Phytochemistry, Vol. 28, No. 11, pp. 2959–2962, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

FOLIAGE VOLATILES OF TWO RICE CULTIVARS

HIDELISA P. HERNANDEZ,* THOMAS C.-Y. HSIEH,† C. MICHAEL SMITH‡ and NIKOLAUS H. FISCHER*§

*Department of Chemistry, Louisiana State University; †Department of Food Science, ‡Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, U.S.A.

(*Received in revised form* 20 *April* 1989)

Key Word Index—*Oryza sativa*; Gramineae; rice; varieties 'Mars' and PI346833; foliage volatiles

Abstract—Volatiles of two rice varieties ('Mars' and PI346833) were collected from seedlings by dynamic headspace sampling and trapping on Tenax TA. Thermal desorption and cryogenic focusing methods were used to introduce the volatiles onto the column for GC-MS analysis. Twenty-eight compounds were identified. Hexanal, (*E*)-2-hexenal, (*Z*,*Z*)- and (*E*,*E*)-2,4-heptadienal represent the major constituents of both varieties. These aldehydes comprise more than 50% of total volatiles within both varieties. The variety 'Mars' contained a larger number and greater quantities (17- to 46-fold) of volatile compounds than the variety PI346833.

INTRODUCTION

Volatile plant constituents play a vital role in plant–insect interactions. Because volatiles are emitted by plants to their surroundings, insects can perceive their presence through sensory cells located on olfactory sensilla [1]. For this reason, volatiles may act as insect attractants or repellents. Secondary plant metabolites also play a role in antibiosis thus affecting the behaviour and physiology of insects [2]. To understand the function of volatiles in plant–insect interaction, it is vital to know the profile of volatiles in plants and their chemical structures.

Little is known about the volatile components of rice. Saxena *et al.* [3] isolated volatile mixtures from rice plants by steam distillation that affected the behaviour of brown planthopper (*Nilaparvata lugens* Stal), striped stemborer (*Chilo suppressalis*), and green leafhopper (*Nephotettix virescens* Distant). However, the components of the steam distillate were not identified. Obata *et al.* [4] identified 27 compounds from a steam distillate of a susceptible rice variety Nihonbare. The mixture of volatiles contained 14 esters, seven carbonyl compounds, five alcohols as well as triallyl isocyanurate. Among the compounds, the methyl and ethyl esters of palmitate, stearate, oleate, linoleate and linolenate represented the major constituents. However, none of the constituents reported by the Japanese authors was detected in our study.

A major disadvantage of obtaining volatiles by steam distillation and organic solvent extraction is the possible production of artifacts due to high temperature heating and the introduction of contaminants from the solvents and drying agents. Steam distillation also requires a large amount of sample, often in the kg range. Moreover, grinding or maceration of leaves can lead to the formation of enzyme-catalysed oxidation products [6].

Recently, dynamic headspace sampling (DHS) has been used in studying volatile compounds in various food samples [7]. This procedure can be used to trap volatiles without laborious sample preparation and does not require extensive sample heating thus, minimizing the formation of artifacts. DHS has been used for collecting volatiles from leaves of wheat [6], potato [1] and strawberry [8]. In these instances, the trapped volatiles were eluted with a solvent for analysis by GC-MS. Moisture interference problems in DHS-GC-MS analysis of high moisture samples have been recently solved by an improved procedure. To avoid the use of elution solvent, thermal desorption and cryogenic focusing was applied to introduce the volatiles to the GC column for GC or GC-MS analysis [9].

Rice variety plant introduction (PI) 346833 is moderately resistant and 'Mars' is susceptible to feeding by fall armyworn (FAW), *Spodoptera frugiperda* (J. E. Smith). In this DHS-GC-MS study, the two rice varieties PI346833 and 'Mars' were analysed for their volatile profiles and composition to be used as a basis for future plant–insect interaction studies.

RESULTS AND DISCUSSION

Dynamic headspace volatiles from the seedling foliage of the rice varieties 'Mars' and PI346833 were purged and trapped in a column of Tenax TA. The trapped volatiles were desorbed in a Tekmar sample concentrator and analysed by GC-MS. The total ion chromatograms (TIC) showed that the two varieties have remarkable qualitative and quantitative differences in their volatile profiles. The susceptible variety 'Mars' contained a greater number of headspace volatiles than the moderately resistant PI346833. It was also apparent that 'Mars' produced larger amounts of volatiles.

