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Brazilian Propolis of *Tetragonisca angustula* and *Apis mellifera*¹

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Abstract – Using electrospray ionization mass spectrometry in negative ion mode, ESI(-)-MS, we characterized and compared the composition of both *Tetragonisca angustula* (Jataí) and *Apis mellifera* (honey bee) propolis from different regions in Brazil. The ESI(-)-MS fingerprints show that the composition of *A. mellifera* propolis is highly region-dependent, whereas that of *T. angustula* propolis is nearly constant through the country, as was confirmed by principal component analysis. The constant chemical composition of *T. angustula* propolis is explained by the collection of resins preferentially from *Schinus terebenthifolius*, a plant source found throughout Brazil. This single source was determined via comparison of the components of the *T. angustula* propolis samples with that of plant extracts.

propolis / electrospray ionization mass spectrometry / *Tetragonisca angustula* / *Apis mellifera* / *Schinus terebenthifolius*

1. INTRODUCTION

The native stingless bee, *Tetragonisca angustula* (Latreille, 1811), known in Brazil as *Jataí*, is found from Mexico to Argentina, with the exception of the Andes mountain range, the scrubland (caatinga) of the Brazilian northeast and some regions of the Amazon (Oliveira et al., 2004). Not only are native stingless bees the natural pollinators of the flora of the Neotropical regions but also are less harmful to humans and domestic animals, and are resistant to the diseases and parasites of honeybees. The propagation of their colonies contributes to the preservation of biodiversity. Nevertheless, there is a poor level of domestication technology for most species, (Heard, 1999), *T. angustula* being one of the notable exceptions. Beekeepers maintain hives of this species,

often alongside hives of the introduced *Apis mellifera* bees, as *T. angustula* honey is sold for higher prices than *A. mellifera* honey. Not enough is known about the trophic niche overlap between introduced and native bee species to determine whether competition for resources may eventually drive the native stingless bees to extinction (Wilms et al., 1996). As the preferred nesting sites for stingless bees are the preformed cavities of live trees found mainly in primary forests, deforestation is also an important factor for the decrease in the density of these eusocial bees (Eltz et al., 2002). For all these reasons, information on the composition of the honey and propolis of these native bees, as well as the plants that are visited as sources of pollen, nectar and resins, are of prime importance. The comparative behavior of native stingless bees and introduced honey bees was

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studied in the south (Toledo et al., 2003) southeast (Wilms et al., 1996) and northeast (Viana et al., 1997) of Brazil. Viana et al. (1997) cited some plants visited for nectar, pollen and resin.

The composition and activity of propolis from native Brazilian stingless bees has not been fully studied. Velikova et al. (2000) studied the composition of ethanolic extracts of samples of propolis from 12 different species of native Brazilian stingless bees and one sample of *A. mellifera* propolis by gas chromatography mass spectrometry (GC-MS), concluding that only *A. mellifera* propolis contained prenylated p-coumaric acid derivatives typical of Brazilian propolis. The chemical composition of the Meliponinae samples was heterogeneous; although the two samples of *T. angustula* propolis were classified together in the "triterpenic" group. Most of the samples analyzed were significantly active against *Staphylococcus aureus*. Miorin et al. (2003) studied the chemical composition of the ethanolic extracts of several samples of propolis of *T. angustula* and *A. mellifera* from the states of Paraná and Minas Gerais in Brazil by high performance liquid chromatography with a diode array detector (HPLC-DAD), identifying high concentrations of several derivatives of cinnamic and p-coumaric acids in propolis from *A. mellifera* but only low concentrations of some of those compounds in the propolis of *T. angustula* from the same regions. As both types of propolis were active against *S. aureus*, the authors concluded that other compounds, not detected by the analytical method used, could be responsible for the antibacterial activity of the *T. angustula* samples. Pereira et al. (2003) studied the chemical composition of the dichloromethane, acetone and methanol fractions of propolis samples of *A. mellifera* and *T. angustula* from São Paulo, Brazil by GC-MS, concluding that the less polar (dichloromethane) fractions were identical, but the other fractions showed significant differences in composition.

It is known that the composition of *A. mellifera* propolis varies in composition depending on its region of origin (Bankova et al., 2000). This variation is less intense in temperate regions, where *A. mellifera* bees find poplar trees, their preferred source of resin (Greenaway et al., 1990). In tropical regions (where poplar trees are not native) introduced *A. mellifera* bees have had to adapt, choosing different plant

sources. A previous study of ethanolic extracts of propolis, using direct insertion electrospray ionization mass spectrometry (ESI-MS) in the negative ion mode, identified five region-dependent types of *A. mellifera* propolis in Brazil (Sawaya et al., 2004).

