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ORIGINAL PAPER

Qualitative assessment of an ultra-fast portable gas chromatograph (zNose™) for analyzing volatile organic chemicals and essential oils in laboratory and greenhouses

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Abstract Qualitative performance of a portable gas chromatograph (zNose™) was assessed by comparing retention indices of major constituents of an essential oil-based insect repellent, and by comparing retention index of limonene, a major chemical in volatile blends of tomato plants, in the laboratory, a research greenhouse and a commercial greenhouse. Effects of temperature and relative humidity on the performance of the device were also assessed. In all experiments, the zNose™ produced consistent results comparable to that of a conventional GC–MS. Our results concur with previous studies confirming the zNose™ as a suitable device for analyzing plant volatiles in the field and for monitoring their rapid changes.

Keywords Gas Chromatograph · Retention Index · zNose™ · Volatile organic chemicals

Introduction

Analysis of herbivore-induced plant volatiles (HIPVs) is a critical component of many research projects that study insect–plant interactions. The scientific literature on HIPVs is vast and continuously growing. In most cases, the researcher does not know the biological activity of the compounds assessed and therefore samples and analyzes a full range of HIPVs. Usually the volatile organic compounds in the headspace of plants that are enclosed in collection chambers are collected through use of an adsorbent material. Subsequently the collected volatiles are eluted and analyzed by gas chromatography (GC) and mass-spectrometry (MS) or a combination of both (GC/MS) (Turlings et al. 1990; Pickett et al. 1999). D'Alessandro and Turlings (2006) examined the most commonly used HIPV collection methods from 1995 to 2004. They found that adsorbent/solvent desorption was the most popular method among the scientists who studied HIPVs in insect-plant interactions (D'Alessandro and Turlings 2006). There are different approaches to volatile analysis depending on the physiological state of the plant, and whether the plant tissue is intact or has been detached from the plant. Choosing the method of analysis that will give the most accurate results takes some careful consideration. For plant-herbivore interaction studies, using intact plants is generally considered to be most rigorous and accurate. Experiments that use detached plant parts often show more fluctuations in volatile profile than intact plants and are often verified against intact plant results to compensate for oscillations caused by the mechanical damage from detachment (Jakobsen 1997).

For studies in chemical ecology, using intact plants is considered the most reliable method but additional considerations must be made to minimize other factors that can affect the accuracy of the results. Such parameters to consider are light intensity, relative humidity (RH), air temperature, and photoperiod (Jakobsen 1997; Gouinguene and Turlings 2002). Recent improvements in analytical tools (development of bio and chemosensors) enable researchers to collect more accurate data about HIPVs and plant systems within their growing environment in short

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periods of time (Kunert et al. 2002; Pasini et al. 2004; Lu et al. 2006; Oh et al. 2008; Zhong et al. 2009).

In a very comprehensive review paper, Tholl et al. (2006) explored different practical methods of plant volatile collection and analysis. In addition to several conventional collection, separation and detection methods reviewed, they also discussed a relatively new instrument, the zNose™ (Electronic Sensor Technology, Newbury Park, CA, USA). They found this instrument a useful tool for fast quantitative estimation of known volatile profiles and for monitoring rapid changes in volatile organic chemical (VOC) emissions. As a portable device, they suggested that it could also be used in field experiments. This system is a miniature, high-speed gas chromatograph (GC). It is based on a six port valve and oven, a preconcentrating trap, a short GC capillary column (DB-5 1 m length, film thickness 0.25 μm, internal diameter 0.25 mm) and a sensitive surface acoustic wave (SAW) quartz microbalance detector, in which VOC analytes are condensed on the surface of an oscillating crystal. The system produces both the retention time and the retention index based on a series of alkanes from C6 to C14 that is used for tuning the device.

zNose™ has been used in different studies. It has been employed for monitoring rhythmic VOC emissions from flowers and induced VOC emissions from herbivore-damaged plants (Kunert et al. 2002), honey classification (Lammertyn et al. 2004), grape aroma classification (Watkins and Wijesundera 2006), detection of lard adulteration in palm olein (Che Man et al. 2005), characterization of vegetable oils (Gan et al. 2005) and monitoring alarm pheromone emission by the pea aphid under predation (Schwartzberg et al. 2008). Part-per-billion sensitivity has been reported with this instrument for volatile compounds (Staples and Viswanathan 2008).

