

The spruce bark volatiles and internal phloem chemical profiles after the forest gap formation: the annual course

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Abstract

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Our study explores the impact of sudden gap formation on the bark volatile and internal chemical profiles of Norway spruce trees during the initial dry year of research plot 2018 following gap formation. We investigated the annual variation in two main physiological traits of Norway spruce trees at the forest edge (FE) and in the forest interior (FI): bark monoterpene (MT) emission spectra and internal phloem MT composition. Given that gap formation increases the solar radiation dose and temperature for trees at the forest edge, we hypothesized that the concentrations of airborne terpenes released from the tree bark and internal phloem terpenes will increase as a consequence of induced tree defenses. Our findings demonstrate significant increases in both airborne terpene concentrations and internal terpene composition in trees at the forest edge compared to the control trees in the forest interior. This study provides novel insights into the annual dynamics of bark monoterpenes following forest edge establishment and underscores the physiological changes experienced by trees in response to the gap formation.

Keywords

forest edge, induced defense, Norway spruce, terpenes, tree predisposition

Introduction

The population of the Norway spruce *Picea abies* (L.) H. Karst, an economically and ecologically important conifer of Central European forests, is declining due to various abiotic and biotic disturbances. Droughts or large wind events are drivers of important biotic disturbance, the infestation by the European bark beetle *Ips typographus* (L.) (Coleoptera: Curculionidae, Scolytinae), which performs better at warmer temperatures (SOUSA et al., 2023; STRÍBRSKÁ et al., 2022). This beetle is responsible for causing epidemic levels of mortality

in Norway spruce (NETHERER and HAMMERBACHAER, 2022). Bark beetle behavior is influenced by olfactory signals (SEYBOLD et al., 2006) composed of terpenoids, especially monoterpenes (MTs), which are found in tree resin oils and in the emission spectra of trees. Beetles use these signals to find suitable host trees, lay eggs and find food (YUVARAJ et al., 2021; JIROŠOVÁ et al., 2022). Diurnal cycle as well as seasonal variability of monoterpene production and evaporation within the year depends on the saturation vapor pressure, which is correlated with temperature (GHIRARDO et al., 2010; KLEIST et al., 2012; SCHÖNWITZ et al., 1990; RABER et al., 2021).

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The main abiotic stresses affecting MT production and correlating with seasonal dynamics are drought (water deficit), increased temperature and increased solar radiation (VAN MEENINGEN et al., 2017; TURTOLO et al., 2003; NIINEMETS, 2015; HOLOPAINEN et al., 2018; NETHERER et al., 2021; YASSAA et al., 2012).

In the first stage of an outbreak in mountainous conditions (incipient epidemic population), *I. typographus* predominantly colonizes windthrown trees or trees at fresh forest edges (damage initiation), and beetles are more likely to subsequently attack trees adjacent to the initial infestation (JAKUŠ et al., 2003; MEZEI et al., 2011). The process of infestation initiation is not yet completely understood. Undisturbed spruce stands are well protected against direct insolation (sun effects) by individual and/or collective shading and are usually not attacked by spruce bark beetles (JAKUŠ et al., 2011a), but natural disturbances such as windthrows or severe droughts can create conditions that favor an attack (JAKUŠ et al., 2011b; KAUTZ et al., 2013). KAUTZ et al. (2013) described the significant susceptibility of trees at forest edges to bark beetle infestation in areas that had been cleared by sanitary logging, particularly in the south-facing sector. However, the edges adjacent to uncleared areas were less susceptible to infestation, although they were still more susceptible than the edges of perennial forests or the interior of the stand. The microclimatic conditions of forest margins and olfactory signals from standing edge trees do favor spruce bark beetle activity (STŘÍBRSKÁ et al., 2022). Once the forest margin has been established, trees that were previously protected by surrounding trees are suddenly unprotected and must immediately respond to increased solar radiation and associated higher bark temperatures (MAREŠOVÁ et al., 2020; STŘÍBRSKÁ et al., 2022). However, under specific conditions, especially in spruce stands at lower elevations, *I. typographus* may not attack spruces at the forest edges (STŘÍBRSKÁ et al., 2022).

