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ELECTROPHORETIC AND BIOCHEMICAL CHARACTERIZATION OF *Tetragonisca weyrauchi* (Hymenoptera, Apidae) STINGLESS BEES ESTERASES

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ABSTRACT - The meliponinae are important pollinators of plant species. *T. weyrauchi* has restricted distribution, is found only in northern Brazil. This study aimed to perform the electrophoretic characterization of esterases from *T. weyrauchi* and check the expression pattern of total proteins. Adult individuals were collected at the entrance of the nests located in Alto Paraíso, state of Rondônia, Brazil and stored at -20 °C. Individual extracts of head and thorax and abdomen of the workers were subjected to electrophoresis on 10% polyacrylamide gel and 5% stacking gel. Extracts from head and thorax of *T. weyrauchi* presented six esterase regions. In abdomen extracts it was only one esterase activity region, EST- 4. The heated samples to 52 °C and 54 °C decrease in the relative activity of esterases 1, 2, 3 and 4 and total degradation of esterases 5 and 6. At 58 °C only esterase 4 presented lower relative activity while the others were totally degraded. On the electrophoretic pattern of the proteins, 24 peptides detected, by molecular weight, their size ranged from 10 to 220 kDa. This study revealed, that number found for esterase species is greater than for other species of the same genus, as well as the number of proteins. The thermostability test showed resistance is associated with thermoregulatory capacity, an important characteristic because this species is found in regions with high temperatures.
Key words: Jataí bees, inhibitors, protein, thermostability.

CARACTERIZAÇÃO ELETROFORÉTICA E BIOQUÍMICA DAS ESTERASES DA ABELHA SEM FERRÃO Tetragonisca weyrauchi (Hymenoptera, Apidae)

RESUMO - Os meliponíneos são importantes polinizadores de espécies de plantas. A *T. weyrauchi* tem distribuição restrita, é encontrada somente na região norte do Brasil. Este estudo teve como objetivo realizar a caracterização eletroforética de esterases em *T. weyrauchi* e verificar o padrão de expressão de proteínas totais. Indivíduos adultos foram coletados na entrada de ninhos localizados na cidade de Alto Paraíso, Rondônia, e estocados a -20 °C. Extratos individuais de cabeça/tórax e abdômen das operárias foram submetidos à eletroforese em géis de poliacrilamida a 10% e gel de empilhamento a 5%. Os extratos de cabeça/tórax de *T. weyrauchi* possuem seis regiões de esterase. Em extratos do abdômen foi observada apenas uma região de atividade esterásica a EST- 4. As amostras aquecidas a 52 °C e 54 °C apresentaram diminuição na atividade relativa das esterases 1, 2, 3 e 4 e degradação total das esterases 5 e 6. A 58 °C somente a esterase 4 apresentou menor atividade relativa, enquanto as demais foram totalmente degradadas. No perfil eletroforético das proteínas foram evidenciados 24 peptídeos de acordo com a massa molecular, o tamanho variou de 10 a 220kDa. Este estudo revelou que o número de esterases encontrada é maior do que para outras espécies do mesmo gênero, bem como o número de proteínas. O teste de termoestabilidade mostrou que a resistência está associada à capacidade de termorregulação, uma característica importante, pois esta espécie é encontrada em regiões com altas temperaturas.
Palavras-chave: abelha Jataí, inibidores, proteína, termoestabilidade.

INTRODUCTION

Indigenous bees are eusocial and are distributed in subtropical and tropical regions of the world and are one of the most diverse and important groups of insects. They occur in South America, Central America, Asia, the Pacific Islands, Australia, New Guinea and Africa. They are taxonomically divided into two tribes: *Meliponini* formed only by the genus *Melipona*, found exclusively in the Neotropical Region (South America, Central and Caribbean Islands) and *Trigonini*, which are distributed throughout the distribution area of the subfamily region (MOURE et al., 2007).