In this study we analyzed a commercial botanical insect repellent that consists mainly of plant essential oils, on a conventional GC–MS and compared it with the zNose™. Further we assessed the performance of the machine in a commercial greenhouse with variable temperature and humidity.

Materials and methods

Qualitative comparison of samples by the zNose™ and a conventional GC–MS

We analyzed a commercial botanical insect repellent (EcoSMART™ Insect Repellent, EcoSMART Technologies, Alpharetta, GA, USA) that consists mainly of plant essential oils, on a conventional GC–MS and compared it with the zNose™ (Electronic Sensor Technology, Newbury Park, CA, USA) by calculating the retention indices (Van Den Dool and Kratz 1963) on both machines using a C6–C14 alkane standard. The active ingredient oils in this insect repellent are rosemary oil, cinnamon leaf oil, lemongrass oil and geraniol. Rosemary oil is assayed by determining the level of the marker compound 1,8-cineole; cinnamon leaf oil was assayed by determining the level of the marker compound, eugenol; lemongrass oil was assayed by determining the level of the marker compound, citral and geraniol, as a pure compound, was assayed directly. Samples were diluted in acetonitrile and analyzed by GC/MS and quantified against known standards. Cinnamic alcohol was used as an internal standard to improve method precision.

Reagents

Cinnamic alcohol (internal standard)–Aldrich (98 + % pure); 1,8-cineole—Aldrich (99 +% pure); eugenol—Supelco (99.6% pure); citral—Aldrich (95.0% pure); geraniol—Aldrich (98.0% pure); C6–C14 alkane standard—Electronic Sensor Technology (98% pure); acetonitrile HPLC grade—VWR/Canlab.

Preparation of internal and external standard solutions

An internal standard concentrate solution containing circa 100 μg/ml of cinnamic alcohol in HPLC grade acetonitrile was prepared. The internal standard concentrate solution was used to prepare all external standards.

A working stock solution containing all reagents except the alkane standard was prepared from analytical grade material by dissolving the following amounts (Table 2.1) in 10 ml of HPLC grade acetonitrile: 1,8-cineole (99%) 0.1004 g; eugenol (99%) 0.1011 g; citral (95%) 0.1039 and geraniol (98%) 0.2071 g.

The alkane standard was used without dilution. A working stock solution of reagents however, was prepared by diluting 0.2 ml of the standard concentrate solution in 10 ml of HPLC grade acetonitrile. A standard curve was then produced by transferring aliquots of the working stock solution together with the internal standard and acetonitrile to septum capped GC vials for analyses.

Sample preparation

An aliquot of sample (0.2 ml), weighed to the nearest 0.1 mg of commercial product was pipetted into a 10-ml volumetric flask and diluted to the mark with HPLC grade acetonitrile. The flask was then shaken and sonicated for 5 min to facilitate complete dissolution. A subsample (0.5 ml) was transferred to a septum-capped GC vial for analysis. To this sample was added 0.5 ml of the internal standard solution.

GC/MS parameters

A Varian 3900 GC system was used employing a Saturn 2100T ion trap, mass selective detector. Data were handled by a PC using Varian GC/MS Workstation software. The GC was used in temperature gradient mode (Table 1). The column used was a FactorFour Capillary column VF-5 ms, 30 m × 0.25 mm ID DF = 0.25 with a low bleed/MS coating (equivalent to a standard J&W DB5 low bleed MS column). Injections were performed by a Varian CP-8410 autosampler with an injection volume of 1 µl. A split ratio of 100:1 was used in the injection port. The carrier gas was 99.999% UHP helium with a column flow rate of 1.0 ml/min. Total run time was 12 min per sample. The MS detector was used in EI (electron ionization) mode with a filament delay time of 2 min. Scan time was 1.0 s per scan with a mass scan range of 40–300 *m/z*. Maximum ionization time in the ion trap was 25 ms. Ion trap temperature was 220°C, manifold temperature was 80°C and transfer line temperature was 300°C.

