

Influence of storage and infection with *Gloeosporium album* Osterw. on release of volatile compounds from different apple cultivars

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*Release of volatile compounds from intact, sound fruit into the surrounding atmosphere was minimal. The predominant compound was butylacetate, but with differences between the five cultivars studied ('Golden Delicious', 'Idared', 'Jonagored', 'King Jonagold' and 'Meteor'). The production of acetaldehyde, ethanol and ethylacetate after 30 days of cold storage was very low. Nineteen volatile compounds were detected for the cultivar 'Meteor' 30 days after harvest. Predominant compounds trapped in a column were esters such as butylacetate (46.7 µg/kg.h), 2-methylbutylacetate (77.7 µg/kg.h) and hexylacetate (30.4 µg/kg.h) and conferred a characteristic 'apple' odour. Other compounds, at lower levels, ranged from 0.1 µg/kg.h for hexylhexanoate to 5.0 µg/kg.h for 2-methylbutan-1-ol. Using discrimination analysis one could identify the different apple cultivars and determine whether they were infected with *Gloeosporium album* or not. This offers a new method for monitoring senescence in stored fruit.*

Key words: apple, storage, volatile compounds, aroma, storage, microbiological decay, *Gloeosporium album* Osterw.

*Einfluss von Lagerung und Infektion mit *Gloeosporium album* Osterw. auf die Freisetzung flüchtiger Substanzen bei verschiedenen Apfelsorten. Die Freisetzung flüchtiger Substanzen aus intakten, gesunden Früchten in die Umgebungsatmosphäre war minimal. Die dominierende Substanz war Butylacetat, aber mit Unterschieden zwischen den fünf untersuchten Sorten. ('Golden Delicious', 'Idared', 'Jonagored', 'King Jonagold' und 'Meteor'). Die Produktion von Acetaldehyd, Ethanol und Ethylacetat nach 30 Tagen Kühlung war sehr niedrig. 30 Tage nach der Ernte wurden für die Sorte 'Meteor' 19 flüchtige Substanzen ermittelt. Die überwiegenden Substanzen, die in einer Säule gebunden wurden, waren Ester, wie Butylacetat (46,7 µg/kg.h), Methylbutylacetat 2 (77,7 µg/kg.h) und Hexylacetat (30,4 µg/kg.h), die einen charakteristischen Apfelduft vermittelten. Andere Substanzen reichten von 0,1 µg/kg.h für Hexylhexanoat bis zu 5,0 µg/kg.h für 2 Methylbutan-1-ol. Mittels Diskriminanzanalyse konnten die verschiedenen Apfelsorten identifiziert und eventuelle *Gloeosporium album*-Infektionen festgestellt werden. Dies bietet eine neue Methode für die Kontrolle des Alterungsprozesses in gelagerten Früchten.*

Schlagwörter: Apfel, Lagerung flüchtige Substanzen, Aroma, Lagerung, mikrobiologischer Verderb, *Gloeosporium album* Osterw.

*L'influence du stockage et de l'infection à *Gloeosporium album* Osterw. sur le dégagement de substances volatiles dans différentes variétés de pommes. Le dégagement de substances volatiles de fruits intacts et sains dans l'atmosphère ambiante a été minime. La substance dominante était l'acétate de butyle, mais il existait des différences entre les cinq variétés examinées ('Golden Delicious', 'Idared', 'Jonagored', 'King Jonagold' et 'Meteor'). La production d'acétaldéhyde, d'éthanol et d'acétate d'éthyle était très faible après 30 jours de stockage au frais. 30 jours après la récolte, 19 substances volatiles ont été déterminées pour la variété 'Meteor'. Les principales substances liées dans une colonne étaient des esters, tels que l'acétate de butyle (46,7 µg/kg.h), l'acétate de méthylebutyle 2 (77,7 µg/*

kg.h) et l'acétate hexylique (30,4 µg/kg.h), qui émettaient une odeur caractéristique de pomme. Les autres substances allaient de 0,1 µg/kg.h pour l'hexylhexanoate jusqu'à 5,0 µg/kg.h pour le 2-méthylbutane-1-ol. L'analyse discriminante a permis d'identifier les différentes variétés de pommes et de constater d'éventuelles infections à *Gloeosporium album*. Cela représente une nouvelle méthode de contrôle du processus de vieillissement des fruits stockés.

