



# Investigating the role of UV-light in volatile pheromone production of *Drosophila melanogaster*

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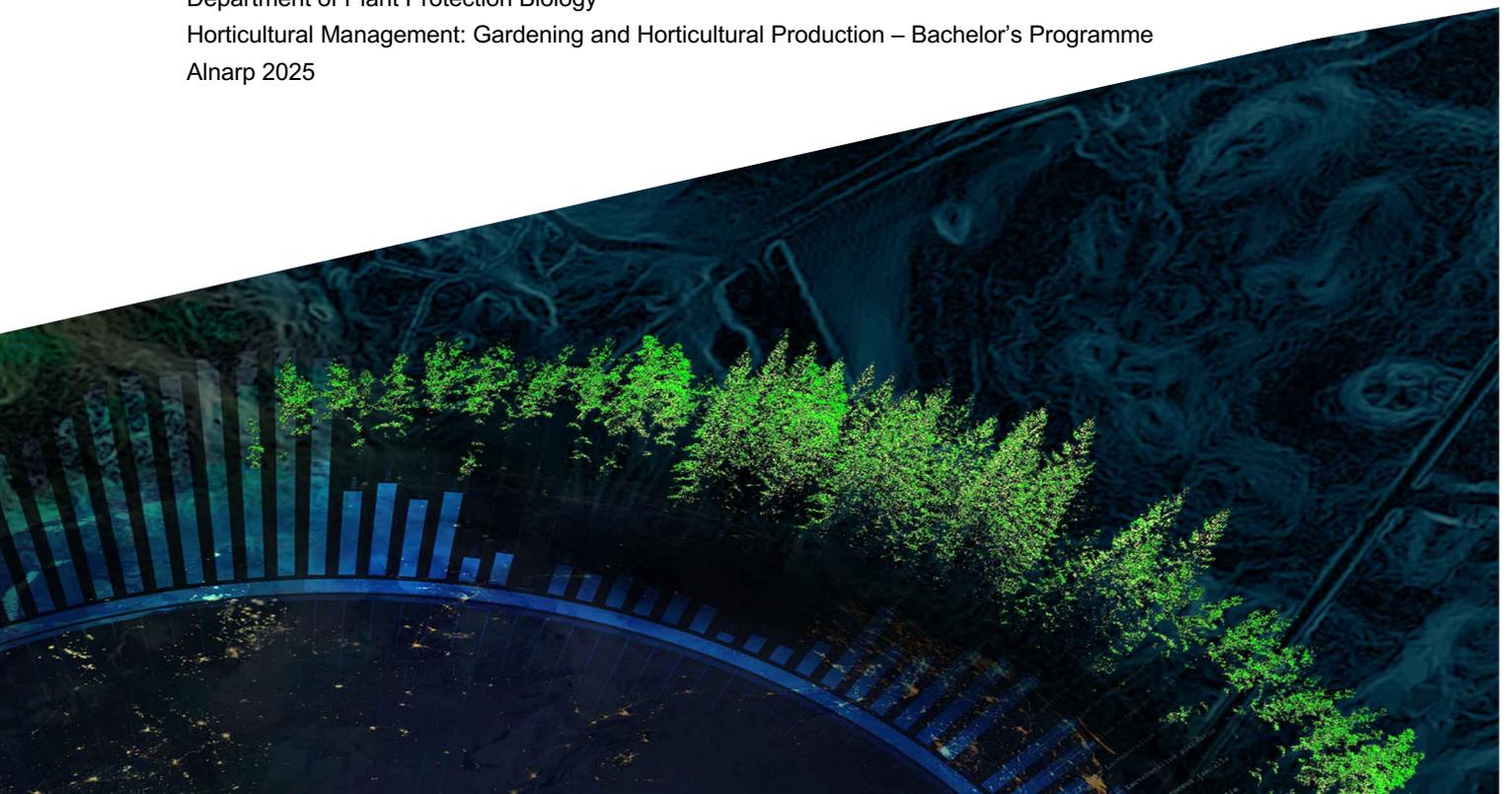
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Swedish University of Agricultural Sciences, SLU

Department of Plant Protection Biology

Horticultural Management: Gardening and Horticultural Production – Bachelor's Programme

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# Investigating the role of UV-light in volatile pheromone production of *Drosophila melanogaster*

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## Abstract

Insects produce an epicuticle comprised of a complex mixture of hydrocarbons and closely related chemical compounds, originally evolved as a mechanism to prevent desiccation but which developed an additional role as a form of chemical fingerprint unique to the species in question. This is useful as both intra- and interspecific communication, however the low volatility of these hydrocarbons permits only short-range communication. One cuticular hydrocarbon produced by *Drosophila melanogaster* females is (Z,Z)-7,11-heptacosadiene, may be spontaneously oxidized at its double bonds. One possible oxidation product is (Z)-4-undecenal, which has recently been shown to function as a long-range pheromone owing to its higher volatility as compared to its hydrocarbon precursor. The aim of this study is to investigate the effects on (Z)-4-undecenal production by ultraviolet light.

This involves investigating different sampling durations of (Z)-4-undecenal, as well as irradiating both synthetic (Z,Z)-7,11-heptacosadiene and live *D. melanogaster* females. Headspace collection is done through solid phase microextraction, and subsequent analysis with gas chromatography-mass spectrometry.

Due to the variability in the results, it was not possible to verify the hypothesis that ultraviolet light increases production of (Z)-4-undecenal in *D. melanogaster*, however the study did provide insights as to which experimental parameters could be modified in future research. Some suggestions are provided.

*Keywords:* *Drosophila melanogaster*, cuticular hydrocarbons, (Z)-4-undecenal, UV, VOC, pheromones, semiochemicals, SPME, GC-MS

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## Abbreviations

7,11-HD	( <i>Z,Z</i> )-7,11-heptacosadiene
CAR	Carboxen
CHC	Cuticular hydrocarbon
cVA	( <i>Z</i> )-11-octadecenyl acetate
DVB	Divinylbenzene
GC	Gas chromatography
MS	Mass spectrometry
PDMS	Polydimethylsiloxane
Q-TOF	Quadrupole time-of-flight mass spectrometer
SPME	Solid phase microextraction
UV	Ultraviolet
UV-A	Ultraviolet-A
WLS-UV	White light-supplemented ultraviolet [lamp]
Z4-11Al	( <i>Z</i> )-4-undecenal

# 1. Introduction

## 1.1 Chemical communication

As a member of the human species, one can perhaps be forgiven for considering sound and sight the foundations of communication; most of us likely think of speech and writing as soon as having registered the word. However, the senses associated with these forms of communication – hearing and seeing – arise from specialized structures containing photoreceptors or systems capable of registering and reporting acoustic waves, and are useful in specific contexts. Something which is common to all organisms, entailed by our shared core projects of sustaining our own existence, is the production of waste. Waste, resulting from assimilation of energy and nutrients from our environments, excreted or otherwise expunged from our respective biochemical systems, can become the basis for both intra- and interspecific communication (Steiger, 2010). As soon as anything was alive, there was a reason to detect the chemical footprints of other organisms: either to stay away, or to give chase.