Calculation of results

The chemicals were quantified against the standard curve on a PC using Varian GC/MS Workstation software. The retention indices were calculated for each compound using the Van Den Dool and Kratz 1963formula.

Preparation of standard solution for the zNose™ and system calibration

A working stock solution was prepared by diluting 1 g (weighed to the nearest 0.1 mg) of each authentic standard into 50 ml of HPLC grade n-pentane solvent. An aliquot of each (100 µl) working stock was pipetted into a 10-ml volumetric flask and diluted to the mark with HPLC grade n-pentane. The flask was then shaken and sonicated for 5 min to facilitate complete dissolution. A subsample (0.5 ml) was transferred to a septum-capped GC vial for calibration. After tuning the machine with alkane standards four different volumes of neat chemical standards were injected into the zNose™ using the zNose™ 3,500 sample injector (0.1 , 0.2 , 0.4 and 0.8 µl—five times per volume). Calibration curves were developed for each chemical.

Table 1 GC–MS temperature profile

Temperature (°C)	Rate (°C/min)	Hold (min)	Total (min)
80	0.0	0.50	0.50
140	8.0	0.00	8.00
300	50.0	0.80	12.00

Sample preparation for the zNose™

Four µl of commercial insect repellent were transferred into a 40-ml glass vial (98 mm L, 28 mm OD) sealed with a screw cap containing a silicon septum. The samples were allowed to equilibrate with the headspace in the vial for 20 min at room temperature (24°C). Fifteen sample vials were prepared the same way. The zNose™ was provided with a 5-cm stainless still needle at the inlet which was used for sampling through the septa of the vials. The sampling was set for 10 s (sample flow 20 ml/min) after which the system switched to 20 s of data acquisition mode.

zNose™ GC parameters

The inlet temperature was 200°C, the valve temperature was 165°C, and the initial column temperature was 40°C. During analysis the column temperature was increased at the rate of 10°C per second to reach a final column temperature of 200°C. The SAW sensor was operated at 60°C and the trap was operated at 250°C. Helium flow was set at 3.00 ccm. After each data sampling period the system needed a 30-s baking period, in which the sensor was heated briefly (~5–8 s) to 150°C and after which the temperature conditions of the inlet, column, and sensor were reset to the initial conditions. In between each sample measurement at least one blank was run to ensure cleaning of the system and a stable baseline.

Retention index variation of limonene in laboratory and commercial greenhouses

In this experiment, we examined the consistency of the zNose™ in the field. It can be difficult to calibrate the machine at the site of analysis. In some situations, the investigator must calibrate the device the day prior to the analysis due to time and logistic limitations. Therefore, it is important to know whether the machine can provide consistent results in the field as it does in the laboratory. The simple act of transportation may cause the machine to go out of tune.

Therefore, we designed an experiment to compare the retention index of limonene as a representative of the compounds that can be found in volatile blends of tomato plants (Baldwin et al. 2008; Mayer et al. 2008; Mathieu et al. 2009) both in the lab and in a commercial tomato greenhouse. As explained before, the retention indices are related to a n-alkane series and they are relatively unique for most compounds unlike retention times that change with chromatographic methods. Therefore, they are a better option for comparing different GC machines. In addition to the retention index, we recorded changes in temperature

and relative humidity as factors that might affect the performance of the device. The environmental factors were recorded using a Fisher Scientific Enviro-Meter™.

The goal was to compare the retention indices both in the lab and greenhouse therefore quantification was not part of this experiment. The experiment had four levels: analyzing (1) pure chemical by GC–MS, (2) pure chemical by the zNose™in laboratory, (3) tomato volatiles by the zNose™ in laboratory and (4) tomato volatiles by the zNose™ in a commercial greenhouse. One set of alkane standards (C6–C14 alkane standard—Electronic Sensor Technology) was used for all analyses. The zNose™ was calibrated with limonene as explained before. 15 samples were prepared and analyzed in the laboratory both by GC–MS and the zNose™ and the retention indices were calculated. Ambient air temperature and relative humidity was recorded in laboratory. The same methods and setting were used for the GC as explained before. Fifteen tomato plants (6 weeks old) were obtained from Houwelings' Hot House (Delta, BC) for this analysis. Plants were kept inside a growth chamber at 23°C with a16/8 light–dark period for 2 days prior to the experiment. Plants were then transferred to the laboratory. The same analysis method was used in the zNose™. Volatiles were collected from the head space above the plants at 10 cm distance. The only difference was the sampling time, which was 30 s (sample flow rate 20 ml/min) as opposed to 10 s for chemicals in vials.

