



# Fertility-related volatiles in higher termites



Klára Dolejšová<sup>1,2</sup>, Jana Krasulová<sup>1,2</sup>, Romain Fougeyrollas<sup>3</sup>, David Sillam-Dussès<sup>4,5</sup>, Robert Hanus<sup>1</sup> & Yves Roisin<sup>6</sup>

<sup>1</sup>Chemistry of Social Insects, IOCB, Prague, Czech Republic <sup>2</sup>Faculty of Science, Charles University in Prague, Czech Republic <sup>3</sup>IEES Paris, Université Paris-Est Créteil, France <sup>4</sup>IEES Paris, IRD, Bondy, France <sup>5</sup>LEEC, Université Paris 13, France <sup>6</sup>Evolutionary Biology and Ecology, ULB, Belgium

**Introduction** Fertility signalling is at the heart of the debate on the evolution and maintenance of social hierarchy and reproductive division of labour in insect societies. While in social Hymenoptera, non-volatile cues on the body surface, the cuticular hydrocarbons, receive an increasing support, the situation in termites is less clear. In primitive termites, living in small societies with a direct contact among nestmates, the cuticular hydrocarbons are likely to be involved in fertility signalling as well. However, in populous colonies of advanced termites, the queens more probably announce their presence using volatiles, as evidenced by the recent discovery of the first termite queen pheromone<sup>1</sup>. Here, we report on identification of volatile compounds emitted by queens of three higher termites *Embiratermes neotenicus*, *Silvestritermes holmgreni* and *S. minutus* (Termitidae: Syntermitinae).



