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Chemical tools to distinguish the fire ant species *Solenopsis invicta* and *S. saevissima* (Formicidae: Myrmicinae) in Southeast Brazil

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ABSTRACT

Morphologically similar fire ants *Solenopsis invicta* and *Solenopsis saevissima* are broadly sympatric in southeastern Brazil. Chemistry from venom (2,6-dialkyl piperidine alkaloids) and cuticular hydrocarbons have been reported as potentially important tools for differentiating *Solenopsis* species. We have analysed two chemical classes in widely separated populations of *S. invicta* and *S. saevissima* and find that both piperidine alkaloids and cuticular hydrocarbons separate the two species. Piperidine alkaloids clustered *S. invicta* but not *S. saevissima*. Cuticular hydrocarbons strongly clustered both *S. invicta* and *S. saevissima*. One population morphologically identified as *S. invicta* presented piperidine alkaloids and cuticular hydrocarbons markedly different from either species. The distinctive piperidine alkaloid differences among populations of *S. saevissima* and the marked difference in piperidine alkaloid and hydrocarbon profiles of the anomalous population of *S. invicta* suggest undescribed species fire ant in southeastern Brazil.

1. Introduction

The ant genus *Solenopsis* has attracted considerable attention because of the pest status of some species, particularly the red imported fire ant, *Solenopsis invicta*, and interest in the biology of it and other species. However, the great morphological similarity of many species has led to doubts concerning species recognition and identification. *S. invicta*, although arriving in North America in the early 20th Century, was misidentified until it was recognized as an undescribed species by Buren in 1972. This ant is native to South America where it co-occurs with behaviourally and morphologically similar species of the *Solenopsis saevissima* complex (Trager, 1991; Tschinkel, 2006), adding to the uncertainty of identifying fire ants from this region.

The morphological distinctions between *Solenopsis* species are often subtle, making them difficult even for specialists to identify. Hybrids intermediate in appearance may additionally complicate identifications (Vander Meer et al., 1985). An important tool that may help overcome these kinds of difficulties is the analysis of potentially diagnostic compounds such as 2,6-dialkyl piperidine alkaloids and cuticular hydrocarbons using gas chromatography/mass spectrometry (GC–MS) (MacConnell et al., 1971; Vander Meer, 1988; Vander Meer and Lofgren, 1988). A number of 2,6-dialkyl piperidine alkaloids are

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present in the venom of fire ants, and different fire ant species have characteristic suites of compounds (MacConnell et al., 1976; Brand et al., 1973a, b). *S. invicta* in North America contain high concentrations of 2-methyl-6-tridecyl piperidine, 2-methyl-6-tridecenyl piperidine, 2-methyl-6-pentadecyl piperidine and 2-methyl-6-pentadecenyl piperidine alkaloids, whereas those of *S. richteri* have mainly 2-methyl-6-undecyl piperidine and 2-methyl-6-tridecyl piperidine; hybrids of the two species contained 2-methyl-6-pentadecyl piperidine and 2-methyl-6-pentadecenyl piperidine characteristic of *S. invicta* and 2-methyl-6-undecyl piperidine characteristic of *S. richteri*, together with 2-methyl-6-tridecyl piperidine and 2-methyl-6-tridecenyl piperidine of both species (Vander Meer et al., 1985). Because cuticular hydrocarbons also reflect genotype, they can provide valuable taxonomic characters (Carlson, 1988; Lockey, 1991; Kaib et al., 1991; Fröhlich et al., 2000), and in *Solenopsis* hydrocarbon composition may be species specific (Vander Meer, 1988). Nelson et al. (1980) discovered two new series of dimethylalkanes in *S. invicta* and *S. richteri* whose abundance patterns are markedly different in the two species.

Morphologically similar *S. invicta* and *S. saevissima* are broadly sympatric in southeastern Brazil (Tschinkel, 2006). Porter et al. (1995) using GC–MS analysis of cuticular hydrocarbons and piperidine alkaloids, and Trager (1991) using morphological characters have not been able to distinguish all Brazilian fire ants as either *S. invicta* or *S. saevissima*. In the present study, we investigate in more detail the 2,6-dialkyl piperidine alkaloids and cuticular hydrocarbons of these two closely related fire ant species and develop chemical criteria for their separation in a roughly 75,000 km^2 area in southeast Brazil.

2. Material and methods

2.1. Collection of fire ant samples in the field

We collected fire ant workers from nest mounds in open disturbed habitats at nine localities, all in São Paulo State, southeast Brazil (see Fig. 1, Table 1). The collection sites were distributed east–west over approximately 500 km following the Tropic of Capricorn, and over approximately 300 km of latitude (Fig. 1). The three southeastern-most localities were in low coastal mountains at elevations between 850 and 1750 m. The remaining six were from lower elevation (500–750 m), more continental sites within southeastern Brazil's interior drainage. Fire ant nests are frequently encountered in disturbed habitats such as pastures, roadsides and city parks throughout study region. Previous surveys suggested that *S. invicta* prevailed at lower elevations in the interior and *S. saevissima* was more frequent in the coastal mountains. Thus, sample sites were selected to include widely separated localities in both geographical zones. To assess intrapopulation chemical variability, ants from four to 10 (mean = 7.6) nests were sampled at each locality. Nests were sampled haphazardly as encountered, generally in areas smaller than 1 ha using mounds far enough apart to guarantee that they belonged to separate colonies. No attempt was made to identify ants in the field. Only one large, aggressive *Solenopsis* species was observed at each study site. All of the field samples obtained during the study were analysed for 2,6-dialkyl piperidine alkaloids and hydrocarbons and the results are presented here.

