

ORIGINAL ARTICLE

The Guianese population of the fire ant *Solenopsis saevissima* is unicolonialAlain Lenoir¹, Séverine Devers¹, Axel Touchard² and Alain Dejean^{2,3}

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Abstract In this study, conducted in French Guiana, a part of the native range of the fire ant *Solenopsis saevissima*, we compared the cuticular hydrocarbon profiles of media workers with previous results based on intraspecific aggressiveness tests. We noted a strong congruence between the two studies permitting us to delimit 2 supercolonies extending over large distances (up to 54 km), a phenomenon known as unicoloniality. *Solenopsis geminata* workers, taken as an out-group for cluster analyses, have a very different cuticular hydrocarbon profile. Because *S. saevissima* has been reported outside its native range, our conclusion is that this species has the potential to become invasive because unicoloniality (i.e., the main attribute for ants to become invasive) was shown at least for the Guianese population.

Key words biological invasions; cuticular hydrocarbons; fire ants; unicoloniality

Introduction

Most ant species have multicolonial and “closed” colonies. Typically, colony mate recognition is based on the chemical cues encoded in a mixture of low-volatile cuticular hydrocarbons (CHs) constituting the “colony odor” which, once learned by the workers, represents a neural template. Discrimination between colony mates and aliens is based on the qualitative and quantitative comparison of the CHs making up the colony odor (the template) with the CHs of encountered individuals, a mismatch usually resulting in aggressiveness (d’Ettorre & Lenoir, 2010 and papers cited therein). Yet, through human conveyance, some ant species were transported to different parts of the world where they thrived, became invasive, and are now recognized as one of the most devastating invaders in the animal kingdom (Holway *et al.*, 2002). Most of these invasive ants develop “open” supercolonies extending over very large areas, a phenomenon known as “unicolonial-

ity” (Holway *et al.*, 2002). Here, intraspecific aggressiveness commonly occurs along the border between the territories of 2 supercolonies due to different CHs (Suarez *et al.*, 2002; Thomas *et al.*, 2007; Moffett, 2012). Unicoloniality has been suggested as being the main attribute in the success of invasive ants because it favors their “ecological dominance” (i.e., both numerically and behaviorally dominant *vis-à-vis* the native ant species of their introduced range) and disrupts arthropod community structure with repercussions for the entire ecosystem (Holway *et al.*, 2002; Helanterä *et al.*, 2009).

On the other hand, it is now recognized that the ecological and/or evolutionary changes underlying biological invasions might occur within the native range of invasive species. For ants, certain of these species also present the supercolony syndrome in their native range, but here the expansion of their territories is much lower due to intra- and interspecific competition (Orivel *et al.*, 2009; Fournier *et al.*, 2012; Moffett, 2012).

Although it is considered a serious problem in invaded regions, the monogyne form of the red imported fire ant (RIFA), *Solenopsis invicta*, is not unicolonial (1 queen per mound; mounds separate from one another as opposed to

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the polygyne form where the mounds are interconnected by galleries) (Tschinkel, 2006). Accordingly, its success in its introduced range could be due to the negative impact of human disturbance on native ants rather than to its superior competitive ability (King & Tschinkel, 2013; but see LeBrun *et al.*, 2012; Stuble *et al.*, 2013). The negative and positive impacts of this species were recently presented (Epperson & Allen, 2010; Drees *et al.*, 2013; Rashid *et al.*, 2013; Vinson, 2013; see also Dejean *et al.*, 2015 for *S. saevissima*).

Therefore, a challenge in estimating the invasive potential of fire ants is to determine their potential to be unicolonial in their native range. Accordingly, this study focused on a population of *Solenopsis saevissima* in French Guiana corresponding to a part of its native range that extends from Suriname to northern Argentina (Antwiki, 2014). Frequently found in human-disturbed areas, *S. saevissima* is considered a major pest in its native range (Taber, 2000; Zeringóta *et al.*, 2014). The population from French Guiana forms “colonial entities” extending over up to 54 km with its mounds interconnected by galleries (Martin *et al.*, 2011). Furthermore, *S. saevissima* was introduced elsewhere, constituting a potential pest (Wetterer, 2014).

