

## Supplementary material:

# Pathogenic fungus uses volatiles to entice male flies into fatal matings with infected female cadavers

### Authors

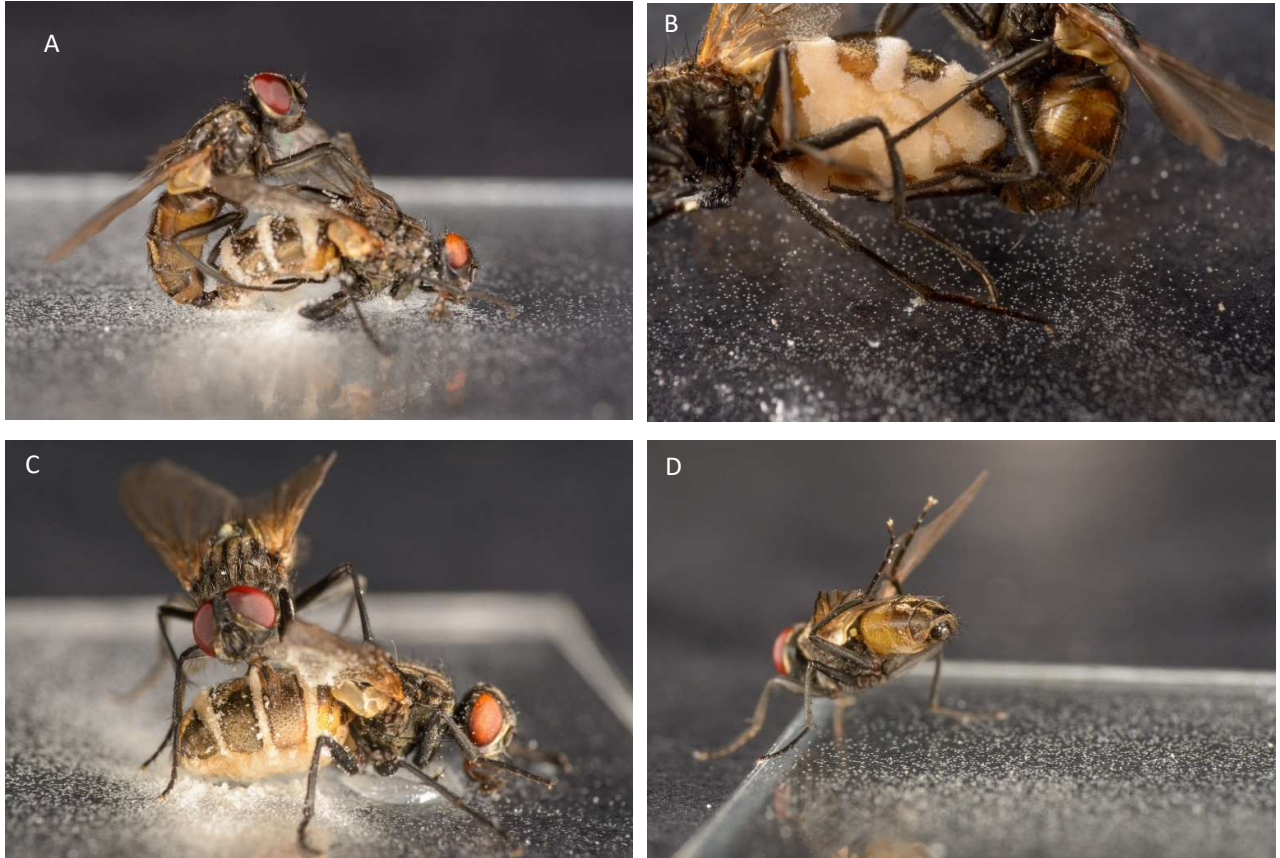
Andreas Naundrup<sup>\*1</sup>, Björn Bohman<sup>2</sup>, Charles A. Kwadha<sup>2</sup>, Annette B. Jensen<sup>1</sup>, Paul G. Becher<sup>2</sup>, Henrik H. De Fine Licht<sup>\*1</sup>

<sup>1</sup>Section for Organismal Biology, Department of Plant and Environmental Science, University of Copenhagen, Denmark

<sup>2</sup>Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

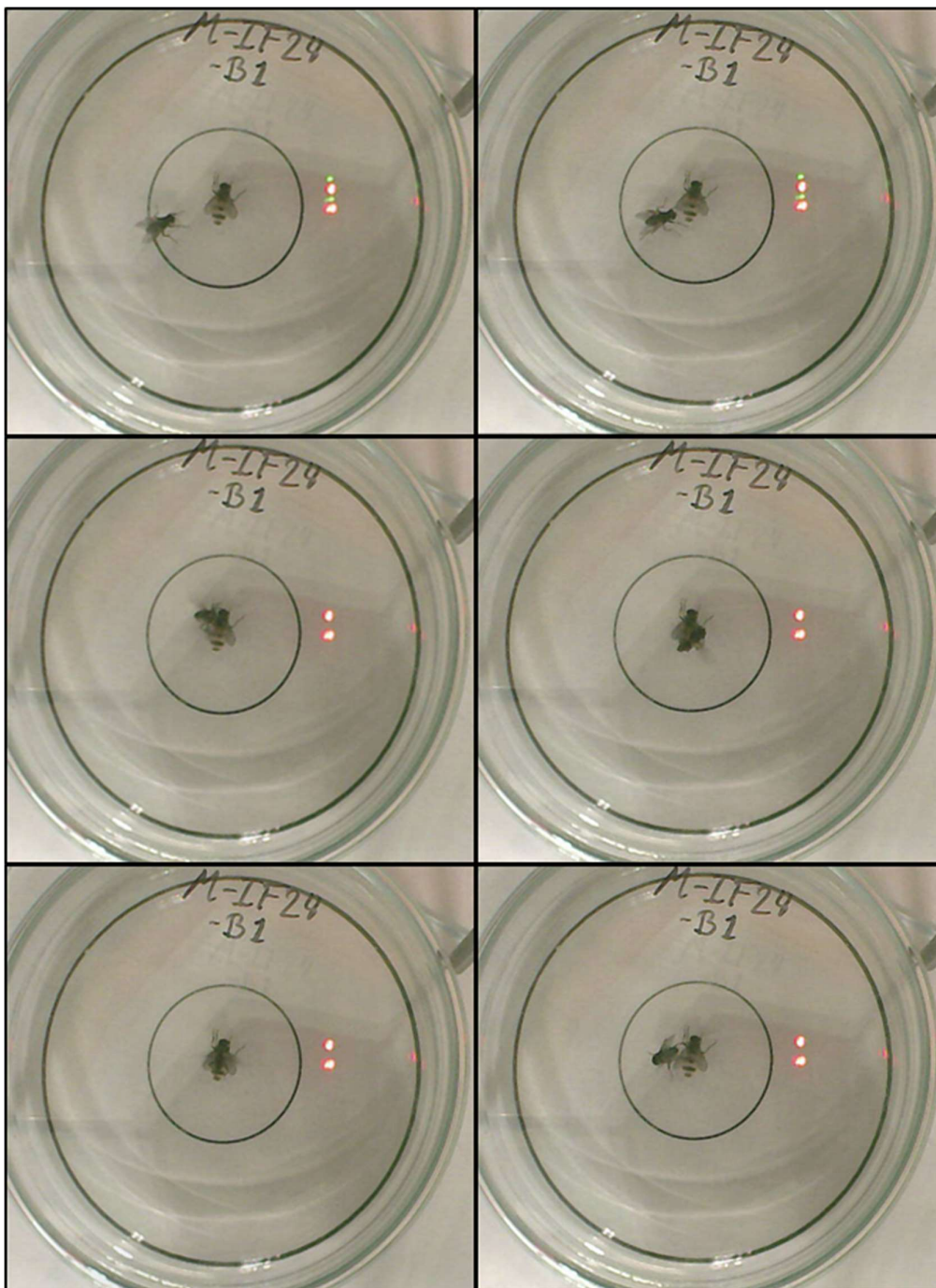
\*Author for correspondence: Email: ah@plen.ku.dk, Tel: +45 42408353, Email: hhdefinlicht@plen.ku.dk, Tel: +45 35320097, Address: Section for Organismal Biology, Department of Plant and Environmental Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

## Supplementary Figure 1



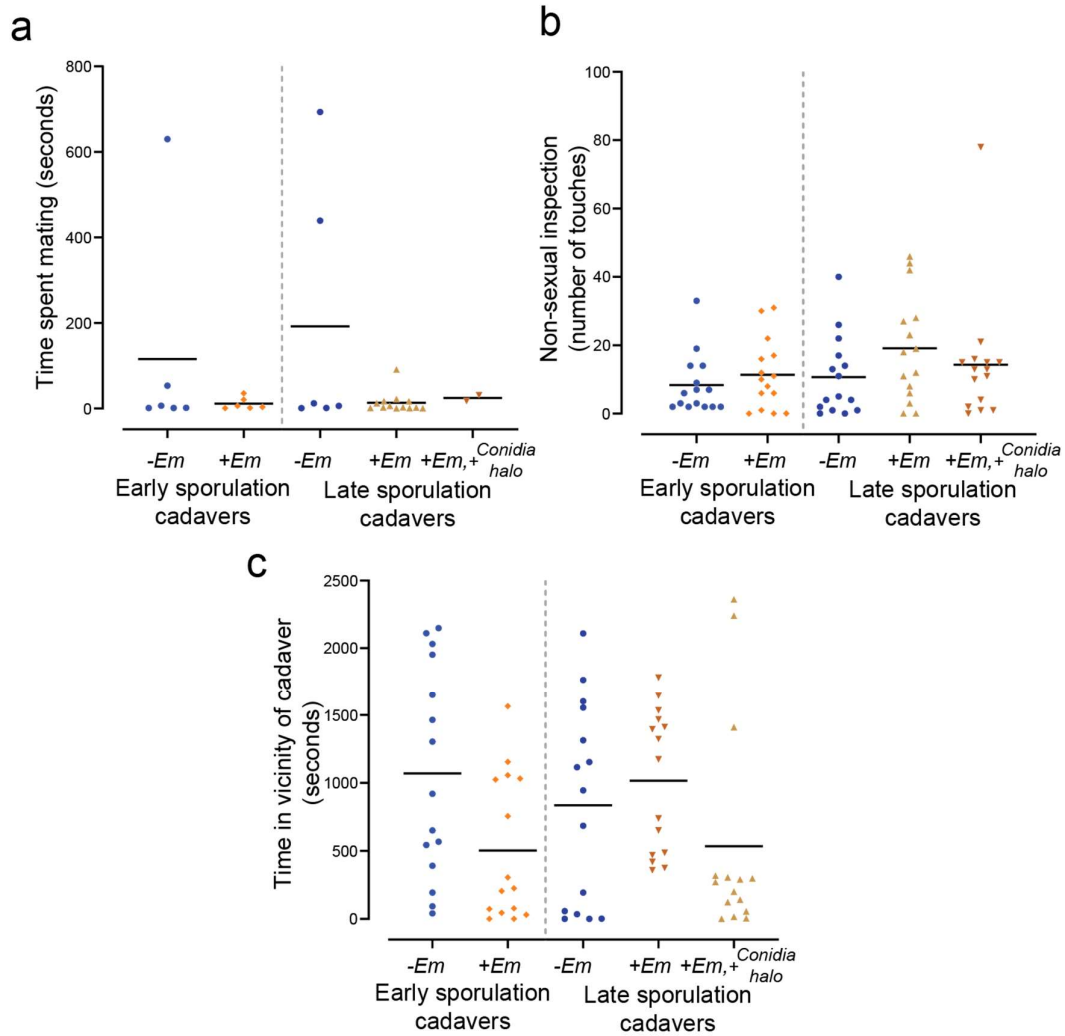
**Supplementary figure 1. Housefly behavior towards *E. muscae* sporulating cadavers.** **a.** A healthy, male housefly attempting to mate with an *E. muscae* sporulating housefly cadaver. **b.** Male attempting to mate with a sporulating female housefly cadaver. The male visibly extends his aedeagus to engage in mating. **c.** A housefly extending its proboscis and inspecting the sporulating cadaver. This behavior was exhibited towards both male and female cadavers and conidia alone. The cadaver is fixated to the glass surface with a small amount of petroleum jelly (Vaseline) on the ventral side of the thorax. **d.** Healthy male housefly engaging in grooming behaviour after being in contact with cadaver and conidia. Here, several conidia have already attached themselves to legs and abdomen. In all photos conidia can be seen as a white powder primarily on surfaces and the cadaver, but also on the male's abdomen and legs. Photos: Filippo Castelucci.

