Digi-Nose Part 1: Characterization of volatile organic compounds (VOCs) emitted by European spruce trees under stress*

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Abstract

In recent years, factors such as heat, drought, storms, and excessive rainfall, which can largely be attributed to man-made climate change, have weakened the European spruce population. Trees, especially during climate-related stress, communicate with each other by exchanging nutrients through their root system or by emitting volatile organic compounds (VOCs) via their needles, leaves, and bark. Some of these VOCs are attractive to pests, such as the bark beetle, as they indicate weakened defense mechanisms. Detection of these VOCs can be attained through gas chromatography-mass spectrometry analysis. Subsequently, a digital "nose" is planned to be developed, utilizing a combination of gas sensors, artificial intelligence, and image recognition to detect vulnerable trees early on. To achieve this, experimental trees were subjected to controlled conditions in a laboratory to simulate various stress situations, such as drought or waterlogging. Pre-filtered ambient air was drawn through ORBO32 sorbent tubes in the sampling set-up, eluted with petroleum ether, and then analyzed using gas chromatography-mass spectrometry (GC-MS). Peak areas of volatile organic compounds were statistically evaluated and compared. The findings suggested that stressed spruce trees emitted higher quantities of volatile organic substances. Particularly noteworthy were alcohols, terpenes, alkanes and alkenes. This work is part of the development of a digital nose to detect tree stress funded by the Austrian Ministry for Agriculture, Forestry, Regions and Water Management.

Keywords

European Spruce, VOCs, GC-MS

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1. Introduction

The following chapters discuss the European spruce, the communication between spruce trees through volatile organic compounds (VOCs) and their detection using GC-MS.

1.1. The European spruce

The European spruce (Picea abies) is an evergreen, shallow-rooted conifer that can grow to a height of around 50 meters and live for 120 years. It is monoecious, which means male and female cones are found on the same tree. The soft, stable and resin-rich wood can be utilized in many ways [1]. Due to its shallow roots, the spruce has a limited resistance to drought and is susceptible to windthrow, which makes it vulnerable to beetle infestations. When attacked, it usually protects itself by increasing resin flow, however, this mechanism is hindered by a lack of water supply. Heat, drought or heavy rainfall trigger stress in plants, which manifests itself in the emission of volatile organic compounds (VOCs), amongst other symptoms. This is largely due to man-made climate change, but monocultures also weaken the natural balance within the forest and thus its defenses [2]. Some of these VOCs are attractive to pests, as they indicate weakened defense mechanisms [3]. Terpenes, however, are in most cases toxic to predators [4].

1.2. Communication between trees

Forests can be understood as social communities. In addition to symbioses between plants and fungi, from which both sides derive benefits [5], other connections are formed. It has been shown that trees in a forest community support each other in emergencies by exchanging nutrients, since the entire forest ecosystem benefits from healthy and strong individuals. Healthy trees, especially those at the forest margins, protect the entire population from wind damage [6]. Many tree species live in symbiosis with fungi, which enables them to expand their root network, which in turn helps with water uptake. In return, the fungus receives nutrients from the tree. Trees also exchange signals or nutrients with each other via this extended root system [7]. However, a faster and more effective form of communication takes place via VOCs, i.e. volatile organic hydrocarbons. These are emitted via the leaves or needles and the bark and are used to transmit signals between trees or within individual specimens [8]. Green leaf volatiles, e.g. α-pinene, β-pinene, camphene and D-limonene, are mainly emitted via the needles of the spruce [9]. Spruce trees also emit the alcohols ethanol, methanol [10] and hexanol [11] as well as acetone, isoprene, monoterpenes [10] and hexanal [11] via the wood, the bark and again via the needles.

1.3. Objective

The bark beetle infestations resulting from drought and heat in recent years, along with the crucial role the spruce plays in forestry in Austria, are reasons why this tree species was chosen for this research. The aim of this project is to establish the differences in volatile organic hydrocarbons emitted by healthy and stressed spruce trees, and to determine the stress level based on the VOC composition (fingerprint). Subsequently, a mobile device will be developed to detect stressed individuals at an early stage using chemical and optical signals.

