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Original article

Electrospray ionization mass spectrometry fingerprinting of propolis of native Brazilian stingless bees*

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Abstract – Stingless bees are found in many tropical and subtropical regions of the word. The knowledge of the composition of their propolis as well as the plants that are visited as sources of resins is therefore of prime importance. Here the negative ion mode electrospray ionization mass spectrometry [ESI(-)-MS] fingerprints of propolis from various species of native stingless bees from different regions in Brazil are compared to determine their composition patterns. The correlation among the propolis samples was investigated via chemometric analysis.

ESI-MS fingerprint / propolis / native stingless bees / Brazil

1. INTRODUCTION

Stingless bees (Hymenoptera, Apidae, Meliponini) are found in many tropical and subtropical regions of the word. They are the major visitors and native pollinators of flowering plants in the tropics, comprising a large group of small to medium sized bees with a level of social organization comparable to that of *Apis mellifera* bees (Heard, 1999). Native stingless bees are, however, less harmful to humans and domestic animals and resistant to the diseases and parasites of honeybees, and the propagation of their colonies contributes to the preservation of biodiversity (Aidar, 1996). Nevertheless, there is a poor level of domestication technology for most species of stingless bees (Heard, 1999). As their preferred nesting sites are the preformed cavities of live trees found mainly in primary forests, deforestation has decreased the density of these eusocial bees (Eltz et al., 2002). Therefore, information on the composition of the honey and propolis of these native bees, as well as the plants they visit as sources of pollen, nectar and resins, are of prime importance. Although studies have been performed on the overlap between native and introduced honey bees visiting flowers is several regions of Brazil (Wilms et al., 1996; Viana et al., 1997; Toledo et al., 2003), few studies have analyzed possible plant sources for the fabrication of propolis by stingless bees.

Bees collect exudates and resins from plants around their hives, adding wax to produce a

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complex mixture of variable chemical composition known as propolis. Bees use propolis to reinforce the combs and to keep the hive environment aseptic. *A. mellifera* propolis has been used by humans for many centuries for its multiple pharmacological properties (Marcucci, 1995), hence its chemical composition and plant sources have been studied for nearly a century. In contrast, the chemical composition of stingless bee propolis has only recently begun to be studied. Bankova et al. (1999) studied the essential oil content of propolis from three native stingless bees via gas chromatography mass spectrometry (GC/MS). Velikova et al. (2000a) studied the composition of ethanolic extracts of several samples of propolis from different species of native Brazilian stingless bees, concluding the chemical composition of the propolis samples analyzed was heterogeneous. Velikova et al. (2000b) isolated three ent-kaurene diterpenoids from a sample of *Melipona quadrifasciata* (*M. quadrifasciata*) propolis. Miorin et al. (2003) compared, by high performance liquid chromatography with a diode array detector (HPLC-DAD), the chemical composition of the ethanolic extracts of several samples of *Tetragonisca angustula* and *A. mellifera* propolis from the states of Paraná and Minas Gerais in Brazil, concluding that the composition *T. angustula* propolis differed from that of *A. mellifera* propolis from the same regions. Pereira et al. (2003) investigated by high temperature – high resolution gas chromatography with mass spectrometry (HT-HRGC/MS) the chemical composition of the dichloromethane, acetone and methanol extracts of one sample of propolis of *A. mellifera* and one sample of *T. angustula* from São Paulo, Brazil, concluding that the less polar (dichloromethane) extracts were identical, but the other extracts showed significant differences in composition. Pino et al. (2006) compared by GC/MS the volatile constituents of one sample of *A. mellifera* propolis and one sample of *Melipona beecheii* propolis from Mexico, concluding that although the flora in the region was similar, the composition of the two samples was different. In a recent study, we (Sawaya et al., 2006) used electrospray ionization mass spectrometry in the negative ion mode [ESI(-)-MS] to compare ethanolic extracts of ten samples *T. angustula* propolis from the south, southeast and northeast of Brazil and compared their ESI(-)-MS fingerprints to the fingerprints of plant extracts from these regions, concluding that the almost constant composition of *T. angustula* propolis in Brazil results from the collection of surface exudates from *Schinus terebenthifolius* as the preferred plant source. This plant, known locally as "aroeira vermelha" can be found throughout South America.