Although GC-MS has been used for many years for the analysis of the main volatile and semi-volatile components of propolis, many components are not volatile enough for direct GC-MS analysis, even upon derivatization. Electrospray ionization has revolutionized the way molecules are ionized and transferred to mass spectrometers for mass and structural analysis, and has greatly expanded the applicability of mass spectrometry for a variety of new classes of molecules with thermal instability, high polarity and high mass. (Cole, 1997) ESI is convenient for direct MS analysis of multi-component polar natural product extracts because most molecules bearing acidic or basic sites will be detected as a single ion, either in their protonated $[M + H]^+$ or deprotonated $[M - H]^-$ forms. Online tandem MS/MS with collision-induced dissociation (CID) of $[M + H]^+$ or $[M - H]^-$ is used for more refined structural elucidation studies. This fast ESI-MS fingerprinting technique has also been successfully applied to complex samples such as plant extracts (Mauri and Pietta, 2000), beer (Araújo et al., 2005), whisky (Möller et al., 2005), vegetable oil (Catharino et al., 2005) and wine (Catharino et al., 2006).

We decided therefore to characterize the composition of *T. angustula* propolis from different regions in Brazil using electrospray ionization mass spectrometry in the negative ion mode, ESI(-)-MS, and to compare the results with those from *A. mellifera* propolis, both from these same regions. The comparison would suggest whether the composition of *T. angustula* propolis is similar or different from *A. mellifera* propolis and whether it is region-dependent or if these bees seek a preferred plant source for their resins. In addition, to statistically establish the correlation among the propolis samples, chemometric principal component analysis (PCA) has been applied. Methanolic and dichloromethane extracts of plants visited by *T. angustula* were also analyzed by ESI(-)-MS to determine the resin-providing plants.

2. MATERIALS AND METHODS

2.1. Propolis samples and extraction procedure

Samples of *Apis mellifera* and *Tetragonisca angustula* propolis were provided by beekeepers from the south, southeast and northeast of Brazil. The ten samples of *Apis mellifera* propolis analyzed were from the following states: 1 from Alagoas collected in July 2002, 3 from Bahia collected in February 2001, 1 from São Paulo collected in April 2000, 1 from Minas Gerais collected in September 2001, 1 from Santa Catarina collected in January 2004 and 3 from Paraná collected in 2002. The ten samples of *T. angustula* propolis analyzed were from the following states: 1 from Bahia collected in August 2004 and 3 from Bahia collected in December 2004, 2 from Minas Gerais collected in August 1998 and 1 from Minas Gerais collected in April 2004, 1 from São Paulo collected in 1998, 1 from Santa Catarina collected in January 2004 and 1 from Paraná collected in 1997.

All samples were frozen and ground prior to extraction. The samples were extracted by maceration for 7 days in a shaker, regulated at a speed of 100 rpm and temperature of 30 °C, with 10 mL of absolute ethanol (Merck, Darmstadt, Germany) for every 3 g of crude propolis. The insoluble portion was then separated by filtration, the filtrates kept in a freezer at -16 °C overnight and filtered again at this temperature to reduce the wax content of the extracts. Solvent was then evaporated on a water bath at a temperature of 50 °C to obtain dry extracts of propolis.

2.2. Plant samples and extraction procedure

The following plants visited by *T. angustula* were initially identified only by their popular names: *casadinha*, *corredeira*, *assa peixe roxo*, *assa peixe branco*, *cajueiro*, *aroeira vermelha*, *atinga de crioulo*, *pega pega*, *cipó uva*, *eucalipto*, *flamboyant*, *mangueira*, *velame*, *jurema*, *bombabo* and *bananaeira*. Samples of the leaves and buds of these plants were extracted by sonication with methanol or dichloromethane for ten minutes in order to obtain the more superficial components. The plant samples were also macerated for 1 week in methanol or dichloromethane to obtain a more complete extract. The solvent was then evaporated to obtain the dry plant extracts.

Only one plant extract, that of *Schinus terebinthifolius* (Radii) known popularly as *aroeira vermelha*, presented compounds in common with *T. angustula* propolis. Therefore, extracts of the fruit

and the flowers were also prepared by the same method as the leaves. A voucher specimen of this plant has been deposited in the herbarium of Universidade São Francisco, Bragança Paulista, SP.