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Stingless bees are among the most common pollinators in tropical environments and in certain regions are the dominant bees, visiting various cultures (MACÍAS-MACÍAS et al., 2009). They play an important ecological role, especially as pollinators of plants that make up the Brazilian forest. They comprise a diverse group of insects that includes more than 400 species with high variability in physiology, morphology and size from 0.2 mm in the genre *Trigonisca* to more than 20 mm in some species of *Melipona* (MOURE et al., 2007).

The catalog of Moure (2007) presented the *Tetragonisca* genus that consists of four species: *T angustula*, *T. fiebrigi*, *T. buchwaldi* and *T. weyrauchi*. Only *T. buchwaldi* is not present in Brazil. *T. weyrauchi* is the species that has the most restricted distribution, it is found only in the northern region in the Brazilian states of Mato Grosso, Rondônia and Acre.

Described the bionomics of Cortopassi-Laurino and Nogueira-Neto (2003), *T. weyrauchi* builds aerial nests often in inclined forks of trees. The nests are cylindrical and vertical, measuring about 60 centimeters in circumference at the thickest point and 35 centimeters long. It is coated with a thin and pliable film of different consistencies.

Several studies are being conducted using species of the genus *Tetragonisca*, especially with *T. angustula* and *T. fiebrigi*, due to its ecological and economic importance, among them, the evaluation of genetic variability and species identification through the use of different markers, Barth et al. (2011), Francisco et al. (2014). Few studies have been conducted with *T. weyrauchi*, because it is a species with a more restricted distribution, nesting area and displaying defensive behavior.

Esterases are the most extensive study family of enzymes, its different forms genetically determined by different loci, and the their differential expression in tissues during the different stages of ontogenetic development, and high frequency of genetic variants usually detected (RUVOLO-TAKASUSUKI et al., 1997).

This study aimed to perform the electrophoretic characterization of *T. weyrauchi* esterases, to identify esterase types through the use of inhibitors, perform thermostability tests and check the expression pattern of total proteins.

MATERIALS AND METHODS

Adult *T. weyrauchi* workers were collected from natural nests located in Alto Paraíso state of Rondônia, Brazil (9° 71'42" S, 63° 34'81" W). After collection, the bees were sacrificed by freezing and kept in a freezer at -20° C.

Preparing the samples and PAGE electrophoresis

Each worker had its head and thorax and abdomen removed, individually homogenized in 1.5 mL polypropylene tubes containing 40 μL of 2-mercaptoethanol plus 10% glycerol solution. Then the samples centrifuged at 12.000 *g* for 10 min at 4 °C.

The vertical electrophoresis were performed using PAGE gels at 10% concentration and stacking gel at 5%. The running buffer was 0.1 M Tris-Glycine, pH 8.3 and gels submitted to electrophoresis at 200 V for approximately 5 hours.

Performing staining, the gel was incubated for 30 min in 50 mL of sodium phosphate buffer solution (0.1 M, pH 6.2). Then the buffer was discarded and added to the staining solution which consisted of 50 mL of sodium phosphate buffer 0.1 M, pH 6.2; 0.03 g of α-naphthyl acetate; 0.03 g of β-naphthyl acetate; 0.06 g of Fast Blue RR Salt for staining (CERON, 1988).

Inhibition testing

The head and thorax extracts from each worker were used twice in the same PAGE gel, the first as control and the second for the inhibition test.

The staining, the gel separated in two pieces: control and inhibition. Each piece was then incubated for 30 min in 50 mL of the sodium phosphate buffer solution (0.1 M pH 6.2). In the incubation buffer for the testing gel it was added the inhibitor to be tested (organophosphate malathion – 1 mM, parachloromercuric benzoate (ρ-CMB) - 0.27 mM of eserine sulfate - 1 mM). After visualization of the bands on the control gel, was compared with the gel containing the inhibitor and an inhibition table was prepared. The inhibitor patterns were analyzed and compared with Healy et al. (1991).

