Hochschule Neubrandenburg
University of Applied Sciences

Bachelor Thesis

„Chemical identification (GC-MS) and sensory physiological responses (GC-EAD) of *Drosophila suzukii* to yeast fermentation products“

conducted at SLU Alnarp, Sweden

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Abstract

The cherry vinegar fly *Drosophila suzukii* is an invasive pest in soft skinned fruit such as berries, peaches, plums and others. This species mostly attacks ripening and ripe fruit, which makes it difficult to control with insecticides. Earlier experiments showed, that *D. suzukii* is highly attracted to fermentation volatiles of the assosiated yeast *Hanseniaspora uvarum*. The aim of this research was to identify the antenally active compounds in the headspaces of *H. uvarum* in minimal medium and of blueberries fermented with *H. uvarum* using GC-MS and GC-EAD. A further goal was to make synthetic blends of antenally active compounds that are as attractive for *D. suzukii* as the original sources. To date I have identified and verified eight compounds in the headspace *H. uvarum* in minimal medium and a total of 17 compounds in the headspace of blueberries fermented with *H. uvarum* that induced antennal responces in *D. suzukii*. In both headspaces those are approximately half the antenally active compounds. Of the identified compounds 3 had not yet been described in any literature currently available on *D. suzukii*. Further research needs to be done to identify the other active compounds. First wind tunnel tests with a synthetic blend of seven identified compounds from *H. uvarum* in minimal medium could not mimic the original source. Future tests will aim at improving the attraction towards synthetic blends.

Content

1 Introduction

1.1 *Drosophila suzukii*

The cherry vinegar fly, *Drosophila suzukii* (Diptera: Drosophilidae), also called Spotted Wing Drosophila (SWD), (Fig. 1), originates from south east Asia, where it was first described by Shonen Matsumura in 1931 (Kanzawa 1939). From Asia it spread to North America, where it was found for the first time on the US mainland in 2008 and around the same time they were also found in southern Europe (Hauser 2010). In Sweden, *D. suzukii* was reported in August 2014 for the first time (Jordbruksverket 2014). The spotted wing drosphila prefers temperate climates and can produce up to 13 generations per year depending on conditions.

Fig. 1: D. suzukii; left: male with the typical dark spot on the wings; right: female with ovipositor visible; both 6 days old

Whereas *Drosophila melanogaster*, the common vinegar fly, can only breed and oviposit on soft fermenting or rotting fruit, females of *D. suzukii* have a serrated ovipositor, which allows them to pierce the skin of ripe and ripening fruit for egg-laying. This serated ovipositor is almost unique in the *Drosophilidae* family. *D. subpulchrella* and *D. pulchrella* also possess a similar ovipositor, but neither are reported as a pest of fruit (Revadi 2015, Attalah 2014). Soft skinned fruit, such as cherries, blueberries, plums, peaches and wine berries are suitable hosts for *D. suzukii.* This oviposition behaviour makes *D. suzukii* a serious pest in fruit-growing regions, causing significant damage and, due to its short generation time, is difficult to control (Baufeld 2010).

In the USA the financial losses for 2008 due to *D. suzukii* in strawberries, black- and raspberries, blueberries and cherries were estimated to 511 million dollars (Bolda 2009). With the ongoing spread in the USA and Canada, this number will rise drastically. In Europe the loss in yield for 2010 was up to 80% depending on the crop and region (Lee 2011).

1.2 *Hanseniaspora uvarum* and *Pichia terricola*

The yeast *Hanseniaspora uvarum* (Niehaus), also known as *Kloeckera apiculata* belongs to the family *Saccharomycodaceae* (For more detailed specification see Fig. 6). The main habitat of *H. uvarum* is fruit, especially grapes, but it can also be found in soil, fresh and salt water and in different animals. *H. uvarum* forms oval, lemon-shaped cells that multiply by forming bipolar budds (Bink 2010).

As it is present on grapes, it plays a role in wine fermentation, especially in the early stages. Alcohol levels up to 3.4% to 6.7% depending on temperature and medium. In wine fermentation *Saccharomyces cerevisiae* usually takes over at around 4% ethanol (wineserver.ucdavis.edu).

Pichia terricola (van der Walt) was isolated from soil, fruit juice, sea water and other such habitats and shows many similarities to *P. kudriavzevii* which accounted for about 30% of the isolated yeasts in a cocoa bean heap fermentation and might be involved in the citrate assimilation during the fermentation (Daniel 2009).

1.3 Connection between *Drosophila suzukii* and yeasts

H. uvarum, *P. kluyveri* and *P. terricola* are the most prevalent yeasts on both *D. suzukii* larvae and adults (Hamby 2012). Larvae need yeasts to process the medium in which they live, to provide necessary proteins and lipids, and yeasts also increases larval resistance to parasitism (Anagnostous 2010). Adult drosophilids prefer yeast inhabited fruit over fruit that is mostly occupied by mould or bacteria (Oakeshott 1989). For adult flies the yeasts in the diet affect among other things egg production, especially the magnitude of eggs (Chippindale 1993, Chippindale 1997).

Previous wind tunnel experiments, performed during my internship, showed, that *D. suzukii* is highly attracted towards *H. uvarum* volatiles and fruit volatiles (Leinweber 2014). Continuing on these results we want to identify the compounds in the headspaces of *H. uvarum* in minimal medium and blueberries fermented with *H. uvarum* using GC-MS. Then, using GC-EAD we want to find out, which of these compounds can be detected by *D. suzukii* and ultimately develope a synthetic blend of the EAD active compounds that is as attractive as the original headspace.

2 Materials and methods

2.1 Flies and yeasts

Drosophila suzukii

For all experiments an Italian strain of *D. suzukii* was used (Revadi 2015). Rearing was conducted under quarantine conditions on semi-artificial drosophila-food substrate (Bloomington see below). If possible, newly hatched flies were harvested twice a day (to prevent uncontrolled mating of young flies) once in the morning and once in the afternoon/evening, anesthetized under carbon dioxide and separated by sex. Males and females were kept separate on drosophila-food substrate until testing.

As *D. suzukii* mate especially in the morning (Revadi 2015), all flies were kept under a controlled light cycle of 16/8h with lights on from 8:00 am till 0:00 am.

The drosophila-food substrate (Bloomington) used for the flies consists of:

1l water, 76.8 ml sugar syrup, 73 g corn meal, 3.8 g plant agar, 20.85 g malt, 17.3 g instant yeast, 10 g soy meal

All ingredients were cooked together and once slightly cooled, 4.74 g propionic acid was added to protect the substrate from moulding. Once the propionic acid was added, the substrate was filled into vials / cups to harden. The cooled vials were covered with cotton plugs and then stored in the fridge until use.

Hanseniaspora uvarum

The *H. uvarum* strain CBS (Centraalbureau voor Schimmelcultures, Netherlands) 2570 was inoculated into 50 ml of minimal medium (Merico et al. 2007) and left to grow for 24 h in a shaking incubator at 260 rpm and 25°C as a pre-culture. Then out of this pre-culture, new minimal medium was inoculated to an optical density of 0.4 at 585 nm and fermented under the same conditions as before.

Blueberries (KRAV svenska ekologisk blåbär (Swedish organic blueberries); frozen (*Vaccinium myrtillus*) were fermented with *H. uvarum* using a pre-culture of *H. uvarum* grown for 24 hours as described above, then 100 g of defrosted blueberries were inoculated with 25 ml and left 22 hours before collecting headspace for 4 hours at room temperature (see 2.2 **headspace collection**).

Pichia terricola

The *Pichia terricola* strain UCDFST (Univerity of California Davis, USA) 11-385 was grown the same way as *H. uvarum* (described above).

2.2 Experimentation setup

Headspace collection

Before sampeling the filter was washed first with 2 ml of hexane and afterwards with 2 ml of redestilled ethanol. The headspace was collected by pumping charcoal filtered air at 0.5 l/min through 100 g of sample in a wash-bottle for 4 hours. The absorbent used was porapak q 80/100 (SIGMA-ALDRICH). The collected volatiles were first eluated with 500 µl of hexane.

SPME (solid phase microextraction)

The fibre (Supleco grey fibre) was conditioned at 250 °C for 20 minutes, then exposed to the sample for 1.5 minutes (pure chemicals for identification/verification) to 5 minutes (collecting yeast / yeast and fruit headspace) at room temperature before heat desorption in the GC-MS.

GC-EAD (Gas chromatography-electroantennographic detection)

On the GC (Hewlett Packard HP6890) a HP-5MS column was used, which has following specifications: 5% phenyl + 95% methyl siloxane; length 30 m; diameter: 0.25 mm; df: 0.25 µm. Hydrogen was used as carrier gas at a flow rate of 35 cm/s. The initial oven temperature was at 40 °C for 3min and was raised at a rate of 10 °C/min to a final temperature of 280 °C and held for 2 min. The postrun temperature was set to 295 °C. The front inlet was run in splitless mode at a temperature of 250 °C and a pressure of 16.74 psi. The total flow was set to 39.2 ml/min. Thirty seconds after injection a purge flow of 30 ml/min for 0.5 min purged the remaining compounds out of the inlet. A constant flow of 3 ml/min at an average velocity of 62 cm/s was applied to the column. The Thermal Aux "Gerstel ODP2" had an initial temperature of 150 °C for 3 min which was raised to 280 °C at a rate of 10 °C/min and held for 10 minutes. A flame ionizing detector (FID) was used with a temperature of 270°C, a nitrogen flow of 30 ml/min and air flow of 400 ml/min. The detector had a makeup airflow of 30 ml/min. The makeup gas at the 4-way cross was nitrogen.