2.3. General experimental procedures

The dry propolis and plant extracts were dissolved in a solution of 70% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA), 30% (v/v) deionized water and 0.1% ammonium hydroxide. The solutions used for ESI(-)-MS analysis contained approximately 50 ng of dry extract. The solutions of propolis and plant extracts were infused directly into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 μ L/min. ESI(-)-MS and tandem ESI(-)-MS/MS were acquired using a hybrid high-resolution and high-accuracy (5 ppm) Micromass Q-TOF mass spectrometer under the following conditions: capillary and cone voltages were set to -3000 V and -40 V, respectively, with a de-solvation temperature of 100 °C. For ESI(-)-MS/MS, the energy for the collision induced dissociations (CID) was optimized for each component. Although fingerprints were acquired in the m/z 100–1000 range, no important ions were observed above m/z 500, therefore results are shown from m/z 100–600.

2.4. Statistical analysis of data

Principal Component Analysis (PCA) was performed using the 2.60 version of Pirouette software from Infometrix, Woodinville, WA, USA. The mass spectra were expressed as the intensities of individual $[M - H]^-$ ions (i.e. variables) of the six most intense ions in the fingerprints of each sample. The data was preprocessed using auto scale and the PCA method was run.

3. RESULTS AND DISCUSSION

Figure 1 shows the ESI(-)-MS fingerprints of the samples of *A. mellifera*, which clearly suggest a highly region-dependent composition. These differences in composition are known to result from different plant origins of the resins used for *A. mellifera* propolis. The main plant source for green propolis from the southeast of Brazil is *Baccharis dracunculifolia* (Banskota et al., 1998; Bankova et al., 1999) resulting in a type of propolis containing many prenylated p-coumaric acid derivatives and caffeic acid derivatives. Brown propolis from the south of Brazil contains flavonoids such as

