

## Original Paper

## Open Access

## Preliminary results of variability in mechanical-induced volatile root-emissions of different maize cultivars

Žiga Laznik<sup>1\*</sup>, Iztok Jože Košir<sup>2</sup>, Ludvik Rozman<sup>1</sup>, Milica Kač<sup>3</sup>, Stanislav Trdan<sup>1</sup>

<sup>1</sup>University of Ljubljana, Biotechnical Faculty, Dept of Agronomy, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia

<sup>2</sup>Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, SI-3310 Žalec, Slovenia

<sup>3</sup>University of Ljubljana, Biotechnical Faculty, Dept of Food Science and Technology, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia

\*Corresponding author: E-mail: [ziga.laznik@bf.uni-lj.si](mailto:ziga.laznik@bf.uni-lj.si)

### Abstract

The current study was conducted to obtain more information on the specificity and variation of the volatile compounds, which are released from the root system of different lines and hybrids of *Zea mays* plants. European (12 genotypes) and North American (5 genotypes) genetic material was included (15 maize hybrids and 2 maize inbreds). The results of our study have shown that roots of European and American maize genotypes secrete different relative amounts of linalool,  $\alpha$ -caryophyllene and  $\beta$ -caryophyllene, which depend on genotype and on the pretreatment of the roots (various mechanical damages).

**Keywords:** *Zea mays*, root system, volatiles, linalool,  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene

### Introduction

Maize, *Zea mays* L, is the world's most abundant harvested crop, followed by rice and wheat (FAO, 2007) and the second largest harvested crop in Europe (FAO, 2007). In most maize-growing environments, pest insects and diseases are considered a limiting factor to maize production. Insect pests cause severe economic losses to farmers by reducing crop yield and quality as well as increasing crop production costs (Heinrichs, 1988). The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is the most severe maize pest in United States and in some parts of Europe (Miller et al, 2005). Rootworm larvae prefer to feed upon succulent new growth of nodal root axes. Very specific nodes of root axes, namely the 3<sup>rd</sup> through 6<sup>th</sup>, produce succulent new growth near the base of the maize plant during the time of egg hatch and larval development. This synchrony of root system growth and larval development enhances feeding of larvae on specific nodes of the adventitious root system and causes damage on the plants (Riedell, 1989). The resulting crop physiological stress often leads to significant grain yield loss (Riedell et al, 1996).

In areas without crop rotation, WCR is exceedingly difficult to control, and insecticide applications are relatively expensive, environmentally unfriendly, and not always effective (Levine and Oloumisadeghi, 1991). The use of genetically modified maize lines that carry bacterial-derived genes coding for Bt toxins shows promise (Romeis, 2006), but resistance traits are likely to develop (Meihls et al, 2008). Plants defend themselves against herbivores either directly using toxins, repellents, and/or morphological structures, or indirectly by attracting the enemies of herbi-

vores (Agrawal, 1998; Dicke and Hilker, 2003).

Reacting to herbivore attack, many plants release volatiles (Paré and Tumlinson, 1999). Volatile organic compounds (VOCs) can attract predatory arthropods (Dicke and Sabelis, 1988; Turlings et al, 1990; De Moraes et al, 1998) and/or repel herbivores (De Moraes et al, 2001) and thus serve as means of plant defense against herbivores (Rasmann et al, 2005). Rasman et al (2005) proved that maize roots respond to WCR larvae attack by releasing (E)- $\beta$ -caryophyllene (E $\beta$ C). Releasing this VOC has been determined as a response to herbivore damage in several wild relatives of maize (Gouinguéné et al, 2001) and in cultivated maize inbreds from European breeding programs, but it is absent from maize inbreds originating from North American breeding programs (Degen et al, 2004). E $\beta$ C is not found in healthy maize roots and is known to attract the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Rasmann et al, 2005).

The current study was aimed to obtain more information on the specificity and variation of the volatile compounds, which are released from the root system of different *Zea mays* lines and hybrids, including European and North American genetic material (12 European and five North American genotypes were included into the study). Further on we would like to test the influence of these volatiles on the entomopathogenic nematodes and to determine their mode of action (attractants, repellents, indifferent compounds).