The 28 compounds identified from both rice varieties are listed in Table 1. Among those identified, fourteen were present in both varieties. Twelve compounds were found only in 'Mars' and two were found only in PI346833. The volatiles were composed of three hydrocarbons, one monoterpene, four aldehydes, two ketones, three enones, two dienones, three alcohols, four enals and six dienals. Sixteen compounds were identified by com-

§Author to whom correspondence should be addressed.

Table 1. Dynamic headspace volatile compounds identified from rice seedling varieties 'Mars' and PI346833

Compound	'Mars' RI*	'Mars' % Peak area	PI346833 RI	PI346833 % Peak area	Standard RI
Pentanal	966	0.30	—	—	982
Hexanal	1085	27.70	1094	20.91	1085
3-Penten-2-one	—	—	1137	0.15	1128
3-Methyl-2-butenal	1146	1.56	1140	2.31	—
Heptanal	1190	1.31	1192	0.75	1189
(*E*)-2-Hexenal	1231	6.33	1228	9.54	1222
6-Methyl-2-heptanone	1240	0.21	—	—	—
3,5-Octadiene	—	—	1270	0.20	—
Limonene	1292	0.16	—	—	1200
5-Nonen-2-one	1314	0.20	—	—	—
2-Heptenal	1331	0.76	—	—	1329
6-Methyl-5-hepten-2-one	1343	0.66	—	—	—
2-Penten-1-ol	1347	2.84	1332	4.54	1318
Nonanal	1387	0.51	—	—	1398
2,4-Hexadienal	1412	1.33	1410	0.84	—
E-2-Octenal	1431	0.68	—	—	1435
7-Octen-4-ol	1457	1.27	1456	1.06	—
(*Z*,*Z*)-2,4-Heptadienal	1477	8.10	1474	11.72	—
(*E*,*E*)-2,4-Heptadienal	1508	9.69	1502	14.19	1500
(*Z*,*Z*)-3,5-Octadien-2-one	1531	6.06	1527	3.79	—
(*E*,*E*)-3,5-Octadien-2-one	1580	2.72	1578	1.62	—
3-Methyl-4-heptanone	1614	3.12	1610	1.93	—
(*Z*,*Z*)-2,4-Nonadienal	1708	0.41	1711	0.44	1710
Naphthalene	1751	1.69	1755	1.92	1746
(*Z*,*Z*)-2,4-Decadienal	1770	0.34	—	—	1778
(*E*,*E*)-2,4-Decadienal	1816	0.26	—	—	1827
1H-Indene-l-ethylidene	1863	0.13	—	—	—
Benzyl alcohol	1890	0.16	—	—	1891

*RI = retention index.

paring their mass spectra and retention indices with those of standard compounds. The other volatiles were tentatively identified by computer matching of their mass spectra with those stored in the mass spectral library.

Based on the per cent peak area of each component, the most abundant compound in both rice varieties are hexanal, (*E*,*E*)-2,4-heptadienal, (*Z*,*Z*)-2,4-heptadienal and (*E*)-2-hexenal. Together, these four compounds comprise more than 50% of the total volatile components in each variety. Hexanal and (*E*)-2-hexenal are known to be components of the 'green leaf odour' of plants [1]. These compounds commonly represent C_6 aldehydes, alcohols and acetates which are produced by the oxidative degradation of unsaturated leaf fatty acids such as, linoleic and linolenic acids [1]. Most of the other volatiles identified are also products of the oxidative degradation of unsaturated fatty acids [12]. Visser *et al.* [1] studied the response of different insects toward C_6 aldehydes, enols and acetate isolated from potato leaves. They found that different insects responded differently to each compound. It is therefore possible that each insect will be attracted or repelled by a plant depending on the composition and relative concentration of each volatile component.

Some of the compounds identified from the rice seedlings have been found as volatile components of other cereal plants. (*E*)-2-Hexenal, nonanal, limonene [6] as well as hexanal, heptanal, (*E*)-2-heptenal and (*E*)-2-octenal [5] were detected in wheat leaves. Volatiles from whole oat leaves were reported to contain heptanal, nonanal, (*E*,*E*)-2,4-decadienal, limonene and 6-methyl-5-hepten-2-one [13]. Likewise, benzyl alcohol has been found in barley leaves [14].

It is of interest to note that in comparison with PI346833, the rice variety 'Mars' produces a larger number of volatile compounds as well as larger amounts. A quantitative comparison of the peak areas of the common volatiles produced by the two rice varieties showed that 'Mars' produces *ca* 17 to 46 times higher quantities than the variety PI346833. For instance, the amount of heptanal is *ca* 46 times higher and (*E*)-2-hexenal is *ca* 17 times higher in 'Mars' than in PI346833.