To obtain ants for chemical analysis, each mound was disturbed with a wooden stick, and fire ant workers crawling onto a glass tube were transferred to glass containers. In collections made near the lab in Campinas, SP in 1998–1999, live ants were immediately frozen and stored at −20 °C until extracted for piperidine alkaloids and hydrocarbons as given below. Fire

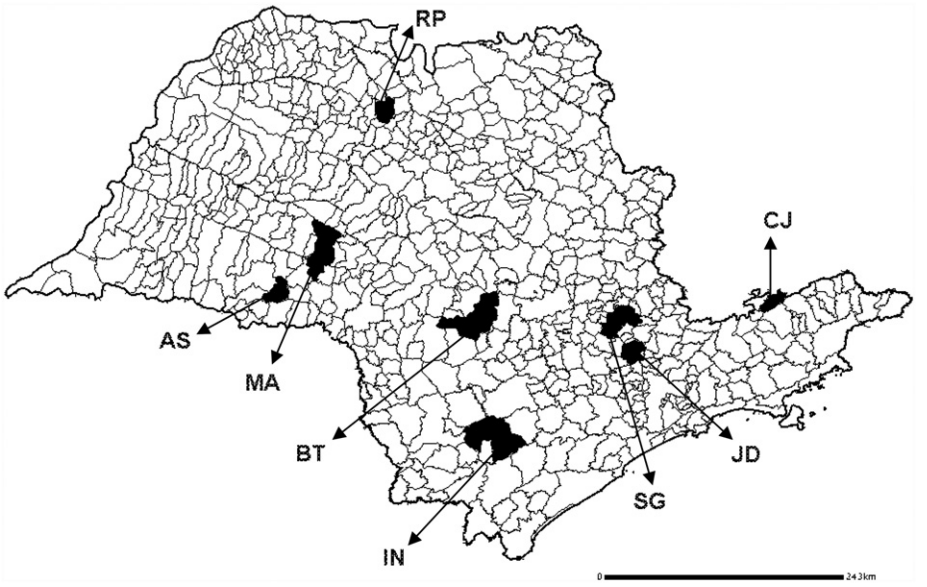

Fig. 1. Map of the São Paulo State, Brazil, showing the municipalities where *Solenopsis* were sampled. See Table 1 for locality codes.

Table 1
Summary of fire ant collection data.

Species and collection site in São Paulo State	Locality Code	No. of Colonies Sampled	Elevation (meters)	Location
Solenopsis invicta				
Instituto Agronômico de Assis, Assis	AS	9	550	22° 37′S; 50° 22′W
Companhia Agrícola Brejo das Almas, Botucatu	BT	10	500	22° 35′S; 48° 09′W
Rural area, Jundiaí	JDi	7	750	23° 17′S; 47° 00′W
Fazenda Experimental/UNIMAR, Marília	MA	5	650	22° 14′S; 49° 58′W
Bosque-Zoológico Municipal, São José do Rio Preto	RP	6	500	20° 46′S; 49° 21′W
Mata de Santa Genebra, Campinas	SG	8	600	22° 49′S; 46° 06′W
Solenopsis saevissima				
Parque Pedra do Baú, Campos do Jordão	CJ	6	1750	22° 40′S; 45° 38′W
Parque Estadual Intervales, Capão Bonito	IN	8	850	24° 16′S; 48° 25′W
Serra do Japi, Jundiaí	JDs	9	1000	23° 16′S; 47° 00′W

ant collections made in 2000 and 2001 were mostly from distant localities, and in these the 30 largest worker ants in each sample were transferred live to a glass vial containing 2 ml of dichloromethane; one day later the ants were extracted and analysed as given below. To evaluate possible systematic bias in the chemical analyses due to the difference in collection procedure, we analyzed paired samples of worker ants from three *S. invicta* colonies from Campinas in April 2000, using both procedures. The paired samples analyzed by gas chromatography (see below) gave virtually indistinguishable results. The 68 fire ant samples were identified by one of us (JCT) as either *S. saevissima* or *S. invicta* using Trager' (1991) key (Table 1).