Mass spectrometry has been used in conjunction with gas chromatography (GC/MS) for many years for the analysis of the main volatile and semi-volatile components of propolis from *A. mellifera* bees, although many components are not volatile enough for direct GC/MS analysis. HT-HRGC-MS was used to analyze the hexane and acetone extracts of a sample of green Brazilian propolis (Pereira et al., 1998) while for the methanolic extracts, prior derivatization was necessary (Pereira et al., 2000).

ESI-MS has revolutionized the way molecules are ionized and transferred to mass spectrometers, greatly expanding the applicability of MS to non-volatile, thermally unstable, heavy and/or polar molecules. Therefore ESI-MS and its tandem version ESI-MS/MS with direct infusion (no previous separation) has been applied to the analysis of a variety of complex natural mixtures such as those found in plant extracts (Mauri and Pieta, 2000; Möller et al., 2007), beer (Araújo et al., 2005), vegetable oils (Catharino et al., 2005; Wu et al., 2004) wine (Catharino et al., 2006; Cooper and Marshal, 2001), whisky (Möller et al., 2005), and even the most complex chemical mixture: petroleum (Hughey et al., 2002).

ESI ionizes more efficiently polar compounds with acid (negative ion mode) or basic sites (positive ion mode) 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 (Cole, 1997). Although the method and sample matrix do not permit the visualization of all the compounds in the samples (as less polar to non-polar compounds do not ionize sufficiently) and the results are of a

qualitative nature, one must bear in mind that it is not necessary to quantify all the compounds in a sample in order to characterize it. ESI(-)-MS fingerprinting focuses on the more polar and acidic components of the sample, which is of special importance for the study of propolis, as most of the pharmacologically active compounds so far identified in propolis are polar, frequently bearing acidic of phenolic sites (Marcucci et al., 1995). Therefore we have successfully applied ESI(-)-MS fingerprinting to typify propolis from different geographical regions, including the variable types of Brazilian propolis (Sawaya et al., 2004), and to compare propolis and plant extracts to determine the plant origins of the propolis samples (Marcucci et al., unpubl. data; Sawaya et al., 2006). These studies show, using standardized conditions (see material and methods), that different types of propolis display unique sets of polar and acid components hence that ESI(-)-MS fingerprinting can be used with confidence for propolis typification. In this study we compare the ESI(-)-MS fingerprints of ethanolic extracts of propolis of several species of native Brazilian stingless bees and some samples of *A. mellifera* propolis to determine patterns of composition for stingless bee propolis from different regions in Brazil. Furthermore, comparison with fingerprints of propolis samples with a known plant origin, permitted the identification of the plant origin of many of the propolis samples studied herein. To evaluate the correlation among the propolis samples, chemometric principal component analysis (PCA) was applied to the ESI(-)-MS data.

2. MATERIALS AND METHODS

2.1. Propolis samples and extraction procedure

Samples of propolis were provided by beekeepers from different regions in Brazil as summarized in Table I. 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 opm 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. General experimental procedures

The dry propolis 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 of propolis were infused directly into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 μL min^{-1}. ESI(-)-MS and tandem ESI(-)-MS/MS were acquired using a hybrid high-resolution and high-accuracy (5 μL/L) 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. Diagnostic ions in the different propolis samples were identified by the comparison of their ESI(-)-MS/MS dissociation patterns with compounds identified in previous studies (Sawaya et al., 2004, 2006; Marcucci et al., unpubl. data). Although fingerprints were acquired in the m/z 100−1000 range, no important ions were observed below m/z 200 or above m/z 650, therefore ESI(-)-MS data is shown in the m/z 200−650 range.

2.3. Chemometric 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 ten 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

ESI(-)-MS fingerprints were collected for each sample and the data was subject to

Table I. Species of bee and place of origin of samples of propolis from Brazil.