Mots clés : pomme, substances volatiles se dégageant au cours du stockage, arôme, stockage, altération microbiologique, *Gloeosporium Album Osterw.*

After harvest the natural disease resistance of fruit gradually declines, leading to inevitable infection and disease. Post-harvest fungus diseases are usually caused by latent infections established in the field or by the infection of wounds received during harvest and handling. However, absence of decay and related disorders, as well as good external appearance and good flavour, are the principal market requirements for quality fresh fruit. Pre-harvest field infections, which occur in surface lesions on the stalks of apples, often enter a latent stage due to the host's resistance to further invasion. Most market fruit spoilage occurs after harvesting, and although fungi most often invade bruised and damaged fruit, others gain entry at specific sites. For example, *Gloeosporium* ssp. invade the lenticels of apples in the pre-harvest stage, to subsequently initiate lenticel rot. The injuries occur at the surface, and while the subsequent progress of infection is sensitive to the composition of the storage atmosphere, the ability to control decay in this manner is limited by the tolerance of fruit to low oxygen or elevated CO₂ levels. Low temperature and/or reduced levels of O₂ in the storage environment suppress decay by prolonging the initial lag phase of lesion development. It has been shown that the growth of *Gloeosporium album* in liquid media is inhibited when O₂ levels are lowered to 2.5 % (LOCKHARDT, 1967). Cold storage also inhibits the onset of rotting, but eventually the fruit tissue loses its resistance. The pathogens ability to secrete various proteolytic and pectolytic enzymes for pectin solubilization, tissue softening and alterations in membrane permeability, makes the host tissues more vulnerable to maceration. Many fungi are well known for producing a variety of secondary metabolites (SUNESSON et al., 1995; SCHOELLER et al., 2002). Analysis of the chemicals in the headspace of *Ceratocystis fagacearum* (oak wilt) revealed 16 compounds which were common fruit-odour constituents (HENGCHEN and LARRY, 1992). The potential for using the volatile compounds excreted by micro-organisms as a rapid and perhaps specific method for detecting microbial activity were investigated. As well as being used for as indicators of fungal growth, there may be chemotaxonomic and other applications.

GIRARD and LAU (1995) reported that the storage regime, followed by harvest date, were the most important factors influencing the release of volatile compounds. Storage under controlled atmosphere (CA) reduced volatile emissions compared to fruit stored in normal air (HANSEN et al., 1992; MATTHEIS et al., 1995). In general, extending storage from 14 to 25 weeks reduced emissions of branched chain esters, with the exception of 2-methylpropyl hexanoate and 2-methylbutyl-2-methylpropanoate (LÓPEZ et al., 2007).

One of the techniques used for the investigation of volatile compounds emitted by fresh fruit is dynamic headspace gas analysis. Volatile compounds from intact fruit can be accumulated by a suitable sorbent packed in a short column. The exact procedure depends on the nature and concentration of the compounds. The individual compounds migrate down the enrichment column at a velocity determined by the sorption capacity of Tenax GC[®] as a sorbent and the velocity of the percolating gas (NOVÁK, 1988; MATTHEIS et al., 1995). Activated charcoal has often been used for the collection of volatile aroma compounds from intact fruit and the volatile compounds can be desorbed by agitation with diethyl ether (LARA et al., 2006). Solvent extraction using dichloromethane, however, subsequently gives a better resolution of the volatiles. AABY et al. (2002) took samples of the cubed flesh of apple fruit for trapping by TenaxGC[®].

The purpose of this study was to examine the volatiles produced by sound apple fruit and those infected by *Gloeosporium album* Osterw., and to determine whether the different types and levels of compounds could be used to identify intact and damaged fruit in five different cultivars. Measuring the levels of volatiles in the ambient atmosphere would offer a method of predicting the rate of senescence of fruit during storage.