Chemical communication, thus arguably a much more fundamental form of communication occurring in animals, plants, fungi, and bacteria (Wyatt, 2014, p. 4; Bradbury and Vehrencamp, 1998, p. 279), is conducted through the detection of semiochemicals. Semiochemicals can be further divided into four categories: pheromones, allomones, kairomones, and synomones. Peter Karlson and Martin Lüscher coined the term *pheromone* in 1959 from the Greek words *pherein* ("to carry") and *hormon* ("to excite") and described them as biologically active substances which serve as means of communication between individuals (Karlson and Lüscher, 1959). For more than half a century, semiochemicals have been of interest in pest management due to their potential use in monitor traps and mating disruption (Karlson and Butenandt, 1959). Today, *pheromone* specifically entails a compound with the purpose of intraspecific communication. *Allomones* are compounds which are produced by members of one species to influence the behavior of members of another species, to the benefit of the producing species (Brown, 1968). *Kairomones* are compounds which are produced by members of one species and, when intercepted by members of another species, provides a benefit to the intercepting species (Brown *et al.*, 1970). A *synomone* is a compound which is produced by members of one species, intercepted by members of another species, to the benefit of both species (Tan and Nishida, 2000), and can be described

as an allomone from the perspective of the producing species, and a kairomone from the perspective of the intercepting species.

It should be noted that many semiochemicals are not by-products of other biological processes but are rather deliberately produced for such purposes (Karlson and Butenandt, 1959).

## 1.2 Cuticular hydrocarbons

The epicuticle of insects consists of a complex mixture of hydrocarbons, methyl esters, long chain-fatty acids, aliphatic alcohols, aldehydes, and ketones (Blomquist and Ginzl, 2021), and is responsible for the low water permeability of the insect cuticle and thus a mechanism to prevent desiccation (Hadley, 1981). Linear *n*-alkanes are ubiquitous in insects, and as their chain length increases, van der Waals forces strengthen to produce better barriers to water loss (Gibbs, 1998; Blomquist and Ginzl, 2021). While *n*-alkanes with a carbon number of 21 or more have a relatively high melting point of 40 °C and above, increasing with chain length by 1-3 °C per additional methylene unit (Gibbs and Pomonis, 1995), the melting points of their methyl-branched counterparts are lower. Shifting the methyl moiety from terminal positions to more medial positions may decrease the melting temperature by more than 30 °C, and addition of a second methyl branch lowers it further. A monounsaturated alkene can have a melting point decreased by more than 50 °C compared to its alkane counterpart. Insects commonly produce a cuticular hydrocarbon (CHC) profile which is biphasic – consisting of both melted and solid compounds – in their natural environments; it has been hypothesized that while a biphasic wax layer offers less waterproofing at any given point on the cuticle as compared to a solid wax layer, it is capable of spreading more evenly over the cuticle to provide an overall better desiccation protection over the entire surface area of the insect (Menzel *et al.*, 2019).

The purpose of the wax layer of insects is not limited to water retention but also comprises a form of chemical signature which can be remarkably complex and range from a few component compounds to more than a hundred (Blomquist and Ginzl, 2021). In this context, CHCs can function as short-range semiochemicals mediating a multitude of interactions in both solitary and social insects (Leonhardt *et al.*, 2016). In the former case, they may mediate mate recognition, courtship, and mate choice (Ferveur, 2005), while in the latter case may serve the function of task-specific signals, fertility cues, and caste, sex, and nest mate recognition pheromones (Lahav *et al.*, 1999; Leonhardt *et al.*, 2016).

As has been found in both the parasitoid wasp *Macrocentrus cingulum* and the Asian long-horned beetle *Anoplophora glabripennis*, desaturated hydrocarbons may be spontaneously oxidized at their double bonds and form aldehydes which are

far more volatile than their CHC precursors, and thus serve as long-range pheromones (Swedenborg and Jones, 1992; Wickham *et al.*, 2012). Factors affecting the rate of autoxidation include heat, metal ions, and light (Ahmed *et al.*, 2016).

Recalling waste products having the potential to become kairomones, the highly specific epicuticular profiles of insects have met a similar fate. As they create a form of species-specific fingerprint, other species have evolved behaviors modulated by certain CHCs or blends thereof. For example, two CHCs found on the predatory backswimmer *Notonecta maculate* which deter the mosquito *Culiseta longioreolata* and repels its oviposition (Silberbush *et al.*, 2010). Conversely, a number of hymenopteran predators and parasitoids detect species-specific CHCs or CHC blends and use these to locate prey (Binz *et al.*, 2016; Koedam *et al.*, 2010; Ranganathan *et al.*, 2015; Rutledge *et al.*, 2014).

Hydrocarbon biosynthesis takes place in secretory cells called oenocytes found in most pterygote insects (Gu *et al.*, 1995, Makki *et al.*, 2014), specifically on the cytoplasmic side of the cell membrane (Kefi *et al.*, 2019) which eliminates the need for cytoplasmic transport proteins (Blomquist and Ginzl, 2021). Newly produced hydrocarbons are then transported by lipophorin through the hemolymph (Gu *et al.*, 1995), although exactly how they are then moved across the cuticle is not yet known (Blomquist and Ginzl, 2021).

### 1.2.1 *Drosophila melanogaster*

*D. melanogaster* has been used in experiments since the beginning of the 20th century, with the ease by which a colony could be kept and its rapid generational cycles proving convenient (Carpenter, 1905). It has been used as a model organism in genetics since 1910, and has been proposed to have potential as the same within cancer research, degenerative brain diseases, and nanotoxicity (Morgan, 1910; Jeibmann and Paulus, 2009; Prüßing *et al.*, 2013; Ong *et al.*, 2014; Mirzoyan *et al.*, 2019).

