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Both Gas Chromatography and an Electronic Nose Reflect Chemical Polymorphism of Juniper Shrubs Browsed or Avoided by Sheep

Gábor Markó [1,2]* · Ildikó Novák[3] · Jenő Bernáth[3] · Vilmos Altbäcker[1]

*1. Department of Ethology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H–1117, Budapest, Hungary, *e-mail: magvacska@hotmail.com*
2. Department of Plant Pathology, Corvinus University of Budapest, Ménesi út 44., H-1118, Budapest, Hungary
3. Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, Villányi út 29-43, Budapest, H-1118, Hungary

Abstract Chemical polymorphism may contribute to variation in browsing damage by mammalian herbivores. Earlier, we demonstrated that essential oil concentration in juniper, *Juniperus communis*, was negatively associated with herbivore browsing. The aim of the present study was to characterize the volatile chemical composition of browsed and non-browsed *J. communis*. Using either gas chromatography with flame ionization detection (GC-FID) or an electronic nose device we could separate sheep-browsed or non-browsed juniper shrubs by their essential oil pattern and complex odor matrix. The main components of the essential oil from *J. communis* were monoterpenes. We distinguished three chemotypes, dominated either by α-pinene, sabinene, or δ-3-carene. Shrubs belonging to the α-pinene- or sabinene-dominated groups were browsed, whereas all individuals with the δ-3-carene chemotype were unused by the local herbivores. The electronic nose also separated the browsed and non-browsed shrubs indicating that their odor matrix could guide sheep browsing. Responses of sheep could integrate the post-ingestive effects of plant secondary metabolites with sensory experience that stems from odor–phytotoxin interactions. Chemotype diversity could increase the survival rate in the present population of *J. communis* as certain shrubs could benefit from relatively better chemical protection against the herbivores.

Keywords Chemical polymorphism · Chemotype · δ-3-Carene · Electronic nose · Essential oil · *Juniperus communis* · Gas chromatography-flame ionization detection (GC-FID) · Odor · α-Pinene · Sheep

Introduction

Almost all plants produce secondary metabolites for their chemical defense against herbivores (Freeland and Janzen, 1974; Miller et al., 2005). The quantity and composition of plant secondary metabolites (PSMs) are key factors in plant chemical defense (Villalba and Provenza, 2005), as well as herbivore sensitivity and their coping capacity against toxins (Freeland and Janzen, 1974; Miller et al., 2005; Iason and Villalba, 2006). Plants that contain higher toxin concentrations benefit from relatively high biochemical protection against herbivores (Bryant et al., 1985) or belong to a relatively new and specific PSM polymorphism (Linhart and Thompson, 1999; Provenza et al., 2003). Chemical polymorphism can be manifested in chemotypes, which represent individuals with similar chemical metabolite composition and are usually designated by their main component (Schmidt et al., 2004).

In general, essential oils have strong antibacterial and antifungal effects (e.g., Angioni et al., 2003; Filipowicz et al., 2003). Such oils were found as repellents in different insect taxons, for instance in moth (Rocchini et al., 2000) and in beetle (Wibe et al., 1998). Feeding big sagebrush, *Artemisia tridentata*, which is high in terpenes, inhibits the cellulolytic activity of rumen microbes (Nagy and Tengerdy, 1968), and accordingly in high volumes it is toxic for ruminants (Schwartz et al., 1980a). Reduced food intake was associated with plant items containing relatively high essential oil yield in the interaction of mule deer, *Odocoileus hemionus*, with different juniper species (*Juniperus deppeana, J. osteosperma, J. scopulorum*) (Schwartz et al., 1980b) or Spanish goats, *Capra bircus* with two juniper species (*J. ashei, J. pinchotti*) (Riddle et al., 1996).

The ability to discriminate among foods is critical for herbivore survival in an environment abundant in poisonous plants (Provenza et al., 1998). Herbivores attempt to avoid or decrease PSM toxicity (e.g., Provenza,, 1995; Dearing et al., 2000; Duncan et al., 2006; Rogosic et al., 2007) and may use the senses of smell and sight before feeding to prevent the harmful consequences (Provenza et al., 1992; Provenza, 1996). Domestic sheep, (*Ovis aries*) were able to avoid food items treated with common monoterpenes and discriminate them by their odor (Narjisse et al., 1996), and the same was observed in female red deer, *Cervus elaphus* (Elliott and Loudon, 1987). Augner and his colleagues (1998) observed that lambs preferred a weaker flavored food, because the more flavored food was associated with a higher toxin level.

