*Journal of Atmospheric Chemistry* **45:** 143–172, 2003.



# Emissions of Oxygenated Volatile Organic Compounds from Plants Part I: Emissions from Lipoxygenase Activity

A. C. HEIDEN [1, 2], K. KOBEL [1], C. LANGEBARTELS [3], G. SCHUH-THOMAS [3, 4] and J. WILDT [1, 5]

[1] *Institut für Chemie der Belasteten Atmosphäre (ICG-2), Forschungszentrum Jülich, D-52425 Jülich, Germany*
[2] *Now at Gerstel GmbH & Co. KG, Aktienstr. 232–234, D-45473 Mülheim/Ruhr, Germany*
[3] *Institut für Biochemischie Pflanzenpathologie, GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Ingolstädter Landstraße 1, D-85758 Oberschleißheim, Germany*
[4] *Present address: Clariant (Schweiz) AG, Rothausstrasse 61, CH-4132 Muttenz 1, Switzerland*
[5] *Present address: Institut Phytosphäre (ICG-III), Forschungszentrum Jülich, D-52425 Jülich, Germany*

(Received: 9 May 2001; accepted: 18 November 2002)

**Abstract.** Emissions of oxygenated volatile organic compounds (OVOC) from several plant species were measured in continuously stirred tank reactors (CSTR). High emission pulses of OVOCs were observed when plants were exposed to stress. Absolute emission rates were highly variable ranging up to $10^{-13}$ mol · cm$^{-2}$ · s$^{-1}$. The temporal shape of these emissions was described by a formalism similar to that of a consecutive reaction of pseudo first order kinetics. The main emitted OVOC was (Z)-3-hexenol together with other $C_6$-aldehydes and alcohols, suggesting that lipoxygenase activity on linolenic acid was mainly responsible for OVOC production. Various stress factors induced lipoxygenase activity and subsequent emissions of OVOCs. These factors were exposure to high ozone concentrations, pathogen attack, and wounding. The pattern of OVOC emissions from tobacco was similar for different stress applications and the same products of lipoxygenase activity were emitted from all investigated plant species. Our results imply that these emissions occur as general response of the plants to stress. Since plants experience various abiotic or biotic stress factors in the environment, OVOC emissions as a response to stress are likely to be of significant importance for atmospheric chemistry.

**Key words:** alcohols, aldehydes, emission, lipoxygenase, ozone, pathogen, wounding.

## 1. Introduction

Global volatile organic compound (VOC) emission from vegetation is estimated to be about an order of magnitude larger than that from anthropogenic sources (e.g., Fehsenfeld *et al.*, 1992; Mueller, 1992; Guenther *et al.*, 1993). Emissions of isoprene and monoterpenes are important with respect to atmospheric chemistry because these compounds are emitted in large amounts from many plant

*Table I.* Oxygenated volatile organic compounds produced in the sequence of lipoxygenase activity (after Hildebrand, 1989; Croft *et al*., 1993; Hatanaka, 1993; Gardner, 1995)

| | Linoleic acid (18:2) | | Linolenic acid (18:3) | |
|---|---|---|---|---|
| Aldehydes | Hexanal | (Z)-3-Nonenal | (Z)-3-Hexenal | (Z)-3, (Z)-6-Nonadienal |
| | | (E)-2-Nonenal | (E)-2-Hexenal | (E)-2, (Z)-6-Nonadienal |
| | | | (E)-3-Hexenal | |
| Alcohols | 1-Hexanol | (Z)-3-Nonenol | (Z)-3-Hexenol | (Z)-3, (Z)-6-Nonadienol |
| | | (E)-2-Nonenol | (E)-2-Hexenol | (E)-2, (Z)-6-Nonadienol |
| | | | (E)-3-Hexenol | |
| | | | (Z)-3-Hexenyl-acetate [a] | |

[a] Hexenylacetate formed in secondary steps.

species. However, besides isoprene and monoterpenes several other VOCs are emitted from plants. Among those are oxygenated volatile organic compounds (OVOCs) such as the $C_6$-aldehydes and -alcohols. The biochemical formation of these OVOCs is well understood (e.g., Hatanaka, 1993; Croft *et al*., 1993; Gardner, 1995). They are derived from the common lipid constituents of plant membranes, linoleic and linolenic acid. Free octadecanoid fatty acids (linoleic acid = 18:2 and linolenic acid = 18:3) are released by phospholipases. Lipoxygenase (LOX) activity produces 9- or 13-hydroperoxylinoleic or -linolenic acid or a mixture of both. A hydroperoxide lyase then catalyzes the breakdown of 13-hydroperoxylinole(n)ic acid to a $C_6$-compound, (Z)-3-hexenal, and a $C_{12}$-product (12-oxo-(Z)-9-dodecenoic acid). (Z)-3-hexenal can give rise to (Z)-3-hexenol, (E)-2-hexenol, (E)-3-hexenol or (E)-2-hexenal in subsequent reactions. The 9-hydroperoxides are converted into nonenal and -ol or nonadienal and -ol, respectively. Table I gives an overview of the compounds produced from linoleic and linolenic acid in this enzyme sequence.

The release of $C_6$-compounds from plants is well known. Arey *et al*. (1991) reported emissions from several plant species with (Z)-3-hexenol or (Z)-3-hexenyl-acetate as predominantly emitted compounds. Large emissions of these OVOCs were also observed after wounding (Buttery and Ling, 1993; Fall *et al*., 1999), during drying (de Gouw *et al*., 1999), and following pathogen attack (Croft *et al*., 1993). However, quantitative data for these emissions and information on the timing of the plants' responses are still sparse. No reliable estimates are available with respect to the impact of LOX products on atmospheric chemistry.

Here we present data indicating that emissions of LOX products occur as a general response of plants to stress. Even though these emissions are transient, during the pulsed emissions the total VOC release from individual plants can increase by orders of magnitude.

## 2. Experiment

VOC emissions were studied in continuously stirred tank reactors (CSTR) similar to that described in Wildt *et al*. (1997). The glass chambers (volume 160 L, 1000 L, or 1500 L, respectively) were mounted in temperature controlled housings. The chambers were supplied with several connections to introduce temperature- and light intensity sensors and to connect the tubings for gas-phase analysis and air supply. In order to minimize wall losses all tubings were either made of Teflon (PFA or PTFE) or of glass.

Up to 12 discharge lamps (Osram HQI 400 W/D) were used for illumination. At full illumination the light flux of photosynthetic active radiation (PAR) at mid-canopy height amounted to 360 (1500 L chamber), 480 (1000 L chamber), or 800 $\mu$mol $\cdot$ m$^{-2}$ $\cdot$ s$^{-1}$ (160 L chamber), respectively. Filters (OptoChem, type IR3) that reflect wavelengths between 750 and 1,050 nm were used as heat shields to avoid overheating of the plants by infrared radiation.

Ambient air was purified by an adsorptive drying device (Zander, KEA 70) and a palladium catalyst (450 °C). The air flow through the chambers was kept constant by mass flow controllers. Between 20 and 200 L $\cdot$ min$^{-1}$ of air were led through the chambers. Teflon fans were used for homogeneous mixing of the chamber air.

The dew point of the air at chamber inlet was reduced to about –18 °C by the adsorptive drying device. To increase the relative humidity of the air in the chamber to about 80%, water vapor was added by diverting a part of the inflowing air in a bypass mode through a water containing glass vessel. If necessary, $CO_2$ from cylinders was added to the air at chamber inlet in order to keep the $CO_2$ concentration at about 350 ppm.

For exposure experiments, $O_3$ was produced in a glas cell outside the CSTR by photolysis of oxygen with a UV light source (Penray, $\lambda = 189$ nm). The ozone containing flow from the cell was mixed to the air entering the chamber.

Mixing ratios of $O_3$, $CO_2$, $H_2O$, NO, and $NO_2$ were measured as described by Schuh *et al*. (1997) and Wildt *et al*. (1997). A detailed description of the GC-MS system used for VOC analysis was given by Heiden *et al*. (1999a). In short, samples for VOC analysis were collected on-line on solid sorbents (Tenax TA/Carbotrap). The thermal adsorption/desorption system (Gerstel online TDS G) was connected to a cooled injection system (Gerstel, KAS 3) where samples were cryofocused before injection into a GC-MSD-system (HP5890 Series II – HP5972A). The sample time was 40 minutes and the time resolution of one analytical run about 60 minutes. A BPX-5 column (SGE, 50 m $\times$ 0.22 mm $\times$ 1 $\mu$m) was used for separation.

Calibration was performed using a permeation source containing pure chemicals in individual vials in combination with a dynamic dilution system. Concentrations of the compounds released from the calibration source were determined from the mass loss rates of the individual compounds and the dilution fluxes. The VOC mixing ratios were in the lower ppb to ppt range.

The reproducibility of VOC concentration measurements was in the range between 10 and 15% and the detection limits of our analytic equipment was between 1 ppt and 5 ppt for individual VOCs.

Identification was based on mass spectra and retention times of pure chemicals (Fluka/Aldrich, purity >93%). Some compounds were only tentatively identified by using reference mass spectra from the NBS75k library. For these compounds no individual calibration was conducted. Nevertheless, we estimated the concentrations of these compounds as follows: The molecular structure is similar for different isomers of a certain compound and therefore, the ionization efficiency for both isomers should be similar. Hence, the relative concentrations of the two isomers could be obtained from the relative peak areas. We measured the peak areas in the single ion mode and therefore, the different fractionation of different isomers had to be taken into account by using the fraction of the peak areas of the respective target peaks over the sum of all peak areas according to the ratio:

$$\frac{Z_T}{\sum_{i=35}^{\mathrm{Mw}+1} Z_i}. \tag{1}$$

In Equation (1), $Z_T$ is the peak area of the target compound, $Z_i$ is the peak area of the peak with $m/z = i$. Summation from $m/z = 35$ to Mw + 1. $m/z = 35$ is the lower limit measured with the mass spectrometer and Mw is the molar weight of the respective compound.

The concentration of the other isomer could be obtained by comparison with the concentrations determined for the calibrated isomer. Experiments using some calibrated compounds showed that the error of the concentrations determined in this way was below 15%.

In some cases the peaks for different compounds were not fully separated. If it was possible to find a fragment in one compound that was not abundant in the other compound (reference mass spectra from the NBS75k library), the error in the quantification of the concentrations was negligible. Problems only occurred in case of (E)-3-hexenal and (E)-3-hexenol where the peaks fully overlapped. Whereas (E)-3-hexenol concentrations could be quantified using $m/z = 67$, those of (E)-3-hexenal ($m/z = 83$) were only measurable when its concentrations were far above those of (E)-3-hexenol.

### 2.1. OZONE REMOVAL BEFORE SAMPLING

To avoid artificial formation of compounds caused by reactions of ozone with other substances on solid sorbents (e.g., Clausen and Wolkoff, 1997) ozone has to be removed before sampling. We tested two different methods.

