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A study on the effect of pest infestation on different vegetables

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Independent project • 15 hp
Swedish University of Agricultural Sciences, SLU
Department of Plant protection
Horticultural Management – Gardening and Horticultural Production
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Abstract

A commonly held belief is that organic vegetables are tastier than their conventionally grown counterparts. Vegetables grown without insecticides are more often attacked by herbivores. This herbivory leads to the emissions of compounds called herbivore induced plant volatiles (HIPVs) that often are perceivable for humans in taste and/or smell. In this study, we hypothesized that part of the difference in perceived quality in organic vegetables is due to the changes in biochemistry following herbivory, that could be detectable for humans. We investigated the difference in volatile emissions and organoleptic qualities between induced and non-induced vegetables. To do this, volatile emissions of three species of vegetables induced by Spodoptera littoralis larvae and their non-induced counterparts were analysed through gas chromatography-mass spectrometry (GC-MS). Thereafter an organoleptic test was held with volunteers to determine the difference in taste. All vegetables displayed a difference in volatile emissions between induced and non-induced plants and endive, Chicorium endivia, displayed a difference in smell. This concludes that herbivores do make a difference in volatile emissions of plants and that these differences also can be smelled in endive.

Keywords: Herbivore-induced plant-volatiles (HIPVs), Organic, Spodoptera littoralis, Volatile organic coumpounds (VOCs), Herbivory

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Abbreviations

GCMS	Gas Chromatography Mass Spectromotry
GLVs	Green Leaf Volatiles
HIPVs	Herbivore Induced Plant Volatiles
SLU	Swedish University of Agricultural Sciences
SPME	Solid Phase Micro-Extraction
VOCs	Volatile Organic Compounds
DMNT	(E)-4,8-dimethylnona-1,3,7-triene

1. Introduction

Home-grown or organically grown vegetables are often regarded as better tasting than those that are conventionally grown (Mditshwa et al. 2017). Why? Could herbivory be one of the reasons for this? If so, how and why does herbivory affect the taste of vegetables?

The scientific consensus as to whether organic produce tastes better is still unclear, and available research on this subject has shown inconsistent results. Schutz & Lorentz (1976) and Zhao et al (2007), for example, suggest that organic cultivation does not inherently mean a better flavor profile in fruits and vegetables. However, the question if organic tastes better is still interesting because of the general conception that organic produce does, in fact, taste better and because of the implications on consumers' motives to buy organic if this sentiment turns out to be true. One of the problems with the label organic is that it is an overarching label that does not take into consideration the almost unlimited number of variables when it comes to growing vegetables. This is why we need to look further into the issue and divide it up into smaller areas of research to pinpoint 1) if there really are differences and 2) what could cause these differences. Because of the decreased use of conventional pesticides in organic farming, a logical assumption would be that herbivory would increase by growing vegetables organically. In this study herbivory was isolated as variable influencing the perceived quality of produce.

It is widely observed that plants emit different types of volatiles following herbivory. These volatile emissions serve as a type of indirect defense, which attracts parasitoids and other carnivorous arthropods to the herbivores that are feeding on the plant (Turlings et al. 1990; Arimura et al. 2009). In their study, War et al. (2011) explored these Herbivore-Induced Plant Volatiles (HIPVs), revealing that these emissions consist of different groups of compounds released at different times. The main groups include terpenoids (such as hemiterpenes, monoterpenes, and diterpenes), benzenoids/phenylpropanoids (including alcohols, aldehydes, and hydroxycinnamic acid), as well as fatty acid derivatives and amino acid derivatives (including alcohols, acids, aldehydes and nitrogen/sulfur containing compounds). Green leaf volatiles (GLVs) are derived from C18 fatty acids and are released immediately after an insect attack around the site of herbivore damage (War et al. 2011). GLVs consist of short carbon chain aldehydes, alcohols and acetates (Turlings et al. 1998). Other volatile compounds are released immediately from plant cells and are emitted for a brief period following cell rupture. In contrast, HIPVs are typically released several hours to a few days after the initial attack and are systemic in the plant, meaning that even leaves that have not sustained any damage undergo the chemical changes that cause these volatile emissions (Turlings et al. 1998)