2.2. Chemical analyses

To extract 2,6-dialkyl piperidine alkaloids and hydrocarbons, samples with 30 large worker ants were manually homogenized for 10 min in a test tube containing sea-sand (Fluka, Geneva, Switzerland) and 2 ml of dichloromethane (residual pesticide grade, Carlo Erba). Because this procedure may extract the hydrocarbon content of the entire ants, an extraction for 10 min in 2 ml dichloromethane without homogenization was carried out in one Campinas colony of *S. invicta*. The later procedure extracts only cuticular hydrocarbons. Since the GC–MS profile of hydrocarbons are similar in both procedures (results not showed), the hydrocarbons of entire ants will hereafter be called cuticular hydrocarbons. Each test tube was centrifuged at 3500 rpm for 10 min and the resulting solution filtered. The volume of each sample was reduced to about 1 ml under a gentle stream of nitrogen prior to injection in the GC–MS. The extract was analyzed by electron impact using a Hewlett Packard-6890 GC system with a fused capillary column (30 m × 0.25 mm × 0.25 μm, HP-5MS, Crossbond 5%-phenyl-95%-dimethylpolysiloxane) directly coupled to a selective mass detector (Hewlett Packard 5973). Conditions of injection: injector temperature 275 °C; oven temperature program 60–320 °C, 2 °C/min; split ratio 10:1; carrier gas He: 1 ml/min, constant flow; sample volume 1 μl. For positive chemical ionization analysis methane was used as ionization gas. Another program temperature (see Geden et al., 1998) was also used for cuticular hydrocarbons. Retention indices (RIs) were calculated according to van den Dool and Kratz (1963). The piperidine alkaloids and cuticular hydrocarbons were tentatively identified comparing the RIs, retention times and mass fragmentation patterns with literature values [for 2,6-dialkyl piperidine alkaloids: MacConnell et al. (1971, 1976), Brand et al. (1972, 1973a, b), Brand (1978), Escoubas and Blum (1990), Blum et al. (1992), and Jones et al. (1996); for cuticular hydrocarbons only *n*- and methyl-branched alkanes were considered: Nelson et al. (1980), Carlson et al. (1998), and Howard et al. (2001)]. In order to confirm the position of the double bond on the alkyl moiety of piperidine alkaloids, we derivatized (alkylthiolation) the samples with dimethyl disulphide as given by Francis and Veland (1981).

2.3. Statistical analyses

For each fire ant sample, the relative abundances (the quantity of each separate compound expressed as percentage of the total occurrence of the class of substance) of piperidine alkaloids and cuticular hydrocarbons were arcsin-transformed to meet the assumptions of normality and subjected to a principal-component analysis (PCA; Legendre and Legendre, 1998) to identify clusters of *Solenopsis* colonies with similar chemical compositions. PCAs were performed separately for hydrocarbons and for alkaloids (MVSP 3.1 package, Kovach Computing Services©). Compounds of both piperidine alkaloids and cuticular hydrocarbons with low relative abundance (<1%) in all samples were left out of the PCA.

3. Results and discussion

3.1. Piperidine alkaloid composition

Nine 2,6-dialkyl peridine alkaloids were tentatively identified in the 68 samples collected in this study (Fig. 2, Table 2). The alkylthiolation of *cis*- and *trans*-$C_{13:1}$ gave the derivatized products with characteristic ions at *m*/*z* 173 and *m*/*z* 200, consistent

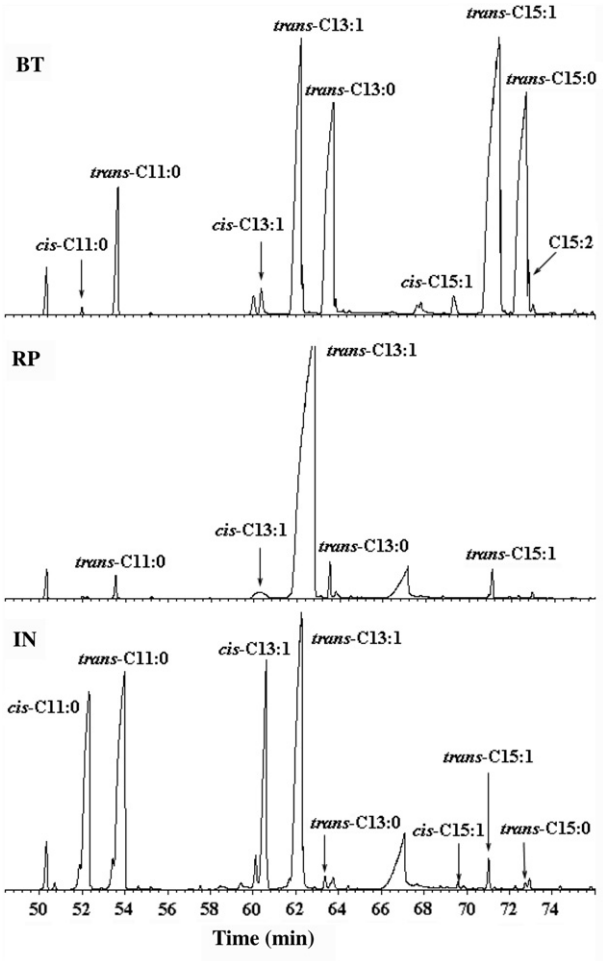

Fig. 2. Gas chromatograph profiles of 2,6-dialkyl piperidine alkaloids of populations morphologically identified as *Solenopsis invicta* (samples from BT and RP) and *S. saevissima* (sample from IN). Abbreviations for compounds given in Table 2.

with the double bond at C_4. The characteristic ions from alkylthiolated products *m*/*z* 326 (M^+-47, 91%) and *m*/*z* 61 ($[CH_2SCH_3]^+$, 11%) were also observed. Similarly, the alkylthiolation of *cis*- and *trans*-$C_{15:1}$ gave characteristic ions at *m*/*z* 173 and *m*/*z* 228 (~1% and 2%, respectively). Some compounds with very low concentrations (<1%), such as isomers of $C_{13:0}$, $C_{13:1}$, $C_{15:0}$, $C_{15:1}$, $\Delta^{1,2}$- and $\Delta^{1,6}$ piredeine alkaloids, were tentatively identified but not used in the PCAs.

