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Bees (Hymenoptera: Apidae) present in the flowers of the balsa wood *Ochroma lagopus* Swartz, 1788

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ABSTRACT. The flower of balsa wood holds about 10 to 15 mL of nectar, which helps attracting pollinating agents, since the genus *Ochroma* is incapable of self-fertilization. However, a high mortality of bees is observed in these flowers. The present study investigated the frequency and constancy of mortality of the individuals of the family Apidae that fed on nectar from the balsa wood. Data was gathered from June to August 2008, in Lavras – Minas Gerais State, Brazil. In addition, the survival of the Africanized bees that fed on the nectar of this flower was compared to those that fed on 50% aqueous solution of honey. Forty flowers were analyzed, and 949 individuals of the orders Hymenoptera (98.1%), Hemiptera (0.95%), Coleoptera (0.74%) and Diptera (0.21%) were collected. Most Hymenoptera individuals were bees of the genera *Partamona* and *Trigona* (677 individuals), which were considered of constant occurrence. Flowers producing up to 16.7 nectar mL were found. The nectar diet contained 16.44% of total sugar, and resulted in low survival of the bees in laboratory (31.32 ± 2.37 hours), compared to a diet of 50% aqueous solution of honey (112.32 ±2.03 hours).

Key words: *Apis*, constancy, mortality, *Partamona*, O. *pyramidale*.

RESUMO Abelhas (Hymenoptera: Apidae) associadas às flores do pau-de-balsa *Ochroma lagopus* Swartz, 1788. A flor do pau-de-balsa produz cerca de 10 a 15 mL de néctar, útil na atração de polinizadores, uma vez que o gênero *Ochroma* é incapaz de fazer autofecundação. É observada intensa mortalidade de abelhas em suas flores. Objetivou-se realizar o levantamento da frequência e constância de mortalidade de indivíduos da família Apidae, sendo os dados levantados no período de junho a agosto de 2008 em Lavras, Minas Gerais, Brasil. Além disso, avaliou-se a sobrevivência de abelhas africanizadas alimentadas com o néctar desta flor quando comparados com aquelas alimentadas com solução aquosa de mel a 50%. Foram analisadas 40 flores e coletados 949 indivíduos das Ordens: Hymenoptera (98,1%), Hemiptera (0,95%), Coleoptera (0,74%) e Diptera (0,21%). Dentre os himenópteros os mais frequentes foram dos gêneros *Partamona* e *Trigona* com 677 indivíduos, sendo estes considerados de incidência constante. Observaram-se flores com até 16,7 mL de néctar. O néctar apresentou 16,44% de açúcares totais e baixa sobrevivência das abelhas em laboratório (31,32 ± 2,37h) quando comparadas com a dieta à base de mel (112,32 ± 2,03h).

Palavras-chave: *Apis*, constância, mortalidade, *Partamona*, O. *pyramidale*.

Introduction

The flower of balsa wood, *Ochroma lagopus* Sw., is a plant belonging to family Malvaceae (SOUZA; LORENZI, 2005), which occurs naturally in the Amazon region, at altitudes varying from 0-1,000 m (LORENZI, 2002). Rainfall ranging of 1,500-3,000 mm, and temperature from 22 to 27°C are the requirements for its growing (CAMPOS; UCHIDA, 2002). This vegetal species grows best when there is intense sunlight, and can tolerate dry conditions very well, as long as air humidity levels do not go lower than 75% (MARENCO et al., 2001). More than 28 genera and 200 species of balsa wood grow in tropical and subtropical regions. The family Bombacaceae has great economic significance because it is used as a construction material for boats, buoys, among other uses (LORENZI, 2002). It is an extremely fast-growing species that can reach up to 30 meters, with trunk diameters of 60-90 cm (PAULA et al., 1998).

The fruits of the balsa wood are formed by an elongated capsule that opens into five valves when the seeds are ripe, releasing cream colored tubes containing plenty of seeds. The flowers provide abundant food for bats, birds and several insects.