Essential oils, such as the commonly produced PSM group of aromatic plants, are very complex natural mixtures (Bakkali et al., 2008). Each component in an essential oil mixture could have a different kind of specific biochemical (toxicological) impact on herbivores (Nagy and Tengerdy, 1968) dependent on its molecule structure and/or its special functional group. Moreover, these components may produce either synergistic or antagonistic interactions with each other (Aparicio et al., 1996; Vokou et al., 2003) influencing the toxicity of the whole mixture. Furthermore, essential oil content shows high diversity with habitat and season (Riddle et al., 1996; Vourc'h et al., 2001; Gomez et al., 2003; Markó et al., 2008) and is also variable among individuals of a particular species (Miller et al., 2005).

Odor or flavor patterns can also be measured by electronic sensory systems. Such devices, also called electronic noses, are rapid, objective and reliable analytical tools for the detection and characterization of volatile and/or odorous compounds (El Barbri et al., 2008). An electronic nose is a detector containing an array of different sensors and software processing sensor data without reference to the exact chemical composition. Until now, electronic noses have been used to detect and identify volatile compounds (Maricou et al., 1998), and to classify the complex aromatic features of herbs (Novák et al., 2001; Baby et al., 2005; Seregély and Novák, 2005). Moreover, their use is widespread in the food and beverage industry (Pearce, 1997b), as well as in quality monitoring (Hobbs et al., 1995; Hogben et al.,

2004). The perception of different chemicals may also induce the same taste (Legin et al., 2003) or flavor (Cleland et al., 2002). As browsing animals are not likely to respond to individual chemical components but rather to differences in odor or taste patterns, one would expect a functional similarity in the odor mixture discrimination by an electronic nose and the herbivore (Pearce, 1997a).

A recent study (Markó et al., 2008) found that both the concentration of PSMs and specific compounds of the essential oil may play a role in selective browsing damage. The main goal of this study was the characterization of the volatile chemical composition of *Juniperus communis,* either browsed or non-browsed by sheep. Firstly, we tried to separate by gas chromatography (GC) the differently browsed shrub groups to see if browsing was affected by the essential oil composition. Secondly, we classified the differently browsed shrub groups with an electronic nose reflecting the odor matrix. Thirdly, we compared the odor matrix with the pattern of essential oil composition. Finally, we investigated which terpenoids reflected the odor matrix best.

Methods and Materials

Sampling and Extraction of Essential Oils The samples originated from the juniper forest of the Kiskunság National Park central Hungary, near the area of Orgovány (46°47’30”N; 19°27’34”E). Heavily browsed (*N*=9) and non-browsed (*N*=9) shrubs of *J. communis* were chosen and all plant material was collected at late winter. The sampling procedure was detailed in Markó et al. (2008). The size (height 2-2.5 m) and sex (female) of the sampled shrubs were similar, reducing the possible impact of these factors. The terminal 10-15 cm of foliage was clipped from several branches from ground level to a height of 2 m. The samples were 100-120 g of foliage after removal of berries. Plant material was gathered in plastic bags that were immediately placed in a cooler (5°C), until they were oven dried at 37±3˚C (Memmert U-type oven). For the extraction of oil yield, the method of water-distillation with a Clevenger-apparatus described by Pharmacopoea Hungarica (Végh, 1986) was used. The content of essential oil was calculated as percentage of the dry mass.

Gas Chromatography The main chemical compounds of essential oils were analyzed by using a Shimadzu 14B GC equipped with a FID detector and SE-30 capillary column (30 m × 0.25 mm, and 0.25 mm film thickness). The carrier gas was nitrogen (1 ml/min), and the split ratio was 75/1. The temperature of the oven was programmed as follows: 110˚C for 3 min, up to 220˚C at 8˚C/min (5 min), 21.75 min analysis time, injector temperature was 220˚C and detector temperature was 250˚C. Data were analyzed by using the Shimadzu Class VP Chromatography Data System (Shimadzu, Kyoto, Japan) software. Relative percentage of the oil constituent was calculated from the GC (FID) peak areas as percent of the total area by using the area normalization method (Novák et al., 2001, 2003). The identification of compounds was performed by peak enrichment with GC-standards from Carl Roth (Karlsruhe, Germany).