### Materials and Methods

*The genetic material included in the study and the growing of plants*

As presented in **Table 1**, 15 maize hybrids and two maize inbreds were included in the study. The plants were grown at the Laboratory of Entomology (Biotechnical Faculty, Dept of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Pasture and Grassland Management, Ljubljana, Slovenia) as follows: seeds were placed on moist filter paper in glass Petri dishes and put into rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia) with a volume of 0.868 m<sup>3</sup> (width x height x depth = 1,000 x 1,400 x 620 mm) and temperature was set on 25°C. To enhance the germination of the seeds, additional moisturizing was provided by a sprayer. The germinating seeds were manually transferred into Styrofoam trays with perlite (Humko doo, Bled, Slovenia), each having 240 holes (14 ml). These were then placed in a floating system

in a glasshouse, the temperature of the water being 25 to 28°C.

The average daily temperature in the compartment was 14±2°C. Relative humidity was maintained at 75±10% using ventilation throughout the growing season. Greenhouse climate was monitored and controlled by a DGT-Volmatic System. The water in the floating system contained nutrients presented in **Table 2**. After two weeks, at an age when all the cultivars carried three developed leaves and the forth was showing and sustainable mass of the root system, the plants were used for the experiment.

#### **Preparation of plant roots for analysis of volatiles**

The root system of 10 plants was treated as a sample. The root samples were studied in three groups: non damaged (ND), sporadically damaged (SD), and quickly damaged (QD). In the first group

**Table 1** - The maize genotypes included in the study

Maize hybrid or inbred	Code	Representative	Breeder
LJ-275	KOLJ	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
LJ-6	LJ6	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
LJ-8	LJ8	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia	Dr. Ludvik Rozman, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
Anjou 280	KOA	Semenarna Ljubljana, doo, Slovenia	Limagrains Genetics BV, Netherlands
Anjou 292	A292	Semenarna Ljubljana, doo, Slovenia	Limagrains Genetics BV, Netherlands
Anjou 400	A400	Semenarna Ljubljana, doo, Slovenia	Limagrains Genetics BV, Netherlands
Anjou 450	A450	Semenarna Ljubljana, doo, Slovenia	Limagrains Genetics BV, Netherlands
PR35D28	PR35D28	Pioneer Semena Holding GmbH, Murska Sobota, Slovenia	Pioneer Overseas Corporation, USA
PR37H24	PR37H24	Pioneer Semena Holding GmbH, Murska Sobota, Slovenia	Pioneer Overseas Corporation, USA
PR38A79	PR38A79	Pioneer Semena Holding GmbH, Murska Sobota, Slovenia	Pioneer Overseas Corporation, USA
PR38P05	PR38P05	Pioneer Semena Holding GmbH, Murska Sobota, Slovenia	Pioneer Overseas Corporation, USA
SAXXOO Ragt	Saxxoo	Agrosaat doo, Ljubljana, Slovenia	Dekalb Genetics Corporation, USA
ZP599	ZP599	KGZ Sloga Kranj zoo, Kranj, Slovenia	Institut za kukuruz »Zemun polje«, Serbia
Cisco	C	Syngenta Agro doo, Ljubljana, Slovenia	Syngenta Seeds AG, Switzerland
Pactol	P	Syngenta Agro doo, Ljubljana, Slovenia	Syngenta Seeds SAS, France
Occitan	O	Syngenta Agro doo, Ljubljana, Slovenia	Syngenta Seeds SAS, France
BC318	BC318	Semevit doo, Lenart, Slovenia	Bc Institut Zagreb, Croatia

(the non damaged roots, ND), the roots were left as they were, without any inflicted injuries. In the second group (sporadically damaged roots, SD) the roots of maize plants were stabbed five times a day with a metal corkborer (diameter 7 mm) for three consecutive days and in the third group (quickly damaged roots, QD) the maize roots were damaged with a metal corkborer one hour prior to washing and subsequent powdering in liquid nitrogen.

Samples (the roots) were washed with water, frozen in liquid nitrogen, and stored in refrigerator at  $-20^{\circ}\text{C}$  till further analysis as this is one of the standard procedures of keeping biotic samples of this kind (Rasmann et al, 2005).