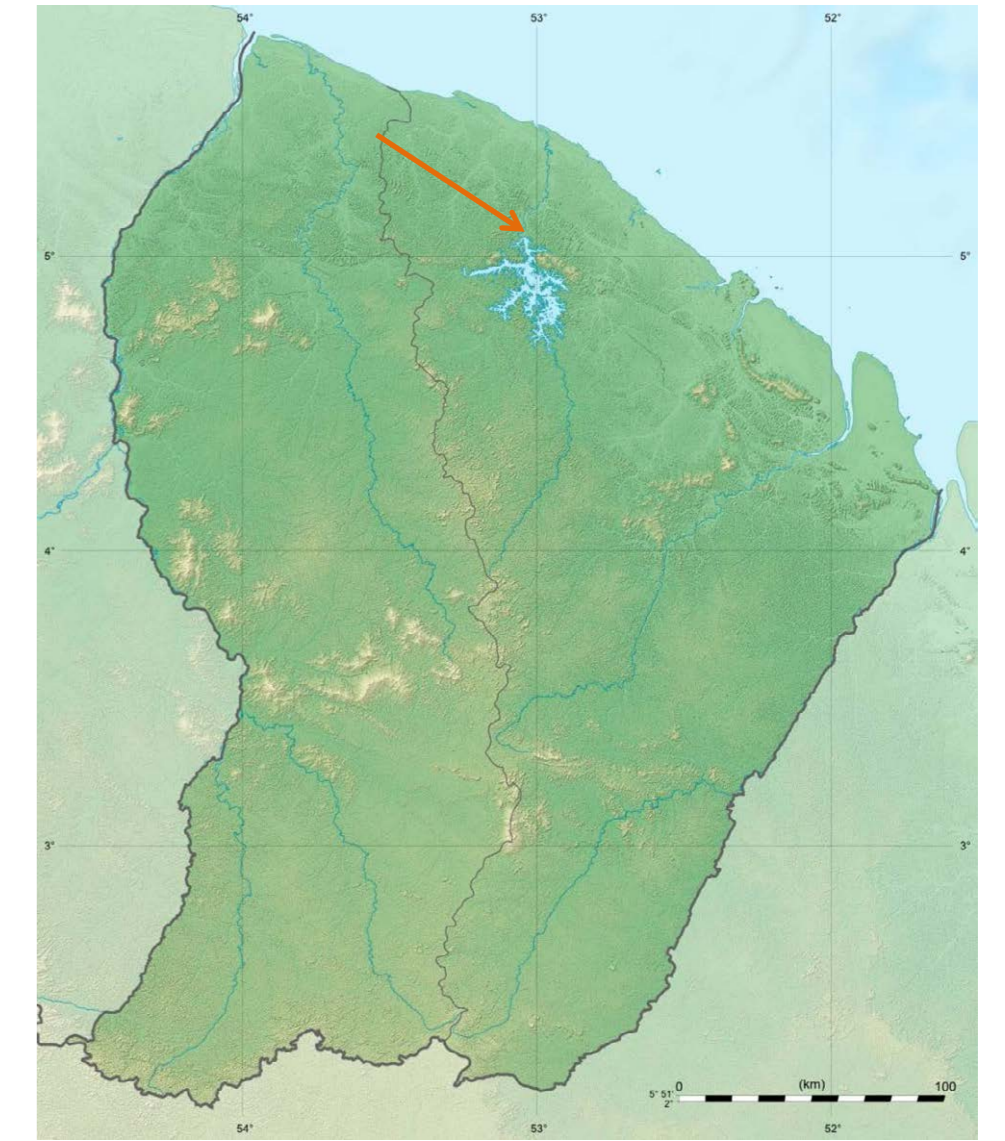
*Embiratermes neotenicus*



*Silvestritermes holmgreni*



*Silvestritermes minutus*

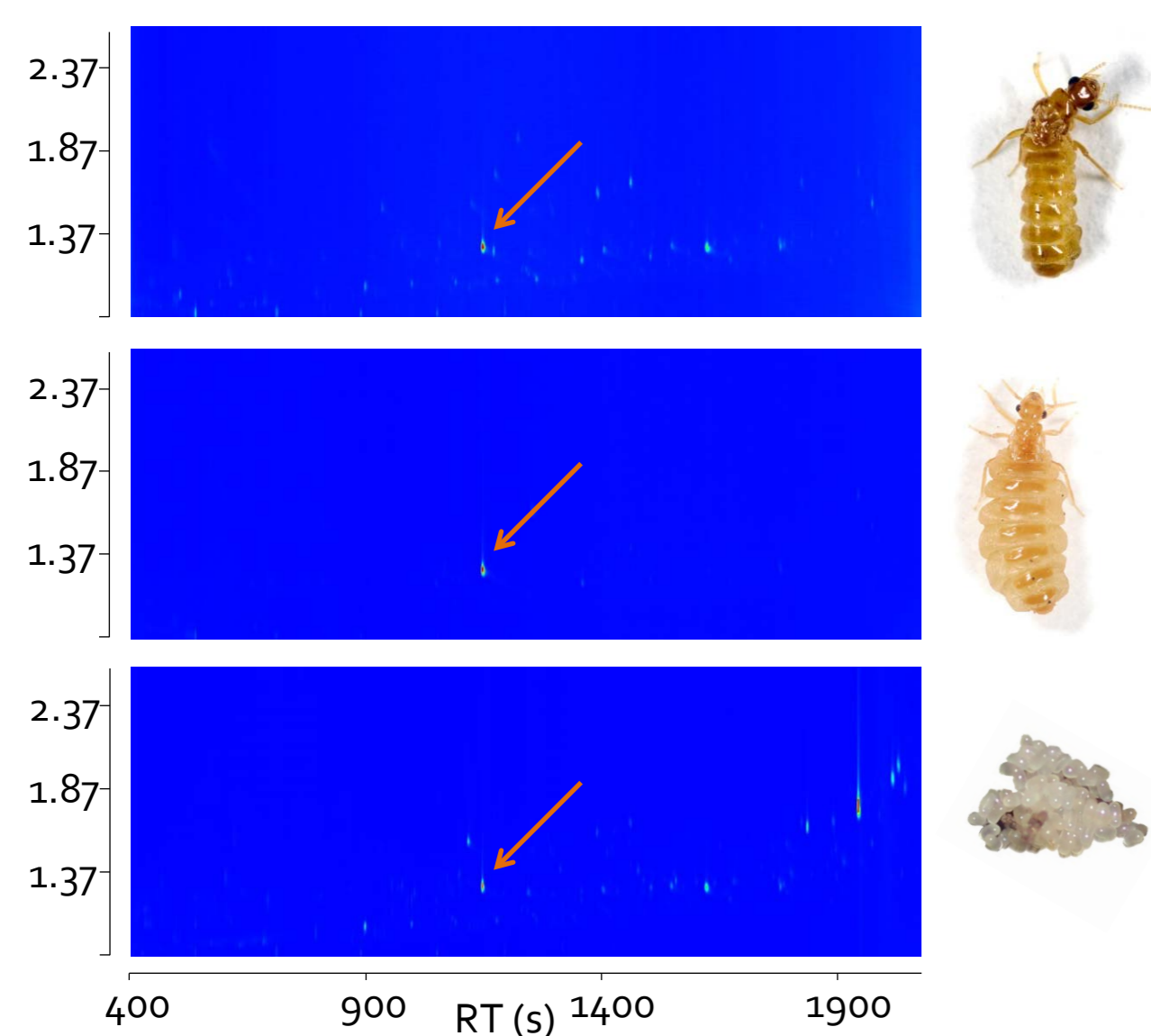


Petit Saut, French Guiana

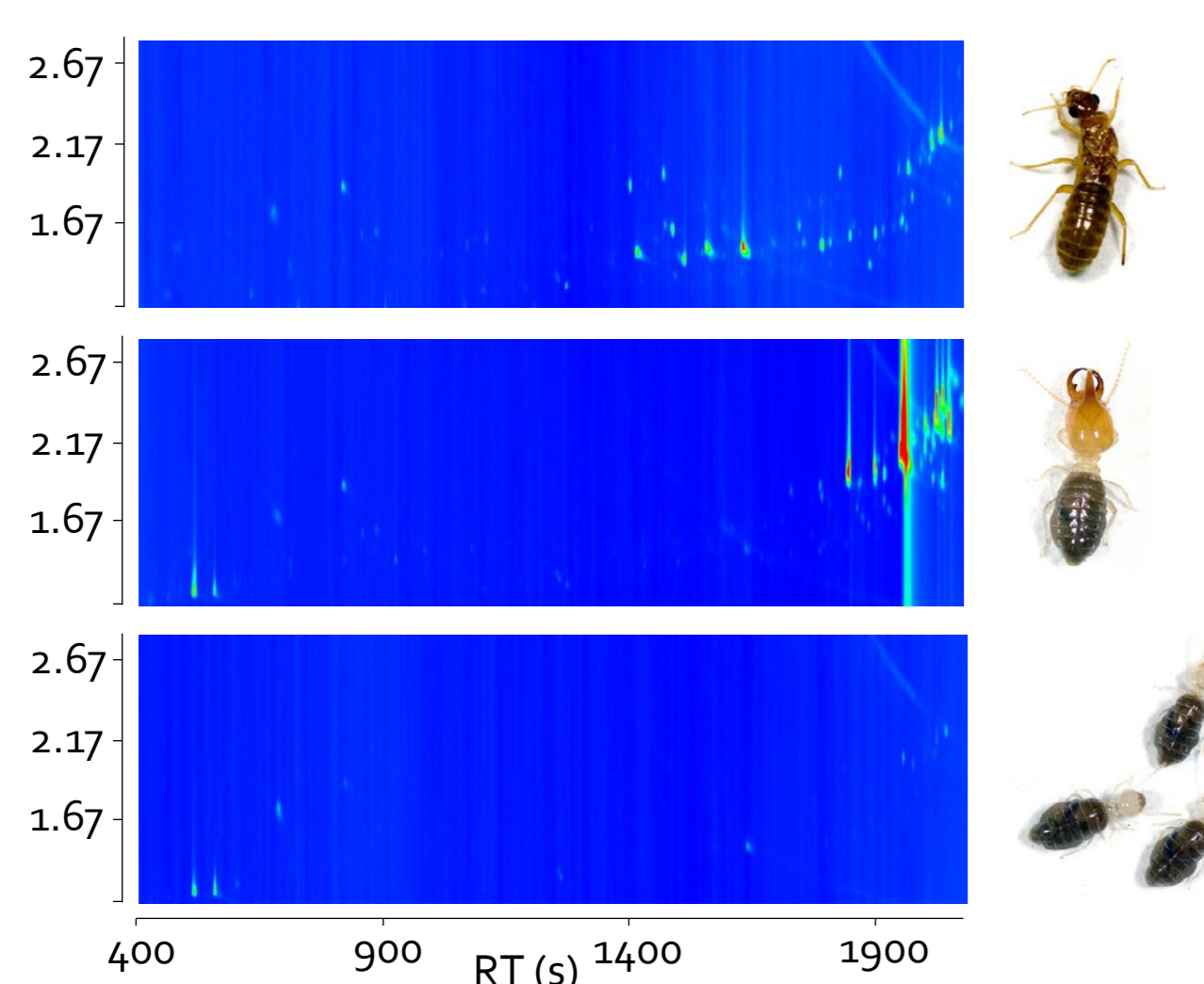
## *Embiratermes neotenicus*

### Chemistry of body washes

In solvent extracts of primary queens, secondary queens and eggs, we detected large quantity of a volatile compound.