Electronic Nose Analysis Electronic nose determinations were performed with an NST 3320 instrument (Applied Sensor, Linköping, Sweden), with a built-in headspace autosampler for 12 samples, a detector unit containing 22 different sensors and software for collecting and processing data from the sensors. The NST 3320 is equipped with 10 MOSFET (metal oxide semiconductor field effect transistor) sensors, 12 MOS (metal oxide semiconductor) sensors and a humidity sensor for measuring relative humidity. Ambient air filtered through a drying column with silica gel and a combined moisture/hydrocarbon filter was used as a clean reference gas for the sensors. The gas flow rate of the dynamic sampling was set to 60 ml/

min. The filtered gas flow to the sensors, and into the headspace of the samples at the same time, finally left the instrument into the ambient air.

The stand-by temperature, at which the samples were kept until their incubation phase, was set to 20°C (5 min). The analysis was started with the incubation at 40°C (5 min). The baseline phase of the measurement cycle timing was set to 10 sec, the sample phase to 30 sec, the recovery phase of the sensors to 260 sec, and the flush time of the gas lines to 60 sec. The total cycle time per sample was 5 min. Signal parameters were collected as follows: "baseline" for 5 sec, "on derivative" (derivative of the sensor signal during adsorption) for 4 sec and "on integral" (integral of the sensor signal during adsorption) for 10 sec, "response" for 5 sec, "off derivative" (derivative of the sensor signal during desorption) for 4 sec and "off integral" (integral of the sensor signal during desorption) for 10 sec. Sample amount and temperature conditions for the electronic nose analysis were optimized before the actual quality evaluations in the storage test.

We used 0.7 g of fresh juniper twigs from the same shrubs as described above for the GC-FID analysis. After removing the berries, the twigs were crushed and put into the labeled vials, which were sealed with a Teflon-coated butyl septum and a screw cap. Electronic nose measurement was conducted at the Department of Refrigeration and Livestock Products Technology, Corvinus University of Budapest. The parallel analysis of samples collected from the same juniper shrubs allowed us to compare the results of the GC and the electronic nose analysis.

Statistical Analyses

Multivariate Statistical Analyses For the evaluation of the electronic nose sensor signal response of the sensor array, principal component analysis (PCA) was performed. The 22 sensor values were reduced to three principal components. Also, PCA was performed using the data set composed of all the essential oil samples transformed into centered and reduced variables (correlation method, varimax rotation). Selected variables consisted of absolute amounts of the identified compounds in the essential oil samples. The PCA is sensitive to rare or missing values, so the components less than 1% of the total samples were excluded from the analysis. The volume of each component was calculated from its relative ratio to the essential oil yield of each shrub. The use of absolute concentrations rather than relative amounts demonstrates that such calculations are not a statistical artifact of compositional data. In this way we corrected for the total essential oil yield which it is more realistic in sheep-juniper interactions.

Factor analysis was used to determine the main variables for each PC of the essential components. The scores from the PCA were inserted to the factor analysis. The first three factor loadings were included based on the eigenvalues (cumulative variance 85.07% in GC-FID).

General Linear Mixed Model After PCA we used general linear mixed models (GLMM) to investigate the relationship between browsing status and the first three PCs of the electronic nose. Browsing status was a dependent variable and showed binomial distribution, while the PCs were used as covariates. We used GLMM to investigate the relationship between browsing status of the juniper shrubs and the first three PCs from GC-FID analysis. Browsing status was a dependent variable and showed a binomial distribution, while the PCs were included as covariates in the model.

In all GLMM models, we employed a stepwise analysis with a backward deletion procedure, removing insignificant effects from the model one by one in decreasing order of statistical significance and re-entering the removed variables to the final model to obtain

relevant statistics. Statistica package (version 8.0, StatSoft, Inc. 2004, Tulsa, OK, USA) was used for all statistical analyses.