Further sample preparation and GC-MS analysis were also performed according to Rasmann et al (2005). The SPME sample preparation of frozen roots was as follows: frozen maize roots were pulverized in a mortar and 0.4 g of each sample were weighed into 40 ml headspace vials, closed with a septum and stored in a freezer at  $-10^{\circ}\text{C}$  until analyzed (prevention of possible evaporation of volatile compounds). The headspace vial was then conditioned in a thermostatic water bath at the temperature of  $40^{\circ}\text{C}$ . A 100  $\mu\text{m}$  polydimethylsiloxane (PDMS) SPME fiber was inserted through the septum and exposed for 60 min. The compounds adsorbed on the fiber were analyzed by GC-MS with a Varian STAR 3400 CX GC system coupled to an ion trap mass selective detector (Varian SATURN 2000); transfer line  $260^{\circ}\text{C}$ , source  $180^{\circ}\text{C}$ , ionization potential 70 eV. The fiber was inserted manually into the injector port ( $180^{\circ}\text{C}$ ), desorbed and chromatographed on a nonpolar column (5% diphenyl and 95% dimethylsiloxane) (HP-5MS, 30 m, 0.25 mm, 0.25  $\mu\text{m}$ ; Agilent Technologies). Helium at a constant pressure of 240 kPa was used as carrier gas. After fiber insertion, the column temperature was maintained at  $50^{\circ}\text{C}$  for 3 min and then increased to  $180^{\circ}\text{C}$  at a rate of  $5^{\circ}\text{C}/\text{min}$  followed by a final stage of 3 min at  $250^{\circ}\text{C}$ . Detector temperature was set to  $280^{\circ}\text{C}$ .

Identification of compounds was performed using standard compounds:  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene and linalool, humulene (all purchased by Fluka)

and by comparison of MS spectra with those from MS library. All analyses were performed in parallel and under the semiquantitative conditions to ensure the relative comparability between the samples.

For the purpose of quantification of volatiles and checking the linearity of the measurements, calibration curves were constructed with the addition of each compound to the sample at the concentrations from 0.125 to 0.500 ng/g. Corresponding calibration coefficients ( $R^2$ ) obtained were 0.9992 for  $\alpha$ -caryophyllene, 0.9957 for  $\beta$ -caryophyllene and 0.9987 for linalool. Repeatability of the measurements was tested with the measurements of six repetitions at the lowest concentration level on the calibration curves. The obtained RSD were as follows: 9.2% for  $\alpha$ -caryophyllene, 9.6 % for  $\beta$ -caryophyllene and 9.7 % for linalool. Obtained RSDs were used as the deviation of the method for all the rest measurements of the samples although in addition all samples were analysed in triplicates.

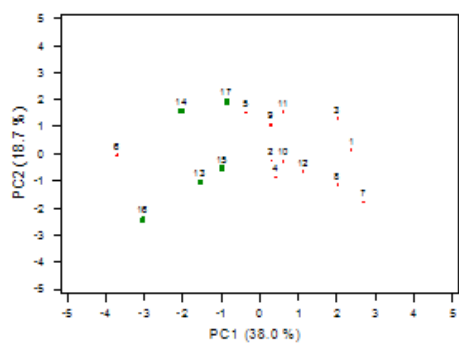
#### Statistical analyses

An ANOVA was conducted in order to establish the differences among European and American genotypes (analysis of pooled results and individual analysis). Before analysis each variable was tested for homogeneity of treatment variances. If variances were non-homogeneous, the data were transformed to log (Y) before conducting the ANOVA. Duncan's multiple range test ( $P < 0.05$ ) was used to separate mean differences among studied parameters in all treatments. In order to examine the differences among maize genotypes in terms of the patterns of their contained and determined organic compounds, principal component analysis (PCA) was applied to the concentration contents (Figure 1) of individual compounds and treatments respectively (reduction of nine dimensional space to a two dimensional one). The PCA scores were estimated from the correlation matrix. The first two components (PC1 and PC2) represented the most prominent differences between-genotypes in the terms of the variance in the pattern of the determined organic compounds. Graphically, PC1 was plotted against PC2 where points on the graph represent maize genotypes. All statistical analyses were

**Table 2** - Chemical characteristics of water in the floating system of the glasshouse, where maize plants for further chemical analysis were grown.