The PCA for alkaloid data showed a distinct cluster of most *S. invicta* samples (populations AS, BT, JDi, MA, and SG), whereas the six samples from RP, the remaining *S. invicta* site, stood as a separate compact outlying cluster (Fig. 3). The most prominent alkaloids from these five similar populations were *trans*-$C_{13:1}$ (~36–57%), *trans*-$C_{13:0}$ (~9–12%), *trans*-$C_{15:1}$ (~19–24%), and *trans*-$C_{15:0}$ (~7–12%) (Fig. 2, Table 2). One *S. invicta* sample from AS showed an atypical pattern that was more similar to *S. saevissima* at JDs than to other samples from AS (Fig. 3). This atypical sample showed a high relative abundance of *trans*-$C_{13:1}$ (81%), and a low relative abundance of *trans*-$C_{15:1}$ (4%) (data not shown).

The populations of CJ, IN and JDs (identified morphologically as *S. saevissima*), rather than form a well-defined cluster, spread out in a linear array with ants from IN being rather distinct from those of the other two areas (Fig. 3). The prominent alkaloids from these *S. saevissima* populations were *cis*-$C_{13:1}$ (~12–29%) and *trans*-$C_{13:1}$ (~25–73%). The samples from IN showed high relative abundances (about 24%) of *cis*- and *trans*-$C_{11:0}$. All other alkaloids detected in the CJ, IN, and JDs samples had abundances lower than 4%.

Fire ant colonies morphologically identical to *S. invicta* from RP, a more tropical locality to the north, gave alkaloid profiles that were more similar to *S. saevissima* (in particular JDs) than to other *S. invicta* populations (Figs. 2 and 3, Table 2). The relative abundances of *trans*-$C_{13:0}$, *trans*-$C_{15:1}$, and *trans*-$C_{15:0}$ are low (about 4%, 3%, and 0.4%, respectively) like in the *S. saevissima* samples. However, the relative abundances of *trans*-$C_{11:0}$ and *cis*-$C_{13:1}$ were similar to that of other *S. invicta*.

Considering all populations except the anomalous *S. invicta* at RP, the most salient qualitative differences in alkaloid composition between *S. invicta* and *S. saevissima* are: (1) the lower abundance of *cis*-$C_{13:1}$ (~0.7–7%) in *S. invicta* when compared to *S. saevissima* (~13–30%), and (2) the greater abundance of *trans*-$C_{13:0}$, *trans*-$C_{15:1}$, and *trans*-$C_{15:0}$ in *S. invicta* (~19–25%, 9–12%, 7–12%, respectively) when compared to *S. saevissima* (1–4%, 2–4%, <0.1%, respectively) (Fig. 2, Table 2).

Table 2
Piperidine alkaloid composition (relative abundance, $\overline{\mathbf{X}} \pm \mathbf{SD}$) of the fire ant samples analyzed in this study. The first column gives the linear retention index for each compound. Locality codes are defined in Table 1. The numbers in parentheses show sample size.

RI	Compounds	Diagnostic ions	*Solenopsis invicta*						*Solenopsis saevissima*		
			AS (9)	BT (10)	JDi (7)	MA (5)	RP (6)	SG (8)	CJ (6)	IN (8)	JDs (9)
1833	*cis*-2-Methyl-6-*n*-undecyl piperidine (*cis*-$C_{11:0}$)	253 (M^+), 252, 238, 98	–	<1.0	–	–	–	–	2.0 ± 0.4	24.2 ± 2.5	0.6 ± 0.2
1869	*trans*-2-Methyl-6-*n*-undecyl piperidine (*trans*-$C_{11:0}$)	253 (M^+), 252, 238, 98	2.0 ± 0.3	2.3 ± 0.6	2.2 ± 0.4	0.9 ± 0.2	2.4 ± 0.3	2.7 ± 0.5	3.0 ± 0.3	24.5 ± 2.8	3.1 ± 0.2
2009	*cis*-2-Methyl-6-(4-*n*-tridecenyl)-piperidine (*cis*-$C_{13:1}$)	279 (M^+), 278, 264, 236, 180, 124, 111, 98	4.5 ± 1.3	6.7 ± 2.7	1.4 ± 0.7	7.0 ± 3.9	1.4 ± 0.1	0.7 ± 0.2	30.2 ± 4.2	20.6 ± 3.1	13.1 ± 3.7
2052	*trans*-2-Methyl-6-(4-*n*-tridecenyl)-piperidine (*trans*-$C_{13:1}$)	279 (M^+), 278, 180, 124, 111, 98	48.8 ± 4.5	37.2 ± 3.4	56.8 ± 3.4	53.9 ± 4.7	87.5 ± 0.8	57.2 ± 4.4	55.3 ± 5.2	26.5 ± 2.7	74.7 ± 3.7
2083	*trans*-2-Methyl-6-*n*-tridecyl piperidine (*trans*-$C_{13:0}$)	281 (M^+), 280, 266, 98	9.7 ± 0.8	11.9 ± 1.1	10.0 ± 0.8	9.1 ± 1.7	4.1.±0.2	9.6 ± 0.8	3.8 ± 0.4	2.4 ± 0.2	4.1 ± 0.2
2221	*cis*-2-Methyl-6-(6-*n*-pentadecenyl)-piperidine (*cis*-$C_{15:1}$)	307 (M^+), 292, 228, 154, 124, 111, 98	1.4 ± 0.4	3.3 ± 1.1	<1.0	<1.0	–	<1.0	1.5 ± 0.6	<1.0	<1.0
2266	*trans*-2-Methyl-6-(6-*n*-pentadecenyl)-piperidine (trans-$C_{15:1}$)	307 (M^+), 292, 228, 154, 124, 111, 98	23.0 ± 2.6	25.0 ± 1.9	21.2 ± 1.9	19.5 ± 3.5	3.5 ± 0.2	20.9 ± 1.7	3.6 ± 0.5	1.0 ± 0.2	3.9 ± 0.4
2298	*trans*-2-Methyl-6-*n*-pentadecyl piperidine (*trans*-$C_{15:0}$)	309 (M^+), 308, 294, 98	9.1 ± 1.3	11.8 ± 1.6	7.9 ± 1.3	7.6 ± 2.3	<1.0	8.4 ± 1.2	<1.0	<1.0	<1.0
2303	2-Methyl-6-pentadidecenyl piperidine	305 (M^+), 290, 154, 124, 111, 98	1.5 ± 0.2	1.4 ± 0.3	<1.0	1.2 ± 0.3	1.0 ± 0.3	<1.0	–	–	–