In our study, we investigated how the disturbance caused by sudden gap creation affects secondary defensive compounds produced by Norway spruce trees in the following year. We selected two main physiological traits of Norway spruce: i) bark MT emission spectra and ii) internal phloem MT composition. Both characteristics influence the behavior of bark beetles during an attack on a host tree (SCHIEBE et al., 2012a; STŘÍBRSKÁ et al., 2022; SOUSA et al., 2023). Our work provides original data on the annual variation of these two physiological traits in two different types of forest stands: i) forest edge (FE) and ii) forest interior (FI). Since the formation of a forest gap will increase the solar radiation dose and temperature for trees at the forest edge, we hypothesized that the concentrations of airborne terpenes from the tree bark and the internal composition of phloem terpenes will increase as a consequence of induced tree defenses. These changes are discussed in the context of ongoing acclimation processes to new light and thermal conditions and the predisposition of trees to bark beetle attack.

Materials and methods

Study area and experimental design

The study site was situated within a 90-year-old, healthy Norway spruce (*Picea abies* (L.) H. Karst.) stand on a slightly south-exposed slope, located at an elevation of 565–575 m above sea level in the forest district of Očová in the Western Carpathians of central Slovakia. The layout of the study site is described in detail in MAREŠOVÁ et al. (2020). The mean tree characteristics ($\pm SE$) were i) diameter at breast height (DBH, cm) of 48.8 ± 1.4 and 42.3 ± 1.3 ($F(1, 10) = 11.72, p < 0.01$); ii) tree height (m) of 32.4 ± 0.4 and 30.1 ± 0.7 ($F(1, 10) = 8.00, p = 0.02$); and iii) crown length (m) of 8.6 ± 0.6 and 8.0 ± 0.8 ($F(1, 10) = 0.45, p = 0.52$) at the forest edge and in the forest interior, respectively. The study site's coordinates were $48^{\circ}38'22.01''N$, $19^{\circ}17'26.67''E$, WGS84. The forest edge was established due to windthrow on 13 August 2018, and windthrown trees were subsequently cleared by foresters. The soil at the site was classified as Albic Luvisols, characterized by shallow covers of loess loams (double substrates), and associated with Eutric Cambisols and local Calcaric Cambisols, which originated from stony, predominantly tertiary sediments. The mean annual climate indicators at the study site included an air temperature range of 4–6 °C, precipitation levels of 700–800 mm, and sums of global radiation ranging between 1,100 and 1,150 kWh m⁻² (LAPIN et al., 2002). The measurements and sampling were conducted on 9 October 2018 (Oct18), 26 April 2019 (Apr19), 19 August 2019 (Aug19) and 14 October 2019 (Oct19) from 10 a.m. to 8 p.m. Two sets of Norway spruce trees were chosen for sampling. The first set comprised six trees situated at a recently established FE (formed two months prior to the commencement of the experiment), while the second set, FI, serving as the control group, consisted of six trees positioned in the fifth row or beyond, moving towards the forest interior.

Sample collection, extraction and analysis

Air samples containing monoterpenes released from spruce bark were collected from individual trees in four analytical replicates. A defined volume of air (69.0 L) was drawn in a Tenax TA 60/80 sorbent tube (200 mg of sorbent, 1/4"×3.5" Glass ATD Tube, CAMSCO, USA) using a pump (AirCheck 2000, SKC Inc., USA) operating at a flow rate of 115 mL min⁻¹ for a standardized time of 600 minutes. Gas flow meters (CNBTR 100–1,000 mL min⁻¹, LZM-6, China) were employed for the airflow standardization. Screw clamps were used to apply pressure to the hoses in the requisite hose sections following the connection of the pumps with the Tenax tubes and the flow meters at the opposite end. Prior to sampling, the flow meters were disconnected, thus leaving only the screw clamps in place. Each device's layout comprised one pump and four Tenax sorbent tubes connected to the pump via 0.5 m long hoses (R3603 TYGON, 6.4 mm (1/4") ID × 1.60 mm) and plastic splitters (polypropylene, PP). The devices were posi-

tioned in close proximity to the spruce trees, at a distance of less than one centimeter from the bark and in an open area devoid of enclosure. This was performed at a height of 2.5 m above the ground on the sunlit side of the tree. On the same day, ambient air within the forest was sampled using the same methodology at a distance of at least 2.0 m from the trees and 2.5 m above the ground. In the ambient air samples, the terpene levels were below the limit of GC–MS quantification.