Parameter	Unit	Quantity	Parameter	Unit	Quantity
pH		7.4	potassium	mg K/l	1.3
electroconductivity	mS/cm	0.486	magnesium	mg Mg/l	31.0
nitrate	mg $\text{NO}_3^-/\text{l}$	3.9	sodium	mg Na/l	1.2
phosphorous	mg $\text{PO}_4^{3-}/\text{l}$	0.25	iron	mg Fe/l	0.036
chloride	mg Cl/l	2.5	manganese	mg Mn/l	0.014
sulphate	mg $\text{SO}_4^{2-}/\text{l}$	7.0	zinc	mg Zn/l	0.031
boron	mg B/l	0.04	copper	mg Cu/l	0.0005
fluoride	mg F/l	0.11	molybdenum	mg Mo/l	0.005
calcium	mg $\text{Ca}^{2+}/\text{l}$	59.0			

done using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp, Manugistics, Inc). The data in Table 3 is presented as means of triplicates.



**Figure 1** - Scatterplot in two dimensional space of PC1 and PC2 for 17 maize genotypes (1-12 European; 13-17 North American) based on variables used to estimate the relationships among organic compound secreted by maize roots due to pretreatment of the roots. The outlier is genotype Saxoo (6).

## Results

### *General comparison of European and American genotypes regarding secreted volatile organic compounds (VOCs)*

In Figure 2 two GC/MS chromatograms of samples bc318 (A) and pr37h24 (B) are presented as a representative of European and American genotypes. In the chromatogram in Figure 2A peaks of linalool,  $\alpha$ - and  $\beta$ -caryophyllene are marked to show the characteristic retention times that were 12.5, 22.8, and 21.9 min respectively.

The results of the analyses showed that the roots of European and North American maize genotypes exhibit some pronounced differences in the secretion of volatiles under investigation, namely: linalool,  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene (Table 3). These differences are in some either due to treatment (ND, QD, SD roots) or to differences in genotype (individual varieties and/or European vs. American group).

The European group of maize genotypes showed no statistically significant differences for the average amount of secreted linalool vs. the American group as far as various treatments of roots are considered (non damaged, sporadically damaged, and quickly damaged). The average values within the European group ranged from 0.013 (QD) to 0.026 (ND) and for the American group from 0.042 (ND) to 0.086 (QD). Although the tendency of less pronounced linalool secretion within the European group compared to the American one is clearly indicated, the statistically significant difference could be confirmed only for the QD treatment (European varieties: 0.013 and American varieties: 0.086).

The studies on the relative  $\beta$ -caryophyllene root secretion led to the following conclusions. Within the European maize genotypes there were no statistically significant differences regarding ND, QD, and SD treatments. The values ranged from 0.283 (SD) to 0.314 (ND). For the American genotypes the statistically significant lowest average relative content of  $\beta$ -caryophyllene was determined for the ND roots (0.264), while the differences for the QD roots (0.302) and the SD roots (0.324) were not statistically significant. Comparison of the European with the American genotypes revealed a statistically significant difference between European (0.314) and American (0.264) genotypes but only in the ND group; the QD and the SD groups showed no such characteristics.

The results for the average secretion of  $\alpha$ -caryophyllene for the European genotypes showed a statistically significant lowest value for the SD group (0.223), while the ND (0.225) and the QD (0.224) groups were not statistically significantly different. No statistically significant differences were observed between the treatments in group of American genotypes. The values ranged between 0.200 (SD) and 0.210 (QD). All the treatments revealed that the relative values for the  $\alpha$ -caryophyllene secretion are significantly higher for the European genotypes compared to the American ones.

The results of the PCA analysis revealed, that the first and the second principal component (PC1, PC2) accounted for 38.0 % and 18.7% of the variance in the data respectively. Graphical representation of the PCA results (Figure 1) in the two-dimensional plot of PC1 and PC2 on two perpendicular axes reveals interesting differences among maize genotypes. PC1 clearly separated European genotypes from the North American ones (with only two exceptions) and the second principal component added to the distribution within (especially European) genotype group due to the substances (VOCs) in question.