Like many other species of *Drosophila*, *D. melanogaster* displays sexual dimorphism in its CHC composition (Khallaf *et al.*, 2021). (*Z*)-11-octadecenyl acetate (*cis*-vaccenyl acetate, cVA) is a male-produced pheromone which modulates oviposition behaviors, sexual receptivity in virgin females, induces aggression in females, and after having been transferred from male to female during mating, reduces the female's attractiveness to other males (Vander Meer *et al.*, 1986; Ha and Smith, 2006; Wang and Anderson, 2009; Ejima, 2015; Duménil *et al.*, 2016).

On the female side, one CHC of particular interest is (*Z,Z*)-7,11-heptacosadiene (7,11-HD, fig. 1), which elicits male courtship behavior. Owing to its low volatility, it is detected by gustatory sensory neurons of the male and thus only effective over very short distances (Thistle *et al.*, 2012; Toda *et al.*, 2012). Autoxidation of 7,11-

HD produces two of four possible aldehydes, depending on which double bond is broken: heptanal and (Z)-4-icosenal, or (Z)-4-undecenal (Z4-11Al, fig. 1) and hexadecanal. Of these autoxidation products, Z4-11Al has been shown to mediate both flight attraction and courtship behavior in *D. melanogaster*, while producing no response in the close relative *D. simulans* (Lebreton *et al.*, 2017).

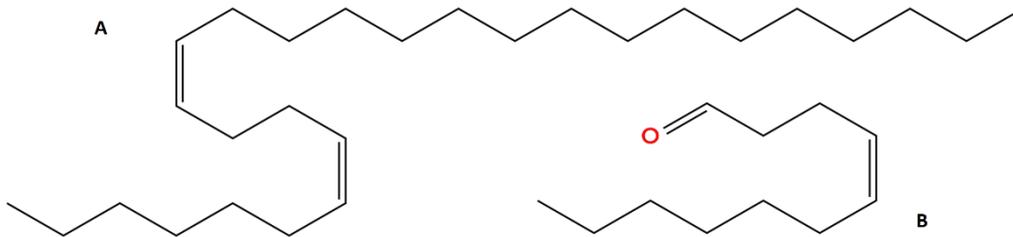


Figure 1. A) Skeletal molecular structure of (Z,Z)-7,11-heptacosadiene, a cuticular hydrocarbon produced by female *D. melanogaster*. B) Skeletal molecular structure of (Z)-4-undecenal, one of the volatile aldehydes formed from autoxidation of (Z,Z)-7,11-heptacosadiene.

### 1.3 Aims and limitations

The experimental aim of this project was threefold:

The first aim was to investigate the volatile collection of the CHC-oxidation product Z4-11Al using three different sampling durations with SPME, and comparing the amount of Z4-11Al collected.

The second aim was to establish if artificial UV-A irradiation can induce oxidation of synthetic 7,11-HD resulting in elevated production of Z4-11Al.

The third aim was to test if the production of Z4-11Al in *D. melanogaster* can be elicited by UV-A and white light exposure by comparing the production of Z4-11Al in live insects exposed to:

- a) UV-A and ambient indoor light,
- b) High intensity white light including UV-A and ambient indoor light,
- c) Ambient indoor light only.

## 2. Material and methods

### 2.1 Insect rearing

*D. melanogaster* (Dalby-HL strain from Dalby, Sweden) were reared on a standard sugar-yeast-cornmeal diet (Appendix I) and kept at room temperature (19-22 ° C) at a natural photoperiod from the 15th of April to the 12th of May 2023. They were kept in 30 ml Plexiglas vials with fresh food, and newly eclosed flies were anesthetized with CO<sub>2</sub> and sexed under an SZX7 microscope (Olympus Corporation, Hachioji, Tokyo, Japan). Virgin flies were identified by the presence of meconium and kept with flies of the same sex eclosed on the same day. Virgin females 3-5 days old were used in the experiments.

### 2.2 Chemicals

Z4-11A1 (a gift from E. Wallin, Mid Sweden University) was diluted in ethanol to a concentration of 1 µg/µl. 7,11-HD (Cayman Chemical Company, Ann Harbor, Michigan, USA) was diluted in hexane to a concentration of 10 ng/µl.

### 2.3 Irradiation experiment

UV treatments were carried out with two different lamps: one UV-only lamp (36-4746 Clas Ohlson, Insjön, Dalarna, Sweden, wavelength 315-380 nm, average 365 nm) at 30 cm distance between bulb and surface, and one Ultra-Vitalux® natural sunlight imitation lamp (Osram, Munich, Germany) at 77 cm distance from the bulb to the surface. The glass vials used were cleaned with acetone and subsequently kept at 400 °C overnight (8 hours) before each use.

Each UV exposure involving live insects used a set of 60 virgin females with three replicates for each treatment. The insects were transferred to empty 30 ml Plexiglas vials 30 minutes before treatment and subsequently transferred to 50 ml glass vials and kept from escaping by two layers of polyvinyl chloride-coated fiberglass mesh. To allow light to enter the vials directly and interact with the glass as little as possible, the vials were placed below the lamp at a straight angle.

Triplicates of empty glass vials were also performed using the Ultra-Vitalux® lamp as described above.

Four sets of 60 virgin females were given an ambient treatment using the method described above, but with only the ambient light of the room.

7,11-HD was applied to 1x1 cm filter paper in 50 ml glass vials and subjected to 1 hour exposure of the Ultra-Vitalux® lamp at 77 cm distance (without mesh).

## 2.4 Volatile collection

After exposure, the fiberglass mesh was removed and replaced with aluminum foil through which a Supelco® (Merck, Darmstadt, Germany) solid phase microextraction (SPME) fiber with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) coating was inserted, conditioned beforehand for 5 minutes at 250 °C.

Z4-11Al was sampled at three different durations: 30 minutes, 1 hour, and 2 hours. Sampling of 7,11-HD and live insect headspace used a 2 hour duration for each replicate.

## 2.5 Gas Chromatography – Mass Spectrometry

Most samples were analysed using combined gas chromatograph and mass selective detector (7890B GC System and 5977A MSD, Agilent technologies, Inc., Santa Clara, CA, USA). The samples were desorbed from the SPME fiber at 225 °C inlet temperature over 60 seconds into a DB-wax capillary column (60 m × 0.25 mm, df = 0.25 µm), with a temperature increase from 40 °C to 225 °C at 8 °C/min, and a final hold time of 10 minutes. Helium was used as mobile phase at 35.182 cm/s, and the MS scanned from m/z 29 to 400 at 5 Hz. The ion source was kept at 250 °C, and the quadrupole mass detector operated at 150 °C and 70 eV.