Solenopsis saevissima belongs to an assemblage of recently diverged taxa, including the well-known invasive fire ants *S. invicta* (RIFA), *S. richteri* and *S. geminata* (Ross *et al.*, 2010).

This species is difficult to distinguish from the RIFA due to similarities in individual morphology, its painful sting and its nesting mode to the point that Dall’Aglío-Hovorcem *et al.*, (2009) difficult to distinguish from the RIFA due to great similarities in morphology and behavior so that Dall’Aglío-Hovorcem *et al.*, (2009) proposed using chemistry to distinguish them.

Also, it was shown that nominal *S. saevissima* comprises cryptic species due to isolation and hybridization with *S. geminata* (Ross *et al.*, 2010; see also Delsinne *et al.*, 2012). Similarly, Fox *et al.*, (2012), based on differences in CHs, distinguished 2 “cryptic species” themselves different from another cryptic species from southern Brazil (Dall’Aglío-Hovorcem *et al.*, 2009) and a fourth one from French Guiana (Roux *et al.*, 2009); they concluded that there were at least 4 cryptic species within nominal *S. saevissima*.

In this study, conducted on the Guianese population of *S. saevissima*, we aimed to verify the results by Martin *et al.*, (2011) showing the existence of vast “entities of mounds” separated by borders. Inside each entity, workers from different mounds are not aggressive towards each other, whereas, when confronted, workers from different entities engage in reciprocal fighting regardless of the

mounds from which they were gathered. We therefore compared the CHs of individuals sampled from mounds belonging to 2 adjacent “colonial entities”.

Materials and methods

The field survey was conducted in French Guiana in November 2013 along the *route de Petit Saut* and Route No. 1 that go from the Petit Saut dam (4°59’N; 53°08’W) to the city of Sinnamary (5°22’N; 52°57’W). The ants were collected from 18 sites along the roadsides; 11 sites corresponded to the “Petit Saut colonial entity” (Colony A) extending over 54 km from Petit Saut to Paracou (05°16’N; 52°55’W), and the 7 others to the “Sinnamary colonial entity” (colony B) extending over 10 km from Paracou to Sinnamary (Martin *et al.*, 2011). We also collected a sample of *S. geminata* to have an out-group for comparison (details in Fig. 1).

Using forceps, we gathered media workers (3.5–4.5 mm) from each mound and put them into glass containers that we transported to the Petit Saut field station where we allowed the workers to calm down. Then, the containers were placed in a freezer at -20 °C for 2 h to keep the workers from secreting compounds during the successive steps of the process. From each container, we took 2 or 3 samples of 10 media workers each and placed them in glass vials along with 1 mL of hexane and kept them there until we transported them to Tours, France for chemical analyses. There, the ants were retrieved from the vials and the solvent evaporated. Then the extract was redissolved in 10 µL of hexane plus 2 µL of hexane containing 400 ng of eicosane (C20) as internal standard. We injected 2 µL of each extract into a Perkin–Meyer GC–MS functioning at 70 eV with a source temperature of 230 °C and equipped with a ZB-5HT column (30 mL × 0.25 mm ID × 0.252 µm df; 5% phenyl and 95% dimethylpolysiloxane). The temperature program was 2 min at 80 °C, and then 10 °C/min until 320 °C, and a 10 min hold at 320 °C (total 36 min). Substances were identified using standard alkanes and comparing the spectra to published data. For the comparisons, we calculated the percentage of each hydrocarbon from the total hydrocarbon content in each ant sample. The data were analyzed using cluster analysis on Euclidian distances and the Ward method (Statistica R, 2012). We compared the Euclidian distances using the Kruskal–Wallis test followed by Dunn’s *post hoc* multiple comparison test (Statistica R, 2012).