2. Materials and Methods

20 spruce trees, 7 to 10 years old, were taken from a forest in the northern Innviertel (Engelhartszell, Austria). The lighting conditions in the laboratory were set to 27 μmol s^{-1} m^{-2} (measured with a PAR meter CaTEC type 060501, PAR probe LI-COR Quantum Q49404) and to a day-night rhythm of 12:12 hours using daylight lamps (SYLVANIA, LUXLINE PLUS, F18W, 865). During the initial measurements, the laboratory maintained an average temperature of 25 °C. After an acclimatization period of two weeks, the experiments began. 4 trees each were selected for drought stress and waterlogging, 8 remained as an indoor control group and 4 trees were placed outdoors not far from the laboratory. Stress was induced by either not watering the trees or placing them in a sealed pot and watering excessively, which caused the roots to grow mold. Measurements were taken at regular intervals to determine how prolonged stress affected the VOC emissions of the spruce trees.

2.1. Sampling method

A display box made of acrylic glass (40 cm * 40 cm * 110 cm) with hose connections was made, in which the spruces (including pot) were placed 15 minutes before the start of the experiment to adjust the vapor equilibrium of the volatile substances. An activated carbon filter (self-made, DARCO, Mesh 4-12) for adsorbing the VOCs of the room air and a commercially available vacuum diaphragm pump (VWR, type PM204005-86.18) were placed before the display box. Previous experiments had shown that the push/pull method, i.e. with additional purge air, is more suitable than the simpler pull-method for measurements of this type due to the lower CO_2 concentration in the display case and thus higher VOC concentrations [12]. The air was drawn at a flow rate of 6 L min^{-1} through inert PTFE tubes, through the display box and then through activated carbon sorbent tubes (ORBO32, Supelco, Mesh 60-80). An identical vacuum pump was used for this purpose. The flow rate was regulated with a variable area anemometer and hose clamps. The pot and soil were sealed with PET roasting foil (Toppits® roasting tube, 3 m), which was baked in advance at 120 °C for 2 h [13], to avoid contamination.
The sample taking set up is visualized in Figure 1 below.

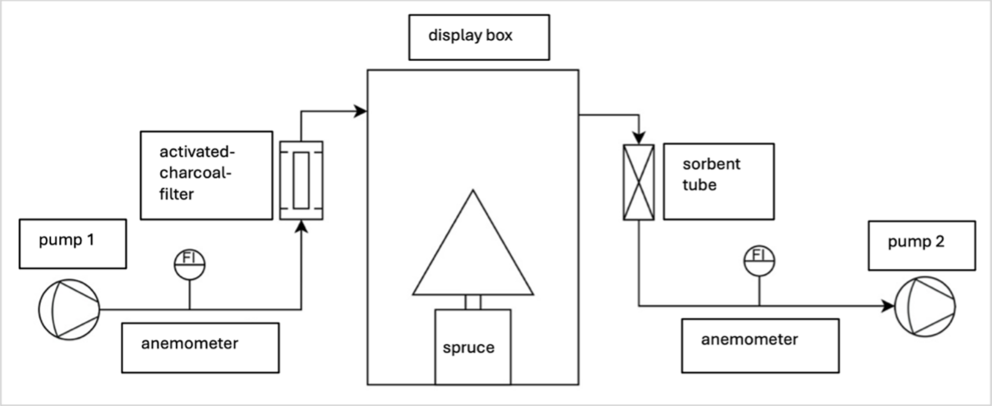

Figure 1: set-up for taking air samples of European spruce trees.

2.2. Sample preparation and GC-MS measurement

After the bound VOCs were eluted from the activated carbon by elution with solvent (petroleum ether (40-60 a.r., CHEM-LAB), this eluate was transferred to a GC vial (1,5 ml Rollrandflasche, 32x11,6 mm, Brucker Analysentechnik) [14]. In the gas chromatograph (Shimadzu GC 2010 Plus), 1 μL of each sample was injected in splitless mode with the autosampler (Thermo Scientific, TriPlus RSH) at 200 °C. The temperature gradient of the column (Agilent DB 5 MS 30 m * 0.25 mm * 0.25 μm) started at 40 °C and was held for 5 min. Subsequently, it was first heated to 130 °C at 6 °C min^{-1} and then to 240 °C at 15 °C min^{-1}. Helium (He 5.0) was used as the mobile phase with which the eluate was passed over the column at 3 mL min^{-1} purge flow and a volume flow of 1.51 mL min^{-1}. The temperature in the transfer line to the mass spectrometer (Shimadzu, GCMS-QP2020) was 200 °C and 240 °C for the ionization source. The detection masses were set from 41 m/z to 300 m/z.