## Supplementary Figure 2



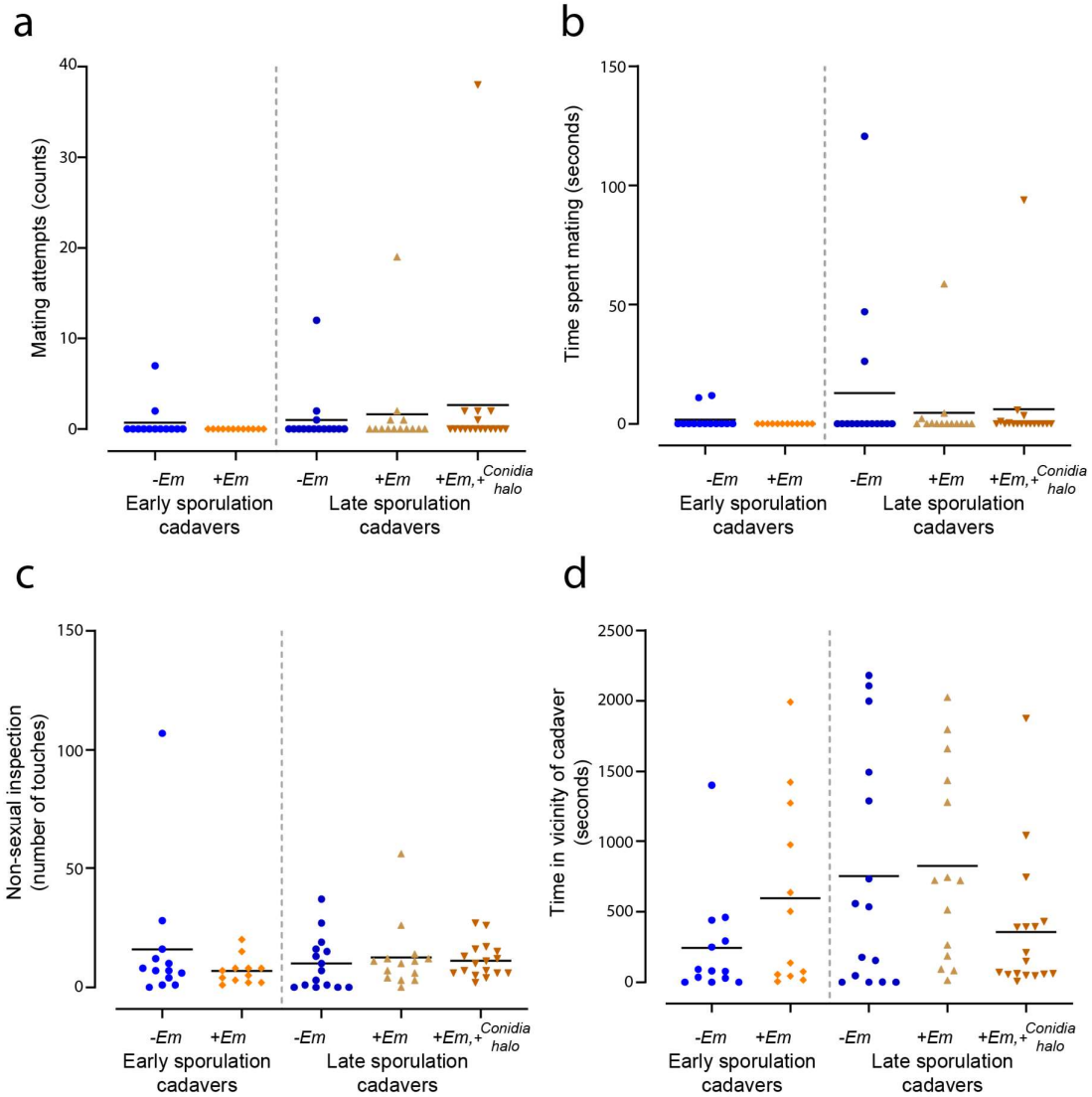
**Supplementary figure 2. Time-course pictures of an attempted mating in the mating activity experiment.** A single cadaver is fixated in the center of a glass arena (Petri dish). The arena is placed on top of a printed sheet of paper, denoting the center (cadaver), an inner ring (radius = 16 mm), which denotes when the subject is “in vicinity” and an outer ring to indicate the outer borders of the arena. After acclimating to the arena, the male will usually first interact with the cadaver by physical contact. A mating attempt occurs when the male approaches the cadaver, usually from the sides or rear, and jumps onto the cadaver, accompanied by excessive wing flicking. Then the male attempts to connect his aedeagus with the tip of the cadaver’s abdomen.

### Supplementary figure 3



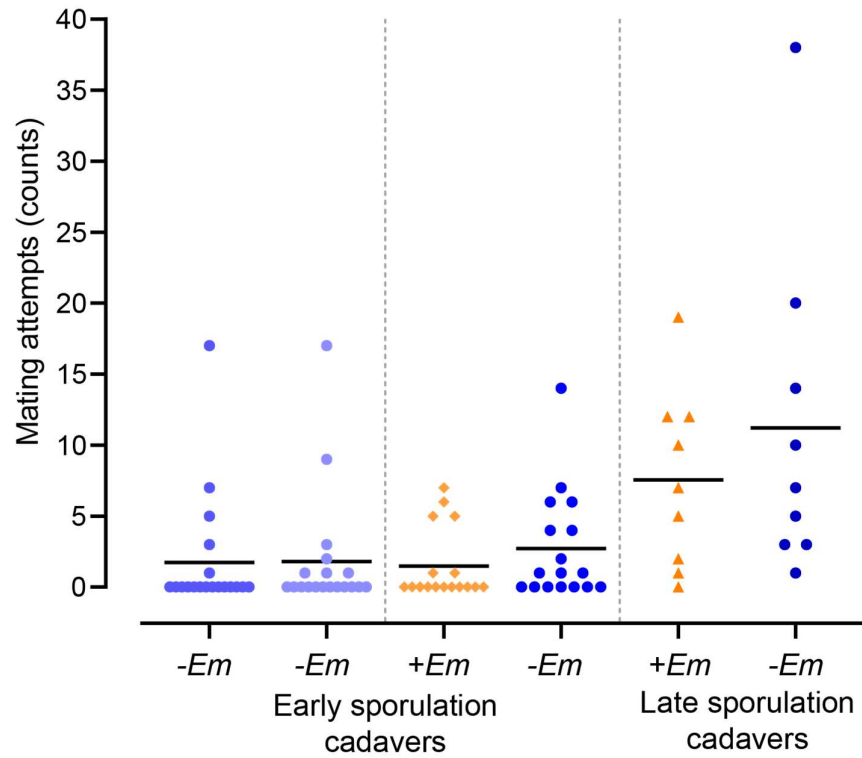
**Supplementary figure 3. Male behaviour towards female cadavers. a.** Time spent mating (seconds). Male on uninfected female early control (-Em, n = 15), +Em: Male on infected female, early sporulation stage (+Em, n = 15), Male on uninfected female, late control (-Em, n = 15), Male on infected female, late sporulation stage (+Em, n = 15), Male on infected female, late sporulation stage with spores around the cadaver (+Em, +*Conidia halo*, n = 15) **b.** Number of non-sexual physical cadaver inspections (number of touches). **c.** Time spent in vicinity of cadaver (radius: 16 mm). Horizontal black lines show the mean.

## Supplementary Figure 4



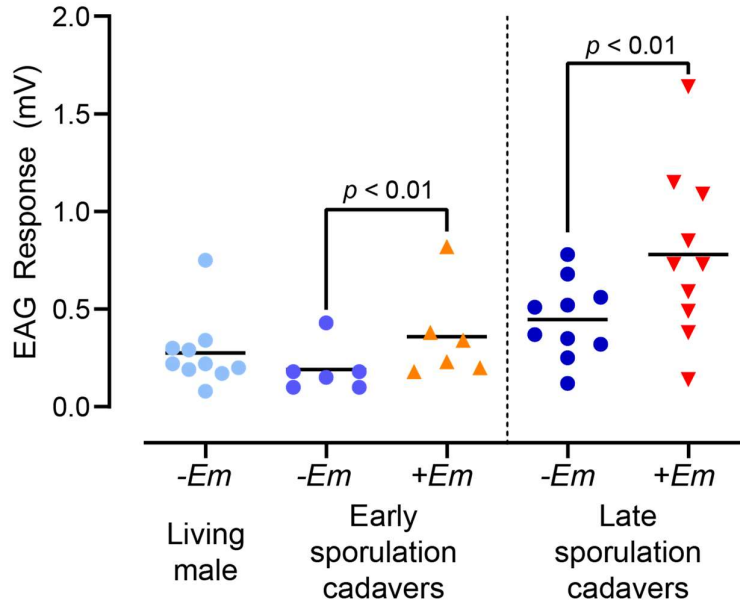
**Supplementary figure 4. Male on male mating behaviour.** **a.** Number of mating attempts measured per trial. Male on uninfected male early control (-Em, n =13), +Em: Male on infected male, early sporulation stage (+Em, n=12), Male on uninfected male, late control (-Em, n=15), Male on infected male, late sporulation stage (+Em, n=14), and Male on infected male, late sporulation stage with halo of conida around the cadaver (+Em, +*Conidia halo*, n=17) **b.** Time spent mating (seconds) **c.** Non-sexual inspections (counts of touches), **d.** Time spent in vicinity (radius: 16 mm) of the male cadaver. Horizontal black lines show the mean.

### Supplementary Figure 5



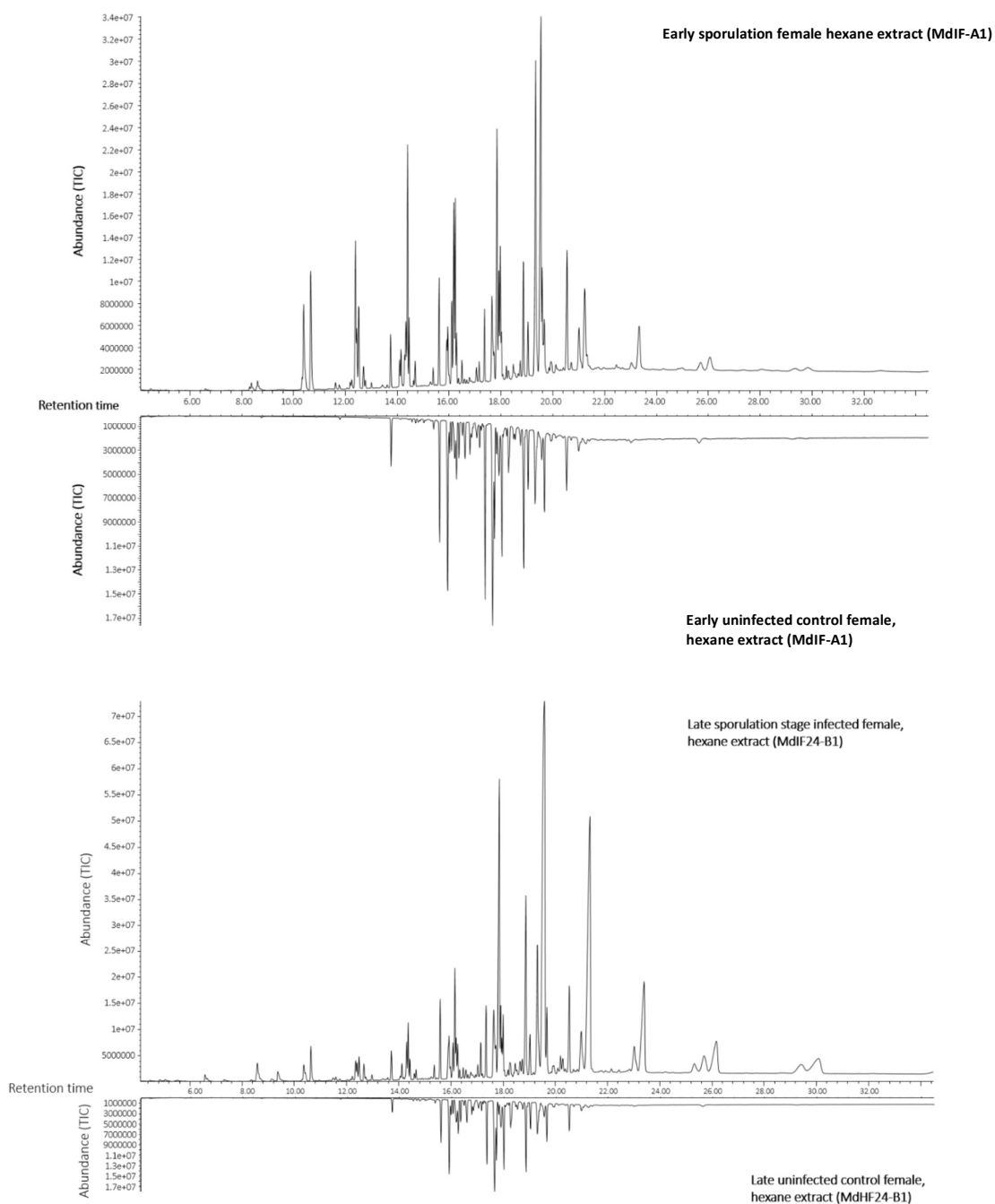
**Supplementary figure 5. Petri dish 2-choice experiment.** One male was allowed to choose between two female cadavers. In the first subset (left), the male had to choose between two uninfected, freeze-killed early controls ( $n = 19$ ). In the second subset (middle), a male was allowed to choose between an uninfected female control ( $-Em$ ), and an *E. muscae*-infected cadaver ( $+Em$ ) in an early sporulation stage ( $n = 17$ ). In the last subset (right), a male was allowed to choose between a freeze-killed, uninfected control ( $-Em$ ) and an *E. muscae*-infected cadaver ( $+Em$ ) in a late sporulation stage ( $n = 9$ ). There was no significant differences in the number of copulation attempts in any of the experiments. Horizontal black lines show the mean.

### Supplementary Figure 6



**Supplementary figure 6. Male EAG response (mV) to volatiles from male cadavers.** EAG responses (mV) to stimulus pipettes containing either uninfected, living males (-Em, n = 10), uninfected (-Em, n = 6) or infected (+Em, n = 6) early sporulation male cadavers, or uninfected (-Em, n = 10) or infected (+Em, n = 10) late sporulation male cadavers. There was a significant effect of treatment on the EAG response when comparing +Em to -Em for both early (linear mixed-effects model (LMM),  $p < 0.01$ ,  $t = 3.835$ ) and late sporulation cadavers (LMM,  $p < 0.01$ ,  $t = 3.887$ ). Horizontal black lines show the mean.