Voucher specimens of the ants were deposited in the *Laboratório de Mirmecologia* collection (acronym CPDC), Cocoa Research Centre (Ilhéus, Bahia, Brazil).

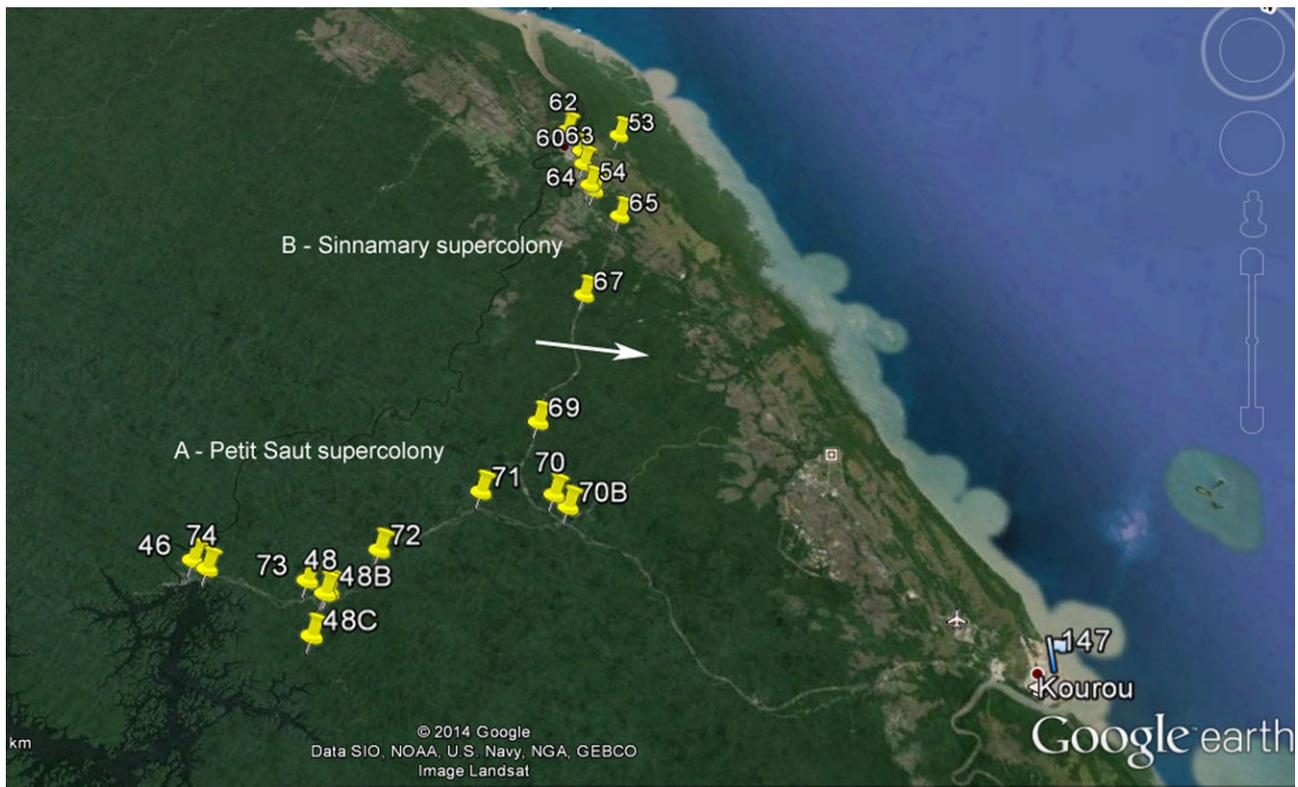


Fig. 1 Google map of the area from where the *Solenopsis saevissima* samples were gathered. Each point corresponds to an ant mound. The 2 colonial entities delimited by Martin *et al.*, (2011) are separated by the white arrow (A and B represent the Petit Saut and the Sinnamary colonial entities, respectively). Sample 53 corresponds to *S. geminata*.