One ambient light sample was run on a combined GC-MS (7890B GC System and 7250 Q-TOF MS, Agilent technologies, Inc., Santa Clara, CA, USA) The sample was desorbed from the SPME fiber at 225 °C inlet temperature over 60 seconds into a fused silica HP-5ms Ultra Inert capillary column (60 m × 0.25 mm, df = 0.25 µm), with a temperature increase from 40 °C to 280 °C at 5 °C/min, and a final hold time of 12 minutes. Helium was used as mobile phase at 34.971 cm/s, and the MS scanned from m/z 29 to 500 at 5 Hz. The ion source was kept at 200 °C, and the quadrupole mass detector operated at 150 °C and 70 eV.

Four replicates of UV-treated 7,11-HD were sampled with SPME and run on the Q-TOF in the same manner as the ambient run, however with a 40 °C initial temperature and 8 °C/min ramp, to a maximum temperature of 225 °C and a hold time of 10 minutes.

A solvent delay of 7.5 minutes was used for samples in ethanol.

### 2.5.1 Data analysis

The volatile data acquired using GC-MS was analysed using Agilent MassHunter Qualitative Analysis Navigator B.08.00 and NIST MS Search 2.4 (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Confirmation of Z4-11A1 and 7,11-HD was verified by the coinjection of synthetic authentic standards.

Calculation of relative peak area followed the formula:

$$A_{rel} = 100 \frac{A_i}{A_{tot}}$$

$A_i$  = area of a given peak

$A_{tot}$  = total area of all peaks above 25 000 total ion count in the chromatogram. This number was chosen as a cutoff point below which the signal-to-noise ratio of such peaks would make them unreliably distinguished from the background.

Welch's t-test was performed by R in RStudio (v. 4.3.1) with the base R function `t.test(group1, group2, var.equal=FALSE)`, following the formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

$\bar{x}_i$  = sample mean of group  $i$

$s_i$  = sample standard variation of group  $i$

$n_i$  = sample size of group  $i$

## 3. Results

### 3.1 Headspace collection duration

Z4-11Al was found in all replicates of all three sampling durations. In the 30-minute sampling replicates, it is present as 0.6%, 2.1%, 5.6%, and 6.0% of total peak area ( $p = 0.037$ ), shown in Figure 2. In the one-hour sampling replicates, it is present as 3.1%, 6.3%, 7.7%, and 20% of total peak area ( $p = 0.042$ ). In the two-hour sampling replicates, it is present as 18%, 24%, 28%, and 41% of total peak area ( $p = 0.006$ ).

Figure 3 illustrates Z4-11Al peaks in three chromatograms, one from each sampling duration, overlaid on top of each other.

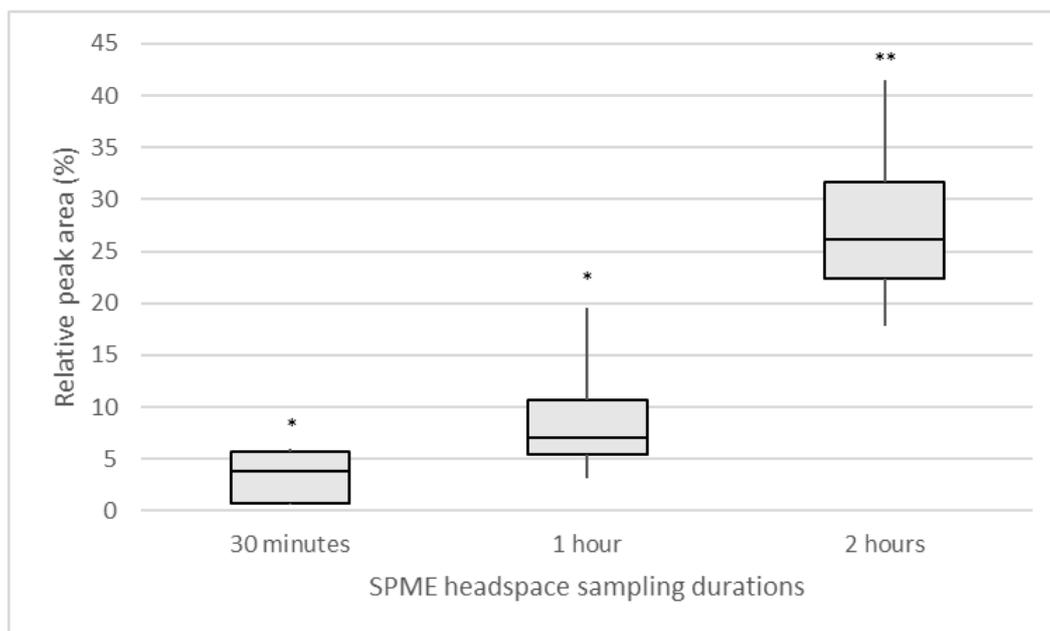


Figure 2. Relative peak area of Z4-11Al as percentages of total chromatogram peak area from the three different sampling times. \* =  $p \leq 0.05$ , \*\* =  $p \leq 0.01$

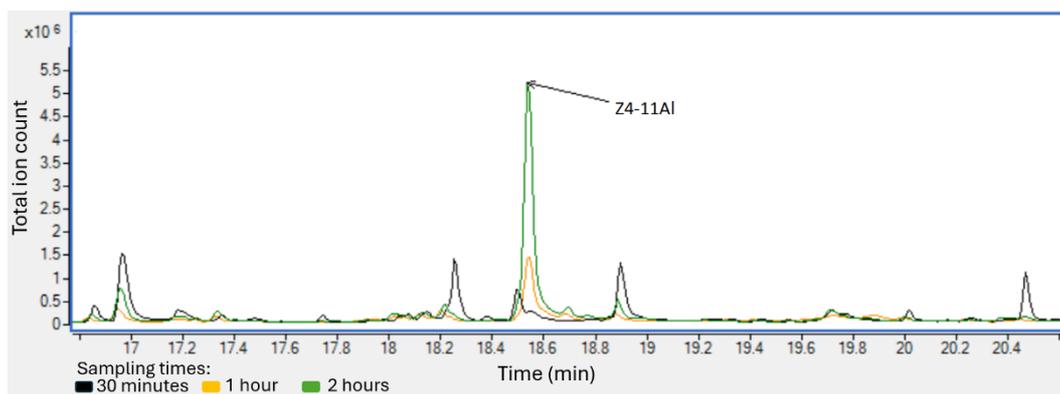


Figure 3. Superimposed sections of three chromatograms from three different headspace sampling duration (polar DB-WAX column). The one-hour and two-hour replicates show clear Z4-11Al peaks at ca 18.55 minutes, while the Z4-11Al peak of the 30-minute replicate appears as a small shoulder to a peak of a different compound at 18.50 minutes.