A virgin female fly (age between 3 and 8 days) was mounted in a truncated pipette tip with the head protruding from the narrow end. The pipette tip was fixed with wax on a stand and on the EAD (Fig. 7). Glass capillaries with a silver wire were filled with Beadle-Ephrussi ringer solution (ref). The recording glass electrode was placed at the tip of the antenna, and the reference electrode at the back of the flies head (Fig. 8). The compounds coming from the GC-column were injected into a stream of 1.3 l/min filtered and humidified air. The fly was positioned in the center of this airstream. The EAG signal was pre-amplified 10x using a Syntech probe (Syntech, Hilversum, The Netherlands). A Syntech I/O box combined signals from different channels (EAG, stimulus, trigger,

GC). After A/D conversion (Syntech IDAC PCI card), the signals were visualized and saved on a PC with Syntech software (Dekker 2006).

Most recordings were run in AC-mode at 0.05 Hz (filtering out frequencies lower than 0.05 Hz). Per headspace sample, recordings on at least 8 different individuals were done. Additionally some recordings were done in DC-mode (no signal filter applied), to help identify responses.

GC-MS (Gas chromatography-mass spectrometry)

Headspace samples of 2 µl were analysed by gas chromatography–mass spectrometry (GC-MS; 6890 GC and 5975 MS; Agilent Technologies Inc., Santa Clara, CA, USA). On the GC an HP-5MS column (Agilent Technologies) was used, which had the following specifications: 5% phenyl + 95% methyl siloxane; length 60 m; diameter: 0.25 mm; df: 0.25 µm. Helium was used as carrier gas at a flow rate of 35 cm/s. The oven temperature started at 40 °C and was held for 2 minutes. Then temperature was increased by 10 °C/min to 250 °C which was held for 10 min. Solvent delay was 9.7 min. The front inlet was set to splitless mode at a temperature of 225 °C and a pressure of 26.36 psi. The total flow was set to 35 ml/min. After 30 seconds a purge flow of 30 ml for 0.5 min to purge remaining compounds frem the inlet. A constant flow of 1.9 ml/min with a pressure of 26.35 psi was applied to the column. The average velocity was at 35 cm/sec. The initial temperature of the Thermal Aux was 125 °C for 13.5 min and was raised to 235 °C at a rate of 8 °C/min.

For SPME the oven temperature started at 30 °C and was held for 2 minutes. Then temperature was increased by 8 °C/min to 250 °C which was held for 1 min. The front inlet was set to splitless mode at a temperature of 225 °C and a pressure of 26.27 psi. The total flow was set to 35 ml/min. The purge flow was set to 30 ml for 0.5 min. A constant flow of 1.9 ml/min with a pressure of 26.29 psi was applied to the column. The average velocity was at 35 cm/sec. The initial temperature of the Thermal Aux was 150 °C for 13.5 min and was raised to 310 °C at a rate of 8 °C/min. The compounds found to be active where identified by matching (sequence and relative peak size) the peaks from the GC-EAD to the correspondent peaks of the GC-MS. The compounds given by the GC-MS database (WILEY 275, Alnarp 11 and NIST 05) were then again tested as pure substances on both GC-MS and GC-EAD.

Identification and verification

To verify the EAD-active compounds in the headspace samples, a mixture of candiate compounds as synthetic compounds (20 ng/ml per compound) in hexane was injected in the GC-MS under the same conditions as the headspace sample. The results were then compared by retention time and

mass spectra. If those corresponded to the compound suggested by the MS-database (Wiley 275, Alnarp 11 and NIST 05) it is most likely the correct compound. The approximate concentrations were calculated by comparing the peak area of the compounds found in the sample and the synthetic standard of known concentration. The mixtures of these compounds in varying concentrations or single compounds were injected in the GC-EAD under the same conditions as the headspace sample to verify that the flies detect these compounds.

For the compounds identified in the hexane based headspaces, quantification was done by comparing peak areas of the synthetic standards with known concentration and the peak area in the headspace.

Compounds

Tab. 1: List of tested compounds

compound	CAS	supplier	purity (acc. to label)
acetic acid	000064-19-7	KEBO	99%
alpha-pinene	000080-56-8	Sigma aldrich	98 %
butyl acetate	000123-86-4	Sigma aldrich	99.5 %
ethanol	000064-17-5	ScanLab	(redestilled)
ethyl acetate	000141-78-6	Sigma aldrich	99.5
ethyl caproate	000123-66-0	Sigma aldrich	99%
ethyl-3-methylbutanoate	000108-64-5	SAFC	98%
hexane	000110-54-3	Merck	98%
1-hexanol	000111-27-3	Sigma aldrich	98%
(Z)-3-hexenol	000928-96-1	Chemika	98%
hexyl acetate	000142-92-7	Sigma aldrich	98%
isoamyl actate	000123-92-2	SAFC	97%
linalool	000078-70-6	Sigma aldrich	97%
2-methyl-1-butanol	000137-32-6	Sigma aldrich	99%
3-methyl-1-butanol	000123-51-3	Fluka	99%
1-pentanol	000071-41-0	ACROS organics	99%
pentyl acetate	000628-63-7	Fluka	98.5%
prenyl acetate	001191-16-8	Sigma aldrich	98%
phenethyl acetate	000103-45-7	Sigma aldrich	99%
2-phenylethanol	000060-12-8	Merck	98%
propyl acetate	000109-60-4	Fluka	99.7%

Windtunnel

The flight experiments were conducted in a glass wind tunnel with 30 x 30 x 100 cm flight section. The air stream (0.25 m/s) was produced by a fan (Fischbach GmbH, Neunkirchen, Germany), which blew air into the tunnel through an array of four activated charcoal cylinders (14.5 cm diam.×32.5 cm long; Camfil, Trosa, Sweden). To evenly diffuse the light in the wind tunnel, the top side of the tunnel was covered with paper. Luminosity in tunnel was 13 lux. At the upwind end of the tunnel, the odour was injected through a glass pipette. At the downwind end of the tunnel, a glass tube with the flies (either one couple or or flies of same sex) was placed in the odour plume. Both ends of the tunnel were sealed by a polyamide mesh (pore size 0.5×0.5 mm; Sintab, Oxie, Sweden). After releasing the flies into the tunnel, they were observed for five minutes. The times for take off, upwind flight, close approach and landing at the source were then registered (Becher et al. 2010).

The prolonged light period (12 hours before to 16 hours now) led to the flies being more active in the early hours of light. Different from earlier wind tunnel experiments (Leinweber 2014), which were done 5 hours after mating, wind tunnel experiments were now done in the morning (around 24 hours after the initial mating). During this time the flies were off food. This might result in increased hunger responce due the change in physiological condition of the flies.

2.3 Statistical Analysis

A generalized linear model (GLM) with a logic link and binomial error distribution was used on wind tunnel data. The number of flies performing each behaviour was the response variable, and sex was the explanatory variables (Burnham and Anderson 2002; Veneables and Ripley 2002). For the statistical analysis of the wind tunnel data two different tests were used. To determine the significance of differences between the different groups for each behavioural step (take off, 1/3,...) the CHI^2 test was conducted. To analyse the differences of the groups in general, a 6 point Likert-scale was formed with 0 = no take off, 1 = take of, … , 5 = landing. Of this set of data the Mann-Whitney U-test, a nonparametric rank sum test, was conducted. Data were also analysed in a generalized linear model (GLM) using R-Studio software (R 2.1.1, R Development Core Team, Free Software Foundation Boston, MA, USA).

