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Mass scans from a proton transfer mass spectrometry analysis of air over Mediterranean shrubland browsed by horses

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Abstract: *Plants usually emit large amount and varieties of volatiles after being damaged by herbivores. However, analytical methods for measuring herbivore-induced volatiles do not normally monitor the whole range of volatiles and the response to large herbivores such as large mammals is much less studied than the response to other herbivores such as insects. In this paper we present the results of using a highly sensitive proton transfer reaction-mass spectrometry (PTR-MS) technique that allows simultaneous monitoring of leaf volatiles in the pptv range. The resulting mass scans in air over Mediterranean shrubland browsed by horses show 70 to 100% higher concentrations of the masses corresponding to mass fragments 57, 43 and 41 (mostly hexenals, acetone and acetic acid) than scans over control non-browsed shrubland. These compounds are biogeochemically active and they are significant components of the volatile organic carbon found in the atmosphere. They influence the performance of living organisms and, the chemical and physical processes of Earth's atmosphere.*

Key words: *Acetic acid, Acetone, Biogenic VOCs, Herbivory, Hexenal, LOX volatiles*
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Introduction

Numerous studies carried out over the last decade have shown that plants release volatiles in response to herbivore attack (Dicke *et al.*, 1990; Turlings *et al.*, 1990; Pare and Tumlison, 1999; Llusia and Penuelas, 2001; Penuelas *et al.*, 2005). Interest in these herbivore-induced volatile emissions has greatly increased in recent years given that these emissions influence neighbouring plants, as well as herbivores and their predators and parasitoids (Turlings *et al.*, 1990; Penuelas and Llusia, 2001; Van Poecke and Dicke, 2004). However, there is another reason why these emissions are worth considering closely: They may also considerably affect atmospheric chemistry and physics (Penuelas and Llusia, 2003).

The analytical methods for measuring herbivore induced volatiles do not usually monitor the whole range of volatiles and the response to large herbivores such as large mammals is much less studied than the response to other herbivores such as insects. Here we used a relatively recent methodology using proton-transfer-reaction mass spectrometry (PTR-MS) that allows monitoring these leaf volatile compounds in the pptv range almost simultaneously. This technique has emerged as a useful tool that allows us to study large numbers of different VOCs accurately with a fast time response (< 1 second) and with a low detection threshold (10-100 pptv) (Lindinger *et al*., 1998; Warneke *et al.*, 2003). Moreover, the lack of sample treatment in PTR-MS facilitates detecting species such as organic acids, peroxides, and doubly oxygenated species that are otherwise difficult to measure. PTR-MS is thus a more general detection method than gas chromatography-mass spectometry (GC-MS), for example, in which different columns may be required to target different classes of compounds.

We used PTR-MS to assess the VOC concentrations in air over Mediterranean shrubland browsed by horses. We analyzed the VOCs in air over the shrubland during horse browsing for four consecutive days to check for possible increases in VOC concentrations resulting from horse browsing.

Materials and Methods

Studied system:

Horses, plants and experimental design: The experiments were carried out with 2 horses both browsing in a 20 by 10 m plot (browsing plot) fenced by an electric wire, and an adjacent (20 m distance) 20 by 10 m plot also wire-fenced without any browsing (control plot) at the same altitude and with the same vegetation: Mediterranean shrubland dominated by *Rosmarinus officinalis, Erica multiflora, Ulex parviflorus* and *Globularia alypum*. Horses were chosen instead of domestic ruminants due to their larger intake rate (Duncan *et al*., 1990), which presumably means a larger effect on plant structure and more continuous browsing activity. The two horses of 300 kg body weight each browsed in the browsing plot for 11 daylight hours every day from the start of the experiment on 25 October 2004 to the end on 28 October 2004. The four days were sunny, with no significant wind, and the temperature ranged between 10.1°C at night and 24.8°C in the afternoon. The horses browsed not only green parts but also woody stems and branches.

We measured the VOC concentration in the air over the browsing plot and the control plot at 1.5 m height, *i.e.* ca. 0.5 m above the canopy before the horses started to browse (pre-treatment values), after 4 and 7 hr browsing on the first day and during the morning of the fourth day of browsing by the horses. We sampled the air in tedlar bags (supelco, bellefonte, pennsylvania) by using a supelco pump (Q-max pump, supelco, bellefonte, pennsylvania) twice every time in each plot. The tedlar bags were lined with teflon and only teflon tubing as connectors. The full tedlar bags, kept at 4°C and in darkness, were transported to the lab (2 hr).

