USING CHEMICAL TOOLS TO DISCRIMINATE *TAPINOMA* SPECIES.

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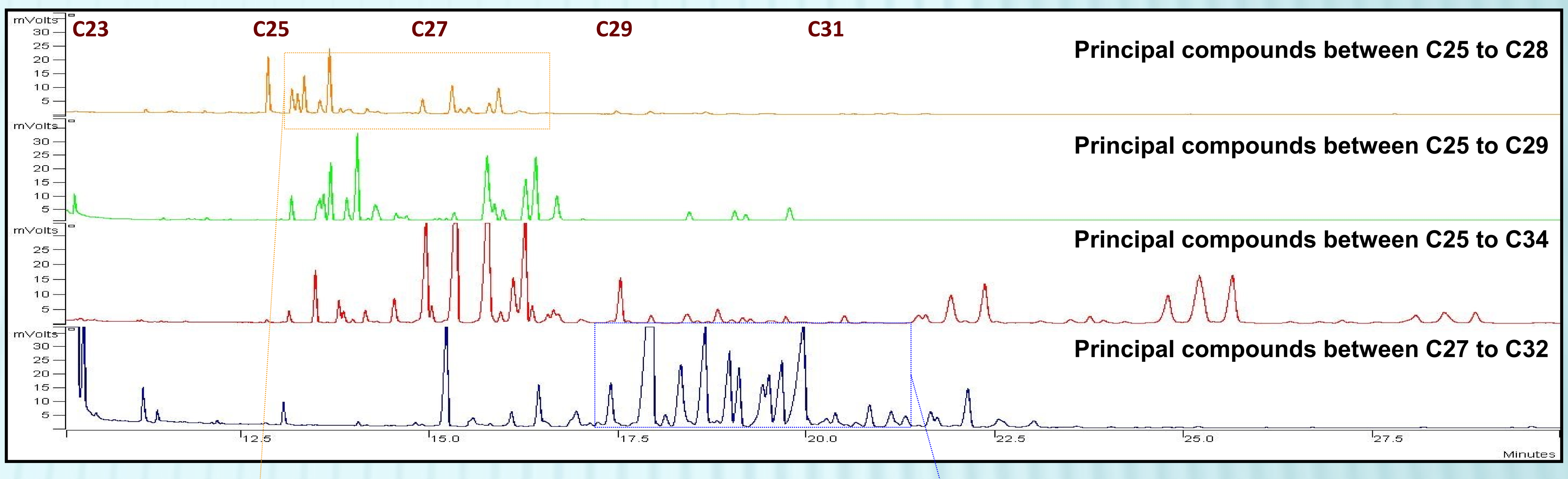
Study context: This study is the latest step in our research on the Argentine ant (*Linepithema humile*) invasion along the Mediterranean coast, with the objective of developing biological tools to contain this invasion. We previously showed that in the laboratory, *T.nigerrimum* workers were able to resist and even in some cases to exterminate the Argentine ant (Blight et al. 2010). In order to contain a potential new settlement of the Argentine ant in the field, we propose erecting a "biological barrier" in the form of *T. nigerrimum* nest transplantations. These transplantations are likely to thrive given that *T. nigerrimum* is native to the zones in question.
However, several species of *Tapinoma*, morphologically not easily distinguishable, are present along the Mediterranean coast and it is difficult to discriminate among *Tapinoma* species, the only variations being in the clypeal cleft shape of queen or workers. Currently, male genitalia analysis provides the only secure method of differentiation.

Study goal: In order to quickly discriminate *Tapinoma* species on the basis of only one worker, we used cuticular hydrocarbon (CHC) profiles to provide a discrimination pattern for each species. Characterization of the HC of each profile completes this study.