3 Results

3.1 GC-EAD and GC-MS

***Hanseniaspora uvarum* in minimal medium**

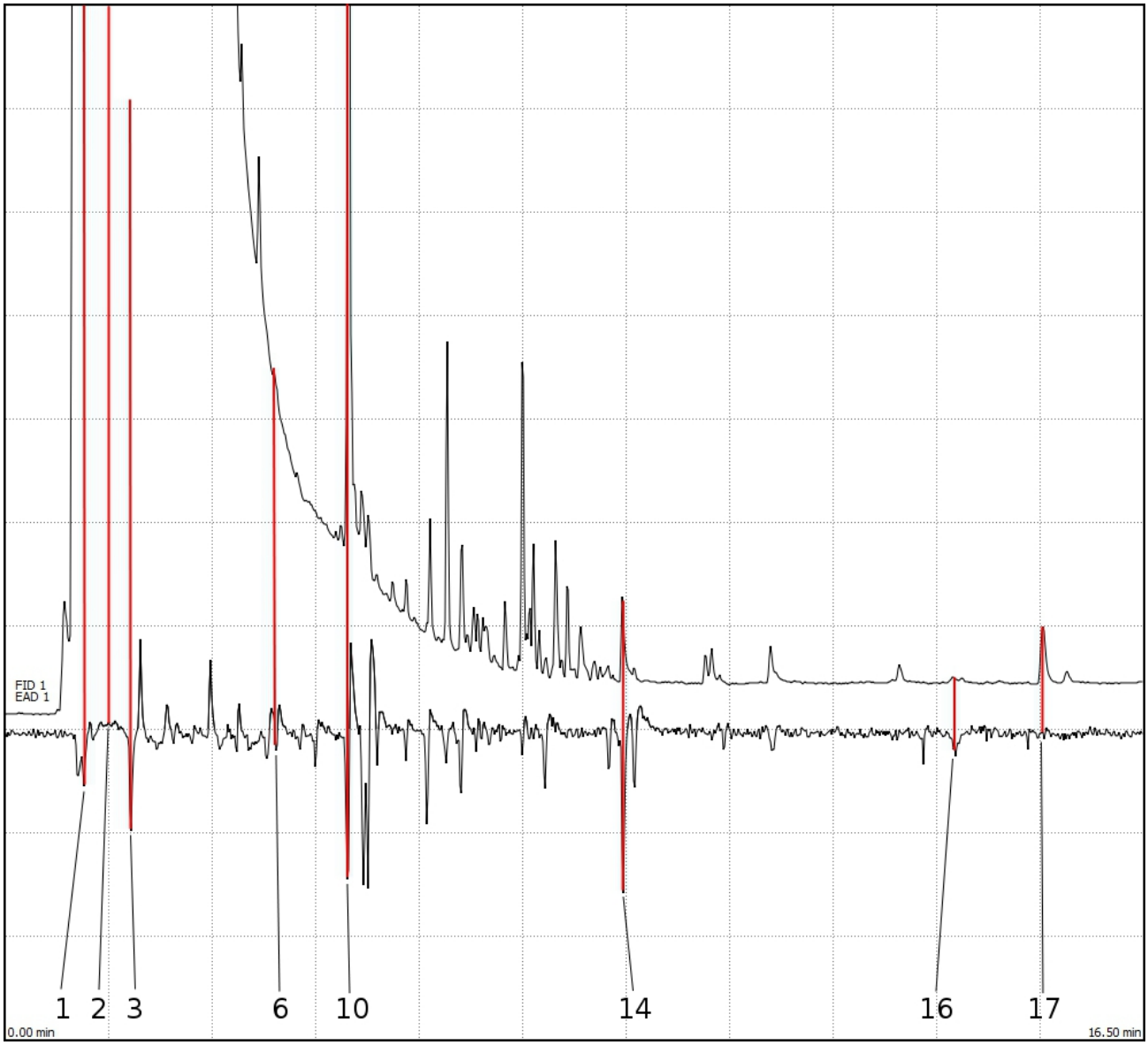

Fig. 2: GC-EAD of H. uvarum in minimal medium (HP5MS) (150115_1)

1 ethanol; 2 acetc acid; 3 ethyl acetate; 6 butyl acetate; 10 isoamyl acetate; 14 hexyl acetate; 16 2-phenyl ethanol; 17 phenethyl acetate

In the headspace of *H. uvarum* grown in minimal medium seven of approximately 20 active compounds were identified, with an eighth compound (phenethyl acetate) being active but below the detection threshold of the antenna. Fig. 2 shows one set of FID and EAD traces recorded from this headspace. The marked peaks show the identified and verified compounds. Tab. 2 shows the identified compounds with retention time, peak are in GC-MS, the approximate concentration and

the number of replicates in GC-EAD. Not included in the table are ethanol, ethyl acetate and acetic acid, as these three compounds are hidden in the solvent peak and had to be identified using SPME. By far the most abundant compound that could be quantified in the hexane dissolved headspace was isoamyl acetate. However comparing the peak areas using SPME of ethyl acetate and isoamyl acetate, the concentration of ethyl acetate was estimated to 5707.0 ng/µl, which is about 19 times as much as isoamyl acetate. For the complete list of compounds see Tab. 7 and 8 in the Annex.

Tab. 2: List of active compounds in headspace of H. uvarum (HP5MS)

Nr	Compound	Ret Time	Area	conc. ng/µl	replicates (x/6)
6	Butyl acetate	7.284	13.308.387	13.9	6
10	Isoamylacetate	9.176	217.168.853	299.1	6
14	Hexyl acetate	11.655	5.265.101	3.8	6
16	2-phenylethanol	13.819	29.909.240	39.7	3
17	phenethyl acetate	15.625	15.557.147	16.1	

Synthetic blend of identified compounds

Stock solutions of 1000 ng/µl of each antenally active compound were diluted in ethanol. Previously we used hexane, but hexane creates a rather diffuse plume, which led to weak responses from the flies as they seem to have problems to follow the odour trail. Ethanol was also used as the main solvent, because it is less toxic than hexane. Furthermore, previous wind tunnel experiments with hexane-based headspace diluted in ethanol showed promise and ethanol might actually be an important compound by itself.

Tab. 3: Formulation for synthetic blend of H. uvarum on minimal medium

compound	conc. ng/µl	conc. ng/µl	amount [µl]
Ethyl acetate	20000	5707.0	286.0
Acetic acid, butyl ester	1000	13.9	13.9
Isoamylacetate	10000	299.5	30.0
Hexyl acetate	1000	3.9	3.9
2-phenylethanol	1000	39.8	39.8
phenethyl acetate	1000	16.1	16.1
solvent (ethanol)			610.0

Due to technical problems with the GC-EAD setup no recordings of the synthetic blend have been conducted so far.

Blueberries fermented with *Hanseniaspora uvarum*

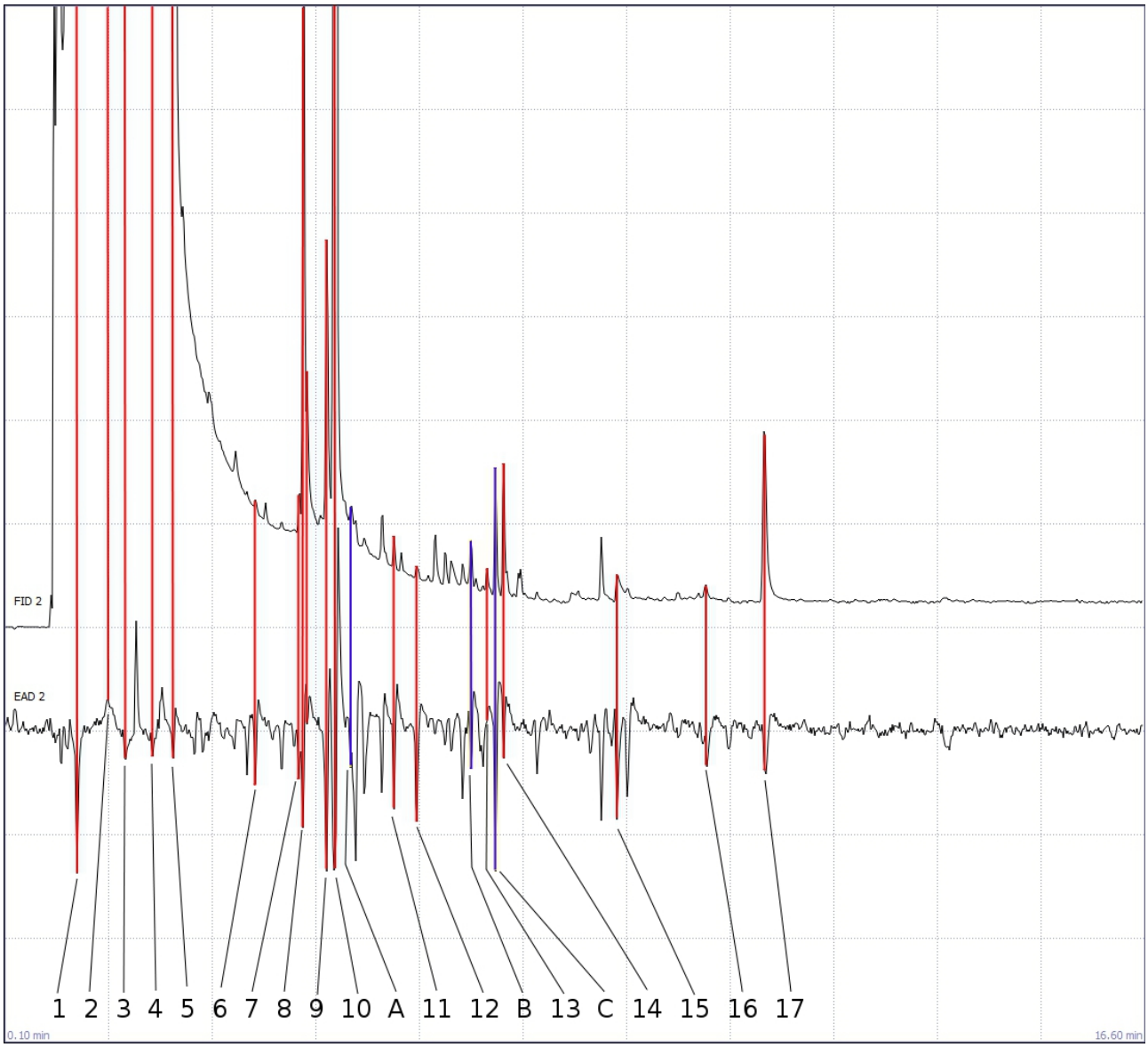

Fig. 3: GC-EAD of H. uvarum on blueberries (HP5MS) (150211_2)

1 ethanol; 2 acetic acid; 3 ethyl acetate; 4 propyl acetate; 5 1-pentanol; 6 butyl acetate; 7 ethyl-3-methyl butanoate (ethyl isovalerate); 8 (Z)-3-hexenol (double peak); 9 1-hexanol; 10 isoamyl acetate; A 2-methylbutyl acetate; 11 pentyl acetate; 12 prenyl acetate; B 6-methyl-5-hepten-2-one; 13 ethyl caproate (ethyl hexanoate); C (Z)-3-hexenyl acetate; 14 hexyl acetate; 15 linalool; 16 2-pheny lethanol; 17 phenethyl acetate;

Numbers mark identified and verified compounds, letters mark potential identificatons or compounds named in other literature.

Fig. 3 shows one set of FID and EAD traces from headspace of blueberries fermented with H. uvarum. The red marked peaks show identified and verified compounds, the peaks marked blue show potentially identified compounds.

In the headspace of blueberries fermented with *H. uvarum* 17 antenally active compounds have

been identified so far. This is approximately half of the compounds that give responses clearly above the noise level. Ten of the identified compounds are esters, seven of these are acetates. Six other compounds are alcohols, the last one is acetic acid (See tab. 4). Not included in the table are ethanol, ethyl acetate and acetic acid, as these three compounds are hidden in the solvent peak and had to be identified using SPME. By far the most abundant compound was isoamyl acetate. Though similar to the *H. uvarum* on minimal medium, the concentration of ethyl acetate could be even higher.