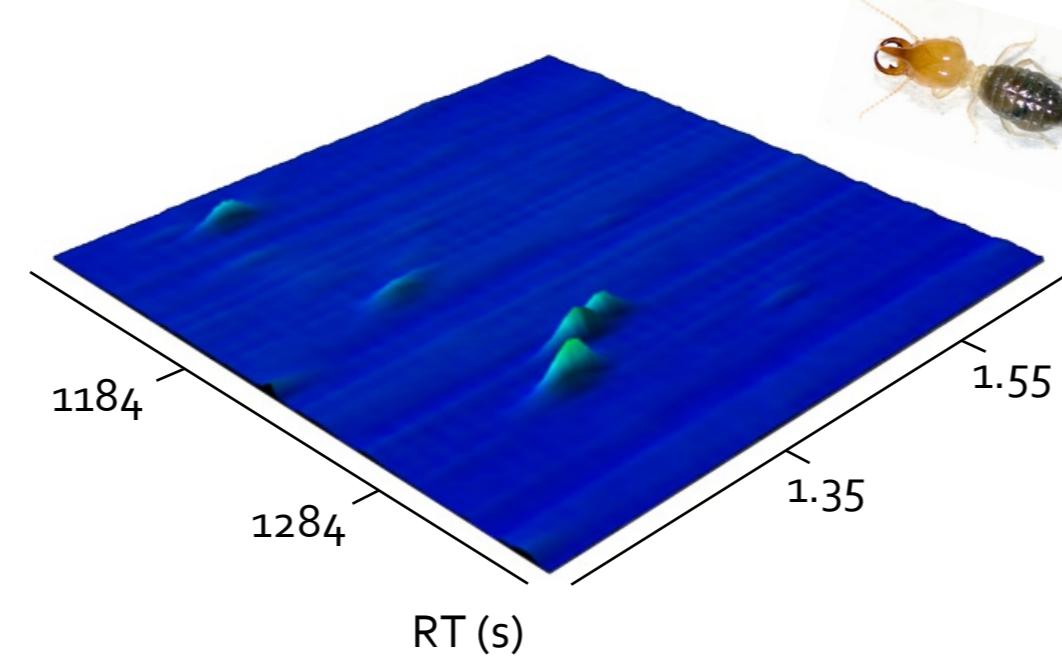
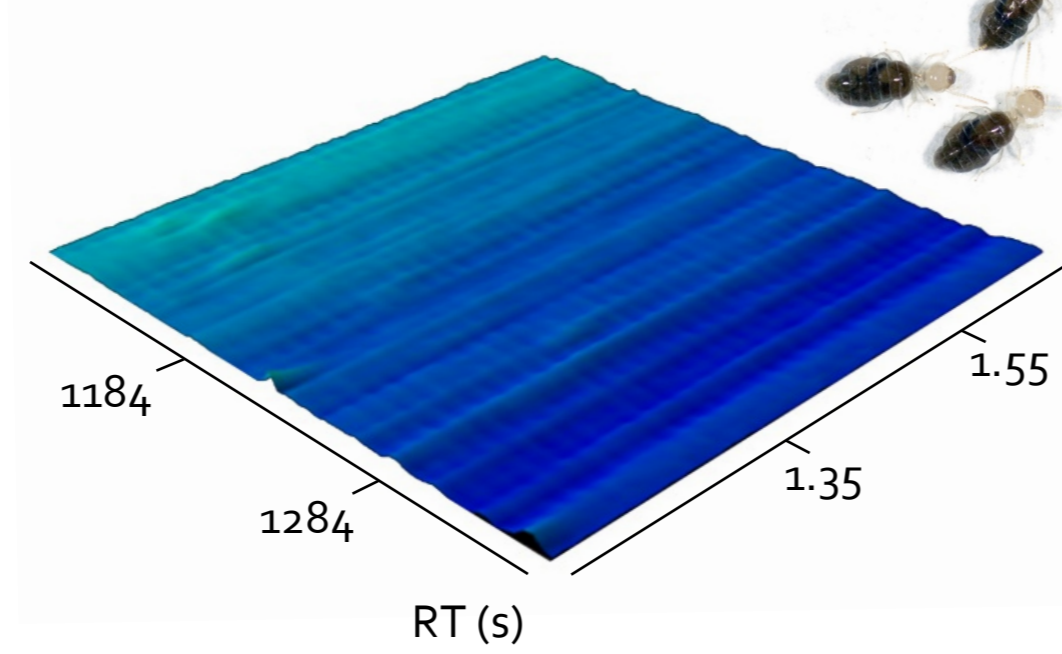
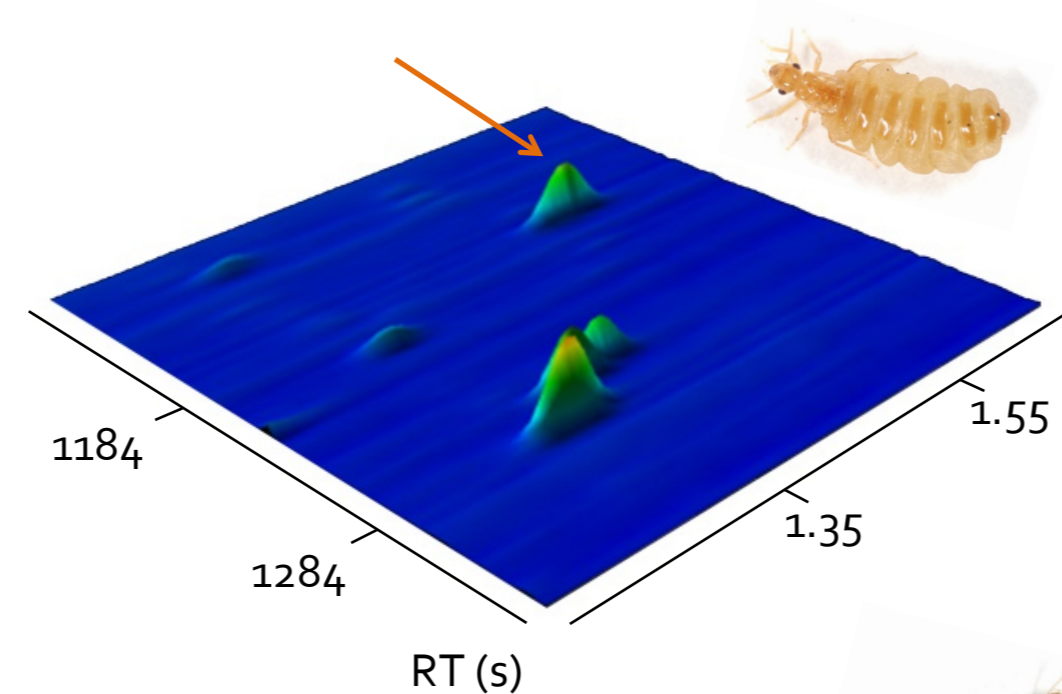


The compound was completely absent in kings and in sterile castes.