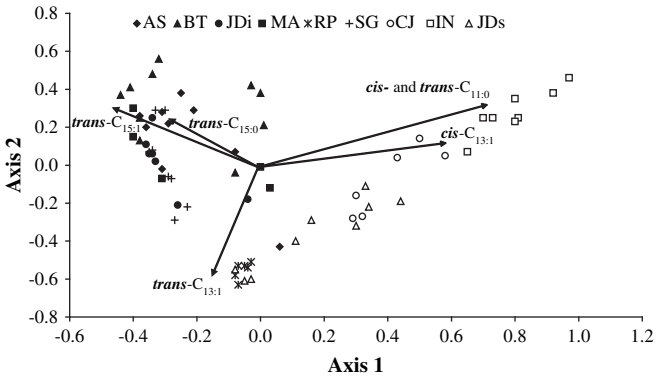

Fig. 3. Ordination diagram based on principal component of relative abundance of 2,6-dialkyl piperidine alkaloids for fire ants identified as *Solenopsis invicta* and *S. saevissima* from nine localities in southeast Brazil. Axes 1 and 2 accounted for 47.1% and 21.5% of total variance, respectively. Each data point represents a different fire ant colony. Filled, cross and asterisk symbols refer to morphological *S. invicta* colonies [asterisks represent *S. invicta* from São Jose do Rio Preto (RP) with anomalous chemical profiles], and open symbols represent *S. saevissima*. The vectors emanating from the center of the graph represent specific 2,6-dialkyl piperidine alkaloids that strongly correlated with PCA patterns. Codes for each locality are given in Table 1.

3.2. Cuticular hydrocarbon composition

Eighteen (C_{25}–C_{31}) alkanes (*n*-alkanes, methyl, dimethyl, and trimethyl-branched alkanes) were tentatively identified in the 68 samples analyzed in this study (Fig. 4, Table 3). As with alkaloids, the PCA for cuticular hydrocarbons in *S. invicta* gave a strong clustering of populations AS, BT, JDi, MA, and SG, while RP samples clustered separately (all identified morphologically as *S. invicta*) (Fig. 5). The most prominent alkanes from the first five localities were C_{25}, 3,7-, 3,9-, and 3,11-DiMe C_{25}, C_{27}, 9-, 11-, and 13-Me C_{27}, 3-Me C_{27}, and 3,7-, 3,9-, and 3,11-DiMe C_{27}. Again as showed for piperidine alkaloids, one sample from AS was atypical (see Fig. 5), having a relatively large amount of 11- and 13-Me C_{25} (6.2%), 3-Me C_{25} (9.3%), and 11,15-, 11,17-, 11,19-DiMe C_{29} (6.1%) compared with traces or absence of these compounds in other samples from AS (data not showed). The lack of 3,7-, 3,9-, and 3,11-DiMe C_{27} also make this sample atypical; other AS samples showed a high relative content of these compounds (23.0–28.0%). The PCA puts this anomalous *S. invicta* sample squarely in the *S. saevissima* cluster (Fig. 5). Two other samples, one from BT and the other from JDi, were also atypical in having a low relative abundance of 3,7-, 3,9-, and 3,11-DiMe C_{27} (6.3% and 6.5%, respectively – data not shown) in comparison with the other samples from these localities (21.0–26.4% and 17.1–29.6%, respectively). These anomalous values are unlike either species.

S. saevissima populations from CJ, IN and JDs produced a well defined cluster for cuticular hydrocarbons (Fig. 5). This stands in stark contrast to the linear array seen in the PCA for alkaloids (Fig. 3). The most prominent hydrocarbons were C_{25}, 11-, 13-Me C_{25}, 3-Me C_{25}, C_{27}, 9-, 11-, 13-Me C_{27}, 11,15-, 13,15-DiMe C_{27}, 3-Me C_{27}, and 11,15-, 11,17-, 11,19-DiMe C_{29} (Fig. 4, Table 3). One sample of *S. saevissima* from JDs showed a high relative abundance of C_{27} (31.2% – data not shown) when compared to other samples from the same site (2.4–6.3%).

The *S. invicta* samples from RP formed a third distinct grouping recognized by PCA (Fig. 5). These alkane profiles, in addition to being homogeneous among themselves, exhibited several clear-cut qualitative differences as well as some interesting similarities with respect to the profiles from other localities. The dominant hydrocarbons at RP were *n*-alkanes (C_{25}, C_{27}, C_{29} and C_{31}). These compounds had much lower abundances or were absent at other localities. The relative amount of 9-, 11-, and 13-Me C_{27} in the RP samples (about 5%) was much lower than in other *S. invicta* samples (20–40%). The abundance of 3-Me C_{27} was also lower (about 2%, against 6–17% at other localities).