Sample	Species	Place of origin	Region
Ta1	*Tetragonisca angustula*	Claudio, Minas Gerais	Southeast
Ta2	*Tetragonisca angustula*	Florianópolis, Santa Catarina	South
Ta3	*Tetragonisca angustula*	Cruz das Almas, Bahia	Northeast
Mq1	*Melipona quadrifasciata*	Amazonas	North
Mq2	*Melipona quadrifasciata*	Amazonas	North
Mq3	*Melipona quadrifasciata*	Cruz das Almas, Bahia	Northeast
Mq4	*Melipona quadrifasciata*	Ribeirão Preto, São Paulo	Southeast
Pr1	*Plebeia remota*	Prudentopolis, Paraná	South
Pr2	*Plebeia remota*	Prudentopolis, Paraná	South
Pr3	*Plebeia remota*	Paraná	South
Pd	*Plebeia droryana*	Atibaia, São Paulo	Southeast
Pd3	*Plebeia droryana*	Ubatuba, São Paulo	Southeast
Ps1	*Plebeia* sp.	Paraná	South
Ps2	*Plebeia* sp.	Itaparica, Bahia	Northeast
Ls	*Lestrimelitta spp.*	Paraná	South
Tc	*Tetragona clavipes*	Paraná	South
Nt	*Nannotrigona testaceicornis*	Minas Gerais	Southeast
Sb	*Scaptotrigona bipunctata*	Paraná	South
Ms1	*Melipona scutellaris*	Bahia	Northeast
Ms2	*Melipona scutellaris*	Cruz das Almas, Bahia	Northeast
Ms3	*Melipona scutellaris*	Sauipe, Bahia	Northeast
Ms4	*Melipona scutellaris*	Amazonas	North
Mf	*Melipona favosa*	Corumbá, Mato Grosso do Sul	Midwest
Am1	*Apis mellifera*	Paraná	South
Am2	*Apis mellifera*	Bahia	Northeast
Am3	*Apis mellifera*	Cruz das Almas, Bahia	Northeast

chemometric analysis. Figure 1 shows the PCA analysis of the ESI(-)-MS fingerprints of all the samples of propolis from stingless bees and *A. mellifera* collected in Brazil. Due to characteristic sets of polar and/or acid components, the samples are clearly divided into three main groups (Fig. 1A) mainly due to several diagnostic ESI(-)-MS ions circled in Figure 1B. A detailed analysis of these ions allows us to indicate the main plant sources of these propolis samples (see below). To illustrate, Figure 2 shows ESI(-)-MS fingerprints of typical samples of each group.

Group 1 is composed of the following nine propolis samples: three of *T. angustula* (from the Santa Catarina, Minas Gerais and Bahia); one of *Nannotrigona testaceicornis* from Minas Gerais; one of *Plebeia sp*. from Itaparica, Bahia; one of *Plebeia droryana* (*P. droryana*) from São Paulo; and the three remaining samples were from Cruz das Almas, Bahia, that is one of *A. mellifera*, one of *Melipona scutellaris* (*M. scutellaris*) and one of *M. quadrifasciata*. Figure 2 shows, as characteristic examples of Group 1, a fingerprint of *T. angustula* propolis from Minas Gerais (Fig. 2A) and a fingerprint of *P. droryana* from São Paulo (Fig. 2B). In a previous study (Sawaya et al., 2006), samples of *T. angustula* propolis from different regions Brazil were found to have the ions of m/z 371, 373, 401, 453, 455, 469 and 471, that are diagnostic for samples of propolis derived from *S. terebenthifolius* resins. The samples of propolis belonging to