### *VOCs for the maize genotypes with non damaged (ND) roots*

For the individual European genotypes the differences in the relative linalool secretion in the ND group were statistically significant (Table 3). The values in question were from 0.0 (LJ6, LJ8, P, A400, BC318, O, A450, C and ZP599) to 0.18 (A292). Varieties remaining KOLJ (0.07) and KOA (0.07) were not statistically significantly different. Among the American genotypes the only variety secreting linalool under the ND root treatment was Saxoo (0.21).

Individual European as well as American maize genotypes exhibited statistically significant differences in the secreted relative amount of  $\beta$ -caryophyllene under ND treatment; the values ranging from 0.27 (P) and 0.36 (A400) for the European and from 0.25 (Pr38A79 and Pr35D28) to 0.29 (Pr37H24) the American ones. Among three European varieties, namely LJ8, KOA and CN were no statistically significant difference.

**Table 3** - Relative contents of  $\alpha$ -caryophyllene,  $\beta$ -caryophyllene, and linalool from different maize genotypes. All concentrations of volatiles are in ng/g fresh root tissue.

	European genotypes										American genotypes						
	Lj6	KOLJ	LJ8	KOA	P	A292	A400	BC318	ON	A450	CN	ZP599	PR38A79	PR38P05	PR37H24	Saxxoo	Pr35D28
	$\alpha$ -caryophyllene																
ND	0.24	0.23	0.25	0.21	0.22	0.20	0.24	0.23	0.22	0.21	0.22	0.23	0.21	0.21	0.19	0.21	0.21
QD	0.25	0.21	0.24	0.23	0.21	0.20	0.24	0.23	0.22	0.22	0.23	0.21	0.22	0.22	0.20	0.20	0.21
SD	0.22	0.23	0.18	0.22	0.22	0.19	0.23	0.22	0.24	0.21	0.30	0.22	0.20	0.20	0.19	0.19	0.22
	$\beta$ -caryophyllene																
ND	0.33	0.30	0.31	0.31	0.27	0.29	0.36	0.35	0.29	0.32	0.31	0.34	0.25	0.26	0.29	0.27	0.25
QD	0.32	0.33	0.31	0.32	0.25	0.28	0.35	0.36	0.27	0.32	0.29	0.32	0.41	0.25	0.29	0.30	0.26
SD	0.30	0.30	0.26	0.34	0.29	0.27	0.37	0.33	0.29	0.32	0	0.33	0.32	0.28	0.37	0.38	0.27
	Linalool																
ND	0	0.07	0	0.07	0	0.18	0	0	0	0	0	0	0	0	0	0.21	0
QD	0	0	0	0	0	0.16	0	0	0	0	0	0	0.12	0.11	0	0.20	0
SD	0	0	0	0	0	0.21	0	0	0	0	0	0	0.22	0.20	0	0	0

Maize roots of all the genotypes included in this investigation secreted  $\alpha$ -caryophyllene under ND treatment, the values ranging from 0.20 (A292) to 0.25 (LJ8) for the European and from 0.19 (Pr37H24) to 0.21 (Pr38A79, Pr38P05, Saxxoo, Pr35D28) for the American genotypes. Among different European and American genotypes exhibited statistically significant differences. In [Figure 3](#) differentiation of both groups of genotypes is clearly seen. PCA was applied to the data matrix formed by concentrations of volatiles ([Table 3](#)) from ND root tissues. On the score plot of the first two PC's, that account for 59.0 % and 27.8 % of total variance a rough clustering of samples can be observed according to the different origin. Results of PCA clearly indicate that the European cluster of samples is separated from the American cluster. Samples number 6 (A292) and 16 (Saxxoo) are outliers from both groups of samples.

#### VOCs for the maize genotypes with quick damaged (QD) roots

Following the effect of the QD root treatment, secreting of linalool was confirmed only for one European maize genotype, namely A292 (0.16), while the results were very variable for the American genotypes, the values ranged from 0.0 (Pr38H24 and Pr35D28) to 0.20 (Saxxoo), the differences between the hybrids Pr38A79 (0.12) and Pr38P05 (0.11) being statistically non significant ([Table 3](#)).

All genotypes (European as well as American) secreted  $\beta$ -caryophyllene under QD treatment, the values ranging from 0.25 (P) to 0.36 (BC318) for the European genotypes and from 0.25 (Pr38P05) to 0.41 (Pr38A79) for the American ones. Among different European and American genotypes exhibited statistically significant differences.