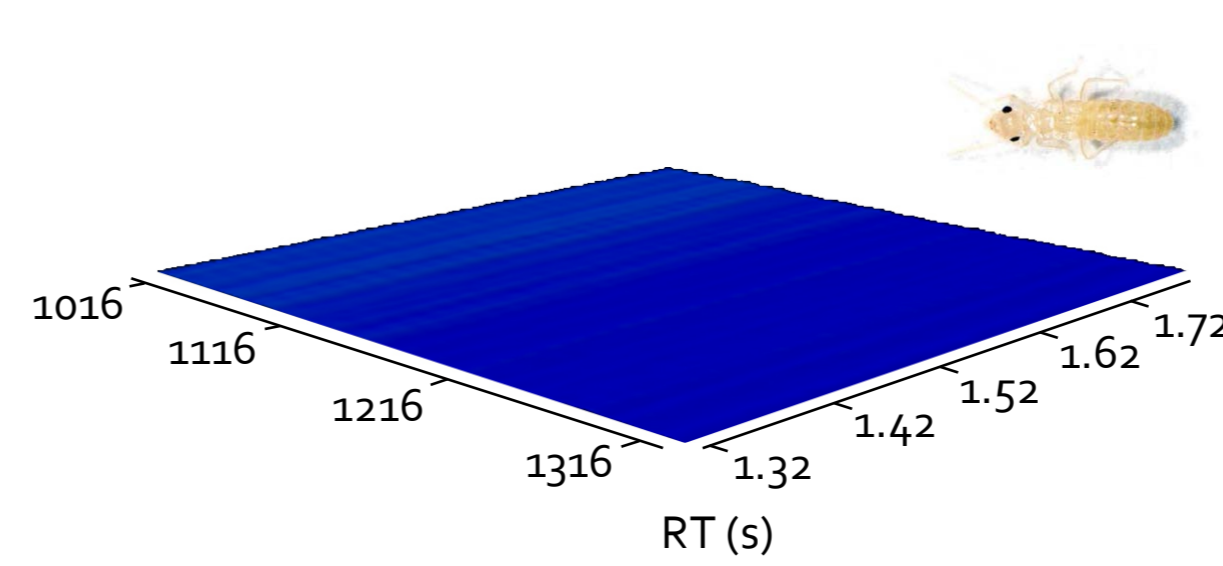
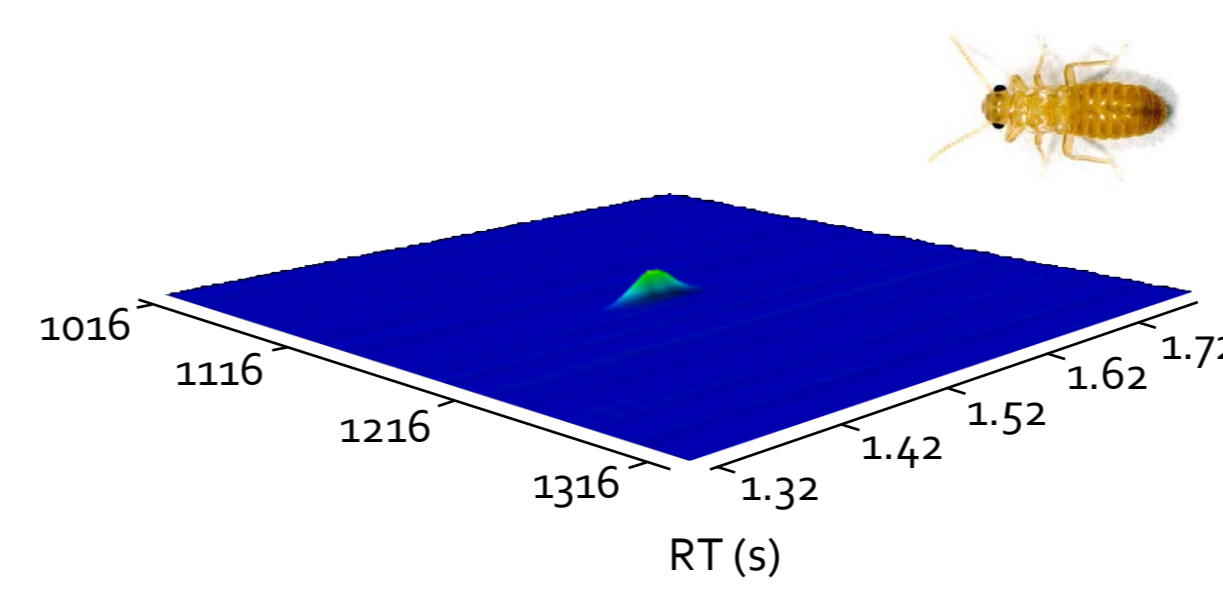
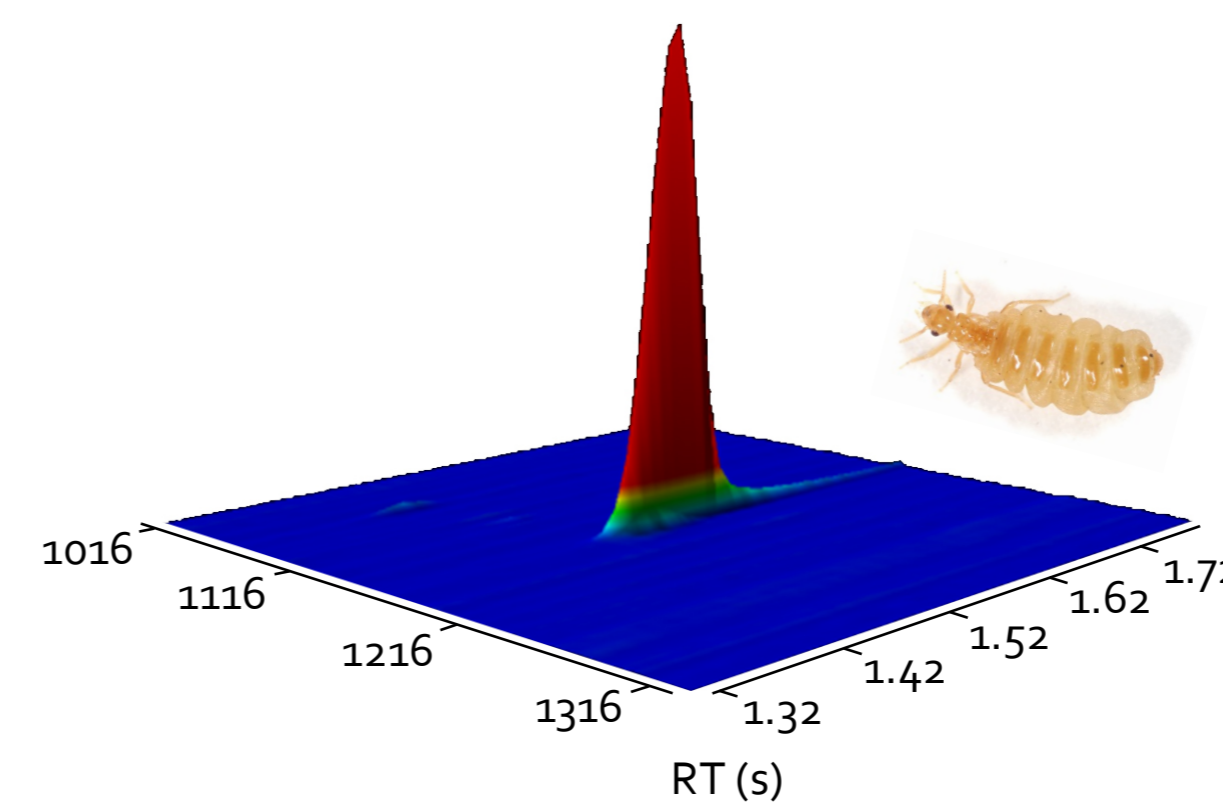
### Headspace analysis

In the next step, we analyzed headspace emanations of the queens and other castes. The same volatile proved to be present in the gaseous emissions of the queens.