2.3. Evaluation

The chromatograms of the stressed spruces were compared to those of healthy spruces to determine which peaks differed in height and area. The detection limit was set at H = 19905. Blank samples were also included to exclude distortions caused by the sampling setting, the adsorbent or the solvent. The values of the areas in TIC (total ion current) of the GC-MS measurements were transferred to Microsoft Excel tables and the percentage distribution was calculated. 18 relevant substances were determined, and the arithmetic mean values of the peak areas were calculated over three measurement rounds with healthy spruce trees and three measurement rounds with stressed spruce trees. The 95 % confidence interval was then calculated according to Formula 1 [15] and a two-sided t-test with unequal variances was carried out to prove that the emission of VOCs during the stress tests differed significantly from the normal state.

$$KI = \overline{x} \pm 1.96 * \sigma/\sqrt{n}. \tag{1}$$

$\overline{x}$ = arithmetic mean value
1.96 = z-value for the 95 % confidence interval
σ = standard deviation
n = number of samples

3. Results and Discussion

First, the VOCs emitted by healthy spruce trees in their normal state were measured by adsorption on activated carbon and subsequent elution with petroleum ether using GC-MS. In the next step, the trees were exposed to stress situations such as drought or waterlogging. They were examined to see whether these emitted substances differed in quantity and distribution depending on the level of stress.

3.1. Listing of VOC emissions

18 chemical compounds were identified that are thought to be associated with stress in spruce trees and are therefore relevant to this research. They are listed in table 1.

Table 1

Listing of relevant VOCs emitted by European spruce trees under stress

Retention time [min]	Substance	Group	Probability of accurate determination [%]
4.782	3-hexanone	ketones	97
4.938	2-hexanone	ketones	96
5.254	3-hexanol	alcohols	90
6.720	2,4-dimethyl-1-heptene	alkenes	96
7.107	butyric-Acid	carboxylic acids	96
9.539	tricyclene	monoterpenes	90
9.936	α-pinene	monoterpenes	96
10.475	camphene	monoterpenes	96
11.396	β-pinene	monoterpenes	96
11.824	2,2,4,6,6-pentamethylheptane	alkenes	97
12.395	3-carene	monoterpenes	90
12.540	4,6-dimethyldodecane	alkanes	92
12.898	o-cymene	aromatic hydrocarbons	92
12.965	2,2,4,4-tetramethyloctane	alkanes	94
13.030	D-limonene, β-phellandrene	monoterpenes	94
13.827	3-ethyl-3-methylheptane	alkanes	93
14.437	1-dodecanol	alcohols	< 90
14.560	3,7-dimethyloctan1-ol	alcohols	< 90

3.2. Emissions of stress exposed spruce trees

4 spruce trees were exposed to dry stress or waterlogging by no or excessive water supply and VOC emissions were measured in 3 rounds (n = 24). Mean, standard deviation and standard error were calculated for all substances to determine the 95% confidence intervals and additional t-tests were performed. The results were then compared to those of the same trees during the initial measurements to determine whether individual substances differ in percentage. These differences were presented in 3 diagrams for better understanding.

Figure 2 shows chemical compounds whose percentage share of VOC emissions decreased during the stress tests. The percentages of the substances in figure 3, on the other hand, increased slightly. The largest and most significant differences in terms of VOC emissions can be seen in figure 4. Figure 2 displays the differences in VOC emissions between healthy and stressed spruce trees for 9 of the 18 substances, along with 95% confidence intervals. The values of the standard measurements were set at 1, and the stress results are presented in relation to these standard measurements. The drop in the percentage share cannot be proven with certainty if the values are within the fluctuation range.