The same as above is true for the  $\alpha$ -caryophyllene secretion. All genotypes (European as well as American) secreted  $\beta$ -caryophyllene under QD treatment, the values ranging from 0.25 (P) to 0.36 (BC318) for the European genotypes and from 0.25 (Pr38P05) to 0.41 (Pr38A79). Among different European and American genotypes exhibited statistically significant differences.

#### VOCs for the maize genotypes with sporadically damaged (SD) roots

Following the effect of the SD root treatment, secreting of linalool was confirmed only for one European maize genotype, namely A292 (0.21) and for two American maize genotypes, namely Pr38A79 (0.22) and Pr38P05 (0.20) ([Table 3](#)). Within other genotypes there were no statistically significant differences.

After the SD root treatment  $\beta$ -caryophyllene was detected in all but one European genotypes. No  $\beta$ -caryophyllene was found in Cisco (C) genotype, while the highest relative value was that for A400 (0.37). For the American genotypes the values ranged from 0.27 (Pr35D28) to 0.38 (Saxxoo). Among different European and American genotypes exhibited statistically significant differences.

Similarly, determining  $\alpha$ -caryophyllene after the SD root treatment all the European genotypes studied secrete it, while the genotype Cisco (C) secreted the relatively highest amount of this substance (0.30). For the American genotypes the relative amounts of this substance found ranged from 0.19 (Pr37H24 and Saxxoo) to 0.22 (Pr35D28). Among different European and American genotypes exhibited statistically significant differences.

## Discussion

The maize hybrids included in this study have not been investigated in the connection with the secretion of volatiles from their roots. Some previous investigations indicated that plants which are damaged either mechanically or by herbivores secrete volatile substances (Turlings et al, 1998; Rasmann et al, 2005), which usually have an impact on natural enemies (predators) of these herbivores. The predators locate their prey more efficiently because of this signaling (Dicke and Sabelis, 1988; Turlings et al, 1990).

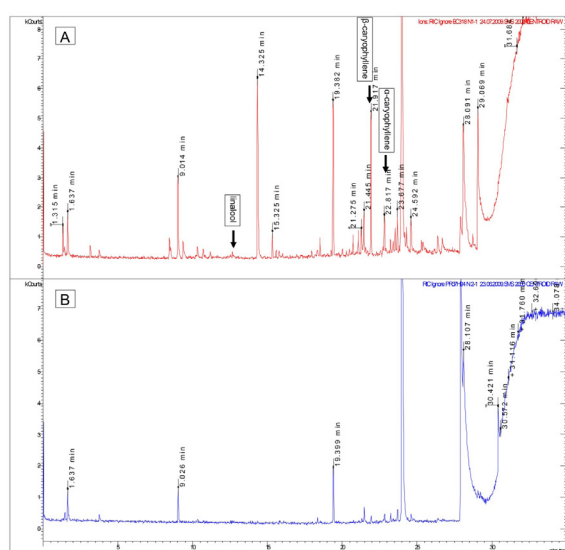
Among the three volatiles measured,  $\beta$ -caryophyllene is quite well known and relatively widely spread compound and has been identified from various plant species (Tholl et al, 2005; Cheng et al, 2007; Helmig et al, 2007). Its function, as for most plant volatiles, remains unclear. Sesquiterpenes in general may have anti-microbial or insecticidal effects (Liu et al, 2006), but at the dose secreted from maize roots it is unlikely to be effective against insects (Rasmann et al, 2005). Interestingly,  $\beta$ -caryophyllene is also emitted from maize silk and has been implicated in the attraction of adult *Diabrotica* beetles (Hammack and Single, 2001), but recent behavioral assays suggest that other plant compounds are considerably more important for this attraction (Tòth et al, 2007).