Since many of the induced organic compounds are an important part in perceived smell and taste properties of vegetables (Bartoshuk & Klee 2013), it is plausible that plants become more or less attractive to us as their volatile profiles change due to herbivory. In his study, Nihlmar (2023) highlights that after infestation of *Spodoptera litteralis* on *Zea mays* 'Delprim', human perception of taste and smell significantly changed. In another report, Bartoshuk and Klee (2013) highlight that while non-volatile macronutrients such as sugar are clear markers for how food tastes, Volatile Organic Compounds (VOCs) can notably intensify sweetness for human perception. For instance, in a comparison between two tomato cultivars, Matina and Yellow Jelly Bean, it was found that despite containing less sugar, Matina was perceived as sweeter than Jelly Bean. This observation was correlated with the higher abundance of volatiles emitted in the headspace of this cultivar (Raghava et al. 2009). Besides affecting the organoleptic qualities in vegetables, Bartoshuk and Klee (2013) also describe how many plant volatiles stem from essential nutrients. This adds even more interest to this area of research since it could have implications for human health as well, thereby potentially adding to consumers' incentive to buy organic.

Thus, the question is raised whether other edible plants than maize will also exhibit a change in chemical and organoleptic properties and if this could be one of the reasons that people regard organic food to be better tasting. The purpose of this study is therefore to determine whether the volatile emissions and taste and smell of plants that have been induced by herbivores differ significantly from those that have not. By understanding these biochemical and sensory differences, we can contribute to a deeper understanding of herbivore-induced impact on the organoleptic quality of organic foods.

In this project, endive, pak choi and maize were grown organically in greenhouse conditions. A selection of plants were induced with the herbivore *Spodoptera littoralis.* The plant volatiles were collected with a Solid Phase Micro-Extraction (SPME) fiber and analyzed with Gas Chromatography- Mass Spectrometry (GC-MS). Additionally, an organoleptic test was conducted on these vegetables.

2. Materials and method

2.1. Materials

Three plant species were used in this study: Pak choi (*Brassica rapa subsp. chinensis*), endive (*Cichorium endivia* ‘Escariol grüner’) and maize of the cultivar Delprim (*Zea mays* ‘Delprim’). Pak choi and endive seeds were provided and planted by Alnarpsfarmen in cell trays measuring 335 x 515 mm. The maize seeds, provided by Ted Turlings, were planted in similar cell trays with two seeds per cell. Thinning occurred 9 days after sowing, leaving one maize per cell to reduce competition. Repotting occurred after 20 days into plastic pots with a diameter of 15 cm. All plants were grown under organic conditions in a greenhouse with the following conditions: a minimum temperature of 20° C during daytime and 18° C during nighttime. Artificial lights were on for 16 hours per day and the plants were watered daily.

2.1.1. Plant induction

Plants for volatile collection

Three maize, two pak choi and two endive plants were selected for herbivore induction. To do this, the plant pots were wrapped in aluminum foil and thereafter put in a plastic oven bag (see figure 1). Four third-instar larvae of *Spodoptera littoralis* were introduced to the maize plants and three larvae to the pak choi and endive plants. The larvae were allowed to feed on the leaves for 12 hours. Following this, the bags were opened, and the larvae were carefully removed. The bags were left open for 24 hours and then resealed for 12 hours prior to the volatile collections. For each species, two non-induced plants were bagged and used as control samples. Furthermore, a blank sample was collected from the headspace of an empty pot with only soil, wrapped in aluminium foil and placed within an oven bag.

Plants for organoleptic tests

Maize plants were subjected to the same procedure as described previously. Individual endive and pak choi leaves were carefully wrapped in smaller bags to have enough non-damaged leaves for the organoleptic test preparations (see figure 1). A single larva was put on every individually bagged leaf, where the larvae were allowed to feed for 12 hours before being removed.

Figure 1. Plants that have individually induced leaves for the organoleptic test to the left and fully induced plants for volatile sampling to the right

2.1.2. Volatile collection and analysis

The volatile samples were collected using SPME fibers (DVB/CAR/PDMS 50/30 µm, Supelco, Sigma-Aldrich, Bellefonte, PA, USA). Prior to each sampling, the SPME fiber was conditioned at 250°C for five minutes and then inserted through a small hole in the plastic bag. It was left to sample the headspace for 30 minutes. Then, the sample was analysed using GC (Agilent Technologies 7890B GC system) coupled with MS (Agilent technology 5977A MSD). The GC was equipped with a high-polar column DB-WAX (60 m x 0.25 mm x 0.25 µm). The temperature of the oven started at 50°C for 1 min, then ramped at 8°C.min^{-1} to 250°C held for 1 min.
Compounds were tentatively identified by comparing the mass spectra in MassHunter software (version B.08.00, Agilent Technologies) and the retention index relative to C6-C30 n-alkanes, with reference data provided in the NIST library.