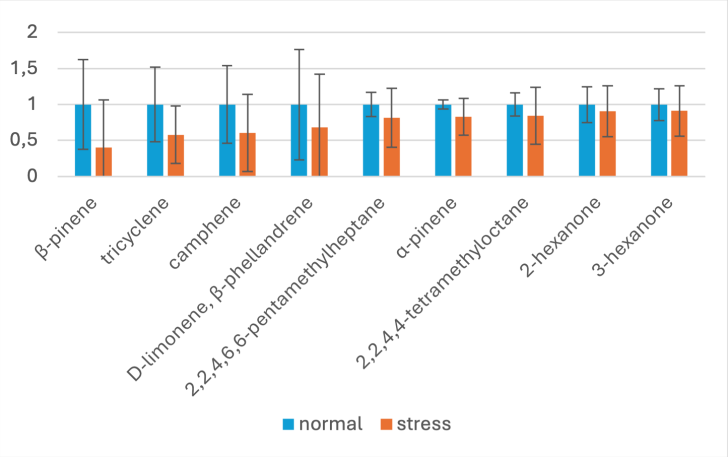

Figure 2: Drop in the percentage of VOCs emitted (β-pinene, tricyclene, camphene, D-limonene/ β-phellandrene, 2,2,4,6,6-pentamethylheptane, α-pinene 2,2,4,4-tetramethyloctane, 2-hexanone, 3-hexanone) by spruce trees under stress.

It was also possible to identify substances whose percentage share decreased.They can be seen in Figure 3 below.

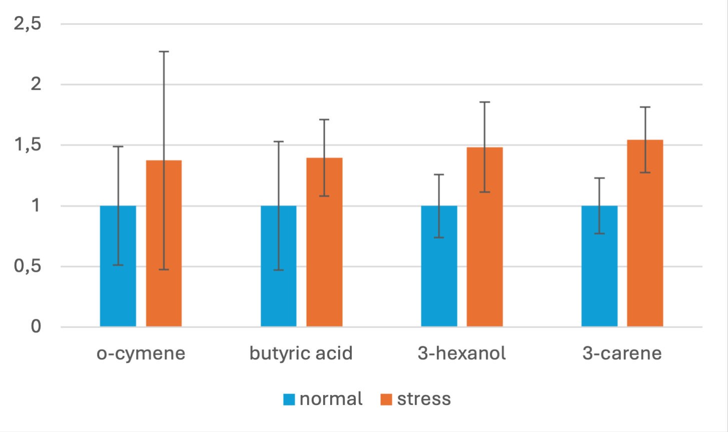

Figure 3: Increase in the percentage of VOCs emitted (o-cymene, butyric acid, 3-hexanol, 3-carene) by spruce trees under stress.

The most significant differences were found in the emissions of 1-dodecanol, 2,4-dimethyl-1-heptene, 3 ethyl-3-methylheptane, 4,6-dimethyldodecane and 3,7-dimethyloctan1-ol, all of which increased by several hundred percentage points. This can be seen in Figure 4.

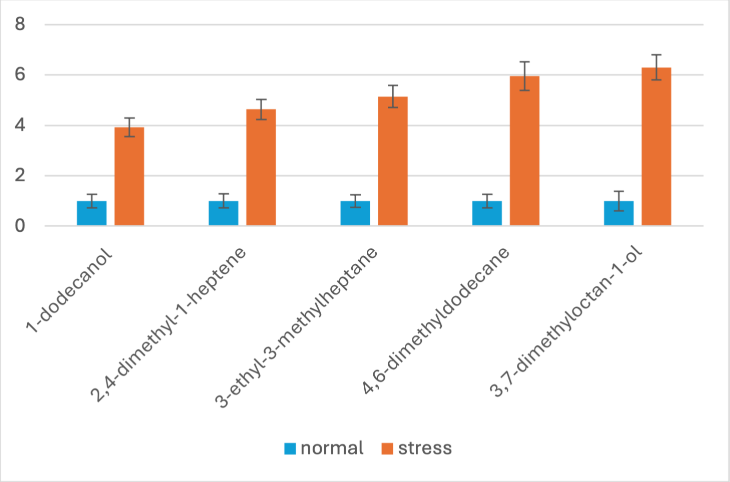

Figure 4: Increase in the percentage of emitted VOCs emitted (1-dodecanol, 2,4-dimethyl-1-heptene, 3 ethyl-3-methylheptane, 4,6-dimethyldodecane, 3,7-dimethyloctan1-ol) by spruce trees under stress.

It was therefore possible to determine that the values of 5 out of a total of 18 substances deviated significantly from the values of the normal measurements during the stress tests. These were identified as the alcohols 1-dodecanol and 3,7-dimethyloctan1-ol, the alkanes 3 ethyl-3-methylheptane and 4,6-dimethyldodecane and the alkene 2,4-dimethyl-1-heptene.