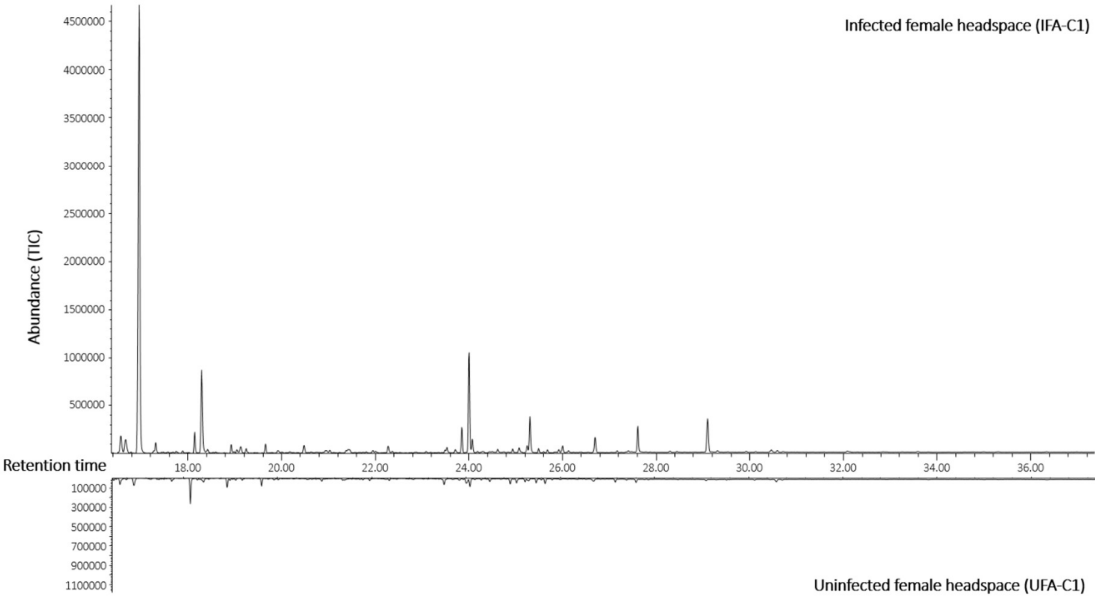
## Supplementary Figure 7



**Supplementary figure 7. Gas chromatography mass spectrometry (GC-MS) representative Total Ion Chromatograms (TIC) of early and late sporulation stage infected female cadavers.** GC-MS analysis conducted on cuticular hexane extracts of *E. muscae* early sporulation stage infected and uninfected female cadavers (top), and late sporulation stage infected and uninfected female control (bottom). Late killed cadavers had been incubated at high humidity for 25-30 hours, to prevent desiccation during sporulation.

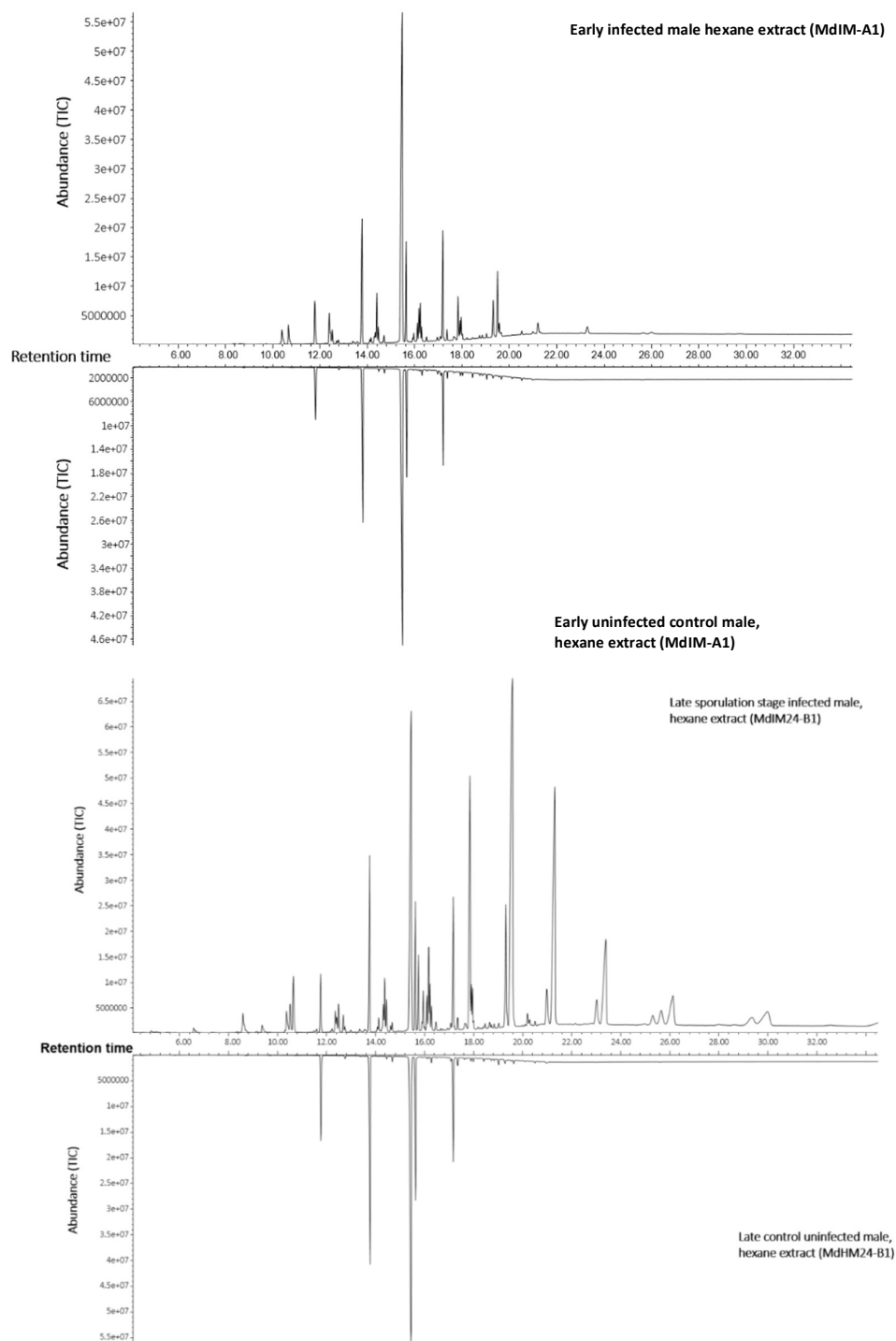


Supplementary figure 8



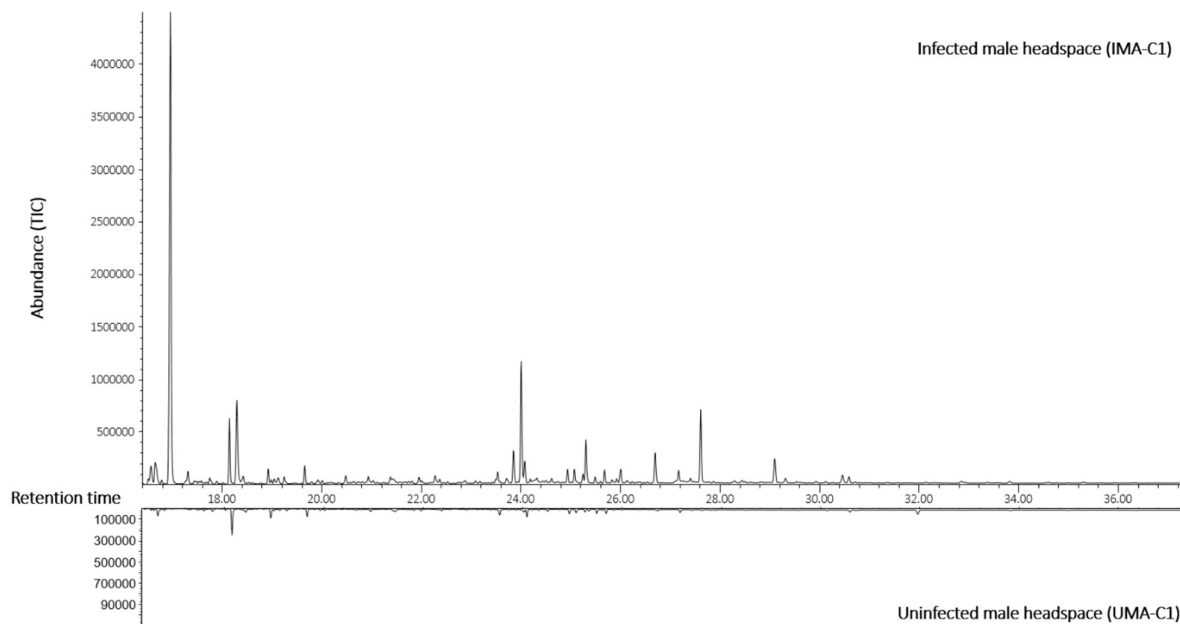
**Supplementary figure 8. Gas chromatography mass spectrometry (GC-MS) representative Total Ion Chromatograms (TIC) of headspace samples from *E. muscae* sporulating females (top) and uninfected females (bottom).** Chromatogram of headspace sampled as an aeration from female, sporulating houseflies (top). Headspace from uninfected female houseflies can be seen in the bottom.

### Supplementary figure 9



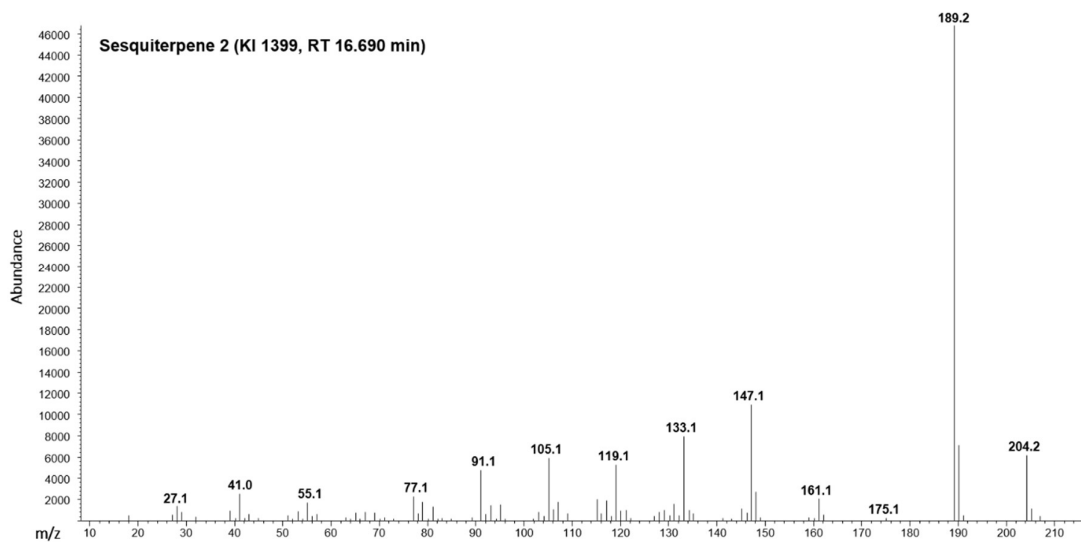
**Supplementary figure 9. Representative Total Ion Chromatograms (TIC) of cuticular extracts of early (top) and late (bottom) sporulation stage infected male cadaver and an uninfected male control. Late killed cadavers had been incubated at high humidity for 25-30 hours, to prevent desiccation during sporulation.**

### Supplementary figure 10



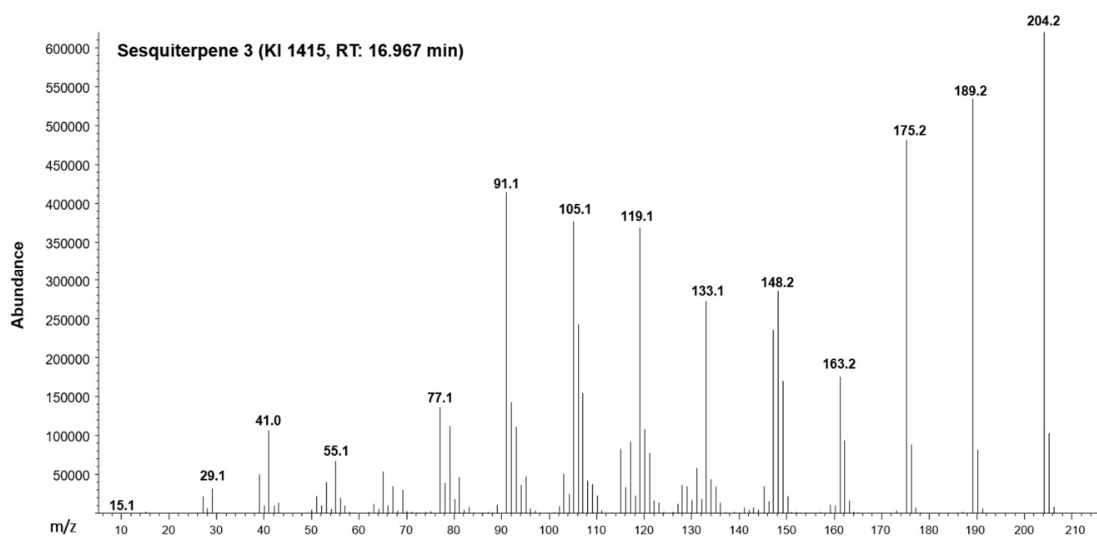
**Supplementary figure 10. Representative Total Ion Chromatogram (TIC) of headspace from male, sporulating houseflies (top) and from uninfected male houseflies (bottom).**