The variety 'Mars' has been found to be highly susceptible to FAW based on its high defoliation rate and FAW's high feeding preference for this rice variety [10]. It is possible that 'Mars' produces large amounts of attractants which makes it a preferred host for this insect pest. As there are 16 compounds (12 identified and 4 unknown) that are present in 'Mars' but not in PI346833, it is possible that a specific constituent and/or a combination of several compounds may act as attractants and/or feeding stimulants of 'Mars' towards certain insect species. Conversely, 3-penten-2-one and 3,5-octadiene, which were not detected in 'Mars' but were present in very small amounts in PI346833, are potential candidates

for laboratory tests on their effect on the behaviour of FAW as feeding deterrents or repellents. Speculation on the possible functions of these volatile leaf components that may cause PI346833 to be more resistant to FAW than 'Mars' will require detailed experimental verification.

Nonanal was found in 'Mars' but not in PI346833. This compound was found to be the chief volatile component in live wheat stem rust spores (*Puccinia graminis* var. *tritici*) [15]. It is also a highly active germination stimulant of wheat stem rust, wheat leaf rust (*P. recondita*), oat crown rust (*P. recondita* F. sp. *avenae*) and common corn rust (*P. sorghi*) [16]. Another interesting study could involve structure–activity relationships of related volatiles such as 6-methyl-5-hepten-2-one and its saturated analog 6-methyl-2-heptanone. 6-Methyl-5-hepten-2-one has been isolated as a minor component of the uredospores of wheat stem rust [15]. This compound stimulated the germination of uredospores of wheat stem rust and oat crown rust [16]. 6-Methyl-5-hepten-2-one is also the alarm pheromone of the *Iridomyrmex* species of ants [17]. Our future studies will be related to the role of these compounds in rice–insect interaction.

EXPERIMENTAL

Plant material. Seeds of rice (*Oryza sativa* L.) variety PI346833 were obtained from the USDA National Small Grain Collection at Beltsville, Maryland. Rice varieties 'Mars' and PI346833 were grown in the Louisiana State University Greenhouse. Seeds were germinated on 19 September 1987 and planted on 22 September 1987. The plants were maintained in a flooded state. The aerial parts were harvested on 13 October 1987 at the 4-leaf stage [11], rinsed with H_2O to remove soil, placed in a plastic bag, frozen, and then kept at $-5°$. Plant samples were analysed for volatiles on 19–20 April 1988.

Collection of volatiles. Whole aerial parts (45.3 g) of frozen rice seedlings were placed in a glass container (23 cm ht × 5.5 cm i.d.) fitted with a Kovar metal–glass connection. The volatiles were collected by passing ultra high purity (UHP) He gas (99.999%, Linde Div., Union Carbide Corp., Danbury, CT) through an O_2 trap, a hydrocarbon trap and then through the sample at a flow rate of 86 ml/min for 16 hr at room temp. (25°). The volatiles were trapped in a 30.48 cm × 0.32 cm o.d. stainless steel column packed with Tenax TA (2,6-diphenyl-*p*-phenylene oxide polymer, 0.24 g, 60–80 mesh, Chrompack, Raritan, NJ). After the collection of volatiles, the trap was purged with UHP He to remove any H_2O until a constant trap weight was obtained according to the method of ref. [9].

Gas chromatography-mass spectrometry. The volatiles were desorbed from the Tenax trap by a Tekmar model 4000 Dynamic Headspace Concentrator at 185° for 15 min into a 60 m length × 0.25 mm i.d. × 0.25 μm film thickness Supelcowax 10 column (Supelco Inc., Bellafonte, PA). Before desorption, a beaker containing a mixture of EtOH and dry ice (−76°) was used as a cryogenic bath inside the chromatograph oven for cryogenic focusing of the volatiles by lowering *ca* 10 cm length of the column at the injector end to form a U-loop and placing the loop in the bath. During desorption, the volatiles were released from the Tenax trap, transferred through the injector and focused into a narrow zone inside the column. At the end of the desorption period, the cryogenic bath was removed from oven and column temperature programming allowed to begin.Cryogenic focusing minimized the diffusion of volatiles inside the column and improved chromatographic resolution. The carrier gas head pressure was 30 psi and the injector split vent was closed during desorption, to facilitate the transfer of volatiles from the trap to the column.