Results and discussion

We found the same *S. saevissima* hydrocarbon profiles described by Roux *et al.*, (2009) for this Guianese population and identified some additional minor ones. The major peaks (C23, C25, 9+11+13C25, 3,7C25 and 3,7,11C25) account for 70%–75% of the hydrocarbons (Table 1). The *S. geminata* profiles are very different as these hydrocarbons accounted for only 32% of the total, whereas they also contain a C27 alkene which represents 26% of the hydrocarbons that are present in only feeble percentages in *S. saevissima* (Table 1 and Fig. S1). As previously noted through interspecific tests of aggressiveness (Roux *et al.*, 2013), *S. geminata* is a real out-group with a very different profile.

Note that fire ant workers have comparatively large quantities of hydrocarbons on their cuticle (Fox *et al.*, 2012). Also, both quantitative and qualitative (i.e., absence of a C23 alkene in media and major workers) differences in CH composition were noted between castes in one Brazilian *S. saevissima* group; there were only quantitative differences in a second group (Fox *et al.*,

2012). Indeed, within-colony variation in CHs in ants is well known; nestmate recognition is generally based on a threshold above which aggressiveness occurs. In other ant species, there is a graded response, while in still other ants only some compounds constitute the nestmate recognition cues (Ichinose *et al.*, 2009; van Zweden & d’Ettorre, 2010; van Zweden *et al.*, 2014).

The cluster analysis (Fig. 2) showed a great difference between *S. saevissima* and *S. geminata* and a high degree of congruency in the hydrocarbon profiles within each *S. saevissima* colonial entity. The Euclidian distances indicated that *S. geminata* profiles differed significantly from those of the 2 *S. saevissima* colonial entities (Petit Saut and Sinnamary). The latter two differed significantly from each other but at a lower level (Fig. S2) confirming the superposition of the profiles (Fig. S1). These results confirm those by Martin *et al.*, (2011) using a method based on intraspecific aggressiveness (i.e., it added the fact that the mounds are interconnected by galleries because it is unfeasible to follow galleries over dozens of kilometers; see Fig. 1). Furthermore, the border between the 2 colonial entities still corresponds to the same area

Table 1 Relative quantities of hydrocarbons for *Solenopsis saevissima* supercolonies A (Petit Saut), B (Sinnamary) and *S. geminata* (Standard Errors [SE] and percentages; *N* = number of chromatograms; in bold major alkanes and alkenes). ?, unidentified compounds; the same values (i.e., C25:1; C34:1; C37:1; C38:1) correspond to alkenes with different position of the double bond.