VOC analysis by PTR-MS: The air from the teflon bags was flowed to the PTR-MS inlet. The PTR-MS apparatus, a highly sensitive proton-transfer-reaction mass spectrometer (PTR-MS-FTD hs) from ionicon analytik, innsbruck, austria, consists of three parts, the ion source where ions are produced by a hollow cathode discharge using water vapour as the molecular source of ions; the drift tube where proton transfer reactions to the trace constituents in the air occur (VOCs with a higher proton affinity than that of water (166.5 kcal mol^{-1}), including most unsaturated and almost all oxygenated hydrocarbons, undergo a proton-transfer reaction with H_3O^+) and finally the ion detector which provides sensitive detection of the mass selected ions that are characteristic of the molecules of interest. PTR-MS and its use in VOC analysis has been described in detail elsewhere (Lindinger *et al*., 1998; Fall *et al*., 1999). Here, the PTR-MS drift tube was operated at 2.1 mbar and 40°C, with a drift field of 600 V cm^{-1} as reported in Penuelas *et al*. (2005). The parent ion signal was maintained at around $3x10^6$ counts per second during the measurements. We conducted scans of all masses between 21 and 205 to determine which masses changed emissions during the different periods after horse browsing.

Statistical analysis: We used repeated measures ANOVA and post-hoc t-tests (Statistica, stat soft inc., tulsa, USA) to compare the air concentration over the browsed plot with those of the control plot in the first hours after browsing started and three days after continuous browsing.

Results and Discussion

The PTR-MS technique revealed that at least 3 masses, 57, 43 and 41 corresponding to fragments mostly of hexenal, acetone and acetic acid presented consistently greater concentration in the browsed plot than the control plot throughout the whole period. The difference was more significant on the fourth day of browsing than the first day (Fig. 1).

The concentrations of mass 57, mostly a fragment of hexenal but also of other compounds such as butanol, reached significant 87-88% increases at the end of the first day and the fourth day of browsing (Fig. 1). The concentrations of these lipoxygenase-derived (LOX) volatile compounds such as hexenal which are primarily responsible for the odour of cut grass were within the range reported in the available literature for example after lawn mowing (Karl *et al.*, 2001).

The concentration of mass 43, a fragment mostly consisting of acetic acid, but also of ethyl acetate, 2, 3 butanedione (Steeghs *et al.,* 2004) or propanol, hydroxyl acetaldehyde and other contributors (Williams *et al*., 2001) was 64-75% greater over the browsed plot than over the control plot (Fig. 1). Acetic acid, with atmospheric concentration between 0.1 and 11 ppbv are of relevance for atmospheric chemistry, as they can have a dominant impact on the acidity within precipitation (Kesselmeier, 2001)

The concentration of mass 41 a fragment of acetone (Steeghs *et al*., 2004) but also of alkanes (Jobson *et al*., 2005) or p-cymene (Tani *et al*., 2003), was 85 to 99% higher in the browsed plot than in the control plot during the first day of browsing, and up to 154% on the fourth day (Fig. 1). Acetone is a ubiquitous component of the