### 3.2 Oxidation of synthetic 7,11-HD

UV exposure of synthetic 7,11-HD produced mixed results on the polar DB-WAX column; Z4-11Al eluted as small peaks in two replicates (1% and 5% of total peak area, respectively), and the third replicate shows a small peak at the expected retention time but barely distinguishable from background.

On the non-polar HP5-ms column, Z4-11Al appeared in three out of four replicates: less than 1% total peak area in two cases, about 2% in the third (Fig. 4).

Several peaks which are not present in the blank control replicates are present in the chromatograms of UV-treated 7,11-HD.

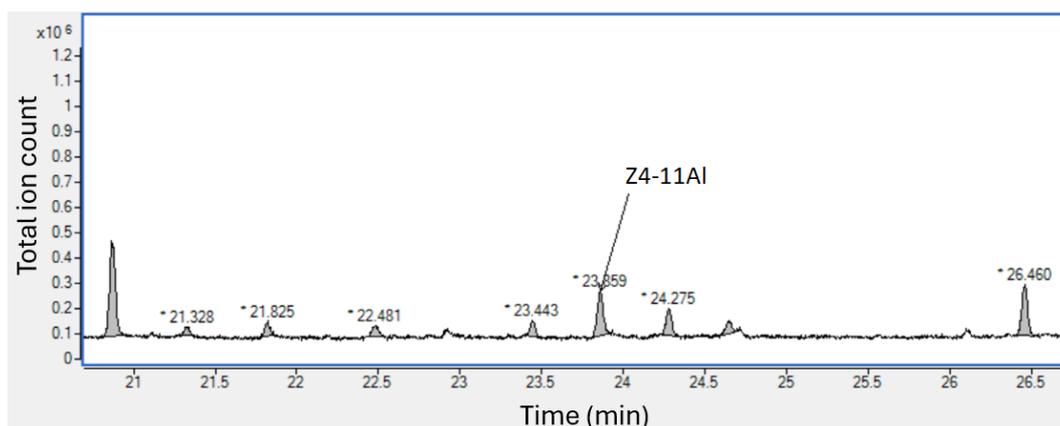


Figure 4. Section of a chromatogram from the polar HP-5ms Q-TOF, with peaks highlighted. Z4-11Al elutes at ca 23.86 minutes.

### 3.3 Irradiation of live insects

Z4-11Al appeared as a clearly distinguishable peak in one replicate of UV-only lamp treatment at 16% of total peak area, and may be present in the second replicate as a shoulder to a larger peak. There is a small but discernible peak in the third replicate, next to another peak of comparable size, at less than 1% of total peak area (illustrated in Figure 5, along with the other treatments as run on the polar column).

In the white-light supplemented UV (WLS-UV) lamp treatment replicates, Z4-11Al appeared as 5% of the total peak area in two out of three replicates. However, it was not distinguishable from the background in the third replicate.

As for the ambient treatment, Z4-11Al appeared in each replicate. In two replicates run on the polar DB-WAX column it appears as small peaks less than 1% in total peak area, and in the third closer to 5%. In the single ambient run on the non-polar HP-5ms column, Z4-11Al eluted at less than 1% of total peak area.

Neither treatment was found to be significantly different in the relative area of Z4-11Al as compared to the ambient control treatments when using Welch's test ( $p > 0.05$ ).

Z4-11Al was not detected in any of the UV-exposed empty glass vial control replicates.

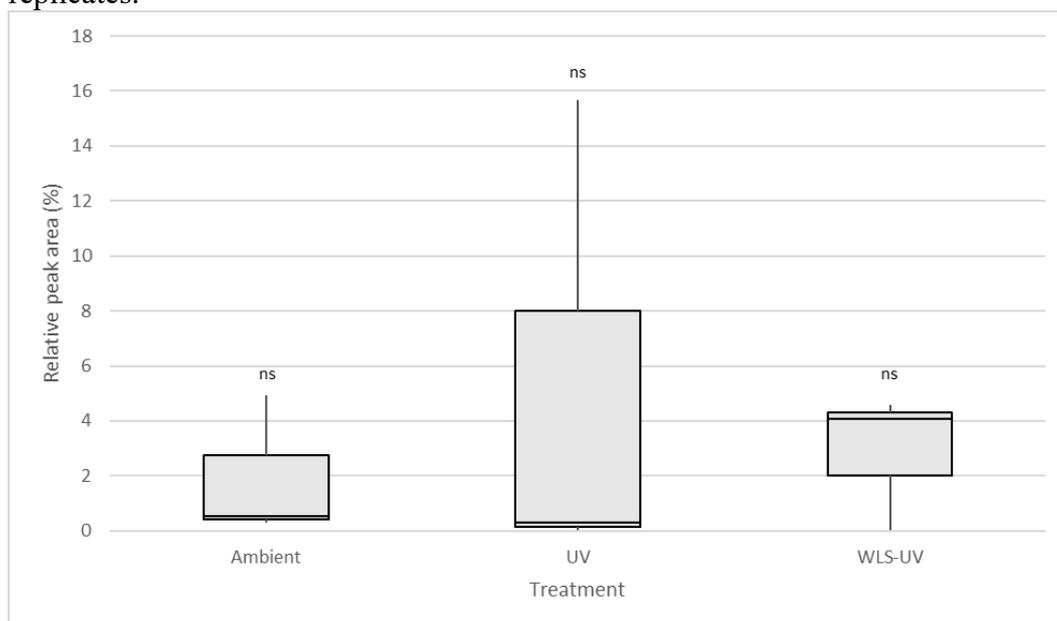


Figure 5. Relative peak area of Z4-11Al in percentages of total peak area of chromatograms, as compared between three different treatments.  $ns = p > 0.05$

## 4. Discussion

### 4.1 SPME sampling times

The different SPME sampling times of Z4-11Al suggested a two-hour duration to be preferable (out of the three durations investigated), however it should be noted that this was a sampling of synthetic Z4-11Al alone. It is possible that the volatile is not preferentially adsorbed in competition with other volatiles readily released from the UV-treated synthetic 7,11-HD or the live insects. One could evaluate different sampling times of the different treatments of both the hydrocarbon and the live flies, although due to time constraints this was outside the scope of this project.

