

Identification of sex pheromones of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) populations from the state of São Paulo, Brazil

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In Brazil, four populations of Lutzomyia longipalpis each producing different sex pheromones are recognised. It has been suggested that these chemotype populations represent true sibling species. In this study we present the results of an analysis, by coupled gas chromatography - mass spectrometry, of the pheromones of males L. longipalpis from two different municipalities of the state of São Paulo. Our study showed that L. longipalpis from these two municipalities produced different sex pheromones from each other. This coupled with the remarkable difference between the epidemiological situation in Araçatuba and Espírito Santo do Pinhal, suggests that the (S)-9-methylgermacrene-B and cembrene-1 populations may have different vectorial capacities.

Key words: *Lutzomyia longipalpis* - sex pheromone - (S)-9-methylgermacrene-B - cembrene-1 - Brazil

The sand fly *Lutzomyia longipalpis* (Lutz & Neiva) is the main vector of *Leishmania (L.) chagasi* (Cunha & Chagas), the causative agent of the American Visceral Leishmaniasis (AVL) in the New World. Males of *L. longipalpis* have secretory glands restricted to a pair of dorsolateral pale patches on tergite 4 (one spot form) or with an additional pair of patches on tergite 3 (two spot form) (Lane et al. 1985). These glands release terpenes, which act as a sex pheromone that is highly attractive to intrapopulation conspecific females (Morton & Ward 1989, Ward & Morton 1991, Hamilton et al. 1994, Kelly & Dye 1997).

Recognition of the members of the *L. longipalpis* species complex is controversial (Lanzaro et al. 1993, Dujardin et al. 1997, Azevedo et al. 2000, Maingon et al. 2003, Watts et al. 2005). Nevertheless, there is evidence that members that produce different sex pheromones are reproductively isolated (Ward et al. 1988, Ward & Morton 1991, Hamilton et al. 2005). Knowing if these sibling species (chemotype populations) have different vectorial capacities is important to understand the epidemiology and therefore the control of AVL in South and Central America. At present four different sex pheromone-producing populations (chemotypes) of *L. longipalpis* are recognised in Brazil based on the main terpene component of their sex pheromone; two are homosesquiterpenes – 3-methyl- α -himachalene, (found in males from Jacobina, state of Bahia) (Hamilton et al. 1996b, 1999a) and (S)-9-methylgermacrene-B (in males from Lapinha Cave, state of Minas Gerais) (Hamilton et al. 1996a, 1999b). The third and fourth

are monocyclic diterpenes partially characterized as cembrene isomers and have been found in males from Sobral, state of Ceará (Hamilton et al. 2005) and males from Jaíba, state of Minas Gerais (Hamilton et al. 2004). There is no correlation between the numbers of spots on the abdomen and the pheromone extracted from them. This study presents data on the type of sex pheromone found in male *L. longipalpis* from two areas of the state of São Paulo: Espírito Santo do Pinhal and Araçatuba. The epidemiology of AVL in Araçatuba is distinctly different to Espírito Santo do Pinhal.

Espírito Santo do Pinhal is situated in the Northeast region of the state (22° 06' 57" S and 46° 40' 58" W) at an altitude of 870 m. According to the Köppen classification, the climate is subtropical warm without a dry season (Cfa). *L. longipalpis* was notified in the Espírito Santo do Pinhal rural area in 1993 (Costa et al. 1997). This species was also observed, at low density (unpubl. observations), in the urban area in sporadic entomological collections, from 2000 to 2004. At the present, no human cases of AVL have been notified in this municipality or region.

Araçatuba is situated in the West region of the state (21° 12' 32" S and 50° 25' 58" W) at an altitude of 390 m. According to the Köppen classification, the climate is tropical with dry winter (Aw). The first notification of *L. longipalpis* in the western region of São Paulo state occurred in the urban area of Araçatuba in 1997 (Costa et al. 1997). Since then this species has been found at high density within the urban area of Araçatuba and other municipalities of this region (unpubl. observation). From 1999 to June 2005 192 human cases, with 20 deaths, were notified in Araçatuba and 335 human cases, with 43 deaths, were notified in the other municipalities in the western region of the state (SES-CVE 2005).

For this study, males of *L. longipalpis* from Espírito Santo do Pinhal were collected with a manual aspirator in a peridomestic chicken shed within the urban area. All males from Araçatuba were from a colony maintained at

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Mogi Guaçu's Laboratory (Sucen-SP) and established from females collected in the city. In both cases males were killed at -20°C for 10 min, placed in a glass vial and then covered with hexane (Hamilton et al. 2004).

Analysis of male sex pheromone extracts was on a HP-5MS capillary column, 30 m x 0.25 mm i.d., 0.25 μm film thickness (Agilent, Stockport, Cheshire) in a Hewlett Packard 5890 II+ Gas Chromatograph coupled to a Hewlett Packard 5972A bench-top mass spectrometer (electron impact, 70 eV, 180°C). Injection and chromatography conditions were as previously described (Hamilton et al. 2005).

The chemical analysis showed that *L. longipalpis* males from Espírito Santo do Pinhal contained the diterpene (C-20) previously characterized as cembrene-1 in *L. longipalpis* from Sobral, CE, Brazil (Hamilton et al. 2005). *L. longipalpis* males from Araçatuba contained (S)-9-methylgermacrene-B, which has previously been found and characterized in males from Lapinha Cave, MG, Brazil (Hamilton et al. 1999). This structural characterization of the sex pheromone of the Araçatuba population may allow us to use the appropriate synthetic male sex pheromone, in highly specific pheromone-baited traps and in the development of novel control strategies (Brazil et al. 1989, Ward et al. 1990, Dye et al. 1991, Kelly & Dye 1997, Kelly et al. 1997).

The remarkable differences between the epidemiological situation and population size of *L. longipalpis* in the urban area of Araçatuba and Espírito Santo do Pinhal, are interesting and suggest that the (S)-9-methylgermacrene-B and cembrene-1 populations may have different vectorial capacities. However in the Northeast of Brazil the cembrene-1 producing type of *L. longipalpis* is abundant and present in areas where AVL is frequently encountered whereas the (S)-9-methylgermacrene-B type is rarely encountered (Hamilton et al. 2005). It is unclear why the epidemiological situation in São Paulo should differ from the Northeastern states of Brazil and is worthy of further investigation.

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