The results of our study showed that there are no differences between the relative amounts of the secreted  $\beta$ -caryophyllene for the damaged and the non damaged roots in the group European genotypes in question, while in the case of American genotypes this amount is higher in the case of damaged roots. According to Turlings et al (1998) though, the damaged leaves of European maize hybrids secrete  $\beta$ -caryophyllene, while the American hybrids do not. According to Rasmann et al (2005),  $\beta$ -caryophyllene

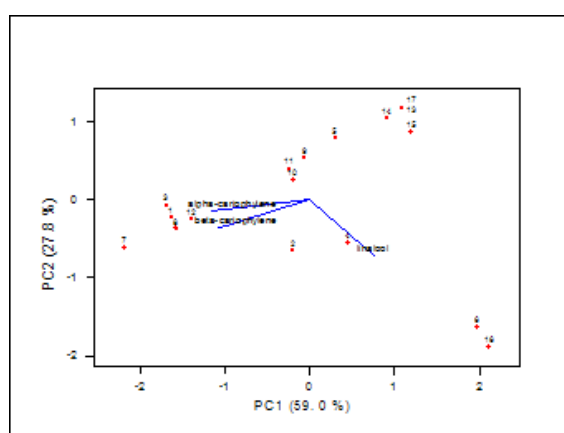
is not secreted by the American hybrids at all, while we have detected this volatile in all American hybrids included in our study.

Kant et al (2004) reports that secretion of linalool by the tomato plants starts after spider mite feeding, while the non damaged tomato plants produce no such substance. The study of Van Schie et al (2007) includes the information that the tomato plant roots secrete no linalool at all. Our study seems to confirm these findings, since the majority of the maize hybrids included in our study secrete no linalool. Turlings et al (1998) reports on a study where the mechanical damage to maize leaves was investigated and secretion of linalool was observed for damaged as well as for non damaged plants for European as well as for American hybrids. Additional information is given by Van Schie et al (2007), claiming that in the case of tomato plants the most linalool is secreted through the trichoma stems. Our results indicate, that damaged American hybrids release more linalool compared to the European ones.

The multivariate technique gave some information about the correlation between the genetic traits of the maize genotypes included in the study from the point of view of their origin and some volatile substances their roots release. As for the contents of  $\alpha$ -caryophyllene in the maize roots our study allows the conclusion that the European hybrids secrete more of this volatile compared to the American ones, but we cannot speculate on reasons for this findings, because the literature data are scarce. In the studies to follow we want to determine if the influence of  $\alpha$ -caryophyllene on the entomopathogenic nematodes is similar to that of  $\beta$ -caryophyllene (Rasmann et al, 2005), since some studies indicate that  $\beta$ -caryophyllene-emitting plants suffered significantly less root damage and had 60 % fewer adults of *Diabrotica*



**Figure 2** - GC/MS chromatograms of two different samples bc318 (A) and pr37h24 (B) as a representative of European and American genotypes.



**Figure 3** - Projection of 17 ND samples including European (1-12) and American genotypes (13-17) represented by three variables ( $\alpha$ -caryophyllene,  $\beta$ -caryophyllene and linalool concentrations) onto the plot defined by the first and the second principal component. The outliers are genotypes Saxxoo (6) and A292 (16).

emerge than those without this terpene (Degenhardt et al, 2009). But terpenes are not the only substances secreted by plants as a response to herbivore attack. Xie et al (1992) reports that the content of hydroxamic acids present in corn rootworm resistant maize lines is higher than in corn rootworm susceptible lines.

As the western corn rootworm is the most severe maize pest in the region where the hybrids under investigation are grown (SE Europe), the information on their chemical signaling is important, since it could have impact on the herbivores and their predators.

### Acknowledgements

This work was carried out within the L4-1013 project funded by the Slovenian Research Agency and Ministry of Agriculture, Food, and Forestry of the Republic of Slovenia and the company Unichem doo. Part of the research was funded within Professional Tasks from the Field of Plant Protection, a program funded by the Ministry of Agriculture, Forestry, and Food of Phytosanitary Administration of the Republic of Slovenia. Suzana Košenina, Robert Hrstar and Jaka Rupnik are acknowledged for technical assistance.