Flowering is seasonal and the flowers last one to two days, holding 10 to 15 mL of nectar per flower (PAULA et al., 1998). This amount of nectar helps attracting pollinating agents with high energy demands, because the *Ochroma* genus is unable of self-fertilization (GRIBEL et al., 1999). According to Opler (1983), the *O. lagopus* is pollinated by bats. In turn, Mora et al. (1999) suggest that a coati species is the pollinator of this Malvaceae species.

The most important pollinating agents belong to the class Insecta, mostly bees of the order Hymenoptera (SANTANA et al., 2002).

One third from the worldwide crop production comes from crops that depend on animal pollination, with the bees accounting for 38% of the pollination of flowering plants, but probably not including *O. lagopus* pollination (KERR et al., 2001).

As for the flower of balsa wood, this insect-plant interaction arouses debate. Paula et al. (1997) identified saccharidosis, glucose and fructose, besides 16 amino acids (lysine, histidine, aspartic acid, asparagine, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleukin, leukin, glutamine and phenylalanine) in the floral nectar of this plant. According to Haydak (1970), the amino acids that play an essential role in the nutrition of bees are: lysine, histidine, threonine, valine, methionine, isoleukin, leukin, phenylalanine arginine and tryptophan. Therefore, all the essential amino acids, except arginine and tryptophan, are contained in the nectar of balsa wood, so it is a very nutritious and attractive food for bees.

However, some researchers noticed a significant amount of bees found dead in the nectar of this plant in the State of São Paulo (NOGUEIRA-NETO, 2002), and in Viçosa, in the region known as *Zona da Mata*, in Minas Gerais State (PAULA et al., 1997). Consequently, new research was conducted to identify the substances that might cause nectar toxicity, such as phenolic compounds, alkaloids and non-protein amino acids. Paula et al. (1997) did not identify any toxic substance in flowers of balsa wood, or any morphological adaptations, such as resin or any sticky substance that might cause bees to be stuck in the plant and die. Adler (2000) reports that some nectar may be toxic to bees due to the presence of microorganisms that produce ethanol. According to Winston (1983), galactosis, manosis and rhamnosus can be toxic to bees or reduce their longevity. Meanwhile, analysis of nectar of balsa wood did not show the presence of the referred substances.

Severe mortality of bees that collected the nectar from balsa wood also occurred in plants at the campus of the Federal University of Lavras, in Southern Minas Gerais State. The present study aimed to investigate the frequency of dead insects found in the nectar of *Ochroma lagopus* flowers in the city of Lavras, Minas Gerais State, and the survival rate of *Apis mellifera* Linnaeus, 1758, in laboratory bioassays, when fed on the nectar of this flower, compared to bees fed on a honey-based diet.

Material and methods

The present study was conducted from June to August 2008, which is the flowering period of the balsa wood, and was based on the observation of some *O. lagopus* trees in the campus of the Federal University of Lavras (UFLA), in Minas Gerais, State, Brazil. The city of Lavras is located at 21°C 14' South latitude, 45°C 00' West longitude and at 910 meters altitude. According to Köppen climate classification, the predominant climate in the region is mild and temperate – mesothermal – rainy, with dry winter and an average temperature below 22°C.

One randomly chosen flower was collected every day, always at 5 p.m., when bees finish foraging, during 40 days. The flowers were taken to the Insect Biology Laboratory of the Department of Entomology, and the insects found inside the flowers were collected, counted and identified. Afterwards, the flowers were placed in an inverted position and the nectar was poured into a glass vial, free from contamination, and immediately quantified.

The bees were identified to genus, as recommended by Silveira et al. (2002), whereas the other insects were identified to order.

The constancy of individuals found dead in the flowers, that is, the percent of taxonomic categories found in the survey was determined (SILVEIRA NETO et al., 1995) by the equation: $C = (P/N) \times 100$, where: C = Constancy, P = number of members of a given taxonomic category collected, N = number of individuals collected. The classes were established by the calculation of the confidence interval (CI) of the mean at a 5% significance level. The taxonomic category was considered to be 'constant' when the percent value of individuals of this category collected was higher than the upper limit of the confidence interval; 'accessory', when the percent value of individuals of the taxonomic category collected fell within the confidence interval; 'accidental' when the percent value of individuals collected was below the lower limit of the confidence interval (LUDWIG; REYNOLDS, 1988).