Thermostability

Test performed for thermostability for esterases by pre-incubating the samples for 5 minutes at a temperature ranging from 52 to 60 °C. After incubation, 20 μL of the supernatant was applied to PAGE gel and subjected to electrophoresis. As control, extracts of head and thorax which were not subjected to heating.

Preparing the samples and SDS PAGE electrophoresis

Extracts of the collected insects were subjected to electrophoresis for protein characterization. The homogenization of the samples in 40 μL of solution containing 2-mercaptoethanol plus 10% glycerol. They were then centrifuged at 12.000 *g* for 10 minutes at 4 °C. On the SDS-PAGE electrophoresis, 20 μL of the supernatant were transferred and added 20 μL of bromophenol blue and 10% SDS. Then, the heat of the tubes at 100 °C for 3 minutes in a water bath and 20 μL which applied onto the gel for electrophoretic run.

The vertical electrophoresis were performed using SDS-PAGE gels at 10% concentration and stacking gel at 5%. The running buffer used was 0.1 M Tris-Glycine pH 8.3 + 10% SDS. The gels were subjected to electrophoresis at a voltage of approximately 90V until the front exit for total protein. The detection of the peptides with Coomassie Brillant Blue staining. The molecular weight standard used was BenchMark™ Protein Ladder.

RESULTS AND DISCUSSION

Esterases

There were six areas of esterase activity in extracts of head and thorax detected. They named numerically according to their electrophoretic mobility. The one which presented the highest migration was EST-1 (Figure 1). The characterization of the EST-5 as β-esterase, it showed red color when stained with α and β-naphthyl acetates together. The others are αβ-esterases, for presenting intermediate color between black and red when stained with α and β-naphthyl acetate together (Table 1).

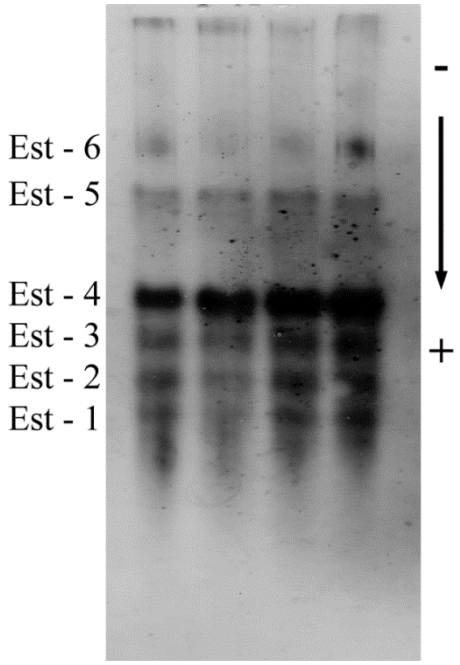

FIGURE 1 - Profile of esterases from *T. weyrauchi* presented six regions.

TABLE 1. Classification and Characterization of esterases in *T. weyrauchi*. (α = α esterase, β = β-esterase; + inhibition, - no inhibition.) [1]parachloromercuric benzoate.

Esterase	(Malathion)	Eserine sulfate	pCMB[1]	Classification	α / β
EST-1	+	-	-	Carboxylesterase	αβ
EST-2	-	+	+	___	αβ
EST-3	-	+	+	___	αβ
EST-4	-	+	-	___	αβ
EST-5	-	+	-	Acetylesterase	β

In abdomen extracts it was observed only one region of esterase activity, EST-4. Differences in the number of isoenzymes and affinities with substrates detected, are probably related to changes in intermediary metabolism and detoxification capacity of these bees.

Out of the various currently available molecular markers, isozymes, for their relative simplicity and analysis speed in relation to other markers, generated a wide range of practical information on identifying hybrid species, natural and cultured populations of many living organisms (TEIXEIRA et al., 2004).