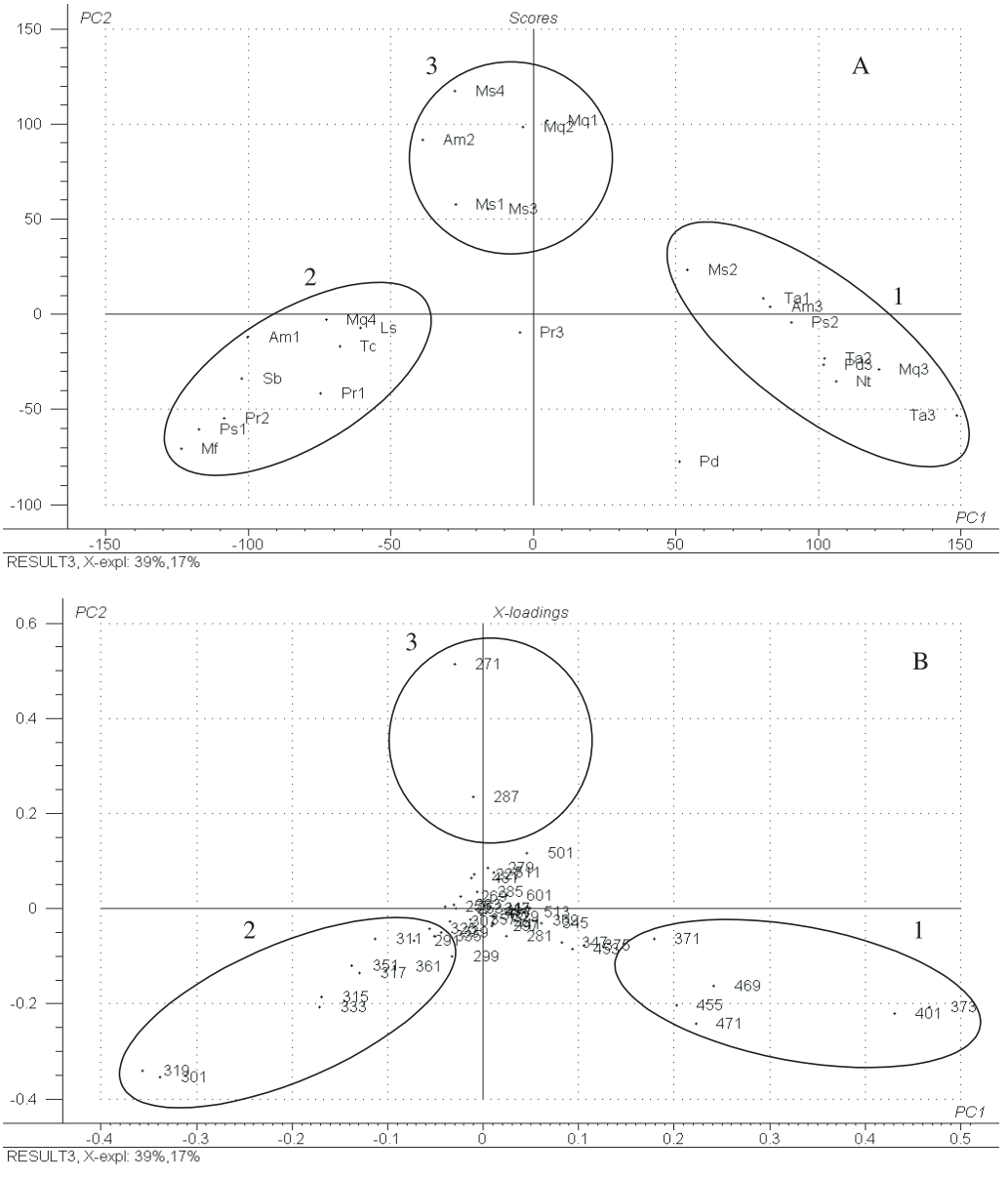

Figure 1. PCA analysis of the ESI(-)-MS fingerprint data for the extracts of *A. mellifera* and native Brazilian stingless bee propolis from the south, southeast, midwest, north and northeast of Brazil (for species see Tab. 1): (A) grouped samples and (B) characteristic ions for each group. Ions circled in (B) are those most characteristic (diagnostic) for each group whereas those more in the center are common to many samples.