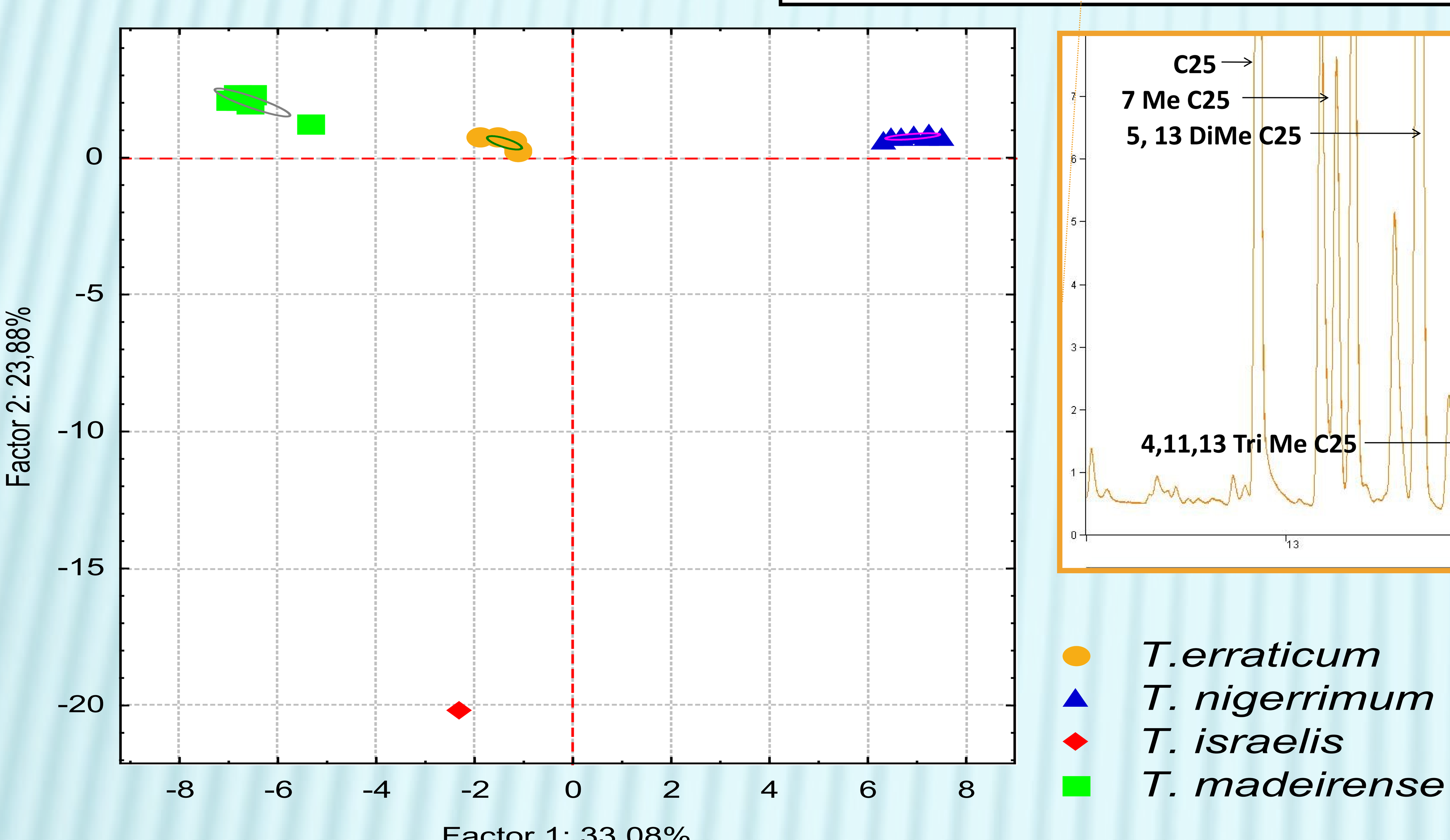
Materials & Methods : Four species of *Tapinoma* (*T. erraticum, T. israelis, T. madeirense* and *T. nigerrimum*) were collected from Israel *(T. israelis)* and continental France (*T. erraticum:* Azay sur Cher, Indre & Loire ; *T. madeirense*: Fréjus, Var and *T. nigerrimum*: Auriol, Bouches-du-Rhone) and were morphologically identified.
We analysed CHC profiles and identified HC by gas chromatography/mass spectrometry using an Agilent 6890N GC equipped with Chrompack CPSil5WCOT apolar capillary column (fused silica, 25m x 0.25 mm; thickness of stationary phase, 0.12µm) and injector in splitless mode. Initial oven temperature was held at 100°C for 1 min and then increased at a rate of 10°C/min to 220°C, then by 3°C/min to 320°C, where it was held constant for 15 min. Compounds were identified on the basis of their mass spectra and comparison with standard data, when available. Individual workers (5 per species) were immersed in 10µl of hexane for 15 min. 2µl extract were injected.
Between-species variability in profile composition was assessed using multivariate principal component analysis (PCA; Statistica v.6) based on the proportions of the 154 major cuticular hydrocarbons.