Tab. 4: List of active compounds in headspace of H. uvarum on blueberries (HP5MS)

Nr	Compound	Ret Time	Area	conc. ng/µl	replicates (x/9)
4	Propyl acetate	6.371	55.257.062	70.3	9
5	1-Pentanol	6.531	41.851.002	53.3	9
6	Butyl acetate	7.987	2.297.825	2.4	9
7	Ethyl-3-Methylbutanoate	8.721	111.924.428	206.9	9
8	(Z)-3-Hexenol	8.801	5.430.473	9.5	9
9	1-Hexanol	9.047	42.948.950	71.1	9
10	Isoamylacetate	9.185	522.050.309	756.0	9
11	Pentyl acetate	9.968	3.159.574	3.0	9
12	Prenylacetate	10.025	6.929.727	6.8	9
13	Ethyl caproate	11.523	18.608.134	20.5	9
14	Hexyl acetate	11.621	29.684.326	21.7	9
15	Linanool	13.150	11.759.645	28.9	9
16	2-phenylethanol	13.872	5.819.575	7.7	7
17	phenethyl acetate	15.632	61.200.431	63.3	9

Replicates = different flies

For the complete list of compounds see Tab. 8 - 11 in the Annex

3.2 Windtunnel

Hanseniaspora uvarum in minimal medium

Earlier wind tunnel experiments showed, that *H. uvarum* is highly attractive to adult *D. suzukii* (Fig. 4) (Leinweber 2014). The Mann-Whitney U-Test shows that in general, *H. uvarum* is significantly more attractive for males than for females (z = -2.965 and p = 0.003). The difference between virgin males and virgin females is also significant (z = -2.585 and p = 0.010) with mated females being more attracted than virgins.

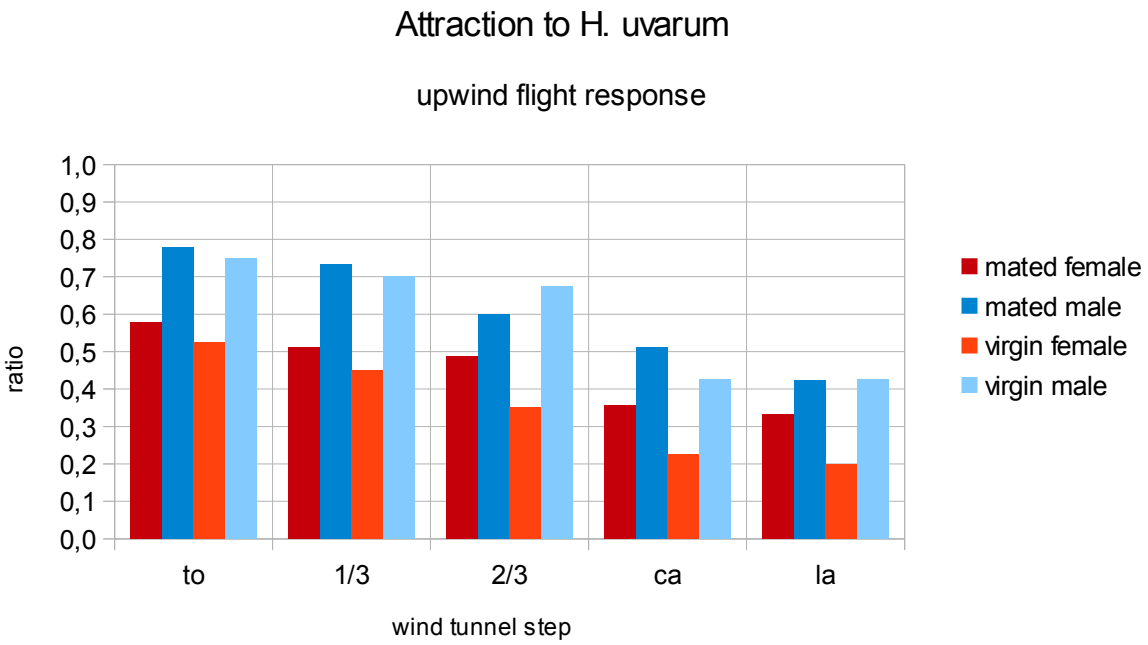

Fig. 4: Wind tunnel results from experiments with H. uvarum in minimal medium as odour source (Leinweber 2014)

Headspace of *Hanseniaspora uvarum* in minimal medium diluted with ethanol

D. suzukii (only mated flies were tested) were attracted to sprayed headspace of *H. uvarum* in minimal medium, diluted in ethanol in the wind tunnel though not as strongly as to the natural odour source.

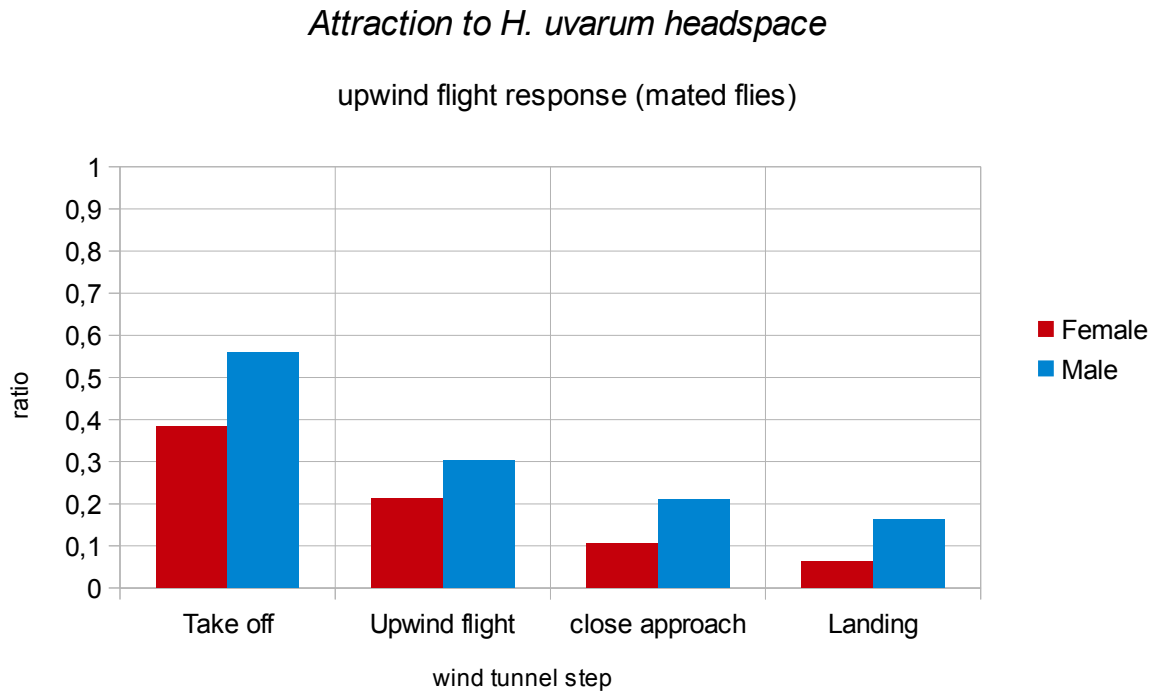

Fig. 5: Wind tunnel results of H. uvarum headspace sprayed into the wind tunnel

Synthetic blend of identified compounds diluted with ethanol

In the first experiments with the synthetic blend there were a few upwind flights towards the synthetic blend, but not more than to the ethanol control.

So far, acetic acid is not included in the synthetic blend, whether that would increase or decrease attraction remains to be seen.

4 Discussion

Drosophila suzukii is an invasive pest insect that lays eggs into soft skinned fruit. *D. suzukii* might become problematic for Sweden, as wild blueberries and other wild berries are found in great abundance. Additionally there is a lot of commercial fruit production in southern Sweden. *D. suzukii* has the potential to spread over large parts of Scandinavia where the climate is suitable. An important goal is to find alterntives to insecticides to reduce the impact of this pestiferous insect. Understanding how *D. suzukii* finds its host is an important step towards this goal.

In earlier wind tunnel experiments *D. suzukii* showed strong attraction towards both *H. uvarum* in minimal medium and to unfrozen blueberries (that presumably conotained some wild yeasts). These experiments were done five to six hours after mating and the flies were starved during this timespan. The virgin flies had the same starvation time as mated flies. Males were on average more attracted than females and mated females more than virgin ones. However it is unclear whether this attraction is in the context of the flies searching for food, mates or oviposition sites. The attraction towards the sprayed headspace was lower than to the fresh sources. This might be due to the hexane, which diffuses the odour plume. For the synthetic blend of compounds more runs will be necessary to have a sufficient set of data.

The yeast *P. terricola* did not induce strong attraction behaviour in the wind tunnel experiments and was therefore not included in any further experiments.

Most papers currently available (Keesey 2015, Abraham 2015, Revadi 2015) focus on fresh fruit volatiles as the main host cue for *D. suzukii* as their shift in host stage is the main difference in comparison to other Drosphilidae. However several of the active compounds identified in fresh fruit headspaces can also be found in yeast headspace. It is possible that the combination of fresh fruit and yeast give the main cues for host finding. Hamby et al. 2012 showed that the presence of *H. uvarum* and a few other yeast species is higher in infested fruit. Hamby et al. (2012) suggest that *H. uvarum* is a good candidate for developing more attractive and selective lure for *D. suzukii*. Therefore identifying fermentation volatiles from these yeasts could be key to finding a highly attractive lure for *D. suzukii*.