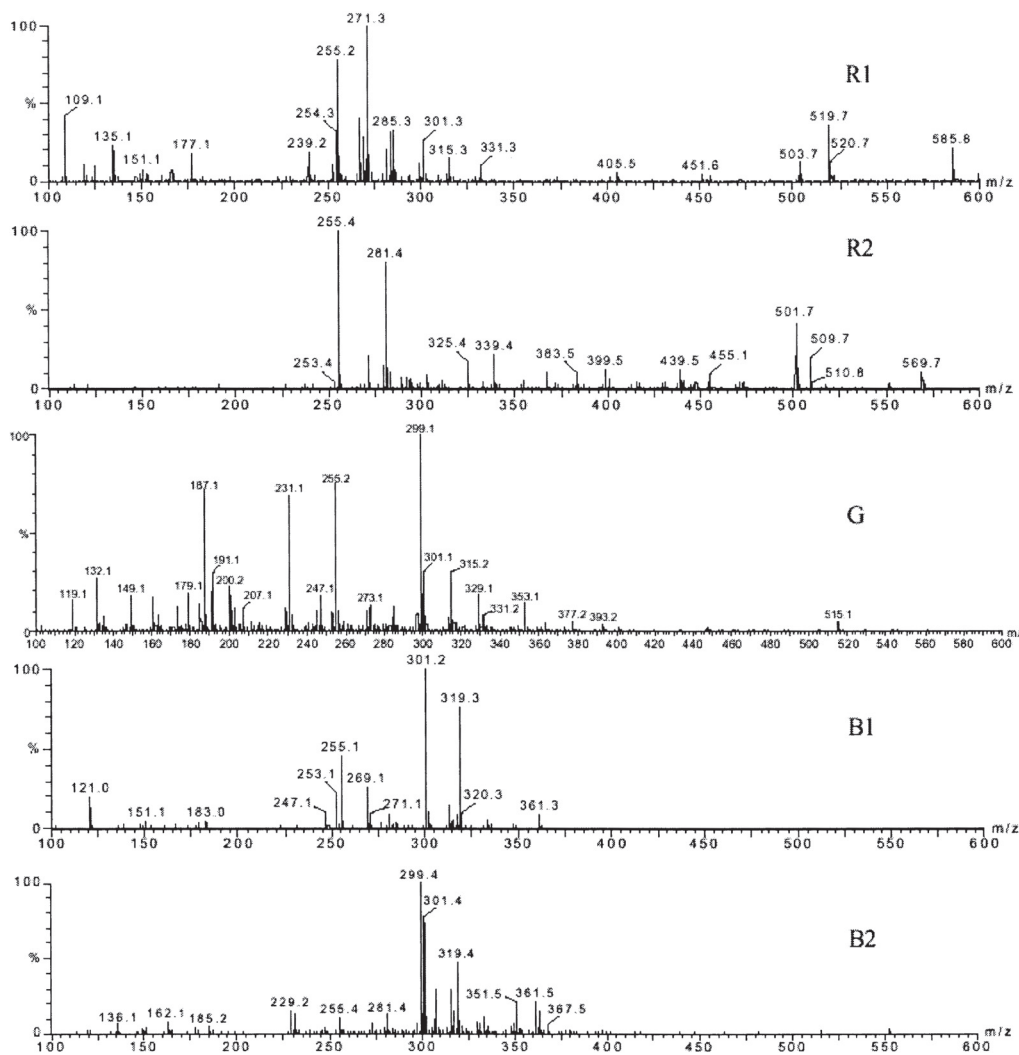


Figure 1. ESI(-)-MS fingerprints of the extracts of *A. mellifera* propolis: red propolis from the state of Bahia (R1 and R2), green propolis from the states of São Paulo and Minas Gerais (G) and brown propolis from the states of Santa Catarina and Paraná (B1 and B2).

chrysin and pinocembrin (Sawaya et al., 2004) also found in propolis from temperate regions in Europe and North America, as well as terpenes which originate from the resins of the native pine trees, *Araucaria angustifolia* (Bankova et al., 1999). Red propolis from the northeast of Brazil has a variable composition and the plant origins of the resins are still under study.

ESI does not have good sensitivity for apolar compounds and compounds with low polarity, and the intensity of the ions observed in the

ESI-MS fingerprints is affected by factors such as ionization conditions, pH of the solution used and matrix suppression. Nevertheless, a previous study demonstrated that ESI(-)-MS fingerprinting was capable of distinguishing between different types of *A. mellifera* propolis (Sawaya et al., 2004). Therefore the same analytical conditions were used for the samples of *T. angustula* propolis to see if there was similarity in the composition of propolis extracts of these two types of bees.