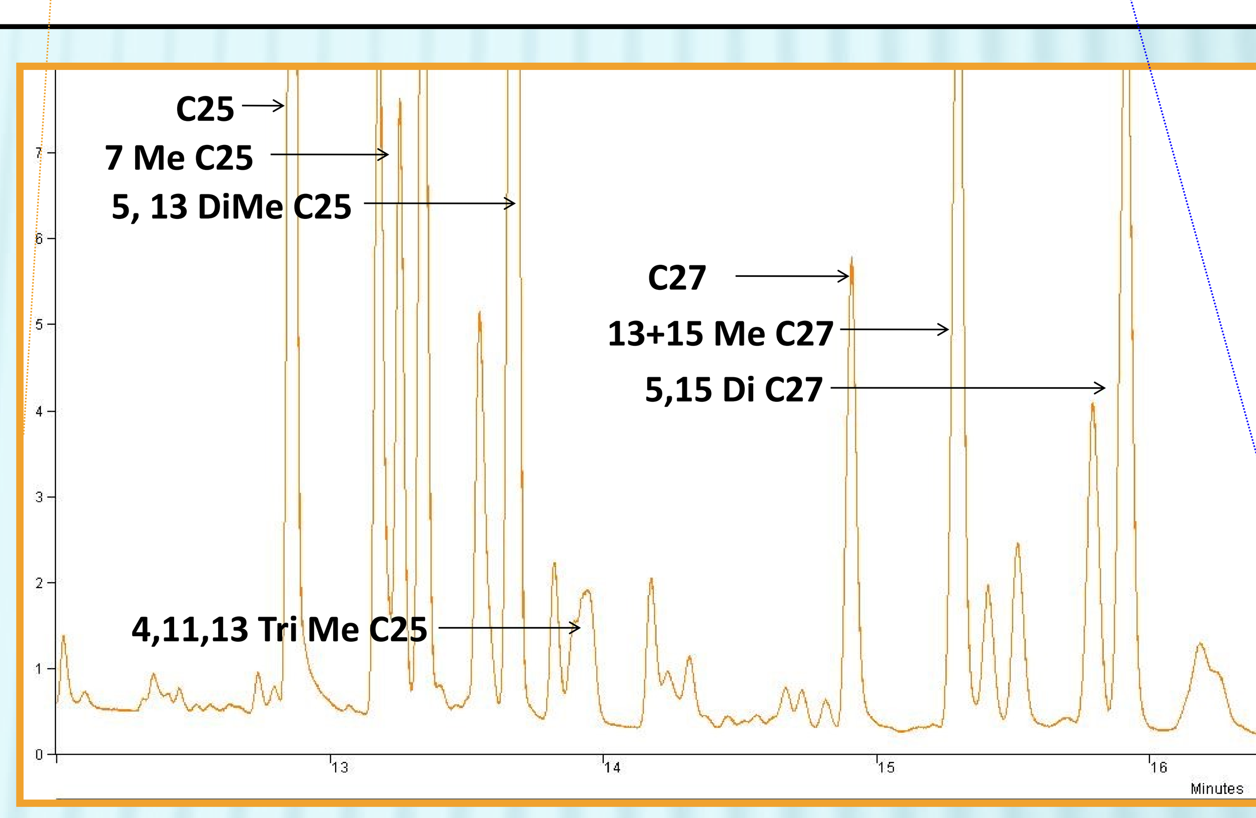
Results :

Cuticular profiles of the four *Tapinoma* species. In orange, CHC profile of *T. erraticum* (Azay sur Cher) – in green, CHC profile of *T. madeirense* (Fréjus) – in red, CHC profile of *T. israelis* (Tel Aviv) and in blue, CHC profile of *T. nigerrimum* (Auriol).