### Supplementary figure 11



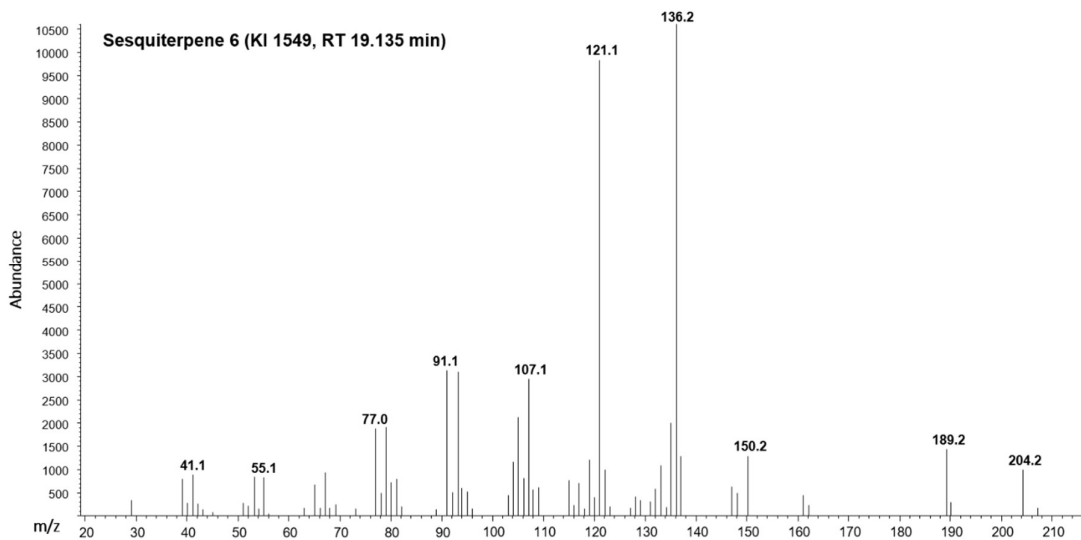
Supplementary figure 11. Mass spectrum of “Sesquiterpene 2”, one of the two unidentified GC-EAD active sesquiterpene compounds.

### Supplementary figure 12



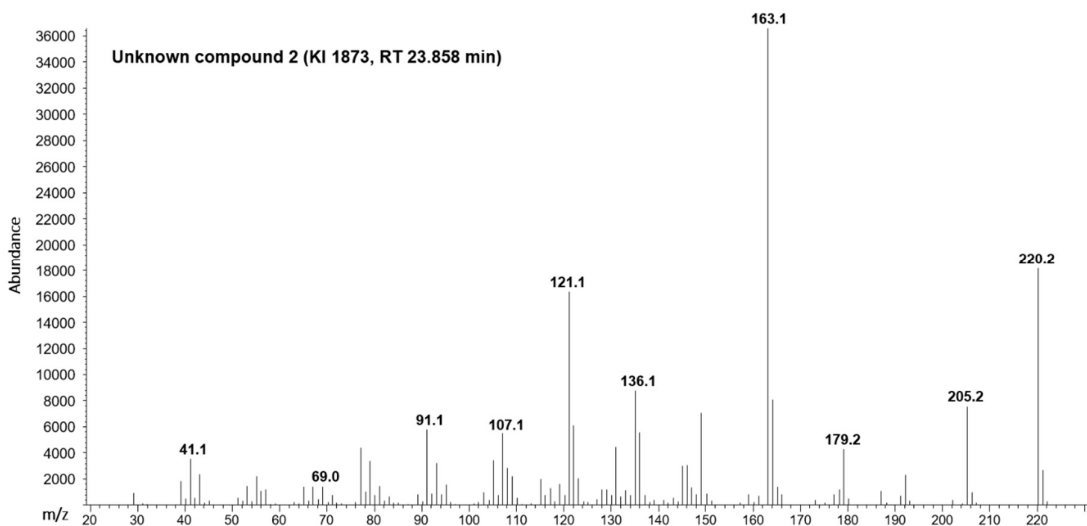
Supplementary figure 12. Mass spectrum of “Sesquiterpene 3”, the largest occurring compound in headspace from any *E. muscae* sample.

### Supplementary figure 13



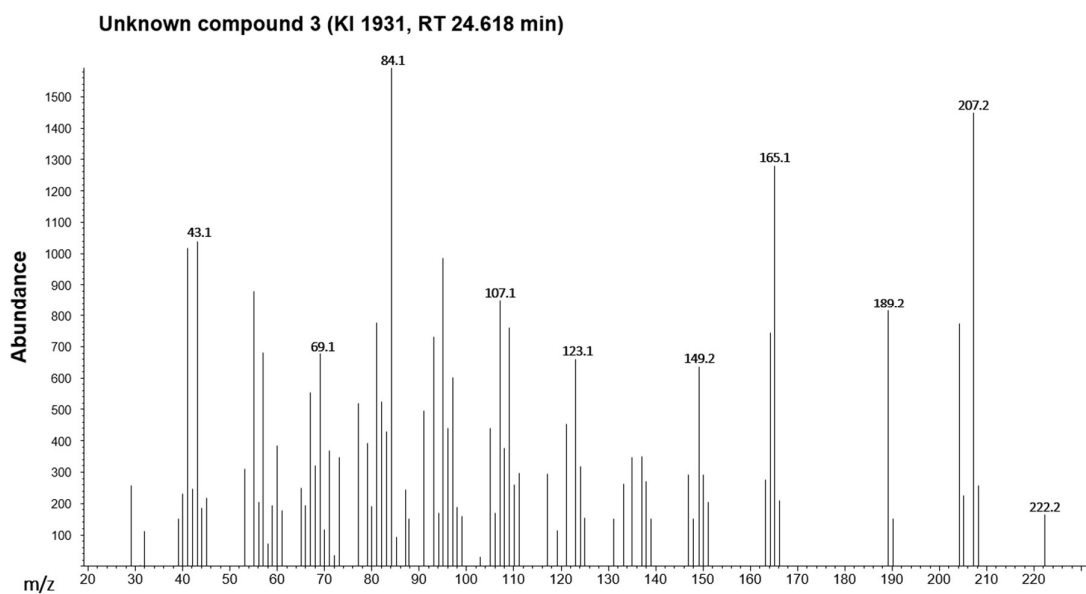
Supplementary figure 13. Mass spectrum of "Sesquiterpene 6", the second GC-EAD active sesquiterpene.

### Supplementary figure 14



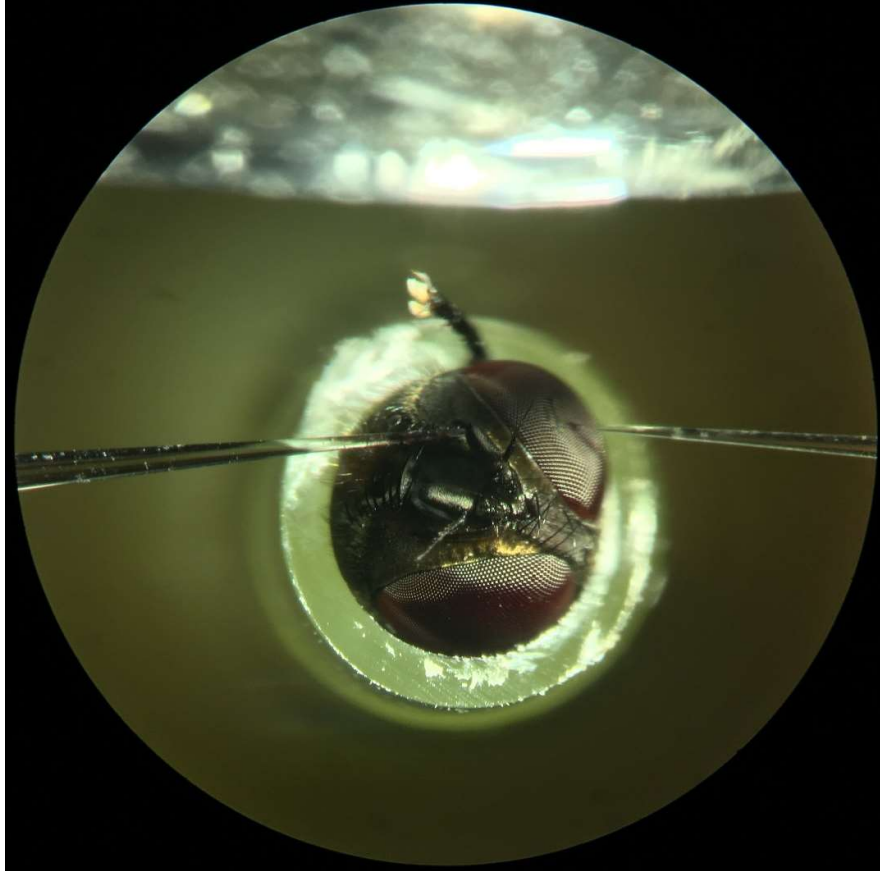
Supplementary figure 14. Mass spectrum of "Unknown compound 2", one of two GC-EAD compounds with unknown structure and identity.

## Supplementary figure 15



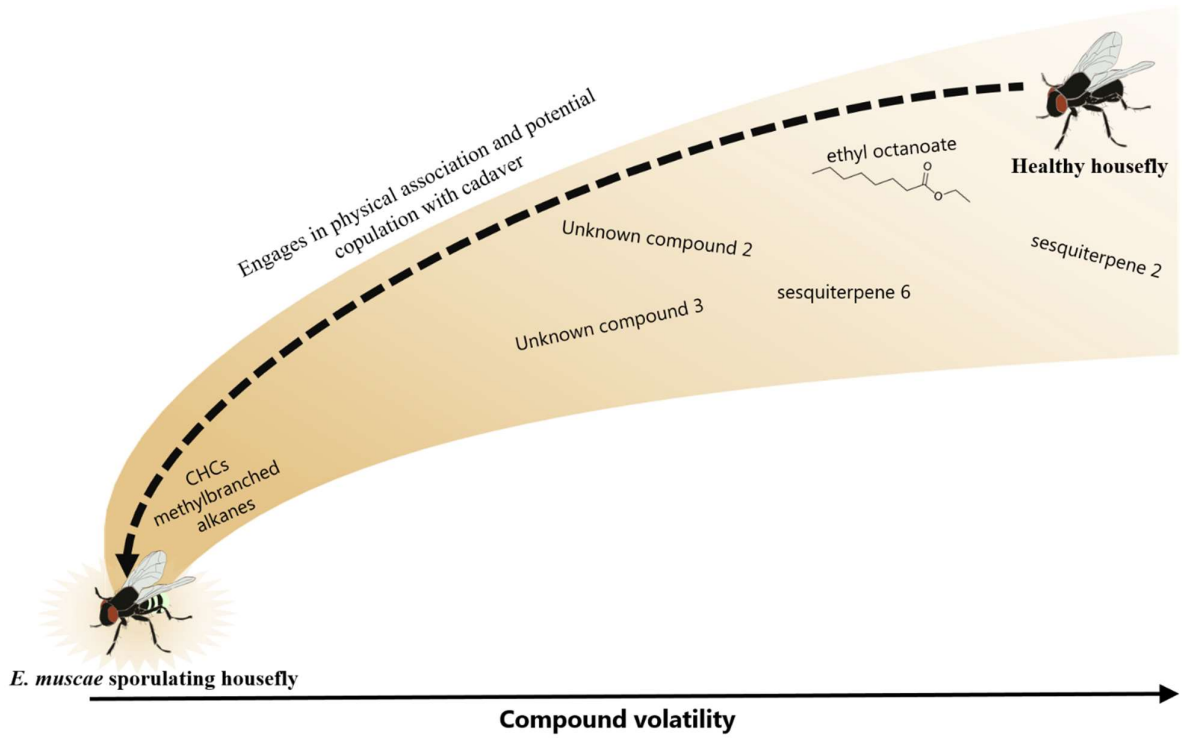
**Supplementary figure 15. Mass spectrum of "Unknown compound 3", the second of two GC-EAD compounds with unknown structure and identity.**

**Supplementary figure 16**



**Supplementary figure 16. GC-EAD setup.** A living, male housefly was fixed in a cut-off pipette tip so that only the head protruded. Electrodes in glass capillaries filled with Ringer solution were placed in the eye (ground electrode) and on the tip of the funiculus (recording electrode) on the living fly. The top, white glass surface is the glass tube that delivered purified and humidified air stream with compounds eluting from the GC column.

## Supplementary figure 17



**Supplementary figure 17. Conceptual model of housefly attraction to *E. muscae* sporulating cadavers.** A healthy housefly male encounters a plume of highly volatile attractants. The male follows this increasing gradient of chemical cues until in vicinity of a sporulating female cadaver (dashed arrow). Here, the changes in the CHC profile and the methylbranched alkanes in particular, stimulate male mating behavior and cause him to engage in copulation with the cadaver.