Experiments with multiple layers of $MnO_2$-coated copper nets (Dasibi Environmental Corp., type Z-0284-S) as ozone scrubbers were unsuccessful. The scrubbers

*Table II.* Decline of the response of our analytical system; $r^2$ = correlation coefficient

| Compound | $X$ [ppb$^{-1}$] | $r^2$ |
|---|---|---|
| Hexanal | 0.0025 | 0.96 |
| Nonanal | 0.0034 | 0.88 |
| (Z)-3-Hexenol | 0.0016 | 0.95 |
| (E)-2-Hexenal | 0.0015 | 0.96 |
| $\alpha$-Pinene | 0.0001 | 0.78 |
| $\beta$-Pinene | 0.0012 | 0.93 |

caused considerable memory effects, especially for compounds as (Z)-3-hexenol, (Z)-3-hexenylacetate or (E)-2-hexenal.

Another recommended method is the ozone-titration with NO. In a series of measurements it was tested if the investigated aldehydes or alcohols were formed due to this method. Alcohols and aldehydes were removed from the permeation source. The air from the permeation source including mono- and sesquiterpenes was led through a reaction vessel (volume: 10 L, residence time: 5 min). NO (1 ppm) and ozone (150 ppb) were added and the mixture was measured for the aldehydes and alcoholes. In no case the OVOCs were detected. The method of ozone titration thus proved to be suitable for ozone removal.

In another series of measurements the effects of this method on the response of the analytical system was tested. NO (1 ppm) and ozone were added to the air from the permeation source, now also including OVOCs. Ten measurements each were conducted adding ozone at concentrations of 0, 10, 20, 60, 130, 170, and 200 ppb, respectively to the glass vessel. The result of this experiment showed an exponential decline of the response of our analytical system with increasing ozone admixture. Equation (2) was used to describe this effect:

$$\ln(R) = \ln(R_0) - x \cdot [O_3]\,. \tag{2}$$

In Equation (2), $R$ is the observed response and $R_0$ is the response without addition of ozone, respectively. $[O_3]$ is the ozone mixing ratio (in units of ppb) and $X$ is the factor describing the decline in response. The value for $X$ was determined by linear regression analysis. Table II shows examples for the results.

Ozone titration at 1 ppm NO occurs within less than a minute implying that the observed losses were due to processes involving the $NO_2$ formed in the titration reaction. Indeed, removing NO and $O_3$ from the reaction vessel and adding $NO_2$ at concentrations of about 100 ppb led to a similar loss in response as 1 ppm NO and 100 ppb $O_3$.

The losses in the response ranged between 1 and 34% at ozone concentrations of 100 ppb. This loss was considered for the determination of VOC concentra-

*Table III.* Conversion factors for emission rates in units of mol $\cdot$ cm$^{-2}$ $\cdot$ s$^{-1}$ to units of $\mu$ $\cdot$ g(dw)$^{-1}$ $\cdot$ h$^{-1}$; Mw = molar weight of the respective compound

| Plant species | Conversion factor |
|---|---|
| Pine | $1.40 \cdot 10^{11} \cdot$ Mw |
| Sunflower | $9.70 \cdot 10^{11} \cdot$ Mw |
| Tomato | $9.20 \cdot 10^{11} \cdot$ Mw |
| Corn | $1.40 \cdot 10^{12} \cdot$ Mw |
| Canola | $1.04 \cdot 10^{12} \cdot$ Mw |
| Tobacco | $1.70 \cdot 10^{12} \cdot$ Mw |

tions during the measurements with plants under ozone exposure using the ozone concentrations measured in the chamber during the time intervals of GC/MS measurements.

This correction procedure may introduce an additional error. However, because of the good correlation between the ozone concentrations and the decline in response (Table II) we are confident that the additional error caused by using the method of ozone titration is less than 10% for measurements at ozone concentrations below 100 ppb. Despite of the loss in response, the method of ozone titration by NO proved to be the most reliable method for ozone removal because no memory effects were found.

### 2.2. DETERMINATION OF EMISSION RATES

The whole leaf area of the plants (one sided) was used as normalization factor for OVOC emissions. This allowed direct comparison of the emission rates to those of other compounds produced via different biochemical pathways. Conversion factors to emission rates in units of $\mu$g $\cdot$ g (dw)$^{-1}$ $\cdot$ h$^{-1}$ are given in Table III.

OVOC emission rates, $\Phi_{\mathrm{OVOC}}$, were determined from the measured concentrations of the individual OVOCs and calculated according to Equation (3):

$$\Phi_{\mathrm{OVOC}} = \frac{F}{A} \cdot [\mathrm{OVOC}] \,. \tag{3}$$

$F$ is the air flow through the chamber (mol $\cdot$ s$^{-1}$), $A$ is the leaf area (cm$^2$), and [OVOC] is the mixing ratio of the individual OVOC at chamber outlet, respectively.

The concentrations of VOCs at chamber inlet were reduced to values below the detection limit by the Pd-catalyst and wall losses in our chamber were negligible (Heiden *et al.*, 1999a). Gas phase reactions of OVOCs with ozone were also neglected. Assuming the rate constants of ozone OVOC reactions to be in the range

between $2.0 \cdot 10^{-18}$ $cm^3 \cdot s^{-1}$ and $6.4 \cdot 10^{-17}$ $cm^3 \cdot s^{-1}$ (Atkinson *et al.*, 1995) the loss of these compounds by gas phase reactions are below 5% in all measurements.

## 2.3. MATERIAL AND METHODS

Experiments were conducted with tobacco (*Nicotiana tabaccum* cv. Bel W3 and Bel B, 4 to 6 plants per CSTR, 23 plants in total), sunflower (*Helianthus annuus* cv. Gigantheus, 1 plant), corn (*Zea mays* L. cv. Dento, 6 plants), tomato (*Lycopersicum esculentum* L. cv. Roma, 6 plants), pine (*Pinus sylvestris* L., 3 experiments with 4 plants each, 1 experiment with a single plant), canola (*Brassica napus* L. cv. Bronovski, 4 plants), and broad bean (*Vicia faba* L. cv. Alfred, 3 plants).

Broad bean, corn, tomato, canola, and sunflower seeds were germinated in moist quartz sand at a temperature of 28 °C and at a relative humidity of 85%. After germination, sunflower, canola, and broad beans were placed into a hydroponic system. The remaining plant species were cultivated in standard substrate (Einheitserde, type ED 73). Nutrient solution (Asher, 1978) was added to the soil every morning. Details regarding the experiments with pine plants are given by Shao *et al*. (2001).

As potential elicitors for the emissions of LOX products we used ozone exposure, infiltration of pathogens, insect attack, wounding, and sudden light-dark transitions.

Ozone exposure was conducted in short pulses (5 h–4 d) at high concentrations. In one experiment the plants were exposed to ozone at lower concentrations for 46 days in total. Pathogen infection was induced by infiltrating $2 \cdot 10^9$/mL colony forming units of *Pseudomonas syringae* pv. *syringae* in a 10 mM $MgCl_2$ solution by a blunt-tip syringe into all middle-aged leaves. Control plants received a treatment with 10 mM $MgCl_2$.

For a set of 4 pine plants that were investigated we observed that about 1% of the needles became yellow within a time period of 3 days (see also Heiden *et al.*, 1999b). Since the plants did not suffer from water stress, were not exposed to ozone, no insects were observable on the plants, and the visible symptoms were identical for the 4 plants we attributed these symptoms to a pathogen attack. The pathogen itself could not be identified.

Wounding was conducted by grinding middle aged leaves of a tobacco plant (cv. Bel W3) with emery paper (approximately 10 $cm^2$ leaf area). To study the effects of insect attack on the emissions we used a single pine plant that was stored outside. This plant showed visible symptoms of insect attack (*pseudococcidae*).

Another elicitor for the emissions of LOX products was found accidentally. Similar to the observations described in Charron *et al*. (1996), switching off of the illumination (480 to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) led to emissions of LOX products. This behavior was not observed when the lamps were turned off sequentially within a time period of 1 hour which was our way to simulate twilight. This behavior was also not observed for all plant species. Pine, canola, tobacco cv. Bel B and cv. Bel

*Table IV.* Overview of measurements with stress applied to plants

| Plant species | *n* | Remarks |
|---|---|---|
| **LOX products observed, *n* = number of observations** | | |
| Tobacco cv. Bel W3, 4 plants | 60 | Ozone exposure, 5 h, 175 ppb |
| Tobacco cv. Bel W3, 4 plants | 77 | Ozone exposure, 11 h, 150 ppb |
| Tobacco cv. Bel W3, 4 plants | 39 | Pseudomonas infection |
| Tobacco cv. Bel W3, 3 plants | 61 | Pseudomonas infection |
| Tobacco cv. Bel W3, 1 plant | 2 | Wounding |
| Corn, 6 plants | 74 | Ozone exposure, 2 d, 200 ppb |
| Pine, 4 plants | 81 | Pathogen attack |
| Tomato, 6 plants | 102 | Ozone exposure, 24 h, 150 ppb |
| Sunflower, 1 plant | 24 | 5 experiments light off , PAR: 480 → 0 |
| Broad bean, 3 plants | 3 | 1 experiment light off, PAR: 480 → 0 |
| **Stress applied to the plants but no observation of LOX products, *n* = number of observations** | | |
| Tobacco cv. Bel W3, 4 plants | 47 | Control experiment/infiltration of solution without pathogens |
| Tobacco cv. Bel B, 4 plants | 18 | Ozone exposure, 5 h, 150 ppb |
| Pine, 1 plant | 119 | Insect attack (fam. *Pseudococcidae*) |
| Pine, 4 plants | 596 | Ozone exposure, 46 d, 5 to 100 ppb |
| Canola, 4 plants | 73 | Ozone exposure, 3 d, 130 ppb |
| Sunflower, 1 plant | 94 | Ozone exposure, 4 d, 40 to 95 ppb |

W3 were not responsive. Sunflower and broad bean emitted large amounts of LOX products after such a fast light-dark transition.

Such sudden light-dark transitions do not occur naturally. Hence, we believe that it is unlikely that these processes occur in nature. We also do not know the internal processes in the plant leading to this behavior. However, we used this effect as a tool to induce emissions of LOX products. No visible damage was observed on the leaves of sunflower and broad bean after such fast light-dark transitions allowing to induce the emissions of LOX products repeatedly.

## 3. Results

Under control conditions the investigated plants emitted mainly mono- and sesquiterpenes. Emissions of LOX products were not detectable. Following several stress applications large OVOC emissions were observed. An overview over the measurements with stress application to plants is given in Table IV.

Emissions of LOX products were frequently found. But there were also cases where stress application did not lead to emission of LOX products. No LOX prod-

ucts were emitted by pine plants exposed to ozone at ambient concentrations over a time period of about 6 weeks (5 to 80 ppb and a maximum pulse of about 100 ppb for 3 hours), the ozone tolerant tobacco variety Bel B, sunflower, and canola plants exposed to ozone at concentrations between 100 and 150 ppb for time periods between 5 hours and 4 days.