b. *a.*

Figure 2. Spodoptera littoralis larvae being placed on pak choi leaves (a) and larvae-induced plants that have been bagged to collect their emitted volatiles for SPME sampling (b)

2.1.3. Smell and taste test

Preparation of samples

For the organoleptic tests, the products were presented in the form of a herbal infusion. Fifty grams of the leaves from each group, i.e., the treatment and control plants, were infused in 1 liter of hot water that was approximately 95°C for 1.5 hours. Subsequently, the resulting infusions were poured in glass bottles and labeled 'A' for the control group and 'B' for the treatment group.

20 participants underwent a taste- and smell test for each vegetable species. The participants were asked to refrain from strongly tasting or smelling foods and beverages 30 minutes before the test. Before and during the test, they were instructed verbally and in writing. To conduct the experiment, we implemented a triangulation test (Marques et al. 2022). For each of the three species, three samples are presented to the assessors at the same time. Two of these samples were the same and one was different. Half of the participants received a combination of two 'A' and one 'B' sample, and the other half received a combination of two 'B' and one 'A'. The samples were provided in wine glasses or cups with a random combination of AAB, ABB, ABA, BAA, BAB and BBA, with A being a non-induced leaf infusion and B an induced leaf infusion. These were numbered 1, 2 and 3 for the assessor. The assessors were asked to determine the odd one out of the three samples and write down their answers on a questionnaire (appendix). Three smell samples of each species were provided first, followed by the taste samples once the participant had completed the smell test. All descriptions, including ones from participants that did not guess the odd one out, were analyzed and included in the results.

The smell samples were presented in blue wine glasses for the participants to fully experience the odor bouquet. The taste samples were presented in blue cups. The use of blue containers was intended to prevent the participants from using visual cues to differentiate the samples.

Figure 3. Prepared leaf infusions that contain non-induced endive, induced endive, induced maize, non-induced maize, induced pak choi and non-induced pak choi from left to right.

Figure 4. Setup of the triangulation test

2.1.4. Statistical analysis

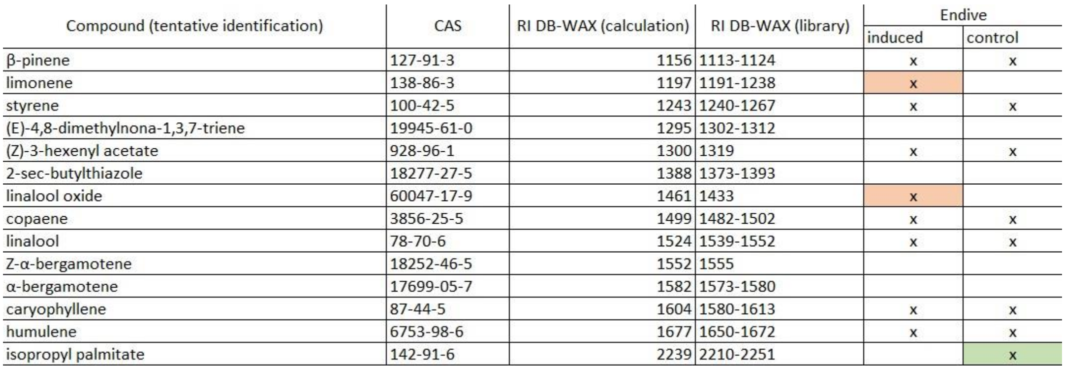
In the triangulation test, the participants were asked to pick the odd one out of the three samples presented to them according to the different combinations. The answers of these tests were categorized as either correctly identifying the odd one out correctly or not. The null hypothesis H_0 is that the volunteer cannot detect any difference in smell or taste. If n subjects participate in the test and X of them correctly identify the odd sample, then under the null hypothesis, X follows a Binomial distribution, $X \sim \text{Binom}(n, 1/3)$. For 20 subjects, H_0 would be rejected at the 5% significance level if 11 or more correct identifications were made. An exact binomial test using the Clopper-Pearson method was applied to the data to calculate the significance of the proportion of correct answers. The calculations were performed using Minitab software (Minitab Inc.)