Supplementary table 1

Tentatively identified compound	KI (DB-1 column)	Uninfected females (5)	Uninfected females, late control (5)	Infected females (5)	Infected females, late sporulation (5)	Uninfected males (5)	Uninfected males, late control (5)	Infected males (5)	Infected males, late sporulation (5)	Conidia from females (2)	Conidia from males (5)
1-Undecyl acetate	1484.9	-	-	-	2.49E+07 ± 2.9E+06	-	-	-	4.65E+07 ± 1.4E+07	1.19E+07	2.69E+07 ± 3.1E+06
1-Tetradecanol	1651.8	-	-	-	8.74E+06 ± 3.3E+06	-	-	-	1.58E+07 ± 5.5E+06	2.93E+07	2.94E+07 ± 3.5E+06
1-Octadecanol	2060.1	-	-	-	5.75E+07 ± 1.1E+07	-	-	-	9.43E+07 ± 2.4E+07	9.97E+07	2.36E+08 ± 3.3E+07
Heneicosane	2094.3	-	-	-	-	2.90E+06 ± 4.3E+05	2.87E+06 ± 6.8E+04	5.58E+06 ± 9.0E+05	3.90E+07 ± 2.9E+07	-	-
Tetradecyl hexanoate	2160.7	-	-	1.88E+08 ± 4.3E+07	1.78E+08 ± 4.8E+07	-	-	1.01E+08 ± 2.2E+07	2.09E+08 ± 5.2E+07	-	-
Octadecyl isocyanate	2171.6	-	-	-	-	-	-	-	-	3.38E+08	1.96E+08 ± 3.7E+07
1-Octadecyl acetate	2184.0	-	-	1.92E+08 ± 4.2E+07	2.34E+08 ± 4.7E+07	-	-	1.16E+08 ± 1.0E+07	4.37E+08 ± 4.5E+07	4.66E+07	1.24E+08 ± 7.7E+06
Tricosene	2268.6	-	-	-	1.82E+07 ± 2.2E+06	-	-	-	2.93E+07 ± 6.9E+06	-	-
Tricosane	2301.1	4.91E+06 ± 1.0E+06	3.32E+06 ± 2.6E+05	1.21E+07 ± 2.6E+06	1.53E+07 ± 2.4E+06	2.48E+08 ± 2.3E+07	3.65E+08 ± 2.0E+07	2.90E+08 ± 3.1E+07	3.37E+08 ± 2.9E+07	-	-
Tetradecyl octanoate	2355.0	-	-	2.14E+08 ± 4.8E+07	1.39E+08 ± 3.0E+07	-	-	1.51E+08 ± 1.8E+07	2.68E+08 ± 5.0E+07	3.42E+07	3.21E+07 ± 4.1E+06
Hexadecyl hexanoate	2359.1	-	-	7.63E+07 ± 1.6E+07	1.05E+08 ± 1.4E+07	-	-	5.03E+07 ± 5.2E+06	1.11E+08 ± 1.1E+07	6.57E+06	6.89E+06 ± 1.6E+06
Octadecyl butyrate	2365.9	-	-	1.10E+08 ± 2.4E+07	1.47E+08 ± 2.8E+07	-	-	7.29E+07 ± 1.1E+07	1.91E+08 ± 2.3E+07	2.66E+07	8.33E+07 ± 6.0E+06
1-Eicosyl acetate	2386.0	-	-	2.91E+07 ± 6.9E+06	8.11E+07 ± 7.9E+06	-	-	2.78E+07 ± 8.3E+06	1.18E+08 ± 1.3E+07	3.36E+07	8.91E+07 ± 3.9E+06
Tetracosane	2393.6	-	-	1.74E+07 ± 3.3E+06	2.62E+07 ± 6.7E+06	9.19E+06 ± 7.4E+05	1.32E+07 ± 4.7E+05	3.84E+07 ± 1.0E+07	4.14E+07 ± 7.0E+06	-	-
Pentacosene	2477.3	-	-	-	-	-	-	6.26E+07 ± 1.8E+07	5.34E+07 ± 1.5E+07	-	-
Pentacosane	2493.4	7.35E+07 ± 9.4E+06	5.65E+07 ± 2.4E+06	1.37E+08 ± 2.4E+07	1.61E+08 ± 1.9E+07	7.23E+08 ± 6.2E+07	1.13E+09 ± 3.5E+07	6.81E+08 ± 6.7E+07	9.46E+08 ± 6.6E+07	-	8.98E+06 ± 1.3E+06
9-, 11-, 13-Methylpentacosanes	2531.8	2.77E+06 ± 4.6E+05	1.97E+06 ± 1.6E+05	-	-	-	-	-	-	-	-
Hexadecyl octanoate	2556.2	-	-	1.23E+08 ± 3.1E+07	2.14E+08 ± 2.7E+07	-	-	6.38E+07 ± 6.6E+06	2.96E+08 ± 4.5E+07	2.01E+07	3.28E+07 ± 3.5E+06
Octadecyl hexanoate	2561.7	-	-	2.79E+08 ± 6.0E+07	2.75E+08 ± 4.9E+07	-	-	2.06E+08 ± 2.2E+07	2.94E+08 ± 2.4E+07	7.72E+06	1.53E+07 ± 2.1E+06
3-Methylpentacosane	2569.1	2.24E+06 ± 3.4E+05	1.58E+06 ± 4.1E+05	-	-	-	-	-	-	-	-
9-Hexacosene <sup>A</sup> + unknown compound <sup>B</sup>	2570.8	-	-	-	-	1.11E+07 ± 1.5E+06 <sup>A</sup>	1.47E+07 ± 1.0E+06 <sup>A</sup>	8.59E+07 ± 1.4E+07 <sup>A,B</sup>	1.93E+08 ± 1.9E+07 <sup>A,B</sup>	-	-
5,9-Dimethylpentacosane	2580.1	6.58E+06 ± 1.2E+06	1.01E+07 ± 3.7E+06	-	-	-	-	-	-	-	-
1-Docosyl acetate	2586.5	-	-	7.40E+06 ± 2.4E+06	5.06E+07 ± 1.5E+07	-	-	8.38E+06 ± 3.6E+06	4.01E+07 ± 6.1E+06	8.74E+06	2.71E+07 ± 1.7E+06
Hexacosane	2594.9	8.48E+06 ± 1.1E+06	1.37E+07 ± 6.4E+06	4.42E+07 ± 6.4E+06	7.02E+07 ± 1.6E+07	1.79E+07 ± 1.4E+06	2.50E+07 ± 1.3E+06	4.76E+07 ± 9.2E+06	6.38E+07 ± 1.1E+07	-	-
3,7- 3,9-, 3,11- and 3,13-Dimethylpentacosanes	2606.7	1.12E+07 ± 2.0E+06	9.95E+06 ± 1.8E+06	-	-	-	-	-	-	-	-
10-, 12-, 13-Methylhexacosanes	2631.8	7.30E+06 ± 1.2E+06	6.45E+06 ± 5.8E+05	-	-	-	-	-	-	-	-
2-Methylhexacosane	2659.6	5.40E+06 ± 1.0E+06	2.66E+06 ± 6.6E+05	1.21E+07 ± 2.3E+06	-	-	-	-	-	-	-
Heptacosene	2672.8	2.31E+07 ± 3.6E+06	3.21E+07 ± 1.2E+07	4.81E+07 ± 8.3E+06	9.39E+07 ± 1.9E+07	1.84E+09 ± 1.9E+08	2.67E+09 ± 1.5E+08	2.95E+09 ± 2.3E+08	3.69E+09 ± 3.0E+08	-	3.86E+07 ± 7.0E+06
Heptacosane	2696.9	2.16E+08 ± 2.4E+07	1.75E+08 ± 1.6E+07	2.75E+08 ± 4.6E+07	3.20E+08 ± 1.5E+07	4.82E+08 ± 5.1E+07	7.69E+08 ± 4.2E+07	4.56E+08 ± 4.2E+07	6.47E+08 ± 4.9E+07	-	-
9-, 11-, 13-Methylheptacosanes	2729.4	3.73E+08 ± 3.7E+07	3.70E+08 ± 2.4E+07	1.60E+08 ± 4.3E+07	1.45E+08 ± 3.6E+07	4.81E+06 ± 6.5E+05	6.01E+06 ± 7.7E+05	1.76E+07 ± 3.9E+06	-	-	-
7-Methylheptacosane	2740.0	5.30E+07 ± 6.4E+06	8.05E+07 ± 2.9E+07	4.93E+07 ± 1.9E+07	4.59E+07 ± 3.6E+06	2.11E+06 ± 4.8E+05	2.43E+06 ± 4.1E+05	3.85E+06 ± 1.5E+06	-	-	-
5-Methylheptacosane <sup>A</sup> + Tetradecyl dodecanoate <sup>B</sup>	2747.5	4.75E+07 ± 5.8E+06 <sup>A</sup>	5.84E+07 ± 1.1E+07 <sup>A</sup>	1.21E+08 ± 2.3E+07 <sup>A,B</sup>	1.86E+08 ± 1.5E+07 <sup>A,B</sup>	-	-	7.97E+07 ± 8.1E+06 <sup>A,B</sup>	2.57E+08 ± 3.9E+07 <sup>A,B</sup>	7.55E+07 <sup>B</sup>	4.80E+07 ± 6.6E+06 <sup>B</sup>
Octadecyl octanoate	2757.3	-	-	2.39E+08 ± 4.7E+07	4.91E+08 ± 6.0E+07	-	-	1.42E+08 ± 1.4E+07	6.21E+08 ± 6.8E+07	4.75E+07	1.02E+08 ± 1.4E+07
9,13-, 11,15-Dimethylheptacosanes	2761.0	1.42E+08 ± 1.6E+07	1.49E+08 ± 2.1E+07	-	-	-	-	-	-	-	-
2-Methylheptacosane	2761.1	-	-	-	-	6.80E+06 ± 7.7E+05	8.91E+06 ± 1.0E+06	-	-	-	-
3-Methylheptacosane <sup>A</sup> + 7,11-Dimethylheptacosane <sup>B</sup> + C28:1 <sup>C</sup> + unknown compound <sup>D</sup>	2771.0	1.52E+08 ± 1.7E+07 <sup>A,B</sup>	1.53E+08 ± 1.7E+07 <sup>A,B</sup>	8.20E+07 ± 1.4E+07 <sup>A,B,D</sup>	1.50E+08 ± 1.1E+07 <sup>A,B,D</sup>	2.31E+07 ± 3.1E+06 <sup>A,B,D</sup>	3.18E+07 ± 2.7E+06 <sup>A,B,C</sup>	8.20E+07 ± 1.1E+07 <sup>A,B,C,D</sup>	1.41E+08 ± 1.4E+07 <sup>A,B,C,D</sup>	-	-
5,9-Dimethylheptacosane	2779.5	7.30E+07 ± 8.1E+06	7.79E+07 ± 1.4E+07	1.88E+07 ± 4.8E+06	4.85E+07 ± 1.5E+07	-	-	-	-	-	-
Octacosane	2794.7	2.94E+07 ± 4.0E+06	4.14E+07 ± 1.4E+07	4.01E+07 ± 6.1E+06	8.18E+07 ± 2.2E+07	3.91E+06 ± 4.6E+05	5.83E+06 ± 4.8E+05	2.36E+07 ± 3.7E+06	5.02E+07 ± 1.7E+07	-	-
3,7-, 3,9-, 3,11-Dimethylheptacosanes	2805.9	1.09E+08 ± 1.2E+07	1.21E+08 ± 2.0E+07	2.26E+07 ± 6.2E+06	6.36E+07 ± 1.8E+07	-	-	-	-	-	-

