters as eggs and they hatch during June. The hatched larvae eat leaves of the birch trees until they have gone through five larval instars and start to pupate in July-August (Kankaanhuhta 2005, Klemola 2009). The larvae crawl to the ground for the pupation. Adult moths emerge after a month and start grouping after emerging (Ossipov et al. 2001, Kankaanhuhta 2005, Klemola et al. 2006).

3.2 POPULATION DYNAMICS

Population dynamics of the autumnal moth show distinctive cyclic patterns in most mountain birch areas. These cycles occur in regular intervals of 9-11 years but in some

regions the amplitude of the cycle is not as clearly recognisable than in others. Furthermore, in some regions of eastern Fennoscandia, the population size fluctuations with regular outbreaks do not occur at all (Neuvonen et al. 2001, Neuvonen et al. 2005, Klemola 2009). The driving force of these cycles is predation, parasitism and induced resistance of plants (Neuvonen et al. 2001). Delayed induced resistance seems to be the factor that keeps the collapsed population low and prolong the time between outbreaks (Ruuhola et al. 2007). Yet, recent studies suggest that the major driving force is specialised parasitism by hymenopteran parasitoids (Klemola 2009).

The population dynamics has some features that seem to be repeating in the areas of distinctive cycle. In fact, there are 1000 to 100 000 fold differences between the low and the high points of the population size in the cycle, and the peak population sizes can vary significantly between cycles (Neuvonen et al. 2001). Poorer conditions of mountain birch forest (poor soil conditions, older stand, etc.) seem to increase the peak larval populations, outbreaks show some synchrony between different areas but sometimes they can move as a wave over the outbreak zones (Neuvonen et al. 2001, Selås et al. 2001, Klemola 2006).

The most effective regulator of expansion in the areas of the autumnal moth outbreaks seems to be extremely cold temperatures and high rates of predation (Neuvonen et al. 2001, Klemola 2009). Some specialised parasitoids are in major role when the decline of the autumnal moth population starts after the outbreak. At the onset of outbreak, parasitism can be almost absent, yet, in high peak and after, the rates of parasitism can reach 100% (Neuvonen et al. 2005, Klemola 2009). Apparently generalist predators, such as birds, mammals, spiders, ants, etc., have more significance in the southern than in the northern Fennoscandia, even though they have a local negative impact on the population size also in the north. The most likely reason why massive outbreaks do not occur in the southern parts is that the generalist predation pressure is higher and more stable (Neuvonen et al. 2005). If the survival rate in all stages of the reproduction cycle of the autumnal moth is high (especially in early larval stage), the probability for the outbreak is also higher. A high survival rate is dependent on the following conditions: a relatively low parasitism rate, a low induced resistance of mountain birch and a relatively high amount of foliage. Nevertheless, these conditional features may vary in different locations and phases of cycle. On many occasions population sizes of the moth had collapsed before the foliage

quantity became a limiting factor (Neuvonen et al. 2001, Klemola 2003 et al., Neuvonen et al. 2005).

Anthropogenic factors have also their own influence on the population size of birch herbivores. Pollution has either a negative or a positive effect on population size, depending on the feeding habits of the herbivore. In case of the autumnal moth, large scale pollution such as acid rain has little effect on the population, however, it has been recorded that in the vicinity of smelters, densities of the autumnal moth have been lower than average (Neuvonen et al. 2001, Tenow et al. 2005).

The global climate change could cause more widespread and drastic changes on the population dynamics of the autumnal moth. Higher winter temperatures favour the survival of over wintering eggs, the eggs of the autumnal moth suffers from substantial mortality in temperatures lower than –36 Celsius. The change in the temperature of the summer period is controversial. There is still uncertainty if the warmer summer temperature decreases population densities via higher predation (Neuvonen et al. 2001, Jepsen et al. 2008). It seems that the autumnal moth has expanded the region of distribution into coldest areas and lost the dominant status in some warmer areas to the less cold tolerant winter moth (*Operophtera brumata*) as a cause of global warming (Jepsen et al. 2008).

3.3 THE IMPACT OF THE AUTUMNAL MOTH ON THE MOUNTAIN BIRCH

One of the major factors limiting the growth of the mountain birch by defoliation is the peak population of the autumnal moth and other similar moths. Those outbreaks can cause deforestation in large areas. For example, 1965 larva of autumnal moth ate 5000 km² in the municipality of Utsjoki in the Finnish Lapland and left 1000 km² deforested (Mikkola et al. 1985). For the autumnal moth, the mountain birch is one of the most valued plant species for food in the region of its distribution (Mikkola et al. 1985, Neuvonen et al. 2001, Neuvonen et al. 2005). It provides the nutritional base for mass outbreaks of the autumnal moth and influences the growth, the behaviour and the survival of the autumnal moth by quality of food available (Haukioja 2003, Lempa et al. 2004). As mentioned earlier, some epiphytic lichens on the mountain birch serve as overwintering places for the autumnal moth’s eggs (Tammaru et al. 2001, Neuvonen et al. 2001).

4. VOLATILE ORGANIC COMPOUNDS

4.1 GENERAL INFORMATION ABOUT VOLATILE ORGANIC COMPOUNDS

Volatile organic compounds (VOCs) can originate from biogenic or anthropogenic sources. The primary sources are emissions from vegetation, biomass burning and human activities. Secondary emissions derivate from the reactions of other organic compounds in the atmosphere (Ruuskanen 2009). The term *biogenic volatile organic compound* includes all organic trace gases found in atmosphere except carbon dioxide and carbon monoxide (Kesselmeier and Staudt 1999). This group consists of large number of subgroups of saturated, unsaturated and oxygenated derivates. Biogenic VOCs include isoprene, terpenes, alkanes, alkenes, alcohols, esters, carbonyls and acids (Kesselmeier and Staudt 1999, Peñuelas et al. 2001).

All plants exchange non-organic volatile compounds during respiration or photosynthesis and most of them have also ability to produce and emit VOCs from different organs (Tholl et al. 2006). The biogenic emissions are estimated to be tenfold compared to anthropogenic emissions and it seems to have great importance on ozone and aerosol formation and methane oxidation (Kesselmeier and Staudt 1999, Simon et al. 2001, Ruuskanen 2009). Concentrations of biogenic VOCs range between ppt and ppb and some of them, such as isoprene, monoterpenes and sesquiterpenes, can be fairly reactive as their chemical lifetime varies between some minutes to hours (Kesselmeier and Staudt 1999). Overall, the global amount anthropogenic emissions are around 149 Tg/year and global biogenic emissions around 1150 Tg/year (Guenther et al. 1995, Hakola 2001) and biogenic VOCs emissions range from 0.2 to 10.0 % of the carbon assimilated by a plant, depending on the conditions (Ruuskanen 2009).

There are total of 1700 volatile compounds that have been found from more than 90 plant families. Plant emitted VOCs are low on molecular weight, and their chemical composition and concentration may contain information about the physiological state of the plant and/or about the stress factors that the plant is currently under (Dudareva et al. 2006). For

example, during herbivore infestation plants tend to emit VOCs as a response to physical damage, protection and/or signalling (Mäntylä et al. 2008, Himanen et al. 2010).