3. Results

3.1. Volatile emissions

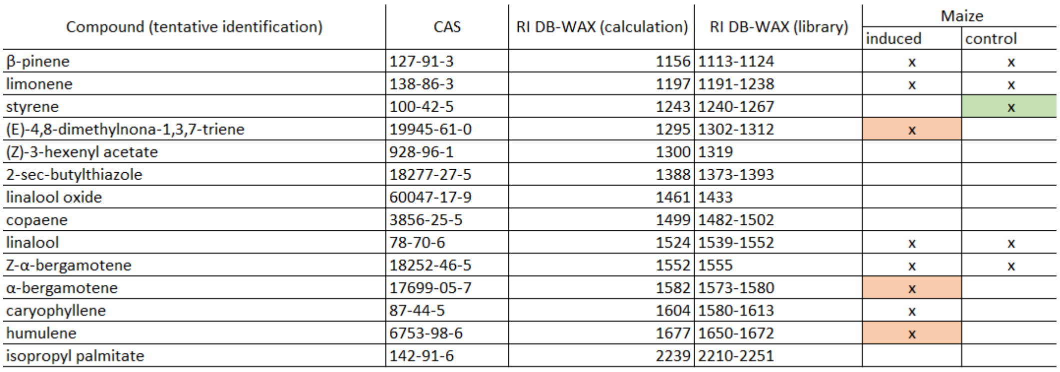
In the headspace of the three species studied, 14 compounds were tentatively identified using GC-MS. Results indicate differences in the volatile composition between induced and non-induced plants for each species (table 1, 2 and 3). Some compounds were common to both induced and non-induced plants, while others were unique to each category. However, for the control group of pak choi, only few compounds were identified, which made comparisons difficult.

Unique compounds identified from the headspace of induced endive were limonene and linalool oxide while isopropyl palmitate is unique for the control samples (table 1). Induced maize emitted three compounds that we were able to identify: (E)-4,8-dimethylnona-1,3,7-triene (DMNT), α-bergamotene and humulene. Of the control samples, styrene was identified (table 2). In the headspace of induced pak choi, 2-sec-butylthiazole and (3Z)-3-hexenyl acetate were identified. The headspace analysis of control samples showed caryophyllene as a unique compound for pak choi (table 3). Volatiles found in the blank sample were removed from the analysis. All identifications are tentative.



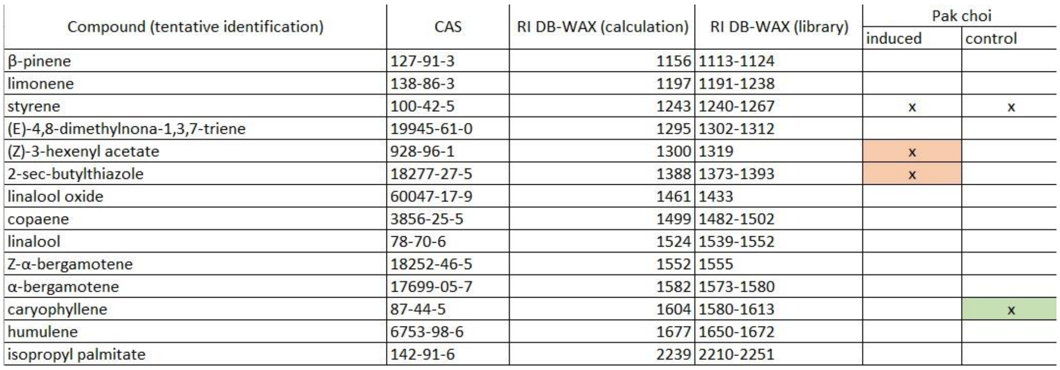
Compound (tentative identification)	CAS	RI DB-WAX (calculation)	RI DB-WAX (library)	Endive	
				induced	control
β-pinene	127-91-3	1156	1113-1124	x	x
limonene	138-86-3	1197	1191-1238	x	
styrene	100-42-5	1243	1240-1267	x	x
(E)-4,8-dimethylnona-1,3,7-triene	19945-61-0	1295	1302-1312		
(Z)-3-hexenyl acetate	928-96-1	1300	1319	x	x
2-sec-butylthiazole	18277-27-5	1388	1373-1393		
linalool oxide	60047-17-9	1461	1433	x	
copaene	3856-25-5	1499	1482-1502	x	x
linalool	78-70-6	1524	1539-1552	x	x
Z-α-bergamotene	18252-46-5	1552	1555		
α-bergamotene	17699-05-7	1582	1573-1580		
caryophyllene	87-44-5	1604	1580-1613	x	x
humulene	6753-98-6	1677	1650-1672	x	x
isopropyl palmitate	142-91-6	2239	2210-2251		x