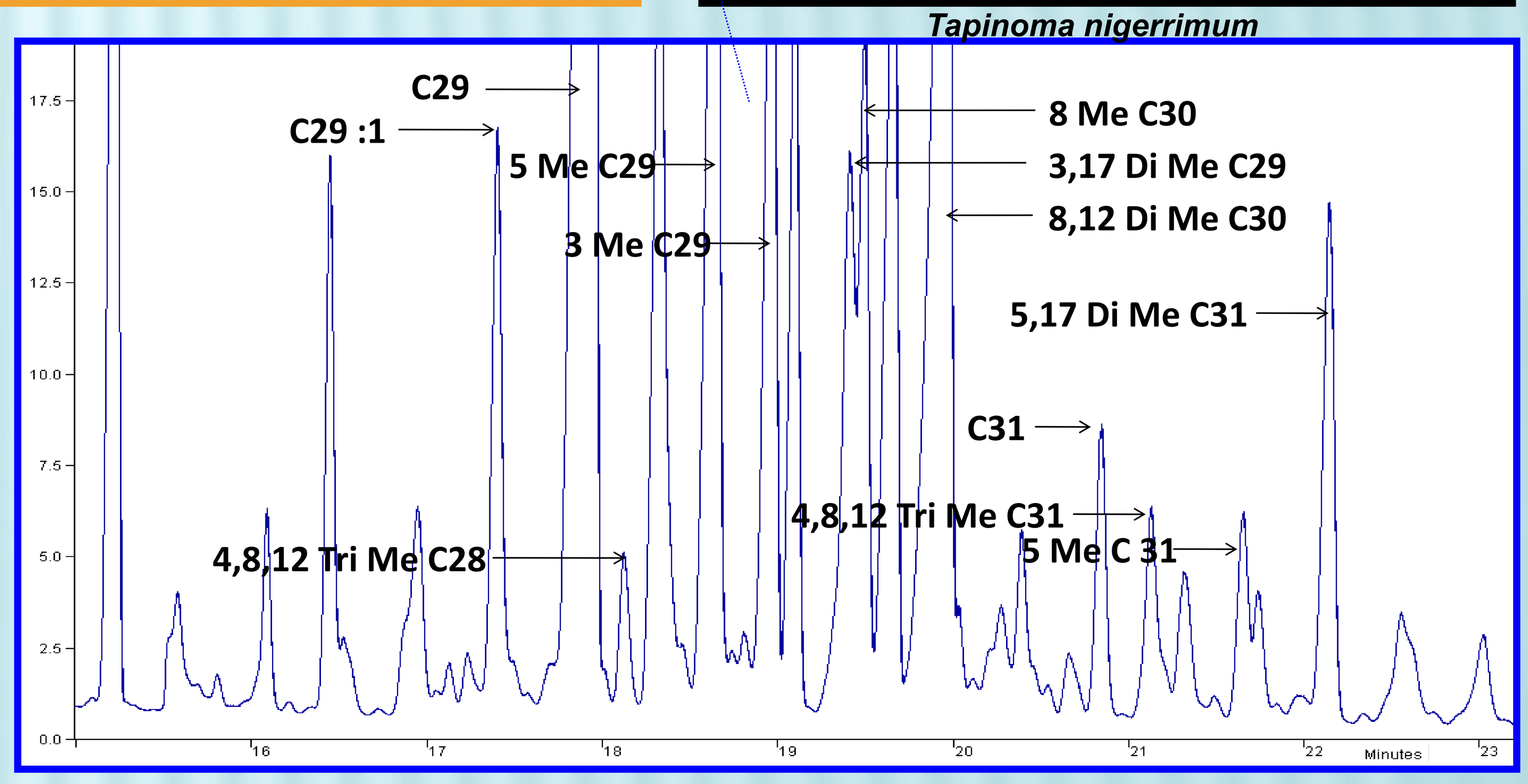


The PCA based on 154 major cuticular hydrocarbons of the four species, and 5 individuals per species revealed clear discrimination among these four species. Chemical profiles between the species differed qualitatively and quantitatively. In orange, *T. erraticum* (Azay sur Cher) – in green, *T. madeirense* (Fréjus) – in red, *T. israelis* (Tel Aviv) and in blue, *T. nigerrimum* (Auriol). Ellipse showed a range of 0.95 % of the distribution of each species.





Tapinoma nigerrimum



On the left: Compound list according to the species.