Hydrocarbon name	<i>S. saevissima</i> ColA Petit Saut		<i>S. saevissima</i> ColB Sinamary		<i>S. geminata</i> Col 53	
	%	SE	%	SE	%	SE
C21	0.01	0.01	0.00	0.00	4.36	0.23
C22	0.01	0.01	0.00	0.00	0.00	0.00
C23	5.99	0.80	12.12	0.81	23.84	0.73
9+11C23	1.65	0.18	1.92	0.16	4.27	0.11
7C23	0.02	0.02	0.00	0.00	0.00	0.00
5C23	0.76	0.13	0.49	0.13	0.00	0.00
3C23	4.17	0.23	3.67	0.25	3.07	0.05
C24	1.56	0.17	1.34	0.22	2.10	0.43
3,7C23	2.57	0.18	1.66	0.12	0.00	0.00
8,12+10,12C24	1.60	0.13	1.49	0.06	0.34	0.15
6C24	0.09	0.05	0.00	0.00	0.00	0.00
4C24	0.85	0.06	0.58	0.02	0.11	0.01
C25:1	3.85	0.19	5.01	0.36	0.53	0.00
C25:1	0.20	0.10	0.00	0.00	0.00	0.00
C25	15.18	1.31	10.23	0.34	5.68	0.17
4,8,10C24	0.02	0.02	0.00	0.00	0.00	0.00
9+11+13C25	6.43	0.32	11.00	0.43	0.48	0.07
7C25	0.10	0.05	0.00	0.00	0.00	0.00
5C25	1.70	0.14	2.16	0.18	2.26	0.07
3C25	13.19	0.30	12.44	0.26	1.52	0.11
5,9+5,11C25	0.20	0.11	0.00	0.00	0.00	0.00
3,7+3,9+3,11C25	22.06	0.70	17.78	0.52	0.83	0.16
3,7,11C25	10.67	0.51	6.68	0.19	0.25	0.08
4C26	0.46	0.05	0.52	0.01	0.13	0.02
8,10C26	0.06	0.03	0.00	0.00	0.00	0.00
6,10C26	0.04	0.02	0.00	0.00	0.00	0.00
C27:1	0.18	0.03	0.57	0.15	26.07	0.50
4,10+4,12C26	2.02	0.12	1.39	0.10	3.57	0.17
C27	0.25	0.11	1.04	0.16	0.00	0.00
4,8,12C26	0.94	0.13	0.56	0.09	0.03	0.01
11+13C27	0.45	0.03	1.50	0.09	1.06	0.04
5C27	0.17	0.01	0.31	0.04	0.00	0.00
3C27	0.30	0.05	1.55	0.09	0.50	0.02
5,9C27	0.04	0.02	0.00	0.00	0.00	0.00
C28	0.77	0.04	1.19	0.17	0.00	0.00
6C28	0.27	0.03	0.15	0.01	0.21	0.02
4C28	0.03	0.02	0.01	0.00	0.09	0.05
C29	0.20	0.04	0.47	0.03	0.74	0.04
11C29	0.03	0.01	0.09	0.01	0.28	0.05
3C29	0.02	0.00	0.16	0.03	0.00	0.00
C30	0.04	0.02	0.07	0.02	0.07	0.01
?	0.14	0.06	0.20	0.07	0.00	0.00
C32	0.00	0.00	0.00	0.00	0.00	0.00
?	0.00	0.00	0.22	0.05	0.61	0.06

(to be continued)

Table 1 Continued.

Hydrocarbon name	<i>S. saevissima</i> ColA Petit Saut		<i>S. saevissima</i> ColB Sinamary		<i>S. geminata</i> Col 53	
	%	SE	%	SE	%	SE
C33:1	0.13	0.04	0.24	0.07	3.00	0.14
C33	0.17	0.11	0.09	0.04	1.04	0.13
?	0.06	0.03	0.06	0.02	0.37	0.04
C34:1	0.01	0.01	0.11	0.06	0.04	0.03
C34:1	0.01	0.01	0.02	0.02	0.22	0.02
C34	0.02	0.02	0.03	0.01	0.05	0.01
?	0.18	0.13	0.24	0.12	2.56	0.17
C35:1	0.00	0.00	0.01	0.01	4.78	0.15
C35	0.02	0.02	0.00	0.00	1.07	0.04
MeC35	0.01	0.01	0.02	0.01	0.32	0.04
diMeC35	0.04	0.01	0.11	0.05	0.62	0.05
C36	0.00	0.00	0.01	0.00	0.00	0.00
C37:1	0.00	0.00	0.01	0.01	0.82	0.09
C37:1	0.01	0.01	0.16	0.15	0.63	0.07
C37:1	0.00	0.00	0.00	0.00	0.14	0.02
C38:1	0.00	0.00	0.09	0.04	0.34	0.04
C38:1	0.02	0.02	0.11	0.07	0.53	0.04
Total	100%		100%		100%	
	<i>N</i> = 17		<i>N</i> = 17		<i>N</i> = 3	
Major alkanes	73.52%		70.24%		32.60%	
Total alkenes	4.42%		6.34%		37.11%	