Of the identified compounds both analysed headspaces (*H. uvarum* grown in minimal medium, blueberries fermented with *H. uvarum*), acetates and other esters made the majority of active compounds. Among these ethyl acetate and isoamylacetate were the most abundant antenally active compounds in headspace of *H. uvarum* on minimal medium and on blueberries. The GC-MS analysis of the headspace of *P. terricola* grown in minimal medium suggestes that at least a few

esters were found, but in difference to the other two headspaces in the headspace of *P. terricola* isoamylacetate was only a minor compound. In the wind tunnel experiments *P. terricola* induced less attraction than *H. uvarum* which seems to produce more isoamylacetate. So isoamylacetate might be an important compound for *D. suzukii* to find its host. Mated female *D. suzukii* are strongly attracted to even low concentrations (10 ng/µl) of isoamylacetate (Revadi 2015). Isoamylacetate is described as a fresh fruit volatile, but yeasts, especially *H. uvarum* produce it in relativly high quantities.

Abraham et al. (2015) published similar research on antenally active fruit volatiles. A few of these were also found in our headspace samples. The compounds that matched were 1-hexanol, (Z)-3-hexenol, linalool and hexanal, which we did not test on the GC-EAD so far. Butyl acetate, acetic acid and ethyl hexanoate where mentioned in this paper, but not in connection with blueberries. This and the other publications might support identification the currently unknown compounds in our headspaces.

Of the 16 antenally active compounds identified from the headspace of *H.uvarum* growing in minimal medium and *H.uvarum* with blueberries, most are also described in other papers on fruit volatiles (Revadi 2015, Keesey 2015). Additionally a few more compounds found in those headspaces, that I could not test and verify yet, were named in literature, for example (Z)-3-hexenyl acetate (Keesey 2015). Another compound that was described in literature was 6-methyl-5-hepten-2-ol (Abrahahm 2015, Keesey 2015). In the yeast on blueberry headspace I found 6-methyl-5-hepten-2-one, the before mentioned compound`s corresponding ketone, which seems to be behavioural antenally active as well. 6-methyl-5-hepten-2-one is known to be antenally active in *D. melanogaster*, with 24 receptors (primarily OR85b and OR67a) detecting this compound (http://neuro.uni-konstanz.de/DoOR/).

Ethanol, ethyl acetate, acetic acid, 1-hexanol, isoamyl acetate and 2-phenyl ethanol were found to be antenally active here and were also found in red wine (Merlot) and rice vinegar headspace (Cha 2012). Currently mixes of wine and vinegar or pure vinegar are used as lure in traps, though there are also blends of few synthetic compounds in use. Both seem to work, but the fermenting food or wine plus vinegar tend to attract more non-target insects (Cha 2013). So synthetic blends of attractive compounds seem to be best way to trap *D. suzukii*.

Comparing with the currently available publications on active compounds in *D. suzukii* (Cha 2012, Abraham 2015, Keesey 2015, Revadi 2015) of the 17 verified compounds found in the two headspace we analysed three were not mentioned in any of the publications. These three compounds are ethyl-3-methylbutanoate (ethyl-isovalerate), prenyl acetate and phenethyl acetate. Phenethyl

acetate is known to be active in D. melanogaster with 24 responsive ORs and OR67a being the strongest of those (see Tab. 5)

Tab. 5: List of active compounds in context of available literature

compound	head space	Keesey 2015	Revadi 2015	Abraham 2015	Cha 2012	DoOR	
(Z)-3-Hexenol	H. u. + BB	-	-	+	-	33	OR67b
1-Hexanol	H. u. + BB	-	-	+	+	53	OR35a
1-Pentanol	H. u. m. m.	-	-	-	-	30	OR35a
2-phenylethanol	both	-	+	-	+	27	OR67a
acetic acid	both	-	+	+	+	28	OR47b
butyl acetate	both	-	-	+	-	33	OR47b
Ethanol	both	-	+	-	+	25	OR47b
Ethyl acetate	both	+	+	-	+	54	OR42b
Ethyl caproate	H. u. + BB	+	-	+	-	24	OR22a
Ethyl-3-Methylbutanoate	H. u. + BB	-	-	-	-	3	OR67b
Hexyl acetate	both	+	+	-	-	33	OR35a
Isoamylacetate	H. u. + BB	-	+	-	+	53	OR47b
Linanool	H. u. + BB	+	+	+	-	31	OR19a
Pentyl acetate	H. u. + BB	+	-	-	-	53	ab5B
phenethyl acetate	both	-	-	-	-	24	OR67a
Prenylacetate	H. u. + BB	-	-	-	-	n. l.	
propyl acetate	H. u. + BB	+	-	-	-	42	OR42a

DoOR shows the number of ORs and the strongest OR in *D. melanogaster.* n.l = not listed

To date only half the active compounds in both headspaces were identified, so identifing the other unknown compounds should be the focus of future work and might help to create better lures for *D. suzukii*. For a list of compounds that were found to be antenally active by other researchers see Tab. 12 – 13 in the annex.

An other focus will be on further wind tunnel experiments with the syntheic blend of antenally active compounds, as the first experiment with the synthetic compounds in ethanol *D. suzukii* did not show attraction behaviour. We will run further experiments with this blend to see whether it is not attractive or whether there was some problem with the flies on that day.

In the wind tunnel both *H. uvarum* and blueberries were highly attractive for *D. suzukii*. Testing blueberries fermented with *H. uvarum* would be the next step in the wind tunnel experiments. This headspace would probably be the closest to the natural source of odours that seem to attract *D. suzukii*. If this odour source performs well, I would like to test the sprayed headspace and then a synthetic blend of this (more complicated) mix of active compounds.

To double-check the concentrations in the synthetic blend of compounds it will have to be run on the GC-EAD. But due to technical problems with this machine this will be done some time in the future.

5 Summary

Earlier wind tunnel experiments showed, that *D. suzukii* is highly attracted to fermentation volatiles from *H. uvarum* and Hamby et. al 2012 suggest that *H. uvarum* would be a good basis to create a highly attractive lure for *D. suzukii*. Using GC-MS and GC-EAD to identify the antenally compounds in headspace, we identified and verified eight out of approximately 20 active compounds in headspace collected of *H. uvarum* grown in minimal medium. One of these compounds (phenethyl acetate) has not yet been described as an active compound for *D. suzukii*. Having quantified the identified compounds, we made a synthetic blend including seven of these compounds and tried it in the wind tunnel. However we need more data to see whether this mix is attractive or not.

A second headspace was collected from blueberries fermented with *H. uvarum*. In this headspace 17 out of approximately 30 antenally active compounds were identified and verified by GC-MS and GC-EAD. In this headspace three compounds (ethyl-3-methylbutanoate (ethyl-isovalerate), prenyl acetate and phenethyl acetate) had not been described in previous publications. So far we have not tested this headspace in the wind tunnel.

More work needs to be done to identify the other antenally active compounds and then to find out which of those are the key behavioural active cues.

6 Annex

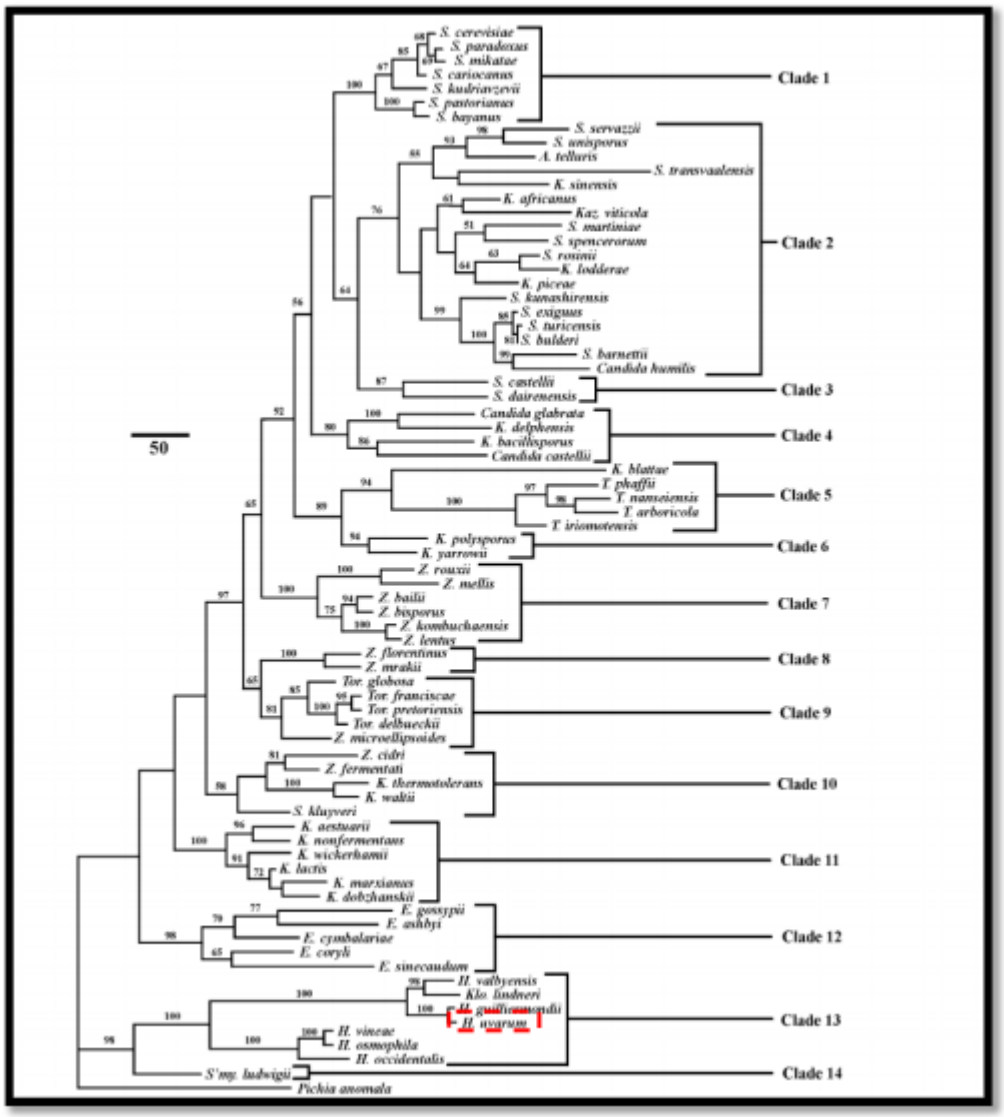

Fig. 6: Phylogenetic classification of H. uvarum (Kurtzman 2003)