In cases where ozone exposure led to the emissions of LOX products necrotic spots (approx. 20% of the leaf area) became visible by bare eye one day after the treatment. In cases where ozone exposure did not lead to the emissions of LOX also no visible symptoms were observed. Both observations suggest a relation between emissions of LOX products and leaf injury.

### 3.1. TEMPORAL SHAPE OF EMISSIONS

The emissions of LOX products were transient. Figure 1 shows the temporal shapes of the (Z)-3-hexenol emissions from different plant species and for different treatments.

The emissions of (Z)-3-hexenol occurred in one large pulse lasting less than 24 h except for corn where a second pulse was observed. However, the amount of emissions during the second pulse was by far lower than that measured during the first pulse.

Differences were observed with respect to the time lag between stress application and beginning of the (Z)-3-hexenol emissions. The emissions occurred with a time lag of up to 15 h after starting the ozone exposure. After a sudden light-dark transition the emissions started without a measurable time delay. Another obvious difference was found for the temporal shape. After ozone exposure or infiltration of tobacco plants with pathogens the emissions increased on a time scale of hours. Such an increase was not measurable within the time resolution of our set up after a fast light-dark transition.

In order to compare the temporal behavior of these emissions quantitatively, the temporal shapes of emissions after ozone exposure or after pathogen attack on tobacco were fitted to Equation (4):

$$\frac{\Phi}{\Phi_{\max}} = A \cdot \left[\exp\left(-k_1 \cdot t\right) - \exp\left(-k_2 \cdot t\right)\right] . \tag{4}$$

In Equation (4), $\Phi$ is the actual emission rate, $\Phi_{\max}$ is the maximum emission rate measured during the pulse, $A$ is a scaling factor used to achieve $\Phi = \Phi_{\max}$ at the time $t$ [h] where the maximum emission was reached. $k_1$ and $k_2$ are empirical parameters that describe the temporal shape of the emission pulse (both in units of $h^{-1}$). The data obtained during the first 10 to 20 hours of the emissions were used for the fits.

Because fast light-dark transitions produced emissions from sunflower and broad bean with maximum rates during the first chromatographic run after the

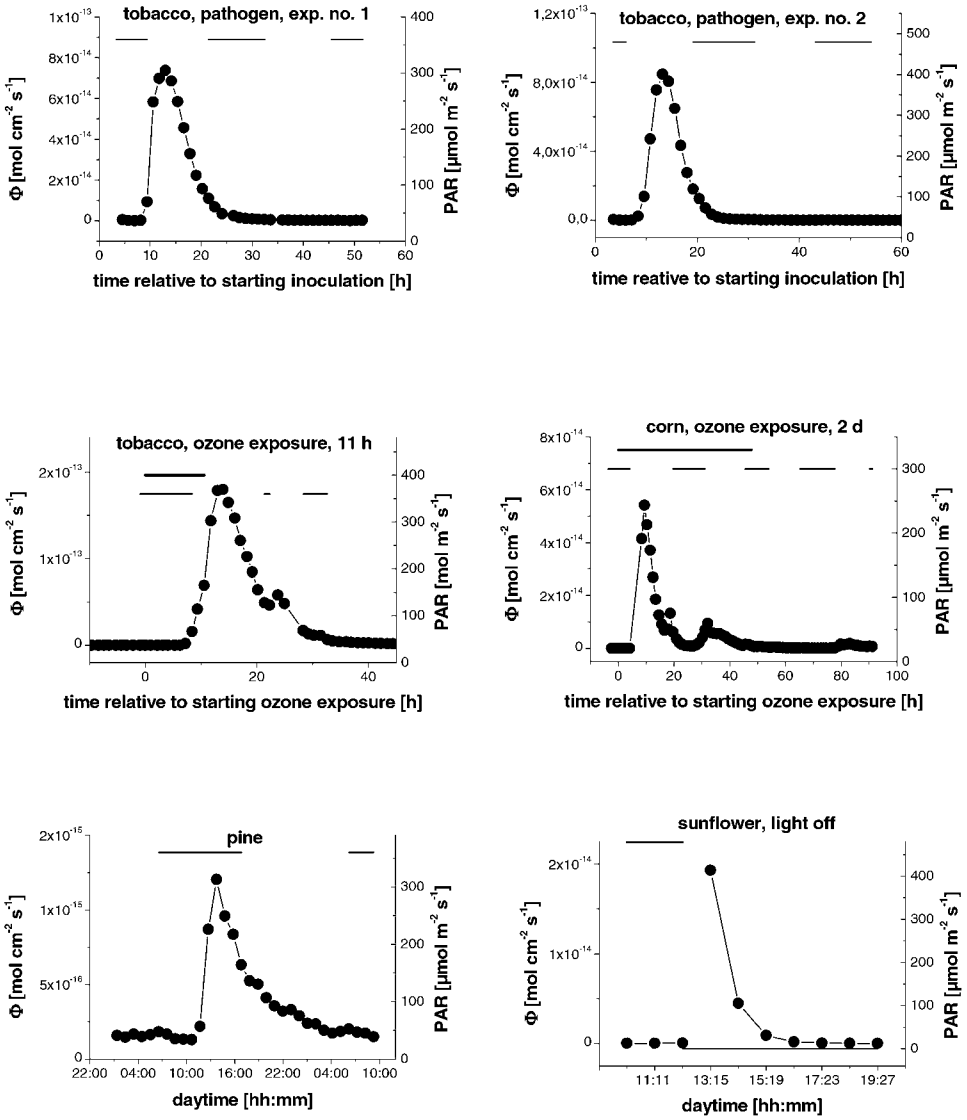


*Figure 1.* Temporal shape of the (Z)-3-hexenol emissions from different plant species. The black bars indicate periods of illumination (right scale). For ozone exposure the duration of the ozone exposure is indicated by the upper black bar.

lamps were turned off, the temporal shape of the decay of these emissions was fitted to a single exponential decay:

$$\frac{\Phi}{\Phi_{\max}} = \exp\left(-k_2 \cdot t\right) \ . \tag{5}$$

In a second step, Equation (4) was used for the fits. $k_1$ was fixed arbitrarily to values between 10 and 100. The results obtained for $k_2$ were the same as those

*Table V.* Results of fits for (Z)-3-hexenol; $N$ = number of data points used for the fits

| Plant species | $k_1$ [$h^{-1}$] | $k_2$ [$h^{-1}$] | $N$ | $R^2$ |
|---|---|---|---|---|
| Tobacco cv. Bel W3, 5 h $O_3$ | $0.25 \pm 0.01$ | $0.27 \pm 0.02$ | 18 | 0.92 |
| Tobacco cv. Bel W3, 11 h $O_3$ | $0.30 \pm 0.005$ | $0.28 \pm 0.005$ | 13 | 0.98 |
| Tobacco cv. Bel W3, pathogen attack | $0.42 \pm 0.01$ | $0.43 \pm 0.01$ | 17 | 0.98 |
| Tobacco cv. Bel W3, pathogen attack | $0.34 \pm 0.013$ | $0.36 \pm 0.014$ | 20 | 0.94 |
| Corn, 2 d $O_3$ | $0.39 \pm 0.02$ | $0.40 \pm 0.02$ | 18 | 0.93 |
| Tomato, 24 h $O_3$ | $0.17 \pm 0.04$ | $0.18 \pm 0.05$ | 16 | 0.47 |
| Pine, pathogen attack, Equation (4) | $1.10 \pm 0.007$ | $0.23 \pm 0.06$ | 20 | 0.92 |
| Pine, pathogen attack, Equation (5) | – | $0.20 \pm 0.007$ | 16 | 0.97 |
| Sunflower, light off | – | $2.10 \pm 0.001$ | 3 | 0.99 |
| Sunflower, light off | – | $1.40 \pm 0.003$ | 4 | 0.99 |
| Sunflower, light off | – | $1.90 \pm 0025$ | 5 | 0.99 |
| Sunflower, light off | – | $1.50 \pm 0.05$ | 5 | 0.84 |
| Sunflower, light off | – | $1.50 \pm 0.014$ | 4 | 0.99 |
| Sunflower, light off | – | $1.80 \pm 0.04$ | 4 | 0.96 |
| Broad bean, light off | – | $1.60 \pm 0.06$ | 5 | 0.98 |

obtained from the fits to a single exponential decay within the error limits. Table V summarizes the results obtained from the fits.

In Equation (4), the parameter $A$ is very sensitive to the difference between $k_1$ and $k_2$. The numerical values of $k_1$ and $k_2$ were very similar causing large error limits in $A$, and vice versa in $k_1$, and $k_2$. The error limits listed in Table V for $k_1$ and $k_2$ were determined after the fits had converged and the numerical value for $A$ was fixed. Data for $A$ are not given here.

Data with respect to the temporal behavior of the emissions after wounding could not be analyzed because the emissions were only observed for 2 chromatographic runs.

In most cases the emission rates of the other LOX products produced from 18:3 showed a similar temporal behavior than those of (Z)-3-hexenol. However, in some cases time shifts were observed between the maximum emission rates of individual OVOCs. Figure 2 shows the temporal shapes of the (Z)-3-hexenol, (E)-3-hexenol, and (E)-2-hexenal emissions from pathogen infected tobacco plants. Probably due to the low time resolution of our experiments these shifts were not always observed. Despite of these time shifts the shapes of the pulses were similar. As an example, Table VI shows the data obtained for tobacco.

We related the emissions of the OVOCs to those of (Z)-3-hexenol. As expected, the emission rates of the other $C_6$-OVOCs listed in Table I correlated very well with those of (Z)-3-hexenol. Figure 3 shows representative examples for (E)-2-hexenal, 1-hexanol and (Z)-3-hexenylacetate. In cases where time shifts were observed for

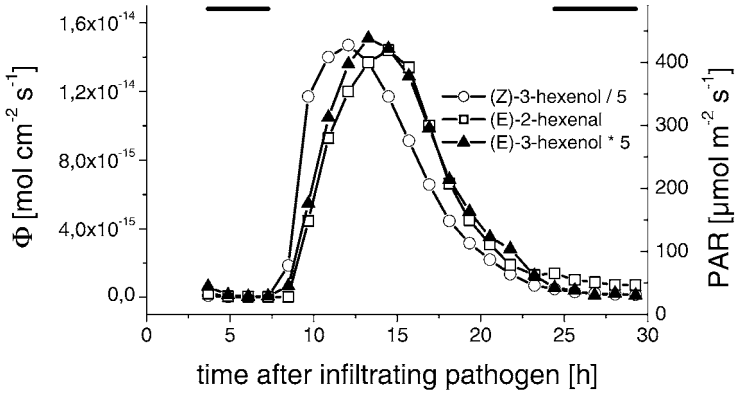


*Figure 2.* Temporal shape of the emissions of (Z)-3-hexenol (open circles, data divided by 5), (E)-3-hexenol (triangles, data multiplied by 10) and (E)-2-hexenal (closed circles). Emissions from pathogen infected tobacco plants. Bars indicate periods of illumination (right scale).