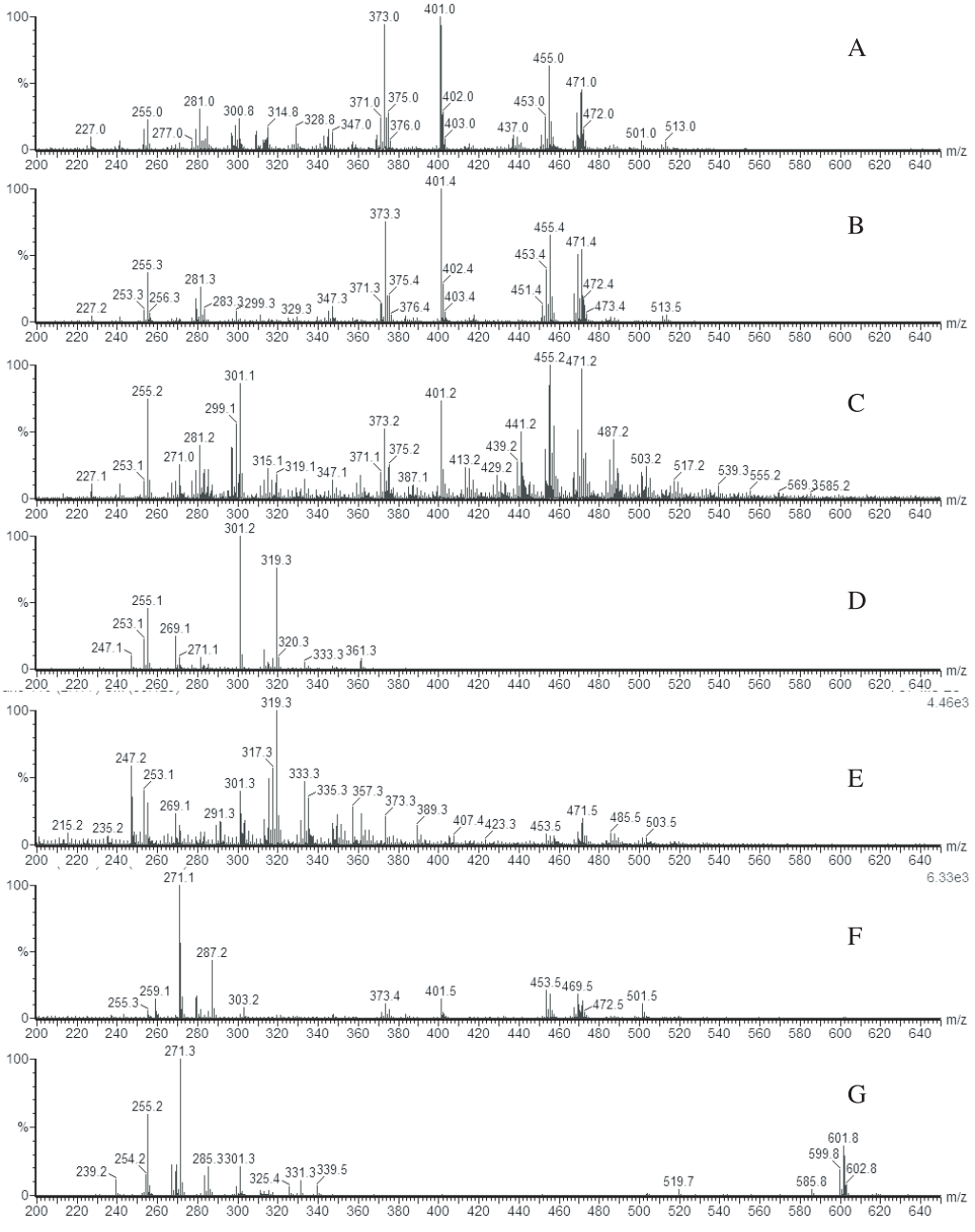

Figure 2. ESI(-)-MS fingerprints of propolis, origin and type: (A) *T. angustula* from Minas Gerais – Group 1; (B) *P. droryana* from Sao Paulo – Group 1; (C) *P. droryana* from Sao Paulo – intermediate Groups 1 and 2; (D) *A. mellifera* from Paraná – Group 2; (E) *P. remota* from Paraná – Group 2; (F) *M. quadrifasciata* from Amazonas – Group 3 and (G) *A. mellifera* from Bahia – Group 3.