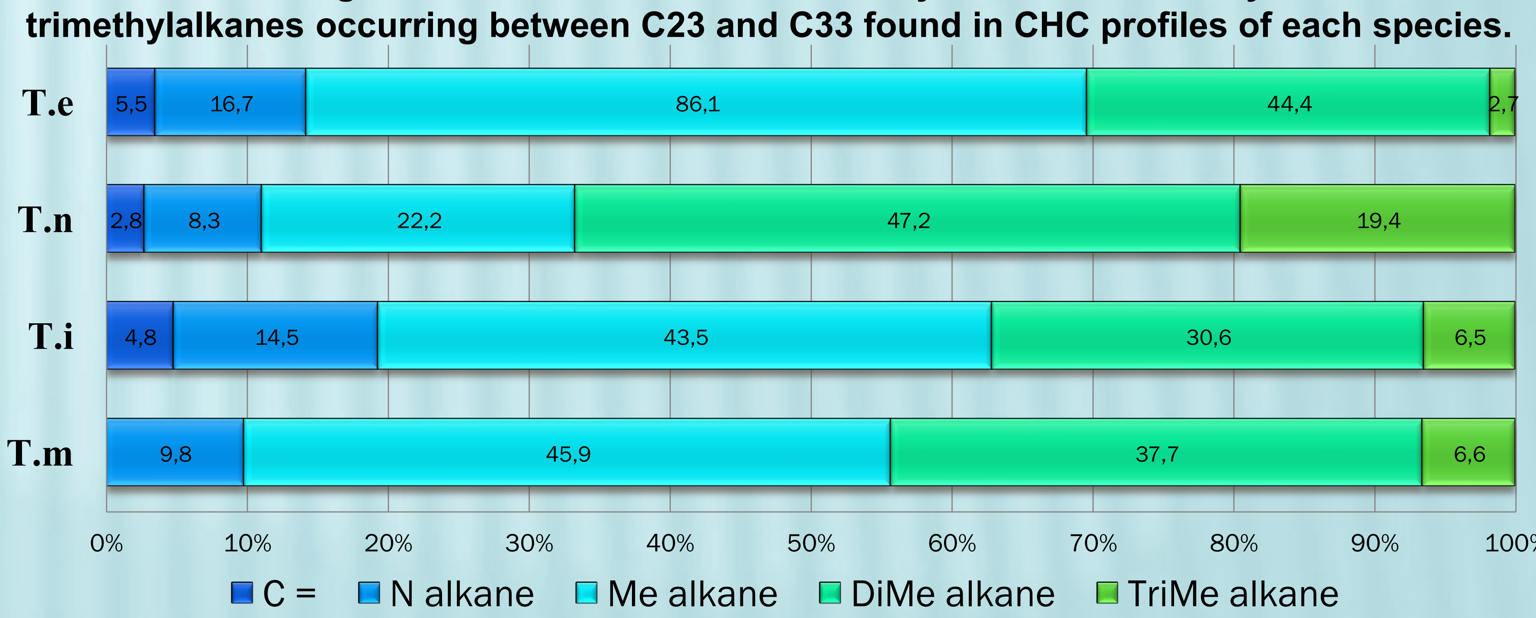
Compounds	T. madeirense	T. israelis	T. nigerrimum	T. erraticum
C23				*
11 C23				*
7 C23				*
5 C23				*
3 C23				*
C 24				*
7 C24				*
8 C24				*
5 C24				*
C25 : 1				*
C25	*	*		*
11 Me C25	*	*		*
13-Me C25	*	*		*
7 Me C25				*
5-Me C25	*	*		
11,13-diMeC25		*		
7,9 Di Me C25				*
3-Me C25	*	*		*
5,13-diMeC25	*	*		*
C26	*	*		*
4,11,13 TriMe C25				*
8 Me C26				*
10-Me C26		*		
12-Me C27		*		
14-Me C28	*	*		
5,9,11-TriMe C25	*			
12,13-diMe C26				*
12,14-diMe C26	*			*
C27:1		*		
8,12-diMe C26	*			
6-Me C26				*
5-Me C26				*
4-Me C26	*			*
2-Me C26	*			
6,12-diMe C26	*			*
5,12-diMe C27	*			
4,10 C26				*
4,12-diMe C28	*			