4.2 FUNCTIONS OF VOCS

As seen in Figure 1, VOCs has multiple functions in plant interactions with the surrounding environment. One of the functions that has been in interest of studies is the VOC relation to pest resistance and repellent properties of VOCs. Most of the terpenes function as pest repellents or as a measure to prevent herbivore infestation (direct defence). Many herbivores also use the plant specific volatile emissions to recognize potential food plants (unintended signalling), further the plant emitted VOCs and it may attract predators and parasites of herbivores (indirect defence) (Holopainen 2001, Unsicker et al. 2009). For example, there is proof that monoterpenes emitted by *Chrysanthemum morifolium* repel ovipositioning females of the *Plutella xylostella* and maybe also the larvae but it was not as effective towards moths experienced with these monoterpenes (Wang et al. 2008). Plant emitted volatiles may also act as defence signals to neighbouring plants (priming) (Pare et al. 2005, Dudareva et al. 2006, Himanen et al. 2010)

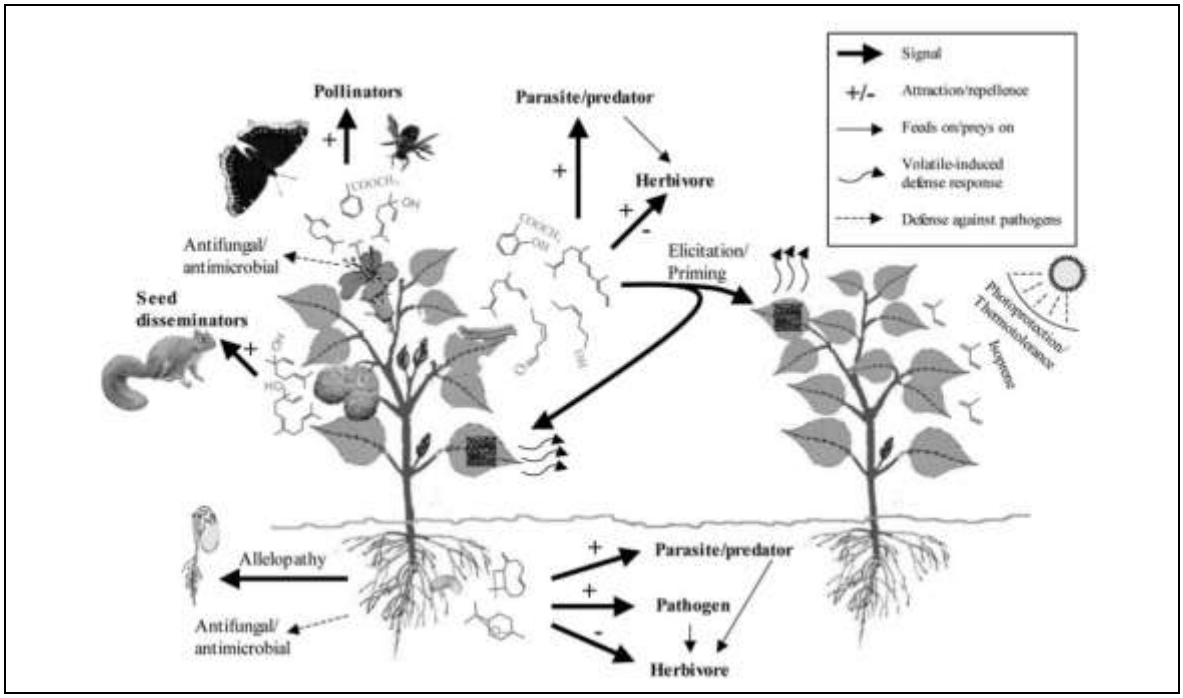

Figure 1. Functions and interactions of the volatile organic compounds in the plants surrounding environment (Original figure Dudareva *et al.* 2006).

Large diversity of flowering plants has made plants maximize their reproduction and find competitive traits and strategies to do so. There are a quarter of million animal pollinated plants and they have to ensure large enough quantity of pollinators and seed disperser in order to secure the survival of future generations. To do so, animal pollinated flowering plants emit certain VOCs to attract needed animals (intended signalling), this blend of volatiles is diverse; there are no two floral scents exactly alike. For example, by providing specific compound mixes, they facilitate the insect's ability to learn particular sources of food. Although there is no certainty if insect pollinators use one or more compounds of floral scent for identification (Dudareva et al. 2006).

4.3 ABIOTIC FACTORS INFLUENCING VOC EMISSIONS

There are many various factors, biotic and abiotic, that influence the production and emission of VOCs. As seen in Figure 1, plants are interacting in many ways with their environment while the environment is simultaneously interacting with plants. Factors, such as, soil water content, air humidity, wind, precipitation, pollution, genetics, physiology, herbivores, etc. may affect the quality and quantity of plant-emitted VOCs. The effect of influence on the emissions of VOCs varies between plant species (Kesselmeier and Staudt 1999, Dudareva et al. 2006).

The influence of light and temperature are the best known abiotic factors that influences the VOC emissions on plants, and in many studies it has been proved that there is clear temperature related dependency on emissions of isoprene, monoterpenes and most likely with other VOCs compound groups (Hewitt et al. 1995, Steiner and Goldstein 2007). Moreover, it has been suggest that global warming would affect the VOC balance by increasing the emissions (Kellomäki et al. 2001, Tiiva et al. 2008).

The characteristics of light (duration, intensity, quality) affect the VOC production and emissions (Linnala and Keskitalo 2001). It has also been shown that isoprene emissions are triggered by light, and there is a strong link between isoprene production and photosynthetically active radiation (PAR) (Hewitt et al. 1995, Kesselmeier and Staudt 1999, Steiner and Goldstein 2007). Isoprene is produced mainly from products related to photosynthesis; in fact there is no large isoprene storage pool. In consequence isoprene synthesis and emissions stop in few minutes subsequently after light is no more available.

Where isoprene is displaying light dependency, monoterpenes do not show it as clearly as the isoprene. Monoterpenes are stored in special organs, and forming fairly large storage pool, compared to the normal emission rates that can be released when needed. Yet, there are some monoterpenes that display light dependency on production and emissions, it has been indicated in many studies that monoterpenes are also emitted from recently synthesized compounds as well as from storage pools (Kesselmeier and Staudt 1999, Sabillon and Cremades 2001, Steiner and Goldstein 2007).

Seasonal cycle can also influence the flux of VOCs. The phenological changes, the forming of the leaves, flowering, ageing, leaf senescence, etc. have their own impact to emissions of VOCs (Hewitt et al. 1995, Sabillon and Cremades 2001, Steiner and Goldstein 2007). Isoprene emission has shown variation regarding the state of leaf maturation, they increase till full maturation and rapidly drop when leaves are facing senescence (Kesselmeier and Staudt 1999, Hakola et al. 2002, Steiner and Goldstein 2007). For example, in Hakola et al. (2001) on the birches (*Betula pendula* and *pubescens*) seasonal VOC emissions variation, monoterpenes of *B. pendula* demonstrated clear seasonal variation.

Other factors influencing VOC emissions that have been of interest are carbon dioxide, ozone and moisture (Hewitt et al. 1995, Kesselmeier and Staudt 1999, Peñuelas and Llusia 2001). There is some indication that elevated CO_2 (Constable et al. 1999, Kesselmeier and Staudt 1999, Vuorinen et al. 2005), nor elevated O_3 levels (Kesselmeier and Staudt 1999, Vuorinen et al. 2005) would not affect VOC emissions. The studies of these factors are not fully conclusive and there are contradictory results found (Kesselmeier and Staudt 1999, Peñuelas and Llusia 2001, Pinto 2010). Moisture content and pressure have some role on the emissions of plant VOCs but it seems to be fractional (Kesselmeier and Staudt 1999).

4.4 TERPENES

4.4.1 General

Terpenes, also sometimes referred as isoprenoids or terpenoids, have many functions and they are present in all living organisms, moreover they are highly diverse among plants.

Terpenes are one of the most abundant and diverse secondary compound group in plants. A plant may contain tens of various terpenes and other secondary compounds. At present there are 30 000 different plant terpenes listed and new terpenes are continuously found (Langenheim 2003, Heldt 2005).

Terpenes are formed from five carbon isoprene units, 2-methyl-1,3-butadiene. Monoterpenes are formed from two isoprene units (10 carbons), sesquiterpenes from three isoprene units (15 carbons), diterpenes from four isoprene units (20 carbons), triterpenes from six isoprene units (30 carbons), tetraterpenes from eight isoprene units (40 carbons) and polyterpenes more than eight isoprene units (Table 1.) (Keskitalo et al. 2001, Heldt 2005). Homoterpene has one more carbon and it does not divide directly to isoprene units (Langenheim 1994). Monoterpenes and sesquiterpenes are etheric oils and they are volatile where diterpenes and triterpenes are not (Langenheim 1994).