The esterase activity regions are different when compared to bees from the same genus. Tetragonisca of three species found in Brazil through the utilization of markers of esterases is possible to separate into distinct groups. Ruvolo-Takasusuki et al. (2006) supported characterization of the esterase activity regions in *T.*

angustula; these *authors* found two regions, named EST-1 (one β-esterase) and EST-2 (one αβ-esterase).

Stuchi et al. (2012) found that the number of regions with esterase activity varied according to the species; in extracts of *T. fiebrigi* three esterases observed which were designated EST-1 (more anodic), EST-2 (intermediate) and EST-4 (less anodic), while in extracts of *T. angustula* two regions of esterase activity, EST-3 (more anodic) and EST-4 (less anodic). EST-4 is common to both species, EST-1 (*T. fiebrigi*) and EST-3 (*T. angustula*) probably differed by mutations throughout the evolution of both species, and EST-2 (*T. fiebrigi*) may have originated by duplication and subsequent mutations.

Utilization of other molecular markers were identified hybrids between species of this genus as Barth et al. (2011) found the presence of B chromosomes only in *T. fiebrigi* and not observed in *T. angustula*. Francisco et al. (2014) by sequencing the regions of mtDNA and microsatellite analysis also verified the presence of hybrids between *Tetragoniscas*.

Inhibition

Esterases characterization based on sensitivity to the organophosphate inhibitors malathion, eserine sulfate and parachloromercuric benzoate (pCMB). These inhibitors allows the classification of esterases of Healy et al. (1991) criteria, into four classes, cholinesterases, carboxylesterases, arilesterases and acetilesterases. According to the inhibition tests, EST-1 was only inhibited by the organophosphorus compound malathion, characterizing a carboxylesterase. The inhibition of the EST-2, 3, 4 by eserine sulfate and EST - 2 and 3 inhibited with pCMB, not being possible their characterization. EST-5 and EST-6 were not inhibited by malathion and pCMB being classified as Acetilesterases (Table 1).

Among the enzymatic systems, esterases are effective for the catalyzing the hydrolysis of a wide range of aliphatic and aromatic esters, amides and thioesters, as well as many different processes. Moreover, these enzymes have a broad tissue distribution during insect development (RUVOLO-TAKASUSUKI et al., 1997).

The esterase family of enzymes hydrolyse ester bonds, which are present in a wide range of insecticides; therefore, these enzymes may be involved in resistance to the main chemicals employed in control programs. Historically, insecticide resistance has driven research on insect esterases and schemes for their classification. The esterase gene family appears to be rapidly evolving and each insect species has a unique complement of detoxification genes with only a few orthologues across species (MONTELLA et al., 2012).

Based on sensitivity to synthetic substrates which esterases hydrolyze *in vitro*, two groups can be distinguished in insects: α-esterases which preferentially hydrolyze α-naphthyl acetate and β-esterase which preferentially hydrolyze β-naphthyl-acetate (OAKESHOTT et al., 1993).

Galego et al. (2006) reported a great variation in banding patterns and esterase activity in different populations and species. Esterases identified and characterized in several species, especially by polyacrylamide gel electrophoresis (PAGE), that is a fast and efficient technique to indirectly verify the genes for this isoenzyme system and detect qualitative and quantitative differences between these isozymes in different populations or species (ROSSITER et al., 2001).

Classes of esterases in insects based on their sensitivity to three inhibitor groups: organophosphorous, eserine sulfate and sulfhydryl reagents. Qualifying criterion, sensitivity to different inhibitors of enzymatic activity and the amino acid residues in the active site, are recognized, the acetilesterases (EC 3.1.1.6), the arilesterases (EC 3.1.1.2) the carboxylesterases (EC 3.1.1.1) and the cholinesterases which include acetylcholinesterases (EC 3.1.1.7) and pseudocholinesterases (EC 3.1.1.8) (HEALY et al., 1991).