The sorbent tubes with sorbed terpenes were transferred in a car refrigerator to the laboratory. Then, they were washed under laboratory conditions with 1.6 mL of n-hexane (99% p.a.), filtered into 2 mL glass vials, and 1 µL was injected using an autosampler for GC-MS. The Coretax Increment Hammer (Haglöf, Sweden) was employed to obtain phloem samples from the trees. For each tree, a phloem sample was prepared from 6 cutting pieces with a diameter 0.3 mm from the north aspect (shaded side). To extract terpenes, the frozen phloem sample (−20 °C) was ground with liquid nitrogen using a mortar and pestle. Subsequently, 2.0 mL of n-hexane was added to 0.15 g of the homogenate. The hexane extracts from both the air and phloem samples underwent analysis to determine their terpene content using gas chromatography–mass spectrometry (GC-MS). The GC–MS system utilized an Agilent setup, comprising a 7890B GC coupled with a 5977A MS instrument (Agilent Technologies, Palo Alto, CA, USA). The GC oven program began at 45 °C for 3.0 minutes and then increased at a rate of 3 °C min^{−1} to 110 °C, followed by an increase at a rate of 20 °C min^{−1} to 220 °C, where it was held for 3 minutes. The injection was performed in the splitless mode with a purge time of 0.35 min and an injector temperature of 250 °C. The carrier gas was helium with a constant flow rate of 0.5 mL min^{−1} and an injection volume was 1 µL. The MS operated with electron impact ionization at 70 eV in the scan mode, covering a mass range of 50–300 m/z. The MS quadrupole temperature was set to 150 °C, the interface temperature to 230 °C, and the ion source temperature to 250 °C. The column used was an HP-INNOWax column (30 m in length, 0.25 mm i.d., and 0.5 µm in film thickness; Agilent, USA). Data analysis of all chromatograms was conducted using the Agilent MassHunter software. Identification was accomplished by comparing the retention times and mass spectra with authentic commercial pure standards (AP—alpha-pinene, BP—beta-pinene, 3C—delta-3-carene, CAM—camphene, MYR—myrcene, LIM—limonene, and BA—bornyl acetate; 99% p.a., Sigma-Aldrich). Monoterpene concentrations were calculated using the closest individual standard concentration (10.0 µg mL^{−1}). Subsequently, the monoterpene concentrations in the phloem samples were recalculated based on the samples' wet weight. The following equation was used to calculate airborne monoterpene concentrations:

$$C = \frac{m}{V},$$

where C represents the monoterpene concentration (ng L^{−1}), m is the quantity of individual terpene in the eluate from the Tenax tube (ng), and V the volume of air drawn into

the tube (L).

Each Tenax sorbent tube drew 69 L of air and was washed with 1.6 mL of n-hexane. Based on the absorbance measured for standard terpene solutions with a known concentration of 1 µL, the unknown concentration of terpenes in the sample was recalculated from the measured sample absorbance with the trinomial while accounting for dilution.

Data processing and statistical analysis

The statistical analysis was performed as follows: on each date, the monoterpene sums ($\pm SE$) in the air and phloem of Norway spruce trees at the forest edge and in forest interior were compared. Considering the non-normal distribution of data and the small sample size, the Mann–Whitney U test ($\alpha = 0.05$) was used to conduct the analysis and interpretation. All statistical evaluations and graphs were performed using the R 3.4.0 statistical software (R Core Team, 2017).

Results

Concentration of airborne terpenes close to the spruce bark

Throughout the first year after the forest gap formation, the mean values of terpene concentrations in the volatile spectra showed that trees at the forest edge contained significantly higher levels of terpenes than trees in the forest interior (Fig. 1a). In October 2018, two months after the forest gap formation, the average airborne terpene concentration (ng L^{−1}; Fig. 1a) at the forest edge was more than 60-fold higher than in the forest interior. Additionally, during the following year, the concentrations of released terpenes at the forest edge were significantly higher than in the forest interior, with ratios of 1.7, 4.4 and 1.6 in April 2019, August 2019 and October 2019, respectively. The maximum value of 9.3 ng L^{−1} was measured in April 2019 (Table 1a).