Precursor	Class	Example	Function
C_5: Dimethylallyl-PP	Hemiterpene	Isoprene	Protection of the photosynthetic apparatus against heat
Isopentenyl-PP		Side chain of cytokinin	Growth regulator
C_{10}: Geranyl-PP	Monoterpene	Pinene Linalool	Defense substance attractant
C_{15}: Farnesyl-PP	Sesquiterpene	Capsidiol	Phytoalexin
C_{20}: Geranylgeranyl-PP	Diterpene	Gibberellin Phorbol Casbene	Plant hormone Defense substance Phytoalexin
C_{30}: 2 Farnesyl-PP	Triterpene	Cholesterol Sitosterol	Membrane constituents
C_{40}: 2 Geranylgeranyl-PP	Tetraterpene	Carotenoids	Photosynthesis pigments
Geranylgeranyl-PP or Farnesyl-PP	Polyprenols	prenylated proteins	Regulation of cell growth
		Prenylation of plastoquinone, ubiquinone, chlorophyll, Cyt *a*	Membrane solubility of photosynthesis pigments and electron transport carriers
		Dolichols Rubber	Glucosyl carrier

Table 1. Terpenes of higher plants (Original table: Heldt 2005).

The plant terpenes can be divided to primary and secondary metabolites. Primary metabolites are found universally in all plants and they include such compound groups as sterols, steroids, carotenoids, various hormones, etc. They function as membrane constituents, photosynthetic pigments, electron transport carriers, growth substances and

plant hormones. They also act as glucosyl carriers in glucosylation reactions and play a part in the regulation of cell growth (Keskitalo et al. 2001, Heldt 2005).

On the contrary, secondary metabolites of terpenes, such as monoterpenes and sesquiterpenes, have more constricted distribution and can be specific for a family or even for a species (Chappel 1995, Heldt 2005). The majority of these plant terpenes are found in resins, waxes and oils. They make a plant toxic or indigestible as a defence measure against herbivores, and they act as protection against pathogenic micro-organisms, give colour and odour for fruits and flowers in order to attract insects, birds and bats to distribute pollen or seeds on their behalf, or to inhibit the germination and growth of other plants (Langenheim 1994, Heldt 2005). From the secondary metabolites of plants, terpenes are the biggest and the most diverse group. With a few exceptions, these secondary compounds consist about 1% of the plants dry weight (Linnala and Keskitalo 2001).

4.4.2 Synthesis of terpenes

The precursors of the synthesis of terpenes are five- carbon isopentenyl pyrophosphate (IPP) and its allylic isomer 1,1 – dimethylallyl pyrophosphate (DMAPP). These precursors are produced in plants via two alternative biosynthesis pathways, the classical mevalonate (MVA) pathway and the methyl-erythritol-phosphate (MEP, also referred as DOXP pathway) pathway (Upper part of Figure 2.) (Kesselmeier and Staudt 1999, Heldt 2005, Dudareva et al. 2006). The biosynthesis of terpenes on MVA pathway proceeds in cytosol. First, mevalonate is formed from three acetyl-CoA molecules by HMG-CoA synthase. Mevalonate pyrophosphorylates via mevalonate kinase and mevalonate phosphate kinase to 5-pyrophosphomevalonate that becomes IPP via pyrophosphomevalonate decarboxylase (Langenheim 2003, Heldt 2005).

The finding that mevilonin inhibits the isoprenoid synthesis in the cytosol but not in the plastids, led to the discovery of the MEP pathway. For the MEP pathway in plastids to produce IPP, the precursors in the process are pyruvate and D-glyceraldehyde-3-phosphate. Pyruvate is decarboxylated via thiamine pyrophosphate and then transferred to D-glyceraldehyde-3-phosphate to yield 1-deoxy-D-xylulose-5-phosphate (DOXP). From DOXP the next step is 2-C-methyl-D-erythritol-4-phosphate by rearrangement and

reduction, followed by dehydration and phosphorylation that leads to formation of IPP. These last steps from DOXP to IPP are not yet fully resolved in detail (Langenheim 2003, Heldt 2005).

The MVA pathway in the cytosol is responsible for the synthesis of sterols, certain sesquiterpenes and the side chain of ubiquinone and MEP pathway in plastids is responsible for the synthesis of hemiterpene isoprene, monoterpenes, diterpenes and tetraterpenes. The MEP pathway is only known in bacteria, algae and plants, where the MVA pathway is also found in animals (Heldt 2005). Although these two pathways are able to function and synthesize IPP independently, it seems that there is metabolic crosstalk between these pathways, especially in the direction from plastids to cytosol (Dudareva et al. 2006).

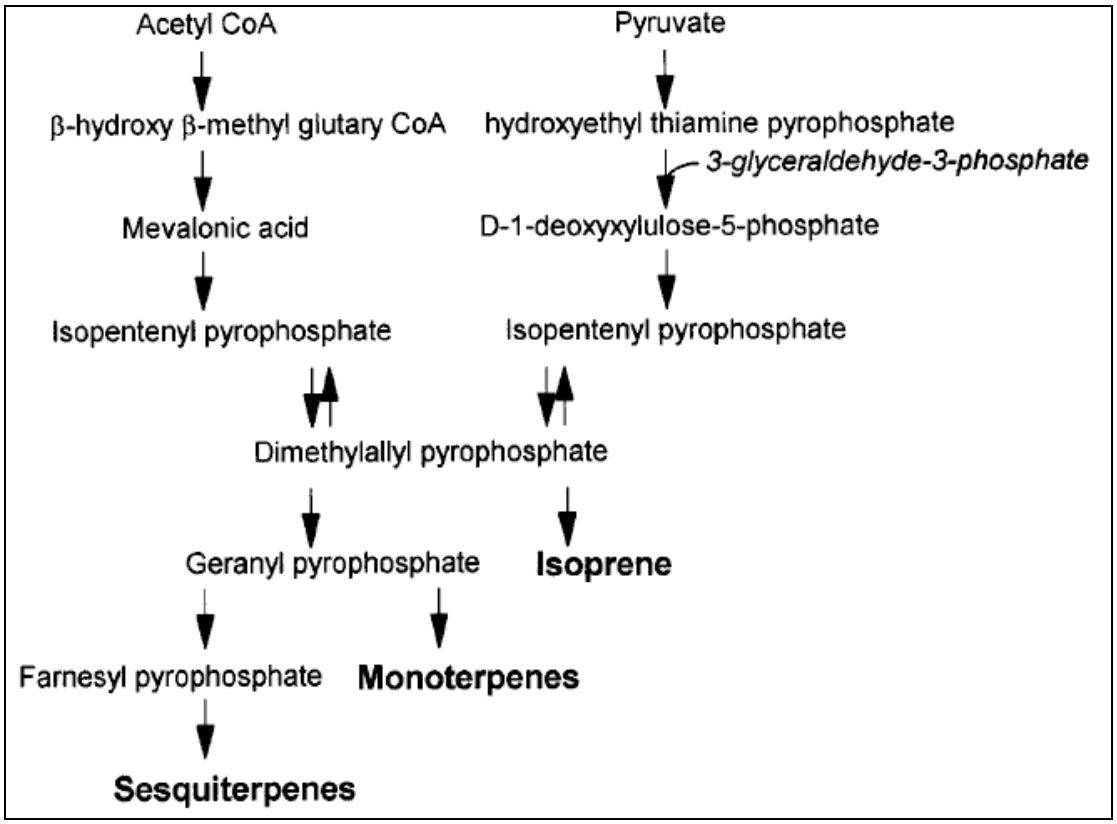

Figure 2. The major reaction steps in formation of isoprenoids (Original figure: Kesselmeier &Staudt 1999).

As mentioned earlier, IPP and DMAPP, works as precursors for the isoprenoid synthesis. By adding units of IPP to DMAPP, the precursors of different isoprenoid groups are

formed in the cytosol and in the plastids. (See Figure 3) When one IPP added to DMAPP geranyl pyrophosphate (GPP) is formed, which is starting unit of monoterpenes, and when two IPP units are added to DMAPP farnesyl pyrophosphate (FPP) is formed, which is starting unit for sesquiterpenes. (Kesselmeier and Staudt 1999, Linnala and Keskitalo 2001, Dudareva et al. 2006). The chaining of IPP units can continue till there is 500- 10 000 IPP units together (Linnala and Keskitalo 2001).