Stuchi et al. (2012) reported the inhibition pattern in *T. fiebrigi* EST-1 and EST-2 can be classified as cholinesterases, and EST-1 is a type I cholinesterase, whereas EST-2 is a type II cholinesterase. EST-4, in both species, is a carboxylesterase. In *T.angustula*, EST-3 is an acetylesterase.

The carboxylesterases and cholinesterases are quite common isoenzymes in insects, possibly because they play key roles in detoxification of xenobiotic compounds, participating in insecticide resistance in several representatives of this class. Regarding organophosphates, such mechanisms involve the increase in metabolic detoxification by hydrolysis or sequestration of these compounds or structural alterations in acetylcholinesterase, primary target for this class of insecticide (HSU et al., 2006).

Fermino et al. (2011) observed changes in *T. angustula* esterases compared to *T. fiebrigi*. The authors reported that when these bees are exposed to insecticides *T. fiebrigi* is more sensitive to the presence of paraquat herbicide than *T. angustula*. The herbicide nicosulfuron, no action upon esterase inhibition (MASSA et al., 2008).

Thermostability

The heated samples to 52 °C and 54 °C showed a decrease in the relative activity of carboxylesterases 1, 2, 3 and 4 and the total degradation of esterases 5 and 6. At 58 °C only esterase 4 showed lower relative activity while the others were fully degraded. At 60 °C no esterase was detected.

The thermostability observed on *T. weyrauchi* bees is greater than the one observed by Stuchi et al. (2012) in *T. angustula* and *T. fiebrigi* which lose activity at 54 °C. Whereas T. *weyrauchi* are restricted to the states of Rondônia, Acre and Mato Grosso, Brazil. These states present high temperatures throughout the year, esterases may have molecular structure more suited to this type of environment because of its role in intermediary metabolism of insects.

The morphology of the nests of *T. weyrauchi*, *T. angustula*, and *T. fiebrigi* present the temperature-related differences. *T. weyrauchi* nests present extensions with several openings and protuberances described by Cortopassi-Laurino and Nogueira-Neto (2003) and called

breathing holes, which increase nest ventilation at the hottest times of the day.

Total Proteins

On the electrophoretic pattern of the proteins, 24 peptides detected by molecular weight, size ranged from 10 to 220 kDa (Figure 2). Peptide 24 showed molecular weight higher than 220 kDa, peptides 6 and 7 (between 25 and 30 kDa) presented differences regarding their presence and absence which suggests polymorphism that requires further studies for confirmation.

The electrophoretic pattern of *T. weyrauchi* total proteins is different from the observed by Stuchi et al. (2012) for *T. angustula* and *T. fiebrigi* which presented 23 peptides with molecular weights ranging from 20 to 120 kDa. *T. weyrauchi* presented 24 peptides which presented higher molecular weights than those detected in *T. angustula* and *T. fiebrigi*.

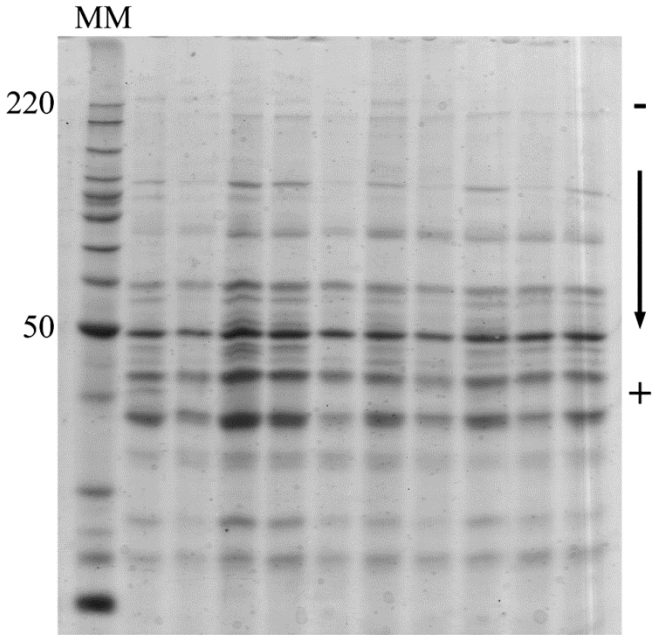

FIGURE 2. Total protein profile of *T. weyrauchi*, MM - molecular marker, molecular weight ranged from 10 to 220 kDa.