Representation of individual terpenes in the airborne spectra

In October 2018, alpha-pinene and beta-pinene were present in the spectrum of evaporated terpenes at the forest edge, with alpha-pinene being the predominant compound (57.3%). In the forest interior, only beta-pinene was present (Table 1a). In April 2019, in addition to alpha-pinene and beta-pinene, camphene, delta-3-carene, myrcene and limonene were additionally found in the spectrum of evaporated terpenes, with limonene being the most abundant terpene, accounting for up to 62.5% and 77.0% at the forest edge and in the forest interior, respectively. In August 2019, the situation changed, and limonene was the least represented terpene in the airborne spectrum. The monoterpene contents decreased in August 2019 in the order of beta-pinene > alpha-pinene > delta-3-carene > myrcene > camphene > limonene for both tree groups. In October 2019, we

Table 1. The mean concentration of individual monoterpenes (MTs): (a) in the air (ng L^{-1} ; $\pm SE$) and (b) phloem ($\mu\text{g g}^{-1}$ wet wt.; $\pm SE$) of Norway spruce trees at the forest edge (FE) and in the forest interior (FI). AP: alpha-pinene; BP: beta-pinene; 3C: delta-3-carene; CAM: camphene; MYR: myrcene; LIM: limonene; and BA: bornyl acetate.

a)

	MT	Oct18		Apr19		Aug19		Oct19	
		FE	FI	FE	FI	FE	FI	FE	FI
Airborne MTs (ng L^{-1})	AP	2.1 ± 0.55	0.0 ± 0.0	1.39 ± 0.1	0.67 ± 0.04	0.78 ± 0.19	0.1 ± 0.01	0.4 ± 0.04	0.23 ± 0.02
	BP	1.56 ± 0.26	0.06 ± 0.03	0.87 ± 0.12	0.2 ± 0.02	0.88 ± 0.14	0.17 ± 0.02	0.6 ± 0.07	0.29 ± 0.04
	3C	–	–	0.7 ± 0.11	0.15 ± 0.03	0.09 ± 0.03	0.05 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
	CAM	–	–	0.6 ± 0.06	0.34 ± 0.05	0.03 ± 0.0	0.01 ± 0.0	–	–
	MYR	–	–	0.07 ± 0.02	0.05 ± 0.0	0.05 ± 0.0	0.01 ± 0.0	–	–
	LIM	–	–	5.63 ± 0.28	3.85 ± 0.13	0.03 ± 0.0	0.01 ± 0.0	–	–

b)

	MT	Oct18		Apr19		Aug19		Oct19	
		FE	FI	FE	FI	FE	FI	FE	FI
Phloem MTs ($\mu\text{g g}^{-1}$ wet wt.)	AP	110.4 ± 17.0	95.6 ± 1.3	51.7 ± 12.5	57.7 ± 6.3	101.2 ± 19.1	66.9 ± 4.8	79.7 ± 16.9	72.3 ± 6.3
	BP	138.6 ± 29.1	111.9 ± 5.4	53.7 ± 15.8	51.3 ± 7.5	76.3 ± 13.1	63.7 ± 9.1	72.3 ± 15.2	65.8 ± 10.1
	3C	12.2 ± 4.0	5.2 ± 1.2	1.7 ± 0.9	6.1 ± 2.3	10.3 ± 4.1	9.6 ± 4.7	5.7 ± 1.9	5.4 ± 2.9
	CAM	–	–	1.4 ± 0.3	2.3 ± 0.4	4.3 ± 1.3	2.6 ± 0.4	2.0 ± 0.3	2.9 ± 0.2
	MYR	18.0 ± 2.8	8.3 ± 2.1	10.0 ± 3.5	8.3 ± 1.5	17.4 ± 3.0	12.3 ± 1.5	17.7 ± 2.9	12.4 ± 0.8
	LIM	5.8 ± 1.1	8.48 ± 2.36	2.55 ± 0.69	6.3 ± 1.8	5.4 ± 1.2	5.8 ± 1.7	4.4 ± 0.8	7.3 ± 2.2
	BA	25.1 ± 12.0	3.78 ± 0.7	4.03 ± 1.13	4.34 ± 0.69	35.8 ± 5.2	33.0 ± 4.7	3.0 ± 0.4	2.6 ± 0.2