Gas chromatography was carried out on a Hewlett Packard 5792 gas chromatograph under the following conditions: carrier gas head pressure, 15 psi; carrier gas linear velocity, 25 cm/sec; carrier gas flow rate, 0.76 ml/min. The column temperature was programmed as follows: initial temp., 40° for 5 min, programming rate, 2°/min: final temp. 175° for 15 min; total run time, 87.5 min. The chromatographic components were analysed on an HP5970B mass selective detector. Electron ionization was carried out at 70 eV and electron multiplier voltage was set at 1 800 V. Solvent delay was set at 2 min.

Calculation of retention indices. The determination of the retention indices of the volatile compounds was performed by adding 200 ng of each normal alkane (C_9–C_{19}) to 45.3 g of 'Mars' seedlings. The same procedure for collection of volatiles and subsequent GC-MS analysis was followed, as described above. The retention index of each compound was calculated according to ref. [18].

GC-MS of standard compounds. Five μl of each standard and of each C8-C22 *n*-alkane were dissolved in 5 ml of hexane. One μl of the standard mixture was injected at a 27:1 split ratio. The solvent delay was set at 6 min with the other chromatographic and MS conditions the same as above. The mass spectra and retention indices of the standards were used to identify the volatiles obtained from the rice samples. When authentic standards were not available tentative identification of some compounds were based on computer matching of the unknown mass spectra with the reference mass spectra of the NBS/NIH/EPA/MSDC Data Base [19] installed on the HP MSD Chem Station. Per cent peak area of each chromatographic peak was calculated by electronic integration with an HP59970C MS ChemStation Program. The baseline threshold was set at 16 and the peak width at 0.40 min.

REFERENCES

1. Visser, J. H. (1983) in *Plant Resistance to Insects* (Hedin, P. A., ed.), p. 215. ACS Symposium Series 208. ACS, Washington, D.C.
2. Saxena, R. C. (1986) in *Natural Resistance of Plants to Pests* (Green, M. B. and Hedin, P. A., eds), p. 142. ACS Symposium Series 296. ACS, Washington, D.C.
3. Saxena, R. C. and Okech, S. H. (1985) *J. Chem. Ecol.* **11**, 1601.
4. Obata, T., Koh, H. S., Kim, M. and Fukami, H. (1983) *Appl. Ent. Zool.* **18**, 161.
5. Hamilton-Kemp, T. R. and Andersen, R. A. (1984) *Phytochemistry* **23**, 1176.
6. Buttery, R. G., Xu, C. and Ling, L. C. (1985). *J. Agric. Food Chem.* **33**, 115.
7. Badings, H. T., de Jong, C., Dooper, R. P. M. and de Nijs, R. M. C. (1984) in *Progress in Flavour Research* 1984 (Adda, J., ed.). Elsevier, New York.
8. Hamilton-Kemp, T. R., Andersen, R. A., Rodriguez, J. G., Laughrin, J. H. and Patterson, C. G. (1988) *J. Chem. Ecol.* **14**, 789.
9. Williams, S. S., Hsieh, T. C.-Y. and Vejaphan, W. (1988) in *Proc. 39th Pittsburgh Conferences and Exposition on Analytical Chemistry and Applied Spectroscopy*, New Orleans, LA, February, 1988.
10. Pantoja, A., Smith, C. M. and Robinson, J. F. (1986). *J. Econ. Entomol.* **79**, 1314.
11. Yoshida, S. (1981) *Fundamentals of Rice Crop Science.* Int. Rice Res. Inst., Los Baños, Laguna, Philippines.
12. Frankel, E. N. (1982). *Prog. Lipid Res.* **22**, 1.

13. Buttery, R. G., Ling, L. C. and Wellso, S. G. (1982). *J. Agric. Food Chem.* **30**, 791.
14. Juneja, P. J., Gholson, R. K., Burton, R. L. and Starks, K. J. (1972). *Ann. Entomol. Soc. Am.* **65**, 961.
15. Rines, H. W., French, R. C. and Daasch, L. W. (1974). *J. Agric. Food Chem.* **22**, 96.
16. French, R. C., Gale, A. W., Graham, C. L. and Rines, H. W. (1975). *J. Agric. Food Chem.* **23**, 4.
17. Crewe, R. M. and Blum, M. S. (1971). *Ann. Entomol. Soc. Am.* **64**, 1007.
18. Van Den Dool, H. and Kratz, P. D. (1963). *J. Chromatogr.* **2**, 463.
19. NBS/NIH/EPA/MSDC Data Base, Copyright Hewlett Packard Co. 1984, 1986.