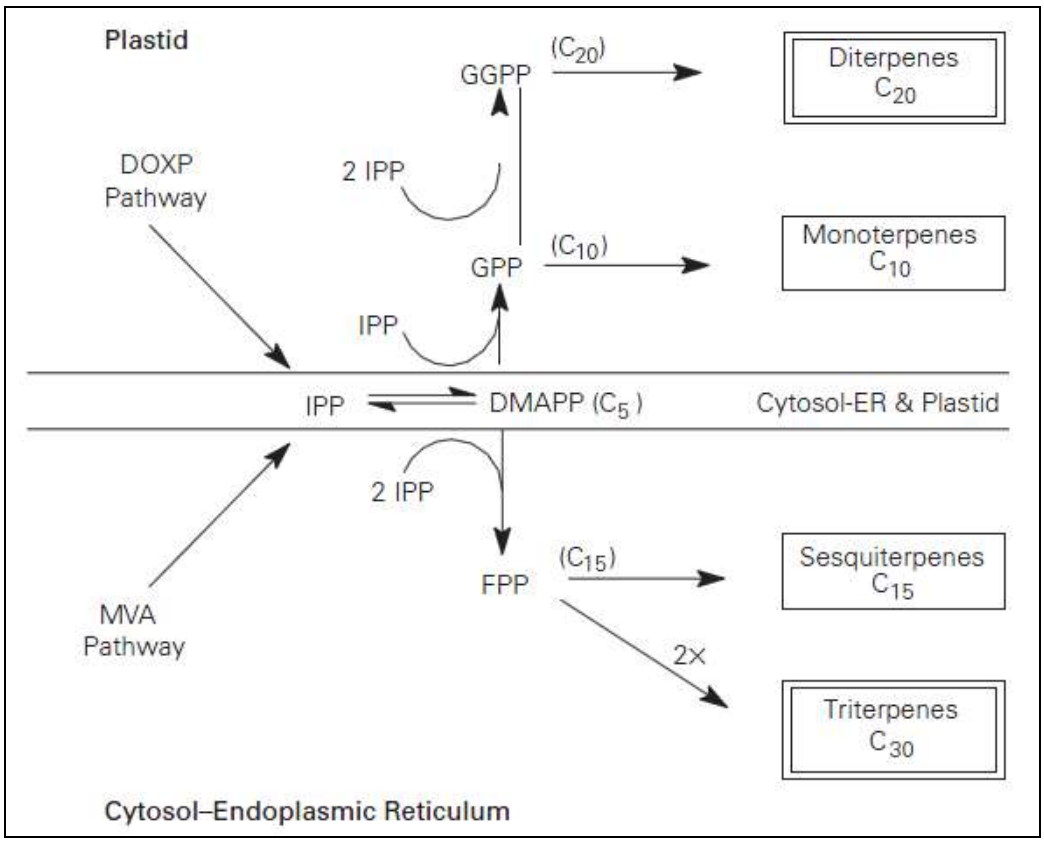

Figure 3. Formation of terpenes (Original figure: Langenheim 2003).

4.4.3 Hemiterpene

The hemiterpene, i.e. isoprene, is formed from dimethyallyl-PP in isoprene synthase where pyrophosphate is released (Heldt 2005). From the plants, isoprene is emitted especially in high temperatures and it can reach several percents of the plants total assimilated carbon (Kesselmeier and Staudt 1999, Heldt 2005, Tiiva et al. 2008). Although only 30% of plants are known to emit isoprene (Kesselmeier and Staudt 1999), the estimation of the total global emissions of isoprene seems to be the same magnitude as the total global methane

emissions (Heldt 2005, Guenther et al. 2006). The significance of these emissions is fairly well realised and recorded but the actual function for the emitting plant is still unclear. There is indication that isoprene stabilize photosynthetic membranes against high temperature damage and oxidative stress (Velikova et al. 2006, Vickers et al. 2009).

4.4.4 Monoterpenes

It is estimated that 11% of the annual global biogenic volatile organic compound flux is caused by monoterpenes (Guenther et al. 1995) and in some ecosystems monoterpenes represent dominant VOC (Constable et al. 1999). Even though there are various monoterpene compounds, it seems that many plants emit only a few in high quantities (Constable et al. 1999, Pressley et al. 2004). In the study of Pressley et al. (2004), it was shown that 64% of monoterpene emissions were comprised from only three different compounds (α-pinene, β-pinene and limonene).

Monoterpenes comprise a large number of open chain (acyclic) and mono-, bi- and tricyclic isoprenoids, mainly constituting of two isoprene units, yet, monoterpenes also consist of compounds that are formed from pyran and furan cycles (Kesselmeier and Staudt 1999, Heldt 2005). These structural cycle molecules are normally linked with hydroxyl-, oxo-, aldehyde-, carboxyl- or esther group (Keskitalo et al. 2001).

The primary function of monoterpenes is the most likely direct plant defence against herbivores and pathogens (Langenheim 1994, Unsicker et al. 2009). In the study of Vourc'h et al. (2002) they tested the repellent qualities of monoterpenes. They made a few different combinations (same plant, different herbivore, different monoterpene) and it showed that in every case the monoterpene functioned as a defence against herbivores, yet, with some variation regarding the species researched. There are also other functions for monoterpenes in plants, such as, to attract predators and parasitoids of herbivores, and to attract pollinators and allelopathy (deterrent or inhibitory compounds) (Langenheim 1994).

4.4.5 Sesquiterpenes

Sesquiterpenes make the largest group of terpenes. They are formed from three isoprene units comprise more than 200 different ring structures, comprising several thousand compounds, and include many aromatic substances such as eucalyptus oil, nevertheless, they are less readily volatile than monoterpenes (Keskitalo et al. 2001, Heldt 2005). Sesquiterpenes can be open chained or cyclic hydrocarbons, alcohols or ketones. They dissolve rapidly when emitted to the air, as seen with monoterpenes (Keskitalo et al. 2001).

Like those of monoterpenes, the functions of sesquiterpenes are related to the defence against herbivores and pathogens. For example in the family of *Asteraceae*, there are sesquiterpene lactoses found that are toxic to butterflies, grasshoppers and beetles (Keskitalo et al. 2001). Those toxic sesquiterpene compounds, found in the family of *Asteraceae*, are also studied for their antiviral and anti-microbial activities in order to find cure to diseases such as malaria or herpes (Özçelik et al. 2009, Milhous and Weina 2010). The study of Himanen et al. (2010) has also found out that there can be inter-species connection with the defence against herbivores. *Rododendron tomentosum* emitted sesquiterpenes can be passively adsorbed by *Betula* spp. and later re-emitted as repellents against herbivores of *Betula* spp.

4.4.6 Homoterpenes

Homoterpenes include two compounds C_{11} 4,8-dimethyl-1,3(E),7-nonatriene (DMNT) and C_{16} (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) that are formed from nerolidol and geranyllinalool respectively (Donath and Boland 1995, Boland et al. 1998).

It seems that homoterpenes are emitted only when a plant is suffers from herbivores and not when it is mechanically damaged (Pare and Tumlinson 1997, Bouwmeester et al. 1999). It has been hypothesized that the trigger for the synthesis of homoterpenes could be β-glucosidases that is found in the saliva of herbivores. The release of homoterpenes took place also when treated with human saliva (Karban and Baldwin 1997). They are also

constituents of the so called “white-floral image” of night-scented flowers (Donath and Boland 1995).

4.5 GREEN LEAF VOLATILES

Green leaf volatiles (GLVs) include six carbon aldehydes, alcohols and their esters (Pare and Tumlinson 1999, Unsicker et al. 2009). Plants produce and emit GLVs almost always when the plant’s cell membranes are broken by mechanical or biological damage. This production (oxidation of leaf lipids) takes place just in few seconds subsequently to the injury (Pichersky and Greshenzon 2002, Schoonhoven et al. 2005). Furthermore, GLVs are emitted under environmental stress, e.g. elevated ozone levels, high temperature, etc., the GLVs are emitted late after stress (Arimura et al. 2009). Green leaf volatiles are emitted in big quantities at the time when needed and can be noticed from the odour, e.g. freshly cut grass or leaves (Steiner and Goldstein 2007). GLVs are easily degraded compounds. Cis-3-hexanol and cis-3-hexenyl acetate are one of the most common plant emitted GLVs and they comprise notable portion of plants VOCs (Kesselmeier and Staudt 1999). GLVs precursors often accounts more than 1% of the dry leaves’ mass (Schoonhoven et al. 2005)

Green leaf volatiles are significant signalling compounds, to the plant itself and to other plants around it. GLVs can act as a priming signal of herbivory to the unharmed neighbouring plants, thus allowing plants to response without an immediate activation of defence signal cascades and the accompanied expenditure of energy for defence mobilisation (Pare et al. 2005, Dudareva et al. 2006). It has been discovered that three GLVs, (Z)-3-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate can give a warning for a neighbouring plant to start producing defence chemicals (Choudhary et al. 2008).