Results

Classification by Essential Oil Patterns We extracted essential oils from the juniper shrubs and identified their components. The PCA distinguished the individual plants based on their browsed level with high power (85.07%) in the first three PCs, which are presented on scatter plots with each other (Fig. 1b). The correlation plot of PC 2 and PC 3, and plot of PC 2 and PC 3 showed well classified groups. The scores of browsed shrubs were more aggregated than the values for the non-browsed junipers, similarly to the electronic nose classification. Furthermore, if we compare the scatter plots of the two patterns (Fig. 1a and 1b), we find the essential oil PCs less aggregated than the dots from the electronic nose analysis.

The GLMM analysis (Table 2) supports the PCA classification of the essential oil pattern. The PC2, PC3 and their interaction were significantly important in discrimination of the juniper individuals, while PC1 was not significant in the model.

Factor Analyses of Gas Chromatography After PCA, reduction factor analyses was used to see which essential oil components belonged to each PC (Table factor analysis). The first factor included almost all monoterpene components, such as, for example, β-myrcene, γ-terpinene, and borneol, while the dominant monoterpenes, such as α-pinene, sabinene, and δ-3-carene, composed the third factor. The second factor grouped all the sesquiterpenes. The essential oil components showed that their retention times were close to each other. The retention time is reflected by the structure and weight of the actual molecule.

Sensory Classification by the Odor Matrix We measured the odor pattern of browsed and non-browsed Juniper shrubs using an electronic nose apparatus and the sensor values were analyzed using PCA. The scatter plots of the first three principal component (PC) scores are shown in Figure 1a. Based on their browsed status, we could classify the odor patterns with high power (77.1%). The scatter plots between PC 1 and PC 3, and between PC 1 and PC 2 express the plant use best. These plots show that the browsed groups were well demarcated from the non-browsed groups, although there were three browsed individuals (identified by numbers 2, 5 and 6) which seemed more similar to the non-browsed groups than to the browsed ones. The plots of PC 2 and PC 3 showed less determinant differences among the groups. Furthermore, the correlation plots showed that scores of browsed juniper individuals were more aggregated than the non-browsed junipers.

The GLMM analysis (Table 1) supported the PCA classification. Every PC was significantly important in the discrimination of the juniper individuals, and none of the interactions was significant in the model.

Discussion

We investigated the essential oil pattern of previously sheep-browsed and non-browsed common junipers and found that both the electronic-nose and GC separated the two groups. Using the e-nose we also found that the juniper shrubs contained significantly different odor pattern depending on their browsed status. For studying the relationship between browsed status and essential oil patterns we reduced the number of components by PCA. The factor analysis suggested that browsed and non-browsed shrubs differed in their mono- and sesquiterpene ratios (see Table 3). We distinguished three different chemotypes, dominated by α-pinene, sabinene and δ-3-carene, respectively, in our juniper population. All shrubs belonging to the α-pinene were browsed, whereas all δ-3-carene chemotype individuals were

unused by the local herbivores. Shrubs containing elevated levels of sabinene were mixed in their browsed status. However, the δ-3-carene chemotype has been reported for Scots pine (Thoss et al., 2007), but to our knowledge this is the first study to identify uncommonly high amount of this monoterpene in *J. communis*.

The observed juniper population was rich in monoterpenes (Markó et al., 2008) similarly to other stands of this species (e.g., Adams and Pandey, 2003; Butkiene et al., 2004). The factor analysis of the essential oil pattern determined by GC resulted in three factors; F1 contained monoterpenes with medium retention times, F2 contained sesquiterpenes, while F3 was related to dominant monoterpenes. The relative importance of the dominant monoterpenes and the sesquiterpenes differed significantly between the two browsed statuses (see Table 2). The first group included almost all monoterpene components, the second group incorporated all identified sesquiterpenes, while the dominant monoterpenes, such as α-pinene, sabinene and δ-3-carene, composed the third group. Essential oil mixtures can show high terpenoid diversity containing 20–60 different components at quite different concentrations (Bakkali et al., 2008) and quite different toxicity (Oh et al., 1967), so grouping the components to find common patterns seems to be necessary to understand their effect in reducing herbivory.

