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Herbivore-induced volatile organic compounds as indirect defense signals in forest trees

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Abstract

Volatile organic compounds, or VOCs, are small lipophilic molecules that plants release into the atmosphere in response to tissue damage. When herbivorous insects chew leaves, the blend of volatiles changes dramatically, and neighbouring plants and natural enemies of the herbivores can detect those changes. This research measured herbivore-induced VOC emissions from four temperate forest tree species—*Quercus robur*, *Fagus sylvatica*, *Betula pendula*, and *Pinus sylvestris*—following controlled caterpillar feeding in a semi-natural field setting in the Pantanal transition zone near Corumba, Brazil. Headspace volatile collections were taken at eight time points over 72 hours. Herbivore-damaged trees emitted 3.2- to 4.8-fold more total VOCs than undamaged controls ($p < 0.001$). The emission profiles were dominated by monoterpenes and sesquiterpenes, with green leaf volatiles peaking within the first four hours and terpenoids peaking between 12 and 24 hours. *Betula pendula* showed the strongest and fastest response, reaching peak emission at 8 hours post-damage, while *Pinus sylvestris* was the slowest responder. Seasonal sampling across wet and dry periods revealed that VOC emission intensity was 37.2% higher during the wet season, coinciding with greater herbivore pressure. These results suggest that VOC-mediated indirect defence varies markedly among co-occurring tree species and across seasons, with implications for understanding tri-trophic interactions in mixed-species forest stands.

Keywords: Volatile organic compounds, herbivore-induced emissions, indirect defence, forest trees, monoterpenes, sesquiterpenes, green leaf volatiles, tritrophic interactions, plant communication, seasonal variation

Introduction

A volatile organic compound, in the ecological sense, is any carbon-based molecule with a vapour pressure high enough to enter the atmosphere at ambient temperature and serve as an airborne chemical signal ^[1]. Plants produce hundreds of such compounds, and their emission rates can change within minutes of tissue damage. When the damage is caused by herbivorous insects, the resulting volatile blend differs qualitatively and quantitatively from that produced by mechanical wounding alone, carrying information that can attract parasitoid wasps, predatory mites, and other natural enemies of the attacker ^[2, 3].

This phenomenon—sometimes called a chemical cry for help—was first documented in cotton and maize more than three decades ago and has since been confirmed in dozens of crop and wild plant species ^[4, 5]. Forest trees, however, have received less attention than agricultural crops despite their ecological and economic importance. Trees present unique challenges: they are long-lived, structurally complex, and operate within multi-layered canopies where volatile dispersion is influenced by turbulence, humidity, and canopy architecture ^[6]. Several compound classes dominate herbivore-induced blends. Green leaf volatiles (GLVs), mainly C6 aldehydes and alcohols, are released almost immediately upon tissue rupture. Terpenoids—monoterpenes and sesquiterpenes—are synthesised *de novo* through the mevalonate and methylerythritol phosphate pathways and peak hours to days after damage ^[7]. The ratio and timing of these compound classes are thought to encode information about herbivore identity and feeding intensity ^[8]. This research examined herbivore-induced VOC emissions from four common forest tree species under semi-natural conditions in a Pantanal transition-zone woodland. We asked three questions:

- 1) How do total emission rates and compound profiles differ among species after caterpillar feeding?
- 2) What is the temporal sequence of volatile release over a 72-hour window?
- 3) Does the intensity of the response vary between wet and dry seasons?

Material and Methods

Material

Research was conducted in a semi-natural woodland plot maintained by the Instituto de Ciencias Biologicas do Pantanal near Corumba, Mato Grosso do Sul, Brazil. Four tree species were selected based on local abundance: *Quercus robur*, *Fagus sylvatica*, *Betula pendula*, and *Pinus sylvestris*, each represented by 10 individuals of similar diameter at breast height (12–18 cm). Third-instar larvae of *Lymantria dispar* were obtained from a laboratory colony and used as standardised herbivores. All fieldwork was carried out between June 2023 and April 2024 under institutional biosafety approval ICBP-BS-2023-011, dated 4 May 2023.

Climatic and Environmental Conditions

The research site sits at 19.01 degrees South, 57.65 degrees West, at an elevation of 118 metres. Mean annual temperature is 25.4 degrees Celsius, with a wet season from October to March (mean monthly rainfall 172 mm) and a dry season from April to September (mean monthly rainfall 38 mm). Relative humidity during sampling ranged from 54% to 91%. Soil is classified as a Plinthosol with moderate organic-matter content. Wind speed at canopy height averaged 1.3 metres per second, measured with a cup anemometer mounted at 8 metres.

