SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

"**Im**pact of **P**henology and **E**nvironmental **C**onditions on B**VOC** Emissions from Forest Ecosystems"

«IMPECVOC»

K. Steppe, M. Šimpraga, H. Verbeeck, J. Bloemen, É. Joó, O.Pokorska, J. Dewulf, H. Van Langenhove, M. Demarcke, C. Amelynck, N. Schoon, J.-F. Müller, Q. Laffineur, M. Aubinet, B. Heinesch, R. Lemeur

ENERGY

TRANSPORT AND MOBILITY

AGRO-FOOD

HEALTH AND ENVIRONMENT

CLIMATE

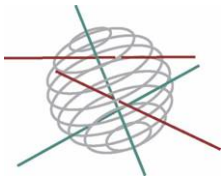
BIODIVERSITY

ATMOSPHERE AND TERRESTRIAL AND MARINE ECOSYSTEMS

TRANSVERSAL ACTIONS

.be

SCIENCE FOR A SUSTAINABLE DEVELOPMENT
(SSD)



Terrestrial Ecosystems

FINAL REPORT PHASE I

"**Im**pact of **P**henology and **E**nvironmental **C**onditions on B**VOC** Emissions from Forest Ecosystems"

«IMPECVOC»

SD/TE/03A

Promotors

Jo Dewulf & Herman Van Langenhove
University of Ghent (UGent)
Environmental Organic Chemistry
and Technology Research Group

Kathy Steppe & Raoul Lemeur
University of Ghent (UGent)
Faculty of Bioscience Engineering

Crist Amelynck, Niels Schoon & Jean-François Müller
Belgian Institute for Space Aeronomie
(BIRA_IASB)

Marc Aubinet & Bernard Heinesch
Faculté Universitaire des Sciences Agronomiques de Gembloux
(FUSAGx)

Authors
KATHY STEPPE, MAJA ŠIMPRAGA, HANS VERBEECK, JASPER BLOEMEN,
ÉVA JOO, OLGA POKORSKA, JO DEWULF,
HERMAN VAN LANGENHOVE, MARIE DEMARCKE,
CRIST AMELYNCK, NIELS SCHOON,
JEAN-FRANÇOIS MÜLLER, QUENTIN LAFFINEUR, MARC AUBINET,
BERNARD HEINESCH, RAOUL LEMEUR

BELGIAN SCIENCE POLICY

Rue de la Science 8
Wetenschapsstraat 8
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be

Contact person: Martine Vanderstraeten
+32 (0)2 238 36 10
Project Website : http://www.impecvoc.ugent.be/

Steppe K, Šimpraga M, Verbeeck H, Bloemen J, Joó E, Pokorska O, Dewulf J, Van Langenhove H, Demarcke M, Amelynck C, Schoon N, Müller J-F, Laffineur Q, Aubinet M, Heinesch B, Lemeur R. ***Impact of Phenology and Environmental Conditions on BVOC Emissions from Forest Ecosystems "IMPECVOC"*** Final Report Phase 1. Brussels: Belgian Science Policy 2009 – 53 p. (Research Programme Science for a Sustainable Development)

PROJECT TEAM

Partner 1 **(Coordinator of phase 1): PE-UG**
Responsible: Prof. dr. ir. Kathy Steppe/ Prof. dr. Raoul Lemeur
Researchers: Msc Maja Šimpraga, dr. ir. Hans Verbeeck
Students: Jasper Bloemen
Institute: Laboratory of Plant Ecology,
Faculty of Bioscience Engineering, Ghent University (PE-UG)
Address : Coupure links 653, 9000 Gent
Phone / fax : +32 9 264 61 12 / +32 9 224 44 10
E-mail : kathy.steppe@ugent.be

Partner 2 **(Coordinator of phase 2): EnVOC-UG**
Responsible: Prof. dr. ir. Jo Dewulf / Prof. dr. ir. Herman Van Langenhove
Researchers: Éva Joó, Olga Pokorska
Students: Lieselotte Schietse, Dora Neina
Institute: Research Group Environmental Organic Chemistry and Technology,
Faculty of Bioscience Engineering, Ghent University (EnVOC –UG)

Partner 3: **BISA**
Responsible: dr. Crist Amelynck/ dr. Niels Schoon / dr. Jean-François Müller
Researchers: Marie Demarcke
Institute: Belgian Institute for Space Aeronomy (BISA)

Partner 4: **FUSAGx - UBP**
Responsible: Prof. dr. Marc Aubinet
Researchers: Quentin Laffineur, dr. Bernard Heinesch
Institute : Faculté Universitaire des Sciences Agronomiques de Gembloux,
Unité de Physique des Biosystèmes (FUSAGx - UBP)

ACRONYMS AND ABBREVIATIONS

BELSPO	Belgian Federal Science Policy Office (Belgisch Federaal Wetenschapsbeleid)
BIRA (BISA or IASB)	Belgisch Instituut voor Ruimte-Aëronomie (BIRA) / Belgian Institute for Space Aeronomy (BISA)/ Institut d'Aéronomie Spatiale de Belgique (IASB) (Partner 3)
BVOC	Biogenic Volatile Organic Compounds
CCI	Chlorophyll Content Index
Co	Coordinator (Partner P1), i.e. Prof. dr. Raoul Lemeur and his team (PE-UG)
$[CO_2]$	Atmospheric CO_2 concentration
Chl	Chlorophyll
Cuv 1	Cuvette 1
Cuv 2	Cuvette 2
Cuv ref	Cuvette reference
DAQ	Data acquisition unit
EC	Eddy-covariance
EnVOC-UG	Research Group Environmental Organic Chemistry and Technology, Ghent University (Partner P2)
EQ	Equitensiometer
FNRS	Fonds National de Recherche Scientifique
FOV	Field of view
FUSAGx	Faculté Universitaire des Sciences Agronomiques de Gembloux (Partner P4)
FWO	Fonds voor Wetenschappelijk Onderzoek
G97	Isoprene emission algorithm (Guenter, 1997)
G06	MEGAN isoprene emission algorithm (Guenter et al., 2006)
G06a	Modified version of MEGAN isoprene emission algorithm
GC-MS	Gas chromatograph – mass spectrometer
I	Irradiance
IMPECVOC	Impact of Phenology and Environmental Conditions on BVOC Emissions from Forest Ecosystems
INBO	Instituut voor Natuur- en Bosonderzoek (Research Institute for Nature and Forest)
IR	Infrared radiation
IRGA	Infrared gas analyzer
IRTC	Infrared thermocouple
ISTD	Internal standard
IWT	Instituut voor de Aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen
KMI	Koninklijk Meteorologisch Instituut van België (Royal Meteorological Institute of Belgium)
LA	Leaf area
LAI	Leaf area index
LVDT	Linear variable displacement transducer
MT	Monoterpenes
NDVI	Normalized difference vegetation index
OVOC	Oxygenated volatile organic compounds
Pn or A	Net photosynthesis or net CO_2 assimilation rate (μmol-CO_2 m^{-2} s^{-1})
P1	Partner 1, i.e. Prof. dr. Raoul Lemeur and his team (PE-UG)
P2	Partner 2, i.e. Dr. Crist Amelynck and his team (BISA)
P3	Partner 3, i.e. Prof. dr. Jo Dewulf and his team (EnVOC – UG)

P4	Partner 4, i.e. Prof. dr. Marc Aubinet and his team (UBP)
PAR	Photosynthetically active radiation (µmol-photons m^{-2} s^{-1})
PE-UG	Laboratory of Plant Ecology, Ghent University (Partner P1)
PPDF	Photosynthetic photon flux density
PTR-MS	Proton transfer reaction - mass spectrometry (spectrometer)
RH	Relative humidity
RRI	Relative retension time
Q	Mass flow
SEF	Standard emission factor
SIM	Selective Ion Monitoring
SPDE	Solid phase dynamic extraction
SQT	Sesquiterpenes
SWC	Soil water content
TDP	Thermal dissipation probe
TIC	Total ion chromatogram
Tl	Leaf temperature
Tr	Transpiration rate
UA	University of Antwerp
UBP	Unit of Biosystem Physics (Unité de Physique des Biosystèmes) Faculté Universitaire des Sciences Agronomiques de Gembloux (Partner P4)
vDEC	virtual Disjunct eddy-covariance
VMM	Vlaamse Milieumaatschappij
VOC	Volatile organic compound
WP	work package
m/e or m/z	mass to charge ratio
ncps	normalized counts per second
ng	nanogram
ppbv	parts per billion (by volume)ppm parts per million
ppmv	parts per million (by volume)
pptv	parts per trillion (by volume)

Table of content

Abstract

Forest ecosystems are known to be important emission sources of Biogenic Volatile Organic Compounds (BVOC). Due to their large emissions and their high reactivity with the main oxidants (OH, O_3, NO_3) in the atmosphere, these BVOCs play an important role in atmospheric chemistry. In order to be able to quantify net formation of oxidants and aerosols from BVOCs, the physicochemical oxidation and aerosol formation and/or growth have to be well understood. Of equal importance, however, is that BVOC emissions need to be well characterized and quantified. Few experimental data are available on the effect of temperature and radiation history on emissions. Measurements are needed to determine the precise dependence of emissions upon radiation and leaf temperature for tree species commonly found in Belgium. The advent of new on-line, rapid and sensitive technologies such as the Proton Transfer Reaction Mass Spectrometer (PTR-MS) has opened new and exciting developments in BVOC emission research. As such direct eddy-covariance BVOC flux measurements at the level of a forest stand became possible. PTR-MS is also very useful to perform long-term continuous BVOC emission measurements from branch enclosures (e.g. leaf cuvettes).

The objectives of the IMPECVOC project are: (1) The collection of BVOC emission data at different levels of biological organisation (leaf emissions from young model trees in climate controlled growth chambers; emissions from horizontal leaf canopy layers observed on the measuring tower at the Aelmoeseneie experimental forest; and emission measurements above the large Vielsalm experimental forest); (2) The validation of new emission algorithms (adaptation of the MOHYCAN canopy model and the MEGAN model which allow spatial upscaling of BVOC emissions from leaf to tree and to stand level); (3) The correction of emission algorithms by inclusion of additional driving variables (e.g. water availability, atmospheric CO_2 concentration and effects of forest functioning (e.g. seasonal leaf area development, leaf age, sunlit and shaded leaves, …); and (4) The estimation of the BVOC emissions from Belgian forests based on the modified emission algorithms and Belgian forest inventories.

During phase 1 of the IMPECVOC-project simultaneous BVOC (PTR-MS and GC-MS), CO_2 and H_2O flux measurements have been carried out on a regular time scale during the branch enclosure experiments in the growth room and at the Aelmoeseneie experimental forest. In order to perform dynamic branch enclosure flux measurements, prototype cuvettes were designed and constructed. In addition, during the first trimester of 2008, in between the experiments in the growth chamber and the field measurements in the Aelmoeseneie forest, laboratory measurements were performed in order to study the influence of instrumental and environmental parameters on the detection of sesquiterpenes with the PTR-MS instrument.

The results showed that beech (*Fagus sylvatica* L.) is a low isoprene emitter and a rather strong monoterpenoid emitter. A clear link was observed between temperature variation and monoterpenoid emissions, linked to net photosynthesis rates. The results revealed that the potted beech tree under well-watered conditions re-emitted a rather low fraction of the assimilated carbon back into the atmosphere as total monoterpenoids. This fraction increased exponentially from 0.01 to 0.10 % with a temperature rise from 17 °C to 27 °C in growth room conditions.

From the results of the drought experiment it was seen that monoterpenoid emissions were linked to tree physiology; more specific to leaf net photosynthesis rate, to stem diameter growth and to sap flux density. Moreover, interdependence between leaf and tree plant processes was observed. Imposed severe drought caused photosynthesis and monoterpenoid emissions to decrease. Upon photosynthesis inhibition, the emissions of monoterpenoids were inhibited most likely due to the photosynthetic origin of the monoterpenoids. Data of the canopy experiment in the Aelmoeseneie forest clearly showed that there was a difference between sunlit leaves and shade-adapted leaves. Diurnal BVOC emission patterns indicated that shade-adapted leaves for a sunny day show a stronger interaction between monoterpenoid emissions and net photosynthesis than for the sunlit leaves. This interaction was even stronger for a cloudy day. It can, hence, be stated that the physiological leaf status plays a major role when considering monoterpenoid emissions, photosynthesis and transpiration rates. The importance of the physiological status of leaves should therefore be emphasized more in the future.

Based on the experimental data, existing emission algorithms could be tested and improved. This work is still ongoing. The observed emissions are more closely approximated using isoprene emission algorithms than by using a light-independent monoterpene emission algorithm developed for

coniferous trees. PTR-MS measurements in the growth room under controlled conditions and in the Aelmoeseneie forest under real outdoor conditions already revealed the effect of light history on monoterpenoid emissions by *Fagus sylvatica* L., which is not correctly incorporated in commonly used emission algorithms. Modifications of these algorithms have been proposed for an accurate description of this effect.
At the end of phase 1, the operational infrastructure at the Vielsalm forest site was established and the stand-scale experiment will be the major focus of phase 2 of the project. This infrastructure includes a meteorological tower fully equipped with adequate sensors, and an equipped shelter. The existing set-up had to be strongly updated for BVOC measurements.

Context

Forest ecosystems are known to be important emission sources of Biogenic Volatile Organic Compounds (BVOC). Due to their large emissions and their high reactivity with the main oxidants (OH, O_3, NO_3) in the atmosphere, these BVOCs play an important role in atmospheric chemistry. In the presence of nitrogen oxides, atmospheric oxidation of BVOCs may for instance result in net oxidant formation that has an important impact on air quality and tropospheric chemistry. Less volatile oxidation products can lead to the formation and/or growth of aerosol particles and, as such, have an important impact on health, visibility and climate (through scattering and absorption of solar radiation and cloud formation). In order to be able to quantify net formation of oxidants and aerosols from BVOCs, the physicochemical oxidation and aerosol formation and/or growth have to be well understood. Of equal importance, however, is that BVOC emissions need to be well characterized and quantified as well.
It is well-established that photosynthetic photon flux density (PPFD) and leaf temperature (Tl) strongly influence BVOC emissions from plant leaves. Few experimental data are available on the effect of temperature and PPFD history on emissions. Measurements are needed to determine the precise dependence of emissions upon PPFD and Tl for tree species commonly found in Belgium.Therefore, commonly used BVOC emission algorithms are mainly a function of these two parameters. Far less is known about the effects of other environmental conditions (e.g. relative humidity of the air, soil water availability, atmospheric CO_2 concentration); and of tree physiology, forest functioning and forest phenology. In order to reduce uncertainties on BVOC emissions from forest ecosystems these effects should be incorporated in BVOC emission algorithms as well. If not, BVOC emission estimates from the simple PPFD and Tl based algorithms will be less precise and include large systematic errors.
The advent of new on-line, rapid and sensitive technologies such as the Proton Transfer Reaction Mass Spectrometer (PTR-MS) has opened new and exciting developments in BVOC emission research. As such direct eddy-covariance BVOC flux measurements at the level of a forest stand became possible. PTR-MS is also very useful to perform long-term continuous BVOC emission measurements from branch enclosures (e.g. leaf cuvettes).
At present, eddy-covariance BVOC flux measurements based on PTR-MS have been carried out by only a limited number of research groups worldwide (e.g. Rinne et al., 2001; Karl et al., 2002; Grabmer et al., 2004; Spirig et al., 2005) but the number of measurement locations in Europe is very small. Also, measurements were only made during a small fraction of the growing season.
The present research program will, in comparison with the previously mentioned studies, also focus on the temporal dynamics of BVOC emissions during a complete growing season, during which forests functioning and forest phenology will be followed at the same time.

Objectives

The objectives of the IMPECVOC project are:
(1) The collection of BVOC emission data at different levels of biological organisation (leaf emissions from young model trees in climate controlled growth chambers; emissions from horizontal leaf canopy layers observed on the measuring tower at the Aelmoeseneie experimental forest; and emission measurements above the large Vielsalm experimental forest);
(2) The validation of new emission algorithms (adaptation of the MOHYCAN canopy model and the MEGAN model which allow spatial upscaling of BVOC emissions from leaf to tree and to stand level;
(3) The correction of emission algorithms by inclusion of additional driving variables (e.g. water availability, atmospheric CO_2 concentration, and effects of forest functioning (e.g. seasonal leaf area development, leaf age, sunlit and shaded leaves, …); and
(4) The estimation of the BVOC emissions from Belgian forests based on the modified emission algorithms and Belgian forest inventories.

The expected outcomes are:
(1) An improved insight in the fundamental plant processes which govern BVOC emissions;
(2) The availability of validated emission algorithms with better precision as more abiotic and biotic driving variables are taken into account;
(3) The establishment of practical calculation procedures for upscaling and estimation of BVOC emissions at stand level; and
(4) The elaboration of regional and national BVOC emission inventories.

Materials and methods

For an extensive description of the materials and methods used in the IMPECVOC project we refer to the previous reports of this project. Nevertheless, several crucial methodological issues are discussed in the results section below.

Results

The results are discrebed below according to each workpackage as described in the IMPECVOC project proposal.