Comparing *S. invicta* (excluding RP) to *S. saevissima*, the differences in hydrocarbon composition can be summarized as follows: (1) the moderate relative abundance of 3,7-, 3,9-, and 3,11-DiMe C_{25} (2–11%) and the high relative abundance of 3,7-, 3,9-, and 3,11-DiMe C_{27} in *S. invicta* (20–27%, respectively) contrasting with the lack of these compounds in *S. saevissima*, and (2) the lower abundance of 11- and 13-Me C_{25} and 3-Me C_{25} in *S. invicta* (<1–2%) when compared to *S. saevissima* (8–18%) (Fig. 4, Table 3).

3.3. Chemotaxonomy considerations

Alkane and alkaloid composition provided two objective and independent separation criteria for most *S. invicta* and *S. saevissima* populations across the region covered in this study. The venoms of *S. invicta* and *S. saevissima* each contain diagnostic amounts of particular 2,6-dialkyl piperidine alkaloids. *S. invicta* can be differentiated from *S. saevissima* by one very conservative pattern in alkaloid profiles: the high relative abundance of *trans*-$C_{15:1}$ in *S. invicta*. The high relative abundance of *cis*-$C_{13:1}$ in *S. saevissima* can also be considered diagnostic, but the high variability across *S. saevissima* populations suggests that the quantity of this compound may not be conservative in this fire ant.

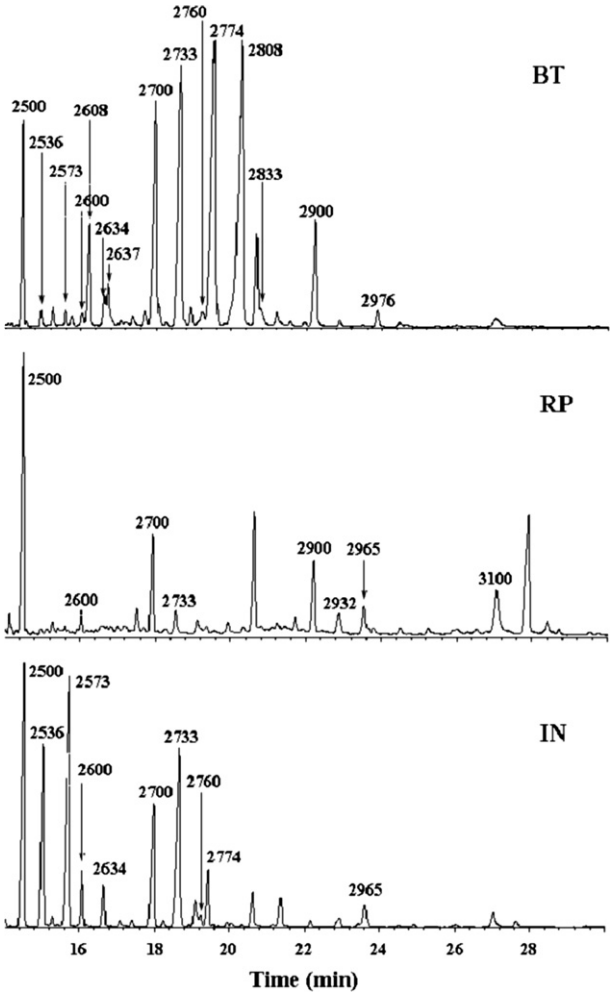

Fig. 4. Gas chromatograph profiles of cuticular hydrocarbons of populations morphologically identified as *Solenopsis invicta* (samples from BT and RP) and *S. saevissima* (sample from IN). The number on each peak corresponds to the retention index given in Table 3. For temperature program for GC–MS see Geden et al. (1998).

The piperidine alkaloid profile of the Brazilian *S. invicta* sampled by us, excepting the population at São José do Rio Preto (RP), is very similar to that found by Vander Meer and Lofgren (1990) for introduced *S. invicta* in the USA. Specimens sampled in Brazil (Mato Grosso State) and at the Argentina–Brazil border share the same profile (MacConnell et al., 1976; Vander Meer and Lofgren, 1990). On the other hand, our results for *S. invicta* at RP matched results of MacConnell et al. (1976) for *S. saevissima* from Buriti, MT, Brazil, but did not correspond to any identified species they sampled in São Paulo or Mato Grosso State, or in the Distrito Federal, in Brazil, or in Tucuman, Argentina.

On the other hand, the single analysis of *S. saevissima* from Buriti (MT) by MacConnell et al. (1976) did not match any of our *S. saevissima* profiles. Alkaloid profiles of the three *S. saevissima* populations here analyzed are also highly variable, although variability seems mostly constrained to a linear array of predictable chemical substitutions between populations. Colonies in one *S. saevissima* population (IN) showed an unusually high relative abundance of *cis*- and *trans*-$C_{11:0}$ when compared to the other two. *S. richteri* from Buenos Aires (Argentina) and *Solenopsis xyloni* and *Solenopsis geminate* from the USA also have a high relative abundance of *cis*- and *trans*-$C_{11:0}$ (MacConnell et al., 1976; Vander Meer and Lofgren, 1990).