Squalene	2814.9	-	-	5.35E+06 ± 1.1E+06	3.40E+07 ± 1.2E+07	-	-	4.51E+06 ± 1.2E+06	1.76E+07 ± 3.6E+06	7.16E+06	8.79E+06 ± 1.2E+06
12-, 13-, 14- Methyloctacosanes	2827.5	6.98E+07 ± 6.6E+06	7.05E+07 ± 1.2E+07	2.36E+07 ± 4.9E+06	5.55E+07 ± 2.0E+07	-	-	-	-	-	-
4-, 6-, 8-, 10- Methyloctacosanes	2835.9	6.14E+07 ± 7.2E+06	7.71E+07 ± 1.8E+07	1.23E+07 ± 2.4E+06	4.09E+07 ± 1.7E+07	-	-	-	-	-	-
2-Methyloctacosane	2859.5	5.36E+07 ± 6.3E+06	5.60E+07 ± 1.9E+07	4.32E+07 ± 6.8E+06	8.22E+07 ± 1.6E+07	-	-	-	-	-	-
Nonacosene	2872.6	4.87E+07 ± 6.3E+06	4.92E+07 ± 8.9E+06	7.91E+07 ± 1.9E+07	1.30E+08 ± 1.6E+07	3.56E+08 ± 4.2E+07	5.60E+08 ± 4.7E+07	5.54E+08 ± 3.5E+07	7.88E+08 ± 7.9E+07	-	-
Nonacosane	2894.6	3.07E+08 ± 4.1E+07	2.59E+08 ± 1.4E+07	2.01E+08 ± 4.2E+07	2.80E+08 ± 4.1E+07	2.79E+07 ± 2.6E+06	4.05E+07 ± 2.8E+06	4.84E+07 ± 3.5E+06	7.90E+07 ± 1.0E+07	-	-
9-, 11-, 13-, 15- Methylnonacosane	2929.4	6.43E+08 ± 5.9E+07	5.74E+08 ± 3.5E+07	3.87E+08 ± 7.5E+07	4.87E+08 ± 5.9E+07	-	-	-	-	-	-
7-Methylnonacosane	2936.1	1.84E+08 ± 1.9E+07	1.81E+08 ± 1.3E+07	1.04E+08 ± 2.6E+07	1.28E+08 ± 2.0E+07	2.44E+06 ± 3.2E+05	2.93E+06 ± 1.4E+05	1.81E+07 ± 1.8E+06	-	-	-
5-Methylnonacosane	2948.3	4.96E+07 ± 6.2E+06	4.91E+07 ± 5.9E+06	-	-	-	-	-	-	-	-
Myristyl myristate	2951.8	-	-	4.03E+08 ± 8.4E+07	1.29E+09 ± 1.8E+08	-	-	2.20E+08 ± 2.4E+07	1.99E+09 ± 3.2E+08	2.46E+09	1.78E+09 ± 2.5E+08
2-Methylnonacosane <sup>A</sup> + unknown compound <sup>B</sup>	2959.8	1.91E+08 ± 2.1E+07 <sup>A</sup>	1.79E+08 ± 1.7E+07 <sup>A</sup>	1.95E+08 ± 2.9E+07 <sup>A,B</sup>	2.97E+08 ± 3.1E+07 <sup>A,B</sup>	-	-	-	-	-	-
3-Methylnonacosane	2971.8	2.88E+08 ± 3.3E+07	2.78E+08 ± 2.1E+07	1.44E+08 ± 3.4E+07	1.99E+08 ± 3.1E+07	1.55E+07 ± 1.6E+06	1.99E+07 ± 2.4E+06	8.37E+07 ± 1.8E+07	1.35E+08 ± 5.2E+07	-	-
Triacosane	2994.8	2.46E+07 ± 3.7E+06	2.56E+07 ± 6.0E+06	2.93E+07 ± 4.8E+06	5.63E+07 ± 1.1E+07	-	-	8.22E+06 ± 7.1E+05	2.28E+07 ± 4.1E+06	-	-
3,7-, 3,9-, 3,11-, 3,13- Dimethyltriacosanes	3003.2	1.53E+08 ± 1.6E+07	1.50E+08 ± 1.8E+07	5.82E+07 ± 1.4E+07	1.19E+08 ± 2.0E+07	-	-	-	-	-	-
10-, 12- Methyltriacosanes	3027.8	3.13E+07 ± 4.6E+06	3.00E+07 ± 7.7E+06	4.74E+07 ± 1.1E+07	6.25E+07 ± 4.4E+06	-	-	-	-	-	-
8-Methyltriacosane	3034.2	3.97E+07 ± 4.7E+06	4.41E+07 ± 9.3E+06	2.99E+07 ± 7.1E+06	2.38E+07 ± 4.9E+06	-	-	-	-	-	-
2-, 4-methyltriacosanes	3059.5	4.92E+07 ± 6.3E+06	5.20E+07 ± 1.2E+07	5.76E+07 ± 9.2E+06	7.62E+07 ± 7.9E+06	1.05E+07 ± 1.4E+06	1.31E+07 ± 1.9E+06	1.56E+07 ± 1.4E+06	3.12E+07 ± 4.5E+06	-	-
Henitriacosene	3074.1	2.72E+08 ± 3.3E+07	2.69E+08 ± 2.4E+07	4.91E+08 ± 1.0E+08	6.20E+08 ± 9.4E+07	8.01E+06 ± 9.8E+05	1.03E+07 ± 1.0E+06	1.85E+07 ± 2.4E+06	4.09E+07 ± 7.7E+06	-	-
Henitriacosane	3096.4	1.19E+08 ± 1.8E+07	1.03E+08 ± 6.6E+06	1.67E+08 ± 4.0E+07	2.08E+08 ± 1.2E+07	1.87E+07 ± 1.7E+06	2.46E+07 ± 1.1E+06	2.13E+07 ± 2.1E+06	5.52E+07 ± 9.8E+06	-	-
9-, 11-, 13-, 15- Methylhentriacosanes <sup>A</sup> + unknown compound <sup>B</sup>	3129.9	2.37E+08 ± 2.9E+07 <sup>A</sup>	3.05E+08 ± 3.5E+07 <sup>A</sup>	7.93E+08 ± 1.1E+08 <sup>A,B</sup>	1.12E+09 ± 1.4E+08 <sup>A,B</sup>	2.05E+07 ± 2.4E+06 <sup>A</sup>	2.68E+07 ± 2.5E+06 <sup>A</sup>	2.29E+08 ± 2.0E+07 <sup>A,B</sup>	-	-	-
7-Methylhentriacosane	3137.7	2.91E+07 ± 3.6E+06	1.08E+07 ± 4.0E+06	6.46E+07 ± 1.4E+07	-	2.81E+06 ± 2.0E+05	3.60E+06 ± 4.6E+05	-	-	-	-
5-Methylhentriacosane	3149.7	1.79E+07 ± 3.2E+06	6.33E+06 ± 2.3E+06	-	-	3.67E+06 ± 3.1E+05	3.63E+06 ± 3.9E+05	-	-	-	-
2- Methylhentriacosane <sup>A</sup> + Octadecyl dodecanoate <sup>B</sup> + Hexadecyl tetradecanoate <sup>C</sup>	3163.6	9.36E+07 ± 1.2E+07 <sup>A</sup>	9.81E+07 ± 1.3E+07 <sup>A</sup>	8.51E+08 ± 1.8E+08 <sup>A</sup>	3.41E+09 ± 5.2E+08 <sup>A,B,C</sup>	8.78E+06 ± 9.2E+05 <sup>A</sup>	9.71E+06 ± 1.1E+06 <sup>A</sup>	3.73E+08 ± 4.0E+07 <sup>B,C</sup>	5.08E+09 ± 7.1E+08 <sup>B,C</sup>	7.67E+09 <sup>B,C</sup>	1.08E+10 ± 9.1E+08 <sup>B,C</sup>
3-Methylhentriacosane	3172.3	1.85E+08 ± 2.7E+07	1.64E+08 ± 1.1E+07	2.46E+08 ± 5.0E+07	2.87E+08 ± 1.7E+07	1.75E+07 ± 1.8E+06	2.13E+07 ± 1.6E+06	2.62E+07 ± 2.3E+06	3.55E+07 ± 3.1E+06	-	-
Dotriacosane	3200.2	5.25E+06 ± 8.8E+05	3.33E+07 ± 4.2E+06	1.99E+07 ± 4.9E+06	4.66E+07 ± 8.6E+06	-	-	-	-	-	-
2-Methyldotriacosane	3260.1	2.64E+07 ± 6.5E+06	2.21E+07 ± 5.7E+06	3.65E+07 ± 6.0E+06	5.61E+07 ± 7.4E+06	-	-	-	-	-	-
Tritricosene	3275.9	1.34E+08 ± 1.9E+07	1.08E+08 ± 9.1E+06	4.69E+08 ± 1.1E+08	5.74E+08 ± 6.3E+07	1.63E+07 ± 2.6E+06	1.28E+07 ± 1.2E+06	3.21E+07 ± 4.6E+06	7.95E+07 ± 1.7E+07	-	-
Tritricosane	3296.6	2.14E+07 ± 6.6E+06	1.67E+07 ± 3.6E+06	5.59E+07 ± 1.4E+07	7.40E+07 ± 9.3E+06	7.42E+06 ± 1.0E+06	4.15E+06 ± 3.5E+05	7.10E+06 ± 1.6E+06	2.70E+07 ± 1.3E+07	-	-
7-, 9-, 11-, 13- and 15- Methyltriacosanes <sup>A</sup> + unknown compound <sup>B</sup>	3329.3	8.33E+07 ± 1.8E+07 <sup>A</sup>	5.99E+07 ± 8.5E+06 <sup>A</sup>	2.34E+08 ± 4.9E+07 <sup>A,B</sup>	4.84E+08 ± 4.1E+07 <sup>A,B</sup>	1.18E+07 ± 2.3E+06 <sup>A</sup>	1.25E+07 ± 1.1E+06 <sup>A</sup>	3.01E+07 ± 4.7E+06 <sup>A,B</sup>	-	-	-
11,15-, 13,17-, 15,19- Dimethyltriacosanes	3356.6	4.21E+07 ± 1.2E+07	2.85E+07 ± 3.4E+06	-	-	-	-	-	-	-	-
3-Methyltriacosane	3365.7	1.67E+07 ± 7.6E+06	8.92E+06 ± 2.1E+06	5.52E+07 ± 2.6E+07	-	-	-	-	-	-	-
Octadecyl tetradecanoate	3376.7	-	-	2.15E+08 ± 4.6E+07	2.21E+09 ± 3.8E+08	-	-	9.64E+07 ± 1.1E+07	3.71E+09 ± 7.4E+08	6.10E+09	1.16E+10 ± 8.2E+08
Pentatriacosene	3477.6	-	-	2.66E+07 ± 8.5E+06	6.06E+07 ± 9.6E+06	-	-	-	-	-	-
11-, 13-, 15- and 17- Methylpentatriacosanes	3533.6	2.05E+07 ± 3.7E+06	1.37E+07 ± 1.9E+06	4.79E+07 ± 8.3E+06	-	-	-	-	-	-	-
Tetradecyl eicosanoate <sup>A</sup> + Octadecyl hexadecanoate <sup>B</sup> + Eicosyl tetradecanoate <sup>C</sup>	3565.0	-	-	1.43E+08 ± 3.6E+07 <sup>A</sup>	9.31E+08 ± 1.5E+08 <sup>A,B,C</sup>	-	-	7.59E+07 ± 4.8E+06 <sup>A</sup>	9.31E+08 ± 1.5E+08 <sup>A,B,C</sup>	2.27E+09 <sup>A,B,C</sup>	4.93E+09 ± 5.5E+08 <sup>A,B,C</sup>
11-, 13- Methylheptatriacosanes	3728.7	3.65E+07 ± 5.7E+06	2.48E+07 ± 3.0E+06	8.71E+07 ± 1.9E+07	2.21E+08 ± 4.0E+07	-	-	8.71E+07 ± 1.9E+07	-	-	-
Hexadecyl eicosanoate	3767.4	-	-	-	5.05E+08 ± 8.2E+07	-	-	-	1.05E+09 ± 2.4E+08	1.15E+09	1.81E+09 ± 1.6E+08

**Supplementary table 1. Compound table of housefly cuticular extracts.** Full table of tentatively identified compounds in cuticular hexane extracts. First column shows compound names. Second columns shows Kovats Retention index. The relative amount of each compound in each sample type is given in peak area abundance  $\pm$  Standard error of the mean. Parenthesis after each sample type is the number of samples. A compound had to appear in all 5 samples to be included. Unidentified compounds were not included.

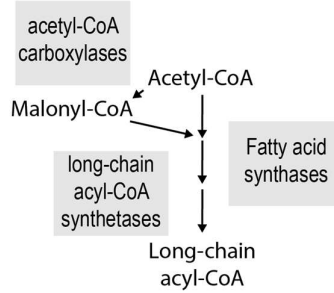
## Supplementary table 2

Compound name	Kovats RI (DB-WAX column)	Uninfected females (5)	Uninfected males (5)	Sporulating females (5)	Sporulating males (5)	Conidia from females (3)	Conidia from males (2)
Sesquiterpene 1	1393	-	-	8.40E+06 ± 3.0E+06	8.56E+06 ± 3.1E+06	-	-
Sesquiterpene 2	1399	-	-	4.99E+06 ± 2.2E+05	5.50E+06 ± 7.2E+05	-	-
Sesquiterpene 3	1415	3.76E+06 ± 1.5E+06	-	1.46E+08 ± 6.2E+07	1.67E+08 ± 5.6E+07	6.75E+06 ± 1.3E06	5.73E+06
Ethyl octanoate	1436	-	-	2.81E+07 ± 2.0E+07	5.76E+06 ± 2.2E+06	-	-
Sesquiterpene 4	1472	-	-	1.31E+06 ± 6.0E+05	1.27E+06 ± 5.5E+05	-	-
Sesquiterpene 5	1496	-	-	1.31E+07 ± 4.0E+06	3.56E+07 ± 1.6E+07	-	-
Sesquiterpene 6	1549	-	-	1.89E+06 ± 8.1E+05	2.38E+06 ± 1.1E+06	-	-
Sesquiterpene 7	1636	-	-	4.44E+06 ± 1.7E+06	2.97E+06 ± 1.2E+06	-	-
Sesquiterpene 8	1673	-	-	3.55E+05 ± 5.0E+04	3.95E+05 ± 5.5E+04	-	-
Unknown compound 1	1758	-	-	5.04E+06 ± 3.4E+06	4.68E+06 ± 2.8E+06	-	-
Unknown compound 2	1873	-	-	1.44E+07 ± 6.9E+06	1.52E+07 ± 6.4E+06	-	-
Unknown	1886	-	-	1.79E+07 ± 4.0E+06	5.19E+07 ± 1.9E+07	-	-
1-Phenylethyl alcohol	1922	-	5.17E+06 ± 3.1E+06	-	-	-	-
Unknown compound 3	1931	-	-	4.73E+05 ± 7.0E+04	3.72E+06 ± 2.0E+06	-	-
Unknown compound 4	1980	-	-	5.38E+06 ± 2.6E+06	1.26E+06 ± 2.5E+05	-	-
Unknown compound 5	1984	-	-	1.85E+07 ± 9.5E+06	2.01E+07 ± 9.1E+06	-	-
Unknown compound 6	2033	-	-	2.55E+06 ± 1.4E+06	5.39E+05 ± 1.0E+05	-	-
Unknown compound 7	2039	-	-	6.69E+06 ± 3.0E+06	6.82E+06 ± 2.6E+06	-	-
Unknown compound 8	2050	-	-	8.79E+05 ± 3.2E+05	4.51E+05 ± 1.3E+05	-	-
1-Tetradecyl acetate	2094	-	-	1.85E+07 ± 1.1E+07	3.68E+07 ± 1.9E+07	1.10E+07 ± 2.9E+06	1.52E+07
1-Tetradecanol	2168	3.91E+06 ± 2.1E+06	2.53E+06 ± 1.3E+06	2.33E+07 ± 1.2E+07	2.49E+07 ± 1.2E+07	8.07E+06 ± 1.5E+06	1.15E+07
Unknown compound 9	2226	-	-	3.90E+05 ± 3.0E+04	4.25E+05 ± 6.7E+04	-	-
Diterpene 1	2286	-	-	2.75E+06 ± 7.9E+05	2.66E+06 ± 8.6E+05	-	-
1-Hexadecyl acetate	2300	-	-	9.10E+06 ± 6.3E+06	2.26E+06 ± 1.1E+06	-	-
1-Hexadecanol	2376	-	-	3.79E+06 ± 1.8E+06	4.30E+06 ± 1.7E+06	-	-

**Supplementary table 2. Full table of tentatively identified compounds in headspace samples.** First column shows compound names. Second column shows Kovats Retention index. The relative amount of each compound in each sample type is given in peak area (TIC) ± Standard error of the mean. Parenthesis after each sample type is the number of samples. Compounds appearing in three out of five samples in a given treatment were included.