After sampling plant volatiles in the laboratory, the zNose™ was then turned off and stored in its carrying case. The greenhouse samples were collected the following day. Fifteen locations were randomly selected within the commercial tomato greenhouse (Houweling's Hot Houses, Delta, BC) and plant volatiles were collected and analyzed by the zNose™ using the same method and settings. Air temperature and relative humidity were recorded at each spot. The same analytical method was used for both the greenhouse and the laboratory samples. HPLC grade n-pentane was used once as a blank between each sample both in the laboratory and in the greenhouse.

Data analyses

Retention index values of 1,8-cineole, eugenol, citral and geraniol on both machines were analyzed by *t*-test (SPSS ver 16). The effect of temperature, relative humidity and method (GC–MS or zNose™ in laboratory or greenhouse) on retention index of limonene were analyzed with linear multiple regression using SPSS software version 16.

Results

Comparing retention indices of selected chemicals analyzed by the zNose™ and conventional GC–MS

The calibration curves of all chemicals analyzed with GC–MS and the zNose™ were linear. 1,8-cineol in GC–MS ($y = 1.3859X + 0.061803$, $R2 = 0.9973$) and in zNose™ ($y = 190.33X + 1504$, $R2 = 0.\ 9721$); eugenol in GC–MS ($y = 2.30343X + 0.0469$, $R2 = 0.9995$ and in zNose™ ($y = 295.07X + 1421$, $R2 = 0.9704$); geraniol in GC–MS ($y = 1.74479X + 0.019488$, $R2 = 0.9993$) and in zNose™ ($y = 260.23X + 88.435$, $R2 = 0.9952$); and citral in GC–MS ($y = 2.03368X + 0.034854$, $R2 = 0.9960$) and in zNose™ ($y = 237X + 10503.3$, $R2 = 0.9985$). No significant differences were found among the retention indices calculated from GC–MS data and the retention indices generated by the zNose™ (Table 2).

Retention index variation of d-limonene in laboratory and commercial greenhouses

According to the results, temperature, relative humidity and analytical method are not significantly correlated with

Table 2 Comparing the retention indices of four chemicals analysed by GC–MS and zNose™ in laboratory and greenhouse

Chemical	Analysis	Mean	SD	SEM	*F*	*F*-sig	*t*	*df*	*t*-sig	95%CI (L-U)	
1,8-Cineole	GC–MS	1041.2	0.414	0.106	0.758	0.391	0.332	28	0.742	−0.2342	0.3249
	zNose™	1041.2	0.328	0.084							
Eugenol	GC–MS	1367.7	0.457	0.118	0.707	0.408	0.418	28	0.679	−0.3931	0.2597
	zNose™	1367.8	0.414	0.106							
Citral	GC–MS	1273.7	0.487	0.125	2.635	0.116	0.807	28	0.426	−0.4718	0.2051
	zNose™	1273.5	0.414	0.106							
Geraniol	GC–MS	1253.3	0.457	0.118	2.611	0.117	0.595	28	0.556	−0.1333	0.2239
	zNose™	1,253.4	0.736	0.196							

SD standard deviation, *SEM* standard error mean, *F* Levene's test for equality of variances, *F-sig* significance of the Levene's test, *t* t-test for equality of means, *df* degree of freedom, *t-sig* significance of the *t*-test, *95% CI (L-U)* 95% confidence interval of the difference with lower and upper intervals

Table 3 Effect of temperature, relative humidity and analyses method (GC–MS + neat chemical, zNose +neat chemical, zNose + plant in laboratory and zNose in greenhouse) on retention index of limonene