The pattern of cuticular hydrocarbons in most of our Brazilian *S. invicta* populations were very similar to that found by Nelson et al. (1980) for USA *S. invicta*. Particularly relevant are the cuticular hydrocarbons 9-, 13-Me C_{27}, 13,15-DiMe C_{27}, 3-Me C_{27}, and 3,7-, 3,9-, 3,11-DiMe C_{27} present in high relative abundance (>10%) in both the USA and Brazil. To our knowledge only Porter et al. (1995) have previously analyzed cuticular hydrocarbons of *S. saevissima*. They found differences between *S. saevissima* and *S. invicta* but did not identify the hydrocarbons.

Our results suggest that cuticular hydrocarbons may be more reliable than alkaloids for separating *S. invicta* from *S. saevissima*. The high relative abundance of 11- and 13-Me C_{25} in *S. saevissima* compared with *S. invicta*, and the high relative abundance of 3,7-, 3,9-, and 3,11-DiMe C_{27} in *S. invicta*, a compound that is absent in *S. saevissima,* can be used as diagnostic

Table 3
Cuticular hydrocarbon composition (relative abundance, $\overline{\mathbf{X}} \pm \mathbf{SD}$) of the fire ant samples analyzed in this study. The first column gives the linear retention index for each compound. Locality codes are defined in Table 1. The numbers in parentheses show sample size.

RI	Compounds	Diagnostic ions[a]	*Solenopsis invicta*						*Solenopsis saevissima*		
			AS (9)	BT (10)	JDi (7)	MA (5)	RP (6)	SG (8)	CJ (6)	IN (8)	JDs (9)
2500	C_{25}	352 (M^+)	5.8 ± 1.4	6.7 ± 0.6	2.4 ± 0.3	3.5 ± 0.2	42.7 ± 1.7	1.7 ± 0.3	9.1 ± 1.0	16.4 ± 1.9	7.8 ± 0.6
2536	11-Me C_{25}	366 (M^+), 351, 225, 169	1.6 ± 0.6	<1.0	<1.0	<1.0	<1.0	<1.0	14.6 ± 1.0	13.7 ± 0.8	14.7 ± 1.0
	13-Me C_{25}	366 (M^+), 351, 197									
2573	3-Me C_{25}	366 (M^+), 337, 309	2.5 ± 0.8	<1.0	<1.0	1.5 ± 0.2	2.6 ± 1.2	<1.0	9.8 ± 0.6	17.7 ± 1.2	8.2 ± 0.7
2600	C_{26}	366 (M^+)	<1.0	<1.0	<1.0	–	2.3 ± 0.4	–	1.2 ± 0.1	3.0 ± 0.5	2.4 ± 0.4
2608	3,7-DiMe C_{25}	380 (M^+), 365, 351, 281, 127	7.5 ± 0.9	6.0 ± 0.7	2.0 ± 0.4	11.0 ± 1.7	–	1.8 ± 0.1	–	–	–
	3,9-DiMe C_{25}	380 (M^+), 365, 351, 253, 155									
	3,11-DiMe C_{25}	380 (M^+), 365, 351, 225, 183									
2634	12-Me C_{26}	380 (M^+), 365, 225, 183	4.2 ± 0.4	3.3 ± 0.6	1.5 ± 0.1	–	–	2.0 ± 0.2	1.5 ± 0.4	3.1 ± 0.4	1.6 ± 0.3
	14-Me C_{26}	380 (M^+), 365, 211, 197									
2637	3,7,11-TriMe C_{25}	394 (M^+), 365, 295, 225, 197, 127	<1.0	<1.0	1.1 ± 0.2	6.1 ± 0.4	<1.0	<1.0	–	–	–
2700	C_{27}	380 (M^+)	9.9 ± 0.4	13.3 ± 1.4	8.5 ± 3.5	7.5 ± 0.8	18.0 ± 0.8	3.7 ± 0.5	7.8 ± 1.8	10.5 ± 1.6	13.7 ± 2.1
2733	9-Me C_{27}	394 (M^+), 379, 281, 141	23.0 ± 0.6	20.9 ± 1.1	33.5 ± 2.2	23.9 ± 0.4	4.6 ± 0.3	38.2 ± 1.3	33.7 ± 2.1	22.6 ± 1.9	31.9 ± 1.3
	11-Me C_{27}	394 (M^+), 379, 253, 169									
	13-Me C_{27}	394 (M^+), 379, 225, 197									
2760	11,15-DiMe C_{27}	408 (M^+), 267, 239, 197, 169	2.2 ± 0.1	1.2 ± 0.5	5.1 ± 1.1	1.9 ± 0.2	2.0 ± 0.8	10.2 ± 1.1	6.9 ± 0.4	3.7 ± 0.9	5.6 ± 0.7
	13,15-DiMe C_{27}	408 (M^+), 211, 239, 197									
2774	3-Me C_{27}	394 (M^+), 379, 365, 337	14.8 ± 0.5	16.9 ± 1.1	15.4 ± 0.9	12.2 ± 0.6	2.0 ± 0.6	12.5 ± 0.5	8.7 ± 0.7	6.4 ± 0.4	10.6 ± 0.4
2808	3,7- DiMe C_{27}	408 (M^+), 393, 379, 351, 309, 127	22.2 ± 2.7	22.4 ± 1.8	21.0 ± 2.7	26.7 ± 0.7	–	22.8 ± 0.8	–	–	–
	3,9- DiMe C_{27}	408 (M^+), 393, 281, 155									
	3,11-DiMe C_{27}	408 (M^+), 393, 379, 253, 183									
2833	3,7,11-TriMe C_{27}	422 (M^+), 393, 323, 253, 197, 127	2.6 ± 0.1	1.9 ± 0.4	3.1 ± 0.7	3.4 ± 0.2	<1.0	2.8 ± 0.2	–	<1.0	<1.0
2900	C_{29}	408 (M^+)	1.5 ± 0.2	3.7 ± 0.6	2.1 ± 0.3	1.2 ± 0.5	11.1 ± 0.7	1.6 ± 0.2	–	–	<1.0
2932	13-Me C_{29}	436 (M^+), 253, 197	<1.0	<1.0	1.2 ± 0.1	<1.0	3.3 ± 0.3	1.2 ± 0.1	–	<1.0	<1.0
	15-Me C_{29}	436 (M^+), 225									
2965	11,15-DiMe C_{29}	436 (M^+), 295, 239, 225, 169	<1.0	<1.0	<1.0	–	6.3 ± 0.5	<1.0	6.7 ± 0.5	1.7 ± 0.6	2.3 ± 0.5
	11,17-DiMe C_{29}	436 (M^+), 295, 267, 197, 169									
	11,19-DiMe C_{29}	436 (M^+), 295,169									
2976	3-Me C_{29}	422 (M^+), 393, 365	<1.0	<1.0	1.0 ± 0.1	–	–	<1.0	–	–	–
3100	C_{31}	436 (M^+)	–	<1.0	–	–	4.4 ± 0.4	–	–	–	–