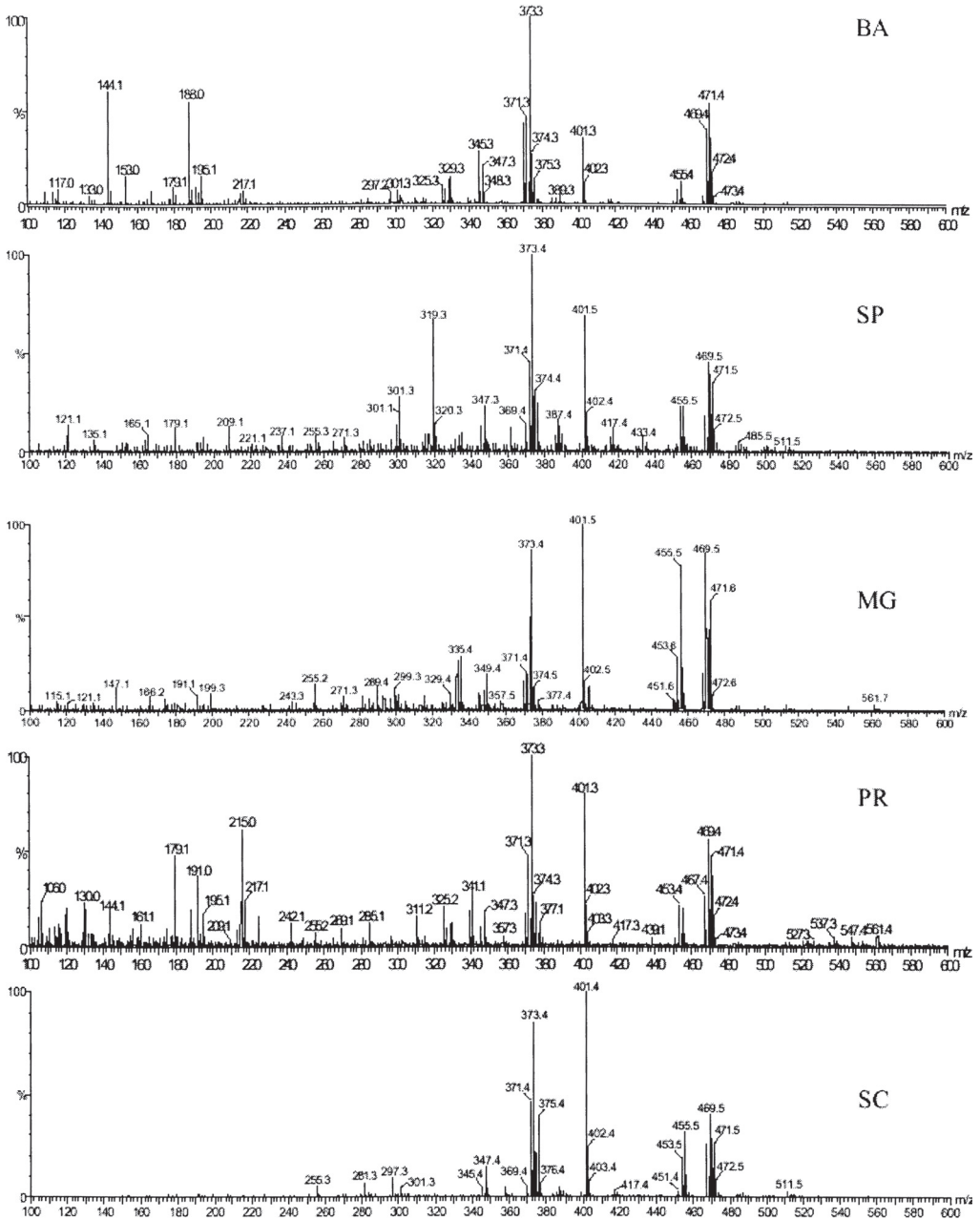


Figure 2. ESI(-)-MS fingerprints of the extracts of *T. angustula* propolis from the state of Bahia (BA), São Paulo (SP), Minas Gerais (MG), Paraná (PR), and Santa Catarina (SC).

The ESI(-)-MS fingerprints of *T. angustula* propolis (Fig. 2) show, however, a nearly constant chemical composition (regardless the geographical origin of the sample) which is

completely different from all the types of *A. mellifera* propolis analyzed so far. This constancy suggests that one plant is the main source of the resins for propolis of this native

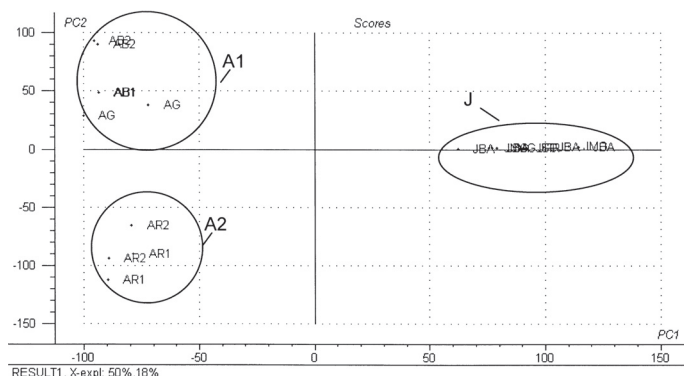


Figure 3. PCA analysis of the ESI(-)-MS fingerprint data for the extracts of *A. mellifera* and *T. angustula* propolis from several regions in Brazil. Group A1: *A. mellifera* from the South and Southeast of Brazil, Group A2: *A. mellifera* from the Northeast of Brazil and group J: *T. angustula* from the Northeast, South and Southeast of Brazil.

bee throughout the northeast, south and southeast of Brazil. Variation in the set and abundances of the minor ions does occur, perhaps due to other eventual plant sources.

The relative intensities of the six most intense ions in the fingerprints of each sample were used as variables for the PCA of the samples of propolis of both types of bees. In order not to bias the results, 10 samples of propolis from each type of bee were analyzed, divided into four samples from the northeast and six from the south and southeast of Brazil. Figure 3 shows the PC1 × PC2 plot that resulted. Note that the *T. angustula* samples are closely grouped, whereas the *A. mellifera* samples are clearly divided into two groups, corresponding to the different geographical regions from which they proceed. This confirms the observation that the composition of *A. mellifera* propolis varies depending on the geographic origin of the samples, whereas *T. angustula* propolis does not.

The extracts of plants indicated by beekeepers as being visited by *T. angustula* bees were analyzed by ESI(-)-MS but only one plant, *Schinus terebenthifolius* (Radii), presented compounds in common with *T. angustula* propolis. *S. terebenthifolius* is a tree 2–6 m high, belonging to the Anacardiaceae family, found throughout Brazil and many parts of South America. It is known in Brazil as “*aroeira vermelha*” or “*aroeira mansa*” and in English as the pink-pepper tree. Its leaves and fruit are popularly used for medicinal purposes (Correa, 1984). Decoctions of the leaves, stalks and flowers have been used for the treatment of tumors and leprosy, whereas recent studies have identified activity against *Candida albi-*

cans yeast in the non-polar fraction of a decoction of the leaves (Schmourlo et al., 2005). References have been found to native bees of the Trigonini family visiting this plant for nectar in the state of São Paulo (Ramalho et al., 1990). In another study, carried out in the south of Brazil, stingless bees were found to be extremely numerous on *S. terebenthifolius* flowers. (Wilms et al., 1997) Therefore it may be that *T. angustula* bees visit this plant looking for resins with antimicrobial activity, which may be necessary for the survival of the colony.