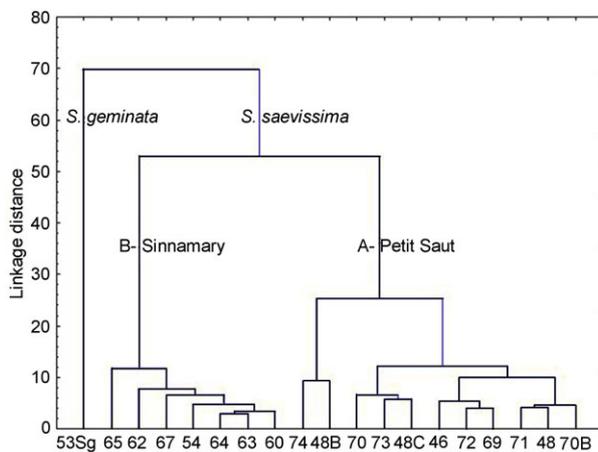


Fig. 2 Dendrogram of *Solenopsis saevissima* cuticular hydrocarbon proportions (A and B represent the Petit Saut and the Sinamary colonial entities, respectively); *S. geminata* is used as an out-group.

along Route No. 1 (i.e., it separates samples 67 and 69; Fig. 1). So, that which was called “colonial entities” by Martin *et al.*, (2011) really corresponds to supercolonies

extending over large distances (up to 54 km). We therefore show that the Guianese population of *S. saevissima* forms supercolonies because the aggressive discrimination of noncolony mates (Martin *et al.*, 2011) is correlated to different hydrocarbon profiles. Our data therefore confirm the presence of at least 4 *S. saevissima* cryptic species based on their CH profiles, 3 from the southern part of the range (Dall’Aglia-Holvorcem *et al.*, 2009; Fox *et al.*, 2012) and a fourth one in French Guiana (Roux *et al.*, 2009; this study). Genetic data are needed to confirm these results. In other invasive ants, this time in their introduced range, different supercolonies also have different CH profiles, and aggressiveness is correlated to these differences (Suarez *et al.*, 2002; Thomas *et al.*, 2007; Drescher *et al.*, 2010).

Due to the development of the global transportation network, *S. saevissima*, which has already been noted outside its native range including Guadeloupe with many transport-related connections with French Guiana (Wetterer, 2014), may become a pantropical invasive pest in tropical areas where its potential threat could be comparable to what is currently happening with regard to *S. invicta* in temperate and subtropical climates (see Taber, 2000).

Acknowledgments

We are grateful to anonymous reviewers who helped to improve the manuscript, to Dr Jacques Delabie for the identification of the ants, to Andrea Yockey-Dejean for proofreading the manuscript, and the *Laboratoire Environnement de Petit Saut* for furnishing logistical assistance. Financial support for this study was provided by a project CNRS-CEBA (*Centre d'Etude de la Biodiversité Amazonienne*) PPAR (Phthalate Pollution in Amazonian Forest).

Disclosure

The authors declare that no competing interests exist.