A drawback of SPME is the appearance of many peaks in the chromatograms that originate from the adsorbent coating of the SPME fibers. These can be excluded by cross-referencing them with empty control runs, where they also appear, or by identification given the fact that they are not volatile compounds that occur in the emissions of living organisms. However, they still run the risk of overlapping with peaks of collected volatile organic compounds with the same retention time, which in turn can be hard to identify. Using SPME fibers of different composition and comparing these could mitigate this issue.

### 4.2 Oxidation products of 7,11-HD

It is unclear what effect the UV had on the production of Z4-11Al: that it would have no effect is unlikely given the documented sensitivity of unsaturated CHCs to oxidative agents such as ozone and UV-light (Hatano *et al.*, 2020; Jiang *et al.*, 2023). More likely is the possibility of over-oxidation, where Z4-11Al is far from the only oxidation product produced by UV exposure. Given the structure of the hydrocarbon, compounds like heptanal, 1-heptanol, undec-4-en-1-ol, and perhaps heptanoic and undec-4-enoic acid, are likely produced as well.

A more in-depth study of the oxidation products of 7,11-HD would benefit from examining each of these degradation products in parallel. Sixteen- and twenty-carbon aldehydes, primary alcohols and carboxylic acids can all potentially be investigated using volatile collection or solvent extraction and GC-MS for chemical analysis.

The use of two different columns was originally intended to increase the certainty of tentative identification of compounds, with the increased sensitivity of the Q-TOF MS detector not being of importance. Time constraints later precluded tentative identification of unknown compounds, and the difference in detection

sensitivity in the Q-TOF MS detector as compared to that of the single quadrupole MS detector makes it impossible to compare peak areas between the two.

### 4.3 Irradiation of live insects

It is noteworthy how Z4-11Al is produced in similar relative amounts in the ambient runs as in the UV exposure treatments, and with high variability within treatments. As with the UV exposure of 7,11-HD, the methodology used here does not permit a clear statement about the effect of the different lamps, neither in comparison with each other nor in comparison with the ambient replicates. The issue lies not only in the possibility of further oxidation of Z4-11Al, but also in the fact that SPME as used here yields qualitative, not quantitative, results. Quantitative analysis of SPME samples is non-trivial (Nolvachai *et al.*, 2022), preferably involving an internal standard, and beyond the scope of this study. Also considering that the power of Welch's t-test decreases with small sample size and high variability (Fagerland and Sandvik, 2009), a higher number of replicates in conjunction with an internal standard should permit a more robust statistical analysis.

But other factors not related to the SPME-fibers can also potentially influence the variability of results. The live insects were observed to exhibit negative phototaxis during UV-exposure, resulting in their clustering at the sides of the glass vials just below the double-layered PVC-coated fiberglass mesh. Since this mesh, which was meant to keep the insects inside the vials while filtering as little UV light as possible, could not feasibly be applied in the exact same way in the different replicates, there is the likelihood of a variation in effective radiation intensity affecting the insects between replicates. Using a specialized quartz crystal lid or vials could minimize UV-absorption.

SPME fibers are furthermore sensitive to factors like heat and humidity (Risticvic *et al.*, 2010), and are subject to general wear and tear with use and conditioning. This is in turn further complicated by the fact that once a sample is extracted from the SPME fiber it is not retrievable and cannot be re-analyzed. As such, replicates may vary depending on the day they were performed, and depending on the number of times the SPME fiber has been used and for what purposes.

To minimize the variation due to individual differences between the experimental insects, further experiments could be done by sampling the headspace of the same insects before and after UV-irradiation. Furthermore, rather than collecting separate sets of insects at different occasions for specific treatments, environmental factors affecting the individuals could be minimized by collecting individuals in a large set first, and splitting this set into groups just prior to treatment. This could give a better idea of an eventual increase in Z4-11Al

production for each treatment and reducing potential effects of comparing insects eclosed on different days.

## 5. Conclusions

Developing protocols for studying CHC-oxidation serves as part of developing monitoring solutions in non-model pest insects, and further research is needed. Such research should compare the adsorption efficiency of Z4-11Al at different sampling durations, both for verified Z4-11Al alone and in competition with the other volatiles produced by the UV-irradiated synthetic 7,11-HD and the live insects, preferably utilizing more than one kind of SPME fibers. The photooxidation of 7,11-HD should also be analyzed more in-depth regarding other potential oxidation products (other aldehydes, alcohols, and carboxylic acids). As for the UV-exposure of live *D. melanogaster*, using an internal standard in the headspace collection would make quantification of Z4-11Al amounts possible. Efforts should be taken to minimize variation in irradiation intensity and individual variation alike, such as specialized UV-inert equipment and headspace collection before and after treatment. Increasing the number of replicates per treatment for a more robust statistical analysis is necessary.