Material and methods

Plant material and storage conditions

Five varieties of apples (*Malus × domestica* Borkh. cv. 'Golden Delicious', 'Idared', 'Jonagored', 'King Jona-

gold', 'Meteor') were harvested at a normal commercial date from 5-year-old trees grown on 'M9' rootstocks in a commercial orchard in Holovousy (Czech Republic). Immediately after harvest, two lots of 60 kg apples from each variety, free from obviously visible defects, were selected with the following characteristics: diameter >70 mm; starch index 5 to 5.8; firmness 720 to 740 kPa. One of these lots was stored at 3 °C and 92 to 93% relative humidity in a cold-storage chamber, and after 30 days was analysed for the amount of aroma volatiles produced. The second of these two lots was divided after 120 days into two groups, sound fruit and fruit infected by *Gloeosporium album* Osterw. The aroma volatiles were measured when the area of the infection lesion was about 100 mm² or more.

Sampling fruit for aroma volatiles measurement

The dynamic headspace method was used to extract volatile compounds from intact apples. A weight (W) of about 0.120 kg of fruit was immediately transferred from the cold storage room and placed in hermetically sealed, spherical jars, with a volume of 0.5 l at a temperature of 20 °C. The volatile compounds released from the fruit were washed out by a stream of gas percolating at a flow rate (F) of 50 ml/min and then trapped in an enrichment column with Tenax GC[®] as a sorbent. After one hour, the incoming flow in the enrichment column had produced a total volume (V) of three litres. Under these suction conditions, aroma volatiles were completely retained in the enrichment column (conservation version).

The compounds collected in the Tenax GC[®] trap were recovered by thermal desorption (Scientific Instrument Services, Inc., Ringoes NJ, USA) and transferred to gas chromatographic (GC) columns using a carrier gas (He). A gas chromatograph with FID (4890D; Agilent Technologies, Inc. Wilmington, USA) was employed. The substances were separated on a DB WAX fused silica capillary GC column (30 m x 0.25 mm; I.D., 0.25 µm; J. & W. Scientific). The GC program was as follows: 1 min at 35 °C, increasing by 3 °C/min to 200 °C, with a hold for 10 minutes. The carrier gas was He at 1.2. ml/min; FID conditions were as follows: H₂ and air were 270 kPa and 120 kPa, respectively. The peak areas were processed by a data station (CSW 1.7). The quantification of the chromatograms was carried out by the external calibration method. Alternatively a SPME technique was used for the identification of apple compounds. A PA 85 µl fibre (Fa. Supelco) was

inserted 3 mm into the headspace of a vial of 20 ml in volume containing a 2 g sample of apple. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as carrier gas. The identity of volatile compounds detected was confirmed by comparing their GC retention indices and their mass spectra with those of an external standard, injected into a gas chromatograph GC (Fa. Hewlett-Packard 5890) with a mass spectrometer (Agilent 5975C GC/MS) under the same conditions as described above. The GC-MS apparatus was equipped with the same capillary column as in the GC-FID analysis.

The production (Gi) of the given compounds is expressed as a function of the velocity of the percolating gas in litres/hour (F), the weight of fruit in kilograms (W) placed in the spherical jar, and the concentration of analytes (ci) in the percolating gas expressed in mg/l, and can be calculated as follows:

$$Gi = ci F/W \text{ µg/kg.h}$$

A statistics package, Statistica (StatSoft[®] CR) version 7.1, was employed for the data analysis. ANOVA was used to determine the significance of variations in the fruit volatiles parameters, and the influence of cultivars, levels of fruit infection and time of storage.

Results and discussion

Volatiles determined in the ambient atmosphere surrounding intact fruit

Samples of volatiles were collected from intact fruit, where the generation and release of such compounds is usually low due to the minimal disruption of tissues. Furthermore, samples collected in this manner do not necessarily reflect actual apple tissue concentrations, since diffusion from the fruit into the surrounding atmosphere may be suppressed. The volatile concentrate from non-infected fruit contained 17 compounds other than ethanol and acetaldehyde. These compounds consisted of one hydrocarbon, six alcohols and eleven esters. Esters represented more than 70% of the total volatile compounds detected. The predominant compound emanating from intact fruit was butyl acetate and can be used to differentiate the various cultivars (Table 1). However, LÓPEZ et al. (1998) showed that the main compound emitted during ripening at 20 °C of sound fruit was hexylacetate, which is predominant in 'Golden Delicious' apples.