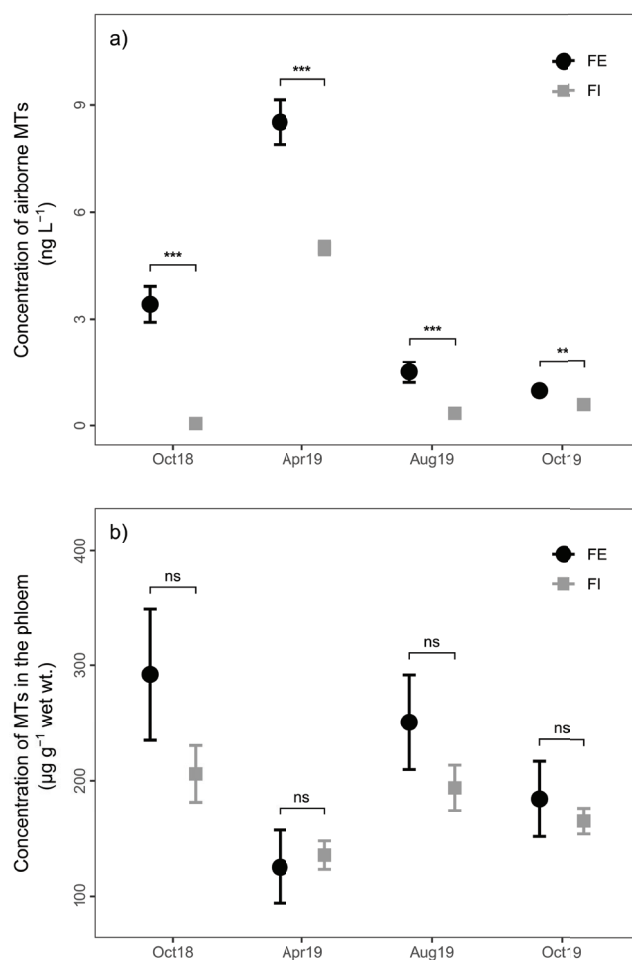


Fig. 1. The mean concentration of monoterpenes (MTs): (a) in the air (ng L^{-1} ; $\pm SE$) and (b) in the phloem ($\mu\text{g g}^{-1}$ wet wt.; $\pm SE$) of Norway spruce trees growing at the forest edge (FE, black color) and in the forest interior (FI, gray color). *** indicates significance at $p < 0.001$, ** indicates significance at $p < 0.01$, and ns indicates not significant.

found only three terpenes in both stands: beta-pinene (FE: 58.5%, FI: 49.3%), alpha-pinene (FE: 34.8%, FI: 37.8%) and delta-3-carene (FE: 6.6%, FI: 1.3%). The most abundant airborne terpenes were alpha-pinene, beta-pinene, delta-3-carene and limonene. Throughout the year, except for October 2018, alpha-pinene was the second most abundant terpene, with a range of 10.0–41.6%. Beta-pinene dominated in October 2018 (100%) and in August and October 2019 in both stands (47–59%). Monoterpene delta-3-carene was the third most abundant terpene at the forest edge in April 2019, and its content was 2.7-fold higher in the trees at the forest edge compared to the trees in the forest interior. In August and October 2019, delta-3-carene was still the third most abundant terpene (1.3–12.7%); however, its concentrations showed no differences across the two forest groups.

Terpenes extracted from the spruce phloem

The mean values of terpene concentrations in the phloem of the trees at the forest edge were higher than those of the trees in the forest interior, but the differences were insignificant (Fig. 1b). The representation of terpenes in the phloem spectra was similar to airborne spectra, and only bornyl acetate was additionally found in the phloem tissues (Table 1a,b). The ratio of the mean terpene concentrations ($\mu\text{g g}^{-1}$, wet wt.) between the forest edge and forest interior ranged from 0.9 to 1.4 across the time periods measured. The highest internal terpene concentration of 310 ± 57 ($\mu\text{g g}^{-1}$, wet wt.; $\pm SE$) in the phloem was detected in October 2018 at the forest edge, and the lowest concentration of 125 ± 32 ($\mu\text{g g}^{-1}$, wet wt.; $\pm SE$) was also detected at the forest edge in April 2019.