Compounds	T. madeirense	T. israelis	T. nigerrimum	T. erraticum
C27	*	*	*	*
11- MeC27	*	*		
13-MeC27	*	*		*
15-Me C27				*
14,15-diMe c28			*	
7-MeC26	*	*		
8,12-diMethyl C28			*	
7- Me C27				*
5-MeC27	*	*		*
C29:1			*	
7,15 - C 27				*
5, 15 - C27				*
11,15-diMeC27		*		
4,12-diMe C27			*	
4,16-diMe C28			*	
3-MeC27	*	*		
5,13-diMeC27	*	*		
C28	*	*		*
14-Me C28	*			*
13-Me C28				*
12-Me C28				*
8-Me C28		*		*
7-Me C28				*
12,14-diMe C28	*			
13, 15 DiMe C28				*
15,15 Di Me C28				*
12,16-diMeC28		*		
8,12-diMe C28		*		
6-Me C28	*	*		
5-Me C28		*		
4-Me C28	*			
C29:1		*		*
6,14-diMe C28	*			
5,13-diMeC28	*			
4,12 -diMe C28	*			
4,14-diMe C28	*			
C29	*	*	*	*
11- MeC29	*	*		

Compounds	T. madeirense	T. israelis	T. nigerrimum	T. erraticum
13- MeC29	*	*	*	*
15-MeC29	*	*	*	*
4,8,12-triMe C28			*	
7-MeC29	*	*		*
2,6,12-triMe C28			*	
5-MeC29	*	*	*	*
11,17-diMe C29			*	
3-MeC29	*		*	
5,13-diMeC29	*			
5,15-diMeC29			*	
5,17-diMeC29			*	
5,19-diMeC29			*	
13,15-diMeC29		*		*
7,15-diMeC29		*		*
5,15 - DiMe C29				*
3,17-diMeC29			*	
5,17-diMeC29		*		
7,15,17-triMeC29		*		
C30		*		
16-MeC30	*			
15-MeC30				*
14-MeC30	*			*
13-MeC30				*
8-Me C30		*	*	
7-Me C30				*
4-Me C30				*
4,8-diMe C30			*	
4,12-diMe C30	*		*	
4,14-diMe C30	*		*	
8,12-diMe C30		*		
8,12-diMe C30	*		*	
10,14-diMe C30	*			
6,14-diMe C30	*			
C31 :1				*
sterol	*			
C31	*	*	*	*
2,8,12-triMe C30	*			
4,8,12-triMe C30			*	

Compounds	T. madeirense	T. israelis	T. nigerrimum	T. erraticum
15-MeC31	*		*	*
13- MeC31	*	*	*	*
11-MeC31	*	*		
7-MeC31	*	*		*
5-MeC31	*		*	
11,17- diMe C31		*		
13,17-diMe C31		*		
13,24-diMeC31			*	
7,15-diMe C31		*	*	*
7,13-diMe C31	*			
7,11-diMe C31	*			
5,13-diMe C31	*			
5,15-diMe C31				*
5,17-diMe C31			*	
7,11,15 -triMe C31			*	
11,15,17 -triMe C31		*		
5,9,13 -triMe C31	*		*	
C32		*		
8,12-diMe C32	*		*	
11-MeC32		*		
5,13-diMe C35	*			
8,12,16-triMeC32			*	
6,10,14-triMeC32			*	
x,y-diMeC32		*		
C33:1		*		
14,18,22-TriMe C32		*		
C33		*		
15-MeC33				*
13-MeC33				*
11-MeC33		*		
7-MeC33		*		
13,15-diMe C33		*		
13,17-diMe C33		*		
7,11-diMe C33		*		
7,15-diMe C33		*		*
11,15,17 -triMe C33		*		

Below: Percentages of n-alkanes, monomethylalkanes, dimethylalkanes and trimethylalkanes occurring between C23 and C33 found in CHC profiles of each species.



Conclusions :

- **Our results demonstrated a clear discrimination among species.**
- **There was a large diversity of specific dimethyl alkanes that makes them likely candidates for nestmate and species discrimination signals.**
- **We found 154 cuticular hydrocarbons occuring between C23 and C33 (n-alkanes, monomethyl alkanes, dimethyl alkanes and trimethyl alkanes).**
- **Cuticular HC profiles are a quick discriminant tool for ecological studies.**

Blight O., Provost E., Renucci M., Tirard A. & Orgeas J. (2010) A native ant armed to limit the spread of the Argentine ant. *Biological Invasions* . Published on line DOI 10.1007/s10530-010-9770-3