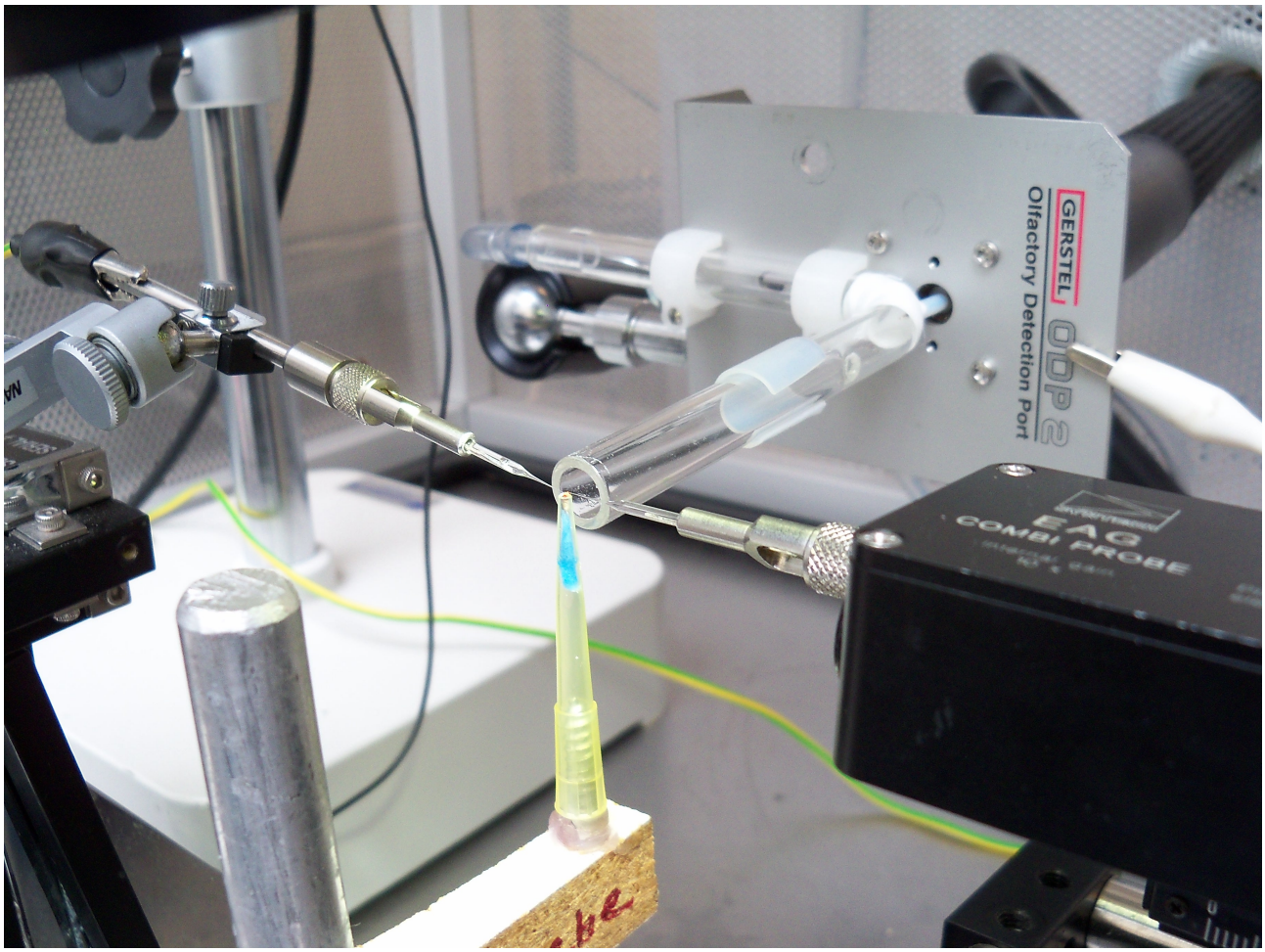

Fig. 7: GC-EAD setup with mounted fly

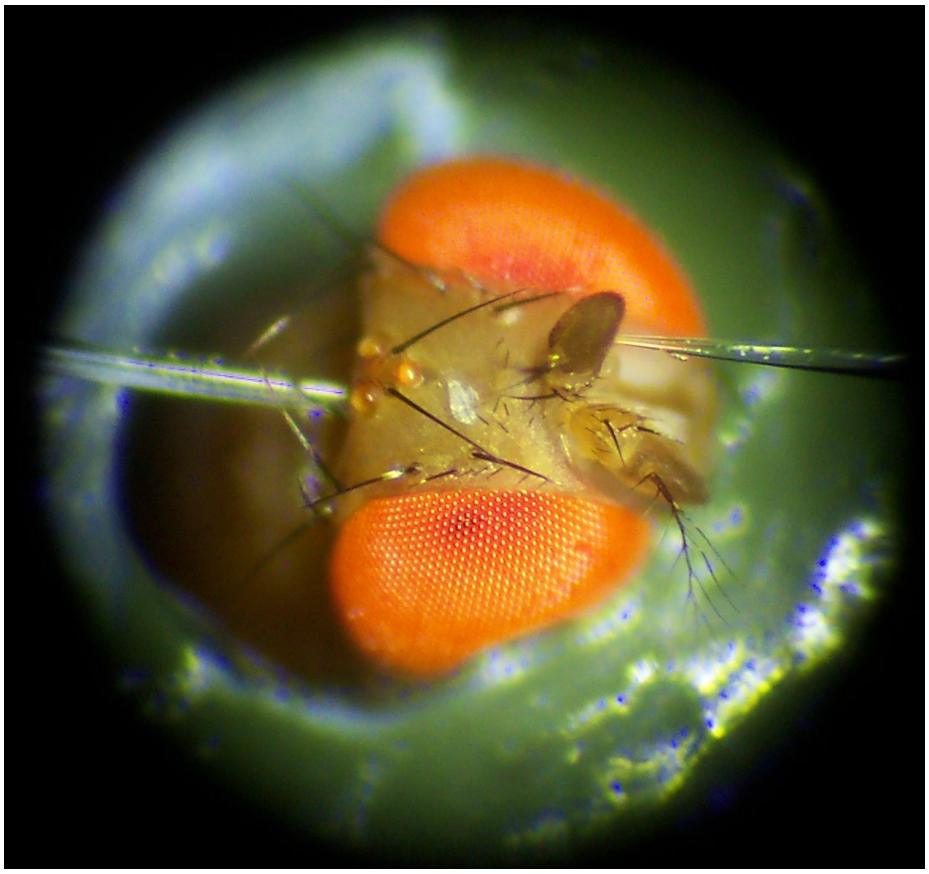
Fig. 8: Close up of mounted D. suzukii

This picture from older experiments shows a recording at the base of the antenna. In the current experiments we recorded at the tip.

Tab. 6: List of compounds found in headspace of H. uvarum in minimal medium

Hanseinaspora uvarum 2µl in hexane 4h

Peak Nr.	Retention time	Compound	CAS#	Quality	Peak area
1	5.750	Pentane, 3,3-dimethyl-	000562-49-2	83	47.611.552
2	5.824	Hexane, 2-methyl-	000591-76-4	64	307.686.979
3	5.846	Pentane, 2,3-dimethyl-	000565-59-3	95	-
4	5.897	Hexane, 3-methyl-	000589-34-4	95	165.941.974
5	6.024	Isooctane	026635-64-3	83	274.972.220
6	6.055	Cyclopentane, 1,2-dimethyl-, trans-	000822-50-4	90	-
		Cyclopentane, 1,2-dimethyl-	002452-99-5	90	
		Cyclopentane, 1,2-dimethyl-, cis-	001192-18-3	87	
7	6.219	Heptane	000142-82-5	91	3.156.177.421
8	6.438	Pentane, 2,2,4-trimethyl-	000540-84-1	72	91.968.044
		Butane, 2,2,3,3-tetramethyl-	000594-82-1	72	
9	6.518	Cyclohexane, methyl-	000108-87-2	94	129.724.374
10	6.582	Ethane, 1,1-diethoxy-	000105-57-7	53	19.964.538
11	6.635	1-Butanol, 3-methyl-	000123-51-3	86	87.875.208
12	6.690	1-Butanol, 2-methyl-, (.+/-.)-	034713-94-5	91	-
13	6.891	Pentane, 2,3,4-trimethyl-	000565-75-3	91	22.491.682
14	6.983	Pentane, 2,3,3-trimethyl-	000560-21-4	83	-
		Hexane, 2,3,4-trimethyl-	000921-47-1	72	
15	7.284	Acetic acid, 2-methylpropyl ester	000110-19-0	72	-
		Acetic acid, butyl ester	000123-86-4	64	13308387
16	7.426	Hexane, 2,2,5-trimethyl-	003522-94-9	83	-
		Hexane, 2,2,4-trimethyl-	016747-26-5	72	
17	9.176	3-Methylbutyl acetate (Isoamylacetate)	000123-92-2	86	217.168.853
18	9.217	1-Butanol, 2-methyl-, acetate	000624-41-9	72	32.860.027
19	9.323	Octane, 2,2-dimethyl-	015869-87-1	50	-
20	9.440	D,L-2,3-BUTANDIOL DIACETATE	-	59	-
21	9.515	Styrene	000100-42-5	90	-
22	9.574	Heptane, 3,3,5-trimethyl-	007154-80-5	64	-
		Hexane, 3,3,4,4-tetramethyl-	005171-84-6	64	
		Octane, 3,3-dimethyl-	004110-44-5	64	
23	9.900	Nonane, 4-methyl-	017301-94-9	70	-
24	10.142	Octane, 2,2,6-trimethyl-	062016-28-8	78	-
25	10.576	Butane, 1,1-diethoxy-3-methyl-	003842-03-3	50	-
		Butane, 1,1-diethoxy-2-methyl-	003658-94-4	42	