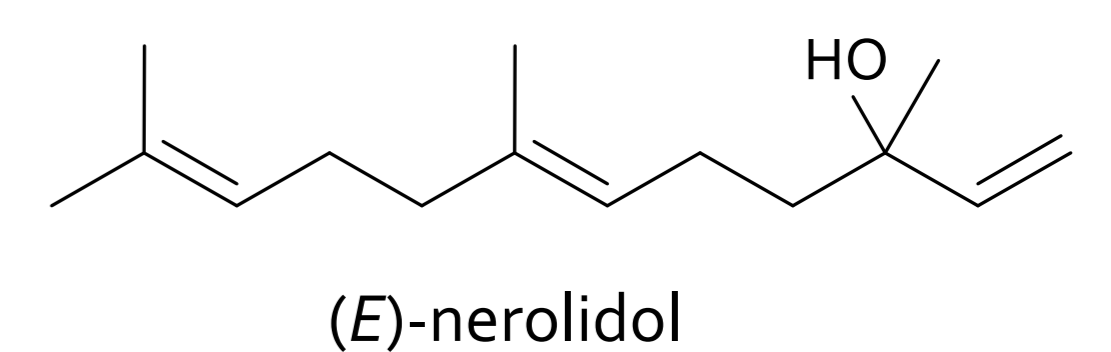
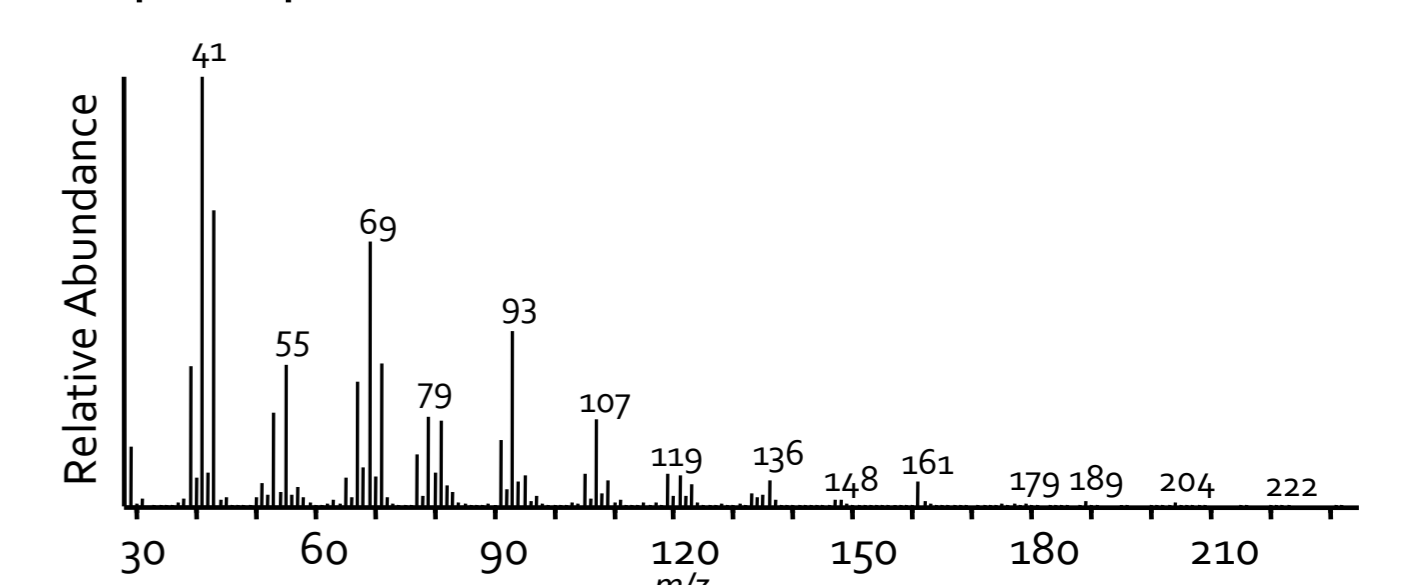
### Fertility-related differences

In young non-breeding queens this volatile was absent and its quantity increased with the level of physogastry of the queens.



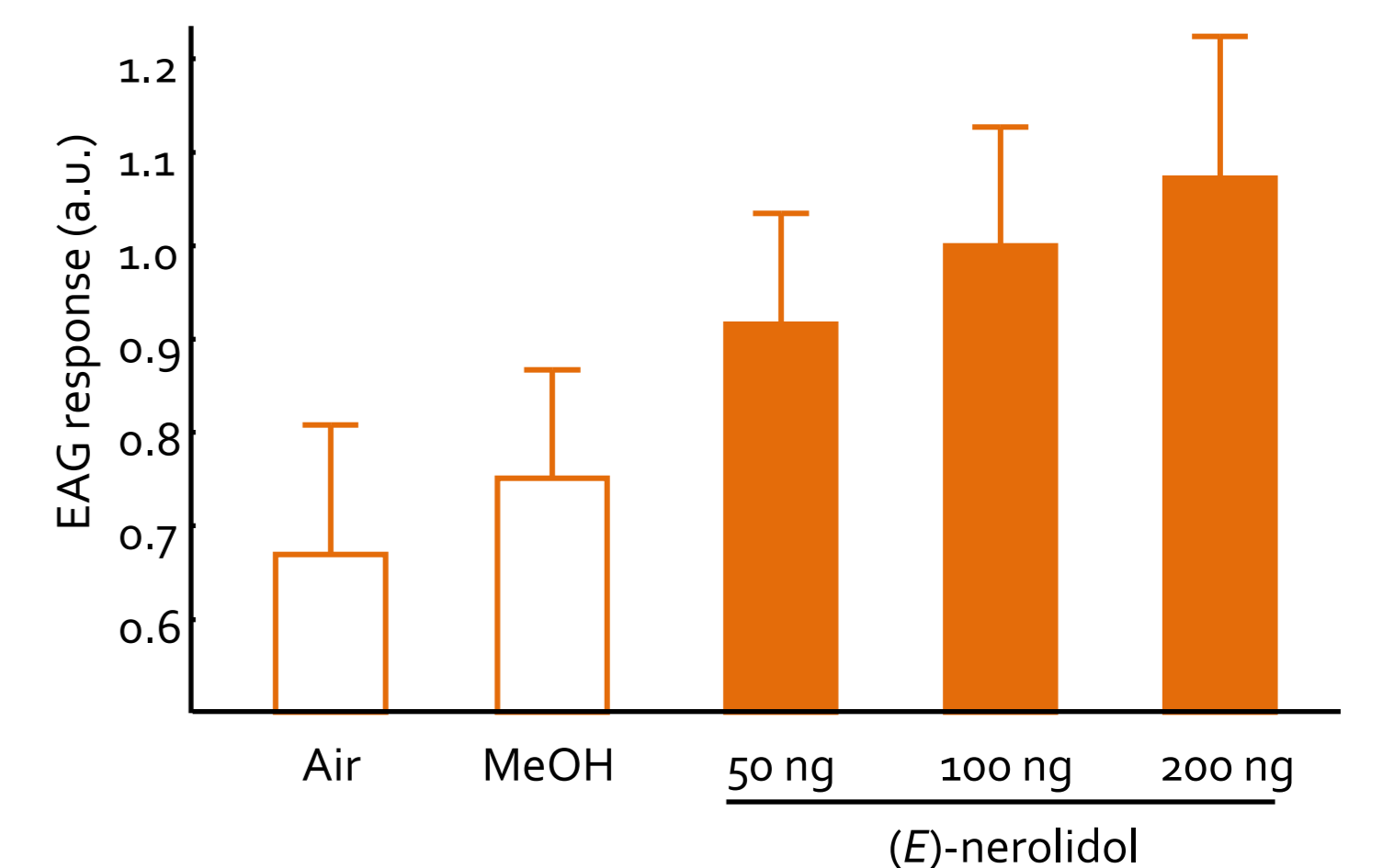
### Chemical identity

Based on its retention characteristics and mass spectra, the volatile compound was identified as sesquiterpene alcohol (*E*)-nerolidol.