Table 1: Production of volatile aroma compounds ($\mu\text{g}/\text{kg}\cdot\text{h}$) from apple cultivars after storage for 30 days at 3°C

	Act	EtOAc	EtOH	PropOAc	IsoBuOAc	PropOH	BuOAc	IsoBuOH
JongHeal	0,1±0.1	0.3±0.1	0.3±0.0	0.6±0.1	1.4±0.1	0.7±0.1	48.5±10.0	6.1±1.8
JongGloeo	4,1±1.7	13.5±5.8	19.7±9.9	1.8±0.4	1.2±0.2	1.1±0.1	68.1±9.8	4.4±0.3
IdarHeal	0.2±0.0	0.5±0.2	0.4±0.1	1.2±0.2	0.7±0.1	1.2±0.1	42.2±4.1	3.2±0.3
IdarGloeo	1.4±0,2	1.4±0.3	2.3±0.4	1.6±0.4	0.6±0.2	1.7±0.2	27.3±4.1	3.3±0.2
GDelHeal	1.1±0,2	4.3±3.3	1.5±0.6	0.8±0.3	1.5±0.3	1.8±0.4	27.9±6.4	4.5±1.0
GDelGloeo	6.3±1.7	22.7±8.6	16.4±7.3	1.7±0.4	1.0±0.2	2.8±0.3	36.7±8.6	5.3±0.9
cultivar	**	**	*	ns	*	**	**	ns
disease	**	**	**	*	ns	**	*	ns

Table continued

	2-MeBuOAc	BuOH	AmOAc	2-MeBuOH	HexOAc	HexOProp	HexOH	FurfOH
JongHeal	9.3±1.4	23.3±4.9	2.2±0.4	1.9±0.3	0.3±0.1	0.2±0.1	8.9±1.9	0.2±0.0
JongGloeo	7.5±2.0	13.6±3.7	2.7±0.5	2.3±0.3	1.0±1.0	0.3±0.1	7.2±1.7	0.2±0.0
IdarHeal	13.0±1.6	34.8±8.7	1.6±0.2	6.1±0.5	0.8±0.1	0.1±0.0	9.5±0.8	0.2±0.1
IdarGloeo	5.8±1.1	9.1±1.0	3.0±0.9	3.7±0.2	0.6±0.1	0.7±0.4	5.2±0.9	0.2±0.0
GDelHeal	3.0±0.6	41.4±14.9	1.6±0.4	1.7±0.3	1.7±0.2	0.1±0.0	12.7±2.9	0.2±0.0
GDelGloeo	2.2±0.7	14.2±2.5	3.5±1.5	2.3±0.5	1.5±0.1	7.5±2.6	6.3±1.3	0.2±0.1
cultivar	*	ns	ns	**	ns	**	ns	ns
disease	**	**	ns	**	ns	**	*	ns

Values are means and standard errors calculated from 10 fruit for each cultivar and infected by *Gloeosporium album* Osterw. ANOVA: ** $P < 0.01$, * $P < 0.05$, ns = not significant

Abbreviations:

JongHeal = Jonagored health

IdarHeal = Idared health

GDHeal = Golden Delicious health

Act = acetaldehyde

IsoBuOAc = isobutyl acetate

2-MeBuOAc = 2-methylbutyl acetate

HexOAc = hexyl acetate

JongGloeo = Jonagored infected by *Gloeosporium album* Osterw.IdarGloeo = Idared infected by *Gloeosporium album* Osterw.GDGloeo = Golden Delicious infected by *Gloeosporium album* Osterw

EtOAc = ethyl acetate

PropOH = propyl alcohol

BuOH = butanol-1

HexOProp = hexyl propionate

EtOH = ethanol

BuOAc = butyl acetate

AmOAc = pentyl acetate

HexOH = hexanol-1

PropOAc = propyl acetate

IsoBuOH = isobutyl alcohol

2-MeBuOH = 2-methylbutyl alcohol

FurfOH = furfuryl alcohol

Volatile emissions at onset of ripening

In the cultivar 'Meteor' nineteen volatile compounds were detected after 30 days of storage. Predominant substances released from intact fruit are the esters butylacetate, 2-methylbutylacetate and hexylacetate at levels of $46.7 \mu\text{g}/\text{kg}\cdot\text{h}$, $77.7 \mu\text{g}/\text{kg}\cdot\text{h}$ and $30.4 \mu\text{g}/\text{kg}\cdot\text{h}$, respectively (Fig. 1). Other compounds (Fig. 2) ranged from $0.1 \mu\text{g}/\text{kg}\cdot\text{h}$ for hexylhexanoate to $5.0 \mu\text{g}/\text{kg}\cdot\text{h}$ for 2-methylbutan-1-ol. One of the most significant changes during the ripening process is the production of aroma compounds. Harvesting fruit at the proper stage of ripeness improves the biosynthesis of esters from short-chain amino acids and the subsequent development of characteristic flavours during storage.