The presence of *Varroa destructor* Anderson and Trueman mite was assessed in *A. mellifera* bees collected and in nectar. Although this ectoparasite occurs worldwide, there is little information on its population fluctuation in Brazil (ANDERSON; TRUEMAN, 2000).

In order to investigate the causes of the survival of bees fed on nectar of balsa wood, and construct the survival function, newly emerged bees from Central Apiary were used and a colony was selected to provide individuals for laboratory trials.

In the Insect Biology laboratory – DEN/UFLA, groups of ten individuals were placed in a 15 cm wide x 10 cm high PVC cage, with a tulle net placed on top and an organza fabric on the bottom, and maintained in an acclimatized chamber at a 29 ± 2°C, UR 70 ± 10% and photoperiod of 12 hours.

The experiment was conducted in a completely randomized design with two treatments and ten replicates, and each experimental unit had two 2 mL eppendorf tubes, one for distilled water and the other for the diets, representing the treatments: 1) 50% aqueous solution of honey; 2) nectar of balsa wood.

In order to quantify the total sugar contents, reducers (monosaccharides) and non-reducers (disaccharides) in the nectar of balsa wood and the 50% aqueous solution of honey used in the laboratory trials, chemical analysis using the Somogyi-Nelson method was performed. This method is based on copper ion reduction where sugar is heated in alkaline solution of arsenic tartrate, producing a blue-colored compound quantified by spectrophotometry at 510 nm (SILVA et al., 2003). This analysis may help to justify the attractiveness of bees to nectar compared to 50% aqueous solution of honey, and was performed in the Analysis Laboratory of the Department of Food Science – UFLA.

The number of surviving bees was counted every 12 hours until 120 hours. Since the survival data is censored, Kaplan-Meier analysis was performed to determine the average life period for individuals and obtain a survival curve for each treatment (COLOSIMO; GIOLO, 2006). The Log-Rank test was performed to compare the treatments.

Results and discussion

Forty flowers were analyzed, and 949 dead individuals of the orders Hymenoptera (98.10%), Hemiptera (0.95%), Coleoptera (0.74%) and Diptera (0.21%) were found. Most Hymenoptera individuals were bees of the *Partamona*, *Trigona* and *Apis* genera (Table 1).

Table 1. Number of insects found dead in flowers of *Ochroma lagopus*, from June to August 2008.

Insects found in the flowers			N° of individuals	Relative frequency (%)	Constancy (%)
Hymenoptera	Apidae	*Partamona*	398	41.94	80.0
		Trigona	279	29.40	77.5
		Apis	219	23.08	72.5
		Tetragonisca	2	0.21	5.0
		Plebeia	1	0.10	2.5
		Total Apidae	899	94.73	
Hymenoptera	Vespidae		30	3.16	45.0
	Formicidae		2	0.21	2.5
Hemiptera			9	0.95	17.5
Coleoptera			7	0.74	17.5
Diptera			2	0.21	5.0
		Total	949	100.00	

No insect was found dead in the nectar of 20% of the flowers. Nevertheless, the average mortality was 23.7 insects per flower, and it is important to stress that 112 individuals were found dead in one flower, 63 of which belonging to the *Partamona* genus and 40 of the *Trigona* genus. It is possible that these Hymenoptera bees are more attracted to the nectar of balsa wood, which allows us to suggest that mortality is caused by some toxic element present in the nectar, or by asphyxia due to drowning in nectar (Figure 1), this latter cause is the most plausible due to the amount of nectar produced by *O. lagopus* flowers.

Figure 1. Flower of *Ochroma lagopus* Sw. with dead insects in the nectar.