Green = verified antenally active compound

Tab. 7: List of compounds found in headspace of H. uvarum in minimal medium

26	10.631	Camphene	000079-92-5	98	22.842.005
27	10.741	Hexane, 2,2,5-trimethyl-	003522-94-9	59	52.890.892
		Hexane, 2,2,5,5-tetramethyl-	001071-81-4	59	
28	10.861	Propanoic acid, pentyl ester	000624-54-4	72	-
29	10.978	Hexane, 2,2,3-trimethyl-	016747-25-4	72	-
		Heptane, 2,2,4,6,6-pentamethyl-	013475-82-6	72	
30	11.086	2,2,7,7-Tetramethyloctane	001071-31-4	72	-
		Octane, 2,2,6-trimethyl- (CAS)	062016-28-8	72	
		Decane, 2,2,7-trimethyl- (CAS)	062237-99-4	72	
31	11.220	Cyclohexene, 4-methyl-1-(1-methylethyl)-	000500-00-5	94	-
32	11.295	3(2H)-Thiophenone, dihydro-2-methyl-	013679-85-1	95	-
33	11.655	Hexyl acetate	000142-92-7	86	5265101
		Decane, 2,2,7-trimethyl-	062237-99-4	72	
34	11.705	delta-3-Carene	013466-78-9	97	-
		alpha-Pinene	000080-56-8	95	
		gamma-terpinene	000099-85-4	90	
35	11.839	Decane, 2,2,9-trimethyl-	062238-00-0	72	44.032.185
		Heptane, 2,2,4,6,6-pentamethyl-	013475-82-6	72	
36	11.941	p-Cymene	000099-87-6	95	34.972.411
37	11.972	Heptane, 2,2,4,6,6-pentamethyl-	013475-82-6	64	-
		Undecane, 2,2-dimethyl-	017312-64-0	59	
38	12.073	Nonane, 3-methyl-5-propyl-	031081-18-2	72	20.373.760
39	12.123	Decane, 2,5,9-trimethyl-	062108-22-9	83	
40	12.411	Heptane, 2,2,4,6,6-pentamethyl-	013475-82-6	59	28.504.909
41	12.449	Hexane, 2,2,5-trimethyl-	003522-94-9	64	-
42	12.534	Heptane, 4-ethyl-2,2,6,6-tetramethyl-	062108-31-0	59	21.183.458
43	12.766	Undecane, 2,8-dimethyl-	017301-25-6	72	-
44	13.819	2-phenylethanol	000060-12-8	95	29909240
45	15.625	phenethyl acetate (2-Phenylethanol acetate)	000103-45-7	90	15557147

Green = verified antenally active compound

Tab. 8: List of compounds found in headspace of blueberries fermented with H. uvarum
Hanseinaspora uvarum on blueberry 2µl in hexane 4h

Peak Nr.	Retention time	Compound	CAS#	Quality	Peak area
1	5.673	Pentane, 3,3-dimethyl-	000562-49-2	83	31.244.393
2	5.747	Hexane, 2-methyl-	000591-76-4	90	174.425.559
3	5.771	Pentane, 2,3-dimethyl-	000565-59-3	95	-
4	5.821	Hexane, 3-methyl-	000589-34-4	91	54.059.365
5	5.952	Pentane, 2,2,4-trimethyl-	000540-84-1	72	117.547.768
6	5.988	Cyclopentane, 1,2-dimethyl-, cis-	001192-18-3	87	-
7	6.094	Heptane	000142-82-5	90	385.697.711
8	6.205	3-hydroxy-2-butanone	000513-86-0	86	-
9	6.239	Propanoic acid, ethyl ester	000105-37-3	86	-
10	6.281	Acetic acid, propyl ester	000109-60-4	54	-
11	6.371	Butane, 2,2,3,3-tetramethyl-	000594-82-1	78	53.753.932
12	6.453	Cyclohexane, methyl-	000108-87-2	96	76.782.658
13	6.531	1-Pentanol	000071-41-0	47	41.851.002
14	6.591	1-Butanol, 3-methyl-, formate	000110-45-2	83	150.909.230
15	6.646	1-Butanol, 2-methyl-	000137-32-6	90	29.889.609
16	6.840	Pentane, 2,3,4-trimethyl-	000565-75-3	90	-
17	7.250	Acetic acid, 2-methylpropyl ester	000110-19-0	72	-
18	7.309	Butanoic acid, 3-methyl-, methyl ester	000556-24-1	83	-
19	7.467	3-Cyclopenten-1-ol	014320-38-8	68	-
20	7.710	Octane	000111-65-9	87	-
21	7.985	Acetic acid, butyl ester	000123-86-4	72	-
22	8.662	Butanoic acid, 2-methyl-, ethyl ester	007452-79-1	95	-
23	8.722	Ethyl 3-methylbutanoate	000108-64-5	96	102.733.360
24	8.796	(Z)-3-Hexenol	000928-96-1	97	-
25	9.048	1-Hexanol	000111-27-3	83	28.194.342
26	9.097	O-Xylene	000095-47-6	90	-
27	9.188	Isoamylacetate	000123-92-9	86	510.808.275
28	9.214	2-Methylbutyl acetate	000624-41-9	83	-
29	9.323	4-Penten-1-yl acetate	001576-85-8	42	-
30	9.440	2-Butanol, 3-methyl-, acetate	005343-96-4	43	-

Green = verified antenally active compound

Tab. 9: List of compounds found in headspace of blueberries fermented with H. uvarum

31	9.849	Pentyl acetate	000628-63-7	90	-
32	9.966	3-Hexanone, 4-hydroxy-2,5-dimethyl-	000815-77-0	37	-
33	10.016	Prenylacetate	001191-16-8	94	-
34	10.326	alpha-Pinene	000080-56-8	95	-
35	10.568	Ethyl 3-hydroxy-3-methylbutanoate	018267-36-2	72	-
36	10.819	Benzaldehyde	000100-52-7	97	-
37	10.835	Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester	002441-06-7	35	-
38	11.195	6-Methyl-5-hepten-2-one	000110-93-0	96	-
39	11.295	3(2H)-Thiophenone, dihydro-2-methyl-	013679-85-1	93	-
40	11.379	Ethyl caproate	000123-66-0	98	-
41	11.479	3-Hexen-1-ol, (E)-	000928-97-2	60	-
42	11.521	E3-Hexenyl acetate	003681-82-1	90	-
43	11.621	Hexyl acetate	000142-92-7	86	18.921.313
44	11.663	E2-Hexenyl acetate	002497-18-9	93	-
45	11.705	delta-3-Carene	013466-78-9	95	-
46	11.939	p-Cymene	000099-87-6	94	-
47	12.014	(R)-(+)- Limonene	005989-27-5	99	-
48	12.081	2-Hydroxy-1,8-cineole	(000470-82-6)	87	-
49	12.766	E-Citral	000141-27-5	46	-
50	13.151	Linanool	000078-70-6	97	-
51	14.100	2-Phenylethanol	000060-12-8	93	-
52	14.362	Ethyl benzoate	000093-89-0	93	-
53	14.621	Octanoic acid, ethyl ester	000106-32-1	90	-
54	14.722	Naphthalene	000091-20-3	50	-
55	14.814	Cyclopentylcyclohexane	001606-08-2	55	-
56	15.633	phenethyl acetate	000103-45-7	90	60.203.591
57	17.455	Decanoic acid, ethyl ester	000110-38-3	92	-
58	18.282	Geranyl acetone	003796-70-1	90	-
59	27.927	Diisooctyl adipate	001330-86-5	91	-

Green = verified antenally active compound

Tab. 10: List fo compounds found in concentrated headspace of blueberries fermented with H. uvarum

Hanseinaspora uvarum on blueberry 2µl in hexane 4h conc.