Representation of individual monoterpenes in the phloem

Throughout the year, the phloem content of individual terpenes showed a very similar trend, with alpha-pinene (34.5–43.9%) and beta-pinene (32.9–51.1%) being the two most abundant terpenes (Table 1b). Alpha-pinene was most abundant at the forest edge in August and October 2019 and in the forest interior from April to October 2019. Beta-pinene was the most abundant terpene at the forest edge in October 2018 and April 2019 and in the forest interior in October 2018, as well as in the airborne spectrum in October 2018.

Other terpenes we analyzed in the phloem were delta-3-carene, myrcene, limonene, camphene, and bornyl acetate, which was not present in the airborne spectrum. Bornyl acetate and myrcene were the third and fourth most abundant terpenes, respectively, with the concentrations of bornyl acetate being 1.5–8.9-fold higher in the forest interior and 6.3–17.0-fold higher in the forest interior in August 2019 compared to the other months. The concentration of myrcene in the phloem was highest in October 2019, with higher levels in the forest interior than at the forest edge in each month. The content of the monoterpene delta-3-carene ranged from 1.4 to 4.9%, with a 1.1–1.6-fold higher content at the forest edge in October 2018 and

October 2019 and a 1.2–3.2-fold higher content in the forest interior in April and August 2019. The content of the monoterpene limonene was 1.1–2.5-fold higher in the forest interior than at the forest edge, but its concentrations represented only 1.8–4.7% of the total terpene composition.

Discussion

Emission spectra of monoterpenes close to the spruce bark

We observed the most significant difference in monoterpene bark evaporation between the forest edge and forest interior in the first months after the gap formation, namely, in October 2018. The highest evaporation in both groups of trees was detected in the following spring in April 2019. Similarly, STŘÍBRSKÁ et al. (2022) found significantly higher abundance of selected bark volatile monoterpenes in the trees at the forest edge compared to the trees in the forest interior during spring months, although the most significant difference between the stands was in August. These authors monitored terpene evaporation after the formation of the forest gap from May to August, and the forest edges were created by harvesting in April.

It seems that the actual timing of forest edge or gap creation influences the pattern of monoterpene dynamics in the following vegetation season. Some authors have reported a peak in terpene emissions during spring and early summer (JANSSON et al., 1993; KEMPF et al., 1996; PERSSON et al., 2016), while others have noted a peak in late summer (STEINBRECHER et al., 1997). JANSSON et al. (1993) identified differences in emissions based on vegetation moisture, whereas JURÁŇ et al. (2017) found that relative humidity did not influence the emission rate of evaporated terpenes. In the study by GRABMER et al. (2006), the maximum monoterpene emission rates of mature Norway spruce trees were during periods of high temperature and high net carbon assimilation and stomatal conductance for water vapor during the day. Several authors argued that there may be large variation in emission intensities, but the main emitted compound remains the same within the season (BÄCK et al., 2012; PERSSON et al., 2016) and the emission patterns are mostly related to genetic predispositions.

The airborne spectra of both tree groups throughout the year showed the predominance of alpha-pinene, beta-pinene, limonene and delta-3-carene. In addition to these four most abundant monoterpenes, we also detected the presence of camphene and myrcene. A similar representation of terpenes evaporated from Norway spruce has been reported by several authors (JANSON et al., 1993; JURÁŇ et al., 2017). GITAU et al. (2013) described alpha-pinene, myrcene, beta-pinene and delta-3-carene as tree-derived kairomones that are important for attracting bark beetles of both sexes. After *Ips* spp. males are exposed to the volatiles released by the trees that they attacked (BYERS and WOOD, 1981), myrcene is converted to the pheromone components ipsdienol and ipsenol, which, together with verbenone, form aggregation pheromones

(BLOMQUIST et al., 2010). Moreover, myrcene enhances bark beetle's response to aggregation pheromones (RAFFA, 2013). Alpha-pinene is the most important terpene for the primary attraction of beetles, and *Ips* spp. males convert it to verbenone in the process of digestion (KLIMETZEK and FRANCKE, 1980). Thus, high concentrations of alpha-pinene and myrcene benefit these beetles.