### Antennal perception of (*E*)-nerolidol

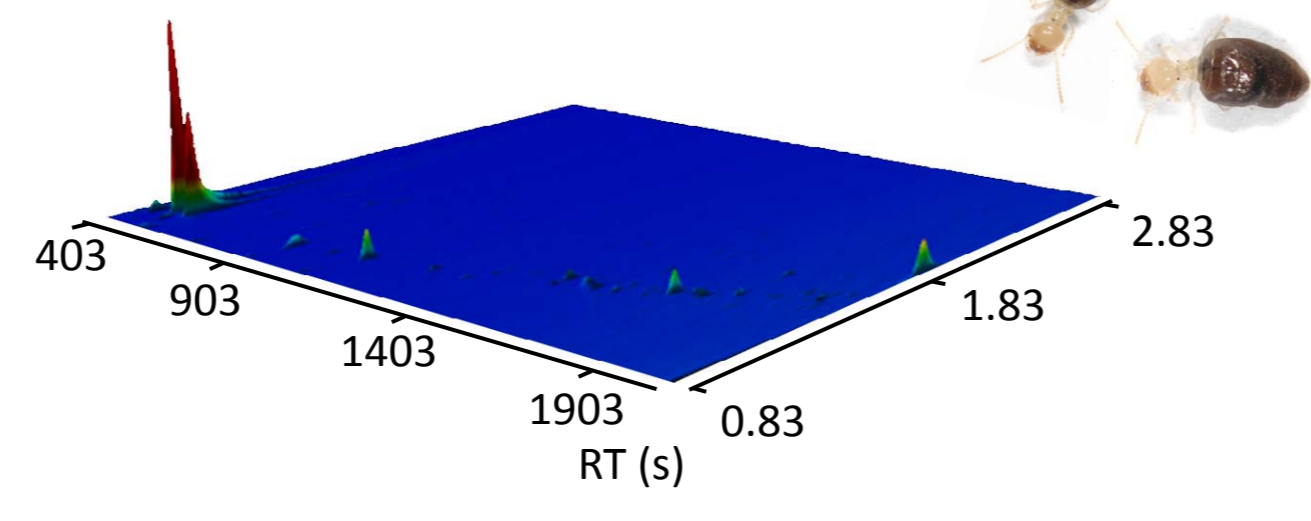
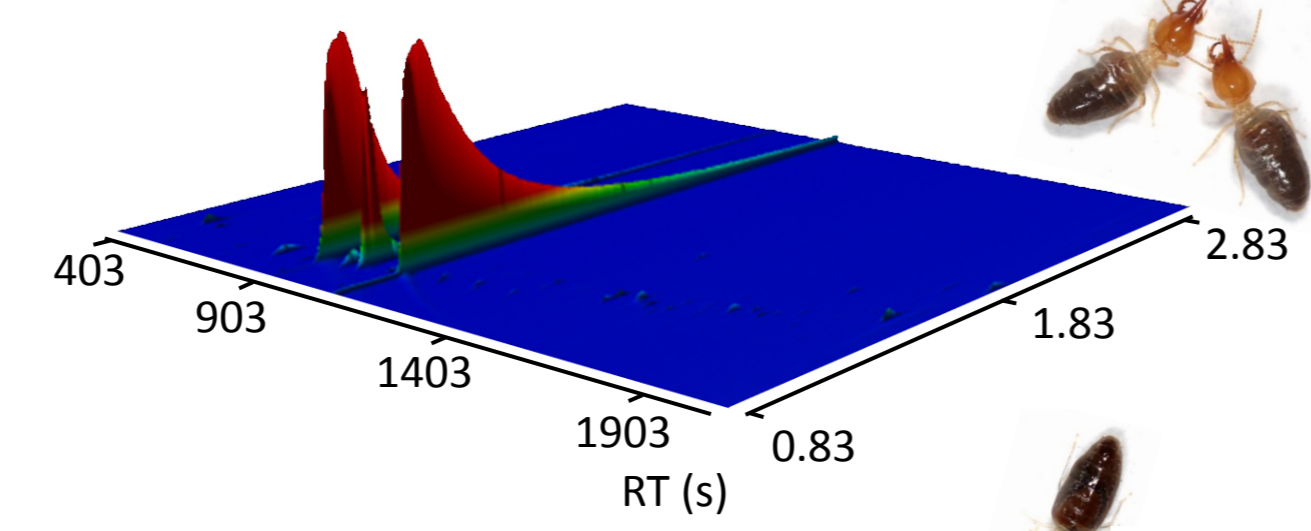
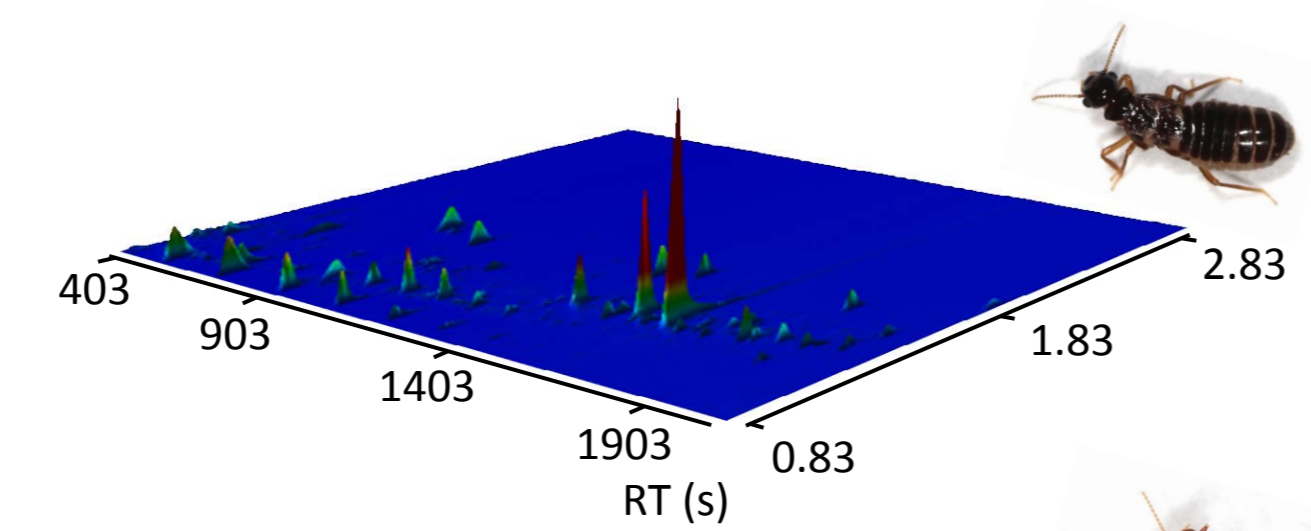
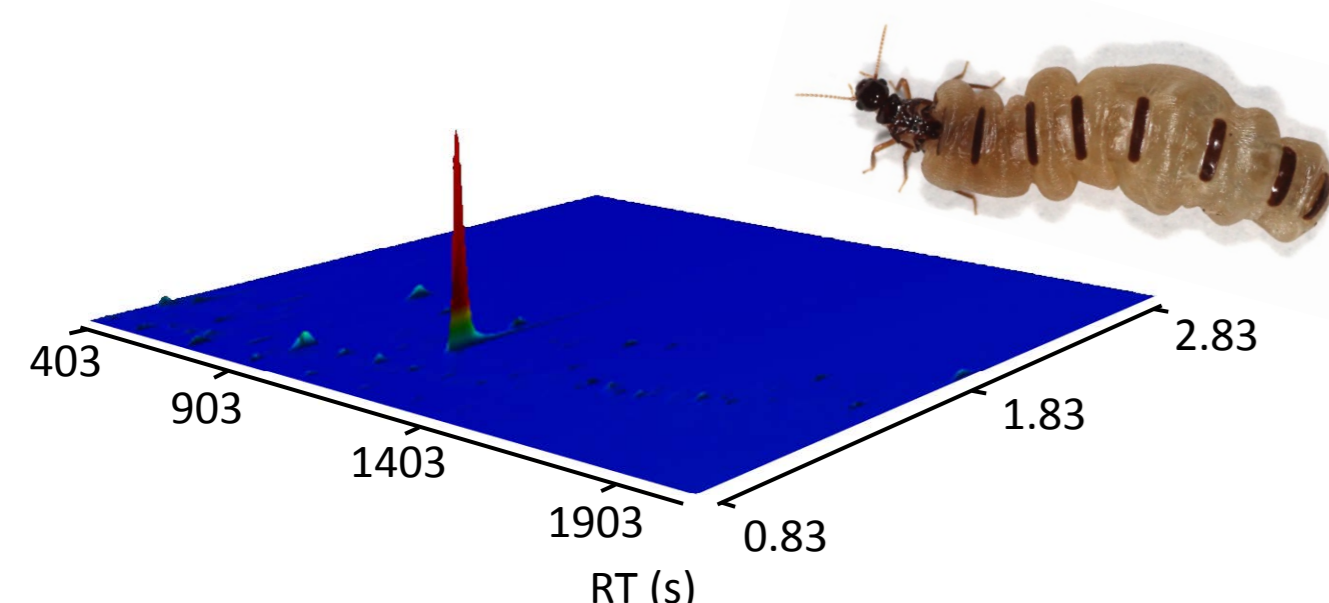
When searching for the function of (*E*)-nerolidol, we performed a series of electroantennographic analyses showing that the compound is perceived by the antennae and brain of workers.