GLVs are produced from fatty acids and acetyl-CoA by peroxidation and by oxidative degradation of cell membrane lipids via lipoxygenase pathway (LOX). The products of the lipoxygenase pathway, including hexanals and hexenols, are not detected in systematically released VOCs (except (Z)-3-hexenyl acetate) (Pare and Tumlinson 1999).

4.6 METHYL SALICYLATE

Methyl salicylate (MeSa) is produced and emitted in many plants after a plant is damaged and also under environmental stress. It is synthesised from salicylic acid on phenyl propanoid pathway (Steiner and Goldstein 2007, Karl et al. 2008). Moreover, it is known as a signal molecule for plants (Shulaev et al. 1997). In the study of Dicke et al. (1999) on the Lima bean response to spider mites herbivory, it was shown that plants with spider mites emitted significant quantities of methyl salicylate. Further, in the study of Blande et al. (2010) with *Betula pendula* and *Alnus glutinosa*, the results indicated that there was a correlation between the duration of aphid infestation and quantity MeSa emitted (longer the time, more MeSa emitted).

5. THE AIM OF THE STUDY

The main aim of this study was to determinate the emissions of VOCs on the mountain birch growing stands on normal conditions and if there was any induction of VOCs when the autumnal moth larvae were defoliating the leaves.

6. MATERIALS AND METHODS

6.1 STUDY SITE AND THE DESIGN OF EXPERIMENT

The experiment site was at the proximity of the Kevo Subarctic Research Station (69°45'N, 27°01'E) in the northern most Finland. The experiments were conducted in June 2007, in the beginning of the growing season. 30 trees of mountain birch (*Betula pubescens* ssp. *Czerepanovii* (Orlova) Hämet-Ahti) were selected by Elina Mäntylä for her research to follow the predation activity of birds on moth-damaged trees. Same trees were used for our experiments as well. The selected trees were in a mountain birch forest close by the station, forming the tree line in this region. In this region the mountain birches forms polycormic stands with multiple stems.

The trees were selected the following criteria (visually inspected): 1. No obvious herbivore damage prior to the experiment, 2. Each selected polycormic bush would belong to same individual and genotype, 3. Pairs (15) formed would be closely located (2-10 meters) and have seemingly same phenotype (two to four meters high, similar trunk diameter). The pairing was made so that from each selected tree one stem was selected, and from these 30 stems, 15 pairs were made and divided two similar groups (group of seven pairs and group of eight pairs). From the tree pairs selected, within a pair, one was randomly chosen to be the tree under herbivory of autumnal moth (*Epirrita autumnata* (Borkhausen)) larvae and the other one was then chosen to be the control tree without larvae. From these selected tree stems three branches were selected and mesh bags (size about 80x35 cm, mesh 0.4 mm) were placed on the branches. 20 laboratory hatched third instar autumnal moth larvae were placed inside the mesh bag on the trees selected to be the herbivory trees. The mesh bags on the control trees were left empty. The mesh bags and the larvae were placed to the first group of pairs (seven pairs) on 8th June. For the second group (eight pairs), the mesh bags and the larvae were placed on 9th June. These dates mark a starting date of the defoliation. They were allowed to feed inside the mesh bags throughout the experiment. The larvae were on their fifth instar when collection of VOCs was made.

6.2 COLLECTING AND ANALYSING VOCS

Volatile organic compounds were collected during two days. VOC samples were collected from the group of seven pairs on the first day, 14th June (defoliation started on 8th June). From the group of eight pairs, the samples were collected on the second day, 15th June (defoliation started on 9th June). One branch with mesh bag was sampled from each stem selected. The mesh bag's outmost end (the tip of the branch) was opened just before the beginning of collecting the samples. The larvae on the branches were gently moved to the back of the mesh bag, and the mesh bag was closed for the end part in order to avoid larvae from escaping. The tip part of the branch was without the mesh bag's cover, ready for the VOC sample collection. The mesh bag was removed partially from the branches without the larvae, as much as from the ones with larvae.

Polyethylene terephthalate (PET) bags (size 45x55 cm, LOOK Terinex Ltd, Bedford, England) were used as collection containers and they were decontaminated before the use in order to prevent any contamination from the bag. This was done by heating the opened bags for one hour in +120°C and subsequently cooled and repacked. These bags were used on field as collection containers, from where the clean sample was taken. The bag was placed carefully (to avoid any damages to foliage) on the opened part of the branch and closed air tight on the bark of the branch with plastic, flexible tape. An air inlet tube and a sensory unit of a HOBO Micro Station Data Logger (MicroDAQ.com Ltd, Contoocook, NH, USA) for recording climatic data were inserted with the support of a tripod after the bag was on its place and one corner of the bag was cut (see Picture 3). The corner was closed with a tape to make it air tight. The bag's air tightness was checked by pumping air inside and then visually checked if there were any air leakages in order to ensure the sample's purity. Then the second corner was cut and the bag was flushed with clean air, at the rate of 600 ml/min, to remove any impurities that were not emitted from the sampled branch. Then the flux of air was dropped to 300 ml/min. The influx air was cleaned with charcoal filter and MnO_2 all the time before entering the sampling bag. A stainless tube was placed and it was sealed with tape to the opened second corner. The collection tube contained approximately 150 mg of Tenax TA-adsorbent (Supelco, mesh 60/80). The sample was taken to the Tenax tube by pulling the air at the rate of 200 ml/min. The clean air was pumped and the sample was pulled with a battery operated sampling pumps

(Rietschle Thomas, Pucheim, Germany). The flow rates of the pumps were controlled with a M-5 bubble flowmeter (A.P. Buck, Orlando, FL, USA). The VOC collection system, including inlet and outlet pumps, clean air filters, HOBO Micro Station Data Logger and batteries, were installed in to a portable plastic toolbox. The toolbox was made to provide equipment for taking two simultaneous samples. During the sampling period of 60 minutes, the temperature, photosynthetically active radiation (PAR) and air humidity inside the plastic bag were monitored with the HOBO Micro Station Data Logger. Two empty VOC collection bags (no source of VOCs inside the bag) were measured to be certain that there were no impurities in the collection systems or any VOCs entering the bag during collection. This procedure was done during both sampling days.

After taking the gas sample to the Tenax tube, the tubes were preserved in a refrigerator and transported to a laboratory of the University of Kuopio. The sample tubes were analysed there with a gas chromatograph-mass spectrometer (Hawlett-Packard GC 6890, MSD 5973). The compounds were desorbed with a thermal desorption unit (Perkin-Elmer ATD400 Automatic Thermal Desorption system) in 250°C for 10 minutes, cryofocused at -30°C, and injected on to a HP-5 capillary column (50 m x 0.2 mm i.d. x 0.5 μm film thickness, Hewlett-Packard) with helium as a carrier gas. The oven temperature program was held in 40°C for one minute and then raised to 210°C at a rate of 5°C/min, and finally further to 250°C at a rate of 20°C/min. The identification of the compounds was performed with comparison to Wiley library and pure standards. The emissions were presented in $ng/cm^2/h$. As many biogenic emissions of VOCs are light and temperature dependent, they were made comparable by standardizing them to temperature of 30°C using the classic algorithm established by Guenther et al. 1993. The temperature coefficient of 0.09 was used for monoterpenes, as recommended by Guenther et al. 1993, and coefficient 0.18 for sesquiterpenes, as recommended by Helmig et al. 2006, as objective being standardise the emissions.