[a] For methyl-branched alkanes, ion clusters occur as even/odd mass pairs depending on the branching point (Nelson et al., 1972). For brevity, except for the ion quasi-molecular, only the odd fragment of each pair is listed.

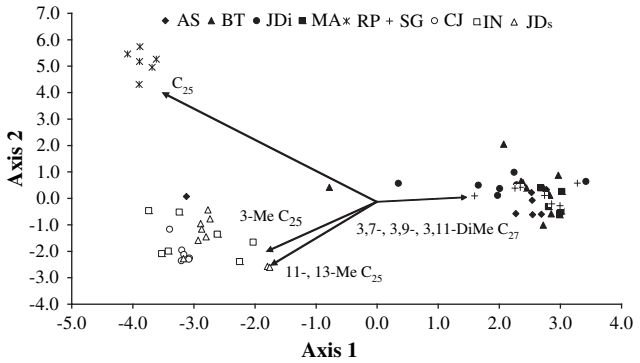

Fig. 5. Ordination diagram based on principal component of relative abundance of cuticular hydrocarbons for fire ants identified as *Solenopsis invicta* and *S. saevissima* from nine localities in southeast Brazil. Axes 1 and 2 accounted for 51.0 and 34.3% of total variance, respectively. Each data point represents one ant colony. Filled, cross and asterisk symbols refer to morphological *S. invicta* colonies [asterisks represent *S. invicta* from São Jose do Rio Preto (RP) with anomalous chemical profiles], and open symbols represent *S. saevissima*. The vectors emanating from the center of the graph show specific cuticular hydrocarbons and its direction that strongly correlated with PCA patterns. Codes for each locality are given in Table 1.

characters to separate the two species. Cuticular hydrocarbons have been used previously to separate *S. invicta* from *S. richteri* (Nelson et al., 1980; Vander Meer and Lofgren, 1990).

One striking aspect of our results is the great between-colony differences sometimes found in local populations of the same species. Almost 5% of the colonies had chemical profiles with highly deviant PCA scores due to one or more compounds falling well beyond typical values for the local population. The commonness of outliers is even more evident when contrasting variation of typical *S. saevissima* and *S. invicta* with the clustering of alkaloid and hydrocarbon PCAs of chemically unique *S. invicta* at RP. Much of the chemical variation documented by us is probably genetic in nature. This suggests that both *Solenopsis* species studied here may have considerable evolutionary potential for varying chemicals used in communication and defense. If chemical profiles are the product of adaptive evolution, we might expect that populations have evolved profiles appropriate to the environments in which they live. To our knowledge, chemical matching with environment has not been investigated in *Solenopsis* or other ants.

A point that remains unexplained in our analysis is the unusual distinctive chemistry of the six chemically homogeneous *S. invicta* nests sampled at São José do Rio Preto (RP). These collections made along open paths in the city's main city park presented every indication of being typical *S. invicta*. The ants look like *S. invicta*, and their unusual alkaloid profile is neither similar to *S. invicta* nor to *S. saevissima*; the hydrocarbon pattern of this population dominated by *n*-alkanes such as C_{25}, C_{27}, C_{29}, and C_{31}, looks very different from populations at other localities, either *S. invicta* or *S. saevissima*.

We conclude that, setting aside the anomalous RP population, *S. invicta* has, with few exceptions, a consistent chemical profile for both class of chemicals here analyzed, whereas populations of *S. saevissima*, while consistent for hydrocarbons, tended to present local differentiation in alkaloids. The relatively great chemical differentiation in the *S. saevissima* populations studied here may be in part due to their occurrence over a wide elevational range in dissected mountainous terrain. The marked chemical differences among populations of *S. saevissima*, the distinctive alkaloid and hydrocarbon profiles of *Solenopsis* from RP, together with earlier suggestions of undescribed species of *Solenopsis* based on chemical and morphological characters [see MacConnell et al. (1976) and Porter et al. (1995)] will require additional study.

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