*Table VI.* Results of fits to Equation (4) for individual LOX products

| Plant species | Compound | $k_1$ [$h^{-1}$] | $k_2$ [$h^{-1}$] | $r^2$ |
|---|---|---|---|---|
| Tobacco, | (Z)-3-Hexenal | 0.40 ± 0.014 | 0.41 ± 0.02 | 0.93 |
| cv. Bel W3 | (Z)-3-Hexenol | 0.30 ± 0.005 | 0.28 ± 0.005 | 0.98 |
| 11 h $O_3$ | (E)-3-Hexenol | 0.44 ± 0.02 | 0.57 ± 0.02 | 0.97 |
| | (E)-2-Hexenol | 0.33 ± 0.014 | 0.03 ± 0.015 | 0.96 |
| | (E)-2-Hexenal | 0.37 ± 0.015 | 0.37 ± 0.015 | 0.91 |
| | Hexanal | 0.27 ± 0.014 | 0.34 ± 0.03 | 0.91 |
| | 1-Hexanol | 0.36 ± 0.015 | 0.37 ± 0.014 | 0.93 |
| Tobacco | (Z)-3-Hexenal | 0.37 ± 0.02 | 0.38 ± 0.02 | 0.89 |
| cv. Bel W3, | (Z)-3-Hexenol | 0.42 ± 0.01 | 0.43 ± 0.01 | 0.98 |
| pathogen attack | (E)-3-Hexenol | 0.32 ± 0.01 | 0.34 ± 0.01 | 0.98 |
| experiment No. 1 | (E)-2-Hexenal | 0.31 ± 0.02 | 0.33 ± 0.02 | 0.96 |
| | (E)-2-Hexenol | 0.29 ± 0.01 | 0.30 ± 0.01 | 0.96 |
| | Hexanal | 0.41 ± 0.03 | 0.43 ± 0.03 | 0.94 |
| | 1-Hexanol | 0.31 ± 0.01 | 0.32 ± 0.01 | 0.98 |
| Tobacco | (Z)-3-Hexenal | 0.68 ± 0.04 | 0.68 ± 0.04 | 0.94 |
| cv. Bel W3 | (Z)-3-Hexenol | 0.34 ± 0.013 | 0.36 ± 0.014 | 0.94 |
| pathogen attack | (E)-3-Hexenol | 0.38 ± 0.015 | 0.38 ± 0.015 | 0.97 |
| experiment No.2 | (E)-2-Hexenol | 0.33 ± 0.013 | 0.33 ± 0.013 | 0.97 |
| | (E)-2-Hexenal | 0.49 ± 0.02 | 0.49 ± 0.02 | 0.96 |
| | Hexanal | 0.38 ± 0.016 | 0.38 ± 0.016 | 0.97 |
| | 1-Hexanol | 0.31 ± 0.02 | 0.31 ± 0.02 | 0.91 |

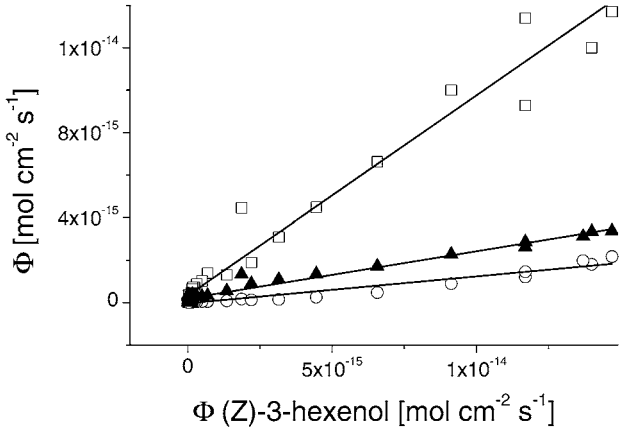


*Figure 3.* Plot of the emission rates of (E)-2-hexenal (open squares), 1-hexanol (triangles), and (Z)-3-hexenylacetate (open circles) versus the emission rates of (Z)-3-hexenol. The data for (E)-2-hexenal are shifted versus those of (Z)-3-hexenol for 1 hour. The lines show results from fits.

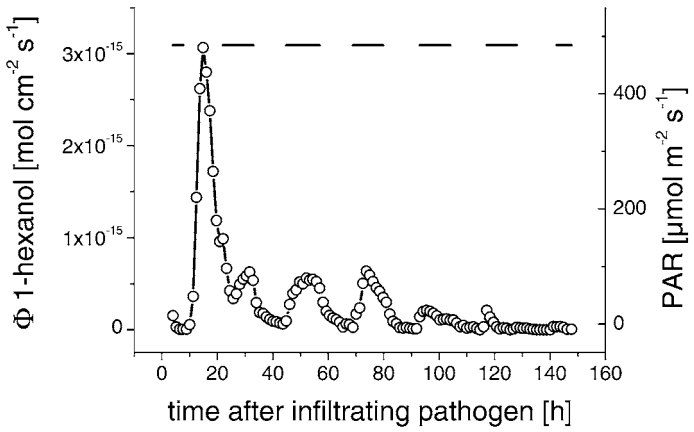


*Figure 4.* Temporal shape of the 1-hexanol emissions over a time period of 6 days. Bars indicate periods of illumination (right scale). Emission from pathogen infected tobacco.

the emissions of individual LOX products, the data measured for these OVOCs were shifted versus those of (Z)-3-hexenol for 1 or 2 hours to obtain optimum correlation.

In some cases differences were observed between the temporal behavior of the emissions of OVOCs produced from linoleic acid (18:2) and linolenic acid (18:3), respectively. Whereas the emissions of compounds produced from 18:3 were nearly zero one day after stress application those of the compounds produced from 18:2 were emitted for a longer time. This behavior was very pronounced for the pathogen infected tobacco plants. Figure 4 depicts the temporal shape of the 1-hexanol emission from pathogen infected tobacco plants.

During the first pulse the shapes of the compounds produced from 18:2 were similar to those produced from 18:3. Thereafter, the emissions of 18:2 products showed a diurnal variation. During daytime (PAR = 480 $\mu$mol $\cdot$ m$^{-2}$ $\cdot$ s$^{-1}$, T = 27 °C) the emissions were larger than during nighttime (T = 23 °C). These diurnal variations implied that after the first pulse the emissions were affected by light intensity or by temperature. Nevertheless, more than 80% of the total emissions of LOX products occurred within 24 h. Therefore, we focused on the first pulse.

### 3.2. ABSOLUTE EMISSION RATES

Because more than 80% of the emissions occurred within one day, a rough estimation for the total amount of an emitted compound was obtained using Equation (6):

$$\Phi_{24} = 3600 \cdot \sum_{24\ \mathrm{h}} \Phi. \tag{6}$$

In Equation (6), $\Phi_{24}$ is the amount of compounds emitted within 24 hours by 1 square centimeter leaf area (units of $\Phi_{24}$: mol $\cdot$ cm$^{-2}$ $\cdot$ 24 h$^{-1}$).

During the first pulse one of the $C_6$-compounds was the predominantly emitted OVOC for a given species. We determined this compound as the main LOX compound. Dependent on the plant species the emission of the main compound contributed between 30 and 76% to the emissions of all $C_6$-compounds.

Table VII lists the results obtained with respect to the maximum of the emission rates for the main LOX compound, $\Phi_{max}$ (mean for the time of one chromatographic run, 1 h), $\Phi_{24}$ for the main LOX compound, for the sum of all $C_6$-compounds, $\Sigma\Phi_{24}$, and the ratio $\Phi_{24}/\Sigma\Phi_{24}$ for the main compound.

As can be seen in Table VII, the range of maximum emission rates as well as the total amount of emitted $C_6$-compounds differed by up to a factor of 30 between the species and treatments. Also for the sunflower treated with fast light-dark transitions the maximum emission rates and the total amount of emissions varied by about a factor of 4.

### 3.3. EMISSION PATTERN

The tobacco variety Bel W3 was exposed to different stress factors, high ozone concentrations, pathogen infection, and wounding. Figure 5 shows examples for chromatograms taken for tobacco during or after stress application. The emission patterns were very similar.

For other plant species the emission patterns were different. Figure 6 shows the pattern of the $C_6$ compounds for tobacco, corn and tomato, respectively. While tomato and corn emitted more aldehydes than alcohols, tobacco emitted more alcohols than aldehydes.

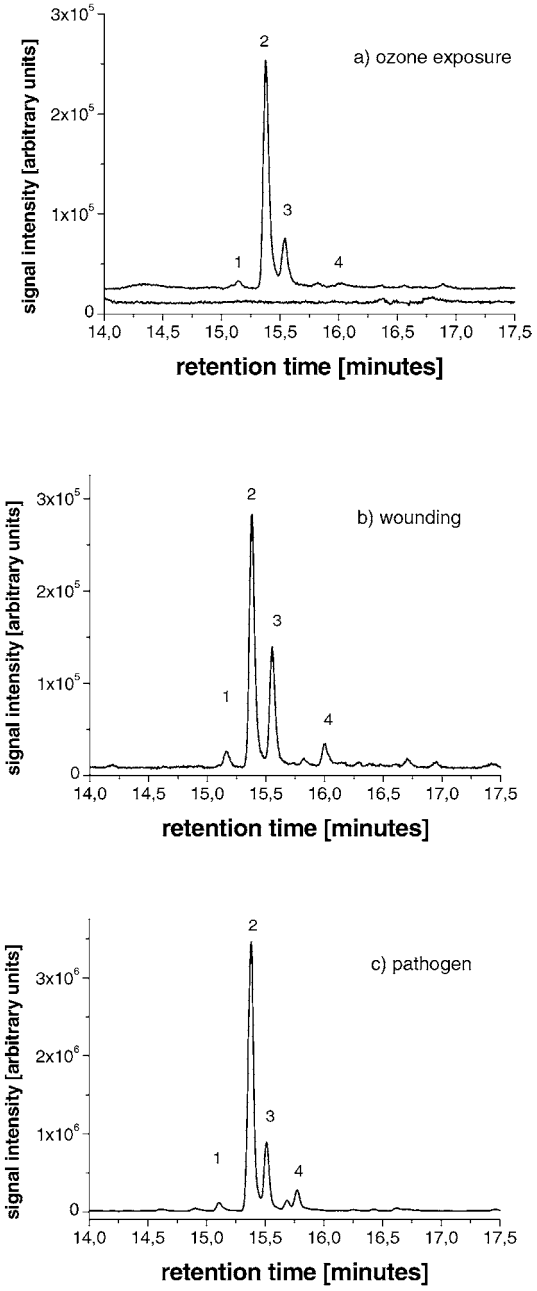


*Figure 5.* Chromatograms taken for tobacco in the total ion mode. 1: (E)-3-hexenol, 2: (Z)-3-hexenol, 3: (E)-2-hexenal, 4: 1-hexanol. (a) ozone exposure (upper trace, shifted for clarity) and chromatogram taken before ozone exposure (lower trace), (b) wounding, (c) pathogen infection.