Picture 3. The sampling bag with HOBO, air inlet tube and Tenax tube with intake tube (Original Picture: Panu Piirtola 2007)

6.3 MEASURING THE LEAVES OF THE MOUNTAIN BIRCH

The emissions from the branches measured need to be comparable to each other, therefore the emission source had to be measured, the leaf area were needed for the algorithm of Guenther et al. (1993). Since, the experiment with selected trees continued after the sampling made for this study, there was no possibility to cut the branches and get exact measurements of the leaves. Instead of cutting the branches and removing the leaves for the measurements, the leaves were counted and photographed. The photographs were taken with millimetre paper on the background, the leaves as close as possible to the background paper and the photo taken directly towards the background paper. In this way it was possible to have the area of the leaves with an accurate ratio with a square on the millimetre paper. The area of leaves in pixels was measured from the photos with the millimetre paper in a photo editor (ImageJ) as well as the pixel size of one square on the millimetre paper.

With these figures, the area of leaves was calculated. The defoliation area was also measured by the same method and it was excluded from the total leaf area of each branch.

6.4 STATISTICAL ANALYSES

The results were analysed by using oneway ANOVA. The statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, Il, USA).

7. RESULTS

7.1 VOLATILE ORGANIC COMPOUNDS (VOCS)

30 independent samples were taken and four extra ones were taken from the empty sample bags. Two samples were destroyed from the 30 samples taken. 14 different VOCs were found from the experiment samples and two compounds were found from the empty bag samples.

7.2 VOC EMISSIONS FROM THE MOUNTAIN BIRCH

The VOCs found from the samples were monoterpenes: α-pinene, β-myrcene, limonene and linalool. One homoterpene was found, (E)-DMNT. Five sesquiterpenes were found: α-copaene, α-humulene, caryophyllene oxide, (E)-β-caryophyllene and β-bourbonene. Alöso following green leaf volatiles were present: cis-3-hexan-1-ol+(E)-2-hexenal, 3-hexen-1-ol acetate, nonanal and cis-3-hexenyl butyrate. The relative emissions of each VOC and VOC group, and their induction can be seen in Figure 4 and 5. Although there are higher levels of emissions on the herbivore samples compared to the control samples, only three compounds had statistical significance, $p<0.05$. These compounds were (E)-DMNT, linalool, cis-3-hexenyl butyrate. From the measured groups of compounds, homoterpene, monoterpenes, green leaf volatiles and total emissions showed statistical significance.

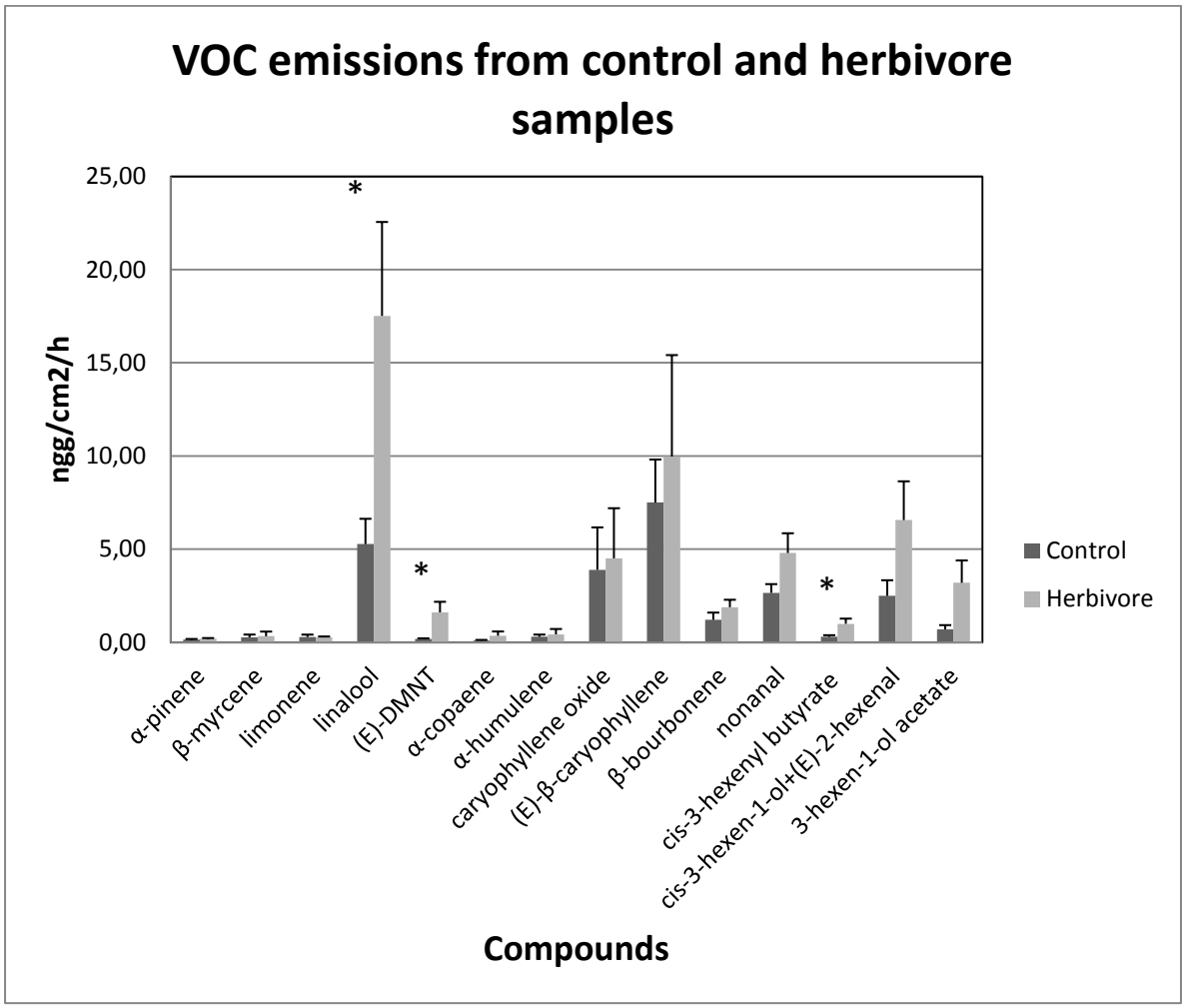

Figure 4. The volatile organic emissions from the studied pairs of birch tree branches, the dark bars represent branches without herbivores and the pale bars represent branches with autumnal moth larvae caused defoliation. P<0.05 = *