The *Juniperus* taxon exhibits chemical polymorphism with geographical variation (Adams et al., 2003). A Swedish population showed high amounts of α-pinene, with moderate amounts of β-pinene, myrcene, limonene, δ-3-carene and β-phellandrene, and low amounts of sabinene (Adams, 1998). Sabinene-type and α-pinene-type junipers have been described for Poland (Filipowicz et al., 2009) and Lithuania (Butkiene et al., 2009; Butkiene et al., 2007). The δ-3-carene was described in many other juniper species (Adams, 1998; Angioni et al., 2003) and also in pines (Hiltunen and Laakso, 2006; Rocchini et al., 2000). PSMs, such as δ-3-carene, could be very effective toxins, having a relatively unique molecular structure, because herbivore only hardly copes with it. Despite of these widely existing chemicals among conifers plants could use them in their defence system. Juniperus oil with high carene content was effective against bacteria and fungi species (Angioni et al., 2003), and the same effect was observed in insects (Almquist et al., 2006; Rocchini et al., 2000). Delta-3-carene had strong inhibition on the rumen microbial activity in sheep (Oh et al., 1967).

PSM production and composition are well studied in conifers. Among conifer taxons, terpenoid biosynthesis shows high similarity and their genetic regulation is also known (Fäldt et al., 2003; Trapp and Croteau, 2001). Terpene production and their relative amounts are genetically determined and expressed through specific enzymes, monoterpene synthetases (Huber et al., 2004; Martin et al., 2004). Thoss and colleagues (2007) found two different chemotypes, either δ-3-carene or α-pinene dominated, for Scots pine (*Pinus sylvestris*). They also found δ-3-carene production to be negatively associated with α-pinene concentration. The same biochemical trade-off between δ-3-carene and α-pinene was found in common juniper (Orav et al., 1997), similarly to junipers of this chemotype in the present study. The δ-3-carene concentration is under strong genetic control (Hiltunen, 1975; Kinloch et al., 1986; Yazdani et al., 1982). In *Pinus taeida*, variation in content of all major monoterpenes except α-pinene, such as β-pinene, myrcene, limonene, β-phellandrene, seems to be controlled by two alleles at a single locus (Squillace et al., 1980).

In the present study, browsed and non-browsed juniper shrubs contained different odor patterns. We found that every δ-3-carene chemotype shrub was untouched. This chemotype contains a special essential oil composition pattern which could be more aversive or toxic to sheep than the other chemotypes, such as α-pinene or sabinene. Chemical quality of the essential oil can be linked to its unique odor matrix (Novák et al., 2001). We should also note that some browsed junipers (shrub ID: 2, 5, 6, see Fig. 1a) showed non-browsed odor characteristics. Supposing that the discrimination ability of sheep and our electronic nose is

similar, there are two alternative hypotheses to explain this category overlap: browsing of junipers could be based on complex odor pattern recognition and selection, or avoidance of one specific component. Neurological bases of mammalian olfaction enabling pattern recognition are well known (Buck and Axel, 1991; Vosshall et al., 2000), and their importance in sheep is also demonstrated (Lledo et al., 2005). Such similarity in the categorization of junipers by sheep and electronic nose suggests that discrimination in both sheep browsing and in the electronic nose analysis may have common bases.

The sensing ability of sheep is crucial for avoiding unpalatable or poisonous plants and for survival (Provenza et al., 1998). Unpalatable food plants often have strong or unpleasant odors and/or taste (Eisner and Grant, 1981). Sheep are able to discriminate different kind of aromatic food items, containing volatile compounds, using their senses. In an aversion learning study Augner et al. (1998) found that sheep preferred weaker flavors over strong ones, because the animals learned to associate the flavor with toxicity and avoided the conditioned flavor depending on its concentration. Narjisse et al. (1996) reported that sheep exposed to the aroma of a monoterpene mixture during feeding discriminated against feed associated with the terpene mixture. Other ruminants are also able to discriminate odors on the basis of the quality of their food. Red deer rejected a pelleted diet while being exposed to the odor of widespread monoterpenes, including α-pinene (Elliott and Loudon, 1987). Monoterpenes were also shown to be related to shrub use by goats (Riddle et al., 1996). Estell et al. (1998) found that the negative influences of specific monoterpene (α-pinene and camphor) concentrations on pellet consumption could explain differential herbivory of individual tarbush plants.