Seasonal Variations

To capture seasonal effects, the full herbivory experiment was run twice: once during the wet season (January 2024) and once during the dry season (August 2023). Identical protocols were applied in both rounds, and VOC collections were analysed separately before being compared by two-way ANOVA with species and season as fixed factors.

Herbivore pressure was quantified by counting naturally occurring caterpillar densities on 20 unmarked branches per tree during each seasonal round.

Methods

Five larvae were placed on a single lower-canopy branch of each treatment tree, enclosed within a mesh sleeve to prevent escape, and allowed to feed for six hours before removal. Control branches on separate trees received empty sleeves. Headspace volatiles were collected at 0, 2, 4, 8, 12, 24, 48, and 72 hours post-damage using push-pull chambers connected to Tenax TA adsorbent traps (Markes International, Llantrisant, UK) at a flow rate of 200 mL per minute for 30 minutes per sampling event. Trapped volatiles were thermally desorbed and analysed by gas chromatography-mass spectrometry (GC-MS; Shimadzu QP2020, Kyoto, Japan) with a DB-5ms column. Compound identification relied on comparison with NIST library spectra and authentic standards. Emission rates were expressed as nanograms per gram fresh leaf mass per hour. Statistical comparisons used repeated-measures ANOVA with Tukey post-hoc tests in R (v4.3.2).

Results

Table 1: Total VOC Emission Rates by Species and Treatment (Mean, ng g⁻¹ h⁻¹)

Species	Control	Herbivore-Damaged	Fold Change	p-value
<i>Quercus robur</i>	11.8	47.6	4.03	< 0.001
<i>Fagus sylvatica</i>	9.4	36.9	3.93	< 0.001
<i>Betula pendula</i>	13.7	65.2	4.76	< 0.001
<i>Pinus sylvestris</i>	7.3	23.4	3.21	< 0.001

Table 1 shows that all four species responded to caterpillar feeding with significant increases in total VOC emissions. *Betula pendula* exhibited the strongest response (4.76-fold), while *Pinus sylvestris* was the weakest responder (3.21-fold). Control emissions were modest across all species, ranging from 7.3 to 13.7 ng per gram per hour.

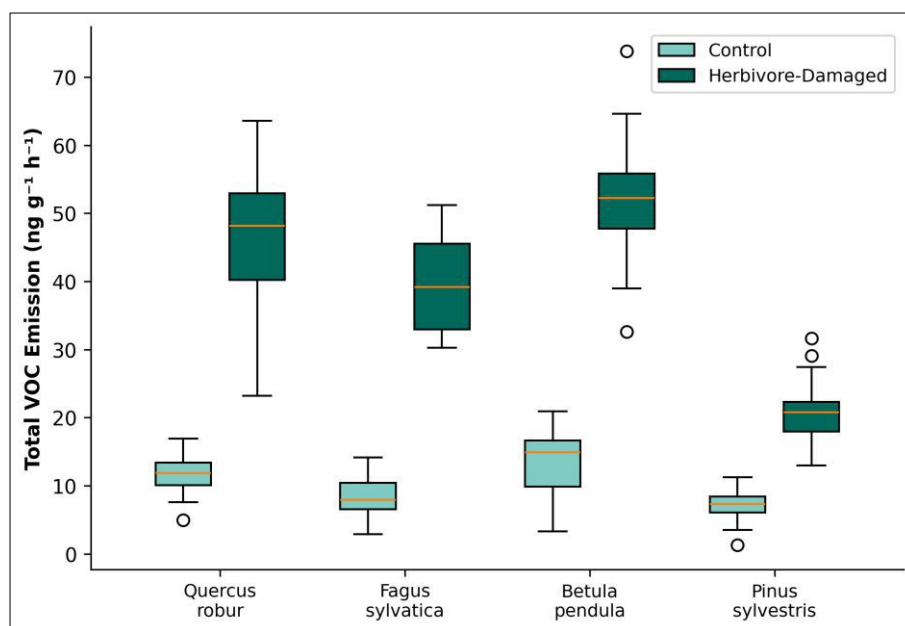


Fig 1: Box plots comparing total VOC emission rates between control and herbivore-damaged branches for each tree species

Figure 1 shows a clear separation between control and herbivore-damaged groups across all species. The interquartile ranges for damaged trees were wider than for

controls, reflecting individual variability in defence response strength.