Descriptive statistics		Mean	SD	*N*
RI		1.0358E3	0.6195	60
Temperature		24.1897	0.77505	60
RH%		42.3542	3.74332	60
Method		2.5000	1.12747	60
Correlations	RI	Temperature	RH%	Method
Pearson correlation				
RI	1.000	−0.054	−0.100	−0.163
Temperature	−0.054	1.000	0.869	0.638
RH%	−1.00	0.869	1.000	0.404
Method	−0.163	0.638	0.404	1.000
Sig. (1-tailed)				
RI		0.340	0.223	0.107
Temperature	0.340		0.000	0.000
RH%	0.223	0.000		0.001
Method	0.107	0.000	0.001	

Model	*R*	*R* square	Adjusted *R* square	Std. error of the estimate
Model summary				
1	0.257[a]	0.066	0.016	0.41215

[a] Predictors: (Constant), method, RH%, Temperature

the retention index. Only 6.6% of the variation of retention index can be explained by temperature, relative humidity or analytical method (Table 3). The regression line predicted by the independent variables (temperature, RH% and method) do not explain a significant amount of variation in the retention index: $F(3, 56) = 1.316$; $P > 0.05$. None of the independent variables are individually significant predictors of the retention index (Table 4).

Discussion

In this study we assessed a relatively new analytical technology. The zNose™ is an instrument for rapid gas chromatography that is capable of repeated quantitative sampling of headspace volatiles (Kunert et al. 2002; Tholl et al. 2006). Our main goal was to investigate the detection and identification capability of the zNose™ as well as its consistency in the field under variable environmental conditions. To do so, we compared retention indices (Van Den Dool and Kratz 1963) of known chemicals analyzed by the zNose™ and compared them with the retention indices of the same chemicals analyzed by a conventional GC–MS. Our results indicate that the zNose™ is capable of correctly identifying the known compounds. The SAW sensor can produce quite consistent results in spite of the environmental conditions which makes this device a good option for field work. Because of the fast analysis time (about 3 min for a complete cycle), it can be used for monitoring rapid changes in volatile emissions of plants and other organisms (Schwartzberg et al. 2008). One of the major shortcomings of this machine is its short column (1 m) which can limit resolution of peaks when there are a large number of peaks in a sample. Based on our personal experience, we do not recommend overloading the system with more than 10 chemicals at one time during calibration. Calibration is another area which needs careful attention.

Table 4 Correlation of limonene retention index with temperature, relative humidity and analyses method (GC–MS + neat chemical, zNose+neat chemical, zNose + plant in laboratory and zNose in greenhouse)

ANOVA: dependent variable = retention index					
Model	Sum of squares	*df*	Mean square	*F*	Sig
Regression	0.671	3	0.224	1.316	0.278[a]
Residual	9.513	56	0.170		
Total	10.183	59			

Coefficients: dependent variable = retention index					
Model	Unstandardized coefficients		Standardized coefficients	*t*	Sig
	B	SE	Beta		
(Constant)	1031.422	3.172		325.145	0.000
Temperature	0.273	0.181	0.509	1.508	0.137
RH%	−0.046	0.032	−0.413	−1.453	0.152
Method	−0.118	0.067	−0.320	−1.756	0.085

[a] Predictors: (Constant), method, RH%, Temperature

Table 5 Feedback to the zNose™ manufacturer

	Recommendations
Hardware	The limitation of peak resolution might be solved with a longer column. It might slightly increase the analysis time but would allow for a greater range of compounds that can be analyzed by the machine
Operation	The cooling fan for the sensor is located on top of the inlet. Collecting samples in the field, might be affected by the fan especially when the operator is analyzing a compound in low concentrations. An attachment for the inlet (like a funnel) can prevent this problem
Software	The method panel and setting panel do not synchronize automatically. The operator must make changes twice to be effective
	Data logging is relatively difficult. It would be very helpful if the operator could extract the information and record it directly into an excel file
	It would be more convenient if the battery charge level was displayed as percentage

We believe that the zNose™ needs to be calibrated for each new analysis to improve the precision of the results.

There is a range of different opinions about this particular instrument among researchers.

Like any new instrument, the zNose™ has some limitations and problems that might be improved over time (Table 5). But these problems won't get addressed unless researchers use this instrument and push it to its limits and provide feedback to the manufacturer. Overall, our results confirmed previous studies and show that the zNose™ can be used for volatile analysis both in the laboratory and the field. With proper care and maintenance it can provide consistent results and enable us to perform rapid volatile analyses in the field.

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