It is noteworthy that the State of Rondônia has a large number of species of stingless bees. Brown and Oliveira (2014) reported 110 species are found in that state, the survey conducted by the authors have collected over 9,555 individuals of 98 species of stingless bees, with the record 10 new species found.

Brown and Oliveira (2014) evaluated the impact of agriculture and deforestation on stingless bees in the state of Rondônia, and the main conclusion was that deforestation affects the quantity, diversity and composition of stingless bees, they claim that deforestation may result in serious consequences for pollination and reproduction of both native and cultivated plants.

In this study, we showed that number found for esterase species is greater than for other species of the same genus, as well as the number of proteins. The thermostability test showed resistance is associated with thermoregulatory capacity, an important characteristic because this species is found in regions with high temperatures.

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REFERENCES

BAITALA, T.V.; MANGOLIN, C.A.; TOLEDO, V.A.A.; RUVOLO-TAKASUSUKI, M.C.C. RAPD polymorphism in *Tetragonisca angustula* (Hymenoptera; Meliponinae, Trigonini) populations. **Sociobiology**, v.48, n.3, p.861-873, 2006.

BARTH, A.; FERNANDES, A.; POMPOLO, S.G.; COSTA, M.A. Occurrence of B chromosomes in *Tetragonisca Latreille*, 1811 (Hymenoptera, Apidae, Meliponini): A new contribution to the cytotaxonomy of the genus. **Genetics and Molecular Biology**, v.34, n.1, p.77-79, 2011.

BROWN, J.C.; OLIVEIRA, M.L. The impact of agricultural colonization and deforestation on stingless bee (Apidae: Meliponini) composition and richness in Rondônia, Brazil. **Apidologie**, v.45, n.2, p.172-188, 2014.

CERON, C.R. **Padrão de esterases no desenvolvimento de *Drosophila mulleri*, *D. arizonensis* e seus híbridos.** 1988, 142p. Tese (Doutorado) Instituto de Biociências, Universidade de São Paulo, 1988.

CORTOPASSI-LAURINO, M.; NOGUEIRA-NETO, P. Notas Sobre a bionomia de *Tetragonisca weyrauchi* Schwarz, 1943 (Apidae, Meliponini). **Acta Amazonica**, v.33, n.4, p.643-650, 2003.

FERMINO, F.; FALCO, J.R.P.; TOLEDO, V.A.A.; RUVOLO-TAKASUSUKI, M.C.C. Isoenzymes and Cytochemical Analysis in *Tetragonisca angustula*. **Sociobiology**, v.58, n.2, p.353-366, 2011.

FRANCISCO, F.O.; SANTIAGO, L.R.; BRITO, R.M.; OLDROYD, B.P.; ARIAS, M. C. Hybridization and asymmetric introgression between *Tetragonisca angustula* and *Tetragonisca fiebrigi*. **Apidologie**, v.45, n.1, p.1-9, 2014.

GALEGO, L.G.; CERON, C.R.; CARARETO, C.M. Characterization of esterases in a Brazilian population of *Zaprionus indianus* (Diptera: Drosophilidae). **Genética**, v.126, n.1-2, p.89-99, 2006.

HEALY, M.J.; DUMANCIC, M.M.; OAKESHOTT, J.G. Biochemical and physiological studies of soluble esterases from *Drosophila melanogaster*. **Biochemical Genetics**, v.29, n.7-8, p.365-387, 1991.