Studies of the fruit, leaves and bark of *S. terebenthifolius* identified several triterpenes: terebinthone and schinol (Kaistha and Kier, 1962a, b), masticadienoic and hydroxymasticadienoic acids, sitosterol, and simiarenol (Campello and Marsaioli, 1974), baruenone and terebenthifolic acid (Campello and Marsaioli, 1975), α-amyrin and α-amyrinenone (Lloyd et al., 1977). Of these components, masticadienoic acid and masticadienolic acid have been found to possess medicinal properties (Jain et al., 1995). The presence of triterpenes in the composition of *T. angustula* propolis has also been characterized. β-amyrine and lanosterol were identified in *T. angustula* propolis from the south and southeast of Brazil (Velikova et al., 2000). α- and β-amyrine, lupenone, lupeol, β-amyrine acetate, lupeol acetate, cycloartenol, lupeol, friedour-7-en-one and friedour-7-en-ol were identified in the dichloromethane extracts of *T. angustula* propolis from São Paulo (Pereira et al., 2003).

Extracts of *S. terebenthifolius* leaves, flowers and fruit obtained by one-week maceration in dichloromethane contain several of the components of *T. angustula* propolis, although

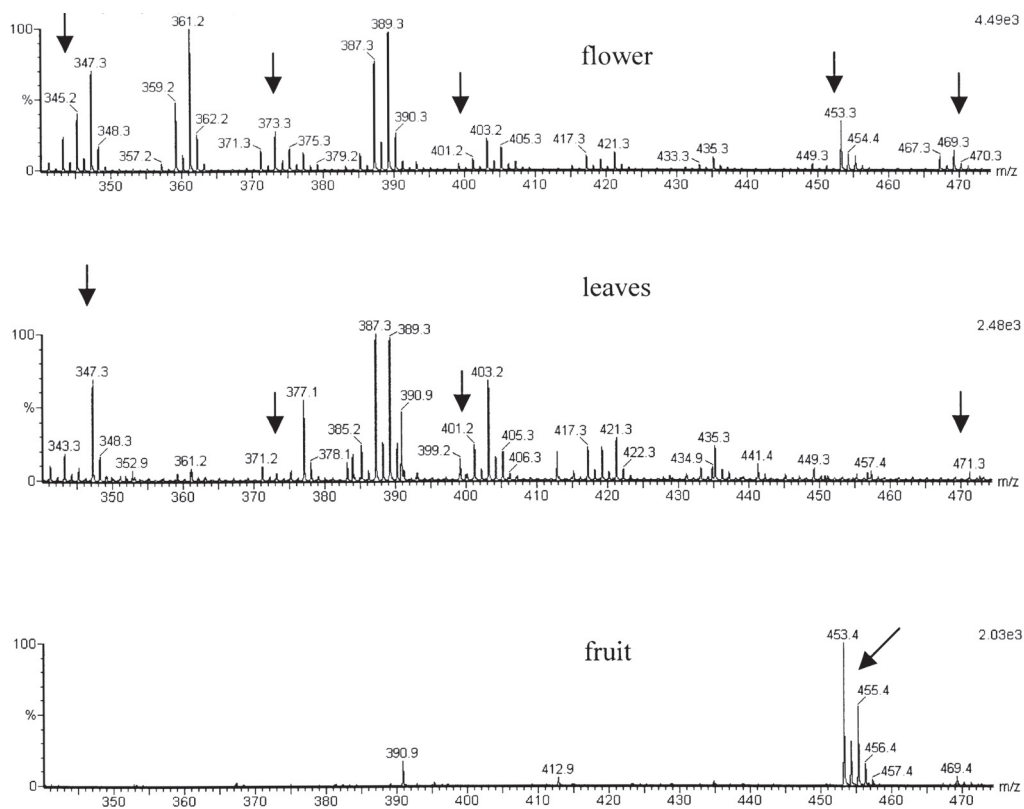


Figure 4. ESI(-)-MS fingerprints of the dichloromethane extracts of *S. terebenthifolius* flowers, leaves and fruit.

many other compounds were also extracted. In spite of this problem, it was still possible to determine the high-resolution mass of the compounds of interest and their dissociation patterns. The ESI(-)-MS fingerprints of these extracts can be seen in Figure 4. Further studies on the best method to extract only the resins of this plant will certainly be needed. Table I gives the fragments observed in the ESI(-)-MS/MS and high-resolution m/z of compounds found in both the extracts of *S. terebenthifolius* and *T. angustula* propolis. Two compounds, found in *T. angustula* propolis as well as in the extracts of flowers and fruit of *S. terebenthifolius*, are probably masticadienoic acid and masticadienolic acid, previously identified in extracts of *S. terebenthifolius* fruit (Jain et al., 1995). The calculated and measured m/z for deprotonated masticadienoic acid was 453.3369/453.3384

($\Delta m/z = 3.3$ ppm) and for deprotonated masticadienolic acid: 455.3525/455.3555 ($\Delta m/z = 6.5$ ppm), with enough accuracy to determine a composition match.