Concerning the greater number of *Trigona* bees compared to the *Apis* genus, according to Morgado et al. (2002), in sunflower crops in Lavras (Minas

Gerais State), *Trigona spinipes* were more abundant than *A. mellifera* in some periods of time, which may be an indication of the high number of individuals of this species in the region, or colonies in nests surrounding those areas. However the study from Gamito and Malerbo-Souza (2006) determined *T. spinipe* as an accessory species, and the *A. mellifera* as a constant species in orange tree flowers.

The *Partamona, Trigona* and *Apis* genera were found as constant, and the others were found to be of accessory incidence. Despite the constancy observed in these insects, they did not interfere with the pollination of this species. No category was found to be accidental, due to the high standard deviation observed.

One significant finding from this study is that the *V. destructor* mites was not found in any of the 219 *A. mellifera* bees collected, not even in the nectar collected, which may indicate a low scale fluctuation in the population of this parasite, during the period of the present study, and that this parasite in some stage of life cycle is a phoretic mites.

Concerning the life period, *A. mellifera* adult bees were found to have an average survival time of 31.32 ± 2.37 hours when fed on nectar of balsa wood, which is significantly lower than the average survival time of 112.32 ± 2.03 hours observed for individuals fed on 50% aqueous solution of honey (Figure 2).

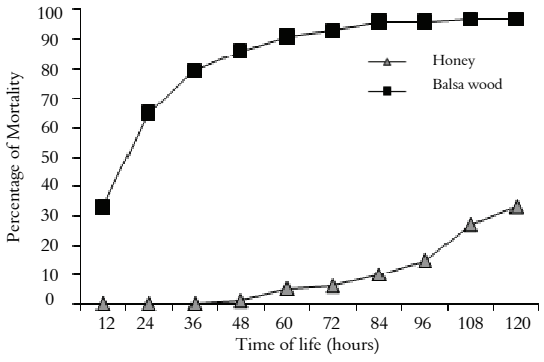

Figure 2. Mortality of *Apis mellifera* bees along the time fed on a diet of 50% aqueous solution of honey and nectar of balsa wood. Temperature of 29 ± 2°C, UR 70 ± 10% and photoperiod of 12 hours.

The functions of survival of *A. mellifera* bees fed on a diet of 50% aqueous solution of honey and on a diet of nectar of balsa wood were drawn (Figure 3). The treatments were found to be significantly different according to Log-rank test at significance level $\alpha = 5\%$, with a p-*value* < 0.001.

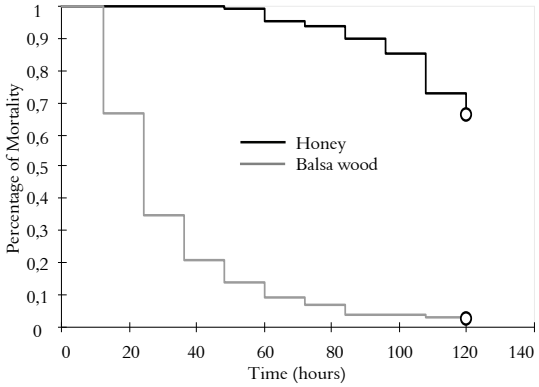

Figure 3. Function of distribution of survival of *Apis mellifera* bees fed on the following diets: 50% aqueous solution of honey, and nectar of balsa wood. Temperature of 29 ± 2°C, UR 70 ± 10% and photoperiod of 12 hours.

The lethal time TL_{50} for 50% of the population fed on a diet of balsa wood nectar was estimated at only 25.03 ± 0.32 hours, whereas for bees fed on a diet of 50% aqueous solution of honey it was longer than 120 hours.

As for the volume of nectar collected, some flowers were found to have none, while others had as much as 16.7 mL of nectar, and the analysis of the nectar collected showed a low sugar content (16.44%) compared to the honey collected in hives of *A. mellifera* from UFLA's Apiary (75.80%) or to Melipona honey (77.88%) (ALVES et al., 2005) (Table 2).

Table 2. Analysis of total sugars, reducers and non-reducers by the Somogyi-Nelson method.