*Table VII.* Compounds emitted in largest quantities (main compound, MC); peak emission rates for these compounds ($\Phi_{max}$(MC) [mol · cm$^{-2}$ · s$^{-1}$] · $10^{15}$); sum of emissions over a time period of 24 hours for the main compound $\Phi_{24}$ (MC) and for the sum of the $C_6$ compounds listed in Table I [mol cm$^{-2}$ · 24 h$^{-1}$] · $10^{10}$ and the ratio $\Phi_{24}$(MC)/$\Phi_{24}$($\Sigma C_6$)

| Plant species | MC | $\Phi_{max}$ (MC) | $\Phi_{24}$ (MC) | $\Phi_{24}$ ($\Sigma C_6$) | $\Phi_{24}$(MC)/ $\Phi_{24}$ $\Sigma C_6$ |
|---|---|---|---|---|---|
| Tobacco Bel W3, $O_3$ | (Z)-3-Hexenol | 92 | 47 | 76 | 0.62 |
| Tobacco Bel W3, $O_3$ | (Z)-3-Hexenol | 40 | 13 | 17 | 0.76 |
| Tobacco, Bel W3, pathogen | (Z)-3-Hexenol | 74 | 17 | 25 | 0.68 |
| Tobacco, Bel W3, pathogen | (Z)-3-Hexenol | 84 | 9.6 | 16.7 | 0.57 |
| Tobacco, Bel W3 wounding | (Z)-3-Hexenol | 29 | 1 | 2 | 0.5 |
| Corn, $O_3$[a] | Hexenylacetate | 124 | 34 | 113 | 0.3 |
| Pine, pathogen/3 days | (Z)-3-Hexenol | 4 | 3.1 | 6.9 | 0.45 |
| Tomato, $O_3$[a] | (E)-2-Hexenal | 47 | 22 | 32 | 0.69 |
| Sunflower, light off | (Z)-3-Hexenol | 34 | 1.4 | 1.9 | 0.74 |
| Sunflower, light off | (Z)-3-Hexenol | 122 | 5.8 | 10.2 | 0.57 |
| Sunflower, light off | (Z)-3-Hexenol | 99 | 4.6 | 8.9 | 0.52 |
| Sunflower, light off | (Z)-3-Hexenol | 41 | 1.9 | 3.8 | 0.5 |
| Sunflower, light off | (Z)-3-Hexenol | 96 | 4.5 | 7.5 | 0.6 |
| Sunflower, light off | (Z)-3-Hexenol | 39 | 1.7 | 5.4 | 0.3 |
| Broad bean, light off[b] | (Z)-3-Hexenal | – | – | – | 0.54 |

[a] Measurements during exposure to high ozone concentrations, errors may exceed 15%.
[b] For broad bean the leaf area was not determined, no absolute emission rates given here.

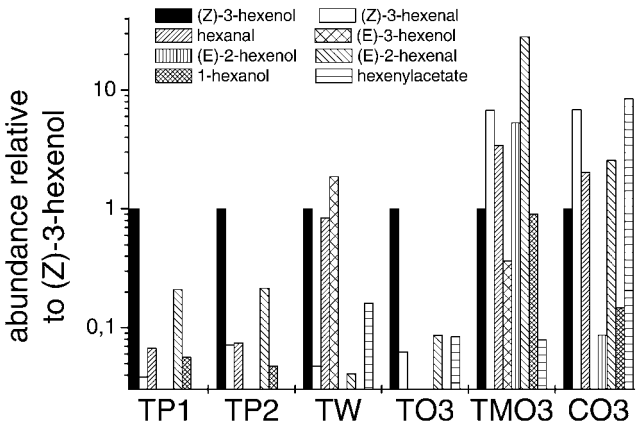


*Figure 6.* Emission pattern of LOX products normalized to the emission of (Z)-3-hexenol (black bars = 1). TP1 = tobacco, pathogen infection, experiment No. 1; TP2 = tobacco, pathogen infection, experiment No. 2; TW = tobacco, wounding; TO3 = tobacco, ozone exposure; TMO3 = tomato, ozone exposure; CO3 = corn, ozone exposure.

*Table VIII.* Relative abundance of 18:3 and 18:2 products

| Plant species | 18:3/18:2 |
|---|---|
| Tobacco cv. Bel W3, 5 h $O_3$ | 52 |
| Tobacco cv. Bel W3, 11 h $O_3$ | 25 |
| Tobacco cv. Bel W3, pathogen | 39 |
| Tobacco cv. Bel W3, pathogen | 18 |
| Tobacco cv. Bel W3, wounding | 7.6 |
| Corn, 2 d $O_3$ | 11 |
| Pine, pathogen | 2 |
| Tomato, 24 h $O_3$ | 10 |
| Sunflower, light off | >50 |
| Broad bean, light off | 17 |

We distinguished between emissions of compounds formed from the fatty acids 18:2 and 18:3, respectively (Table I). The relative emissions of compounds produced from 18:3 and 18:2, respectively were determined using the ratio:

$$\frac{\sum_{18:3} \Phi_{24}}{\sum_{18:2} \Phi_{24}}, \tag{7}$$

where $\sum_{18:3}$ is the sum of the compounds formed from 18:3 and $\sum_{18:2}$ is the sum of the compounds formed from 18:2, respectively. Table VIII shows the results. Nearly all plant species with the exception of pathogen-infected Scots pine (ratio 2) and wounded tobacco (ratio 7.6), yielded a high abundance (between 10 and 50-fold) of 18:3 to 18:2 products. Nevertheless, the ratios given in Table VIII show a high variability from plant species to plant species and also within the same species.

The relative emissions of alcohols and aldehydes was determined according to the ratios (8) and (9) for the compounds formed from 18:2 and from 18:3, respectively. The amounts of emitted (Z)-3-hexenylacetate were added to those of the alcohols because it is produced from the corresponding alcohol.

$$\frac{\Phi_{24}^{\text{hexanal}}}{\Phi_{24}^{1-\text{hexanol}}} \tag{8}$$

$$\frac{\sum_{\text{aldehyde}} \Phi_{24}}{\sum_{\text{alcohol + acetate}} \Phi_{24}}. \tag{9}$$

*Table IX.* Ratio of emission rates of aldehydes/alcohols produced from 18:2 and from 18:3 calculated using ratios (8) and (9), respectively

| Plant species | 18:2 | 18:3 |
|---|---|---|
| Tobacco cv. Bel W3, 5 h $O_3$ | 3.3 | 0.38 |
| Tobacco cv. Bel W3, 11 h $O_3$ | 0.9 | 0.18 |
| Tobacco cv. Bel W3, pathogen | 0.88 | 0.36 |
| Tobacco cv. Bel W3, pathogen | 1 | 0.13 |
| Tobacco cv. Bel W3, wounding | 9.5 | 1.4 |
| Corn, 2 d $O_3$ | 13 | 1.1 |
| Pine, pathogen | 1.6 | 0.26 |
| Tomato, 24 h $O_3$ | 3.1 | 17 |
| Sunflower, light off | – | 1.1 |
| Broad bean, light off | 4.6 | 2.1 |

$\sum_{\text{aldehyde}}$ represents the sum of the aldehydes formed from 18:3 and $\sum_{\text{alcohol + acetate}}$ represents the sum of the alcohols and acetate formed from 18:3, respectively. As shown in Table IX, the relative abundance of aldehydes over alcohols was high for 18:2 products in wounded tobacco and ozone-treated corn plants. Similar values for ozone- and pathogen-treated plants were found for the 18:3 products, with the exception of a high proportion of aldehydes emitted from ozone-exposed tomato.

Although the emission pattern differed significantly for different plant species, the same LOX products were emitted from all plants. Besides emissions of these $C_6$ compounds also those of LOX products containing 9 C-atoms should be expected. However, the $C_9$ compounds listed in Table I were only emitted in trace amounts. But several other OVOCs not listed in Table I were emitted. Because the emissions of the $C_6$ compounds occurred as a pulse and the temporal shapes of these pulses were very similar it was possible to estimate which OVOC emissions may be related to this pathway.

We correlated the peak areas of these tentatively identified compounds to those of (Z)-3-hexenol (Table X). Using the data obtained during the first emission pulse, the emissions of all compounds correlated with that of (Z)-3-hexenol. For the data of some of these compounds it was again necessary to introduce a time shift to obtain optimal correlation. The good relations suggest a connection between these emissions and lipoxygenase activity or, more general, with lipid peroxidation under abiotic and biotic stress.

No calibration was conducted for most of the compounds listed in Table X. However, assuming a similar response of our analytical system for these compounds and the $C_6$-OVOCs, we estimated the emission strength of these compounds in relation to those of (Z)-3-hexenol. During the first pulse, the emission

*Table X.* List of compounds coemitted with (Z)-3-hexenol; $r^2$ = correlation coefficient; $\Delta t$ = time shifts needed for optimal correlation coefficients; A = amount of emissions estimated for these compounds given in % of the (Z)-3-Hexenol emissions (first pulse, tobacco, pathogen attack, 2nd experiment); except of (E,E)-2,4-hexadienal the compounds are only tentatively identified

| Compound | $r^2$ | $\Delta t$ [h] | A [%] |
|---|---|---|---|
| 2-Ethylfuran | 0.97 | +1 | 3.0 |
| (E,E)-2,4-Hexadienal | 0.95 | 0 | 3.1 |
| Hexadienal isomer | 0.94 | 0 | 6.9 |
| 3-Hexenylbutanoate | 0.91 | +1 | 0.2 |
| Methylcyclohexenone | 0.92 | +1 | 0.3 |
| 1,3-Pentadiene | 0.75 | +1 | 0.7 |
| 1,4-Pentadiene | 0.76 | +1 | 0.3 |
| 1-Penten-3-ol | 0.89 | 0 | 6.9 |
| 1-Penten-3-one | 0.90 | 0 | 2.1 |
| 2-Pentenal-isomer No. I | 0.94 | 0 | 0.3 |
| 2-Pentenal-isomer No. II | 0.94 | +1 | 1.6 |
| 2-Penten-1-ol | 0.92 | +1 | 2.1 |
| 4,4-Dimethyl-(E)-2-hexene | 0.94 | 0 | 0.5 |

rates of the compounds listed in Table X would contribute less than 25 % to the total emissions of LOX products. Therefore, we concentrated our efforts on the $C_6$-OVOCs listed in Table I.

It has to be mentioned that in case of pathogen infected tobacco plants it was observed that especially the emissions of $C_5$ compounds lasted longer than those of the $C_6$ compounds produced from 18:3. After the first pulse the emissions of the $C_5$ compounds showed a pronounced diurnal variation similar to that of hexanal and 1-hexanol. Because the emission rates of the $C_6$ compounds produced from 18:3 were near to zero one day after the large pulse, the emissions of $C_5$ compounds exceeded those of the $C_6$ compounds. As an example Figure 7 shows the emissions of 2-pentenal isomers from pathogen infected tobacco.