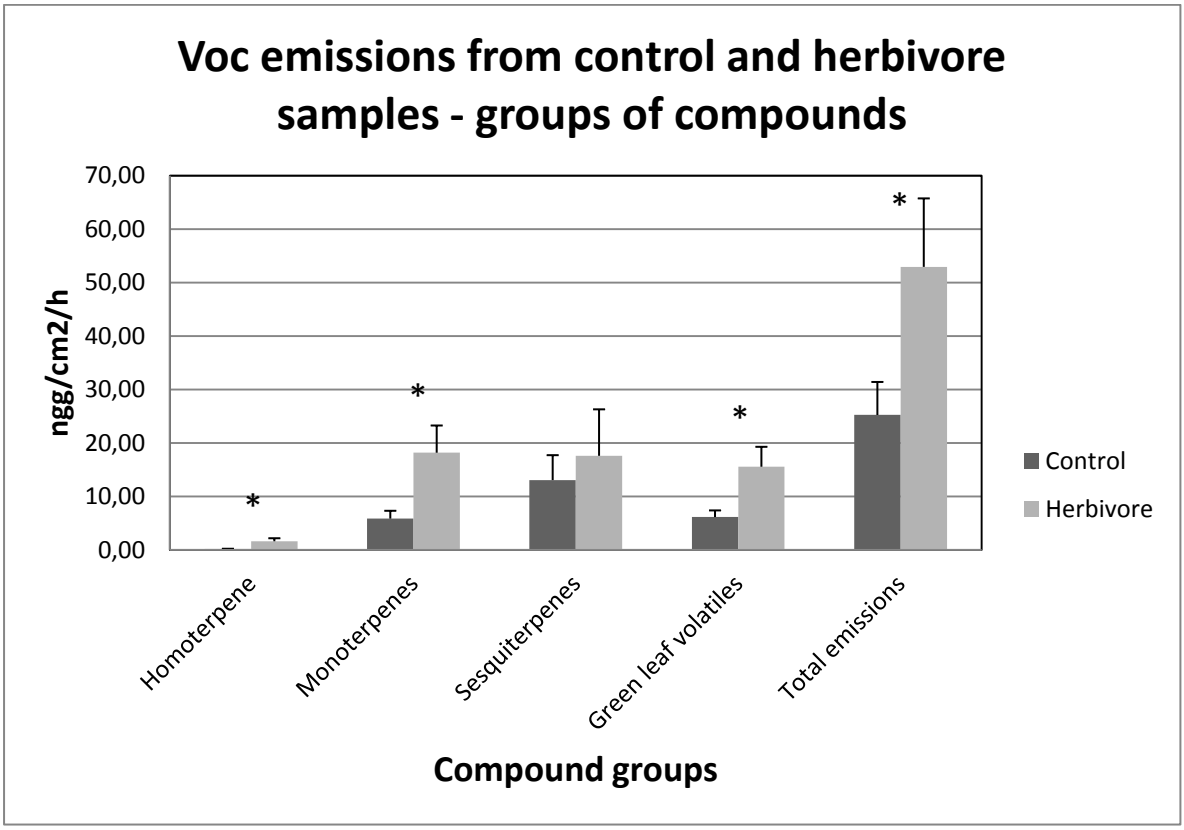

Figure 5. The volatile organic compound emissions as groups of compounds from the studied pairs of birch tree branches; the dark bars represent branches without herbivores and the pale bars represent branches with autumnal moth larvae caused defoliation. P<0.05 = *

In Figure 6 the relative emissions of VOCs are represented in percentage of total 100%. The relative portions of the emissions of homoterpene, monoterpenes and green leaf volatiles increased. On the contrary sesquiterpenes decreased on the branches sampled that were defoliated by the larvae when compared to the samples of control branches.

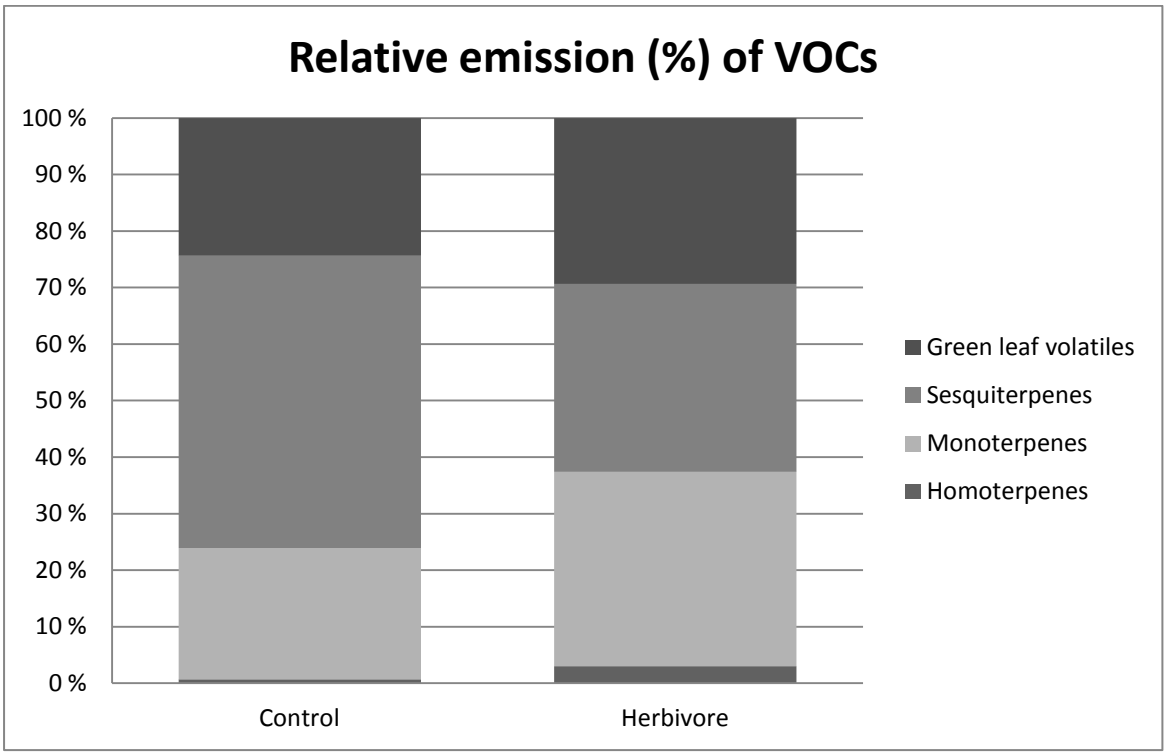

Figure 6. Relative portions (in percentage) of volatile organic compound groups: homoterpene, monoterpenes, sesquiterpenes and green leaf volatiles (GLVs), according to the treatment, control= no defoliation and herbivore= defoliation by autumnal moth larvae.

As mentioned in section Materials and methods, the raw emission data had to be converted to comparable results by the algorithm of Guenther et al. (1993). There is an effect of the algorithm on mean emissions seen in the Figure 7 (normalised=mean emissions of a sample after the use of the algorithm, unadjusted=mean emissions of a sample from raw data).

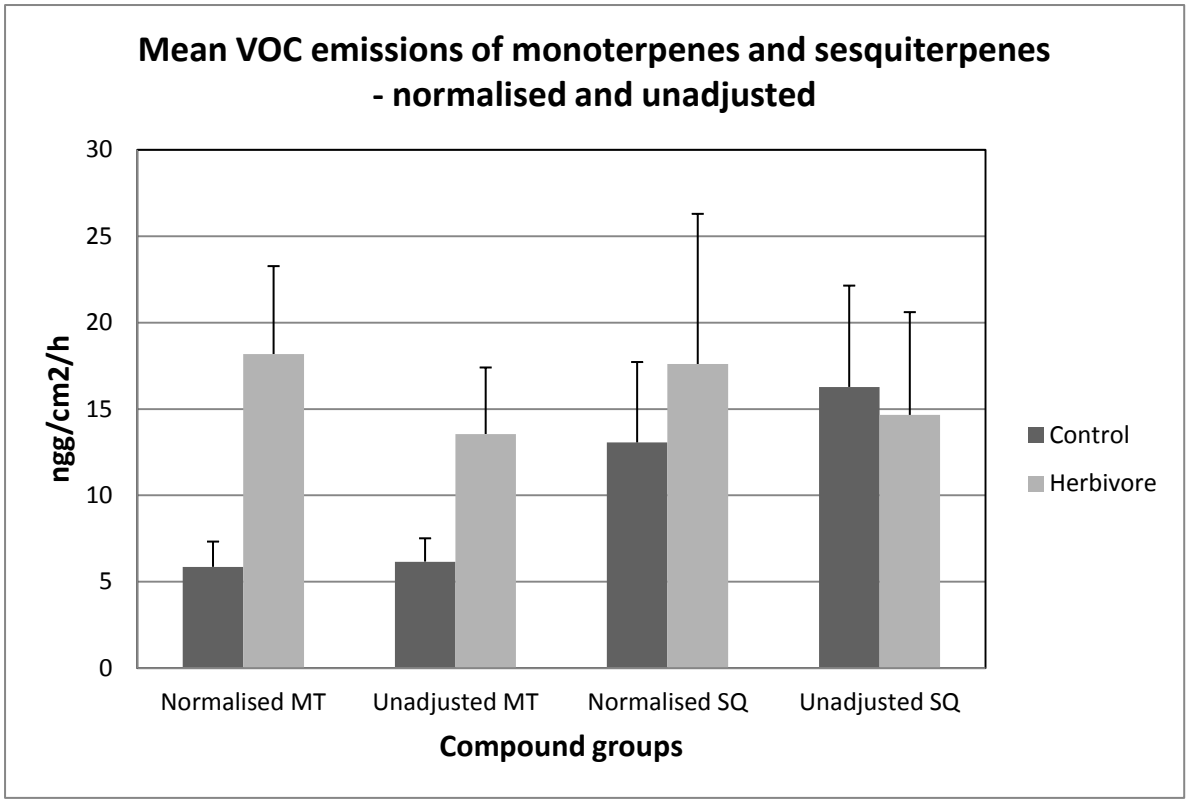

Figure 7. Comparison of mean emission (+SE) regarding to Guenther *et al.* 1993 algorithm. Normalised= algorithm used to adjust raw emissions values, unadjusted= raw emission values. MT=monoterpenes and SQ=sesquiterpenes. Control= no defoliation and herbivore= defoliation by autumnal moth larvae.