Group 1 contain these same diagnostic ions (Fig. 1B), indicating that they derive their resins mainly from *S. terebenthifolius,* a tree 2–6 m high, belonging to the Anacardiaceae family, known in Brazil as "aroeira vermelha". The leaves and fruit of *S. terebenthifolius* are popularly used for medicinal purposes and contain substances with known medicinal properties (Jain et al., 1995; Schmourlo et al., 2005). References have been found to several species of native bees visiting this plant in the state of São Paulo (Ramalho et al., 1990) and in the south of Brazil (Wilms et al., 1997). Due to the wide geographic distribution of *S. terebenhtifolius*, it is hardly surprising that propolis samples from different regions (south, southeast and northeast of Brazil) use this plant source. The samples of *T. angustula* propolis analyzed in the present study were not the same ones analyzed in the previous paper (Sawaya et al., 2006), which reinforces our findings that *S. terebenhtifolius* is a major resin source for *T. angustula*.

Group 2 is also composed of 9 propolis samples: two of *Plebeia remota* (*P. remota*), one of *Plebeia* sp., one of *Lestrimelitta* sp., one of *Tetragona clavipes,* one of *Scaptotrigona bipunctata* (*S. bipunctata*) and one of *A. mellifera* from Paraná; one of *Melipona favosa* from Mato Grosso do Sul and one of *M. quadrifasciata* from São Paulo. Figure 2D shows a fingerprint of *A. mellifera* propolis from Paraná whereas Figure 2E shows that of *P. remota* from Paraná, both characteristic fingerprints of group 2. The ions of m/z 301, 315, 317, 319, 333, and 361 that are the most diagnostic for these samples (Fig. 1B), which are characteristic of brown *A. mellifera* propolis from the south of Brazil. These ions are also found in resins of *Araucaria* pine trees and were characterized by comparison of their ESI-MS/MS (Marcucci et al., unpubl. data). Most of the samples of Group 2 come from the state of Paraná, where *Araucaria* trees are common and these resins are apparently the main source of propolis for bees in the south of Brazil. Furthermore, ions of m/z 253, 255 and 269 found in the fingerprints of both samples of propolis (Fig. 2D–E) are also characteristic of brown propolis from the south of Brazil and were identified by ESI-MS/MS as flavonoids commonly found in poplar type propolis in Europe (Sawaya et al., 2004). Although the plant source for these flavonoids in southern Brazil has not been determined yet it is noteworthy that both native (*P. remota*) and introduced (*A. mellifera*) bees take resins from this source. The sample of *P. remota* propolis from the south of Brazil (Fig. 2E) also contains compounds derived from *S. terebenthifolius*, as revealed by the detection of ions of m/z 453 and 471 in the ESI(-)-MS fingerprint, albeit with low intensity. Due to matrix suppression, one cannot affirm that the intensity of an ion is proportional to its concentration.

Two samples of propolis (*P. droryana* from São Paulo and *P. remota* from Paraná) were placed between Groups 1 and 2 in the PCA analysis (Fig. 1A), which indicates that both *S. terebenthifolius* and *Araucaria* were used as plant sources. Figure 2C shows the fingerprint of such a sample (*P. droryana* propolis from São Paulo) where the ions of m/z 301 and 319 (characteristic of Araucaria) as well as m/z 373, 401, 455 and 471 (characteristic of *S. terebenthifolius*) are observed.