Peak Nr.	Retention time	Compound	CAS#	Quality	Peak area
1	5.645	Cyclopentane, methyl-	000096-37-7	91	453.421.338
2	5.758	Pentane, 3,3-dimethyl-	000562-49-2	83	2.017.338
		Sulfurous acid, hexyl 2-pentyl ester	001185-33-7	56	
3	5.826	Hexane, 2-methyl-	000591-76-4	90	19.075.750
4	5.900	Hexane, 3-methyl-	000589-34-4	95	6.416.332
5	6.022	Butane, 2,2,3,3-tetramethyl-	000594-82-1	78	20.528.211
6	6.148	Heptane	000142-82-5	86	60.199.553
7	6.264	2-Butanone, 3-hydroxy-	000513-86-0	80	5.275.223
8	6.300	Propanoic acid, ethyl ester	000105-37-3	87	7.338.549
9	6.342	Acetic acid, propyl ester	000109-60-4	50	2.748.690
10	6.426	1-Butanol, 3-methyl-	000123-51-3	86	22.614.214
11	6.508	Isoamylacetate ?	000123-92-9	53	49.405.589
12	6.580	Ethane, 1,1-diethoxy-	000105-57-7	80	136.274.290
13	6.665	3-methyl-1-butanol	000123-51-3	80	244.575.463
14	6.711	2-methyl-1-Butanol	000137-32-6	86	55.912.073
15	7.045	Propanoic acid, 2-methyl-, ethyl ester	000097-62-1	70	1.563.467
16	7.286	Isobutyl acetate	000110-19-0	83	14.783.691
17	7.341	Butanoic acid, 3-methyl-, methyl ester	000556-24-1	80	3.768.910
18	7.495	3-Cyclopenten-1-ol	014320-38-8	64	1.841.115
19	7.567	Hexanal	000066-25-1	52	-
20	7.766	2-butylmethyl ether	000584-03-2	59	11.333.626
21	7.917	2-Propanol, 1-(2-propenyloxy)-	021460-36-6	50	2.352.187
22	8.007	Acetic acid, butyl ester	000123-86-4	72	4.476.080
23	8.179	1,3-Dioxane, 2,4,6-trimethyl-	019145-91-6	91	5.618.755
24	8.415	Hexane, 2,4-dimethyl-	000589-43-5	53	1.810.107
25	8.681	Butanoic acid, 2-methyl-, ethyl ester	007452-79-1	95	16.035.814
26	8.747	Ethyl 3-methylbutanoate	000108-64-5	96	157.499.741
27	8.862	Butanoic acid, 3-methyl-	000503-74-2	74	7.622.095
28	8.905	(Z)-3-Hexenol	000928-96-1	97	14.809.296
		(E)-3-Hexen-1-ol	000928-97-2	91	
29	9.004	3-Hexen-1-ol	000544-12-7	92	21.881.433
		3-Hexen-1-ol, (Z)-	000928-96-1	86	
		Cis-Hexenol	000928-96-1	80	
30	9.271	Isoamylacetate	000123-92-2	90	1.532.274.417

Green = verified antenally active compound

Blue = Compounds described as active in literature and not tested here

Red = Tested compounds that did not induce antenna responces

Hexanal was described as active by Abraham et al. 2015

Tab. 11: List fo compounds found in concentrated headspace of blueberries fermented with H. uvarum

31	9.522	Butyric acid, 2-hydroxy-3-methyl-, methyl ester	017417-00-4	37	1.165.175
32	9.862	Pentyl acetate	000628-63-7	90	3.561.910
33	9.981	3,5-Dimethyl-5-hexen-3-ol	-	37	2.179.879
		3,4-Dimethyl-5-hexen-3-ol	-	33	
34	10.034	Prenylacetate	001191-16-8	95	3.962.784
35	10.343	alpha-Pinene	000080-56-8	95	1.253.393
36	10.446	2-Heptanone, 3-methyl-	002371-19-9	53	1.336.281
37	10.575	Ethyl 3-hydroxy-3-methylbutanoate	018267-36-2	91	1.714.164
38	10.818	Benzaldehyde	000100-52-7	96	-
39	10.853	Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester	002441-06-7	53	24.734.388
40	11.023	Octanoic acid	000124-07-2	59	4.489.268
41	11.198	6-Methyl-5-hepten-2-one	000110-93-0	96	9.662.933
42	11.304	3(2H)-Thiophenone, dihydro-2-methyl-	013679-85-1	90	4.909.062
43	11.386	Ethyl caproate	000123-66-0	98	7.793.479
44	11.489	3-Hexenoic acid, ethyl ester	002396-83-0	49	1.258.316
45	11.524	E3-Hexenyl acetate	003681-82-1	90	5.030.768
		(Z)-3-Hexenyl acetate	003681-71-8	90	
46	11.623	Hexyl acetate	000142-92-7	80	11.425.084
47	11.704	delta-3-Carene	013466-78-9	96	-
		alpha-Pinene	000080-56-8	94	
48	11.938	p- Cymene	000099-87-6	90	-
49	12.021	(R)-(+)- Limonene	005989-27-5	99	1.987.029
		(S)-(-)-Limonene	005989-54-8	99	
50	12.088	2-Hydroxy-1,8-cineole	-	91	3.618.249
		1,8-Cineole (1,8-Eucalyptol)	000470-82-6	76	
51	13.150	Linanool	000078-70-6	96	15.689.242
52	13.466	2-phenylethanol	000060-12-8	95	29.007.091
53	14.186	Octanoic acid	000124-07-2	95	5.499.664
54	14.353	Ethyl benzoate	000093-89-0	97	5.436.971
55	14.619	Ethyl caprylate	000106-32-1	98	8.846.100
56	14.710	.ALPHA. TERPINEOL	000098-55-5	64	3.221.410
		.BETA. FENCHYL ALCOHOL	000470-08-6	64	
57	15.629	phenethyl acetate	000103-45-7	90	83.403.463

Green = verified antenally active compound

Blue = Compounds described as active in literature and not tested here

Red = Tested compounds that did not induce antenna responces

(Z)-3-Hexenyl acetate was described as active by Keesey 2015

Tab. 12: List of compounds found to be antenally active by other researchers

Compound	Paper	Source
Butyl acetate	Abraham 2015	homogenized raspberry
Hexanal	Abraham 2015	homogenized raspberry
2-Heptanone	Abraham 2015	homogenized raspberry
3-Methyl-1-butanol	Abraham 2015	homogenized raspberry
trans-2-Hexenal	Abraham 2015	homogenized raspberry
3-Methyl-2-butenyl acetate	Abraham 2015	homogenized raspberry
2-Heptanol	Abraham 2015	homogenized raspberry
1-Hexanol	Abraham 2015	homogenized raspberry
cis-3-Hexenol	Abraham 2015	homogenized raspberry
6-Methyl-5-hepten-2-ol	Abraham 2015	homogenized raspberry
Linalool	Abraham 2015	homogenized raspberry
Methyl butyrate	Keesey 2015	ripe strawberries
methyl isovalerate	Keesey 2015	ripe strawberries
butyl acetate	Keesey 2015	ripe strawberries
isopropyl butyrate	Keesey 2015	ripe strawberries
isopentyl acetate	Keesey 2015	ripe strawberries
2-butoxy ethanol	Keesey 2015	ripe strawberries
methyl hexanaote	Keesey 2015	ripe strawberries
ethyl hexanoate	Keesey 2015	ripe strawberries
hexyl acetate	Keesey 2015	ripe strawberries
linalool	Keesey 2015	ripe strawberries
benzylacetate	Keesey 2015	ripe strawberries
methyl salicylat	Keesey 2015	ripe strawberries
(Z)-3-hexenol	Keesey 2015	leaves
(E)-2-hexenol	Keesey 2015	leaves
1-octen-3-ol	Keesey 2015	leaves
6-Methyl-5-hepten-2-ol	Keesey 2015	leaves
(Z)-3-hexenyl acetate	Keesey 2015	leaves
(E)-2-nonenol	Keesey 2015	leaves
phenyl ethanol	Keesey 2015	leaves
2-nitrophenol	Keesey 2015	leaves
methyl salicylate	Keesey 2015	leaves
β-cyclocitral	Keesey 2015	leaves
eugenol	Keesey 2015	leaves
β-ionone	Keesey 2015	leaves

Tab. 13: List of compounds found to be antenally active by other researchers

Compound	Paper	Source
acetic acid	Revadi 2015	fruit headspaces
hexanoic acid	Revadi 2015	fruit headspaces
ethanol	Revadi 2015	fruit headspaces
hexanol	Revadi 2015	fruit headspaces
(Z)-3-hexen-1-ol	Revadi 2015	fruit headspaces
1-octanol	Revadi 2015	fruit headspaces
1-octen-3-ol	Revadi 2015	fruit headspaces
β-phenylethanol	Revadi 2015	fruit headspaces
(E)-2-hexenal	Revadi 2015	fruit headspaces
nonanal	Revadi 2015	fruit headspaces
2-heptanone	Revadi 2015	fruit headspaces
ethyl acetate	Revadi 2015	fruit headspaces
hexyl acetate	Revadi 2015	fruit headspaces
isoamyl acetate	Revadi 2015	fruit headspaces
ethyl butanoate	Revadi 2015	fruit headspaces
ethyl hexanoate	Revadi 2015	fruit headspaces
ethyl octanoate	Revadi 2015	fruit headspaces
methyl hexanoate	Revadi 2015	fruit headspaces
methyl octanoate	Revadi 2015	fruit headspaces
(Z)-3-hexenyl acetate	Revadi 2015	fruit headspaces
methyl salicylate	Revadi 2015	fruit headspaces
Norisoprenoids	Revadi 2015	fruit headspaces
(α)-ionone	Revadi 2015	fruit headspaces
α-phellandrene	Revadi 2015	fruit headspaces
β-phellandrene	Revadi 2015	fruit headspaces
limonene	Revadi 2015	fruit headspaces
p-cymene	Revadi 2015	fruit headspaces
(±)-linalool	Revadi 2015	fruit headspaces
(E)-caryophyllene	Revadi 2015	fruit headspaces
Acetoin	Cha 2012	red wine / rice vinegar
Ethyl butyrate	Cha 2012	red wine / rice vinegar
Ethyl lactate	Cha 2012	red wine / rice vinegar
1-hexanol	Cha 2012	red wine / rice vinegar
Isoamyl acetate	Cha 2012	red wine / rice vinegar
2-methylbutyl acetate	Cha 2012	red wine / rice vinegar
Grape butyrate	Cha 2012	red wine / rice vinegar
Methionol	Cha 2012	red wine / rice vinegar
Isoamyl lactate	Cha 2012	red wine / rice vinegar
Ethyl sorbate	Cha 2012	red wine / rice vinegar
2-phenylethanol	Cha 2012	red wine / rice vinegar
Diethyl succinate	Cha 2012	red wine / rice vinegar
Acetic acid	Cha 2012	red wine / rice vinegar
Ethyl alcohol	Cha 2012	red wine / rice vinegar
Ethyl acetate	Cha 2012	red wine / rice vinegar

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