WP1 Comparison GC-MS and PTR-MS

1.1 Experimental setup for coupling PTR-MS and GC-MS

For GC-MS analysis of BVOCs pre-concentrated on adsorbent sampling tubes were used (Figure 1). The tubes were selected by considering three major points as: (i) complete adsorption of VOCs in a qualitative and quantitative way should be obtained; (ii) artefacts which are formed on the tube, especially when ozone is present during sampling, should be avoided; and (iii) interference with water during the analysis should be eliminated. Therefore, the decision – based on literature (Helmig, 2006; Dominguez-Taylor, 2007; Ormeno, 2007; Tiiva, 2007; Liakakou, 2007) – was taken to use multiple adsorbent tubes containing Tenax TA (35 m²/g, MARKES) and Carbotrap (100 m2/g, 20-40 mesh, MARKES) with a ratio of 50:50 packed in glass tubes (O.D. 0.25-inch, length 3.5 inch). Analyses were performed with an on-line coupled Unity thermal desorber and air server (MARKES International, Pontyclum, UK) and a GC Trace 2000 gas chromatograph (ThermoFinnigan, Milan, Italy) (Figure 7b) connected to a MS Trace DSQ WE-250 mass spectrometer (ThermoFinnigan, Austin, TX, USA). After the desorption of the concentrated compounds, they were separated on a DB-1 (30 m x 0.25 mm x 1 µm) column placed inside the GC and then ionized and detected in the MS. In some cases the Ultra Autosampler (QUI-0006 3.1) was used instead of the mentioned thermal desorber. The whole system is controlled from a personal computer (Compaq PC-EVO-310) with Unity 1.2.0 (MARKES International) and XCalibur 1.3 (ThermoFinnigan, Austin, TX, USA) software installed.

Figure 1. From left to right: (a) adsorbent sampling tubes, (b) GC-MS and (c) scheme of GC-MS work principle

In order to perform accurate BVOC concentration measurements with the PTR-MS instrument (Figure 2a) an additional experimental set-up for determining the instrumental background and for calibrating the PTR-MS for the BVOCs of interest has been designed and constructed at BISA (Partner P3). This set-up mainly consists of a catalytic converter for generating a zero-VOC air flow, a calibration gas mixture containing trace amounts of methanol, ethanol, acetone, isoprene and monoterpenes (sabinene and α-pinene) diluted in N_2 (Apel-Riemer Inc., Denver, CO), a set of mass flow controllers and three-way solenoid valves, a diaphragm pump for generating a gas flow, and power supplies for the flow controllers and solenoid valves. To enable alternate sampling from multiple cuvettes in growth chamber experiments, as well as in the canopy experiment, a gas multiplexer (Figure 2c) has also been constructed. All gas lines and the inner surfaces of the solenoid valves (in the calibration set-up as well as in the multiplexer) are made of Teflon and PFA (polyfluoro alkoxy Teflon) to avoid losses of BVOCs. The output direction of the three-way valves and the setpoints of the flow controllers are set through a HP DAQ (Figure 2b), which also serves to log the valve positions and the flow controller output values continuously. The DAQ in turn is controlled by a LabVIEW based software program (developed at BISA); and both this program and the PTR-MS software program can be run from any location by using remote PC control. PTR-MS calibration and background measurements, as well as alternate BVOC emission measurements from different cuvettes can be performed in an automatic mode by using scripts (set of command lines). The entire set-up is constructed in such a way that it can easily be transported between the growth chambers and the two forest sites. Close to the location where the PTR-MS capillary inlet line is connected to this set-up, an additional inlet (which can be shut off with a PFA stop valve) is foreseen for Partner P2 (ENVOC) in

order to use the same calibration standard as BISA and to perform simultaneous measurements. The above described additional experimental set-up works successfully.

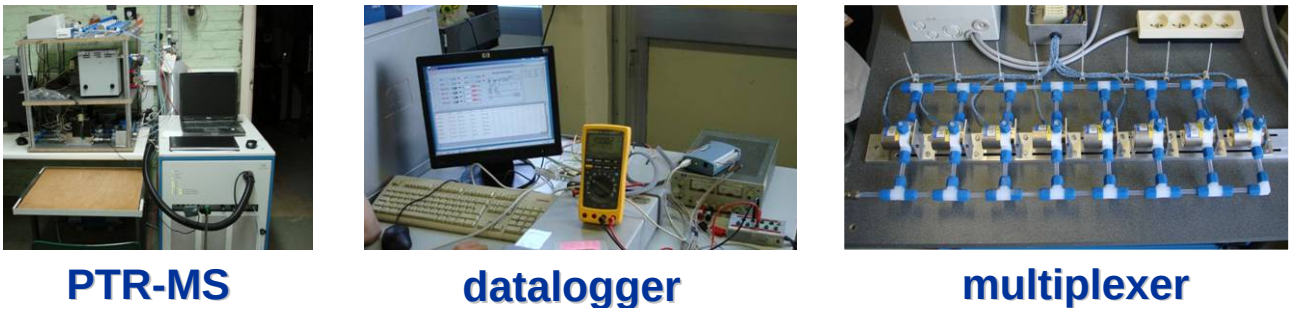


Figure 2: From left to right: (a) PTR-MS set-up, (b) DAQ and (c) multiplexer.

1.2 Calibration PTR-MS and GC-MS

The deliverable of this workpackage (tested and validated calibration method for the analytical instruments) was achieved by Partners P2 and P3. Calibration of the PTR-MS for isoprene, acetone and the sum of monoterpenes (sabinene + α-pinene) has been carried out regularly, i.e. once or twice a week during active measuring periods. The BVOC mixing ratios were obtained by means of a dynamic dilution system (see WP 1 -1.1). Perfectly linear calibration curves (of normalized BVOC ion signal versus mixing ratio) were obtained for all considered compounds over the entire range of mixing ratios as is shown in Figure 3.

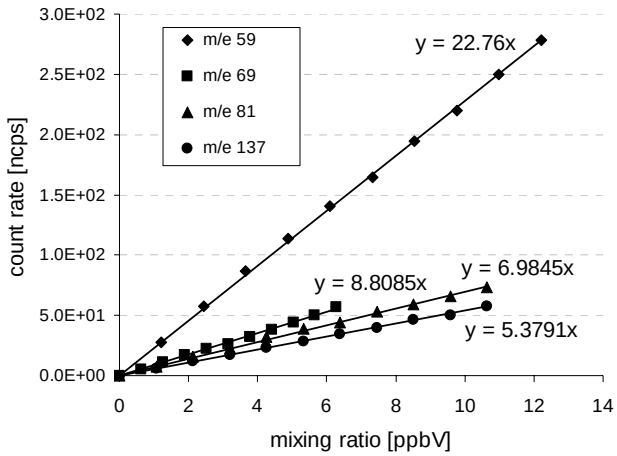


Figure 3: Calibration curve for the PTR-MS ion signals related to monoterpenes (m/z 81 and 137), acetone (m/z 59) and isoprene (m/z 69).

Calibration for GC-MS was performed with a mixture from an aluminium gas cylinder (Luxfer, Inc., Riverside, CA, N150, 1800 psig) containing methanol (1.01 ppmv), ethanol (1.01 ppmv), acetone (1.01 ppmv), isoprene (0.52 ppmv), α-pinene (0.47 ppmv) and sabinene (0.41 ppmv), with an uncertainty of <5 %. Flow coming directly from the calibration bottle was then diluted for the different concentrations by zero-air, purified by IEC 1010 Parker Balston Zero Air Generator. Measurements were done from the same calibration mixture that was used by BISA. Calibration was done regularly during the sampling period. For the quantification of terpenes that were not available in the gas standard, response factors of sabinene were used. Below on Figure 4, an example of a calibration curve is given for a wide range of expected concentrations. The coefficient of determination R^2 shows

a good fit with a value of 0.996 for sabinene. The graph shows the ratio of the integrated terpene and the internal standard vs. the specified amount of terpene used for the calibration.

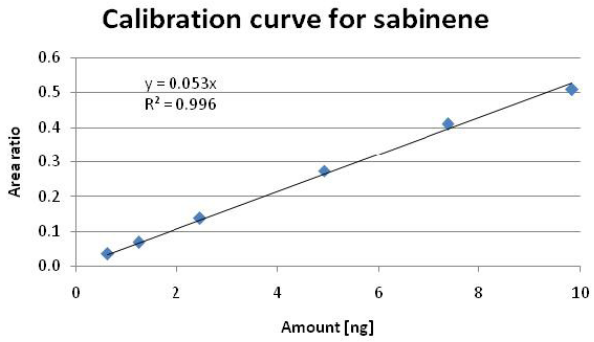


Figure 4: Calibration curve for one of the monoterpenes used in GC-MS analysis

Reliable and accurate quantification was set as a target of these experiments. Quantification is based on the internal standard (ISTD) methodology, which was used during the experiments in order to correct fluctuation caused by MS detector and give a feedback about the whole sampling and analyzing procedure. Toluene-D8 was chosen as an ISTD in our situation.

1.3 Intercomparison growth chamber

PTR-MS and GC-MS data were intercompared and validated in the growth chamber experiment. The analysis of this intercomparison is described under 1.5.

1.4 Intercomparison forest sites

PTR-MS and GC-MS data were intercompared and validated in the forest experiment. The analysis of this intercomparison is also described under 1.5.

1.5 Data analysis

Simultaneous PTR-MS and GC-MS measurements have been carried out on a regular time scale during the branch enclosure experiments in the growth room (2007; WP 1 1.3) and at the Aelmoeseneie experimental forest (2008; WP 1 1.4).
The introduction of monoterpenes in a PTR-MS instrument results in ion signals at m/z 81 and m/z 137, and the detection sensitivity is only slightly dependent on the nature of the monoterpenes. In the absence of other compounds which also result in product ions at the same m/z values, the ion signals at m/z 81 and m/z 137 are expected to be directly proportional to the sum of the emissions of the individual monoterpenes emitted by the tree. Analysis of the GC-MS data revealed, however, that, next to monoterpenes (mainly sabinene), significant amounts of the oxygenated monoterpene linalool and of the sesquiterpene α-farnesene are emitted by *Fagus sylvatica* L. Both compounds have PTR-MS signatures at m/z 81 and m/z 137. Consequently it is impossible, based on the PTR-MS ion signal at m/z 137 or m/z 81, to differentiate the monoterpenes from the monoterpenoid alcohol linalool or the sesquiterpene α-farnesene. Therefore the PTR-MS signal at m/z 137 is rather a measure of the emission of "monoterpenoid" compounds than a measure of the emission of the monoterpenes solely. This will be discussed and illustrated in detail in a paper, which is currently in preparation (Joó et al. "Quantification of BVOC using PTR-MS and GC-MS techniques in the view of quality control approaches"). The intercomparison also revealed that the PTR-MS signal at m/z 81 is not suited to

characterize the emission of monoterpenoid compounds, due to the presence of unidentified compounds, which have a PTR-MS signature at m/z 81.
Notwithstanding the fact that the PTR-MS can not differentiate monoterpenoid compounds based on the m/z 137 signal, it should be kept in mind that this technique is the only available one to perform fast and sensitive on-line BVOC measurements, as for instance is required for stand scale eddy covariance measurements.

Laboratory experiments to characterize the PTR-MS instrument for the detection of sesquiterpenes

During the first trimester of 2008, in between the experiments in the growth chamber and the field measurements in the "Aelmoeseneie" forest, laboratory measurements were performed in order to study the influence of instrumental and environmental parameters on the detection of sesquiterpenes with the PTR-MS instrument. Apart from the fact that the outcome of these studies is of general interest for the PTR-MS community, the observation of sesquiterpene emissions during the growth chamber experiments by Partner P2 motivated us to undertake these laboratory experiments.
A set-up was constructed to produce controlled and pure sesquiterpene flows, diluted in ozone-free and VOC-free air of varying relative humidity. Sesquiterpene product ion distributions were obtained as a function of the drift field in the PTR-MS reactor at different relative humidities. A peer-reviewed paper on the experimental results has been published (Demarcke et al. (2009) "Laboratory studies in support of the detection of sesquiterpenes by Proton-Transfer-Reaction-Mass-Spectrometry", Int J Mass Spectrom 279:156-162) and these results should lead to a more sensitive detection of sesquiterpene fluxes by PTR-MS in the future.

WP2 Growth chamber experiment

2.1 Tree cuvette

Design of an optimal branch cuvette for measurement of BVOC, CO_2 and H_2O vapour exchange from leaves

In order to perform dynamic branch enclosure BVOC, CO_2 and H_2O flux measurements, prototype cuvettes were designed and constructed (deliverable) in the mechanical workshop of BISA. In order to minimize losses of BVOCs to the walls, all cuvette parts that are in contact with the BVOCs are made of PFA or Teflon. The cuvettes consist of a transparent plexi glass base plate and three support rings. Two rings are held together by aluminium bars and serve as support structure of a cylindrical 50 µ thick PFA foil with a transparency to PAR of 95 %. The base plate is also covered with PFA foil and contains an inlet and outlet opening in PFA and Teflon, a Teflon ventilator to homogenize the BVOC-enriched air inside the cuvette, and a Teflon structure for passing electrical wires (for the fan, thermistor, leaf temperature sensor, …) without creating air leaks. One of the rings of the cylindrical support structure is clamped to the base plate. Opposite to the base plate the branch enters the cylindrical enclosure and the cuvette is closed by means of a second cylindrical PFA envelope. On one end, the envelope is fixed to the support structure with a third aluminium ring and associated clamps. On the other end, it is wrapped and tightened around a branch of a tree. VOC-free air is pumped in the cuvette where it is enriched with BVOCs emitted by the leaves of the enclosed branch. Part of the BVOC-enriched air leaving the cuvettes is pumped towards the analytical instrumentation through PFA tubing. The rest is sent into the growth chamber. Three of these cuvettes have been manufactured and used in the course of the reporting period (Figure 5).

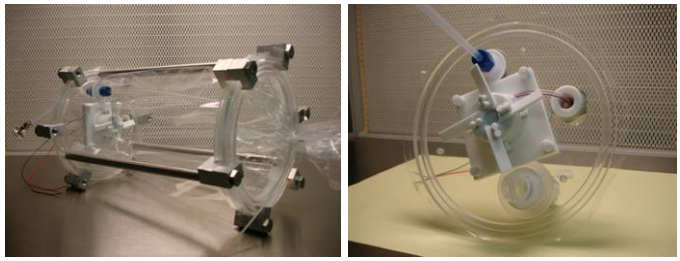


Figure 5: Prototype cuvette for inclusion of tree branches (left) and base plate with input and output connections (right)

To minimize BVOC losses only components in PFA or Teflon were used for the construction of the cuvettes and of the multiplexer. Possible BVOC losses (losses in the cuvette or in the PFA tubing in between the cuvette and the multiplexer or in the multiplexer itself, …) were characterized by introducing into the cuvettes a mixture with known concentration of monoterpenes, acetone and isoprene in VOC free air and by measuring the concentration of these compounds at different locations in between the cuvette and the PTR-MS (just after the cuvette, just in front of the multiplexer, after the multiplexer). It was found that the loss of BVOCs in the complete system is negligible (less than 2%).

Design of an inlet system of purified air into the cuvettes

To perform measurements of BVOC emissions with high accuracy purified air input lines for the cuvettes were necessary. Therefore, specific precautions had to be taken: (1) the removal of ozone present in ambient air; (2) the elimination of background VOCs; and (3) the adsorption of dust particles. As a solution, air was continuously taken from outside through an inlet line with a flow rate of 13.5 l/min using a special membrane pump (MVP 055-3 diaphragm vacuum pump, PFEIFFER VACUUM, Aachen, Germany). To compensate for [CO_2] fluctuation in the inlet air flow, a 70 l buffer vessel was installed. A dust filter was added as well as MnO_2 as ozone scrubber. Two active coal filters were included for this purpose (DESOTEC N.V.-S.A.) and were placed in series, loaded by AIRPEL 10 and ORGANOSORB 10-CO in ascending order of specific area. Ensuring the absence of carry-over of carbon powder, a second dust filter was put before the flow splitting. Flow meters

(5860S BROOKS Instruments) with valves were installed in each input line going to the cuvettes. All parts of this inlet line were connected by inert ¼ inch Teflon tubing. To ensure the quality of measurements and the data collected, ozone levels were checked monthly from the inlet air of cuvettes by a Z ECC Ozonesonde. This sensor is routinely used by the Royal Meteorological Institute of Belgium for atmospheric ozone measurements. Quantitative analysis is based on electrochemical reactions between ozone and potassium-iodide. It was found to be < 2 ppbv at all times.

2.2 Growth chamber measurements at optimal conditions

In 2007, simultaneous measurements (PE-BISA) were done during exp p1, exp p2, exp p3 and exp p4. In 2008, this was repeated during exp p2 (Table 1). The aim was to study BVOC emissions (P3) and CO_2 and water vapour exchange (P1) in different conditions. A level of 21°C was chosen as an optimal reference air temperature and PPFD was changed in a stepwise pattern (see paragraph 2.3). Prior to the growth chamber experiments, the trees were grown under constant regime until new shoots were fully developed and mature. The total time needed to develop new shoots was between 1-1.5 months. The trees adapted well to the growth conditions in the growth chamber. When the leaves reached their mature stage, the experiments with varying temperature and, later on, with soil water content availability started. In early spring, before leaf sprouting (26/03/08), the branches were respiring and did not show any net photosynthetic activity, which is a normal developmental process (Figure 6). When fully expanded leaves were present, net photosynthetic assimilation (P_n or A_n) was positive during the daytime and negative during the night (R_d). Leaves reached their highest seasonal P_n at high light conditions with a mean of 10 µmol CO_2 m^{-2} s^{-1} for tree 1 and a mean of 12 µmol CO_2 m^{-2} s^{-1} for tree 2. Early spring net assimilation of tree 1 was slightly higher when compared to tree 2. Regarding beech BVOC emissions in the beginning of the growing season, emission started 15 days later.

Partner P2 joined to the continuous measurements at the beginning and at the end of experimental period, and also for measurements at different temperatures. Samples were taken 15 min after PAR changes, which is the time necessary to reach constant IRGA and BVOC signal (checked by Partner P3). From the ozone measurements it was found that the constructed system is suitable for BVOC analysis, since O_3 presence was below 2 ppbv. BVOCs were successfully detected as it is shown in Figure 7.