Supplementary table 3

A



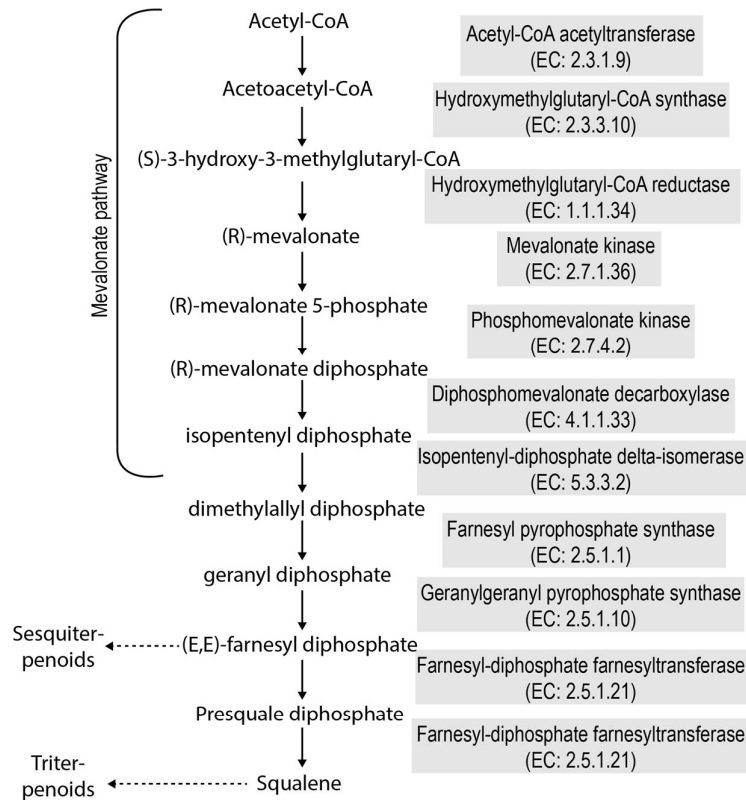
B

	TranscriptID	BLASTPtophit	%Identity	e-value	Kegg	EC	log2FC	FDR_p
Acetyl-CoA carboxylase	TRINITY_DN20838_c2_g2	ACAC_SCHPO	39.3	8.14e-95	K11262	6.4.1.2, 6.3.4.14	2.46	0.0000
	TRINITY_DN9224_c0_g1	ACAC_SCHPO	66.7	6.98e-19	K11262	6.4.1.2, 6.3.4.14	4.19	0.0000
	TRINITY_DN11999_c0_g1	ACAC_SCHPO	37.7	2.04e-61	K11262	6.4.1.2, 6.3.4.14	2.66	0.0000
	TRINITY_DN8745_c0_g1	ACAC_YEAST	70.3	1.59e-117	K11262	6.4.1.2, 6.3.4.14	3.46	0.0001
	TRINITY_DN1774_c0_g1	ACAC_YEAST	44.0	5.42e-49	K11262	6.4.1.2, 6.3.4.14	2.56	0.0002
	TRINITY_DN6921_c0_g1	ACAC_SCHPO	62.7	2.77e-41	K11262	6.4.1.2, 6.3.4.14	4.06	0.0010
	TRINITY_DN5496_c0_g1	ACAC_YEAST	54.6	3.58e-62	K11262	6.4.1.2, 6.3.4.14	3.76	0.0024
	TRINITY_DN10159_c0_g1	ACAC_SCHPO	58.6	3.63e-49	K11262	6.4.1.2, 6.3.4.14	1.38	0.0035
	TRINITY_DN20838_c1_g1	ACAC_SCHPO	31.7	2.92e-15	K11262	6.4.1.2, 6.3.4.14	-0.76	0.0047
	TRINITY_DN21736_c0_g1	ACAC_SCHPO	62.1	3.35e-70	K11262	6.4.1.2, 6.3.4.14	1.69	0.0059
	TRINITY_DN1062_c0_g1	ACAC_SCHPO	53.3	4.62e-40	K11262	6.4.1.2, 6.3.4.14	1.50	0.0174
	TRINITY_DN21389_c0_g1	ACAC_SCHPO	24.1	2.56e-22	K11262	6.4.1.2, 6.3.4.14	0.47	0.0195
	TRINITY_DN20838_c2_g1	ACAC_SCHPO	50.6	0	K11262	6.4.1.2, 6.3.4.14	0.39	0.0568
	TRINITY_DN2573_c0_g1	ACAC_SCHPO	61.3	5.9e-28	K11262	6.4.1.2, 6.3.4.14	1.78	0.0591
	TRINITY_DN2535_c0_g1	ACAC_YEAST	79.2	6.31e-64	K11262	6.4.1.2, 6.3.4.14	2.31	0.0741
TRINITY_DN5348_c0_g1	ACAC_YEAST	75.4	5.07e-66	K11262	6.4.1.2, 6.3.4.14	2.37	0.0925	
Fatty acid synthases FAS1/FAS2	TRINITY_DN21615_c2_g1	FAS2_PENPA	54.4	0	K00667	2.3.1.86	2.45	0.0000
	TRINITY_DN20289_c0_g1	FAS2_EMEND	52.5	0	K00667	2.3.1.86	3.43	0.0000
	TRINITY_DN21615_c2_g3	FAS2_YEAST	65.3	6.1e-36	K00667	2.3.1.86	3.13	0.0000
	TRINITY_DN16765_c0_g1	FAS2_EMEND	64.4	1.62e-133	K00667	2.3.1.86	2.25	0.0000
	TRINITY_DN21615_c0_g1	FAS2_LACKL	53.6	2.14e-35	K00667	2.3.1.86	3.07	0.0014
	TRINITY_DN7801_c0_g1	FAS2_CANAX	81.0	1.63e-39	K00667	2.3.1.86	1.59	0.0017
	TRINITY_DN1128_c0_g1	FAS2_PENPA	76.1	2.49e-32	K00667	2.3.1.86	3.30	0.0154
	TRINITY_DN2844_c0_g1	FAS2_SCHPO	69.6	9.34e-32	K00667	2.3.1.86	2.24	0.1581
	TRINITY_DN21615_c2_g4	FAS1_SCHPO	49.4	0	K00668	2.3.1.86	3.33	0.0000
	TRINITY_DN17570_c0_g1	FAS1_SCHPO	58.1	9.62e-149	K00668	2.3.1.86	3.99	0.0000
	TRINITY_DN12197_c0_g1	FAS1_YEAST	49.3	4.23e-83	K00668	2.3.1.86	3.75	0.0000
	TRINITY_DN17406_c0_g1	FAS1_SCHPO	51.5	4.78e-115	K00668	2.3.1.86	4.82	0.0000
	TRINITY_DN15402_c0_g1	FAS1_YARLI	39.8	1.57e-61	K00668	2.3.1.86	4.65	0.0000
	TRINITY_DN14812_c0_g1	FAS1_CANAX	36.2	1.26e-37	K00668	2.3.1.86	3.98	0.0002
	TRINITY_DN3608_c0_g1	FAS1_YEAST	50.7	3.45e-20	K00668	2.3.1.86	2.57	0.0831
TRINITY_DN5417_c0_g1	FAS1_SCHPO	63.6	4.8e-37	K00668	2.3.1.86	2.93	0.1534	
long-chain acyl-CoA synthetase	TRINITY_DN17778_c0_g2	LCF2_YEAST	31.1	3.06e-49	K01897	6.2.1.3	1.49	0.0000
	TRINITY_DN25342_c0_g1	LCF2_SCHPO	34.8	1.13e-136	K01897	6.2.1.3	1.70	0.0000
	TRINITY_DN19153_c0_g2	LCF2_SCHPO	38.3	4.99e-164	K01897	6.2.1.3	0.87	0.0000
	TRINITY_DN17671_c0_g1	LCF2_YEAST	25.7	1.99e-43	K01897	6.2.1.3	0.54	0.0000
	TRINITY_DN17778_c0_g1	LCF2_YEAST	35.6	2.46e-39	K01897	6.2.1.3	-0.50	0.0002
	TRINITY_DN19147_c0_g1	LCF2_YEAST	35.6	3.89e-110	K01897	6.2.1.3	-0.28	0.0004
	TRINITY_DN20446_c3_g2	LCF2_SCHPO	33.3	1.76e-121	K01897	6.2.1.3	4.30	0.0008
	TRINITY_DN21065_c0_g2	LCF2_YEAST	31.4	3.05e-95	K01897	6.2.1.3	0.61	0.0047
	TRINITY_DN20446_c3_g1	LCF1_YEAST	37.1	6.00e-106	K01897	6.2.1.3	4.21	0.2630
	TRINITY_DN19131_c0_g1	LCF2_YEAST	33.4	5.81e-88	K01897	6.2.1.3	-0.12	0.5653

Supplementary table 3. Fatty acid gene expression in *E. muscae*. **A.** Schematic drawing of the general fatty acid synthesis pathway in fungi. **B.** Expressed *E. muscae* transcripts annotated as either Acetyl-CoA carboxylase, Fatty acid synthases, or long-chain acyl-CoA synthetase enzymes. Fungal transcripts written in bold are significantly higher expressed in late-stage sporulating cadavers vs. early-stage sporulating cadavers.

**Supplementary table 4**

**A**



**B**

TranscriptID	BLASTPtophit	%identity	e-value	Name	Kegg	EC	log2FC	FDR_p
TRINITY_DN14635_c0_g1	THIL_SCHPO	61.3	6.31e-82	Acetyl-CoA acetyltransferase	K00626	2.3.1.9	0.86	0.0031
TRINITY_DN2357_c0_g2	THIL_YARLI	52.1	4.89e-123	Acetyl-CoA acetyltransferase	K00626	2.3.1.9	-0.10	0.6482
TRINITY_DN18770_c0_g1	THIL_YARLI	40.7	1.47e-73	Acetyl-CoA acetyltransferase	K00626	2.3.1.9	-0.37	0.0500
TRINITY_DN14635_c0_g2	THIA_CANTR	55.5	3.63e-67	Acetyl-CoA acetyltransferase	K00626	2.3.1.9	-0.62	0.0312
TRINITY_DN2357_c0_g1	THIL_YARLI	50.6	1.08e-119	Acetyl-CoA acetyltransferase	K00626	2.3.1.9	-1.63	0.0000
TRINITY_DN17308_c1_g1	HMCS_YEAST	51.4	4.33e-154	Hydroxymethylglutaryl-CoA synthase	K01641	2.3.3.10	0.41	0.0000
TRINITY_DN20557_c0_g1	HMDH2_YEAST	44.1	0	3-hydroxy-3-methylglutaryl-coenzyme A reductase 2 (hydroxymethylglutaryl-CoA reductase (NADPH))	K00021	1.1.1.34	0.50	0.0001
TRINITY_DN18020_c0_g1	KIME_SCHPO	33.2	2.17e-36	Putative mevalonate kinase	K00869	2.7.1.36	-0.50	0.0004
TRINITY_DN15321_c0_g1	ERG8_SCHPO	33.2	1.96e-50	Probable phosphomevalonate kinase	K00938	2.7.4.2	0.57	0.0000
TRINITY_DN27786_c0_g1	MVD_GANLU	56.8	9.41e-139	Diphosphomevalonate decarboxylase	K01597	4.1.1.33	-0.04	0.7858
TRINITY_DN17598_c0_g1	IDI1_SCHPO	58.7	7.94e-87	Isopentenyl-diphosphate Delta-isomerase	K01823	5.3.3.2	0.02	0.9016
TRINITY_DN16421_c0_g1	FPPS_KLULA	55.5	1.78e-140	Farnesyl pyrophosphate synthase	K00787	2.5.1.1, 2.5.1.10	0.24	0.0926
TRINITY_DN18257_c1_g1	GGPPS_MUCCL	52.7	7.51e-108	Geranylgeranyl pyrophosphate synthase	K00804	2.5.1.1, 2.5.1.10, 2.5.1.29	0.16	0.1063
TRINITY_DN14336_c0_g1	GGPPS_MUCCL	64.2	5.27e-42	Geranylgeranyl pyrophosphate synthase	K00804	2.5.1.1, 2.5.1.10, 2.5.1.29	0.01	0.9633
TRINITY_DN16883_c0_g1	FDFT_SCHPO	52.1	5.65e-123	Farnesyl-diphosphate farnesyltransferase	K00801	2.5.1.21	-0.46	0.6100

**Supplementary table 4. Mevalonate and terpenoid synthesis gene expression in *E. muscae*.** **A.** Schematic drawing of the general terpenoid synthesis pathway in fungi. **B.** Expressed *E. muscae* transcripts annotated as enzymes in the general terpenoid synthesis pathway in fungi. Annotation, blastp results, and expression in late stage sporulating cadavers vs. early stage cadavers are given.