## 6. References

- Ahmed, M., Pickova, J., Ahmad, T., Liaquat, M., Farid, A., & Jahangir, M. (2016). *Oxidation of lipids in foods*. Sarhad Journal of Agriculture, 32(3), 230–238. <https://doi.org/10.17582/journal.sja/2016.32.3.230.238>
- Binz, H., Kraft, E. F., Entling, M. H., & Menzel, F. (2016). *Behavioral response of a generalist predator to chemotactile cues of two taxonomically distinct prey species*. Chemoecology, 26(4), 153–162. <https://doi.org/10.1007/s00049-016-0215-z>
- Blomquist, G. J., & Ginzl, M. D. (2021). *Chemical ecology, biochemistry, and molecular biology of insect hydrocarbons*. Annual Review of Entomology, 66(1), 45–60. <https://doi.org/10.1146/annurev-ento-031620-071754>
- Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication* (1<sup>st</sup> ed). Sunderland, MA: Sinauer Associates Incorporated. ISBN-13: 9780878930456
- Brown, W. L. (1968). *An hypothesis concerning the function of the metapleural glands in ants*. The American Naturalist, 102(924), 188–191. <https://doi.org/10.1086/282536>
- Brown, W. L., Eisner, T., & Whittaker, R. H. (1970). *Allomonones and kairomones: transspecific chemical messengers*. BioScience, 20(1), 21–22. <https://doi.org/10.2307/1294753>
- Carpenter, F. W. (1905). *The Reactions of the Pomace Fly (Drosophila ampelophila Loew) to Light, Gravity, and Mechanical Stimulation*. The American Naturalist, 39(459), 157–171. <https://doi.org/10.1086/278502>
- Duménil, C., Woud, D., Pinto, F., Alkema, J. T., Jansen, I., Van Der Geest, A. M., Roessingh, S., & Billeter, J. (2016). *Pheromonal Cues Deposited by Mated Females Convey Social Information about Egg-Laying Sites in Drosophila Melanogaster*. Journal of Chemical Ecology, 42(3), 259–269. <https://doi.org/10.1007/s10886-016-0681-3>
- Ejima, A. (2015). *Pleiotropic actions of the male pheromone cis-vaccenyl acetate in Drosophila melanogaster*. Journal of Comparative Physiology A, 201(9), 927–932. <https://doi.org/10.1007/s00359-015-1020-9>

- Fagerland, M. W., & Sandvik, L. (2009). *Performance of five two-sample location tests for skewed distributions with unequal variances*. *Contemporary Clinical Trials*, 30(5), 490–496. <https://doi.org/10.1016/j.cct.2009.06.007>
- Ferveur, J. (2005). *Cuticular hydrocarbons: Their evolution and roles in drosophila pheromonal communication*. *Behavior Genetics*, 35(3), 279–295. <https://doi.org/10.1007/s10519-005-3220-5>
- Gibbs, A. G. (1998). *Water-Proofing properties of cuticular lipids*. *American Zoologist*, 38(3), 471–482. <https://doi.org/10.1093/icb/38.3.471>
- Gibbs, A., & Pomonis, J. (1995). *Physical properties of insect cuticular hydrocarbons: The effects of chain length, methyl-branching and unsaturation*. *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology*, 112(2), 243–249. [https://doi.org/10.1016/0305-0491\(95\)00081-x](https://doi.org/10.1016/0305-0491(95)00081-x)
- Gu, X., Quilici, D., Juarez, P., Blomquist, G., & Schal, C. (1995). *Biosynthesis of hydrocarbons and contact sex pheromone and their transport by lipophorin in females of the German cockroach (Blattella germanica)*. *Journal of Insect Physiology*, 41(3), 257–267. [https://doi.org/10.1016/0022-1910\(94\)00100-u](https://doi.org/10.1016/0022-1910(94)00100-u)
- Ha, T. S., & Smith, D. P. (2006). *A pheromone receptor mediates 11-Cis-Vaccenyl Acetate-Induced responses in Drosophila*. *Journal of Neuroscience*, 26(34), 8727–8733. <https://doi.org/10.1523/jneurosci.0876-06.2006>
- Hadley, N. F. (1981). *Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition, and waterproofing function*. *Biological Reviews/Biological Reviews of the Cambridge Philosophical Society*, 56(1), 23–47. <https://doi.org/10.1111/j.1469-185x.1981.tb00342.x>
- Hatano, E., Wada-Katsumata, A., & Schal, C. (2020). *Environmental decomposition of olefinic cuticular hydrocarbons of Periplaneta americana generates a volatile pheromone that guides social behaviour*. *Proceedings of the Royal Society B Biological Sciences*, 287(1921), 20192466. <https://doi.org/10.1098/rspb.2019.2466>
- Jeibmann, A., & Paulus, W. (2009). *Drosophila melanogaster as a Model Organism of Brain Diseases*. *International Journal of Molecular Sciences*, 10(2), 407–440. <https://doi.org/10.3390/ijms10020407>
- Jiang, N., Chang, H., Weißflog, J., Eberl, F., Veit, D., Weniger, K., Hansson, B. S., & Knaden, M. (2023). *Ozone exposure disrupts insect sexual communication*. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-36534-9>

- Karlson, P., & Butenandt, A. (1959). *Pheromones (Ectohormones) in insects*. Annual Review of Entomology, 4(1), 39–58.  
<https://doi.org/10.1146/annurev.en.04.010159.000351>
- Karlson, P., & Lüscher, M. (1959). '*Pheromones*': a New Term for a Class of Biologically Active Substances. Nature, 183(4653), 55–56.  
<https://doi.org/10.1038/183055a0>
- Kefi, M., Balabanidou, V., Douris, V., Lycett, G., Feyereisen, R., & Vontas, J. (2019). *Two functionally distinct CYP4G genes of Anopheles gambiae contribute to cuticular hydrocarbon biosynthesis*. Insect Biochemistry and Molecular Biology, 110, 52–59. <https://doi.org/10.1016/j.ibmb.2019.04.018>
- Khallaf, M. A., Cui, R., Weißflog, J., Erdogmus, M., Svatoš, A., Dweck, H. K. M., Valenzano, D. R., Hansson, B. S., & Knaden, M. (2021). *Large-scale characterization of sex pheromone communication systems in Drosophila*. Nature Communications, 12(1). <https://doi.org/10.1038/s41467-021-24395-z>
- Koedam, D., Morgan, E. D., Nunes, T. M., Patricio, E. F. L. R. A., & Fonseca, V. L. I. (2010). *Selective preying of the sphecid wasp Trachypus boharti on the meliponine bee Scaptotrigona postica: potential involvement of caste-specific cuticular hydrocarbons*. Physiological Entomology, 36(2), 187–193.  
<https://doi.org/10.1111/j.1365-3032.2010.00769.x>
- Lahav, S., Soroker, V., Hefetz, A., & Vander Meer, R. K. (1999). *Direct behavioral evidence for hydrocarbons as ant recognition discriminators*. The Science of Nature, 86(5), 246–249. <https://doi.org/10.1007/s001140050609>
- Lebreton, S., Borrero-Echeverry, F., Gonzalez, F., Solum, M., Wallin, E. A., Hedenström, E., Hansson, B. S., Gustavsson, A., Bengtsson, M., Birgersson, G., Walker, W. B., Dweck, H. K. M., Becher, P. G., & Witzgall, P. (2017). *A Drosophila female pheromone elicits species-specific long-range attraction via an olfactory channel with dual specificity for sex and food*. BMC Biology, 15(1).  
<https://doi.org/10.1186/s12915-017-0427-x>
- Leonhardt, S. D., Menzel, F., Nehring, V., & Schmitt, T. (2016). *Ecology and evolution of communication in social insects*. Cell, 164(6), 1277–1287.  
<https://doi.org/10.1016/j.cell.2016.01.035>
- Makki, R., Cinnamon, E., & Gould, A. P. (2014). *The development and functions of oenocytes*. Annual Review of Entomology, 59(1), 405–425.  
<https://doi.org/10.1146/annurev-ento-011613-162056>