## 4. Discussion

Following abiotic or biotic stress situations, typical LOX products were emitted from several plant species. The biosynthetic pathways leading to these emissions are well known (e.g., Hatanaka, 1993; Croft *et al.*, 1993; Gardner, 1995). Figure 8 shows a schematic of the processes leading to the plant internal formation of the $C_6$ compounds produced from 18:3.

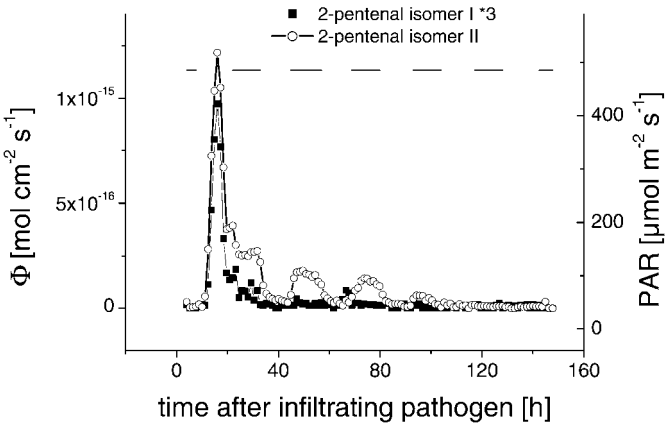


*Figure 7.* Temporal shape of 2-pentenal emissions of over a time period of 6 days. From our data it was impossible to distinguish between both isomers. Bars indicate periods of illumination (right scale). Emission from tobacco cv. Bel W3 infected by pathogen. No direct calibration was performed for these compounds and therefore the error in the absolute emission rates may be up to 50%.

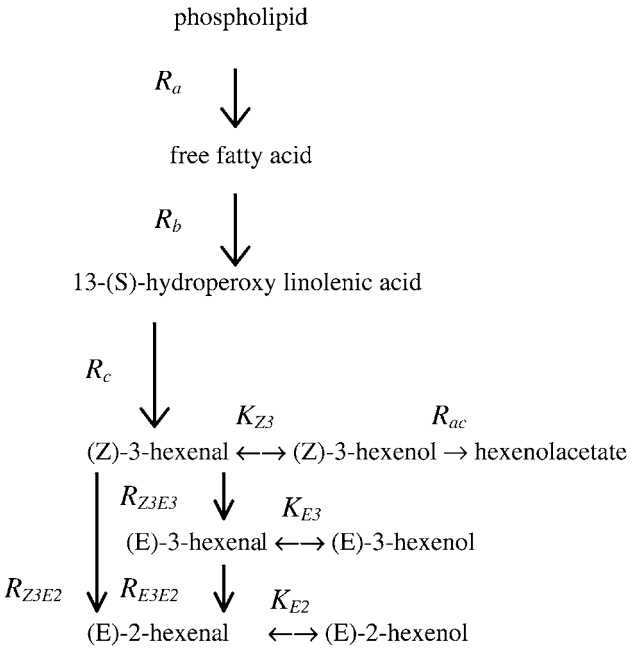


*Figure 8.* Schematic of the processes leading to the emissions of $C_6$ compounds produced from 18:3 (adapted from Croft *et al.*, 1993). Instead of the enzymes, reaction rates are introduced. $R_a$ = production of free 18:3; $R_b$ = oxidation, $R_c$ = decomposition, $R_{ac}$ = transformation of (Z)-3-hexenol to hexenylacetate. $K_{Z3}$, $K_{E3}$, and $K_{E2}$ = equilibrium constants between aldehydes and the respective alcohols, $R_{Z3E3}$, $R_{Z3E2}$, and $R_{E3E2}$ = isomerization rates.

Differences were observed in the emission patterns for different plant species. Qualitatively, these differences can be understood. Free 18:2 and 18:3 are produced from phospholipids. For different plant species, these phospholipids can contain different amounts of 18:2 and 18:3. Moreover, stress can induce different LOX isoforms, which also can be different for different plant species (e.g., Vick and Zimmerman, 1987; Hildebrand, 1989). Hence, different plant species may emit different relative amounts of compounds produced from 18:2 and 18:3, respectively.

The relative amounts of aldehydes and the respective alcohols are determined by the activities of the enzymes converting the aldehydes to the alcohols and vice versa. Also the activity of these enzyme systems can be different in different plant species and therefore, different plant species can emit different relative amounts of aldehydes or alcohols.

We also observed time shifts between the maxima of the emissions of individual LOX products. As described by Croft *et al*. (1993), (Z)-3-hexenal is the $C_6$-aldehyde produced in the first step from 18:3. Thereafter, (E)-3-hexenal as well as (E)-2-hexenal are produced in parallel from (Z)-3-hexenal by different isomerase enzymes. The transformation of one compound into another is dependent on the transformation rate (Figure 8: $R_{Z3E3}$, $R_{Z3E2}$, and $R_{E3E2}$). Hence, also the differences in the temporal behavior of the emissions of individual LOX products (Figure 2) are understandable.

In order to compare the temporal shapes of the OVOC emissions we used Equation (4). Equation (4) is similar to the formula describing the concentration of an intermediate product in a consecutive reaction according to pseudo first order kinetics.

The emission of the primary $C_6$ products (hexanal and (Z)-3-hexenal for 18:2 and 18:3, respectively) is a reaction sequence consisting of 4 steps: Formation of free fatty acids ($R_a$), oxidation of free fatty acids ($R_b$), decomposition of the hydroperoxide formed by this oxidation ($R_c$), and the emission process of the primary product. These steps may be considered as consecutive reactions. Hence, the use of Equation (4) is related to the sequential enzymatic processes in the plants that lead to the OVOC emissions.

Since there are more than 2 steps in the reaction sequence, our description according to Equation (4) is a simplification. However, in order to keep Equation (4) as simple as possible we use this formulation. Figure 9 shows the temporal shape of the (Z)-3-hexenol emission from ozone exposed tobacco plants and the result from the fit.

Equation (4) cannot describe the smooth increase of the emission rates during the first hours of emissions. Nevertheless, the main part of the temporal behavior of the emissions is described quite well.

The secondary $C_6$ products are formed by isomerization steps and by alcohol dehydrogenase enzymes. These additional steps can produce the observed differences between the temporal behavior of the emissions.

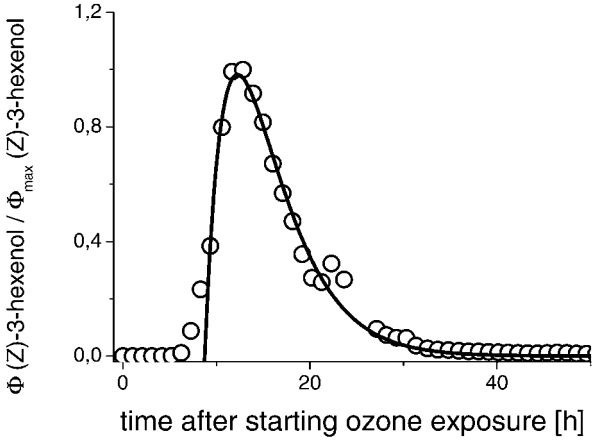


*Figure 9.* Temporal shape of normalized (Z)-3-hexenol emissions from ozone exposed tobacco plants. The line is the result from a fit according to Equation (4).

A different temporal behavior between emissions of individual LOX products has been observed before (Hatanaka, 1993; Fall *et al.*, 1999). Fall *et al.* 1999 show very different temporal shapes for the primary product (Z)-3-hexenal and the secondary LOX product (Z)-3-hexenol after cutting (therein Figure 2). The emissions of (Z)-3-hexenal occur rapidly, decrease to low amounts on a time scale of minutes and thereafter decrease on a much longer time scale. Contrary, the emissions of (Z)-3-hexenol increase slower than those of (Z)-3-hexenal. They increase on a time scale similar to that of the first decrease of the (Z)-3-hexenal emissions. This temporal behavior is understandable because (Z)-3-hexenol is formed from (Z)-3-hexenal. The first fast decrease of the (Z)-3-hexenal emissions can be considered as a relaxation to an equilibrium between the plant internal concentrations of (Z)-3-hexenal and (Z)-3-hexenol ($K_{Z3}$).

Our observations on the emissions from plants under pathogen attack or ozone exposure were somewhat different. However, the temporal increase of the emissions observed during our experiments with ozone exposure and pathogen attack occurred on a time scale much longer than the time needed to form an equilibrium between the concentrations of both compounds. Thus, the differences in the temporal behavior of the emissions of these compounds were smeared. In such a case, the ratio of the emissions of (Z)-3-hexenal over those of (Z)-3-hexenol is mainly determined by $K_{Z3}$.

(Z)-3-hexenal is also the precursor for the other secondary LOX products. Therefore, the ratio of the emissions of (Z)-3-hexenol over those of (Z)-3-hexenal is also determined by $R_{Z3E3}$ and $R_{Z3E2}$. Only if the relaxation to the equilibrium occurs much faster than the isomerization steps, $K_{Z3}$ will mainly determine the ratio of the (Z)-3-hexenol emissions over those of (Z)-3-hexenal.

Such a behavior was found for tobacco. The temporal difference between the maxima of the emissions of (Z)-3-hexenol and those of the other secondary LOX

products imply that the isomerization steps are slower than the formation of the equilibrium. Close to equilibrium the concentrations of (Z)-3-hexenol are higher than those of (Z)-3-hexenal and thus, (Z)-3-hexenol is emitted in higher amounts than (Z)-3-hexenal.

Because the isomerization steps are slow compared to the formation of the equilibrium, the yield of (Z)-3-hexenol formation should be higher than the yield for the other secondary LOX products. This behavior was also found for the emissions from tobacco. For tobacco, (Z)-3-hexenol was the main LOX product contributing to more than 50% to the emissions of all $C_6$ compounds.

If the isomerisation steps are slow and furthermore, the conversion of (Z)-3-hexenol to hexenylacetate is very efficient ($R_{ac} \ll R_{Z3E3} + R_{Z3E2}$), hexenylacetate will be the compound emitted in the largest amounts. This behavior was observed for corn. If the isomerization rates are fast i.e., if either $R_{Z3E2}$, or both, $R_{Z3E3}$ and $R_{E3E2}$ are higher than the formation rates of the equilibria, (E)-2-hexenal can be the main LOX product. This was observed for the emissions from tomato.

In summary, the emission pattern of LOX products is determined by the kinetics of the processes in the plants. If there is a long lasting production of the primary product, the differences in the temporal behavior of the emissions will be smeared and the shapes will be similar for individual LOX products. This temporal shape is quite well described by Equation (4).

If both, the production of the primary product and the transformation to secondary LOX products occur on a similar time scale, the temporal behavior of the emissions can be complicated by the transformation steps. However, lumping the emissions of individual LOX products avoids these complications. After lumping it is possible to describe the temporal shapes of short lasting emissions by Equation (4).