Table 1. Tentatively identified volatile compounds in endive. Cells marked in orange indicate compounds found only in induced endive (limonene, linalool oxide). The cell marked in green indicates a unique compound found in control samples (isopropyl palmitate)



Compound (tentative identification)	CAS	RI DB-WAX (calculation)	RI DB-WAX (library)	Maize	
				induced	control
β-pinene	127-91-3	1156	1113-1124	x	x
limonene	138-86-3	1197	1191-1238	x	x
styrene	100-42-5	1243	1240-1267		x
(E)-4,8-dimethylnona-1,3,7-triene	19945-61-0	1295	1302-1312	x	
(Z)-3-hexenyl acetate	928-96-1	1300	1319		
2-sec-butylthiazole	18277-27-5	1388	1373-1393		
linalool oxide	60047-17-9	1461	1433		
copaene	3856-25-5	1499	1482-1502		
linalool	78-70-6	1524	1539-1552	x	x
Z-α-bergamotene	18252-46-5	1552	1555	x	x
α-bergamotene	17699-05-7	1582	1573-1580	x	
caryophyllene	87-44-5	1604	1580-1613	x	
humulene	6753-98-6	1677	1650-1672	x	
isopropyl palmitate	142-91-6	2239	2210-2251		

Table 2. Tentatively identified volatile compounds in maize. Cells marked in orange indicate compounds found only in induced samples (DMNT, a-bergamotene, humulene). The cell marked in green indicates a unique compound found in control samples (styrene)



Compound (tentative identification)	CAS	RI DB-WAX (calculation)	RI DB-WAX (library)	Pak choi	
				induced	control
β-pinene	127-91-3	1156	1113-1124		
limonene	138-86-3	1197	1191-1238		
styrene	100-42-5	1243	1240-1267	x	x
(E)-4,8-dimethylnona-1,3,7-triene	19945-61-0	1295	1302-1312		
(Z)-3-hexenyl acetate	928-96-1	1300	1319	x	
2-sec-butylthiazole	18277-27-5	1388	1373-1393	x	
linalool oxide	60047-17-9	1461	1433		
copaene	3856-25-5	1499	1482-1502		
linalool	78-70-6	1524	1539-1552		
Z-α-bergamotene	18252-46-5	1552	1555		
α-bergamotene	17699-05-7	1582	1573-1580		
caryophyllene	87-44-5	1604	1580-1613		x
humulene	6753-98-6	1677	1650-1672		
isopropyl palmitate	142-91-6	2239	2210-2251		

Table 3. Tentatively identified compounds in pak choi. Cells marked in orange indicate compounds found in induced samples ((Z)-3- hexenyl acetate, 2-sex-buthiazole). The cell marked green indicates a unique compound found in control samples (caryophyllene).

3.2. Organoleptic tests

The organoleptic test results of maize were 7 correct answers out of 20 for smell and 2 out of 20 for taste. The p-values were 0.519 and 0.997, respectively. Consequently, the null hypothesis was accepted for both smell and taste, indicating that participants could not distinguish significantly between the induced and non-induced preparations.

Despite this, participants provided descriptive feedback on the infusion samples they believed to be different, whether they guessed correctly or not. These answers were taken into account in order to have an overview of the general descriptions of the smell and taste of the induced and non-induced samples. It was known if the sample they gave a description of was induced or not.

Assessors described the smell of induced maize as more woody, floral, acidic, and generally stronger. In contrast, non-induced samples were noted for having rubbery tones and rancid odors. For the taste test, induced samples were described as more floral, sulfurous, buttery, umami, and savory, while control samples were characterized as more fruity and woody.

Maize	Total answers	Number of correct answers	Correct answers out of total answers (probability)	p-value	95% for lower bound p
Smell	20	7	0.350	0.519	0.177
Taste	20	2	0.100	0.997	0.018

Table 4. Descriptive statistics for maize organoleptic test. The table includes the number of total answers, the number of the correct answers, the percentage of right answers out of total answers and the 95% for lower bound p.