Diet	Total sugars (%)	Reducer sugars (%)	Non-reducer sugars (%)
Nectar of balsa wood	16.44	14.63	1.73
50% aqueous solution of honey	37.70	17.82	1.05

Bioassays to assess the toxicity of *O. lagopus* nectar in *Partamona* and *Trigona* were conducted by Paula et al. (1997). However, no increase was found in the mortality rate of bees fed on nectar compared to the control bees fed on a saccharose solution. Another hypothesis to explain the high mortality of bees found in balsa wood nectar would be the drowning in the nectar, but this hypothesis is refuted by an experiment conducted by Paula et al. (1997) where nectar of some flowers was collected and replaced by almost identical concentrations of saccharose, and the authors observed that bees that fell on this solution managed to get out and fly again. On the other hand, when bees fell on balsa wood nectar they were unable to fly, and died within 30 seconds. However, it is worth stressing that the

breathing tubes of bees are connected to the outside through spiracles in their exoskeleton and when these insects are foraging their breathing is increased, so their abdomen is rhythmically contracted to improve gas exchange (WINSTON, 1983). In this case, when bees dive deep into the nectar, which has a different viscosity from the solution tested by Paula et al. (1997), asphyxia may occur due to the presence of some types of amino acids that obstruct the spiracles, and lead these insects to die. The addition of amino acid to the solution proposed by Paula et al. (1997) was not yet tested. The most likely cause of death of bees is asphyxia by obstruction of air sacs and spiracles by one of the amino acids present in the balsa wood.

Conclusion

The dead insects found in the nectar of balsa wood belonged to the orders Hymenoptera, Coleoptera, Diptera and Hemiptera, with great predominance of Hymenopterous species.

The *Partamona* and *Trigona* genera were constant categories among the individuals collected in *Ochroma lagopus* flowers, in the city of Lavras, and were attracted by the great amount of nectar in these flowers.

The longevity of *Apis mellifera* adult bees fed on nectar of balsa wood flower was shorter than when these insects were fed on a 50% aqueous solution of honey.

The total sugar contents in the nectar of balsa wood was significantly lower than the one found in the 50% aqueous solution of honey, however the amount of reducer sugars was the same.

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References

ADLER, L. S. The ecological significance of toxic nectar. **Oikos**, v. 91, n. 3, p. 409-420, 2000.

ALVES, R. M. O.; CARVALHO, C. A. L.; SOUZA, B. A.; SODRÉ, G. S. S.; MARCHINI, L. C. Características físico-químicas de amostras de mel de *Melipona mandacaia* Smith (Hymenoptera: Apidae). **Ciência e Tecnologia de Alimentos**, v. 25, n. 4, p. 644-650, 2005.

ANDERSON, D. L.; TRUEMAN, J. W. H. *Varroa jacobsoni* (Acari:Varroidae) is more than one species. **Experimental and Applied Acarology**, v. 24, n. 3, p. 165-189, 2000.

CAMPOS, M. A. A.; UCHIDA, T. Influência do sombreamento no crescimento de mudas de três espécies amazônicas. **Pesquisa Agropecuária Brasileira**, v. 37, n. 3, p. 281-288, 2002.

COLOSIMO, E. A.; GIOLO, S. R. **Análise de sobrevivência aplicada**. São Paulo: Edgar Blucher, 2006.

GAMITO, L.; MALERBO-SOUZA, D. Visitantes florais e produção de frutos em cultura de laranja (*Citrus sinensis* L. Osbeck). **Acta Scientiarum. Animal Sciences**, v. 28, n. 4, p. 483-488, 2006.

GRIBEL, R.; GIBBS, P. E.; QUEIROZ, A. I. Flowering phenology and pollination biology of *Ceiba pentandra* (Bombacaceae) in Central Amazonia**. Journal of Tropical Ecology**, v. 13, n. 3, p. 247-263, 1999.

HAYDAK, M. H. Honey bee nutrition. **Annual Review of Entomology**, v. 15, p. 143-156. 1970.