Among the more common „impact compounds“ in the flavour and aroma of 'Golden Delicious' apples, DRAWERT et al. (1973) and SONG and BANGERTH (1996) found the four compounds butylacetate, hexylacetate, 2-methylbutylacetate and ethyl-2-methylbutanoate. The compounds contributing mostly to the characteristic aroma of 'Fuji' apples were ethyl 2-methylbutanoate, 2-methylbutyl acetate and hexylacetate, and their concentra-

tions were higher the first day after being taken out of storage after 5 months (ECHEVERRIA et al., 2004). The production of acetaldehyde, ethanol and ethylacetate

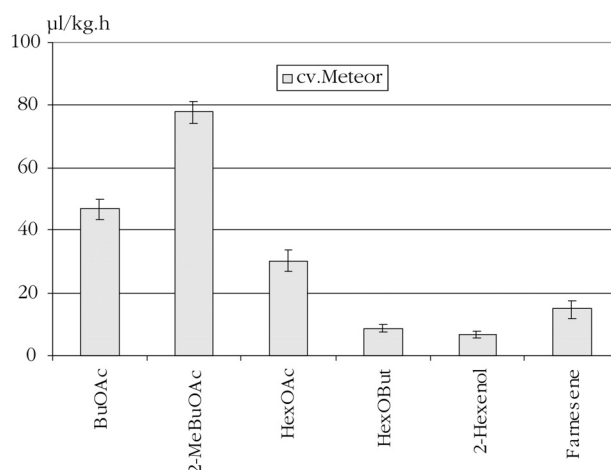


Figure 1: Major volatile compounds produced by the apple cultivar 'Meteor', held at 3°C for 30 days (mean of ten replicates \pm S.E.)

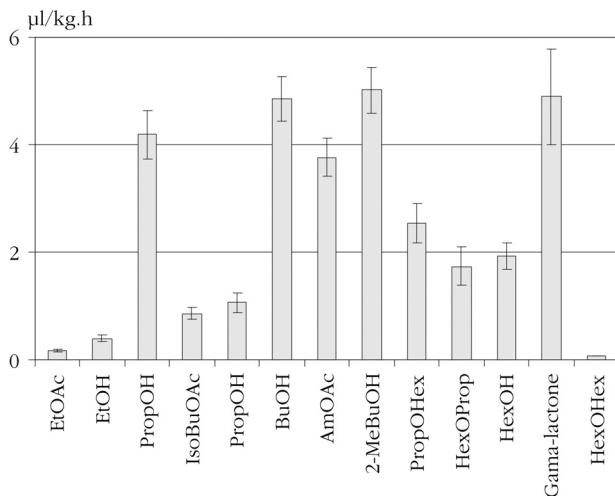


Figure 2: Minor volatile compounds produced by the apple cultivar 'Meteor', held at 3°C for 30 days (mean of ten replicates \pm S.E.)

in the first 30 days of cold storage was very low (Table 1), and so they belong to the minority of volatile compounds.

As a result of infections by *Gloeosporium* ssp. and the macerating effect of its enzymes, undamaged but infected fruit become a source of volatiles. These volatiles also occur in sound fruit at even much higher levels, but normally they are not released into ambient atmosphere.

Survey of volatiles from *Gloeosporium* ssp.