### References

- Agrawal AA, 1998. Induced responses to herbivory and increased plant performance. *Science* 29: 1201-1202
- Cheng A-X, Xiang C-Y, Li J-X, Yang C-Q, Hu W-L, Wang L-J, Lou Y-G, Chen X-Y, 2007. The rice (E)- $\beta$ -caryophyllene synthase (OsTPS3) accounts for the major inducible volatile sesquiterpenes. *Phytochemistry* 68: 1632-1641
- Degen T, Dillmann C, Marion-Poll F, Turlings TCJ, 2004. High genetic variability of the herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol* 135: 1928-1938
- Degenhardt J, Hiltbold I, Köllner TG, Frey M, Gierl A, Gershenzon J, Hibbard BE, Ellersieck MR, Turlings TCJ, 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proc Natl Acad Sci USA* 106: 13213-13218
- De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH, 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393: 570-573
- De Moraes CM, Mescher MC, Tumlinson JH, 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410: 577-580
- Dicke M, Hilker M, 2003. Induced plant defences: from molecular biology to evolutionary ecology. *Basic Appl Ecol* 4: 3-14
- Dicke M, Sabelis MW, 1988. How plants obtain predatory mites as bodyguards. *Neth J Zool* 38: 148-165
- FAO, 2007. web site: <http://www.fao.org/>
- Gouinguéné S, Degen T, Turlings TCJ, 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11: 9-16
- Hammack L, 2001. Single and blended maize volatiles as attractants for diabroticite corn rootworm beetles. *J Chem Ecol* 27: 1373-1390
- Heinrichs EA, 1988. Global food production and plant stress, pp 1-34. In: *Plant Stress-Insect Interactions*. Heinrichs EA ed. John Wiley and Sons, New York
- Helmig D, Ortega J, Duhl T, Tanner D, Guenther A, Harley P, Wiedinmyer C, Milford J, Sakulyanontvittay T, 2007. Sesquiterpene emissions from pine trees - Identifications, emission rates and flux estimates for the contiguous United States. *Environ Sci Technol* 41, 1545-1553
- Kant MR, Ament K, Sabelis MW, Haring MA, Schuurink RC, 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiol* 135: 483-495
- Levine E, Oloumisadeghi H, 1991. Management of diabroticite rootworms in corn. *Ann Rev Entomol* 36: 229-255
- Liu CH, Mishra AK, Tan RX, 2006. Repellent insecticidal and phytotoxic activities of isoalantolactone from *Inula racemosa*. *Crop Prot* 25: 508-511
- Meihls LN, Higdon ML, Siegfried BD, Miller NJ, Sappington TW, Ellersieck MR, Spencer TA, Hibbard BE, 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proc Natl Acad Sci USA* 105: 19177-19182
- Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, Derridj S, Kim KS, Reynaud P, Furlan L, Guillemaud T, 2005. Multiple transatlantic introductions of the western rootworms in corn. *Science* 310: 992
- Paré PW, Tumlinson JH, 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol* 121: 325-331
- Rasmann S, Köllner TG, Degenhardt J, Hiltbold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ, 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434: 732-737
- Riedell WE, 1989. Western corn rootworm damage in maize: Green house technique and plant response. *Crop Sci* 29: 412-415
- Riedell WE, Schumacher TE, Evenson PD, 1996. Nitrogen fertilizer management to improve crop tolerance to corn rootworm larval feeding damage. *Agron J* 88: 27-32
- Romeis J, Meissle M, Bigler F, 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat Biotechnol* 24: 63-71
- Tholl D, Chen F, Petri J, Gershenzon J, Pichersky E, 2005. Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from Arabidopsis flowers. *Plant J* 42: 757-771

- Tòth M, Vuts J, Szarukán I, Juhász I, Manajlovics F 2007. Preliminary study of female-targeted semi-chemical baits for the western corn rootworm in Europe. *J Appl Entomol* 131: 416-419
- Turlings TCJ, Lengwiler UB, Bernasconi ML, Wechsler D 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207: 146-152
- Turlings TCJ, Tumlinson JH, Lewis WJ, 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253
- Van Schie CCN, Haring MA, Schuurink RC, 2007. Tomato linalool synthase is induced in trichomes by jasmonic acid. *Plant Mol Biol* 64: 251-263
- Xie Y, Arnason JT, Philogene B Jr, Olechowski HT, Hamilton R, 1992. Variation of Hydroxamic Acid Content in Maize Roots in Relation to Geographic Origin of Maize Germ Plasm and Resistance to Western Corn Rootworm (Coleoptera: Chrysomelidae). *J Econ Entomol* 85: 2478-2485