- Menzel, F., Morsbach, S., Martens, J. H., Räder, P., Hadjaje, S., Poizat, M., & Abou, B. (2019). *Communication vs. waterproofing: the physics of insect cuticular hydrocarbons*. *Journal of Experimental Biology*.  
<https://doi.org/10.1242/jeb.210807>
- Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (2019). *Drosophila melanogaster: A Model Organism to Study Cancer*. *Frontiers in Genetics*, 10. <https://doi.org/10.3389/fgene.2019.00051>
- Morgan, T. H. (1910). *Sex Limited Inheritance in Drosophila*. *Science*, 32(812), 120–122. <https://doi.org/10.1126/science.32.812.120>
- Nolvachai, Y., Amaral, M. S., Herron, R., & Marriott, P. J. (2022). *Solid phase microextraction for quantitative analysis – Expectations beyond design?* *Green Analytical Chemistry*, 4, 100048. <https://doi.org/10.1016/j.greeac.2022.100048>
- Ong, C., Yung, L. L., Cai, Y., Bay, B., & Baeg, G. (2014). *Drosophila melanogaster as a model organism to study nanotoxicity*. *Nanotoxicology*, 9(3), 396–403.  
<https://doi.org/10.3109/17435390.2014.940405>
- Prüßing, K., Voigt, A., & Schulz, J. B. (2013). *Drosophila melanogaster as a model organism for Alzheimer's disease*. *Molecular Neurodegeneration*, 8(1).  
<https://doi.org/10.1186/1750-1326-8-35>
- Ranganathan, Y., Bessière, J., & Borges, R. M. (2015). *A coat of many scents: Cuticular hydrocarbons in multitrophic interactions of fig wasps with ants*. *Acta Oecologica*, 67, 24–33. <https://doi.org/10.1016/j.actao.2015.05.007>
- Risticvic, S., Lord, H., Górecki, T., Arthur, C. L., & Pawliszyn, J. (2010). *Protocol for solid-phase microextraction method development*. *Nature Protocols*, 5(1), 122–139. <https://doi.org/10.1038/nprot.2009.179>
- Rutledge, C. E., Silk, P. J., & Mayo, P. (2014). *Use of contact chemical cues in prey discrimination by *Cerceris fumipennis**. *Entomologia Experimentalis Et Applicata*, 153(2), 93–105. <https://doi.org/10.1111/eea.12233>
- Silberbush, A., Markman, S., Lewinsohn, E., Bar, E., Cohen, J. E., & Blaustein, L. (2010). *Predator-released hydrocarbons repel oviposition by a mosquito*. *Ecology Letters*, 13(9), 1129–1138. <https://doi.org/10.1111/j.1461-0248.2010.01501.x>
- Steiger, S., Schmitt, T., & Schaefer, H. M. (2010). *The origin and dynamic evolution of chemical information transfer*. *Proceedings of the Royal Society B Biological Sciences*, 278(1708), 970–979. <https://doi.org/10.1098/rspb.2010.2285>

- Swedenborg, P. D., & Jones, R. L. (1992). *(Z)-4-Tridecenal, a pheromonally active air oxidation product from a series of (Z,Z)-9,13 dienes in Macrocentrus grandii Goidanich (Hymenoptera: Braconidae)*. *Journal of Chemical Ecology*, 18(11), 1913–1931. <https://doi.org/10.1007/bf00981916>
- Tan, K., & Nishida, R. (2000). *Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *Bactrocera* fruit flies via a floral synomone*. *Journal of Chemical Ecology*, 26(2), 533–546. <https://doi.org/10.1023/a:1005477926244>
- Thistle, R., Cameron, P., Ghorayshi, A., Dennison, L., & Scott, K. (2012). *Contact Chemoreceptors Mediate Male-Male Repulsion and Male-Female Attraction during *Drosophila* Courtship*. *Cell*, 149(5), 1140–1151. <https://doi.org/10.1016/j.cell.2012.03.045>
- Toda, H., Zhao, X., & Dickson, B. J. (2012). *The *Drosophila* Female Aphrodisiac Pheromone Activates *ppk23+* Sensory Neurons to Elicit Male Courtship Behavior*. *Cell Reports*, 1(6), 599–607. <https://doi.org/10.1016/j.celrep.2012.05.007>
- Vander Meer, R. K., Obin, M. S., Zawistowski, S., Sheehan, K. B., & Richmond, R. C. (1986). *A reevaluation of the role of *cis-vaccenyl acetate*, *cis-vaccenol* and *esterase 6* in the regulation of mated female sexual attractiveness in *Drosophila melanogaster**. *Journal of Insect Physiology*, 32(8), 681–686. [https://doi.org/10.1016/0022-1910\(86\)90109-5](https://doi.org/10.1016/0022-1910(86)90109-5)
- Wang, L., & Anderson, D. J. (2009). *Identification of an aggression-promoting pheromone and its receptor neurons in *Drosophila**. *Nature*, 463(7278), 227–231. <https://doi.org/10.1038/nature08678>
- Wickham, J. D., Xu, Z., & Teale, S. A. (2012). *Evidence for a female-produced, long range pheromone of *Anoplophora glabripennis* (Coleoptera: Cerambycidae)*. *Insect Science*, 19(3), 355–371. <https://doi.org/10.1111/j.1744-7917.2012.01504.x>
- Wyatt, T. D. (2014). *Pheromones and animal behavior: Chemical Signals and Signatures*. Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9781139030748>

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# Appendix 1

*Drosophila melanogaster* sugar-yeast-cornmeal diet recipe:

Water	6330 ml
Corn meal (polenta)	462 g
Agar (plant agar)	24 g
Malt	132 g
Yeast	109.5 g
Soy meal	63.24 g
Sugar syrup	486 ml
Propionic acid	30 ml

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