The endive organoleptic test results show a 12 out of 20 and 4 out of 20 correct answers, respectively, for smell and taste. The corresponding p-values were 0.013 and 0.0939. The null-hypothesis is therefore rejected in the endive smell test and accepted in the taste test. This indicates that subjects could discriminate the smell of induced from non-induced endive.

For the smell test, three participants described induced samples to be more acidic, more fruity and weaker. The control samples were described as fruitier and more sulfurous. For the taste test, induced samples were described as more acidic, woody, sweet and sulfurous. Control samples were described as tasting stronger, more savory and more umami.

Endive	Total answers	Number of correct answers	Correct answers out of total answers (probability)	p-value	95% for lower bound p
Smell	20	12	0.600	0.013	0.177
Taste	20	4	0.200	0.939	0.018

Table 5. Descriptive statistics for the endive organoleptic test. The table includes the number of total answers, the number of the correct answers, the percentage of right answers out of total answers and the 95% for lower bound p.

The pak choi organoleptic tests resulted in 9 correct answers out of 20 for smell and 3 correct answers out of 20 for taste. The p-values were 0.190 for smell and 0.939 for taste, the null hypothesis is accepted for both tests, indicating that subjects were not able to differentiate induced and non-induced by taste or smell.

Induced samples were described as having a stronger cabbage smell, being more sulfurous, and less fruity and floral. In the taste test, induced samples were described as more fruity, sulfurous, acidic, sweet, umami, and savory, but slightly weaker overall.

Pak choi	Total answers	Number of correct answers	Correct answers out of total answers (probability)	p-value	95% for lower bound p
Smell	20	9	0.259	0.45000	0.190
Taste	20	3	0.042	0.150000	0.939

Table 6. Descriptive statistics for pak choi organoleptic test. The table includes the number of total answers, the number of the correct answers, the percentage of right answers out of total answers and the 95% for lower bound p.

4. Discussion

The results of this study provide interesting findings on the effect of herbivory on the volatile profile and organoleptic qualities of endive, maize and pak choi. We observed that herbivory-induced plants present different volatile emissions compared to the non-induced. However, the organoleptic tests indicated no statistically significant differences in human perception of smell and taste between non-induced and induced plants, except for the smell of endive.

4.1. Volatile analysis

GC-MS analysis results show that there are differences between the volatile composition of the induced and non-induced plants of the different species used in this experiment. Collected volatiles consist of β-pinene, limonene, copaene, linalool, α-bergamotene and Z-α-bergamotene, caryophyllene, humulene, styrene, (E)-4,8-dimethylnona-1,3,7-triene (DMNT), (Z)-3-hexenyl acetate, 2-sec-butylthiazole, linalool oxide and isopropyl palmitate.

4.1.1. Endive

In the headspace of induced endive, limonene and copaene were found as unique compounds, while isopropyl palmitate was found in the headspace of control sample. These results are intriguing since not much research, if any at all, has been done on this species. Limonene has been discussed as being released 24h after herbivory as a response to herbivore attacks in plants in general (War et al. 2011). Results in this study support those findings. Copaene is another compound that has been found to be emitted by maize and desmodium plants (Erdei et al. 2024). *Psidium salutare* fruits emit isopropyl palmitate according to Pino et al. (2002), but further research on this compound in relation to plants has been hard to find. The limited research on endive makes it complicated to make any further conclusions.

4.1.2. Maize

Induced maize samples emitted (E)-4,8-dimethylnona-1,3,7-triene (DMNT), α-bergamotene and humulene as unique compounds. Control maize samples emitted styrene.

α-bergamotene has previously been found 4-5 hours after herbivory in herbivore induced maize seedlings (Turlings et al. 1998). Along with this, caryophyllene and humulene were found, which also are compounds that have been discussed to only be present for a short while after herbivory since they are not systematically synthesized afterwards (Turlings et al. 1998). Turlings et al. (1995) and Ramadan et al. (2011) found that DMNT, besides other terpenes, also was emitted by maize after a herbivore attack.