Table 2 represents the emission means (+SE) of all individual compound emissions, VOC group emissions (monoterpenes, homoterpene, sesquiterpenes and green leaves volatiles) and all the other emissions. There were three compounds that showed statistical significance ($p<0.05$), (E)-DMNT, linalool and cis-3-hexynyl butyrate, that can be noted from the Table 2. There was statistical significance on monoterpenes, homoterpenes ((E)-DMNT), Green leaf volatiles and All emissions. The two compounds also found from the empty bag samples were nonanal and β-myrcene.

Compounds	Control	Herbivore	P-value
	n=14	n=14	
Monoterpenes			
α-pinene	0.14±0.03	0.19±0.03	0.31
β-myrcene	0.27±0.14	0.33±0.24	0.83
limonene	0.29±0.13	0.27±0.05	0.89
linalool	5.27±1.36	17.52±5.04	**0.03**
Homoterpenes			
(E)-DMNT	0.18±0.04	1.61±0.57	**0.02**
Sesquiterpenes			
α-copaene	0.10±0.03	0.36±0.23	0.28
α-humulene	0.30±0.12	0.43±0.29	0.68
caryophyllene oxide	3.89±2.27	4.51±2.69	0.86
(E)-β-caryophyllene	7.50±2.31	9.98±5.44	0.68
β-bourbonene	1.21±0.39	1.89±0.40	0.23
Green leaf volatiles			
nonanal	2.66±0.47	4.80±1.06	0.08
cis-3-hexenyl butyrate	0.30±0.08	1.00±0.28	**0.03**
cis-3-hexen-1-ol+trans-2-he	2.49±0.84	6.56±2.08	0.08
3-hexan-1-ol acetate	0.70±0.29	3.20±1.20	0.06
Monoterpenes	5.85±1.46	18.18±5.01	**0.03**
Homoterpenes	0.18±0.04	1.61±0.57	**0.02**
Sesquiterpenes	13.06±4.66	17.60±8.69	0.65
Green leaf volatiles	6.14±1.24	15.55±3.72	**0.02**
All emissions	25.23±6.18	52.94±12.81	**0.05**

Table 2. Mean (±SE) emission rates (ng/cm2/h) of volatile organic compounds emitted by control branches and branches under herbivory. N= number of replicates. P-value is based on oneway ANOVA. Statistically significant p-values are bolded.

8. DISCUSSION

8.1 VOLATILE ORGANIC COMPOUND EMISSIONS

The results show clearly that there is an induction of volatile organic compounds caused by the autumnal moth. The emissions of the compounds that where statistically significant (Figure 4 and Table 2), linalool (monoterpene), (E)-DMNT (homoterpene) and cis-3-hexenyl butyrate (GLV) are such compounds that are normally emitted when the plant is defoliated by herbivores. (E)-DMNT is particularly emitted under the herbivore's damage. Linalool, as a monoterpene, is most likely acting as an indirect defence compound. Moreover cis-3-hexenyl butyrate, as a GLV, is also most likely emitted in higher quantities under stress conditions, as seen in this case. The emission of groups of compounds supports this finding; homoterpene, monoterpenes, GLV's and total emissions showed statistical significance (Figure 5, 6 and Table 2).

The same composition of compounds were emitted regardless the treatment. This might indicate that there is always some stress that causes the emissions or it is rather strongly related to the existing environmental conditions rather than stress caused by other sources. In general, the results related to compound composition and induction of compounds followed the other studies made. When this study was conducted in 2007, there was no information available of the basic VOC emission of the mountain birch in a forest site. Haapanala et al. (2009) they studied VOCs of mountain birch as well. They found high quantities of sabinene and α-farnesene, yet, α-farnesene was absent during the second year of the study. Other compounds found by Haapanala et al. (2009) that did not occur in this study, were trans-ocimene and terpinolene. The difference of the compound composition could be explained with different environmental conditions, collection and measurement techniques used and with the differences in the mountain birch pheno- and genotype. Further Vuorinen et al. (2007) studied *Betula pendula* Roth: the autumnal moth larvae induced VOCs were similar to this study. The compounds found to be induced by the autumnal moth were methyl salicylate, (*Z*)-ocimene, (*E*)-β-ocimene and DMNT

In this study, the highest quantities of emissions were measured on monoterpene linalool and with sesquiterpenes caryophyllene oxide and (E)-β-caryophyllene. These three

compounds comprised more than a half of the total emissions despite to the treatment. All compounds studied showed an increase on the emissions when herbivores were causing defoliation, however, statistical significance was found only for the three compounds, as mentioned earlier. This was most likely consequence from the high within treatment variance and relatively low sample size.

Two compounds, nonanal and β-myrcene, were also found in the empty sample bags, as in actual sample bags. They had no clear source for emissions. This observation makes it unclear if the mountain birch itself emits these compounds at all. This finding could have been caused by the impurities in the collection system or by the emission from other sources of VOCs.

8.2 IMPLICATION OF VOCS

VOC properties can be multiple. In the studies of Carroll et al. (2006 and 2008), (E)-DMNT and linalool were not only functioning as an indirect defence but seemed to attract more herbivores. (E)-DMNT seems to give just indication of stress (presence of herbivores), where other induced compounds may also provide more information (such as quantity of herbivores) to predators and parasitoids (Copolovici et al. 2011). The studies of Mäntylä et al. (2008 and 2011) provides also information on birds possibly being able to react to induced VOCs and their presence having a positive influence on the plants.

There can be other indirect implications from the VOCs emitted. For instance, aerosol formation has a direct link to biogenic VOCs, the oxidation of VOCs produce aerosols. Aerosols play a key role in radiation budget. The possible warming effect of the global climate change can increase the emissions of biogenic VOCs by increasing induction caused by herbivores (more favourable conditions) and environmental stress (temperature dependency of some VOCs, other environmental stresses) (Tunved et al. 2006, Pinto et al. 2010).

In my study, the induction was caused by herbivores. The induced compounds indicate that trees had activated their indirect defence. Further, in Mäntylä et al. (2008) suggested that it also worked. Herbivores have most likely influenced also the long term biochemistry of the

trees (immunological memory), as speculated in Haapanala et al. (2009). There is also possibility that increased amount of VOCs have influenced the local aerosol formation.

8.3 SOURCES OF ERROR

The experiments and collection of VOCs were made on field which most likely caused the biggest source of error. The selection of the study trees and branches were made only visually and left a lot of place for variance on pheno- and genotype and growing conditions. This might have led to selection of pairs that might have been incomparable to each other by the factors that affect the emissions of VOCs. It is also slightly unclear how big is the role of past events. The influence of immunological memory effect was suggested to be the cause of variation of VOC composition in the study of Haapanala et al. (2009).

The sample collection system might have caused an error with nonanal and β-myrcene, or those compounds might have entered the collection system from surrounding emissions. At the time when collections were made, there were, for example, strong odours coming from wild rosemary (*Rhododendron tomentosum*).

Other sources of error, than the ones related to field experimenting, are minor. The algorithm (Guenther et al. 1993) used for temperature correction might cause some error but as the main one used in many studies and no resources for experimenting and adjusting the algorithm, the error is acceptable, and most likely not influencing the results significantly (Figure 7).

8.4 FUTURE ASPECTS OF STUDY

This study is one of the very few studies made on mountain birch's VOCs and it was made on field conditions. More perspective could be obtained by making similar experiments in laboratory conditions with clones of mountain birch in stable conditions, and on field during different years, areas and seasons. That knowledge would be useful for any future research made with mountain birch related studies or ecology of subarctic vegetation. Since the mountain birch is the dominant tree species in the northern most and elevated areas of

northern Europe, there is reason for a further study on the magnitude of the possible connection to aerosol formation.

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