A chromatogram of volatile compounds from sound and infected fruit from apples of cultivar Jonagored is shown in Figure 4. Generally from apples infected with *Gloeosporium* the release of acetaldehyde, ethanol, ethylacetate, propylacetate and propanol was higher than from sound apples. The production of isobutan-1-ol, 2-methylbutan-1-ol, isobutylacetate, amylacetate and furfural was practically unchanged. Also from other quantitative important esters like butylacetate, hexan-1-ol, hexylacetate and hexylpropanoate the release was comparable in infected and sound apples (Table 2). The observed variation in volatiles supports the idea that headspace analysis can be used to identify indicator substances for this and other *Gloeosporium* ssp. However, it appears to be unlikely that one or a few compounds can be used as indicators of certain classes of microorganisms in a mixed microflora. SCHÖLLER et al. (2002) showed that alcohols are most frequently produced, which could be formed by trans-esterification (or reduction) of the activated acyl-CoA derivati-

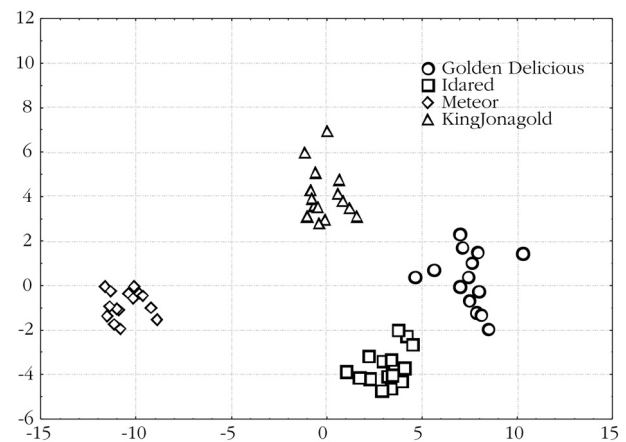


Figure 3: Discriminant analysis of nineteen volatile compounds collected from five apple cultivars 'Golden Delicious', 'Idared', 'Jonagored', 'King Jonagold' and 'Meteor' held at 3°C for 30 days (means of fifteen replicates \pm S.E.)

ves from the amino acids, followed by additional reduction reactions.

Volatile production in cold storage

Ester compounds like butylacetate, 2-methylbutylacetate, hexylacetate, hexylhexanoate, hexylbutanoate and hexylpropanoate were largely predominant in the head space aroma profile of the examined apple cultivars and conferred a characteristic 'apple' odour. After 120 days in cold storage the main impact odour compounds were lower and hexylpropanoate, hexylbutanoate and

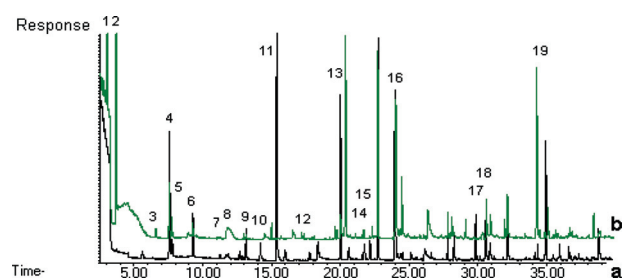


Figure 4: Chromatograms obtained from the apple cultivar Jonagored for (a) healthy fruit and (b) fruit infected by *Gloeosporium album* Osterw. Some volatile compounds identified by SPME-GC-MS: 1 methanol, 2 ethanol, 3 propanol, 4 butylacetate, 5 hexanal, 6 2-methylbutylacetate, 7 amylacetate, 8 butan-1-ol, 9 hexanal, 10 amylalcohol, 11 hexylacetate, 12 heptenal, 13 hexan-1-ol, 14 hexylbutanoate, 15 hexan-1-ol, 16 furfural, 17 hexylhexanoate, 18 ethyleneglycol, 19 farnesene

Table 2: Production of volatile aroma compounds ($\mu\text{g}/\text{kg}\cdot\text{h}$) from healthy and infected apple cultivars after storage for 120 days at 3°C

	EtOAc	EtOH	ProOAc	IsoBuOAc	PropOH	BuOAc
Golden Delicious	1.7 \pm 0.2	0.4 \pm 0.0	5.4 \pm 0.3	1.2 \pm 0.1	0.4 \pm 0.0	216.5 \pm 16.9
Idared	0.6 \pm 0.1	1.0 \pm 0.3	2.1 \pm 0.4	0.4 \pm 0.1	0.4 \pm 0.0	65.2 \pm 8.9
Jonagoldred	0.8 \pm 0.3	0.8 \pm 0.1	3.8 \pm 0.6	0.9 \pm 0.1	0.2 \pm 0.0	180.8 \pm 21.0
Cultivar	**	ns	*	**	**	**
Time	*	ns	**	*	**	**
Cultivar x time	ns	*	*	ns	*	**