As an example, Figure 10 shows the temporal behavior of the emissions of (Z)-3-hexenal, (Z)-3-hexenol, and for the sum of (Z)-3-hexenal, (Z)-3-hexenol, and hexenylacetate. The (Z)-3-hexenal emissions decrease somewhat faster than those of (Z)-3-hexenol. Furthermore, the decay does not strictly follow an exponential behavior. Both is caused by the relaxation of (Z)-3-hexenal and (Z)-3-hexenol concentrations to an equilibrium. Nevertheless, the sum of the emissions of (Z)-3-hexenal, (Z)-3-hexenol, and hexenylacetate shows a good exponential shape ($r^2 = 0.98$) and the decay of the emissions follows a first order kinetics. This can be described by Equation (4).

The emissions of (Z)-3 hexenol also follow a first order kinetics. This behavior is caused by the fact that (Z)-3-hexenol is the main LOX compound. Disturbances of the exponential shapes of the (Z)-3 hexenol emissions by secondary steps are low because the yield of formation for other secondary LOX products is small compared to that of (Z)-3-hexenol.

In the following we neglect the details and look to the integrated temporal behavior of the OVOC emissions. At first, we look to the lag times between stress application and emissions. The data given by Fall *et al.* (1999) show that the emis-

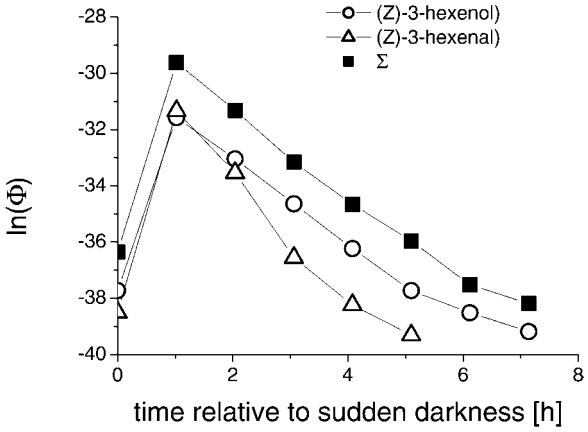


*Figure 10.* Decay of the emissions of (Z)-3-hexenol (open cirles), (Z)-3-Hexenal (open triangles, and of the sum of (Z)-3-hexenol, (Z)-3-hexenal, and hexenylacetate (squares, data shifted by +1 on the y-axis for clarity).

sions occur nearly directly after cutting. Possibly, the response of the plants after a sudden light-dark transition occurs on a similar time scale. The emissions caused by pathogen attack and ozone exposure were observed after a lag time.

LOX enzymes can be constitutive or can be induced (e.g., Rosendahl, 1996) and therefore, emissions caused by different isoforms of the LOX enzymes may show a different temporal behavior. From the quick response of plants to cutting it seems improbable that the emissions caused by cutting are due to an enzyme system that has to be activated. We assume that it is a constitutive LOX enzyme causing the fast response of the plants to cutting.

After ozone exposure or pathogen attack the enzyme system may be induced. The time needed to induce the enzyme system may cause the observed lag times between stress application and emissions. However, it may also be a slow and delayed formation of free fatty acids causing the time delay and the relatively slow increase. From our data we cannot distinguish between these possibilities.

In principle, the time constants $k_1$ and $k_2$ (Equation (4)) given here for the increase and decrease of the emissions of individual LOX products may be determined by the temporal changes of the activities of any of the involved processes. But, from the data given by Hatanaka (1993) and Fall *et al.* (1999) it appears that $R_a$, $R_b$, $R_c$, and the velocity of the emission process itself are very high compared to $k_1$ and $k_2$, respectively at least for the emissions induced by ozone exposure or pathogen attack. Hence, $R_a$, $R_b$, $R_c$, should not determine $k_1$ and $k_2$ in these cases. It seems more probable that temporal changes in $R_a$, $R_b$, or $R_c$ determine $k_1$ and $k_2$.

If $k_1$ and $k_2$ are determined by $\frac{\partial R_a}{\partial t}$, $\frac{\partial R_b}{\partial t}$, or $\frac{\partial R_c}{\partial t}$, the simple model of the emissions as a intermediate product of a consecutive reaction is incomplete. Hence, other formalisms may describe the temporal shape of the emissions better than

Equation (4). For example, a description considering the real shape of $\frac{\partial R_a}{\partial t}$, $\frac{\partial R_b}{\partial t}$, and $\frac{\partial R_c}{\partial t}$ should also describe the smooth temporal increase of the emissions such as observed in some experiments. Nevertheless, such a description would include more parameters than a description using Equation (4) and we do not have sufficient knowledge about the detailed behavior of $\frac{\partial R_a}{\partial t}$, $\frac{\partial R_b}{\partial t}$, and $\frac{\partial R_c}{\partial t}$ to obtain more important information from fits to other functions. In addition, Equation (4) describes the temporal shapes of the emissions quite well and was therefore used.

For the individual sunflower treated repeatedly with the same elicitor of LOX emissions, the emissions decrease on a time scale of about half an hour. The variability in the numerical values determined for $k_2$ is only about 30%. Contrary, the total amount of emissions varies by about a factor of 4. We found no relation between $k_2$ and the total amount of emitted LOX products ($r^2 = 0.25$) implying that, to a good approximation, the decay time of the emissions is independent of the total amount of emitted LOX products.

A consistent explanation for these observations would be:

1. The absolute amount of emitted OVOCs is mainly determined by the amount of free fatty acids produced by the stress application.
2. $\frac{\partial R_a}{\partial t}$, $\frac{\partial R_b}{\partial t}$, or $\frac{\partial R_c}{\partial t}$ which are reflected by $k_2$ are independent of the amount of free fatty acids produced by stress application.

Fall *et al.* (1999) show that the amount of emissions is proportional to the amount of wounding. If the amount of free fatty acids produced by wounding is proportional to the amount of wounding, point 1 would be reasonable.

Arguments in favor of point 2 may be obtained from the results obtained during our measurements. Besides the low variability determined for $k_2$ after sudden light-dark transitions also the values determined for $k_1$ and $k_2$ under ozone exposure and pathogen attack show a fairly low variability. While $k_1$ and $k_2$ vary by less than a factor of 4 and 3, respectively, the absolute amount of emissions varies by more than a factor of 30 (Table VII). Considering the different biological systems and different stress applications it is somewhat surprising that the variability in $k_1$ and $k_2$ is not much higher. However, these observations imply a response of the plant that is somehow adapted to the amount of free fatty acids produced by stress.

De Gouw *et al*. (1999) measured the emissions of LOX products after wounding and drying and showed a biphasic emission kinetics. After a first maximum following wounding, the concentrations decrease for some hours. Thereafter, they increase again and a second maximum occurs about 9 hours after cutting.

A second burst of OVOC emissions after wounding was not observed in our experiment. Only small parts of the leaves were wounded and the plants did not show any symptoms of drying. Since de Gouw *et al*. attribute the second maximum of the emissions to the process of drying, it is understandable that we did not observe a second emission maximum.

Two emission maxima of LOX products such as observed by de Gouw *et al*. cannot be described by Equation (4). But, assuming that LOX activity was induced

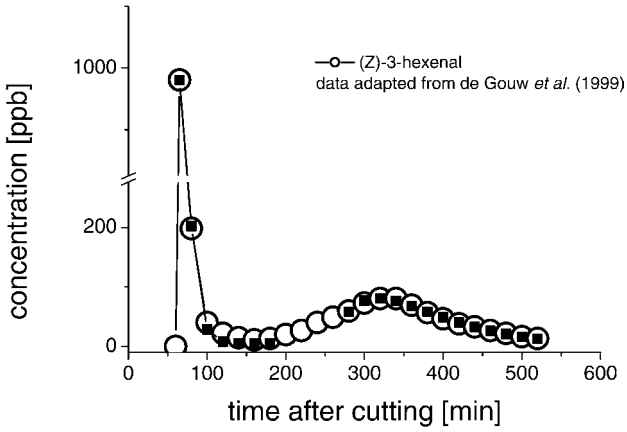


*Figure 11.* Emissions of (Z)-3-hexenal after cutting and during drying (open circles, data adapted from de Gouw *et al.*). The squares give the data points calculated from Equation (4).

twice in the experiments of de Gouw *et al.*, Equation (4) also has to be used twice. Figure 11 shows the temporal shape of the concentrations of (Z)-3-hexenal as given by de Gouw and fitted data according to Equation (4).

Similar to the result shown in Figure 9, the smooth increase before the second maximum cannot be described. However, the main part of the temporal shapes of the emission pulses can be described by using Equation (4) repeatedly. The numerical values obtained for the decay of the emissions after cutting are: $k_2 = 16.8 \pm 0.7$ [$h^{-1}$], and those for the increase and decrease of the emissions caused by drying are: $k_1 = 0.6 \pm 0.05$ and $k_2 = 0.66 \pm 0.04$ [$h^{-1}$], respectively.

The time resolution of our set up is not good enough to evaluate data with respect to cutting. But, the data obtained for the temporal behavior of the emissions after ozone exposure or pathogen attack are comparable to those obtained by de Gouw *et al*. during drying.

While Equation (4) describes the emissions of 18:3 products quite well, the emissions of hexanal and 1-hexanol (Figure 4) and those of the $C_5$ compounds (Figure 7) occurring after the first pulse cannot be described. These emissions show a diurnal variation implying that temperature or photosynthetic active radiation influence these emissions. Neither an influence of temperature nor an influence of photosynthetic radiation on these emissions is considered in Equation (4).

With respect to atmospheric chemistry the total amount of emissions is important. We found that the main amount of OVOCs were emitted within a time period of about 1 day and therefore, we focused our efforts on the first large emission pulse.

We show data obtained from experiments where the plants were exposed to a single and short stress situation. In the environment various environmental stress factors may occur. The effects of these different stress factors may overlap in their

kinetics and their impact on different plant species. Hence, the temporal behavior of the emissions of LOX products from plants covering a large area will be smeared.

Fall *et al*. (2001) measured high atmospheric concentrations of LOX products after a hard freeze event. They show concentrations in the range of several ppb over a time period of 5 days. For a compound produced from 18:3 such as (E)-2-hexenal, this time period is much longer than the emissions observed during our experiments after a single stress application. Possibly, there were several events inducing emissions of LOX products during the measurement period shown by Fall *et al*. which may explain the long lasting emissions.

Fall *et al.* (2001) show also diurnal variations for the concentrations of LOX products including those of $C_5$ compounds. Maximum concentrations were measured during darkness. In many cases we also found the first pulse during the first night after stress application. We cannot explain this behavior, but this behavior could explain the higher atmospheric concentrations of these compounds during darkness. However, concentrations of trace compounds in the atmosphere are determined by the emission strengths, by the mixing of air, and by the removal of the compounds caused by oxidation. During daytime the decrease of OVOC concentrations caused by a fast mixing of air masses and a fast oxidation by hydroxyl radicals may overcompensate higher emission strengths from vegetation.