4.1.3. Pak choi

Induced pak choi emitted (3Z)-3-hexenyl acetate and 2-sec-butylthiazole as unique compounds, and control samples caryophyllene. (3Z)-3-Hexenyl acetate is a green leaf volatile that is found to be emitted after herbivore damage by several different plants (Loughrin et al. 1994; Frontini et al. 2022; Riahi et al. 2022). This same compound was also found in non-induced pak choi by Liu et al. (2018), raising the question whether this really is a HIPV or just a VOC that is always emitted by this plant that did not get absorbed by the SPME fiber during the volatile collections of control samples. Caryophyllene is typically, as mentioned earlier, a compound that is released shortly after herbivory.

All these results are presented cautiously since only few repetitions were used for the sampling, making it hard to make any confident statements based on them. However, they can provide a tentative insight into how herbivore induced plants and non-induced plants differ in their volatile emissions.

4.2. Organoleptic test results

Organoleptic tests revealed significant differences in the smell of induced endive, while no significant differences were detected for maize and pak choi.

4.2.1. Endive

Volunteers perceived a significant difference in smell, while no statistically significant difference in taste was experienced. The difference in smell is very interesting and shows that humans can sense differences in volatiles emitted by herbivore induced endive plants. The absence of research in this area makes it hard to confirm these findings, but it will hopefully inspire further exploration of this phenomenon.

Participants described the smell of induced endive as more acidic and fruitier, while the control samples were noted to have more sulfurous notes. Although the taste tests did not show significant differences, the feedback from participants suggested potential changes in flavor profiles, such as stronger woody and umami characteristics in the induced samples. These results indicate that there might be a link between flavor and herbivory. A potential utilization of this mechanism could be an increased influence over the taste of vegetables. Using herbivory to affect aroma profiles is used in the cultivation of tea and wine (Cai et al. 2014; Mditshwa et al. 2017) and could possibly be an interesting practice for vegetables as well. For this, however, more research is necessary.

4.2.2. Maize

An unexpected result is the lack of significant sensory differences between induced and non-induced maize plants, contrasting previous research (Nihlmar 2023). In the first days following induction, a noticeable difference in smell was noted in the headspace of the induced and non-induced plants while handling them. This observation, combined with established research on HIPVs in maize, built up to the expectation that induced and non-induced maize would show a greater difference in the organoleptic test. One possible explanation could be abiotic stresses. The temperature was not entirely controlled throughout this experiment, leading to fluctuation on sunny days which caused drying out of the plant substrates. This may have led to drought induced responses that altered the plants' secondary metabolites (Chávez-Arias et al. 2022), potentially affecting the organoleptic properties. Another possible factor influencing the results could be how humans perceive taste and smell. The are many variables that go into this, for example, differences in human physiology, sex, age and genes might contribute to our ability to sense taste and smell, making it complex to test (Zhang & Firestein 2007; Demattè et al. 2011; Sorokowski et al. 2019).

4.2.3. Pak choi

The absence of significant differences in taste between herbivore induced and non-induced pak choi was also somewhat unexpected. This because induced and non- induced plants emitted smelled differently while handling them, which created an expectation that there would be a greater difference between the different taste and and smell samples in the organoleptic test. It is possible that the same abiotic stresses as maize were present in pak choi, potentially masking the difference in taste and smell caused by herbivory.

4.3. Conclusions

To conclude, this study set out to determine whether herbivory induced changes in the volatile emissions of endive, maize and pak choi and if these changes can be perceived by humans. Volatile profiles were found to differ between induced and non-induced plants, and the smell of endive was significantly affected.

For further research, I suggest increasing the sample size of the volatile collection and the organoleptic tests for more reliable results. Additionally, experimenting with the duration of induction of the larvae is needed to optimize the methodology. Standardizing plant care measures, possibly using heat and moisture sensors combined with an automated irrigation system could minimize the impact of abiotic stressors. The method of collecting the plant volatiles is another area of improvement. I used SPME in this study, which worked well for the short in which the study was carried out. However, to improve the volatile collection, I suggest trying out another technique such as using volatile traps (filled with porapak Q adsorbent) together with SPME together or instead of SPME. Doing this will take more time but give more reliable results. Using two different GCMS columns would also be beneficial. A polar column (DB-WAX) was used in this study, but using a non-polar column as well will help to identify more compounds found in the plant headspace.