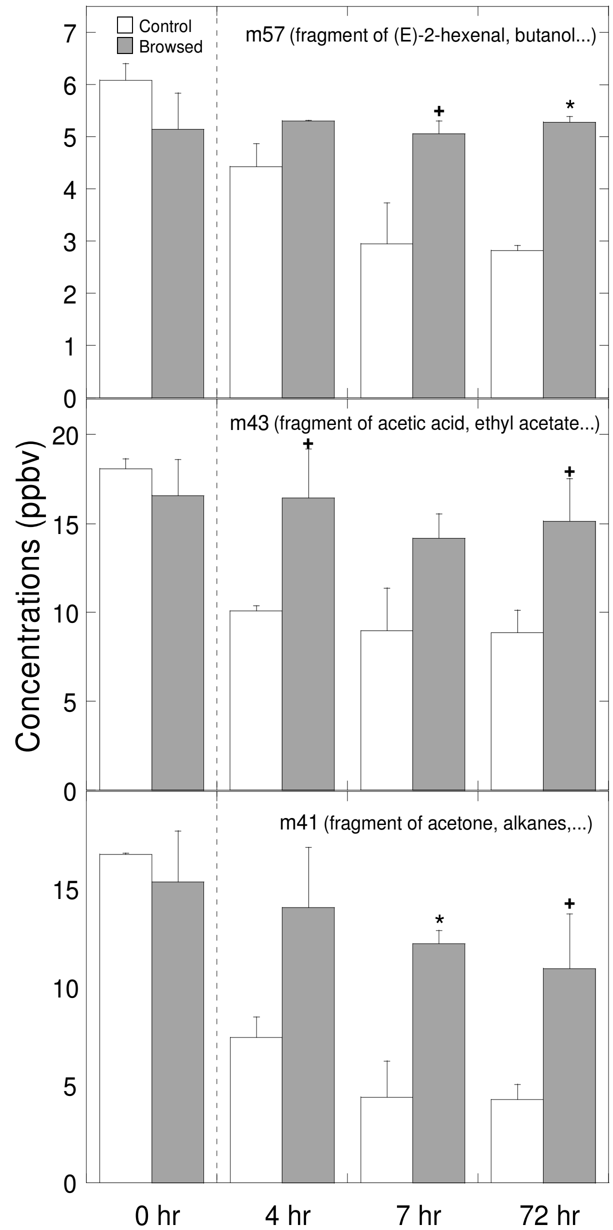

Fig. 1: Air concentrations of masses of volatile organic compounds that were different over the horse-browsing plot to over the control plot without horses in different times throughout the four days browsing. Repeated measures ANOVA. Significant differences between browsed and control plot : + = $p<0.10$, * = $p<0.05$. SDs are indicated by vertical lines on the bars, '...' = Suspension points

atmosphere produced as a byproduct of the plant cyanogenic pathway, which is activated to deter herbivores (Fall, 2003). It is also produced in other metabolic processes such as decarboxilation of acetoacetate resulting in considerable direct plant emissions (Janson and De-Serves, 2001). This decarboxilation of acetoacetate also occurs in soil microorganisms and animals and thus these components of the browsed shrubland system could also be responsible for the increased concentration of mass 41. Acetone has also been found to be emitted in response to cutting alfalfa (De Gouw *et al.*, 2000), which represents a physical stress somewhat similar to browsing by horses. Acetone is also a secondary product of oxidation of monoterpenes and methyl butenol (Goldstein and Schade, 2000; Singh *et al.,* 1994).

Biological functions and possible atmospheric effects: These volatile compounds emitted *de novo* or in greater amounts after horse browsing are commonly emitted by many plants as a response to both biotic (herbivory, pathogen attack) and abiotic (ozone, wounding) stressors (Turlings *et al.*, 1990; Fall *et al.*, 1999; Penuelas and Llusia, 2001; Van Poecke and Dicke, 2004; Penuelas *et al.,* 2005).

The increased emission of the volatiles reported here, such as those of acetone and the lipoxygenase-derived volatiles, might have biological effects on the other plants and organisms (Penuelas and Llusia, 2003, 2004; Owen and Penuelas, 2005; Engelberth *et al.*, 2004). Moreover, if they were generalized they might also significantly affect local oxidative tropospheric chemistry and play an important role in perturbing local ozone dynamics (Litvak *et al.,* 1999; Janson and Serves, 2001; Heiden *et al.*, 2003). Furthermore, all of these volatiles might actually be emitted at different rates at different hours after the stress (Heiden *et al.*, 2003; Wildt *et al.*, 2003). This possibility could not be monitored by our experimental set up, which only monitored twice on the first day and once on the fourth day after the horses started feeding. Unfortunately, as we did not analyze the air continuously for the whole period, we cannot know whether a peak of any of these or other volatile emissions occurred between the first minute and several days after browsing started. Finally, the actual amount of volatiles emitted by this (and other possible) plant communities and therefore their overall atmospheric effects, will depend on the browsing intensity and therefore on the farming management.

Further studies are warranted by the results reported here. In spite of the necessary caution implicit in a study of such limited scope dealing with only one plant community and one mammal herbivore species, the results reported here show that it is worth considering herbivore-induced emissions in our continuing efforts to quantify VOC emissions (Guenther, 2002) in the Earth's major ecosystem. Thus, these preliminary studies should be repeated more intensively and extensively and for other herbivores. The controls over most of these emissions have yet to be determined, and there is no current basis for mechanistic inventory development. Incorporating mechanistic biological controls in future VOC inventories would further improve their capacity to predict emissions in different ecosystems and environmental conditions.

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