## *Silvestritermes holmgreni*

### Chemistry of body washes

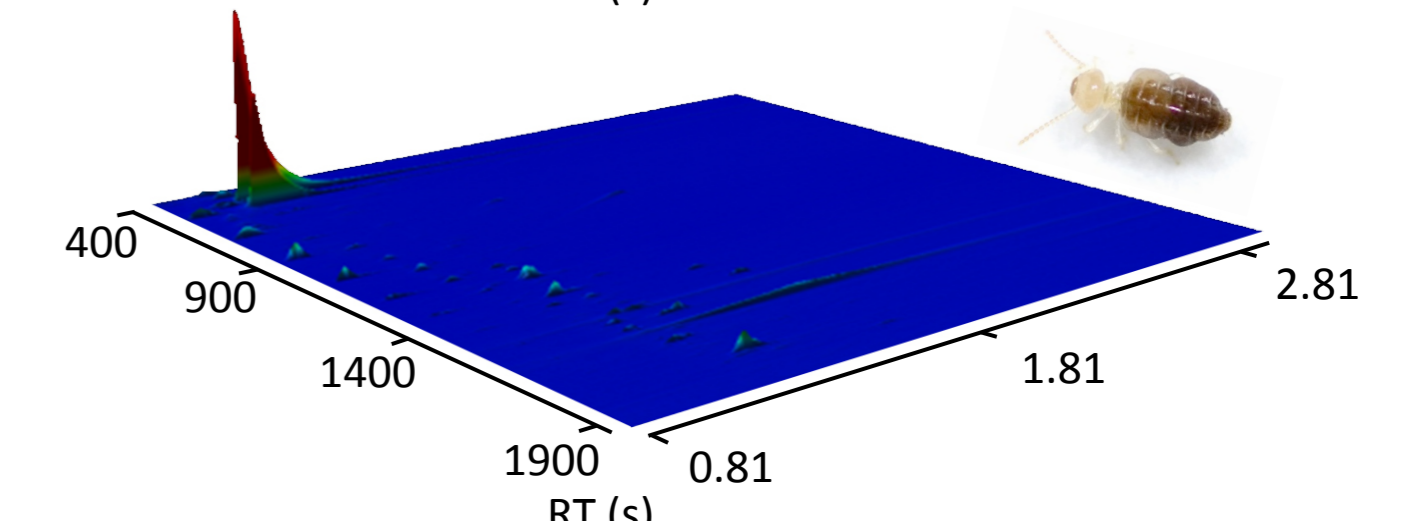
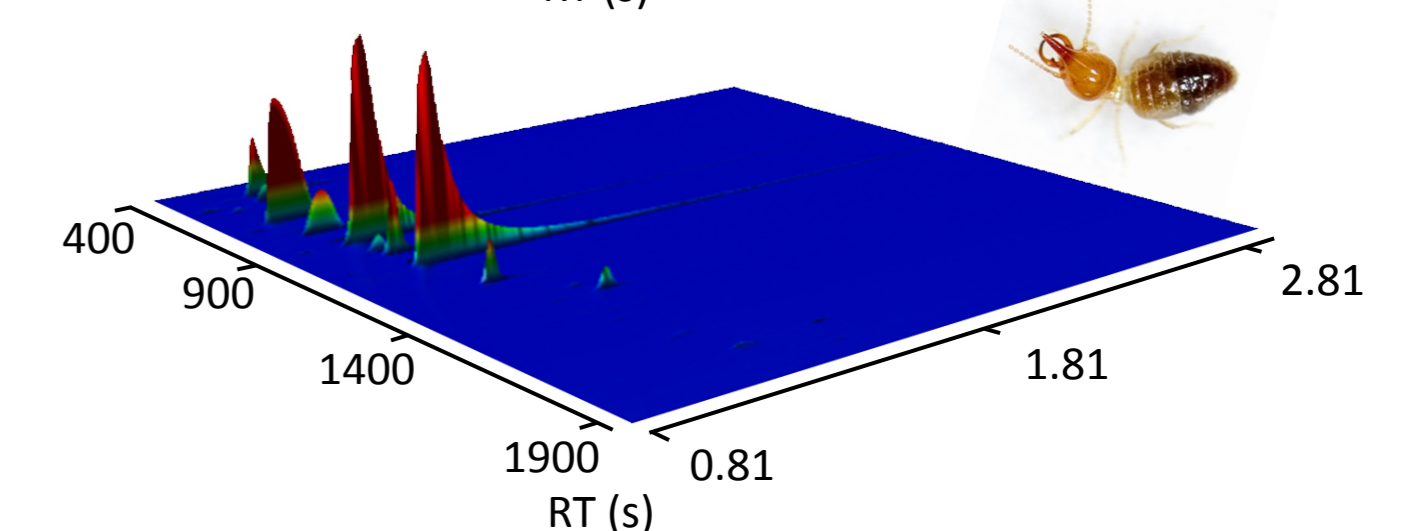
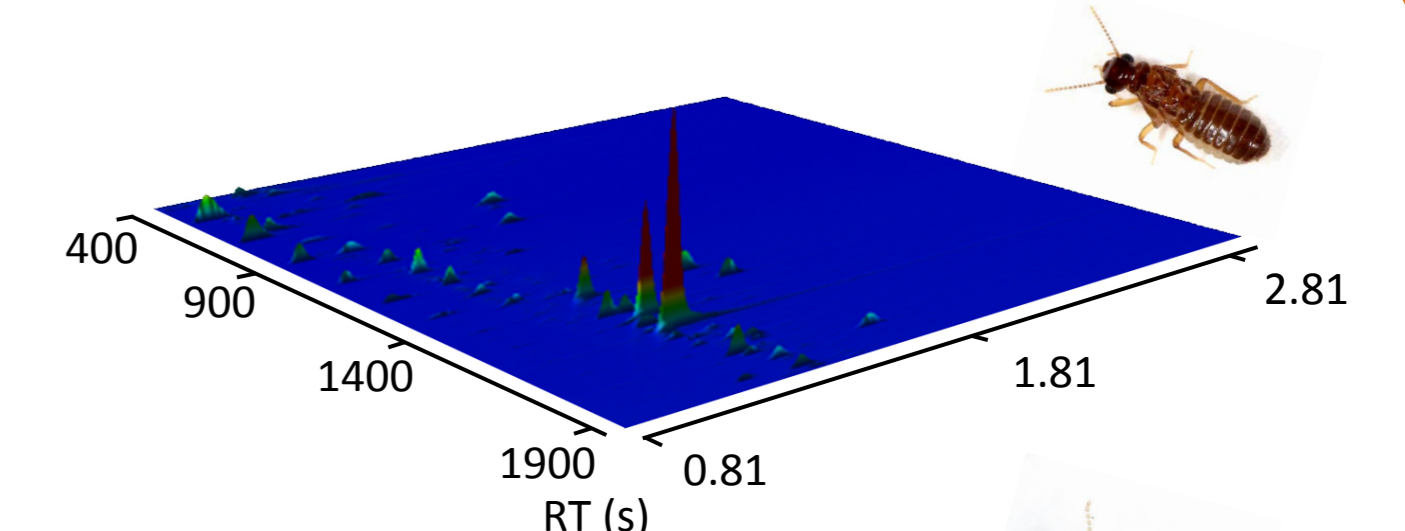
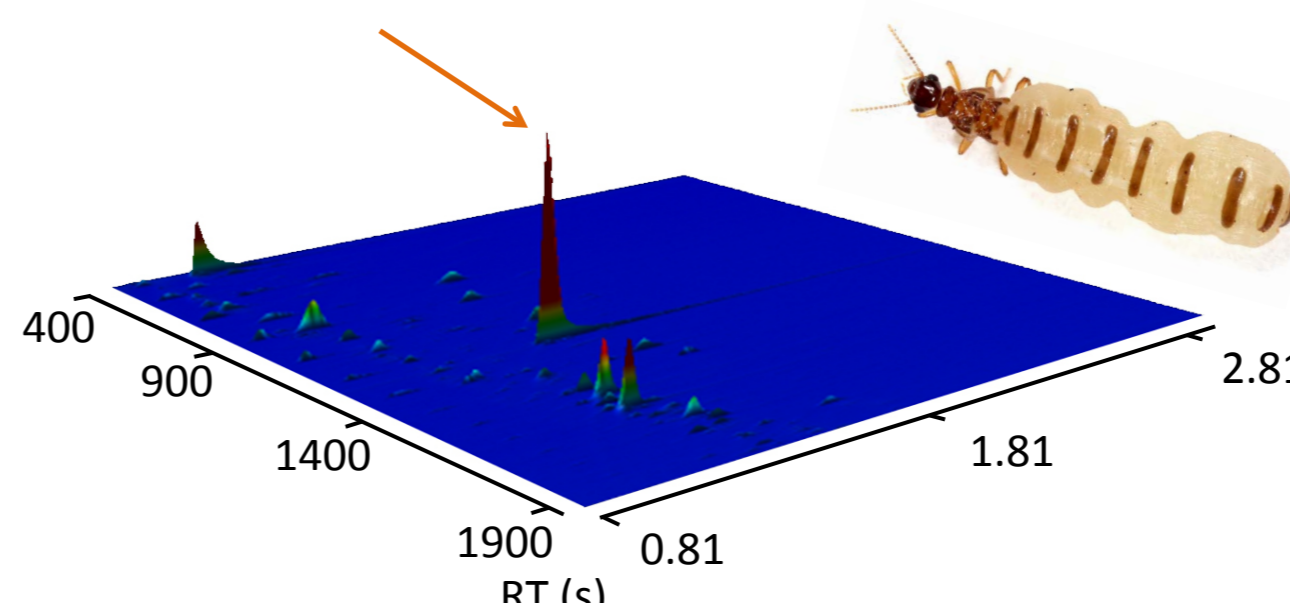
To our surprise, we discovered the same compound, (*E*)-nerolidol, to be produced and emitted exclusively by queens of another syntermitinae, *Silvestritermes* (*Armitermes*) *holmgreni*.



## *Silvestritermes minutus*

### Chemistry of body washes

Another volatile compound, not yet fully identified, has been detected to be produced by the queens of a related species *Silvestritermes* (*Armitermes*) *minutus*. Its retention characteristics and mass spectra suggest that it differs, but not dramatically, in its structure from (*E*)-nerolidol.



**Conclusions** Primary and secondary queens of *Embiratermes neotenicus* produce large amounts of (*E*)-nerolidol, a sesquiterpene alcohol, which is absent in all other castes. The quantity of the compound emitted by the queens appears to be correlated with their reproductive status; it increases with the level of physogastry and thus fertility of the queens while it is absent in young non-breeding queens. Interestingly, we identified this same compound also in the extract of eggs. Our electrophysiological experiments suggest that the compound is perceived by *E. neotenicus* workers. We observed this same volatile to be produced by the queens of another syntermitine, *S. holmgreni*. The queens of the third studied species, *S. minutus*, also produce large amounts of a volatile compound, not yet fully identified, close but not identical in its structure with (*E*)-nerolidol. Multiple functions can be hypothesized for these queen- and egg-specific volatiles, ranging from queen and egg recognition signal to primer pheromone function preventing the nestmates from reproduction. These putative functions are not mutually exclusive and should all be considered in the future research.

**References** <sup>1</sup> Matsura, K., Himuro, C., Yokoi, T., et al. 2010. Identification of a pheromone regulating caste differentiation in termites. PNAS 107: 12963-12968.

**Acknowledgements** We are grateful to the Czech Science Foundation (14-12774S), to the Charles University Grant Agency (580413) and to FNRS Belgium (FRFC grant 2.4594.12).