It is also important to note that only leaves were used in both volatile and organoleptic tests. Since maize leaves are not so commonly eaten, investigating the differences in the taste of maize kernels would make the research more relevant to farmers and consumers. Finally, presenting samples in the form of infusion may not fully capture the sensory experience of vegetables. Sampling actual leaves or maize kernels could provide a more accurate assessment of herbivore-induced changes in taste, smell and overall experience.

HIPVs and organoleptic properties were two variables highlighted in this study, and there is likely much more to be discovered. Research is providing a greater confidence that herbivory does change volatile emissions in plants, and the results of the organoleptic test in this study might suggest that there is more to be discovered about the human perception of these changes. Further investigations can hopefully be a driver to change in consumer opinions where currently only flawless is good enough. Broadening the view to see that herbivores are not inherently bad and that they could even offer direct utilizations to the quality of the food that is produced such as increased health benefits, and possibly also an increased hedonic value.

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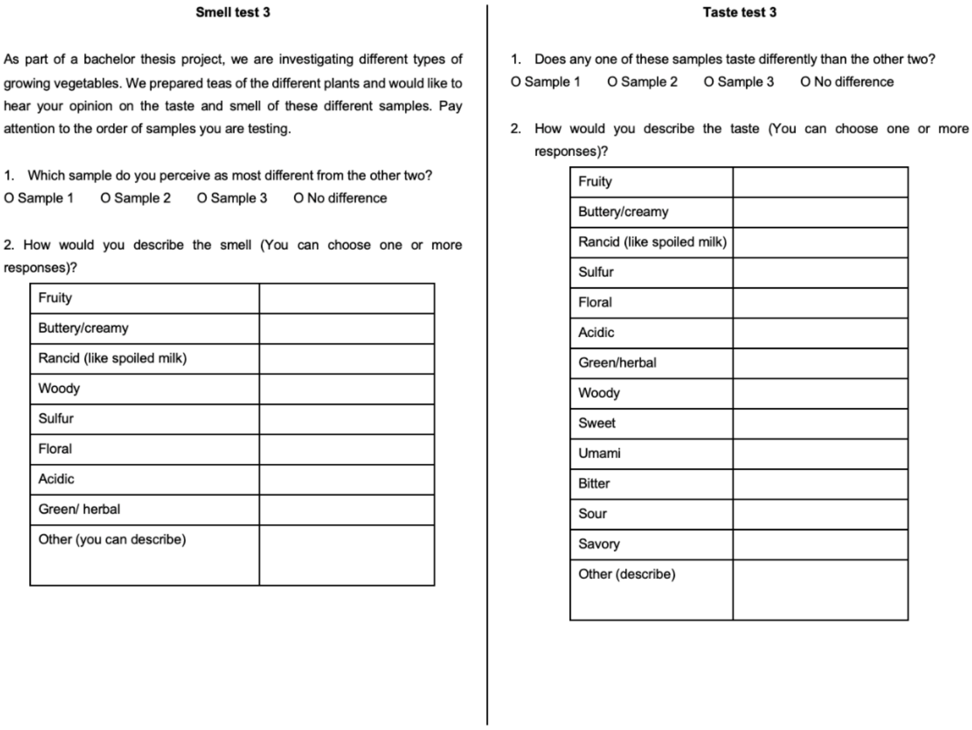
Most of all, I want to give my greatest thanks to God who is my source of strength, comfort and hope. He has provided for me in all things and I will give Him all due glory and honour.

Appendix 1

Questionnaire of the organoleptic triangulation test



Smell test 3

As part of a bachelor thesis project, we are investigating different types of growing vegetables. We prepared teas of the different plants and would like to hear your opinion on the taste and smell of these different samples. Pay attention to the order of samples you are testing.

1. Which sample do you perceive as most different from the other two?

O Sample 1 O Sample 2 O Sample 3 O No difference

2. How would you describe the smell (You can choose one or more responses)?

Fruity	
Buttery/creamy	
Rancid (like spoiled milk)	
Woody	
Sulfur	
Floral	
Acidic	
Green/ herbal	
Other (you can describe)	

Taste test 3

1. Does any one of these samples taste differently than the other two?

O Sample 1 O Sample 2 O Sample 3 O No difference

2. How would you describe the taste (You can choose one or more responses)?

Fruity	
Buttery/creamy	
Rancid (like spoiled milk)	
Sulfur	
Floral	
Acidic	
Green/herbal	
Woody	
Sweet	
Umami	
Bitter	
Sour	
Savory	
Other (describe)	