	2-MeBuOAc	BuOH	AmOAc	2-MeBuOH	HexOAc	HexOProp	HexOH
Golden Delicious	25.3 \pm 2.0	4.0 \pm 0.5	6.0 \pm 0.5	5.5 \pm 0.8	46.9 \pm 4.5	0.5 \pm 0.1	0.8 \pm 0.0
Idared	24.4 \pm 2.9	4.5 \pm 0.5	3.1 \pm 0.4	2.0 \pm 0.3	27.0 \pm 4.0	0.3 \pm 0.0	1.6 \pm 0.2
Jonagoldred	23.8 \pm 8.0	3.5 \pm 0.4	4.2 \pm 0.3	4.0 \pm 0.4	50.7 \pm 4.6	0.2 \pm 0.0	1.1 \pm 0.1
Cultivar	ns	ns	**	*	**	ns	ns
Time	**	**	**	*	**	*	**
Cultivar x time	**	ns	**	**	*	*	ns

Table continued

Values are means and standard errors calculated from 10 fruit for each cultivar and infected by *Gloeosporium album* Osterw.
ANOVA: **P<0.01, *P<0.05, ns = not significant

Abbreviations:

EtOAc = ethyl acetate

EtOH = ethanol

PropOAc = propyl acetate

IsoBuOAc = isobutyl acetate

PropOH = propyl acetate

BuOAc = butyl acetate

2-MeBuOAc = 2-methylbutyl acetate

BuOH = butano-1

AmOAc = pentyl acetate

2-MeBuOH = 2-methyl butanol-1

HexOAc = hexyl acetate

HexOProp = hexyl propionate

HexOH = hexanol-1

hexylhexanoate were not detectable by headspace technique. Therefore the number of volatile compounds in Table 1 is much lower and the typical compounds that create apple odour and taste are missing. ECHEVERRIA et al. (2004) suggested that decreased amounts of esters might arise from the reduced oxidation of lipids and

the consequent lack of precursors for these esters. On the contrary ethylbutanoate could be used as a ripeness-indicator for the cultivar 'Gravenstein' due to its increasing concentration during storage and its high aroma value (AABY et al., 2002).

Statistical analysis of cultivars, sound fruit and infected fruit

Discrimination analysis was able to differentiate the different cultivars and also the level of microbiological decay. Figure 3 illustrates the differences in content of volatile compounds between sound fruit of five apple cultivars after 30 days of cold storage. A characteristic aroma chromatogram produced by apples of the cultivars 'Jonagored'; 'Idared' and 'Golden Delicious' after 120 days in cold storage for both sound and infected fruit with *Gloeosporium album* Osterw. is shown in Figure 5. Discrimination analysis grouped the apple cultivars into discrete clusters, thereby demonstrating quantitative and qualitative differences. The results for three cultivars, using both sound fruit and fruit infected by *Gloeosporium* ssp., showed good resolution between the volatiles collected from intact fruit (Fig. 2).

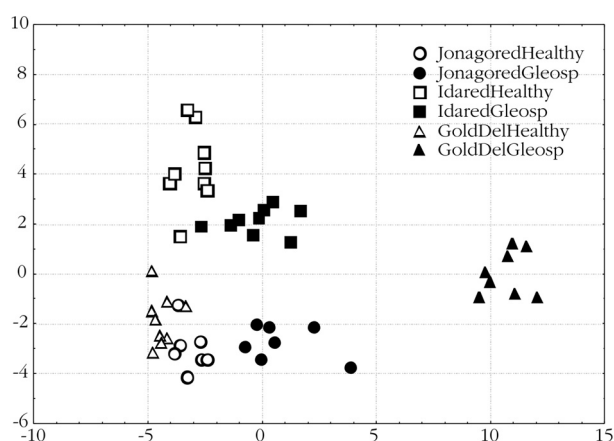


Figure 5: Seventeen volatile compounds collected from three apple cultivars: 'Jonagored', 'Idared' and 'Golden Delicious', both healthy and infected by *Gloeosporium album* Osterw., held at 3°C for 120 days (means of eight replicates \pm S.E.)

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