Such an overcompensation can also explain the differences between the diurnal variations of the atmospheric concentrations measured by Fall *et al*. (2001) and the diurnal variations we observed for the emissions after the first large pulse. After the first pulse the emissions during illumination were higher than during darkness for hexanal, 1-hexanol, and for the $C_5$ compounds.

Although the data given by Fall *et al.* (2001) show that emissions of LOX products from vegetation can lead to high concentrations of these OVOCs very little is known about the impact of emissions from LOX activity on atmospheric chemistry.

During several experiments the emissions of LOX products were not observed although the plants were exposed to stress situations (Table IV). Hence, it requires a certain threshold level of stress to induce these emissions. However, from our data such threshold levels cannot be given. For example, it is not easy to quantify the amount of pathogen attack necessary to induce LOX activity. Also for the abiotic elicitor for these emissions, ozone exposure, general threshold levels are not easy to determine. We exposed plants of the tobacco varieties Bel W3 and Bel B to similar ozone concentrations. Although the uptake rates were similar for both species, Bel W3 did emit LOX products but Bel B did not. Threshold levels for ozone exposure thus depend on the sensitivity of the plant species.

Another reason that complicates extrapolations of our data is the variability in the absolute emission rates (Table VII). This variability may be caused by the normalization procedure. We normalized the absolute emission rates to the total leaf area of the plants. This normalization procedure is arbitrary and is certainly not useful if the emissions predominantly occur from the damaged parts of the leaves.

Free fatty acids are produced at the damaged parts of the leaves and therefore, it would be better to use the amount of free fatty acids produced by the stress for normalization. But this procedure would not help for upscaling. It will not be easy to find out the total amount of free fatty acids produced by stress inside plants covering a large area. Before quantitative information can be given with respect to the impact of LOX products on atmospheric chemistry, more information on the degree, respective threshold levels of stress and an improved normalization factor for the emissions will be required.

With respect to a modeling of these emissions some information can be obtained from our data. The emissions occur as pulses and their shapes are described quite well with Equation (4). With respect to atmospheric chemistry the total amount of emitted compounds is more important than the detailed kinetic steps causing differences in the temporal behavior of the emissions. Hence, for a modeling of the temporal behavior of the emissions lumping different compounds may be allowed.

We lumped data with respect to the precursors of the emitted compounds. The reactivity of the compounds is different for saturated and unsaturated OVOCs, respectively. Saturated and unsaturated OVOCs are produced from 18:2 and 18:3, respectively. Table VIII gives the ratio for these data. Furthermore, alcohols and aldehydes will be emitted in relatively constant amounts if the emissions last much longer than the transformation steps. These ratios are given in Table IX where the data reflect the equilibrium between the emissions of alcohols and aldehydes.

## 5. Summary and Conclusions

While the fundamental biological processes leading to the emissions of LOX products seem to be understood quite well not much is known with respect to the impact of these emissions on atmospheric chemistry. Reasons therefore are: The emissions are transiently induced by stress effects, it requires a hitherto unknown threshold of stress to induce the emissions, and a usable scale up procedure is missing.

Nevertheless, compared to monoterpene emission rates the maximum emission rates of LOX products can be larger by more than 2 orders of magnitude. Within one day, the total amount of emissions of LOX products can be as large as monoterpene emissions from agricultural used plant species over a time period of several weeks. Such large emissions cannot be neglected in emission models.

We found emissions of LOX products from different plant species after different stress applications. There were differences in the emission patterns and in the temporal behavior of the emissions. But all these differences are qualitatively understandable from the basic processes inside the plants. The emissions of these OVOCs are due to a highly regulated process and not to an unspecific lipid peroxidation. We believe that the fundamental processes leading to the emission of LOX products as a response of the plants to stress are basically the same for many plant species and stress factors.

Finally, the similarities between ozone and pathogen responses found here are worth noting. Obviously, ozone exposure of sensitive plants leads to processes which resemble those following pathogen attack in triggering 'hypersensitive' cell death (Croft *et al.*, 1993; Sandermann *et al.*, 1998; Langebartels *et al.*, 2000a, b). The trace gas composition of the atmosphere can alter the behavior of plants in a way similar to that of an attack from the biosphere.

## Acknowledgements

This work was financially supported by the German Ministry for Education, Science, Research and Technology within the project 'Spurenstoffkreisläufe' (grant No. A2b-1) and within the Tropospheric Research Program (grant No. 07TFS23). J. W. thanks Fonds der Chemischen Industrie for financial assistance.

## References

Arey, J., Winer, A. M., Atkinson, R., Aschman, S. M., Long, W. D., and Morrison C. I., 1991: The emissions of (Z)-3-hexen-1-ol, (Z)-3-hexenylacetate and other oxygenated hydrocarbons from agricultural plant species, *Atmos. Environ.* **25**, 1063–1075.

Asher, C. J., 1978: Natural and Synthetic Culture Media for Spermatophytes, in *Handbook Ser. Nutr. Food Sect. G3*, CRC Press Inc., Boca Raton, pp. 575–609.

Atkinson, R., Arey, J., Aschmann, S. M., Corchnoy, S. B., and Shu, Y., 1995: Rate constants for the gas phase reactions of cis-3-hexen-1-ol, cis-3-hexenylacetate, trans 2-hexenal, and linalool with OH and $NO_3$ radicals and $O_3$ at 296 $\pm$ 2 K, and OH radical formation yields from the $O_3$ reactions, *Int. J. Chem. Kinet.* **27**, 941–955.

Buttery, R. G. and Ling, L. C., 1993: Volatile components of tomato fruit and plant parts: Relationship and biogenesis, in R. Teranishi, R. G. Buttery, and H. Sugisawa (eds), *Bioactive Volatile Compounds from Plants, Amer. Chem. Soc.*, Washington, D.C.

Charron, C. S., Cantliffe, D. J., Wheeler, R. M., Manukian, A., and Heath, R. R., 1996: Photosynthetic photon flux, photoperiod, and temperature effects on emissions of (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate from lettuce, *Journal of the American Society for Horticultural Science* **121**, 488–494.

Clausen, P. A. and Wolkoff, P., 1997: Degradation products of Tenax TA formed during sampling and thermal desorption analysis: Indicators of reactive species indoors, *Atmos. Environ.* **31**, 715–725.

Croft, K. P. C., Jüttner, F., and Slusarenko, A. J., 1993: Volatile products of the lipoxygenase pathway evolved from phaseolus vulgaris (L.) leaves inoculated with pseudomonas syringae pv. phaseolicola, *Plant Physiology* **101**, 13–24.

Fall, R., Karl, T., Hansel, A., Jordan, A., and Lindinger, W., 1999: Volatile organic compounds emitted after leaf wounding: On-line analysis by proton-transfer-reaction mass spectrometry, *J. Geophys. Res.* **104**, 15,963–15,974.

Fall, R., Karl, T., Jordan, A., and Lindinger, W., 2001: Biogenic $C_5$ VOCs: Release from leaves after freeze-thaw wounding and occurrence in air at a high mountain observatory, *Atmos. Environ.* **35**, 3905–3916.

Fehsenfeld, F., Calvert, J., Fall, R., Goldan, P., Guenther, A. B., Hewitt, N., Lamb, B., Liu, S., Trainer, M., Westberg, H., and Zimmerman, P., 1992: Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry, *Glob. Biogeochem. Cycles* **6**, 389–430.

Gardner, H. W., 1995: Biological roles and biochemistry of the lipoxygenase pathway, *Hort. Science* **30**, 197–205.

de Gouw, J. A., Howard, C. J., Custer, T. G., and Fall, R., 1999: Emissions of volatile organic compounds from cut grass and clover are enhanced during the drying process, *Geophys. Res. Letts.* **26**, 811–814.

Guenther, A. B., Zimmerman, P. R., Harley, P. C., Monson, R. K., and Fall, R., 1993: Isoprene and monoterpene emission rate variability: Model evaluation and sensitivity analysis, *J. Geophys. Res.* **98**, 12,609–12,617.

Hatanaka, A., 1993: The biogeneration of green odour by green leaves, *Phytochemistry* **34**, 1201–1218.

Heiden, A. C., Hoffmann, T., Kahl, J., Kley, D., Klockow, D., Langebartels, C., Mehlhorn, H., Sandermann Jr., H., Schraudner, M., Schuh, G., and Wildt, J., 1999a: Emission of volatile organic compounds from ozone-exposed plants, *Ecol. Appl*. **94**, 1160–1167.

Heiden, A. C., Kobel, K., Komenda, M., Koppmann, R., Shao, M., and Wildt, J., 1999b: Toluene emissions from plants, *Geophys. Res. Letts.* **26** (9), 1283–1286.

Hildebrand, D. F., 1989: Lipoxygenases, *Physiologia Plantarum* **76**, 249–253.

Langebartels, C., Schraudner, M., Ernst, D., Heller, W., and Sandermann, H., 2000a: Oxidative stress and defense reactions in plants exposed to air pollutants and UV-B radiation, in D. Inzé and M. Van Montagu (eds), *Oxidative Stress in Plants*, Harwood, Amsterdam, pp. 105–135.

Langebartels, C., Thomas, G., Vogg, G., Ernst, D., Wildt, J., and Sandermann, H., 2001: Ozone induction of signal and defence pathways leading to the emission of plant volatiles, in R. Gaschke, H. Papen, and H. Rennenberg, (eds), *Trace Gas Exchange in Forest Ecosystems. Fundamental an Applied Tree Physiology*, Vol. 1, Kluwer, Dordrecht.

Mueller, J.-F., 1992: Geographical distribution and seasonal variation of surface emissions and deposition velocities of atmospheric trace gases, *J. Geophys. Res.* **97**, 3787–3804.

Rosendahl, S., 1996: Lipoxygenases in plants – their role in development and stress response, *Z. Naturforsch.* **51c**, 123–138.

Sandermann, H., Ernst, D., Heller, W., and Langebartels, C., 1998: Ozone: An abiotic elicitor of plant defense reactions, *Trends Plant Sci.* **3**, 47–50.

Schuh, G., Heiden, A. C., Hoffmann, T., Kahl, J., Rudolph, J., and Wildt, J., 1997: Emission of volatile organic compounds from sunflower and beech: Dependence on temperature and light intensity, *J. Atmos. Chem.* **27**, 291–318.

Shao, M., v. Chapiewski, K., Heiden, A. C., Kobel, K., Komenda, M., Koppmann, R., and Wildt J., 2001: VOC emissions from Scots pine: Mechanisms and description by algorithms, *J. Geophys. Res.* **106**, D17, 20,483–20,492.

Vick, B. A. and Zimmerman, D. C., 1987: Oxidative systems for modification of fatty acids: The lipoxygenase pathway, in P. K. Stumpf and E. E. Conn, (eds), *The Biochemistry of Plants. A Comprehensic Treatise, Vol. 9, Lipids: Structure and Function*, Academic Press, New York, pp. 53–90.

Wildt, J., Kley, D., Rockel, A., Rockel, P., and Segschneider, H. J., 1997: Emission of NO from several higher plant species, *J. Geophys. Res.* **102**, 5919–5927.