Herbivores may have different kinds of effective, non-exclusive mechanisms to avoid poisoning. We propose several alternative explanations for the avoidance of δ-3-carene chemotype individuals. The first could be that sheep food choice is conservative, thus the new chemical variant could be an unfamiliar food option for them. As Van Tien and his colleagues (1999) demonstrated, sheep readily eat foods with a familiar odor, but if an unfamiliar food is presented, the animals show aversion. The second explanation could be that the δ-3-carene dominated individuals are relatively new phenotypic variants among junipers in our stand. Therefore, herbivores have not yet developed defense mechanisms to cope with this special and potent toxin mixture. The chemical polymorphism in the studied juniper population might indicate an ongoing adaptation against local herbivory (Markó et al., 2008), because it might provide relatively higher protection against local herbivores unique in consuming junipers.

The various chirality ratio of certain monoterpenoids could be a third alternative explanation for the relationship of differential browsing and odor characteristics. Several studies proved the importance of enantiomers on the effect of odors (Friedman and Miller, 1971; Laska et al., 1999; Leitereg et al., 1971) or their influence on other biological activities (Filipowicz et al., 2003; Wibe et al., 1998; Norin, 1996). Enantiomer ratio of monoterpenes has been investigated in common juniper and α-pinene, sabinene and myrcene were found as the most varied components (Ochocka et al., 1997). In other juniper populations, the chiral analysis of δ-3-carene detected an entirely (+)-enantiomer, while other monoterpene components' ratio was highly varied (Filipowicz et al., 2006; Hiltunen and Laakso, 1995). The same pattern was found in *Pinus sylvestris*, where large differences were found in the enantiomeric ratios among monoterpenes, but in δ-3-carene only the (+)-enantiomer form could be detected (Sjödin et al., 1996).

Nutrients and PSMs do not act in isolation in determining the level of herbivory (Villalba and Provenza, 2005). Partial preferences for certain plant species have been attributed to the inability of herbivores to discriminate among related plant species (Illius et al., 1999), limited perception (Berec, 2000), attempts to meet nutritional needs (Westoby, 1978), or reducing intake of poisonous food (Freeland and Janzen, 1974). The emerging

evidence which integrates biochemical interplay between plant species and browsers suggests that varied diets result from the experience with nutrients and toxins (Provenza, 1995; Provenza et al., 2003). Responses of herbivores integrate the post-ingestive effects of PSMs with sensory experience that results in odor–nutrient–phytotoxin interactions.

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Table legends

Table 1. F–statistics of GLMM analyses of electronic nose.
F–statistics of GLMM analyses are shown. Principal components (PC) are from the PCA of the electronic nose. Stars indicate significance the level among factors (* - $P<0.05$; ** - $P<0.01$). The model had the statistics $F=11.48$, d.f.=3,14, $R^2=0.649$, $P<0.001$.

Table 2. F–statistics of GLMM analyses of gas–chromatography.