Several authors reported that alpha-pinene and limonene are the dominant terpenes released by Norway spruce (KEMPF et al., 1996; PERSSON et al., 2016; RABER et al., 2021). Limonene is highly toxic to bark beetles (RAFFA, 2013), and SCHÜTTE (1984) described limonene as an intermediate compound leading to oxygenated terpenes, which are also of great importance for attracting bark beetles in low concentrations (SCHIEBE, 2012b). We found that limonene is the most abundant terpene found in the airborne spectra in the spring season (April 2019, Table 1a) in both tree stands. In the forest interior, limonene represented almost 80% of the entire airborne terpene spectra. Interestingly, in August 2019, limonene was found in minor quantities compared to the predominant terpenes alpha-pinene, beta-pinene and delta-3-carene. It is known that limonene represents the main starting intermediate for the formation of all terpenes. Since we also found significant amounts of limonene in the interior stand in April 2019, we assume that its high concentrations during this period of the year are related to phenology (genetic factors) and not to stress from sudden increased solar radiation. MOUKHTAR et al. (2006) found that limonene was the most abundant monoterpene emitted, followed by camphene and alpha-pinene. The emissions of these monoterpenes varied depending on the micrometeorological conditions. Limonene, alpha-pinene and camphene emissions were temperature-dependent. Other compounds we found with high emission rates were delta-3-carene and camphene in April 2019 and delta-3-carene in August 2019.

Interestingly, the abundance of delta-3-carene at the forest edge was almost 3-fold higher than in the forest interior in April 2019. However, from August 2019 to October 2019, significantly higher abundance was found in the forest interior. JURÁŇ et al. (2017) found that delta-3-carene emission is induced by solar radiation and air temperature, while the monoterpenes alpha-pinene, beta-pinene, limonene and camphene are more affected by solar radiation than temperature. Significant induction of delta-3-carene has also been observed after fungal infection or methyl jasmonate (MJ) treatment in conifers (MADMONY et al., 2018; ZHAO et al., 2010; ZULAK et al., 2009). On the basis of previous findings, ZHAO et al. (2010) proposed that delta-3-carene might be a useful chemical marker of conifer resistance. Most of the monoterpenes we detected in the airborne spectra have been found to be over-produced after a bark beetle attack (e.g., GHIMIRE et al., 2015). It is known that low concentrations of evaporated monoterpenes are attractive for bark beetles. However, after their overproduction to high concentrations, they become toxic to beetles. It looks like, after attracting bark beetles due to their lower concentrations, terpenes start to overproduce to such high concentrations after the attack that they become toxic to beetles. The

most toxic/repellent terpenes in high concentrations appear to be the monoterpenes delta-3-carene and limonene (PHILLIPS and CROTEAU, 1999).

STŘÍBRSKÁ et al. (2022) recorded 3–6-fold higher abundance of monoterpene volatiles from the bark of Norway spruce at the forest edge than in a closed forest stand (May–August). Conifers have two types of chemical defenses with an expected trade-off system (RASMANN et al., 2015; HUANG et al., 2018). The first type is constitutive defenses, which are present in the tissues all the time and would be most needed during high stress. The second type is induced chemical defenses, which are activated in response to sudden stress events (FRANCESCHI et al., 2005; CELEDON and BOHLMANN, 2019) and would be most cost-effective at low stress levels (NYBAKKEN et al., 2021). Therefore, we hypothesize that, after the forest gap formation, sudden stress from solar radiation represents moderate stress for trees at the forest edge and elevated airborne emission of monoterpenes constitutes a part of their induced defenses.

Several authors found that moderate water deficit causes induced resistance to future stress challenges because of increased monoterpene production and evaporation (TURTOLO et al., 2003; HOLOPAINEN et al., 2018). In our experiment, moderate stress from sudden solar radiation caused an increased tree temperature at the forest edge compared to that in the forest interior (MAJDÁK et al., 2021, MEZEI et al., 2019). Subsequently, trees at the forest edge responded with increased terpene evaporation. We likely captured the process of acquisition of plant stress tolerance in response to previous stress events (TURTOLO, 2003; NIINEMETS, 2010; HOLOPAINEN et al., 2018); in our case, it was tolerance to elevated solar radiation. We can assume that the trees were attempting to defend themselves against other potential damaging agents, including bark beetles, through increased terpene evaporation. In terms of the predisposition of trees at forest edges to bark beetle infestation, some authors asserted that stand edges are more susceptible to bark beetle infestations than forest interiors (KAUTZ et al., 2013; MAREŠOVÁ et al., 2020), while others argued the opposite (STŘÍBRSKÁ et al., 2022). We surmise that previous studies neglected the effects of different environmental conditions in their works, since terpene emission rates also depend on the timing, duration and severity of stress (MARCHESE et al., 2010; NIINEMETS, 2015; SZABÓ et al., 2017). However, the precise mechanism of the internal defense systems that release terpenes as deterrents or toxins after tissue damage (insect feeding, oviposition or pathogen colonization) remains largely unknown (CELEDON et al., 2020).