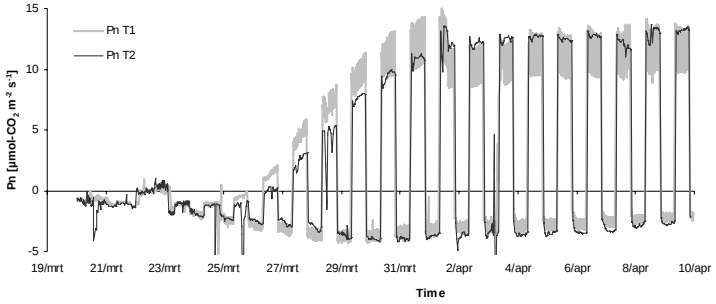


Figure 6. Net photosynthesis measured in the beginning of the season in the growth chamber 2008 experiments (T1=tree 1; T2=tree 2)

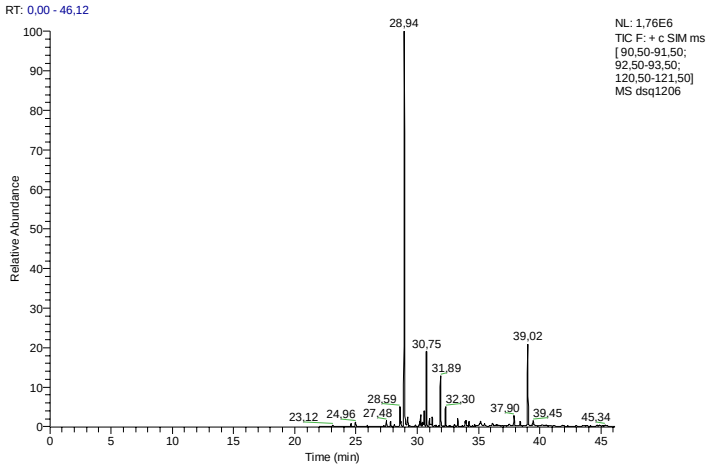


Figure 7. SIM (Selective Ion Monitoring) chromatogram using 91, 93, 121 as detected masses. The highest peak at retention time of 28.94 min is identified as sabinene.

2.3 Growth chamber measurements at varying environmental conditions

Partner P1 collected the ecophysiological data of the trees grown under controlled conditions. In addition to seasonal sampling, two detailed experiments were performed: (1) study of the impact of varying temperature conditions (perfomed during 2007) and (2) study of the impact of varying soil water content conditions (perfomed during July 2008). The data are shown in paragraph 2.4.

Three-year-old beech trees (*Fagus sylvatica* L) of 1.00-1.25 cm height were purchased in containers. This tree species was studied in the 1st and 2nd year of the project, as this it is a common species found in the Flanders (e.g. Aelmoeseneie forest) and in the Walloon region (e.g. Vielsalm), where the IMPECVOC field experiments are performed from year 2 on. The container trees are considered as a model system and their BVOC emissions can be accurately linked with the set points of PPFD and temperature selected in the growth chambers of Partner P1 (PE-UG). The growth room experiments also allowed exploratory experimentation for fine tuning the IRGA, PTR-MS and GC-MS analysis protocols. The below summarized timing was followed once the growth rooms were operational and leaf cuvettes and analysis equipment were installed (Tables 1 and 2).

Table 1: 2007 experimental scheme of growth chamber experiments, indicating the time period, the model trees and branch/leaf cuvettes used.

Experiment period 1 **(07 exp p1)**	Experiment period 2 **(07 exp p2)**	Experiment period 3 **(07 exp p3)**	Experiment period 4 **(07 exp p4)**
13/07/07 – 23/08/07	24/08 – 19/09/07	20/09/07 – 08/10/07	09/10/07 – 26/10/07
Beech tree 1, cuv1	Beech tree 2, cuv1	Beech tree 2, cuv1, cuv2	Beech tree 2, cuv1, cuv2

Table 2: 2008 experimental scheme of IMPECVOC 2008 experiments, indicating the time period, and the sampling location.

Experiment period 1 **(08 exp p1)**	Experiment period 2 **(08 exp p2)**	Experiment period 3 **(08 exp p3)**	Experiment period 4 **(08 exp p4)**
20/03/08 – 26/06/08; Growth chamber	26/06/08 – 29/07/08 Growth chamber (drought stress)	29/07/08 – 04/09/08 Growth chamber	04/09/08 – 21/11/08 Growth chamber

The first experimental period in 2007 represents the preliminary test phase. During this period, beech tree number 1 was used, together with branch cuvette 1 (cuv1) and an empty reference cuvette (cuv ref). During period 07 exp p2, a new beech tree (number 2) was used together with cuv1 and cuv ref. This time, cuv1 contained a horizontal grid to keep the leaves in horizontal position and to avoid mutual shading during the measurements (see Figure 8). During period 3 a second cuvette (cuv2) was installed on the same tree (number 2). This was done on 19/09/07.

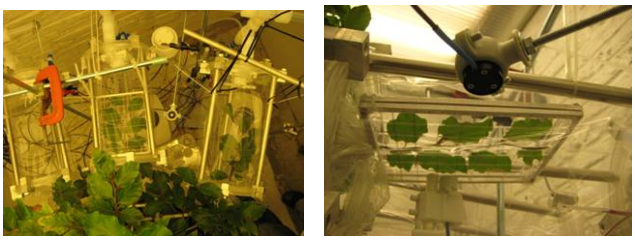


Figure 8: left: view of branches enclosed in the 2 measuring cuvettes and of the empty reference, right: detail of the grid for horizontal and non-overlapping positioning of leaves.

In the first experimental period (07 exp p1) four light steps were chosen in the growth chamber. From experimental period 2 on, eight different light intensities were selected, including complete darkness. The light intensity was varied every hour in ascending order starting from 8 h in the morning reaching maximum at 14-16 h. From 16 h on the light intensity was decreased until 22 h, when darkness started (Figure 9). In 07 exp p2 and 07 exp p3 the following temperatures in the growth room were used: 15, 17, 21 (reference), 23, 25 and 27°C.

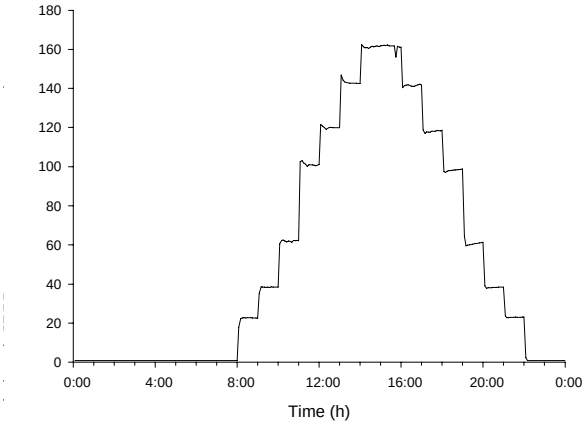


Figure 9: Stepwise variation of PPFD in the growth room simulating the natural daylight in ascending and descending order.

During the summer of 2008 an experimental period was scheduled (08 exp p2), during which one of two beech trees was subjected to drought stress. During this experimental period only one light step was selected (complete darkness between 20h and 8h, maximum PPFD (300 µmol-photons m^{-2} s^{-1}) between 8h and 20h) and the temperature in the growth room was set at 21°C. The drought stress was initiated on July 4th 2008 and the stressed beech tree was rewatered on July 22nd 2008.

Response of BVOC emissions to variations of PPFD at fixed temperature conditions

To see the relationship between BVOC emission and PPFD, samples were taken at different PPFD levels during the chosen days. The curve obtained is shown in Figure 10 with the conditions of air temperature equal to 27 °C and PPFD in the range from 0 to 150 µmol-photons m^{-2} s^{-1} (data for 31-08-2007).

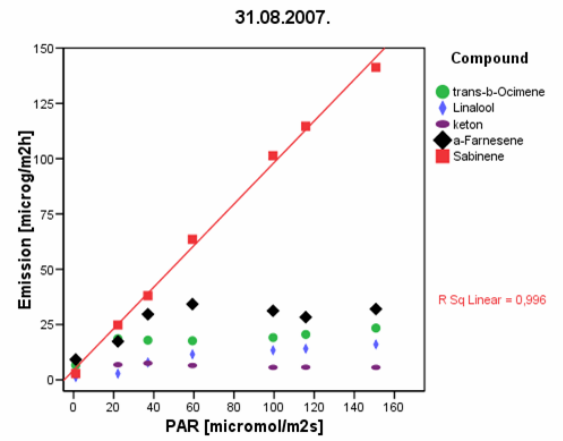


Figure 10. Light response for BVOC emission (different compounds) at 27°C leaf temperature and different PPFD values.

Six compounds were found to be emitted, mostly monoterpenes and one sesquiterpene, identified as α-farnesene. The highest emission is related to sabinene as it was expected from previous results. For sabinene a linear correlation can be seen on the graph with a coefficient of determination R^2=0.996, which is not the case for other terpenes. From full day measurements a difference in emissions, with lower amount in the mornings, was obtained at same conditions as in the afternoons. This effect was observed by Partner P3 as well.

Response of BVOC emissions to temperature variations at constant daily PPFD pattern

Temperature dependence of BVOC emission was performed and compared at the same (maximum) PPFD-level, where a linear correlation is found for sabinene, but not for the other terpenes. When all the available and comparable data were put to one graph (Figure 11), two outliner points were found. The outliners are the last samples that have been taken, which might be explained by senescence.

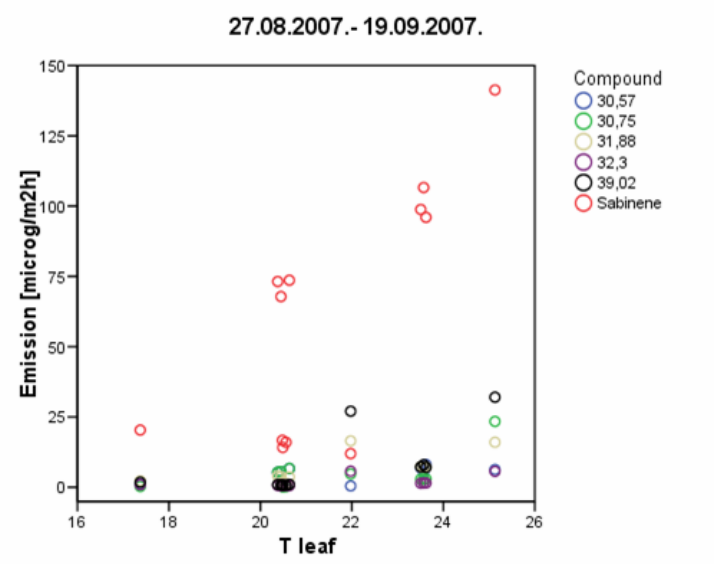


Figure 11: Temperature response for BVOC emission (different compounds) at constant PPFD value

2.4 Data analysis

General BVOC emissions patterns

Ion signals related to monoterpenoid (m/z 81 and 137), isoprene (m/z 69) and acetone (m/z 59) emissions have been measured continuously with the PTR-MS during the five experimental periods in 2007. The data show that beech (*Fagus sylvatica* L.) is a low isoprene emitter and a rather strong monoterpenoid emitter. Therefore data-analysis mainly focused on monoterpenoid emissions.

As an example, the monoterpenoid emission rates, calculated based on the m/z 137 PTR-MS ion signal, are shown in the lower panel of Figure 12 for cuvette 1 during period 07 exp p2 and period 07 exp p3. During these periods the beech trees were subjected to a constant daily PPFD pattern (see Figure 9). The variation of the leaf temperature during these experimental periods (as a consequence of the different settings of the growth room temperature) is shown in the upper panel of Figure 12.

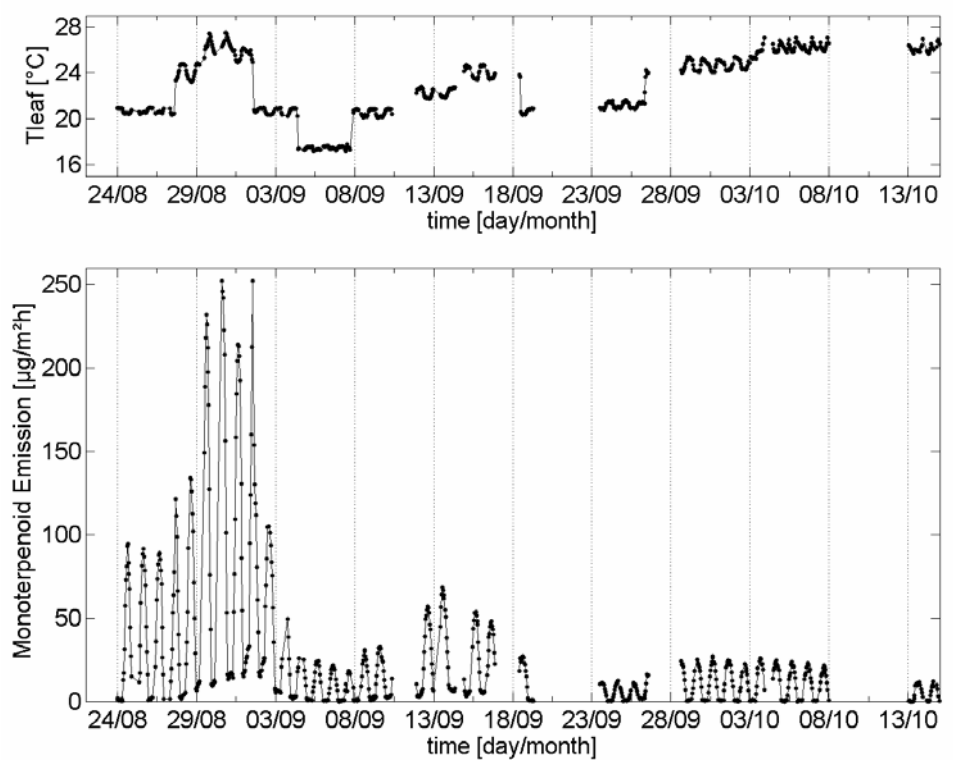


Figure 12: Variation of the leaf temperature during exp p2 and exp p3 2007 (upper panel) and monoterpenoid emission rates (m/z 137) (lower panel).

On several days a (sometimes huge) transient emission was observed at m/z 81 in the evening when the lights in the growth chamber went off (Figure 13).

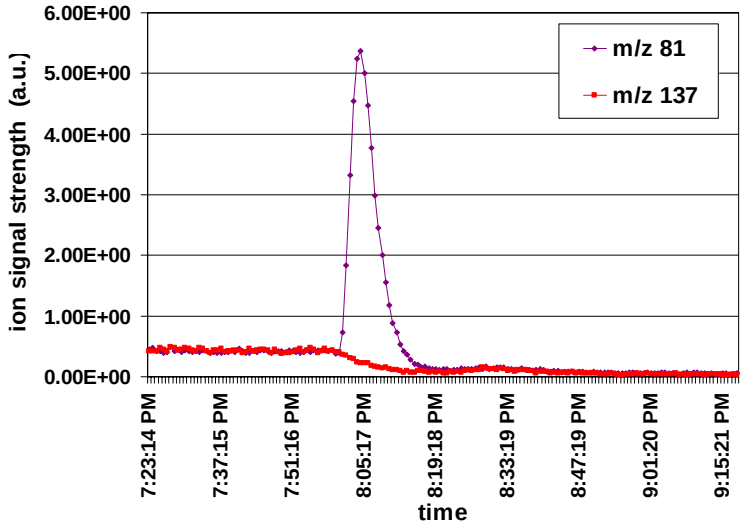


Figure 13: Observed transient phenomenon of the PTR-MS signal at m/z 81 when the lights in the growth chamber were switched off (8 August 2007).

This phenomenon has recently been reported in the literature for poplar trees (Graus et al. 2004). The emissions at m/z 81 have been attributed to hexenals, and they are accompanied by transient emissions of acetaldehyde, hexenols and hexenyl acetates. By monitoring the PTRMS ion signals at masses which are attributed to these species, we were able to follow the time evolution of these compounds. Since the biochemical origin of the light-dark transition phenomenon is not yet fully elucidated (Loreto et al. 2006), further analysis of the prevalence and of the intensity of the transient peaks as a function of environmental parameters might reveal some information about possible driving factors.

Leaf infection by mites

During the growth chamber experiment (PPFD, temperature controlled) of 2008 (08 exp p1-p4), 33 samples have been analyzed by TD-GC-MS. 16 compounds were detected, including 10 monoterpenes (MT), 2 oxygenated-MTs, 2 sesquiterpenes (SQT), isoprene and methyl salicylate. Sabinene showed the highest emission, in an agreement with previous studies (Moukhtar et al. 2005; Holzke et al. 2006). Quantifiable emission appeared 21 days after budburst, and reached the highest level at the beginning of summer. MT emissions showed a clear trend in following each other. As an illustration the trend of sabinene and limonene emission is presented (Figure 15).

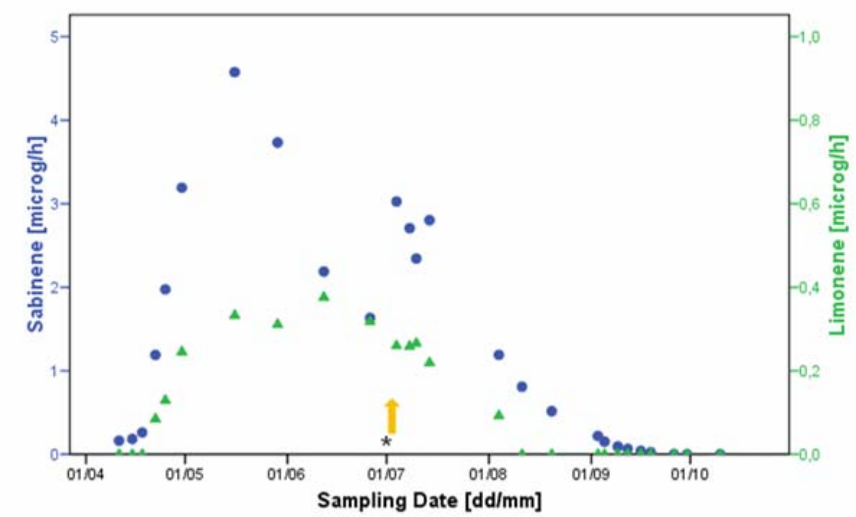


Figure 15: Variation of sabinene and limonene mass flows over a total vegetation period. Experiments were perfomed in the growth chamber and lasted from March to November 2008. PPFD was kept constant from 8 am till 8 pm and temperature was set at 21°C. * Jump in the emission is due to the installation of a new lamp with increased light intensity.