589 F–statistics of GLMM analyses are shown. Principal components (PC) are from the PCA of
590 gas–chromatography. Stars indicate the significance level among factors (* - $P<0.05$; ** -
591 $P<0.01$). The model had the statistics $F=7.591$, d.f.=2,15, $R^2=0.436$, $P=0.0052$.
592
593 Table 3. Composition of the essential oils of the three factors of *Juniperus communis* from
594 Hungary.
595 The components are quoted by their retention order of GC analysis. The most important
596 factors are indicated by stars (*) in each essential oil component.
597
598 Table 4. Composition of the essential oils of the three chemotypes of *Juniperus communis*
599 from Hungary.
600 Columns contain the mean values, standard deviations (SD) and confidence intervals (CI) of
601 each chemotypes, which were calculated from the given sample size (N). The rows contain
602 the main essential oil components.
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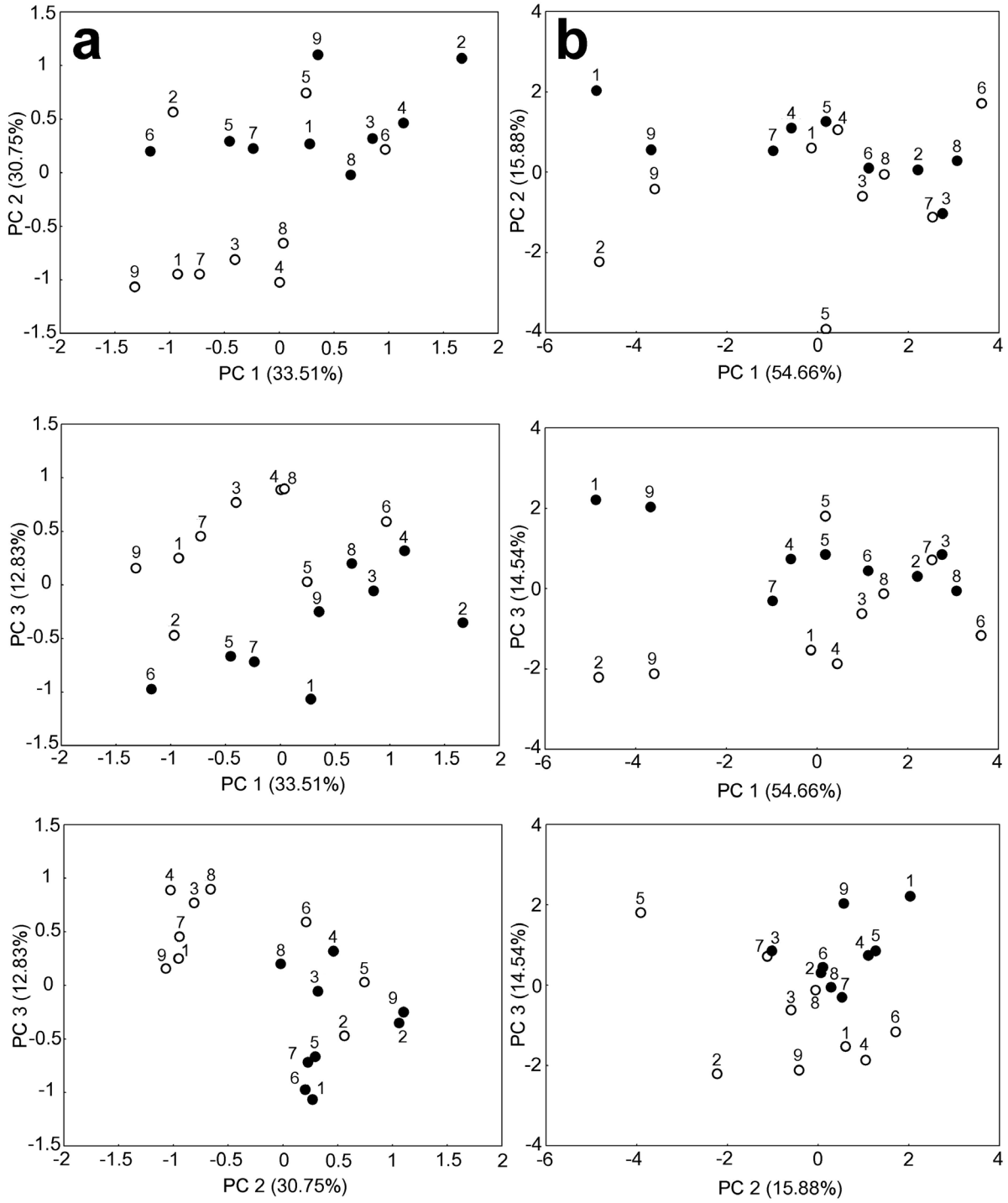
606 **Figures**



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608 Figure 1. Scatter plots of principal components (PC) showing the electronic–nose (a) and GC
609 (b) discrimination of the juniper shrubs. The lines enclose the browsed (empty circles) and
610 non–browsed (full circles) individuals, respectively; labels describe samples. The cumulative
611 power of electronic–nose was 77.1% for the first three factors, while it was 85.1% for the GC.
612 The browsed groups were well demarcated from the non-browsed groups, but some browsed
613 shrubs (ID: 2, 5, 6) showed non-browsed odor characteristics in both classification methods.
614

Table 1. F–statistics of GLMM analyses of electronic nose.