Internal monoterpenes extracted from the spruce phloem

The representation of individual monoterpenes in the phloem samples (Table 1b) showed that their composition in Norway spruce does not significantly change throughout the year, irrespective of whether it is trees at a forest edge or in an interior stand. Similarly, VON RUDLOFF (1975) reported that the composition of monoterpenes in conifers does not change from late summer to late winter. The content of

monoterpenes in the phloem (Fig. 1b) showed a different trend throughout the year compared to the airborne spectra (Fig. 1a). While the evaporation of terpenes near the bark surface was highest in April 2019, at the same time we found the lowest concentration of terpenes in the phloem among the year. BAIER et al. (1999) also concluded that the composition of emitted monoterpenes in Norway spruce does not reflect its internal composition. Except for April 2019, the mean terpene concentrations in the tree phloem were higher at the forest edge than in the forest interior, but the differences were insignificant (Table 1b). Similarly, STŘÍBRSKÁ et al. (2022) found insignificant differences in the mean amounts of selected monoterpenes between stand treatments in the early summer season, although the correlation between evaporated and internal monoterpenes from May to June was positive in their study.

We found the highest terpene concentrations in the phloem in both tree groups in October 2018, 2 months after the formation of the forest edge (Table 1b). We hypothesize that the initial process of increased defense started in the phloem tissues in our study trees based on the elevated terpene concentration found at the forest edge compared to the forest interior, despite the insignificant difference. On the contrary, STŘÍBRSKÁ et al. (2022) stated that trees at a forest edge responded immediately to changing environmental conditions by increasing their defenses only in terms of resin flow but not phloem terpene content. Several authors found that the most abundant monoterpenes in the internal *P. abies* phloem composition are beta-pinene, alpha-pinene and limonene (BUFLER et al., 1990; TURTOLO et al., 2003; RABER et al., 2021). Our results confirmed the most abundant terpenes in the phloem throughout the whole year were alpha-pinene and beta-pinene (Table 1). Of all the monoterpenes, alpha-pinene and beta-pinene represented up to 44% and 51%, respectively (Table 1b). BUFLER et al. (1990) reported that, in the bark, alpha-pinene and beta-pinene represented up to 70% of the total terpenes found and the ratio was 2:3. In their work, they only cut tree tissues after 5 years, regardless of the time of the year.

Other phloem monoterpenes we detected were bornyl acetate, myrcene, delta-3-carene, limonene and camphene. Except for bornyl acetate, DUAN et al. (2020) found a similar terpenoid pattern of terpenes in the phloem tissues of Norway spruce sampled in September, which showed the following order: beta-pinene, alpha-pinene, limonene/beta-phellandrene, myrcene, delta-3-carene and camphene. In terms of the difference between the forest stands, each monoterpene showed a very different pattern across the seasons (Table 1b). Only the monoterpene myrcene, with high abundance across the seasons, and bornyl acetate, with high abundance in August 2019, had higher abundance at the forest edge than in the forest interior (Table 1).

Conclusions

Our investigation showed that trees located at a recently formed forest edge experienced physiological changes throughout the year following the gap formation. This was

indicated by the increased concentrations of monoterpenes in both the airborne spectra and internal terpene composition. This was in contrast to the control trees situated within the forest interior. We assume this increase in the concentrations of secondary metabolites produced by trees at the forest edge can signify the initial process of their increased defenses. Analyses of plant metabolites represent one approach to investigate plant physiological traits. Their monitoring can provide information on the health status of trees and their ability to respond to stress. Our results may be useful in monitoring the adaptation mechanisms of spruce and its defense mechanisms and may help decision-making processes in forest management.

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