Group 3 is composed of the following six propolis samples: two of *M. scutellaris* from Bahia and one of *M. scutellaris* from Amazonas; two of *M. quadrifasciata* from Amazonas and one of *A. mellifera* from Bahia. By far the major diagnostic ion responsible for grouping these samples is that of m/z 271 (Fig. 1B), and this ion is very characteristic for the ruby red*A.*mellifera propolis from the northeast of Brazil (Sawaya et al., 2004). Although the plant source of this type of propolis has not been determined yet, it is apparently a plant source for propolis of several species of bees in the tropical region of Brazil. Figure 2F shows the ESI(-)-MS fingerprint of ruby red *A. mellifera* propolis from Bahia. The ion of m/z 601 is characteristic of ruby red propolis from the coastal regions of the state of Bahia and was observed with less intensity in several samples of stingless bee propolis from this state (data not shown). Figure 2G shows a fingerprint of *M. quadrifasciata* propolis from Amazonas where the ion of m/z 271 is the most intense, but less intense ions which are characteristic of *S. terebenthifolius* (m/z 373, 401, 453 and 469) are also

Figure 3. Map of Brazil indicating the geographic origin of the propolis samples. Sample names abbreviated as in Table I.

observed. Both fingerprints are characteristic of Group 3 samples.

Most of ESI(-)-MS fingerprints of the samples of propolis from native Brazilian stingless bees contained ions characteristic of *S. terebenthifolius*, whereas only three of the samples analyzed contained no trace of these ions (two samples of *M. scutellaris* from Bahia and Amazonas, and one of *S. bipunctata* from Paraná). This finding is consistent with reports in which numerous stingless bees were found visiting *S. terebenthifolius* flowers (Ramalho et al., 1990; Wilms et al., 1997). These results indicate that *S. terebenthifolius* is an important plant source for propolis of native Brazilian stingless bees. In regions where other plant sources are present, stingless bees can adapt and use different plants (such as *Araucaria* in the southern regions of Brazil).

A map of Brazil indicating the states and regions in which the samples of propolis were collected can be seen in Figure 3. Most of the samples were collected in tropical regions, with a wide variety of vegetation. The samples collected in the south of Brazil, states of Paraná and Santa Catarina, show the influence of the vegetation, with *Araucaria* trees being an important plant source for these samples. In the southeast of Brazil, *A. mellifera* bees use *Baccharis dracunculifoia,* as the main plant source for propolis, resulting in the world famous green Brazilian propolis (Bankova et al., 1999; Marcucci et al., unpubl. data). Diagnostic ions of the prenylated phenolic compounds found in green *A. mellifera* propolis (Sawaya et al., 2004) were not found in any of the fingerprints of native stingless bees, in spite of containing both acid and phenolic sites and ionizing well in the negative mode. Therefore we observed that, even in the southeast of Brazil where this shrub is common, native bees do not use *B. dracunculifoia* as a plant source for their propolis.

4. CONCLUSIONS

ESI(-)-MS fingerprints of the samples of native Brazilian stingless bee propolis allow us to conclude that *S. terebenthifolius* is an important plant source for these stingless bees. The diagnostic ions of *S. terebenthifolius* resins, those of m/z 371, 373, 401, 453, 455,

469 and 471, are the most intense in the fingerprints of all the samples of *T. angustula* and *N. testaceicornis* analyzed. These ions are also present in all the samples of propolis from the species of *Plebeia* analyzed, and in several samples of the other stingless bee species (eg. Fig. 2F). Stingless bees can however use many of the same plant sources as *A. mellifera*; in the fingerprints of samples of stingless bee propolis from the south of Brazil, the characteristic ions of *Araucaria* resins (m/z 301, 319) and some flavonoids (m/z 253, 255 and 269) are observed that are common in *A. mellifera* propolis from this area. What is more surprising, is that sometimes native bees seem to simply ignore a potential plant source used by *A. mellifera.* For example, none of the characteristic ions of green *A. mellifera* propolis are observed in the fingerprints of any of the samples of stingless bee propolis from the southeast of Brazil, indicating that *Baccharis dracunculifolia* was not used as a source of resins by any of the stingless bees studied.