In the middle of autumn phytophaga infection was observed on the tree induced by two-spotted mite (*Tetranychus urticae*). New compounds appeared as a result of infection (linalool, methyl salicylate, (E,E)-α-farnesene, unknown oxygenated-MT, unknown SQT) and became dominant over sabinene, explained by the low MT emissions at this time of the year.

These observations point at the importance of further investigation of BVOC emissions (especially SQTs and oxygenated-MTs) and the need for a proper quantification system of these compounds. A peer-reviewed paper on these experimental results has been submitted (Joó et al. "Variation in biogenic volatile organic compound emission pattern of *Fagus sylvatica* L. due to aphid infection").

Impact of air temperature variation

The impact of temperature variation was studied in the period 07 exp p2. Temperature was constant during the day, and varied from 17-27°C between days. The daylength in the growth room was 14 h, representing the mean daylength of June/September months. Moreover, this intermediate photoperiod was optimal for obtaining vigorous vegetative growth. The transition days (when temperature was changed) were not taken into account in the analysis, unless specified. Days with electricity problems or

instrumentation problems were removed from the analysis. The temperature variation applied was random. A temperature of 21°C was taken as reference temperature, because a beech temperature optimum of net CO_2 assimilation was found to be around 21°C in previous experiments.
Mean diurnal patterns for each temperature were calculated for photosynthesis and monoterpenoid emissions. Pronounced diurnal dynamics were observed (Figure 16 a, b). A clear link was observed between temperature variation and the monoterpenoid emissions. When the tree was exposed to higher temperatures (up to 27°C), monoterpenoid mixing ratio was higher. At 27°C emissions reached a peak value of 257 µg m^{-2} h^{-1}. Thus, the monoterpenoid flux was enhanced almost 5 times higher during the period of higher temperature exposure compared to the reference temperature of 21°C. Monoterpenoids did not exhibit a temperature optimum (within the range of 17-27 °C). The response of monoterpenoid emissions was dramatically attenuated when exposing the tree to lower temperatures. At temperatures of 17°C, peak emissions obtained were only 24 µg m^{-2} h^{-1}. However, the net photosynthesis appeared not to be affected by the temperature range used in the experiment. This indicated optimal conditions for photosynthesis. The net CO_2 assimilation maximum ranged from 5.22 to 6.26 µmol m^{-2} s^{-1}, between 14 and 16 h when intensity was highest. The negative values of the CO_2 assimilation during darkness indicated dark respiration.

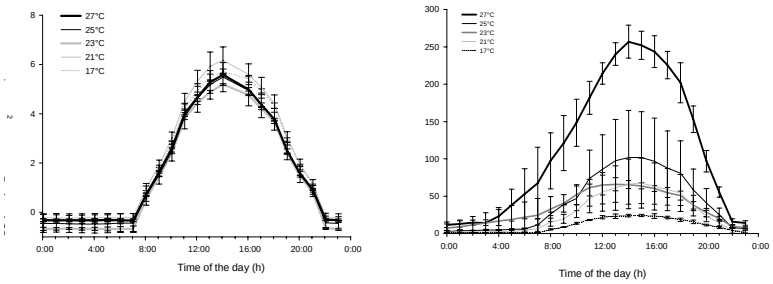


Figure 16. (a) Mean diurnal patterns of net photosynthesis for each imposed temperature. Negative values represent the night-time dark respiration. (b) Mean diurnal patterns of monoterpenoids for each imposed temperature. Low night-time emissions were observed.

Net photosynthesis rates and monoterpenoid emissions showed pronounced diurnal dynamics as the lights were gradually switched on/off. The fraction of assimilated C re-emitted back to atmosphere through monoterpenoid emission was represented by the C ratio between PTR-MS total emitted monoterpenoids and the net photosynthesis. The results revealed that the potted beech tree under well-watered conditions re-emitted a rather low fraction of the assimilated carbon back into the atmosphere as total monoterpenoids. This fraction increased from 0.01 to 0.10 % with a temperature rise from 17°C to 27°C in growth room conditions (Figure 17). This MT/Pn ratio clearly showed an exponential increase with increasing temperatures. We obtained the following relationship: y = 0.003 * exp (0.2149 * x). The coefficient of variation (R^2) was 0.8749 (P-value < 0.01).

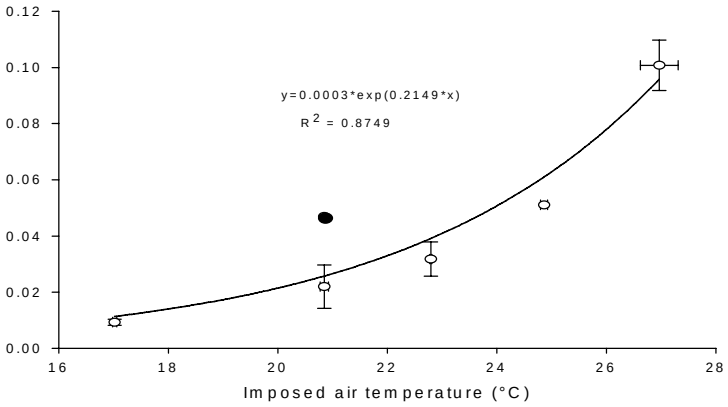


Figure 17. Exponential relationship of the total monoterpenoid/net photosynthesis (MT/Pn) ratio in function of temperature. Temperature transition days are not included. The full black circle represents the ratio measured the day after transition from 27 to 21°C. This day is not included in the mean value for 21°C.

The full black dot in Figure 17 represents the day after temperature was changed from 27 to 21°C. The beech leaves needed a 1-day adaptation time and showed a higher calculated ratio compared to other days of 21°C. Therefore, this day was considered as an outlier, and was not included in the calculation of the mean value. A peer-reviewed paper on these experimental results is in preparation (Šimpraga et al. "Leaf-level temperature response of BVOC emissions and net photosynthesis of a young beech tree (*Fagus sylvatica* L.)").

Drought stress experiment

Up till now it is known that BVOC emissions are driven by light and temperature. Regarding drought effects, literature is conflicting. Some authors indicate that drought stress enhances (Sharkey et al. 1993), while others say it reduces the emissions (Bertin et al. 1996). As drought is worldwide the most significant limiting factor for plant growth, a combination of plant metabolic processes and abiotic drought stress was investigated. Bio-sensors were used to yield extra information on the physiological status of the model trees. The objectives were (1) to investigate the effect of drought stress on BVOC emissions and (2) to relate the BVOC emissions to some main ecophysiological plant processes (i.e. net photosynthesis, internal water transport, stem diameter variations). The results are shown in Figure 18. From the results we can see that monoterpenoid emission was linked with tree physiology; more specific with leaf net photosynthesis rate, with stem diameter growth and with sap flux density as well as an interdependence between leaf and tree plant processes was observed. Imposed severe drought caused Pn and MT emissions to decrease. Upon Pn inhibition, the emission of MT is inhibited most likely due to the photosynthetic origin of the MT.

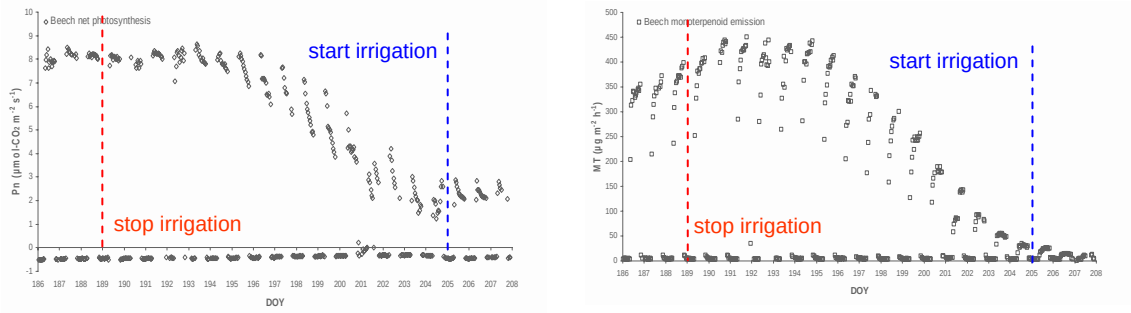


Figure 18: Leaf-level processes measured on beech during the drought stress experiment: photosynthesis (left) and monoterpenoid emissions (right)

Severe drought stress limited and had a negative effect on the overall tree physiology as well as on the monoterpenoid emissions. Stem diameter growth indicated a decrease in stem

growth, while sap flux density almost completely stopped (Figure 19). After re-watering a sharp increase in stem diameter growth was observed followed by a slight increase in sap flux density. After re-watering MT emissions did not recover, while Pn slightly recovered. In the end, one can conclude that drought stress limitation on these processes might become significant in changing global climate conditions.

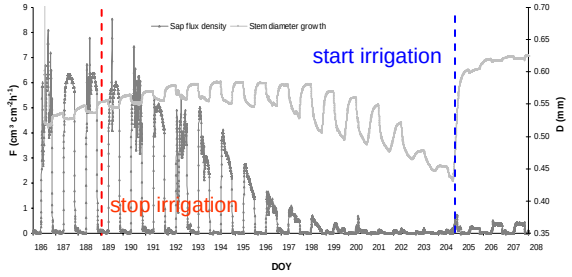


Figure 19. Tree-level processes (sap flow, stem diameter variation and leaf water potential) during the drought stress experiment showing decrease in tree processes and recovery upon re-watering

A peer-reviewed paper on the experimental results is currently in preparation (Šimpraga et al. "Drought stress effects on the link between BVOC emissions and photosynthesis rates"). A repetition of this experiment will be perfomed in the August 2009 (phase 2).

WP3 Canopy experiment

3.1 Branch cuvette

The branch cuvettes that were used for the canopy experiment in the Aelmoeseneie forest are of similar design as the ones used in the growth chamber experiments, except that they are somewhat larger. Most components of these cuvettes have been manufactured by the mechanical workshop of Partner P3. The plexi glass base plates and three support rings were manufactured by a specialized company. The robustness of the cuvettes in real outdoor conditions and of the experimental set-up has been tested in December 2007 before the start of the measurement campaign in the Aelmoeseneie forest (paragraph 3.2).

3.2 Experimental set-up gas exchange

The canopy experiment was conducted in 2 large measurement campaigns (Table 3).

Table 3: 2008 experimental scheme of IMPECVOC 2008 campaigns in the Aelmoeseneie experimental forest.

Experiment campaign 1 (08 exp c1)	Experiment campaign 2 (08 exp c2)
26/05/08 – 26/06/08	04/08/08 – 01/12/08

In order to be ready for the measurements in the forest at the beginning of the 2008 growing season, a lot of preparatory activities were already performed in the fourth trimester of 2007 by Partner P3, such as the installation of insulated heated outlet lines (PFA) and several electrical cables from the third floor (22.5 m) of the measuring tower to the log cabin in the Aelmoeseneie forest, of connection boxes (for a pump, fans, flow meters, micrometeorological sensors), of an ADSL line (for remote communication with the instrumentation) and of an air-cooling unit in the log cabin. To avoid practical problems at a later stage (e.g. related to power consumption, temperature control in the log cabin, remote communication with the analytical instrumentation, heating of the outlet lines, robustness of the branch enclosure systems during bad weather conditions, …), performance tests of the complete experimental set-up under real outdoor conditions were performed in December 2007. During these tests a branch of a solitary potted coniferous Pseudotsuga menziezii [Mirb.] Franco tree, located on the third platform, was enclosed in a prototype 27 litre cuvette, manufactured in the mechanical workshop of BISA. A second identical, but empty, cuvette was installed close to the first one to determine possible background emissions. PTR-MS, IRGA and gas sampling for off-line GC-MS analysis were carried out simultaneously and no major technical problems were encountered.
During the first five months of 2008 the installation in the forest has been completed by Partner P3. From the measuring tower branches at different heights in the canopy of an 80 years old *Fagus sylvatica* L. tree are accessible. Six identical cuvettes have been installed: 2 branch cuvettes and 1 reference cuvette on the third platform (22,5 m), 1 branch cuvette and 1 reference cuvette on the second platform (15 m) and 1 branch cuvette on the first platform (7.5 m) (Figures 20 and 21). All cuvettes were equipped with a thermistor (to measure air temperature) and a relative humidity sensor. All branch cuvettes were also equipped with an IR-thermocouple (to measure leaf temperature) and PAR sensors (2 per cuvette on the third platform, 1 per cuvette on the other platforms). Two systems for VOC- and ozone-free air supply to the cuvettes were installed as well, identical to the one used in the growth chamber.
The IRGA of Partner P1 was coupled with the multiplexer of the PTR-MS analysis circuit in order to allow simultaneous measurements of net photosynthesis and transpiration rate.

Figure 20: Experimental set-up at the Aelmoeseneie tower on the 3rd and 2nd floor.

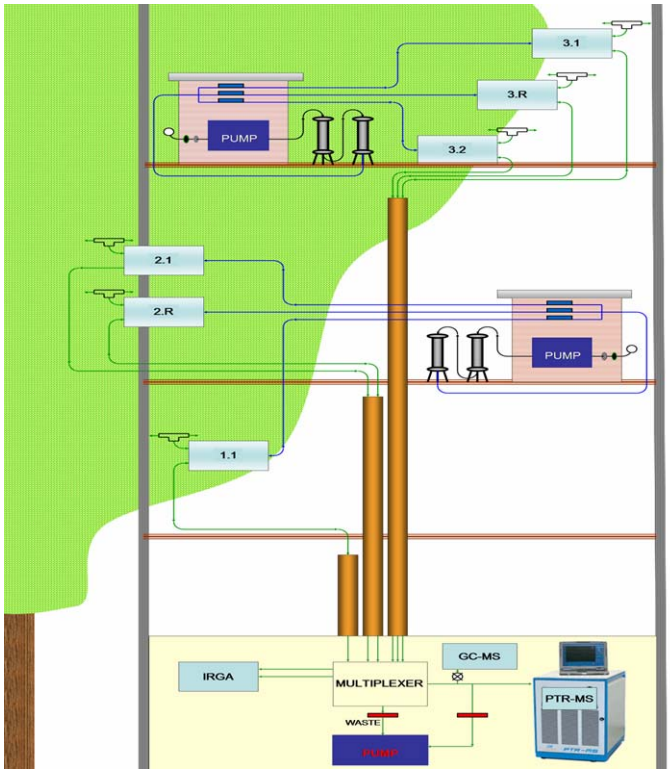


Figure 21: Schematic representation of the experimental set-up in the Aelmoeseneie forest.

In the Aelmoeseneie forest in 2008, CO_2 and H_2O gas fluxes from the canopy were performed by the characterisation of the different canopy layers *in situ* using two types of IRGAs (Figure 22 and 23): (1) stationary gas exchange measuring system IRGA ADC 2250, an NDIR analyzer (Non-Dispersive Infra-Red) operating in the differential mode under steady-state conditions and (2) portable gas exchange measuring system IRGA LI-6400 equipped with LED (Light Emitting Diodes) light source operating under set environmental conditions. Both systems' calibration were performed manually by passing known CO_2 concentration through both the reference cell and the analysis cell simultaneously.

The gas exchange was measured in the sun-layers and the shade-layers of the canopy including all 3 tower platforms. Data of stationary IRGA were continuously recorded at 1-min intervals. Leaves were measured at ambient CO_2 concentrations.

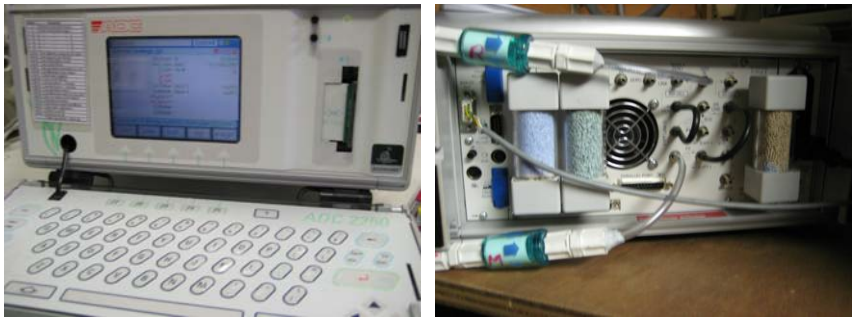


Figure 22: Stationary ADC 2250 IRGA to measure online photosynthesis and transpiration rates

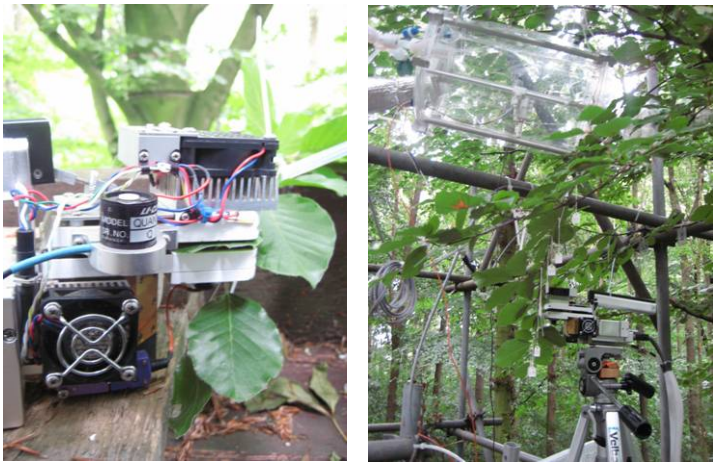


Figure 23: Portable LI-6400 IRGA in the Aelmoeseneie forest (9 July 2008) to measure offline photosynthesis and transpiration rates

3.3 Canopy measurements during different phenological phases

PTR-MS measurements were started on May 7th 2008 in the Aelmoeseneie forest and IRGA measurements on May 26th 2008. Continuous PTR-MS and IRGA measurements went on until June 26th 2008. On June 26th the PTR-MS was moved to the growth chambers to be included in the drought stress experiments. Continuous measurements in the Aelmoeseneie forest were resumed on August 8th until November 28th 2008. On a regular time scale samples for GC-MS analysis were collected by Partner P2. Measurements from the two branch cuvettes on the third platform (horizontal scan) should allow us to study the branch-to-branch intervariability of BVOC emissions and of CO_2 and H_2O vapour exchange at the same altitude level in the canopy of the adult beech tree. Alternate measurements from branch cuvettes at the different platforms (vertical scan) should result in information on the emissions as a function of the vertical position of the branches in the canopy (sunlit versus shaded leaves). Horizontal scans during one day have been alternated by vertical scans the next day. Results of the BVOC and CO_2 measurements are discussed under paragraph 3.4. Different methods for the leaf area evolutions are explained below.