The ESI(-)-MS/MS (Tab. I) frequently show a dissociation by the initial neutral loss of 44 Da (CO_2) or loss of 46 Da (CH_2O_2) common for deprotonated carboxylic acids. These losses are logical for both masticadienoic acid and masticadienolic acid. The similarity of the dissociation patterns of the compounds in Table I leads us to suggest that the other compounds observed in both the extracts of *S. terebenthifolius* and *T. angustula* propolis are also terpenes with an acid group, possibly similar in structure to masticadienoic and masticadienolic acids. Further studies are being undertaken to isolate and confirm the identity of these compounds.

Table I. High-resolution m/z and ESI(-)-MS/MS fragments for dissociation of compounds found in both the extracts of aerial parts of *S. terebenthifolius* and of *T. angustula* propolis.

High-resolution m/z	Source*	Collision energy (V)	Main fragments m/z (relative abundance %)
345.2510	p, fl	25	345 (15), 301 (100), 119 (5), 106 (5)
347.2631	p, fl, l	25	347 (15), 303 (100), 106 (5)
371.2644	p, fl, l	30	371 (10), 327 (100), 119 (10), 106 (10)
373.2755	p, fl, l	30	373 (10), 329 (100), 119 (5), 106 (5)
401.3181	p, fl, l	30	401 (10), 357 (100), 119 (5), 106 (5)
453.3384	p, fl, fr	50	453 (100), 423 (10), 407 (40), 391 (20) 137 (20)
455.3555	p, fl, fr	50	455 (100), 437 (20), 409 (15), 407(25)
469.3395	p, fl, l	45	469 (10), 451 (15), 439 (20), 423 (10), 407 (100), 391 (15)
471.3592	p, fl, l	40	471 (100), 453 (30), 441(50), 425 (60), 407 (80), 393 (15)

* p = propolis, fl = flower, fr = fruit, l = leaf.

4. CONCLUSION

A previous study of the propolis of native stingless bees, which analyzed several different species together, found a variable composition and concluded that this variance was due to the short foraging range of these bees, which led them to collect resins from the first plant exudates encountered during their flights (Velikova et al., 2000). We, by focusing on only one species of stingless bee and on the acid components, observed quite the opposite, a characteristic composition of the propolis of *T. angustula* from samples collected throughout Brazil, confirmed by PCA. Also, in contrast, the composition of Brazilian *A. mellifera* propolis was found by ESI(-)-MS to be very region-dependent. The characteristic composition of *T. angustula* propolis suggests the collection of resins from a preferred Brazilian plant source. By comparing ESI-MS from both propolis samples and *S. terebenthifolius* extracts, we concluded that this plant is the main source of resins for *T. angustula* propolis. This conclusion is also supported by reports from beekeepers, which have witnessed native bees visiting *S. terebenthifolius*. The behavior of *T. angustula* in Brazil is parallel to that of *A. mellifera* in temperate regions where honeybees have evolved to use primarily poplar tree resins, whenever they can be found. Although compound identification was not crucial for the

present comparative study, two compounds were tentatively identified, and further studies are being undertaken to fully identify the main acid components of *T. angustula* propolis as well as to determine by ESI-MS the propolis composition from the many other Brazilian native stingless bees.

ACKNOWLEDGEMENT

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Résumé – La propolis brésilienne de *Tetragonisca angustula* et d'*Apis mellifera*. Le but de cette étude était de caractériser la composition chimique de la propolis de l'abeille sans aiguillon *Tetragonisca angustula* (Hymenoptera, Apidae) de diverses régions du Brésil au moyen de la spectrométrie de masse par ionisation avec électronébulisation en mode ionique négatif (SM(-)-ESI) et de la comparer à la propolis d'*Apis mellifera* des mêmes régions. Les informations sur la composition de la propolis de ces abeilles sans aiguillon, et sur les plantes qu'elles visitent pour récolter des résines, sont primordiales pour la survie de cette espèce indigène. Les spectres SM(-)-ESI de la propolis d'*A. mellifera* (Fig. 1) montrent une composition qui dépend largement de la région. Par contre celles de *T. angustula* (Fig. 2) montrent une composition caractéristique pratiquement constante. L'analyse en composantes principales des données de la SM(-)-ESI confirment ces conclusions (Fig. 3). La composition caractéristique de la propolis de *T. angustula* s'explique par