HSU, J.C.; HAYMER, D.S., WU, J.W.; FENG, H.T. Mutations in the acetycholinesterase gene of *Bactrocera dorsalis* associated to organophosphorus insecticides. **Insect Biochmistry and Molecular Biology**, v.36, n.5, p.396-402, 2006.

MACIAS-MACIAS, O.; CHUC, J.; ANCONA-XIU, P.; CAUICH, O.; QUEZADA-EUAN, J.J.G. Contribution of native bees and Africanized honey bees (Hymenoptera: Apoidea) to solanaceae crop pollination in tropical Mexico. **Journal of Applied Entomology**, v.133, n.6, p.456-465, 2009.

MASSA, R.; BLEVINS, S.; CHAO, S.L. Role of acetylcholinesterase and glutathione Stransferase following exposure to nicosulfuron and diazinon in *Helicoverpa zea*. **Ecotoxicology and Environmentl Safety**, v.71, n.1, p.230-235, 2008.

MONTELLA, I.R.; SCHAMA, R.; VALLE, D. The classification of esterases: an important gene family involved in insecticide resistance - A Review. **Memórias do Instituto Oswaldo Cruz**, v.107, n.4, p.437-449, 2012.

MOURE, J.S.; URBAN, D.; MELO, G.A.R. Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical region. Sociedade Brasileira de Entomologia, Curitiba, 2007, 1058p.

OAKESHOTT, J.G., PAPENRECHT, E. A.; BOYCE, T.M.; HEALY, M.J.; RUSSEL, R.J. Evolutionary genetics of *Drosophila* esterases. **Genetics**, v.90, n.2-3, p.239-268, 1993.

OLIVEIRA, R.C.; NUNES, F.M.F.; CAMPOS, A.P.S.; VASCONCELOS, S.M.; ROUBIK, D.; GOULART, L.R.; KERR, W.E. Genetic divergence in *Tetragonisca angustula* Latreille, 1811 (Hymenoptera, Meliponinae, Trigonini) based on RAPD markers. **Genetics and Molecular Biology**, v.27, n.2, p.181-186, 2004.

ROSSITER, L.C.; CONYERS, C.M.; MACNICOLL, A.D.; ROSE, H.A. Two qualitatively different B- esterases from two organophosphate-resistant strains of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and their roles in fenitrothion and chlorpyrifos-methyl resistance. **Pesticide Biochemistry and Physiology**, v.69, n.2, p.118-130, 2001.

RUVOLO-TAKASUSUKI, M.C.C.; DEL LAMA, M.A.; SOARES, A.E. Genetic characterization of a new *Apis mellifera* esterase. **Apidologie**, v.28, n.5, p.259-267, 1997.

RUVOLO-TAKASUSUKI, M.C.C.; VIANA, L.H.O.; BAITALA, T.V.; NICOLIN, K. C.; TOLEDO, V.A.A. 2006. Characterization of esterases in *Tetragonisca angustula* and *Tetragona clavipes* (Hymenoptera; Meliponinae). **Brazilian Journal of Morphological Science**, v.23, n.3-4, p.431-434, 2006.

STUCHI, A.L.P.B.; TOLEDO, V.A.A.; LOPES, D.A.; CANTAGALLI, L.B.; RUVOLO-TAKASUSUKI, M.C.C. Molecular Marker to Identify Two Stingless Bee Species: *Tetragonisca angustula* and *Tetragonisca fiebrigi* (Hymenoptera, Meliponinae). **Sociobiology**, v.59, n.1, p.123-134, 2012.

TEIXEIRA, A.S.; CHAVES, L.S.; YUYAMA, K. Esterases no exame da estrutura populacional de camucamu (*Myrciaria dúbia* (Kunth) Mc Vaugh-Murtaceae). **Acta Amazonica**, v.34, n.1, p.89-96, 2004.