Non-destructive leaf area measurements using LI-3000

Non-destructive leaf area measurements were performed in the growth room and in the Aelmoeseneie forest, respectively. Side branches were carefully chosen in order to represent the branches enclosed into the cuvettes. Leaf area of the side branches was quantified regularly (bi-weekly) by drawing the leaf circumferences on paper and by subsequent measurement of the cut paper areas of reference branches (Figure 24). This way the cuvettes could remain closed throughout the measurement campaign. The LI-3000 leaf area meter (Licor, Lincoln, NE, USA) coupled with the LI-3050A transparent belt conveyer was used for leaf area determination.

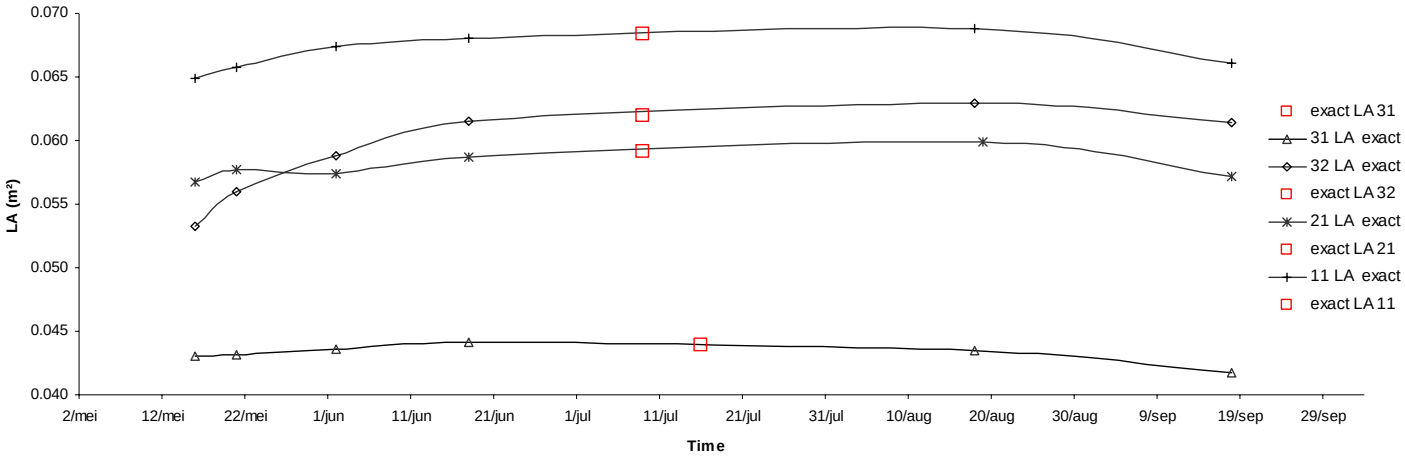


Figure 24: Beech tree leaf area evolution in the Aelmoeseneie forest experiment

Hemispherical photography

As leaf area is a key variable for regional and global models describing biosphere/atmosphere exchange of energy, CO_2, water vapour and other materials, a clear distinction has to be made between leaf area index (LAI) and leaf area development (see above). The first one considers a dimensionless variable and the latter one is a non-dimensionless variable. LAI seasonal dynamics can be evaluated from light penetration measurements. Vegetation LAI depends on species composition, development stage and seasonality (Jonckheere et al. 2004). The hemispherical data collection used in this project consisted of: (1) image acquisition and (b) image analysis. Five different methods were assessed: (1) LAI(Bonhom)-Lin (cal at 57.5°); (2) LAI(2000)-Lin (5 rings with 148° FOV); (3) LAI(2000G)-Lin (zenith rings as sky grid setup); (4) LAI(sphere)-Lin (spherical leaf angle distribution); (5) LAI(ellips)-Lin (zenith rings as sky grid setup). Preliminary data for one sampling point in the experimental forest Aelmoeseneie are presented in Figure 25. Further analysis is still ongoing.

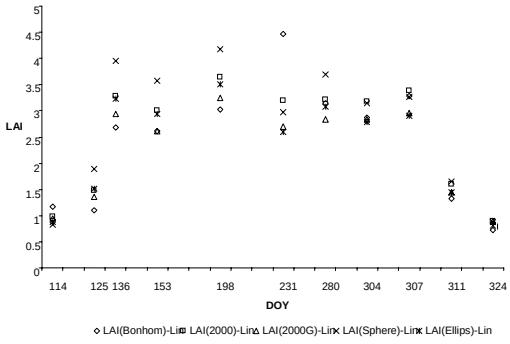


Figure 25: Leaf area index (LAI) evolution in the forest experiment using five different algorithms

Specific leaf area

During the different experiments, leaf samples were collected randomly from the trees in the growth room and in the forest Aelmoeseneie on the three platforms. Leaf samples were collected and when sampled in the forest enclosed into aluminium foil and immediately transported to the laboratory of Partner P1. Petioles were removed prior to the measurements (Vande Walle 2007) and the individual leaf area was measured as described above. At the end of experiment, destructive measurements were performed in order to determine leaf fresh weight. After the leaves were dried at 50°C until constant weight, leaf dry weight was determined (Vande Walle 2007). Specific leaf area (SLA) was calculated. SLA is an important parameter because it describes the allocation of leaf biomass per unit leaf area (results available but not presented here).

3.4 Data analysis

Ion signals related to monoterpenoids (m/z 81 and 137), isoprene (m/z 69), acetone (m/z 59), acetaldehyde (m/z 45), methanol (m/z 33) and water (m/z 39) have been measured continuously with the PTR-MS during the measurement campaign in the forest Aelmoeseneie. The huge amount of PTR-MS measurements and of micrometeorological data has been stored by Partner P3 in an extensive database, which is accessible for the different partners in the project. Quality check of the stored data is still ongoing (75% processed). As an example of the data analysis, the calculated monoterpenoid emission rates for cuvette 3.1 (third platform, sunlit leaves) are shown in Figure 26 for the months August and September 2008.

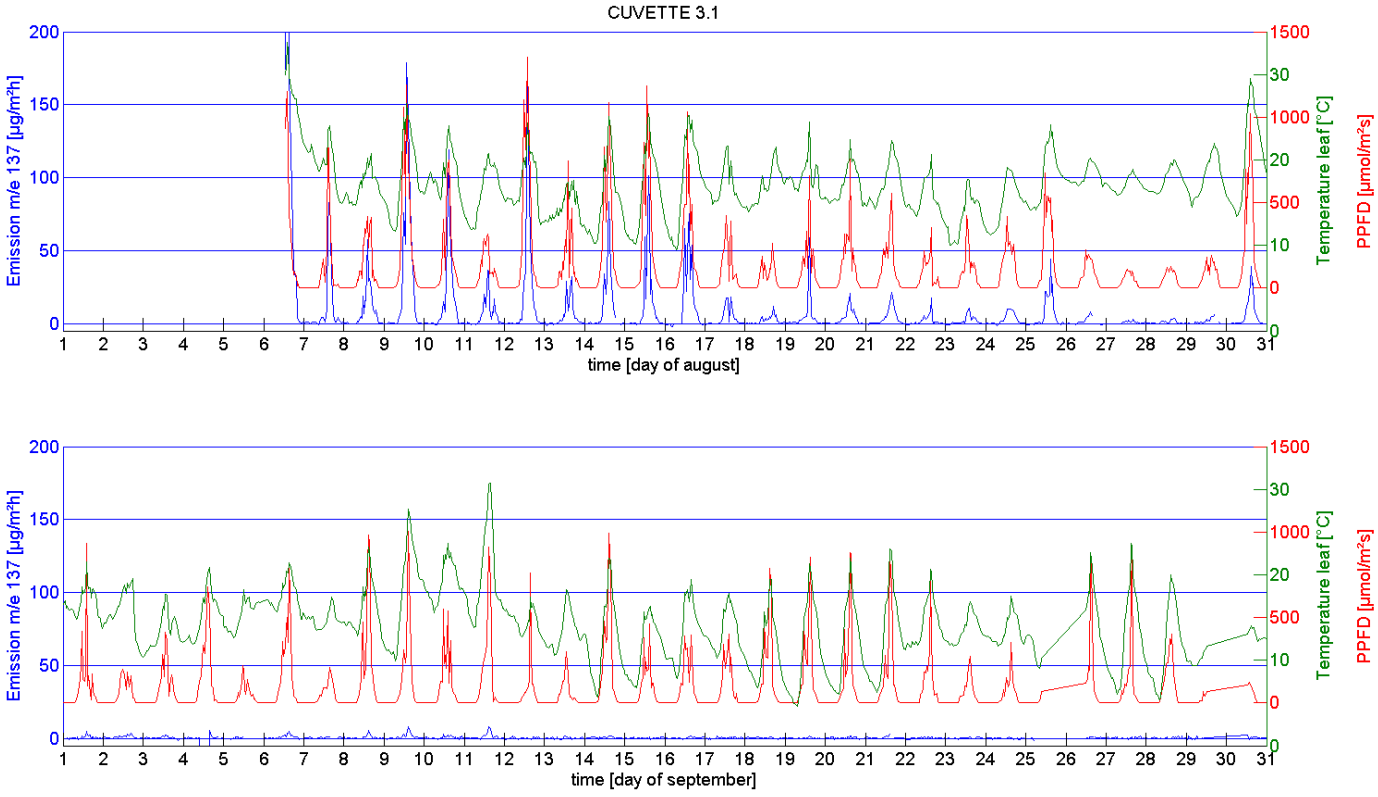


Figure 26: Blue: calculated monoterpenoid emission rates for a sunlit branch at a height of 25 m in the canopy of an 80 years old beech tree during the months August and September 2008. Red: the variation in PPFD. Green: the variation of the leaf temperature during these months.

Preliminary results of the analysis of the horizontal scan are shown under WP5 (integration). The analysis of the vertical scan is still ungoing. Nevertheless below some preliminary results are shown for the vertical scan.
It is known that average irradiance deceases exponentially through the plant canopy, with the extent of light attenuation depending on both the amount and the arrangement of leaves (Monsi & Saeki, 1953). In order to prove this, we performed on the three different tower platforms (pl3, pl2 and pl1) light response curves with a LI-6400 photosynthesis system. An average of two different leaves of the same platform is presented. Data clearly show that there is a difference between sunlit leaves and shade-adapted leaves depending on the platform. The 3rd platform that receives the most light intensity, clearly showed the highest net photosynthesis (Figure 27), while intermediary examined platform (2nd) showed intermediary values. The lowest platform indicated the lowest photosynthetic activity of the examined leaves.

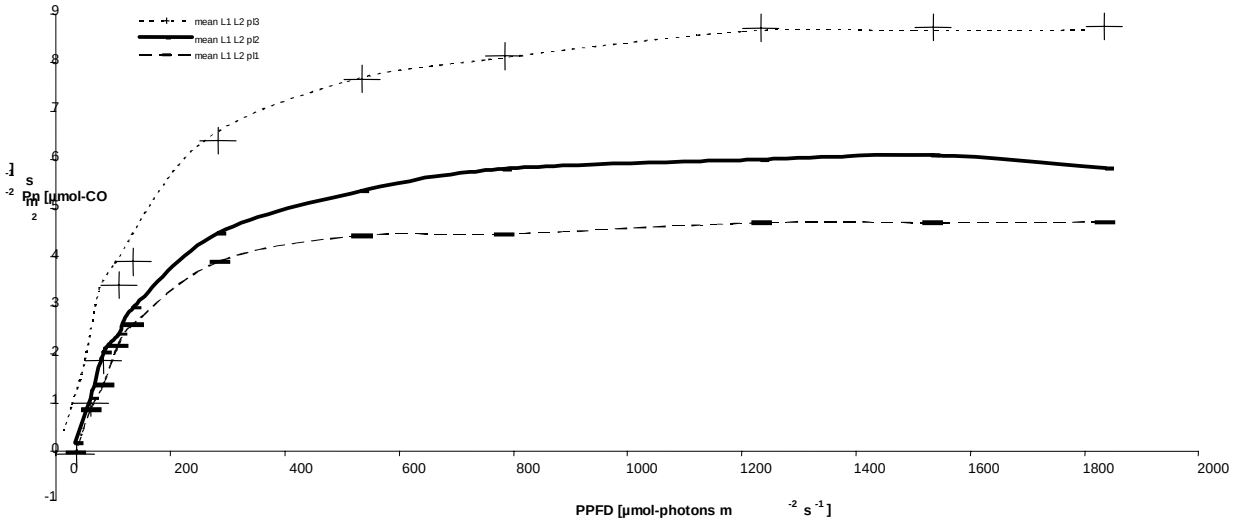


Figure 27: Light response curves of an adult beech tree measured on the three platforms of the tower in the experimental forest Aelmoeseneie showing a clear differences between sun and shade adapted leaves.

WP4 Stand level experiment in Vielsalm

4.1 Preparation experimental site

This workpackage has been shifted to the beginning of phase 2 of the project, due to the delay of the installation of the new measuring tower in the Vielsalm forest which is still going on.
The operational infrastructure needed at the Vielsalm forest site includes a meteorological tower fully equipped with adequate sensors, and an equipped shelter. The existing set-up had to be strongly updated for BVOC measurements.

Tower
The boundary layer theory specifies that, in the first hundred meters of the atmosphere the size of eddies (which are the main responsible transport entities) increases with height. As a consequence, the concentration fluctuation frequencies measured by an eddy covariance system are higher close to the surface. The tower height of the Vielsalm site was chosen in order to comply with these constraints and the IRGA (CO_2 and H_2O analyser) performances. As the PTR-MS analyser is characterized by a lower acquisition frequency, this constraint is no more satisfied and it is expected that the loss of signal in the high frequency range will be too important to allow reliable BVOC measurements. In order to solve this problem, it is necessary to increase the tower height. One consequence will be an enlargement of the system footprint. However, this problem is not critical as the fetch (distance between the tower and the forest edge) in the two dominant wind directions (SW and NE, respectively) is large enough to ensure that the footprint will still be inside the target ecosystem. The present tower has thus be replaced by a new one of 50m height using funding obtained from Belgian national agency (FNRS) (Figure 28).

Shelter and basic equipment
A new and bigger shelter has been built and equipped close to this tower to allow the hosting of the PTRMS analyser in the beginning of phase II. The installation of additional power lines and communication tools were necessary to upgrade the remote control of the instruments which is crucial for the continuity of the BVOC measurements.

Sensors
The tower has been re-equipped with an extensive set of meteorological sensors. We took this opportunity to upgrade the whole site installation that was running continuously since 12 years. Additional sensors have also been installed in the frame of other projects. Dry and wet nitrogen deposition measurements are now performed and an NDVI sensor has been installed on the top of the tower that allows a qualitative monitoring of the state of vegetation. Sapflow measurements together with profiles of soil moisture and litter fall bags will also start during this summer 2009. All these devices will allow better analysing the responses of BVOC fluxes to climate, season and phenology.

Tubing
The tubing for BVOC as well as for CO_2 and H_2O sampling above the forest have been recently installed in insulated and heated protections. Beforehand, the computation of the aerodynamic requirements have been performed in order to select optimal system dimensions (pumping rate, tube size and filter characteristics) in taking PTR-MS constraints (inlet pressure, response time) into account. This was made in collaboration with Partner P3.

Figure 28: Left: the new shelter and the bottom of the new tower. Right: the new tower equipped with tubing for BVOC sampling at the top of the tower (orange tube).

4.2 Preliminary tests

Operational coupling of PTR-MS to eddy-covariance set-up

Computation of eddy covariance flux requires treatment of several hundred thousands data per half hour. In the absence of ready-to-use logging system, an interface and a logging software that allows data acquisition, storage and treatment has been realized. The software collects and synchronises high frequency datastreams coming from the sonic anemometer and the PTR-MS and performs the flux computation. We decided to log the the datastreams from the two instruments on a single computer. This logging strategy presents three major advantages. (i) a single computer being used, perfect synchronisation between the two datastreams is guaranteed, this point being crucial for the flux computation. (ii) the EC-BVOC logging system is completely independent of the traditional CO_2-H_2O eddy-covariance and of the proprietary logging system of the PTR-MS, allowing a better flexibility. (iii) this flexibility will allow handling complex PTR-MS sequences in avoiding complicated post-processing of data, the number of scanned masses and the integration time for each mass being taken as parameters of the logging system.