le fait que ces abeilles récoltent les résines préférentiellement sur une plante qui pousse dans tout le Brésil. Des échantillons de feuilles et de bourgeons des plantes visitées par *T. angustula* ont été extraits au méthanol et au dichlorométhane et analysés par SM(-)-ESI. La comparaison des spectres des échantillons de propolis de *T. angustula* avec ceux des extraits de fleurs, de fruits et de feuilles de *Schinus terebenthifolius* suggère que cette plante est la source principale de propolis pour *T. angustula* au Brésil (Fig. 4). Cette conclusion est confirmée par les dires des apiculteurs qui ont observé des abeilles indigènes visiter *S. terebenthifolius*. Le tableau I donne les fragments observés en SM(-)-ESI et la résolution élevée *m/z* des composés trouvés à la fois dans les extraits de *S. terebenthifolius* et dans la propolis de *T. angustula*. Deux de ces composés ont été identifiés comme étant l'acide masticadiénoïque et l'acide masticadiénolique, déjà trouvés dans des extraits du fruit de *S. terebenthifolius*. Le comportement de *T. angustula* est semblable à celui d'*A. mellifera* dans les régions tempérées, où les abeilles domestiques ont évolué pour utiliser principalement les résines de peupliers, chaque fois qu'elles en trouvent.

Tetragonisca angustula* / *Apis mellifera* / propolis / spectrométrie de masse par ionisation avec électrobulbation / *Schinus terebenthifolius

Zusammenfassung – Brasilianischer Propolis von *Tetragonisca angustula* und *Apis mellifera*. Die chemische Zusammensetzung des Propolis der stachellosen Bienenart *Tetragonisca angustula* (Hymenoptera, Apidae) aus verschiedenen Regionen Brasiliens wurde massenspektrometrisch mittels Elektrospray-Ionisierung und Detektion im Negativ-Ionen Modus (ESI(-)-MS) untersucht. Die Proben wurden mit Propolis von *Apis mellifera* aus denselben Regionen verglichen. Informationen über Zusammensetzung des Propolis dieser Stachellosen Bienen und über die Pflanzen, von denen sie das Harz sammeln, sind sehr wichtig für das Überleben dieser einheimischen Art.

Die ESI(-)-MS Spektren vom *A. mellifera* Propolis (Abb. 1) zeigen eine Zusammensetzung, die zum großen Teil von der Region abhängig ist. Im Gegensatz dazu zeigen die Spektren von *T. angustula* (Abb. 2) eine typische, fast konstante Zusammensetzung. Eine PCA (Faktoren Analyse) der Daten der ESI(-)-MS bestätigen diese Schlussfolgerungen (Abb. 3). Die charakteristische Zusammensetzung des Propolis von *T. angustula* erklärt sich durch die Tatsache, dass diese Bienen bevorzugt nur von einer Pflanze Harz sammeln, die in ganz Brasilien vorkommt. Proben von Blättern und Knospen der von *T. angustula* besuchten Pflanzen wurden in Methanol und Dichloromethan extrahiert und ebenfalls mit ESI(-)-MS analysiert. Der Vergleich der Spektren von *T. angustula* Propolisproben mit den Extrakten der Blüten, Früchte und Blätter von *Schi-*

nus terebenthifolius lassen vermuten, dass diese Pflanze die Hauptquelle für Propolis bei *T. angustula* in Brasilien ist (Abb. 4). Diese Schlussfolgerung wird durch Berichte von Imkern gestützt, die einheimische Bienen auf *S. terebenthifolius* beobachtet haben.

Tabelle I listet Fragmente der ESI(-)-MS/MS Spektren auf sowie die Hochauflösung *m/z* der Fragmente, die gleichzeitig im Extrakt von *S. terebenthifolius* und im Propolis von *T. angustula* gefunden wurde. Zwei dieser Komponenten sind als masticadiénoische Säure und masticadiénolische Säure bestimmt worden, die bereits in Extrakten der Früchte von *S. terebenthifolius* gefunden wurden. Das Verhalten von *T. angustula* ähnelt dem von *A. mellifera* in temperierten Zonen, wo sich bei Honigbienen eine Bevorzugung des Harzes von Pappeln entwickelt hat, wenn sie Pappeln im Flugbereich finden können.

Tetragonisca angustula* / *Apis mellifera* / Propolis / (ESI(-)-MS) / *Schinus terebenthifolius

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