	F value	d.f.	P
PC 1	8.29	1,14	0.012 *
PC 2	10.76	1,14	0.005 **
PC 3	15.37	1,14	0.001 **
PC 1:PC 2	0.24	1,13	0.628
PC 1:PC 3	1.00	1,13	0.334
PC 2:PC 3	0.04	1,13	0.828
PC 1:PC 2:PC 3	0.49	1,13	0.492

F–statistics of GLMM analyses are shown. Principal components (PC) are from the PCA of the electronic nose. Stars indicate significance the level among factors (* - P<0.05; ** - P<0.01). The model had the statistics F=11.48, d.f.=3,14, R^2=0.649, P<0.001.

Table 2. F–statistics of GLMM analyses of gas–chromatography.

	F value	d.f.	P
PC 1	0.02	1, 14	0.873
PC 2	4.64	1, 15	0.047 *
PC 3	10.53	1, 15	0.005 **
PC 2:PC 3	1.24	1, 14	0.232

F–statistics of GLMM analyses are shown. Principal components (PC) are from the PCA of gas–chromatography. Stars indicate the significance level among factors (* - P<0.05; ** - P<0.01). The model had the statistics F=7.591, d.f.=2,15, R^2=0.436, P=0.0052.

Table 3. Composition of the essential oils of the three factors of *Juniperus communis* from Hungary.

Ret. time	Component	Terpene group	Factor 1	Factor 2	Factor 3
8.75	Unknown	Monoterpene?	–0.964 *	0.080	–0.044
9.02	α–Pinene	Monoterpene	–0.284	0.267	–0.660 *
10.08	Sabinene	Monoterpene	–0.433	–0.473	–0.705 *
10.15	δ–3–Carene	Monoterpene	–0.569	0.474	0.620 *
10.44	β–Myrcene	Monoterpene	–0.985 *	0.076	0.021
11.14	Unknown	Monoterpene?	–0.988 *	–0.059	0.002
11.23	Unknown	Monoterpene?	–0.662 *	–0.311	–0.445
11.51	γ–Terpinene	Monoterpene	–0.659 *	0.487	–0.266
12.22	Unknown	Monoterpene?	–0.888 *	0.083	0.280
12.98	Unknown	Monoterpene?	–0.966 *	–0.008	0.118
18.03	Borneol	Oxigenated monoterpene	–0.883 *	–0.259	0.191
23.61	trans–Nerolidol	Oxigenated sesquiterpene	–0.471	–0.701 *	0.310
24.76	γ–Muurolene	Sesquiterpene	0.180	–0.792 *	0.269
	Expl. variance		7.105	2.064	1.889
	Prp. total		0.546	0.158	0.145

The components are quoted by their retention order of GC analysis. The most important factors are indicated by stars (*) in each essential oil component.

Table 4. Composition of the essential oils of the three chemotypes of *Juniperus communis* from Hungary.

Components	Chemotype δ–3–carene (N=4)			sabinene (N=13)			α–pinene (N=1)		
	Mean (µl)	± SD	CI	Mean (µl)	± SD	CI	Mean (µl)	± SD	CI
α–Pinene	41.36	± 5.61	3.18 – 20.93	46.33	± 37.78	27.1 – 62.38	81.63		
Sabinene	0		0 – 0	121.20	± 92.03	66 – 151.93	3.04		
δ–3–Carene	239.9	± 117.64	66.65 – 438.66	3.54	± 2.45	1.76 – 4.05	0		
β–Myrcene	24.16	± 9.49	5.38 – 35.39	13.70	± 8.47	6.08 – 13.99	3.77		
γ–Terpinene	20.28	± 4.31	2.44 – 16.09	14.68	± 6.42	4.61 – 10.61	21.05		
Borneol	21.65	± 8.51	4.82 – 31.74	14.68	± 8.57	6.15 – 14.15	0		
cis–Nerolidol	0		0 – 0	1.80	± 2.62	1.89 – 4.34	0		
trans–Neroidol	9.04	± 6.13	3.48 – 22.89	7.90	± 6.31	4.53 – 10.42	1.95		
γ–Muurolene	0		0 – 0	3.74	± 5.11	3.67 – 8.44	0		
Total volume of the essential oil	431.06	± 170.06	96.34 – 634.09	283.36	± 162.92	116.83 – 268.95	121.65		

Columns contain the mean values, standard deviations (SD) and confidence intervals (CI) of each chemotypes, which were calculated from the given sample size (N). The rows contain the main essential oil components.