A logging test in real conditions was realized in early June 2008 in situ at the Aelmoeseneie site (Figure 29). The aims of this test were (1) to assess the quality of our acquisition system and (2) to obtain real raw data to test the data treatment tools that are under development.

The sampling sequence of the PTR-MS is given in Table 4 giving a total cycling time of 1.8 s. This sequence and the choice of compounds is probably very close to what will be done routinely in Vielsalm during the phase II of the project. A sonic anemometer (Young 81000) was installed above the canopy, at the top of the tower (35 m) and was run with a 16 Hz sampling frequency. A PTR-MS sampling line (PFA tubing of 6.4 mm inner diameter) was extended from the third floor and air was drawn to the PTR-MS with a 9 l min^{-1} flow rate from an inlet placed close to the sensing volume of the sonic anemometer. The system was run during a whole hot and sunny day, giving eleven half-hour of usable synchronised raw data.

Figure 29: Left: sonic anemometer installed at the Aelmoeseneie forest. Right: logging of EC-BVOC data.

Table 4: Description of the PTR-MS sequence during the eddy-covariance test on 09/06/2008.

Compound	Production mass [amu]	Dwell time [s]	Calibration factor [ncps/ppbv]
H_30^+ Primary ion	21	0.23	-
Oxygen	32	0.23	-
Water cluster	39	0.23	-
Acetaldehyde	45	0.23	-
Acetone	59	0.23	19.098
Isoprene	69	0.23	6.5998
Monoterpene fragment	81	0.23	5.6694
Sum of monoterpenes	137	0.23	3.95

Fluxes and mixing ratios of compounds for which calibration factors were available are given in Figure 30. The error bars represent the detection limits computed, following Spirig et al. (2005), as the standard deviation of the covariance function in a time-lag window far away from the true lag. This quantity corresponds to the lag-independent noise in the covariance function. Despite important detection limit ranges, an obvious dependence of the fluxes on PPFD was found for isoprene and monoterpenes indicating reliability in the data acquisition and the flux computation.

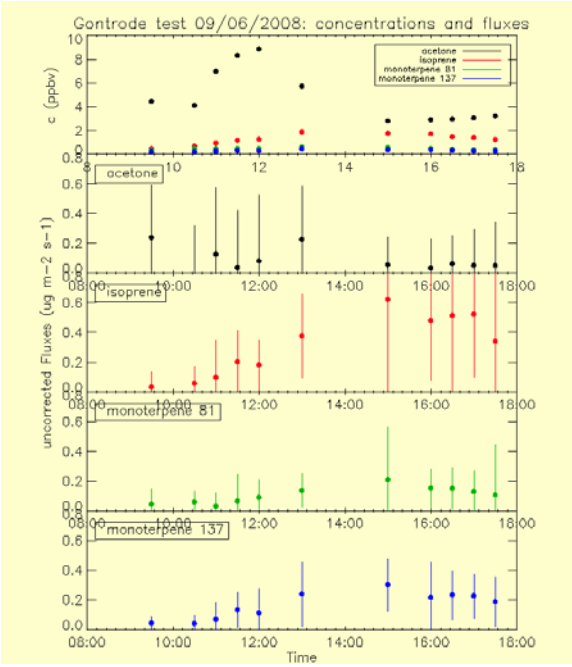


Figure 30: (a) BVOCs mixing ratios as determined from PTR-MS measurements. (b-c-d-e) Fluxes of BVOCs. These fluxes are not corrected for high-frequency damping. Error bars are the precision of individual flux measurements.

Methodology of flux computation

An extensive bibliographic research on eddy-covariance BVOC measurements has been made and contacts with experts have been established in order to identify the methodological problems specific to eddy covariance measurements of BVOC fluxes and to consider the solutions that have been brought.

In particular, the method called "virtual disjunct eddy covariance" (vDEC) was identified as the most appropriate to BVOC fluxes. Rather than using high frequency concentration and velocity measurements as EC (around 10Hz), this method sub-samples the data series at lower frequency, pairing-up each concentration measurement with the associated wind measurement. This allows working with lower frequency analysers and, in the case of BVOCs, to scan a larger spectrum of components at the price of a limited information alteration.

However, the flux quality alteration depends on the spectral content of the turbulence which can be site-specific. In addition, different vDEC procedures has been proposed in the literature. We thus developed a data analysis in order to test the impact of disjunct analysis on the Vielsalm data. To this end, the existing time series obtained with conventional EC for CO_2/H_2O were used and artificially resampled in order to simulate the effect of a vDEC treatment (Figure 31). Comparison with these results and original EC fluxes is used in order to optimise the acquisition software in finding the best compromise between the quality of the flux and the number of masses that are scanned. Results shown in Figure 32 suggest almost no alteration of the fluxes for a disjunct time interval of 1 s (1 Hz), this time interval being representative for a scan on 5 masses, a situation that seems realistic for a long-term run of EC-BVOC above a forest. This situation will even improve due to the increase in measurement height for the real campaign in 2009, allowing the possibility to increase the number of compounds studied.

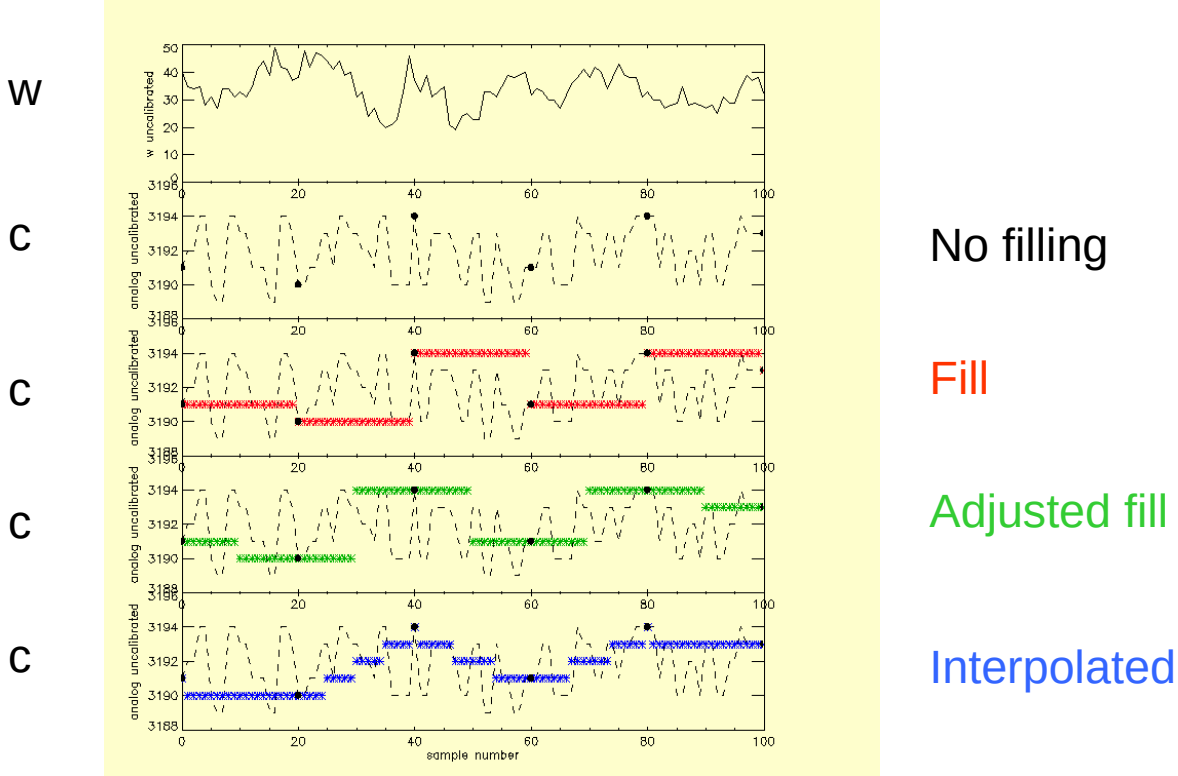


Figure 31: Case example of high frequency CO_2 eddy-covariance data (solid black for vertical wind speed component and dashed black for CO_2 concentration) together with different artificial sub-sampling procedures.

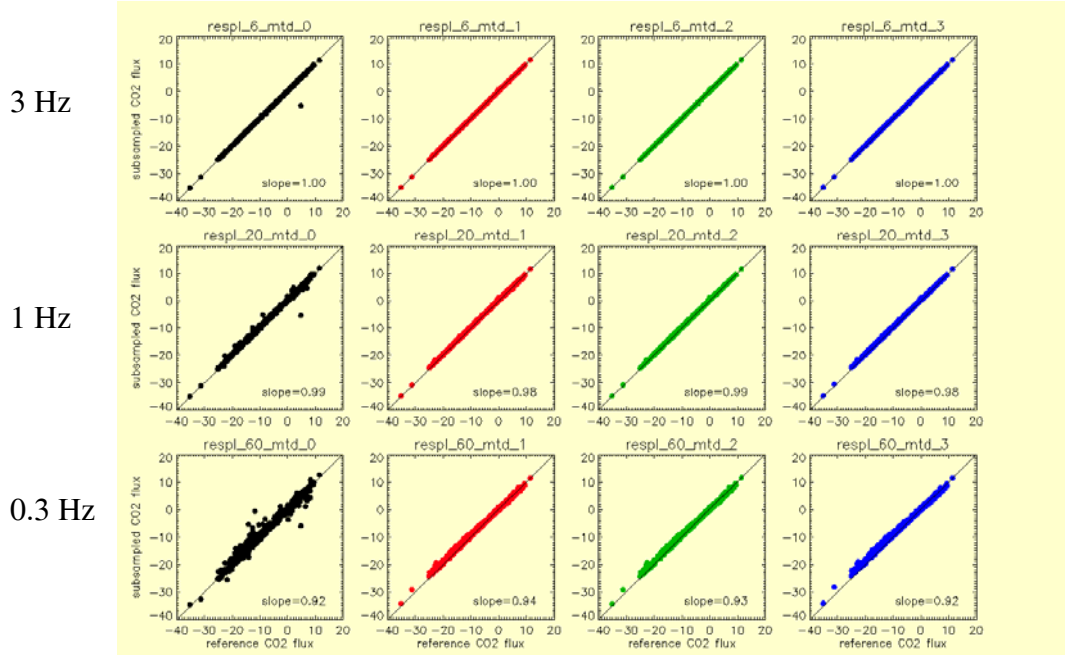


Figure 32: Comparison of the CO_2 flux, subsampled with different procedures, to the reference CO_2 flux computed with the non-subsampled high frequency dataset. This test is performed for three subsampling frequencies.

Another major point for accurate estimation of BVOC fluxes is to be able to correct for damping of fluctuations in the sampling tube. Indeed, a part of the turbulent signal is lost during the transport of

the air from the sampling point to the analyser due to air mixing in the filters and the tube. This part of the signal can be recovered through application of correction factors deduced from spectral analysis. Partner P4 has investigated this problem for water vapour using data from Vielsalm (Deligne et al. "New transfer functions for correcting turbulent water vapour fluxes" submitted to Boundary-Layer Meteorology) and proposed new transfer functions to deal with the damping of fluctuations. This study will be useful for BVOC flux measurements because the same methodology can be applied to derive correction factors for vDEC of BVOCs. In addition, a possible way to assess the reliability of the vDEC PTR-MS derived fluxes is to compare this technique with traditional EC on a common gas. Water vapour is a good candidate because both analysers can measure this gas. So that accurate measurements of water vapour fluxes with traditional EC can be very useful for BVOC flux estimations.

WP5 Integration

5.1 BVOC emission leaf/branch

The results of the canopy experiment in the forest Aelmoeseneie were split into (1) long-term (entire measurement campaign) and (2) short-term (diurnal patterns) results. From the emitted BVOC compunds only the monoterpenoids were studied in detail. Online long-term results indicated that net photosynthesis, PPFD and leaf temperature alone could not explain the higher monoterpenoid emissions observed for the shade adapted leaves. Using offline measurements Partner P1 found that two types of leaves were selected on the third platform: sun adapted and shade adapted leaves. Both types of leaves have different physiological characteristics and were exposed to different radiation and temperature regimes.
Short-term results indicated that shade adapted leaves for the sunny day showed a stronger interaction between monoterpenoid emissions and photosynthesis than for the sun adapted leaves. During the cloudy day this interaction was even stronger for the shade adapted leaves. It can therefore be stated that the physiological leaf status plays a major role in explaining how monoterpenoid emissions are linked to net photosynthesis and transpiration rates. The importance of the physiological status of the leaves should hence be emphasized more in future research, willing to explain BVOC emissions.
An example of a sunny day (30/08/08) is presented in Figure 33. On this day, leaves from the branch enclosed in cuvette 2 (lower cuvette on platform 3) assimilated less CO_2 (Pn max = 5 µmol m^{-2} s^{-1}) in comparison with the leaves in cuvette 1 (Pn max = 6 µmol m^{-2} s^{-1}). On the contrary, monoterpenoid emissions were much higher for the leaves in cuvette 2 (MT = 650 µg m^{-2} h^{-1}) in comparison with the leaves in cuvette 1 (MT = 50 µg m^{-2} h^{-1}). In general one can say that the sunny day measurements and all prevailing conditions (higher temperature, higher PPFD and higher Pn) are more advantageous for cuvette 1 in order to synthesise higher amounts of monoterpenoids and emit them into the atmosphere. But the monoterpenoids emissions from these sun adapted leaves remained lower than for the shade adapted leaves. This example demonstrates that it is thus of utmost importance to consider also the leaf physiology to better understand the story behind BVOC emissions.

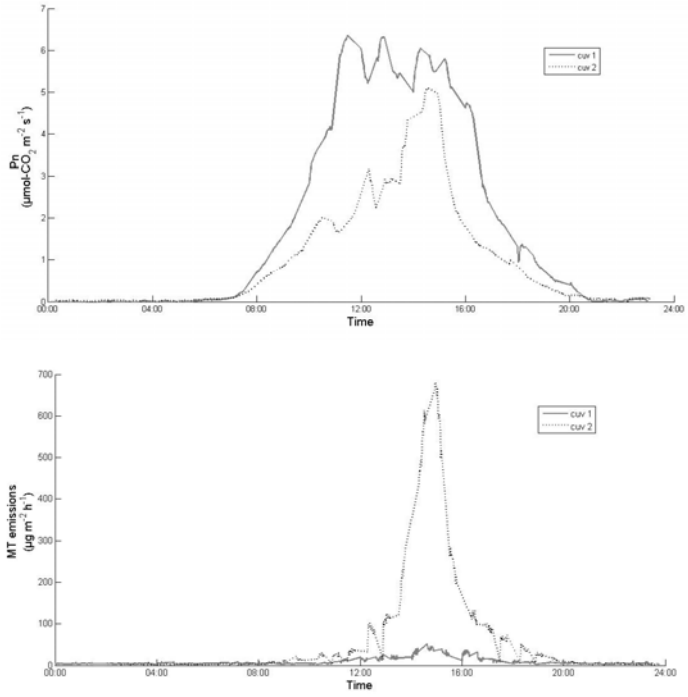


Figure 33: (a) Diurnal patterns of net photosynthesis (Pn) for cuvette 1 and cuvette 2 on 30/08/2008. Negative values represent night-time dark respiration. (b) Diurnal patterns of total monoterpenoid emission (MT) for cuvette 1 and cuvette 2 on 30/08/2008.

Monoterpenoid compounds emitted in cuvette 1 and cuvette 2 on a sunny day (22/08/08) are shown in Figure 34. The same monoterpenoid compounds were reported by Schuch et al. (1997) and Tollsten et al. (1996). The most important message from this figure is that, besides quantitative, also qualitative differences exist in both cuvettes with sun and shade adapted leaves, respectively. The monoterpenoid emissions in cuvette 2 consists of more identified compounds in comparison to cuvette 1. As mentioned in more recent literature, it is shown that beech is a monoterpenoid-emitting tree species.

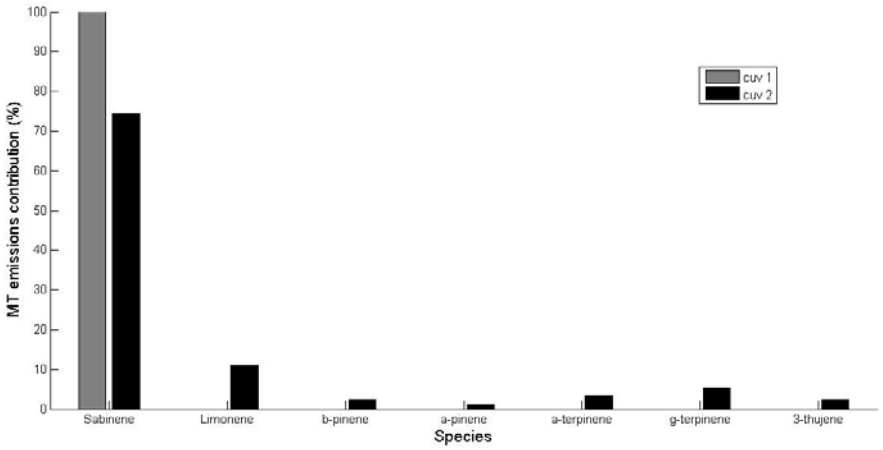


Figure 34: Monoterpenoid compound composition detected by GC-MS in the forest Aelmoeseneie on 20/08/2008 on a beech tree.