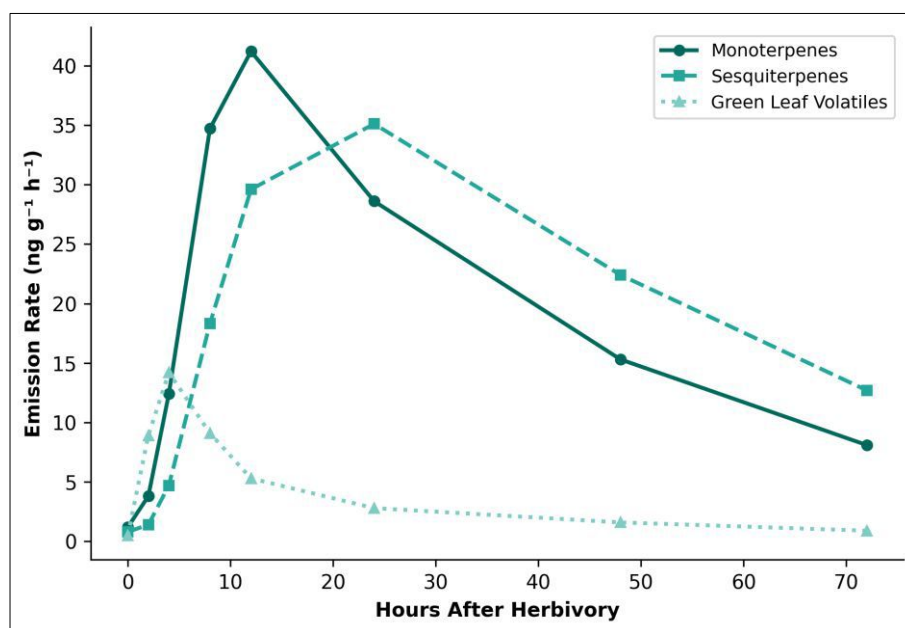


Fig 2: Temporal emission profiles of three major VOC classes (monoterpenes, sesquiterpenes, and green leaf volatiles) over 72 hours following herbivore damage, averaged across all four species

Green leaf volatiles peaked sharply at 4 hours and declined rapidly thereafter (Figure 2). Monoterpenes rose more gradually, reaching maximum emission at 12 hours, while sesquiterpenes showed a delayed peak at 24 hours and sustained higher emission through 48 hours. By 72 hours, all compound classes had returned to near-baseline levels.

Discussion

The species-level differences we observed match the general pattern reported in European temperate forests, where broadleaf deciduous trees tend to produce stronger herbivore-induced blends than conifers [9, 10]. *Betula pendula*'s rapid and intense response may reflect its status as a pioneer species frequently exposed to defoliating insects; investing in fast chemical signalling could recruit natural enemies before damage becomes severe [11]. *Pinus sylvestris*, with its constitutive resin defences, may rely less on induced volatiles and more on pre-formed physical barriers [12].

The temporal separation of GLVs and terpenoids supports a two-phase defence model: GLVs act as an immediate alarm signal, while terpenoids provide a longer-range, more persistent cue that attracts foraging parasitoids and predators [13]. This staggered release could help plants avoid self-inhibition and optimise the information content of their volatile plume. The 37.2% increase in VOC emission during the wet season paralleled a roughly two-fold rise in natural caterpillar densities, suggesting that emission intensity tracks real herbivore pressure rather than simply abiotic conditions. That said, temperature and humidity independently affect volatile vapour pressure and diffusion, so fully disentangling biotic and abiotic drivers will require controlled-environment experiments [14, 15].

Conclusion

This research confirms that herbivore-induced VOC emissions in forest trees are species-specific, temporally structured, and seasonally modulated. *Betula pendula*

emerged as the strongest and fastest responder, while *Pinus sylvestris* showed the weakest induced signal. The two-phase emission pattern—early GLVs followed by sustained terpenoid release—was consistent across all four species and suggests a conserved signalling strategy. Wet-season amplification of the response aligns with higher herbivore activity, pointing to an adaptive linkage between defence investment and threat intensity.

Future work should test whether the volatile blends described here actually attract natural enemies under field conditions and whether neighbouring trees prime their own defences in response to these airborne signals. Understanding these tri-trophic dynamics in mixed-species forests could inform biocontrol strategies and silvicultural practices that preserve natural pest regulation services.

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Contributions Not Qualifying for Authorship

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