3.3. Discussion

The measurements took place between 22.05.2023 and 17.07.2023. As the temperatures both indoors and outdoors rose continuously due to the season, the trees reacted accordingly with increased emissions of VOCs. At the beginning of the measurement series, the temperatures were around 20 °C, whereas they approached 30 °C over the course of the experiment. The vapor pressure of VOCs increases with temperature [16], which could be an explanation for the increased emission of volatiles. To truly verify these peaks, comparisons with standards and retention indices are sought in the future. Currently, this set of experiments is being repeated under more controlled conditions. The trees are kept in grow tents, which are isolated, ventilated and illuminated and the temperatures are being kept steady at 25 °C (except for the trees under drought stress). Stress caused by drought and waterlogging can also be determined by measuring the rate of photosynthesis. The aim of this research is to scientifically prove the increased VOC emissions of spruce trees under stress once more. Additional information about the gathered data (e.g. data distributions) will be generated, and a machine learning aspect will be incorporated. The continuation of this research can be found in "Enhancing accuracy and efficiency of a digital nose system with sensor technology for early detection of changes in the forest" by authors Leo Biljesko, Georg Roman Schneider, Claudia Probst.

References

[1] G. Schwedt, Forstbotanik: Vom Baum zum Holz, Springer Berlin/Heidelberg, 2021.

[2] S. Krengel, P. Seidel, Über die zunahme thermophiler schadorganismen in wäldern am beispiel der borkenkäfer, Warnsignal Klima: Die Biodiversität (2016) 184–189.

[3] N. Korolyova, A. Buechling, F. Lieutier, A. Yart, P. Cudlín, M. Turčáni, R. Jakuš, Primary and secondary host selection by ips typographus depends on norway spruce crown characteristics and phenolic-based defenses, Plant Science 321 (2022) 111319.

[4] E. Breitmaier, Terpene - Aromen, Düfte, Pharmaka, Pheromone, Wiley-VCH Verlag GmbH & Co. KGaA, 2005.

[5] G. Witzany, Bio-communication of plants, Nat. Price (2007).

[6] S. W. Simard, D. A. Perry, M. D. Jones, D. D. Myrold, D. M. Durall, R. Molina, Net transfer of carbon between ectomycorrhizal tree species in the field, Nature 388 (1997) 579–582.

[7] C. J. Rhodes, The whispering world of plants:'the wood wide web', Science Progress 100 (2017) 331–337.

[8] M. Lüpke, M. Leuchner, R. Steinbrecher, A. Menzel, Impact of summer drought on isoprenoid emissions and carbon sink of three scots pine provenances, Tree physiology 36 (2016) 1382–1399.

[9] R. J. Fischbach, I. Zimmer, R. Steinbrecher, A. Pfichner, J.-P. Schnitzler, Monoterpene synthase activities in leaves of picea abies (l.) karst. and quercus ilex l., Phytochemistry 54 (2000) 257–265.

[10] W. Grabmer, J. Kreuzwieser, A. Wisthaler, C. Cojocariu, M. Graus, H. Rennenberg, D. Steigner, R. Steinbrecher, A. Hansel, Voc emissions from norway spruce (picea abies l.[karst]) twigs in the field—results of a dynamic enclosure study, Atmospheric Environment 40 (2006) 128–137.

[11] I. Filella, M. J. Wilkinson, J. Llusia, C. N. Hewitt, J. Peñuelas, Volatile organic compounds emissions in norway spruce (picea abies) in response to temperature changes, Physiologia Plantarum 130 (2007) 58–66.

[12] D. Sorg, Methodenentwicklung zur messung von vocs der fichte mittels gaschromatographie, 2023.

[13] A. Stewart-Jones, G. M. Poppy, Comparison of glass vessels and plastic bags for enclosing living plant parts for headspace analysis, Journal of Chemical Ecology 32 (2006) 845–864.

[14] R. A. Raguso, O. Pellmyr, Dynamic headspace analysis of floral volatiles: a comparison of methods, Oikos (1998) 238–254.

[15] M. Wirtz, C. Nachtigall, Deskriptive Statistik, Beltz Juventa, 1998.

[16] J. Kesselmeier, M. Staudt, Biogenic volatile organic compounds (voc): an overview on emission, physiology and ecology, Journal of atmospheric chemistry 33 (1999) 23–88.