5.2 Improvement of emission algorithms

Critical analysis of the BVOC emissions observed in the 2007 growth chamber experiments and evaluation of improved emission algorithms

The data show that beech (*Fagus sylvatica* L.) is a low isoprene emitter and a rather strong monoterpenoid emitter, with measured fluxes corresponding to standard emission factors (SEF) (cf. Eq. 1, where the G97 algorithm is adopted for C_L and C_T) of up to 1500 µg m^{-2} h^{-1}, in agreement with recently published field measurements on this tree species (Holzke et al. 2006; Dindorf et al. 2006). Data-analysis therefore mainly focused on the emissions of monoterpenoids (mainly sabinene and linalool, as determined with GC-MS by Partner P2). Both isoprene and monoterpene emissions from broadleaved trees are light- and temperature-dependent, with little or no emission at night (Kesselmeier 2004). The observed emissions are therefore more closely approximated using isoprene emission algorithms (Guenther 1997; Schuh et al. 1997 and Guenther et al. 2006, which will be referred to as G97, S97 and G06, respectively) than by using a light-independent monoterpene emission algorithm developed for coniferous trees. However, since small, yet significant, emissions have been detected during the night, it is necessary to separate the light-dependent and light-independent components of the observed emissions, based on the night time measurements (temperature dependent night time emissions were observed since August 29th 2007, as can be seen in Figure 12).

The light-dependent component is tentatively described using the formula:

$$F = SEF \times C_{age} \times C_L \times C_T, \qquad \text{(Eq. 1)}$$

where F is the emission rate (µg m^{-2} h^{-1}), SEF is the standard emission factor for this tree (standard conditions are 1000 µmol m^{-2} s^{-1} PPFD and 30 °C), C_{age} incorporates the effect of leaf age, and C_L and C_T are the light and temperature activity factors, respectively. Neither G97 nor G06 include

appropriate parameterization for the strong impact of leaf senescence observed in our experiments. Here C_{age} is assumed to decrease continuously during the senescence phenophase:

$$C_{age} = \exp(-(t-t_0)/t_s), \qquad \text{(Eq. 2)}$$

where t is time, t_0 is the start of the senescence phase, and t_s is a characteristic time for the effect of senescence. t_0 and t_s are fitted from the measurements. Four algorithms are tested for C_L and C_T:

- G97 and S97, driven by the instantaneous values of leaf temperature and PPFD
- G06 (MEGAN), where the emissions depend also on past temperatures and PPFD levels; and
- G06a, a modified version of MEGAN fitted to our measurements.

In G06 and G06a, C_L depends on the past PPFD averaged over the last n_1 hours (n_1=24 in G06), whereas C_T depends on the past temperatures averaged over the last n_2 and n_3 days (=1 and 10 in G06). In G06a, n_1, n_2 and n_3 are fitted from the data. The response of the emissions to past PPFD levels during the last days cannot be inferred from our data, since a constant diurnal course was used every day for PPFD. Preliminary results of this analysis are presented in the next paragraph.

Response of monoterpene emissions to variations of PPFD at fixed temperature conditions

Most experiments were performed with a constant diurnal cycle of PPFD (Figure 9). Monoterpenoid emissions in the morning at a given PPFD value were generally found to be lower than in the afternoon at the same PPFD, which results in a hysteresis curve of monoterpenoid emissions versus PPFD in the course of a day, as shown in Figure 35. This phenomenon is not accounted for in commonly used emission algorithms. To our knowledge, it has never been reported in previous experimental studies. The observed diurnal cycle of emissions during the first days of the 2nd experimental period could be nicely reproduced by the modified MEGAN algorithm (G06a), when assuming a dependence of the emissions on PPFD levels averaged over the last n_1=3 hours. However, the modified algorithm is probably not suitable in real forest conditions, due to the much higher PPFD levels experienced by sunlit leaves in outdoor conditions.

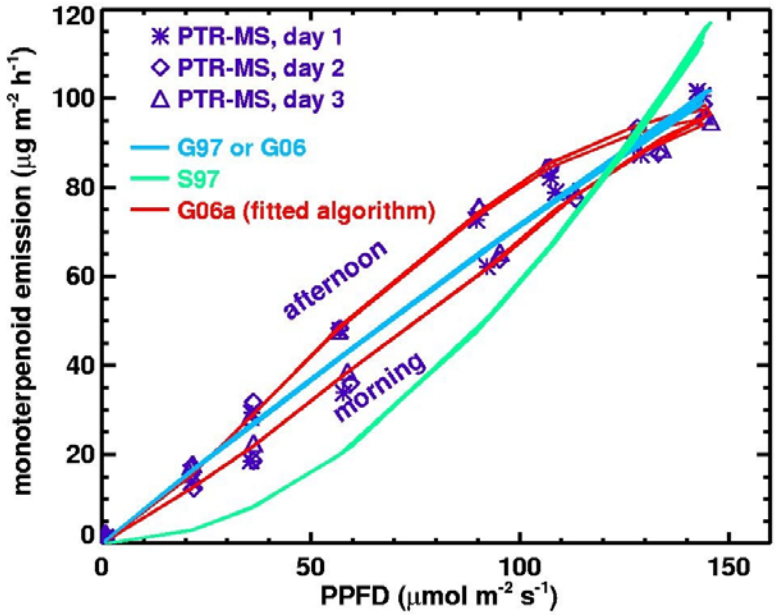


Figure 35: PPFD response curves of monoterpenoid emissions (m/z 137) measured on August 24-26, 2007. Temperature in the growth chamber is 21°C.

Response of monoterpenoid emissions to temperature variations at constant daily PPFD pattern

In order to study the response of monoterpene emissions to variations in temperature, the temperature in the growth chamber was varied as illustrated in Figure 36.

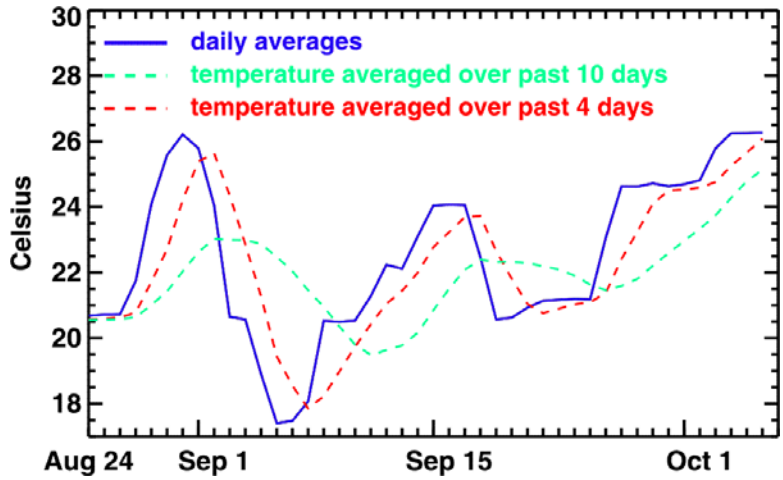


Figure 36: Time evolution of daily averaged leaf temperature during the 2nd and 3rd experimental period in 2007. Temporal averages, used in the emission algorithms, are also shown.

In order to compare emissions at different temperatures, the emission data for each day were fitted to the commonly used G97 algorithm (Eq. 1 with C_{age}=1) to obtain a Standard Emission Factor (SEF). The G97 model ignores the response of emissions to factors such as senescence and the past temperature and radiation levels experienced by the leaves. The variation of this SEF as a function of time in the second and third experimental period for the same branch is shown in Figure 37.

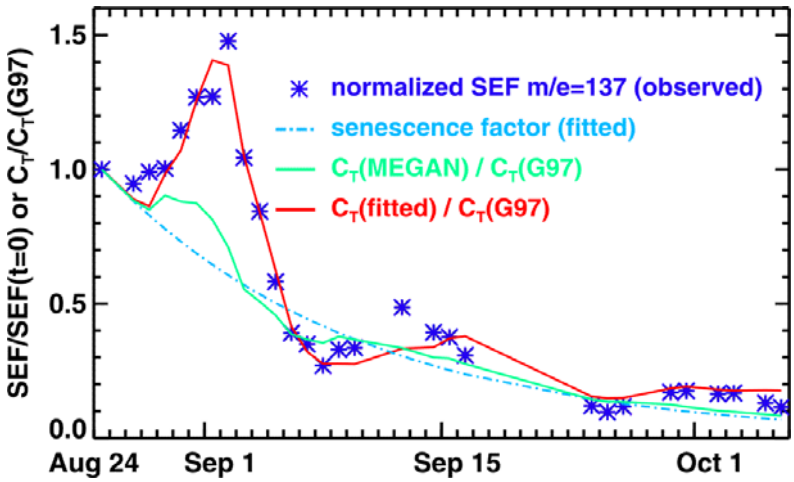


Figure 37: Time evolution of SEF factors derived from the PTR-MS measurements (normalized by the value on Aug. 24th). The senescence factor inferred from these data is also shown.

This plot reveals strong temporal variations of the SEF, which are most probably due to a combination of two factors: a dependence on the recent temperature history, and senescence, which causes a general decrease of the emissions as a function of time. The observations suggest a much stronger dependence on past temperatures than in the MEGAN algorithm, which fails to reproduce the observed SEF variations. The fitted algorithm (G06a) reproduces very well these temporal variations (Figure 37). This algorithm includes a strong dependence of the emissions on temperatures averaged over the past 4 days. The value of the characteristic time for the effect of senescence (t_s), which best reproduces the observations, is 15 days. Senescence is found to have a much stronger impact on

monoterpenoid emissions (an order of magnitude decrease during the period) than on the net photosynthesis rate (see Figure 20 IMPECVOC annual report 2007) or the chlorophyll content index (see Figure 5 IMPECVOC annual report 2008). The observed daily averaged monoterpenoid emissions are compared with the G97, G06 and G06a models in Figure 38.

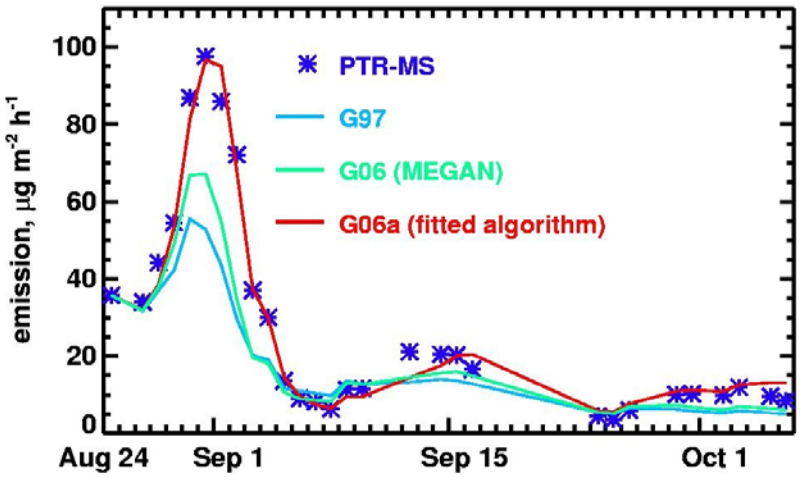


Figure 38: Daily averaged monoterpenoid emission (m/z 137) observed by PTR-MS (symbols) and comparison with three emission algorithms.

A paper about above mentioned results with working title "Measurements of BVOC emissions from *Fagus sylvatica* L. in controlled environmental conditions" is in preparation.

Preliminary analysis of the BVOC emissions observed in the 2008 canopy experiment and evaluation of improved emission algorithms

Although quality check and analysis of the data of the campaign in the Aelmoeseneie forest is still ongoing, some preliminary results are presented below.

Seasonal variation of the standard emission factor

Daily standard emission factors (SEF) values have been obtained for the different cuvettes on the third platform of the measuring tower. The daily SEF is determined from a linear fit through the experimental monoterpenoid emission rates as a function of the product C_T x C_L, with C_T and C_L the G97 temperature and light response functions, respectively. The variation of the SEF during the campaign is shown in Figure 39 for cuvette 3.1 (25 m, sunlit leaves) and for cuvette 3.2 (24 m, shaded leaves). Some literature data are also plotted.

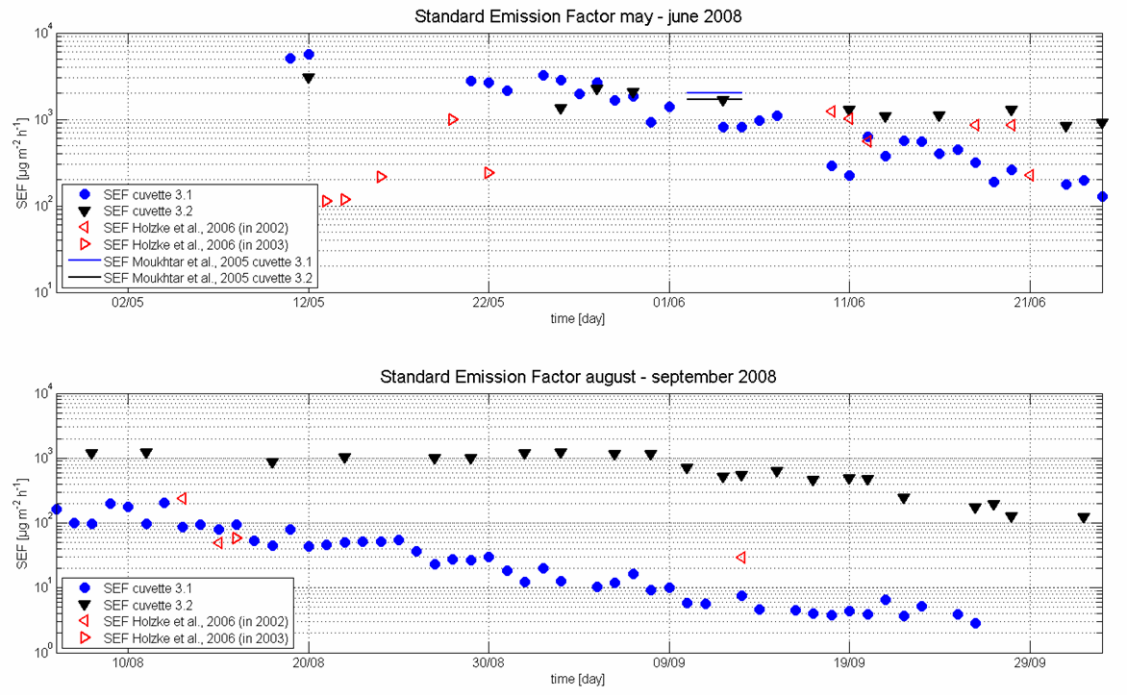


Figure 39: Seasonal variation of the standard emission factor (SEF) for beech leaves in cuvette 3.1 and cuvette 3.2 on the third platform of the experimental tower in the forest Aelmoeseneie.

From this figure it is clear that the G97 SEF exhibits a strong seasonal variation. The SEF values are in reasonable good agreement with literature data. However, pronounced differences are noticed after bud break. The seasonal variation of the SEF depends on the position of the branch in the canopy (cuvette 3.1: sunlit leaves ↔ cuvette 3.2: shaded leaves) and is probably related to the physiological status of the leaves.
No significant monoterpenoid emission rates were measured in the lowest layers of the canopy (cuvette on first (7.5 m) and second (15 m) platform), where light penetration was limited.

Comparison of measured and modeled emission rates
Figure 40 shows the diurnal monoterpenoid emission rate (m/z 137) for cuvette 3.1 (height: 25 m, sunlit leaves) averaged over the entire measurement campaign. This figure clearly shows a discrepancy between the data and the emissions calculated with the G97 (in blue) and G06 (MEGAN, in green) algorithms. Both algorithms overestimate the emissions in the morning, and underestimate the emissions in the afternoon. The observed behaviour is therefore qualitatively similar to the apparent hysteresis of emissions observed in the growth chamber experiment under controlled conditions (see above, Figure 35).

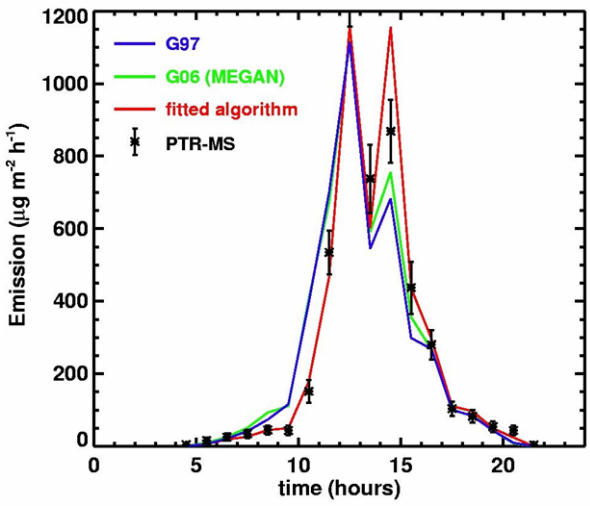


Figure 40: Averaged diurnal monoterpenoid emission rate for beech leaves in cuvette 3.1 on the third platform and comparison with different algorithms.

In order to obtain a better agreement with the experimental data, several parameters appearing in the MEGAN G06 algorithm were optimized by minimizing the overall bias between the model and the data, i.e. the cost function J, given by:

$$J = \frac{1}{2}\sum_i \left(\frac{E_i^{fit} - E_i^{obs}}{\Delta E_i} \right)^2 \qquad \text{(Eq. 3)}$$

with i the observation index, E_i^{fit} the fitted monoterpenoid emission rate, E_i^{obs} the measured emission rate and ΔE_i the estimated error on the measured emission rate. E_i^{fit} is given by:

$$E_i^{fit} = \varepsilon_{iday}^{fit} \times \gamma_T^i \times \gamma_P^i \qquad \text{(Eq. 4)}$$

with ε_{iday}^{fit} the SEF for each day (derived by fitting the G97 algorithm to the experimental determined emission rates for the corresponding day, see previous paragraph), γ_T^i and γ_P^i respectively the temperature and light response function. The following expression for the temperature response function γ_T^i is used:

$$\gamma_T^i = \gamma_{T,G93}^i \times e^{\alpha_1 \times \left(\overline{T_n} - 297\right)} \qquad \text{(Eq. 5)}$$

with γ_T^i the temperature response function according to Guenther et al. (1993) and $\overline{T_n}$ the leaf temperature averaged over the past **n** hours (**n** and $\boldsymbol{\alpha_1}$ to be fitted). The light response function γ_P^i is given by the following formula:

$$\gamma_P^i = e^{\alpha_2 \times \left(\overline{P_n} - P_0\right)} \times \frac{\alpha \times P_i}{\sqrt{1 + \alpha^2 P_i^2}} \qquad \text{(Eq. 6)}$$

with $P_0 = 200$ µmol m^{-2} s^{-1}, P_i the PPFD of observation i in µmol m^{-2} s^{-1}, $\overline{P_n}$ the PPFD averaged over the past **n** hours (**n** and $\boldsymbol{\alpha_2}$ to be fitted). For the parameter α the value according to the G06 algorithm is used.