## Supplementary table 5

A

TranscriptID	BLASTPtophit	Query/Hit_coverage	%Identity	e-value	Name	Kegg	EC	log2FC	FDR_p
TRINITY_DN19029_c0_g1	PANB_EMENI	Q:736-1566,H:62-340	51.613	3.02e-84	3-methyl-2-oxobutanoate	K00606	2.1.2.11	-0.38	0.0617
<b>TRINITY_DN8643_c0_g1</b>	<b>YM60_YEAST</b>	<b>Q:925-362,H:207-397</b>	<b>27.586</b>	<b>1.36e-16</b>	<b>Putative esterase</b>	<b>K07019</b>	-	<b>2.96</b>	<b>0.0000</b>
TRINITY_DN19826_c0_g1	YM60_YEAST	Q:1519-410,H:8-413	29.71	2.82e-52	Putative esterase	K07019	-	0.42	0.0007
TRINITY_DN18612_c0_g1	YM60_YEAST	Q:314-1420,H:33-413	29.095	1.32e-42	Putative esterase	K07019	-	0.21	0.2334

B

Query = <i>S. cerevisiae</i> eeb1 (GU471249.1)		
TranscriptID	Score (bits)	e-value
TRINITY_DN18612_c0_g1	102.0	2.00e-38
TRINITY_DN19826_c0_g1	91.8	1.00e-33
TRINITY_DN8643_c0_g1	46.4	9.00e-12

Query = <i>S. cerevisiae</i> mgl2 (YMR210W)		
TranscriptID	Score (bits)	e-value
TRINITY_DN19826_c0_g1	106.0	3.00e-46
TRINITY_DN18612_c0_g1	105.0	3.00e-40
TRINITY_DN8643_c0_g1	52.4	2.00e-13

Query = <i>S. cerevisiae</i> eht1 (AB012577.1)		
TranscriptID	Score (bits)	e-value
TRINITY_DN19826_c0_g1	96.4	3.00e-30
TRINITY_DN18612_c0_g1	97.3	6.00e-29
TRINITY_DN19029_c0_g1	88.1	3.00e-17
TRINITY_DN8643_c0_g1	49.6	5.00e-07

### Supplementary table 5. Expressed *E. muscae* transcripts with homology to ethyl ester biosynthesis genes.

A. Four *E. muscae* transcripts with homology to the yeast *Saccharomyces cerevisiae* ethyl ester biosynthesis genes eht1 and eeb1 specifically involved in ethyl octanoate biosynthesis. Results of Blastp searches and expression in late-stage sporulating cadavers vs. early-stage sporulating cadavers are shown. **A.** Results of Blastp searches with the yeast ethyl ester biosynthesis genes eht1, eeb1, and mgl2 against the *E. muscae* translated transcripts

Supplementary table 6

Late sporulation vs. Late control	RefSeq Acc.	log2FC	padj
Musca domestica cytochrome P450 4g1-like (LOC101887882), mRNA	NM_001286897.1	-12.16	1.89259E-22
PREDICTED: Musca domestica fatty acid synthase (LOC101893120), mRNA	XM_005175727.3	-11.28	3.58E-12
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101894220), mRNA	XM_005186257.3	-10.17	1.79111E-08
PREDICTED: Musca domestica apolipoporphins (LOC101887937), mRNA	XM_005177378.3	-10.13	5.80409E-17
PREDICTED: stearoyl-CoA desaturase 5 [Musca domestica]	XM_020035426.1	-9.27	6.09374E-20
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101894391), mRNA	XM_011295377.2	-9.11	2.84943E-06
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101891740), mRNA	XM_005176936.3	-7.91	8.26901E-23
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X1, mRNA	XM_005188297.3	-7.90	0.00013416
PREDICTED: acetyl-CoA carboxylase isoform X4 [Musca domestica]	XM_011293554.2	-7.86	2.16833E-05
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X2, mRNA	XM_011296188.2	-7.77	0.000253628
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X3, mRNA	XM_020038489.1	-6.56	0.0269019
PREDICTED: acyl-CoA Delta(11) desaturase isoform X2 [Musca domestica]	XM_011295002.2	-5.92	0.03097972
PREDICTED: stearoyl-CoA desaturase 5 [Musca domestica]	XM_005179649.3	-5.91	1.74128E-07
PREDICTED: acetyl-CoA carboxylase isoform X5 [Musca domestica]	XM_020036198.1	-5.02	0.156581617
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101900313), mRNA	XM_005180061.3	-4.39	0.005026714
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_005185154.3	-4.19	4.06585E-07
PREDICTED: acyl-CoA Delta(11) desaturase isoform X1 [Musca domestica]	XM_020037419.1	-4.11	0.001316354
PREDICTED: Musca domestica cytochrome P450 4g1 (LOC101887550), mRNA	XM_005176292.3	-3.86	0.040126949
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101895407), mRNA	XM_005175978.3	-3.71	8.97821E-05
PREDICTED: Musca domestica cytochrome P450 4g1-like (LOC101888923), mRNA	XM_005176300.3	-3.57	0.47584952
PREDICTED: acetyl-CoA carboxylase isoform X1 [Musca domestica]	XM_005181944.3	-3.43	0.161531896
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900725), mRNA	XM_005182153.3	-2.99	0.061633958
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900725), mRNA	XM_005182153.3	-2.99	0.061633958
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101898308), mRNA	XM_005180049.3	-2.97	2.07232E-06
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101890000), mRNA	XM_005188530.2	-2.77	0.407822353
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101890887), mRNA	XM_005183981.3	-2.52	0.047549704
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101896175), mRNA	XM_005184011.3	-2.42	6.67739E-06
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101889437), mRNA	XM_005189195.3	-1.93	0.108928013
PREDICTED: Musca domestica fatty acid synthase (LOC101888614), mRNA	XM_020038884.1	-1.80	0.000896846
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895587), transcript variant X1, mRNA	XM_005175979.3	-1.75	0.210803962
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101889946), mRNA	XM_005189198.3	-1.40	0.000416809
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101898132), mRNA	XM_005180048.3	-1.39	0.014521176



PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101900903), transcript variant X2, mRNA	XM_011293655.2	-1.32	0.602050254
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101889237), mRNA	XM_005182172.3	-1.19	0.442596133
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X4, mRNA	XM_011296189.2	-1.03	0.545062285
PREDICTED: acetyl-CoA carboxylase isoform X3 [Musca domestica]	XM_011293553.2	-0.96	0.046922987
PREDICTED: Musca domestica cytochrome P450 4g1 (LOC101889105), mRNA	XM_005176301.3	-0.93	0.298771972
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900323), mRNA	XM_011293999.2	-0.91	0.539496046
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101892601), mRNA	XM_005176941.3	-0.27	0.366099761
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101892601), mRNA	XM_005176941.3	-0.27	0.366099761
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_005185155.3	0.12	0.792109614
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_020037334.1	0.14	0.792109614
PREDICTED: Musca domestica farnesyl pyrophosphate synthase (LOC101895546), mRNA	XM_005182378.3	0.36	0.107435616
PREDICTED: acetyl-CoA carboxylase isoform X2 [Musca domestica]	XM_020036197.1	0.46	0.556112607

<b>Early sporulation vs. Early control</b>	<b>RefSeq Acc.</b>	<b>log2FC</b>	<b>padj</b>
PREDICTED: Musca domestica apolipoporphins (LOC101887937), mRNA	XM_005177378.3	-8.75	9.31909E-12
Musca domestica cytochrome P450 4g1-like (LOC101887882), mRNA	NM_001286897.1	-8.49	8.14829E-11
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101894220), mRNA	XM_005186257.3	-7.20	0.00027315
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X2, mRNA	XM_011296188.2	-6.98	0.002849196
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101894391), mRNA	XM_011295377.2	-6.79	0.001633083
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101900313), mRNA	XM_005180061.3	-6.71	2.42625E-05
PREDICTED: acyl-CoA Delta(11) desaturase isoform X2 [Musca domestica]	XM_011295002.2	-6.41	0.041009908
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101891740), mRNA	XM_005176936.3	-5.56	3.50215E-16
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101895407), mRNA	XM_005175978.3	-4.96	2.37481E-07
PREDICTED: stearoyl-CoA desaturase 5 [Musca domestica]	XM_020035426.1	-3.73	1.53157E-09
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101900903), transcript variant X2, mRNA	XM_011293655.2	-3.36	0.217299133
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101890000), mRNA	XM_005188530.2	-3.30	0.445818878
PREDICTED: acetyl-CoA carboxylase isoform X5 [Musca domestica]	XM_020036198.1	-3.23	0.511742809
PREDICTED: stearoyl-CoA desaturase 5 [Musca domestica]	XM_005179649.3	-3.22	0.006325195
PREDICTED: acyl-CoA Delta(11) desaturase isoform X1 [Musca domestica]	XM_020037419.1	-3.18	0.015854541
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_005185154.3	-2.89	0.001904492
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101889237), mRNA	XM_005182172.3	-2.64	0.112915655
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101898308), mRNA	XM_005180049.3	-1.91	0.003587708
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101896175), mRNA	XM_005184011.3	-1.72	0.003019074
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101890887), mRNA	XM_005183981.3	-1.68	0.331271747

PREDICTED: Musca domestica cytochrome P450 4g1 (LOC101887550), mRNA	XM_005176292.3	-1.64	0.558297926
PREDICTED: acetyl-CoA carboxylase isoform X4 [Musca domestica]	XM_011293554.2	-1.41	0.554000921
PREDICTED: acetyl-CoA carboxylase isoform X1 [Musca domestica]	XM_005181944.3	-1.38	0.708316717
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900725), mRNA	XM_005182153.3	-1.37	0.543449079
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900725), mRNA	XM_005182153.3	-1.37	0.543449079
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101892601), mRNA	XM_005176941.3	-1.22	2.26354E-05
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG8306 (LOC101892601), mRNA	XM_005176941.3	-1.22	2.26354E-05
PREDICTED: Musca domestica fatty acid synthase (LOC101888614), mRNA	XM_020038884.1	-1.10	0.09881781
PREDICTED: Musca domestica cytochrome P450 4g1 (LOC101889105), mRNA	XM_005176301.3	-0.99	0.397097265
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895587), transcript variant X1, mRNA	XM_005175979.3	-0.95	0.610179693
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X1, mRNA	XM_005188297.3	-0.74	0.818216359
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_020037334.1	-0.74	0.223020832
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101889437), mRNA	XM_005189195.3	-0.42	0.8363287
PREDICTED: acyl-CoA Delta(11) desaturase [Musca domestica]	XM_005185155.3	-0.31	0.604796698
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101898132), mRNA	XM_005180048.3	-0.23	0.806483462
PREDICTED: acetyl-CoA carboxylase isoform X2 [Musca domestica]	XM_020036197.1	-0.20	0.876850597
PREDICTED: Musca domestica fatty acyl-CoA reductase wat (LOC101889946), mRNA	XM_005189198.3	-0.04	0.960622847
PREDICTED: Musca domestica cytochrome P450 4g1-like (LOC101888923), mRNA	XM_005176300.3	0.00	1
PREDICTED: Musca domestica farnesyl pyrophosphate synthase (LOC101895546), mRNA	XM_005182378.3	0.03	0.948399537
PREDICTED: acetyl-CoA carboxylase isoform X3 [Musca domestica]	XM_011293553.2	0.06	0.952812318
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X3, mRNA	XM_020038489.1	0.10	0.99267009
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101895795), transcript variant X4, mRNA	XM_011296189.2	0.38	0.893147991
PREDICTED: Musca domestica fatty acid synthase (LOC101893120), mRNA	XM_005175727.3	0.41	0.886139873
PREDICTED: Musca domestica putative fatty acyl-CoA reductase CG5065 (LOC101900323), mRNA	XM_011293999.2	0.64	0.772041035

**Supplementary table 6. Housefly gene expression of selected cuticular hydrocarbon biosynthesis related genes.** Tables are sorted by log<sub>2</sub> Fold Change. Top table: Late *E. muscae* sporulating female house flies compared to late uninfected control female house flies. Bottom table: Early sporulation *E. muscae* female house flies compared to early uninfected control female house flies.

### **Supplementary video 1**

[https://sid.erda.dk/share\\_redirect/hIekF7iOhD](https://sid.erda.dk/share_redirect/hIekF7iOhD)

Timelapse video of sporulating female house fly. The video spans 24 hours.

To view video: Follow the link. This will download the videofile.  
This file can be played with a media player (tested with VLC media player & Windows media player).

### **Supplementary video 2**

[https://sid.erda.dk/share\\_redirect/bJf0Bner0Q](https://sid.erda.dk/share_redirect/bJf0Bner0Q)

Video of escaped house flies feeding off conidia on a petri dish lid and bottom. The conidia can be seen as a white powder-like substance in the top of the lid.

To view video: Follow the link. This will download the videofile.  
This file can be played with a media player (tested with VLC media player & Windows media player).