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Spectrométrie de masse par ionisation avec électronébulisation, une méthode par fingerprint pour caractériser la propolis des abeilles sans aiguillon indigènes du Brésil.

propolis / spectrométrie de masse par ionization avec électronébulisation / abeille sans aiguillon / Apidae / Meliponini / Brésil / *Schinus therebentifolius*

Zusammenfassung – Elektrospray-Ionisations-Massenspektrometrie, eine Methode zur Fingerabdruckanalyse von Propolis brasilianischer Stachelloser Bienen. Stachellose Bienen kommen in vielen tropischen und subtropischen Regionen der Welt vor und sind in diesen Regionen wichtige Bestäuber. Nichtsdestotrotz ist die Haltungstechnologie für die meisten Arten Stachelloser Bienen noch auf einem relativ niedrigen Niveau. Obwohl für Brasilien bereits verschiedene Studien zur Nischenüberlappung von einheimischen Bienen mit den eingeführten Honigbienen vorliegen, gibt es nur wenig Informationen zu Pflanzen, die von Stachellosen Bienen als Harzquelle für die Herstellung von Propolis genutzt werden. Propolisproben, die von Imkern in verschiedenen Regionen Brasiliens gewonnen wurden (zusammengestellt in Tab. 1), wurden eingefroren und für die Extraktion zermahlen. Die mazerierten Proben wurden auf einem Schüttler während sieben Tagen in Alkohol extrahiert und anschliessend im negativen Ionenmodus per Elektrospray-Ionisations-Massenspektrometrie [ESI(-)-MS] in einem Q-TOF-Massenspektrometer (Micromass) analysiert. Mittels einer chemometrischen Hauptkomponentenanalyse (PCA) wurden statistisch signifikante Korrelationen in diesen Fingerabdrucksanalysen der Propolisproben aufgedeckt. Abbildung 1 zeigt die PCA-Ergebnisse der ESI(-)-MS Fingerabdrücke von Propolisproben Stachelloser Bienen und von Honigbienen. Die Proben teilen sich anhand ihrer charakteristischen Ionen klar in drei Gruppen auf (Abb. 1A, B). Abbildung 2 zeigt ESI(-)-MS-Spektren typischer Proben aus jeder dieser Gruppen. Gruppe 1 besteht aus neun Propolisproben, für die die Ionen m/z 371, 373, 401, 453, 455, 469 und 471 für die Gruppierung verantwortlich sind. Diese Ionen sind charakteristisch für *Tetragonisca angustula* Propolis, die diese Bienen in ganz Brasilien überwiegend von *Schinus terebenthifolius* sammeln. Gruppe 2 besteht ebenfalls aus neun Propolisproben, mit den Gruppierungsionen m/z 301, 315, 317, 319, 333, and 361. Diese sind für die braune *A. mellifera* Propolis charakteristisch, die vor allem aus Südbrasilien stammt und in der die Bienen Harze der *Araucaria* Tanne verarbeiten. Zwei Propolisproben (*P. droryana* aus São Paulo und *P. remota* aus Paraná) lagen zwischen diesen beiden Gruppen (Abb. 1A), was darauf hinweist, dass diese Bienen sowohl *S. terebenthifolius* als auch *Araucaria* Harze sammelten. Gruppe 3 besteht aus sechs Propolisproben, für die das Hauption (m/z 271) für die Gruppierung verantwortlich zeigt. Dieses ist charakteristisch für rote Robinienpropolis von *A. mellifera* aus dem Nordosten Brasiliens. Die meisten Fingerabdrücke von Propolisproben der einheimischen Stachellosen Bienen zeigten die für *S. terebenthifolius* charakteristischen Ionen. Diese in ganz Brasilien vorkommende Pflanze enthält medizinisch wirksame Substanzen und wird häufig von Stachellosen Bienen besucht. Unsere Ergebnisse zeigen, dass *S. terebenthifolius* eine wichtige Quelle für die Propolisgewinnung darstellt, dass Stachellose Bienen aber auch andere Pflanzen, insbesondere Aurakarien nutzen können.

ESI-MS Fingerabdruck / Propolis / Stachellose Bienen / Brazil

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