KERR, W. E.; CARVALHO, G. A.; SILVA, A. C.; ASSIS, M. G. P. Aspectos pouco mencionados da biodiversidade amazônica. **Parcerias Estratégicas**, n. 12, p. 1-41, 2001.

LORENZI, H. **Árvores brasileiras**: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Nova Odessa: Plantarum, 2002. v. 1.

LUDWIG, J. A.; REYNOLDS, J. S. **Statistical ecology**: a primer on methods and computing. New York: John Wiley & Sons, 1988.

MARENCO, R. A.; GONÇALVES, J. F. C.; VIEIRA, G. Photosynthesis and leaf nutrient contents in *Ochroma pyramidale* (Bombacaceae). **Journal Photosynthetica**, v. 39, n. 4, p. 539-543, 2001.

MORA, J. M.; MÉNDEZ, V. V.; GÓMEZ, L. D. White-nosed coati *Nasua narica* (Carnivora: Procyonidae) as a potential pollinator of *Ochroma pyramidale* (Bombacaceae). **Revista Biologia Tropical**, v. 47, n. 4, p. 719-721, 1999.

MORGADO, L. N.; CARVALHO, C. F., SOUZA, B.; SANTANA, M. P. Fauna de abelhas (Hymenoptera: Apoidea) nas flores de girassol *Helianthus annuus* L., em Lavras-Minas Gerais. **Ciência e Agrotecnologia**, v. 26, n. 6, p. 1167-1177, 2002.

NOGUEIRA-NETO, P. Inbreeding and building up small populations of stingless bees (Hymenoptera: Apidae). **Revista Brasileira de Zoologia**, v. 19, n. 4, p. 1181-1214, 2002.

OPLER, P. A. Nectar production in a tropical ecosystem. In: BENTLEY, B.; ELIAS, T. S. (Ed.). **The biology of nectaries**. New York: Columbia University Press, 1983. p. 39-79.

PAULA, V. F.; BARBOSA, L. C. A.; DEMUNER, A. J. Entomotoxicity of the nectar from *Ochroma lagopus* Swartz (Bombacaceae). **Ciência e Cultura**, v. 49, n. 4, p. 275-277, 1997.

PAULA, V. F.; BARBOSA, L. C. A.; PILÓ-VELOSO, D.; DEMUNER, A. J.; HOWARTH, O. Constituintes químicos da casca *Ochroma lagopus* Swartz (Bombacaceae). **Eclética Química**, v. 23, p. 45-57, 1998.

SANTANA, M. P.; CARVALHO, F. C.; SOUZA, B.; MORGADO, L. N. Abelhas (Hymenoptera: Apoidea)

visitantes das flores do feijoeiro, *Phaseolus vulgaris* L., em Lavras e Ijaci – MG. **Ciência e Agrotecnologia**, v. 26, n. 6, p. 1119-1127, 2002.

SILVA, R. N.; MONTEIRO, V. N.; ALCANFOR, J. D. X.; ASSIS, E. M.; ASQUIERI, E. R. Comparação de Métodos para determinação de açúcares redutores e totais em mel. **Ciência e Tecnologia Alimentos**, v. 23, n. 3, p. 337-341, 2003.

SILVEIRA, F. A.; MELO, G. A. R.; ALMEIDA, E. A. B. **Abelhas brasileiras**: sistemática e identificação. Belo Horizonte: Fernando A. Silveira, 2002.

SILVEIRA NETO, S.; MONTEIRO, R. C.; ZUCCHI, R. A.; MORAES, R. C. B. Uso da análise faunística de insetos na avaliação do impacto ambiental. **Scientia Agricola**, v. 52, n. 1, p. 9-15, 1995.

SOUZA, V. C.; LORENZI, H. **Botânica sistemática**: guia ilustrado para identificação das famílias de angiospermas da flora brasileira. Nova Odessa: Plantarum, 2005.

WINSTON, M. L. **The biology of the honey bee**. Cambridge: Harvard University Press, 1983.

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