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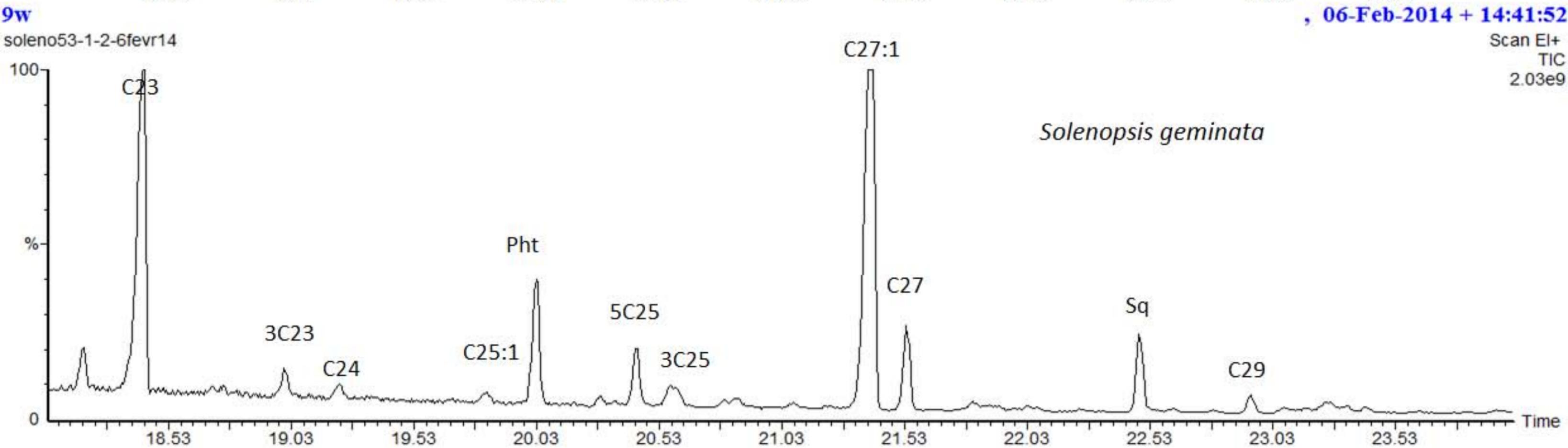
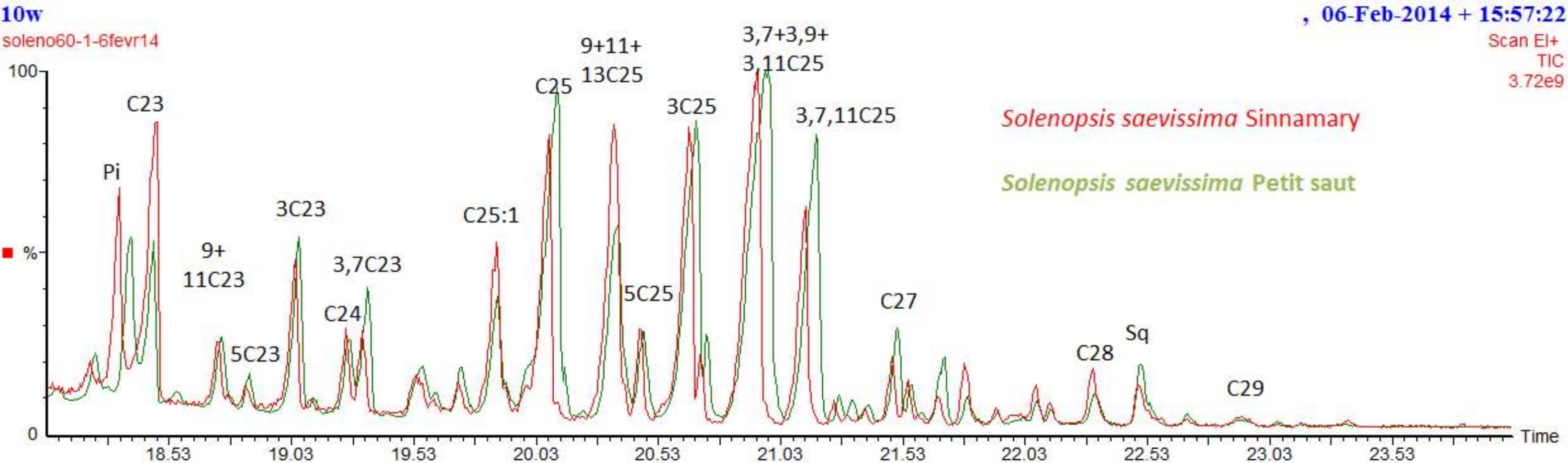
Accepted March 27, 2015

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Fig. S1. Chromatograms of *Solenopsis saevissima* colonial entities (Petit Saut in green and Sinnamary in red in overlay) and *S. geminata*. Sq, Squalene; Pht, Phthalate contaminant; Pi, Piperidine.

Fig. S2. Median Euclidian distances between the different *Solenopsis* entities: S, *S. saevissima* from Sinnamary; PS, *S. saevissima* from Petit Saut; Sg, *S. geminata*. Statistical comparisons: Kruskal–Wallis test: $H_{3,170} = 100$; $P < 0.0001$; Dunn’s multiple comparison test: different letters indicate significant differences at $P < 0.001$.



Euclidian distances