Minimizing the cost function J given by Eq. 3, resulted in the following optimized set of values for the parameters **n**, α_1 and α_2**:**

n	18 hours
α_1	0.11
α_2	0.0043

Except for the beginning of May, the modified MEGAN algorithm, taking into account the light history over the past 18 hours, reproduces the experimental dataset well, as is illustrated in Figure 41. The time delay of the emissions and the resulting light hysteresis are appropriately taken into account.

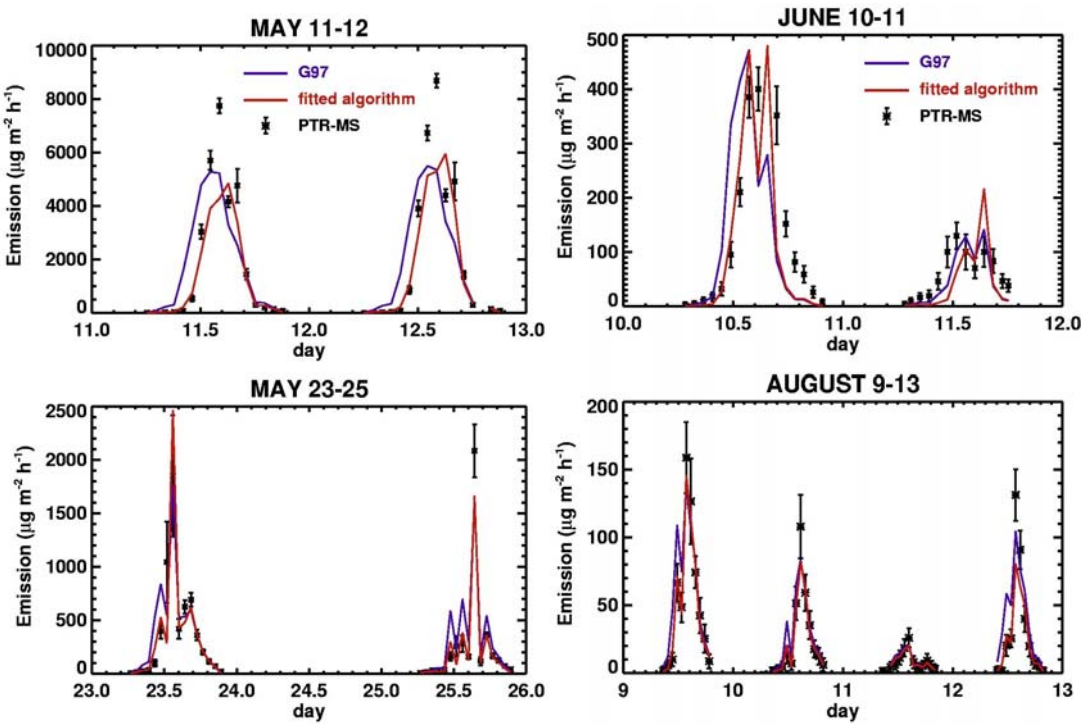


Figure 41: Emission rates for cuvette 3.1 in comparison with the G97 (blue) and the fitted algorithm (red).

WP6 Scientific dissemination

6.1 Follow-up committee

The IMPECVOC network met the members of the follow-up committee during the meetings of 14-3-2007 (kick-off meeting at BISA, Brussels), 6-11-2007 (Faculty of Bioscience Engineering, Ghent University) and 7-7-2008 (Aelmoeseneie experimental forest, Ghent University). At these occasions, detailed discussions were held on the methodology used by the IMPECVOC partners. The results obtained in 2007, and the first ones in 2008, were critically analyzed during respectively the second and third meeting. It appears that the follow-up committee has a profound interest in the IMPECVOC activities and particular interest is expressed for the translation of the scientific results into policy guidelines. Ms. E. De Brabander of the "Vlaamse Milieumaatschappij, Afdeling Meetnetten en Onderzoek", together with Dr. A. Guns of the "Ministère de la Région Wallonne, Direction Générale des Ressources Naturelles", are keen to cooperate with IMPECVOC in order to establish accurate data for the regional and national inventories of BVOC emissions by Belgian forests. It might be expected that the IMPECVOC network will need their experience in order to develop scaling-up procedures for further integration of leaf and stand scale data to the regional and the national level. Therefore, a close collaboration is suggested during the second phase of the IMPECVOC-project.
A problem which needs remediation is the observation that the committee members from abroad (Dr. Christoph Spirig of the Air Pollution and Climate Research Group, Zürich, Switzerland, and Dr. J. Rinne, Dept. of Physical Sciences, University of Helsinki, Helsinki, Finland) have in the beginning not been able to assist the first two committee meetings. They have both considerable experience with BVOC research and, hence, their scientific evaluation of IMPECVOC activities is very important. The solution is that invitations for these members are launched with financial coverage in order to be able to visit the Belgian experimental set-ups (measuring tower in the Aelmoeseneie forest and at Vielsalm, growth rooms and labs for chemical analyses).
This was done for the 3rd meeting of the follow-up committee for which all partners equally supported the travel and lodging costs of Dr. C. Spirig and Dr. J. Rinne. The morning programme consisted of an informal meeting held at the Laboratory of Plant Ecology (Ghent University) during which the preparation of the eddy-covariance measurements at the Vielsalm site was discussed. The afternoon session was held at the Aelmoeseneie site and consisted of the formal meeting of the follow-up committee.
More details can be fould in the reports of the follow-up committee (see Annex 3 of 2008 annual report).

6.2 Dissemination in the scientific community

Peer reviewed publications

1. Demarcke M, Amelynck C, Schoon N, Van Langenhove H, Dewulf J (2009). Laboratory studies in support of the detection of sesquiterpenes by Proton-Transfer-Reaction-Mass-Spectrometry. Int. J. Mass Spectrom. 279: 156-162.

2. Müller J-F, Stavrakou T, Wallens S. De Smedt I, Van Roozendael M, Rinne J, Munger B, Goldstein A, Guenther A (2008). Global isoprene emissions estimated using MEGAN, ECMWF analyses and a detailed canopy environmental model. Atmos. Chem. Phys. 8: 1329-1341.

3. Joó É, Van Langenhove H, Šimpraga M, Steppe K, Amelynck C, Schoon N, Müller J-F, Dewulf J (2009). Variation in biogenic volatile organic compound emission pattern of *Fagus sylvatica* L. due to aphid infection. Atmos. Environ. (under review; Manuscript Number: ATMENV-D-09-00248R1).

4. Deligne A, Heinesch B, Aubinet A (2009). New transfer functions for correcting turbulent water vapour fluxes. Submitted to Boundary-Layer Meteorology.

5. Demarcke M, Amelynck C, Schoon N, Müller J-F, Dewulf J, Van Langenhove H, Steppe K, Lemeur R (2009). Measurements of BVOC emissions from *Fagus sylvatica* L. in controlled environmental conditions. In preparation.

6. Šimpraga M, Steppe K, Verbeeck H, Demarcke M, Amelynck C, Schoon N, Dewulf J, Van Langenhove H, Lemeur R (2009). Leaf-level temperature response of BVOC emissions and net photosynthesis of a young beech tree (*Fagus sylvatica* L.). In preparation.

7. Šimpraga M, Steppe K, Verbeeck H, Demarcke M, Amelynck C, Schoon N, Dewulf J, Van Langenhove H, Lemeur R (2009). Drought stress effects on the link between BVOC emissions and photosynthesis rates. In preparation.

8. Bloemen J, Šimpraga M, Verbeeck H, Amelynck C, Schoon N, Dewulf J, Van Langenhove H, Lemeur R, Steppe K (2009). How do sun and shade adapted leaves influence volatile organic compounds re-emitted back into the atmosphere? In preparation.

9. Joó É, Demarcke M, Amelynck C, Dewulf J, Schoon N, Müller J-F, Steppe K, Van Langenhove H. Quantification of BVOC using PTR-MS and GC-MS techniques in the view of quality control approaches. In preparation.

Other presentations (oral presentation, posters, abstracts)

1. C. Amelynck, N. Schoon, E. Debie and P. Bultinck. Ion/molecule reaction studies in support of the detection of sesquiterpenes by CIMS, in the Proceedings of the 3rd International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications (Obergurgl, Austria), pp. 127-131, Editors: A. Hansel, T.D. Märk, Innsbruck University Press, 2007.

2. M. Demarcke, C. Amelynck, N. Schoon, J.-F. Müller, É. Joó, J. Dewulf, H. Van Langenhove, M. Šimpraga, K. Steppe, R. Samson and R. Lemeur. Measurements of BVOC emissions from *Fagus sylvatica* L. in controlled environmental conditions: preliminary results. Geophysical Research Abstracts, Vol. 10, CD-Rom EGU2008-A-02589, EGU General Assembly, Vienna, Austria, April 13-18 2008.

3. M. Šimpraga, K. Steppe, M. Demarcke, C. Amelynck, N. Schoon, É. Joó, J. Dewulf, H. Van Langenhove, R. Samson, J.-F. Müller and R. Lemeur. Preliminary observations of temperature effects on carbon loss through BVOC emissions in *Fagus sylvatica* L. Geophysical Research Abstracts, Vol. 10, CD-Rom EGU2008-A-09084, EGU General Assembly, Vienna, Austria, April 13-18 2008.

4. M. Demarcke, C. Amelynck, N. Schoon, J.-F. Müller, É. Joó, J. Dewulf, H. Van Langenhove, M. Šimpraga, K. Steppe, R. Samson, R. Lemeur, M. Aubinet, B. Heinesch. Impact of Phenology and Environmental Conditions on Biogenic Volatile Organic Compounds (BVOC) Emissions from Forest Ecosystems (IMPECVOC): preliminary results. University Day, BISA, Brussels, November 28 2008 (poster).

5. M. Demarcke, C. Amelynck, N. Schoon, J. Dewulf and H. Van Langenhove. Investigations on the influence of drift field and humidity on sesquiterpene product ion distributions in a PTR-MS instrument and implications for sesquiterpene detection sensitivity, in the Proceedings of the 4th International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications (Obergurgl, Austria), pp. 170-173, Editors: A. Hansel, J. Dunkl, Innsbruck University Press, 2009.

6. Šimpraga M., Steppe K., Demarcke M., Amelynck C., Schoon N., Samson R., Lemeur R. How does drought stress in a potted beech tree affects the relationship between photosynthesis and BVOC emissions? Studiedag starters in het bosonderzoek, 19 March 2009, Brussel,, Belgium.

7. Bloemen J, Šimpraga M, Verbeeck H, Amelynck C, Steppe K. 2009. Dynamische interactie tussen fotosynthese en BVOS emmissies in bosecosyetemen. Studiedag starters in het bosonderzoek, 19 March 2009, Brussel, Belgium.

8. M. Demarcke, C. Amelynck, N. Schoon, J.-F. Müller, É. Joó, J. Dewulf, H. Van Langenhove, M. Šimpraga, K. Steppe, R. Lemeur, and R. Samson. Branch enclosure BVOC flux measurements from *Fagus sylvatica* L. in a natural forest environment: preliminary results. Geophysical Research Abstracts, Vol. 11, EGU2009-2344-4, EGU General Assembly 2009, Vienna, Austria, April 19-24 2009.

9. M. Demarcke, C. Amelynck, N. Schoon, J. Dewulf, and H. Van Langenhove. Characterization of Proton Transfer Reaction Mass Spectrometry for the detection of sesquiterpenes. Geophysical Research Abstracts, Vol. 11, EGU2009-4044, EGU General Assembly 2009, Vienna, Austria, April 19-24 2009.

10. É. Joó , H. Van Langenhove , L. Schietse, O. Pokorska , M. Šimpraga , K. Steppe , M. Demarcke , C. Amelynck , N. Schoon , J.-F. Müller , R. Samson and J. Dewulf. Changes in BVOC emission pattern from *Fagus sylvatica* L. measured by thermal desorber GC-MS. Geophysical Research Abstracts, Vol. 11, EGU2009-672-2, EGU General Assembly 2009, Vienna, Austria, April 19-24 2009.

11. É. Joó, L. Vandermeersch, K. De Roo, J. Dewulf, H. Van Langenhove. Geuren natuurlijk: De geur van bomen. Wetenschapsweek, 23-24 Oktober 2008.

12. É. Joó, O. Pokorska, H. Van Langenhove, J. Dewulf. Measurement of biogenic volatile organic compounds from forest ecosystems. Opendeurdag Ghent University, 14 March 2009.

13. C. Amelynck, N. Schoon, M. Demarcke, F. Dhooghe, posters presented during the open days of BISA, Brussels, October 6-7 October 2007.

14. Šimpraga M., Steppe K., Verbeeck H., Lemeur R. **The IMPECVOC project** (**Im**pact of **P**henology and **E**nvironmental **C**onditions on B**VOC** Emissions from Forest Ecosystems). CES newletter. Center of Environmental Sanitation, Ghent University. 2009.

Master thesises

1. Neina, D. Biogenic volatile organic compounds from some tree species, Master thesis, 87 pp.

2. Bloemen, J. Dynamische interactie tussen fotosynthese en BVOS emissies in bosecosystemen (Dynamical interaction between photosynthesis and BVOC emissions in the forest ecosystems), Master thesis, 113 pp.

3. Schieste, L. Emission of biogenic volatile organic compounds from European beech related to biotic stress and from Dougles fir subjected to varying environmental conditions, Master thesis, 87 pp.

WP7 Dissemination for policy makers and the professional field

7.1 Flyer

See annex 1 of annual report 2007.

7.2 Website

www.impecvoc.ugent.be

Recommendations for policy makers

Policy makers bring the framework to have proper environmental living conditions for the people. In this sense they rely on scientific data to establish a proper policy for a healthy atmosphere. To that extent, European countries like Belgium are forced to make inventories about compounds emitted by mankind and nature that influence the atmospheric quality. It turns out that for the category of volatile organic compounds with quite different impacts such as contribution to tropospheric ozone formation, global warming and human toxicity, forests are quite important contributors. In this sense emission inventories of this group of compounds, i.e. biogenic volatile organic compounds (BVOCs), are essential in proper decision making. The emission inventory of BVOCs currently rely on models that are based on measurements and models from the early 1990s.

Our project results of the first phase provide measurements and first model trials that are a first step towards a better estimation of BVOC emissions. First, we learn that there are more factors than tree species, light and temperature that determine the emission of BVOCs. For example, seasonality, the physiological status of the leaves and infection influence the emissions. Second, we learn that a number of compounds are emitted which do not get proper attention in emission inventories and that may be of interest in further atmospheric chemistry, in particular in ozone formation and particulate matter development. In a further stage, these findings could be the base of a refined BVOC emission estimation providing a more sound basis for proper policy with respect to atmospheric quality.

If the preliminary relation of BVOC emissions and temperature (growth room experiment) will be confirmed by the canopy and stand level experiments, these results might have important consequences in the framework of global climate change, since models predict a further 1.5-5.5°C warming for this century as a results of increased atmospheric CO_2 and other trace gasses concentrations (IPCC 2007). Thus, when ambient temperatures will rise, the monoterpenoid emissions will rise as well.

References

Bertin et al., Oecol. 107 (1996) 456 – 462.

Dindorf et al., J. Geophys. Res. 111 (2006) D16305, doi : 10.1029/2005JD006751

Dominguez-Taylor, Atmos. Environ. 41 (2007) 2780-2790.

Graus et al., Plant Phys. 135 (2004) 1967-1975.

Guenther et al., J. Geophys. Res. 98 (1993) 12609-12617.

Guenther, Ecol. Appl. 7 (1997) 34-45.

Guenther et al., Atmos. Chem. Phys. 6 (2006) 3181-3210.

Helmig, Atmos. Environ. 40 (2006) 4150-4157.

Holzke et al., J. Atm. Chem. 55 (2006) 81-102.

IPCC 2007. Observed and projected changes in climate as they relate to water. Intergovernmental Panel on Climate Change.

Jonckheere et al., Agri. Forest Meteo. 121 (2004) 19-35.

Kesselmeier, Atmos. Chem. Phys. Disc. 4 (2004) 528530.

Liakakou, Atmos. Environ. 41, (2007) 1002-1010.

Loreto et al., Plant Cell and Env. 29 (2006) 1820-1828.

Monsi and Saeki, Jap. J. of Bot. 14 (1953) 22-52.

Moukhtar et al., Atm. Env. 39 (2005) 3535-3547.

Ormeno, Atmos. Environ. 41 (2007) 629-639.

Schuh et al., J. Atm. Chem. 27 (1997) 291-318.

Sharkey et al., Oecol. 95 (1993) 328-333.

Spirig et al., Atmos. Chem. Phys., 5 (2005) 465-481.

Tiiva, Atmos. Environ. 41 (2007) 3819-3828.

Tollsten et al., Phytochem. 43 (1996) 759-762.

Vande Walle, PhD dissertation (2007) Ghent University.