

Sociobiology An international journal on social insects

SHORT NOTE

Observation of *Trigona recursa* Smith (Hymenoptera: Apidae) Feeding on *Crotalaria micans* Link (Fabaceae: Faboideae) in a Brazilian Savanna Fragment

TMR SANTOS¹, JT SHAPIRO¹, PS SHIBUYA¹, C AOKI²

- 1 Universidade Federal de Mato Grosso do Sul, Campo Grande MS, Brazil
- 2 Universidade Federal de Mato Grosso do Sul, Aquidauana MS, Brazil

Article History

Edited by:

Celso F. Martins, UFPB - Brazil
Received 17 January 2013
Initial acceptance 26 February 2013
Final acceptance 16 March 2013

Keywords

Entomotoxicity, Feeding behavior, Geographic distribution, Monocrotaline, Stingless bee

Corresponding author

Julie Teresa Shapiro
Laboratório de Zoologia
Universidade Federal de Mato Grosso
do Sul, Cidade Universitária s/n,
Campo Grande, MS, Brazil
79090-900
julie.teresa.shapiro@post.harvard.edu

Abstract

In this paper we present observations of individuals of the bee species *Trigona recursa* feeding on the fruits of *Crotalaria micans*. This plant, which contains pyrrolizidine alkaloids, is known to be toxic to humans, mammals and poultry. Over the course of three days, we observed a large number of bees feeding on many individual *Crotalaria micans* plants in an urban fragment of Brazilian Savanna. The bees preferred greener fruits, which are the softest and most toxic. Consumption of the plant had no immediately apparent fatal effect on the bees, since we did not find any dead individuals near the observation site. Some insect species are known to use pyrrolizidine and alkaloids for defense by incorporating them into their body or using them as precursors to pheromones. *Trigona recursa* and other bee species have not been previously recorded consuming *Crotalaria micans* and it is unclear what their motivation may be. We present these observations as a novel finding of the feeding behavior of *Trigona recursa*.

Introduction

Plants of the genus *Crotalaria* (L.) contain pyrrolizidine alkaloids, which are known to be toxic to humans and animals, particularly livestock and poultry (Rose et al., 1957; Alfonso et al., 1993). Monocrotaline, the primary toxin of this genus, has been shown to damage hepatocytes, astrocytes, and glial cells, interfere with cell growth, cytoskeleton protein expression, and ATP production, damage DNA and cause apoptosis (Silva-Neto et al., 2010;Pitanga et al., 2011).

Trigona jurine 1807 is a Neotropical stingless bee genus that occurs from Mexico to northern Argentina, Paraguay and Uruguay (Camargo & Pedro, 2012). The greatest diversity of the genus is found in the Amazon and the central region of Brazil and a total of 19 species can be found within Brazilian territory (Rebêloet al., 2003). Relatively little is known of the feeding behavior of *T. recursa*. Individuals mark their paths and food sources for their nestmates to follow, using pheromones produced in the labial glands (Jarau et al., 2003).

They tend to exploit floral resources, although they have also been observed using non-floral sources and gathering sweat (Lorenzon & Matrangolo, 2005). Other species of the genus are known to be generalists feeding on pollen from a variety of plants (Oliveira et al., 2009). Three Neotropical Trigona species are necrophagous (Noll et al., 1996).

Previous studies of *Crotalaria retusa* have found that few insects visit these plants, which contain toxins throughout all their parts, (Kissmann & Groth, 1999) with two carpenter bee species, *Xylocopa grisescens* and *X. frontalis* making up for 90% of visits (Jacobi et al., 2005). *Trigona spinipes* has been observed visiting occasionally in order to obtain nectar by perforating the base of the flowers (Jacobi et al., 2005).

Materials and Methods

During three consecutive days in February 2012, individuals of the bee species *T. recursa* were observed eating the fruits of *C. micans* in an urban Cerrado fragment on the campus of the Universidade Federal de MatoGrosso do Sul (Federal University of Mato Grosso do Sul), Campo Grande, Mato Grosso do Sul, Brazil (20° 30' S, 54° 36' W).

The first observation was carried out in the afternoon before sunset at approximately 18:00. On the following days, the observations were repeated twice per day, the first at 12:00 and the second at 18:00. Each observation period lasted 30 minutes.

At the end of the second day of observation, seven individual bees, as well as a sample from the plant were collected. These specimens were processed and given to specialists for identification. The bees were deposited in the Entomological Collection Pe. Jesus S. Moure (DZUP) of the Department of Zoology at the Universidade Federal do Paraná (Federal University of Paraná).

Results

We observed the presence of a large number of individuals of *T. recursa* (n>100) consuming the fruit of several dozen individual *C. micans* plants during all observations. Apparently, the number of *T. recursa* was lower during the observations at 12:00 than at 18:00. However, because of their large numbers and frequent, fast movements around and between the plants, it was not possible to quantify the exact number of individuals and therefore it is impossible to be certain of the quantity of individuals consuming the fruits during the different hours of observation.

The fruits of *C. micans* are dry. The bees scraped the velvety-textured external layer of the fruits with their tongues, apparently marking them (Fig. 1).

The bees demonstrated a preference for the younger



Fig. 1.Bees feeding on the fruit of Crotalariamicans. The arrow indicates the scraped portion of the fruit.

and softer fruits. Older and drier fruits were discarded after partial consumption. Each plant had over one hundred fruits and the ratio between consumed and not consumed fruits was approximately 1:1, although a few individual plants had almost 100% of their fruits consumed. All plants had at least some of their fruits eaten.

We did not observe any indication of fatal intoxication in the bees. Over the three consecutive days of observation, no dead bees were found in the area of the plants. Possible intoxication later could not be analyzed.

Discussion

Our observations of *T. recursa* feeding on plants of *C. micans* are notable because it is not a known food source for this bee speciesand toxins are found throughout the entire plant, including the fruit (Kissmann&Groth, 1999). Although *T. spinipesis* known to occasionally collect nectar from *Crotalaria* species (Jacobi et al., 2005), the individuals of *T. recursa* observed did not appear to collect nectar. Furthermore, the scraping method that we observed them using has not been previously recorded. It is possible that the bees scraped the fruits in order to differentiate the consumed fruits from those that had not yet been consumed. They may have also been marking them to recruit nest-mates to this food source (Jarau et al., 2003).

Studies have shown that secondary metabolites of plants can be toxic to bee species. For example, nicotine at high levels can reduce bees' fitness, although at lower, naturally occurring levels, there are no apparently detrimental effects and the substance can even be beneficial (Kohler et al., 2012). Other toxins produced in flowers have also been shown to reduce the life span of bees in laboratory settings, depending on the dose (Santoro et al., 2004; Rother et al., 2009; Rocha-Neto et al., 2011). Rother et al. (2009) tested the effects of ricinine (a toxic compound of Ricinus communis - Euphorbiaceae) on bees of the species Apis mellifera and Scaptotrigona postica and observed that A. mellifera had a high mortality within 72 hours for two of three concentrations tested (0.05 and 0.1%), while S. postica presented high mortality rate only after 14 days. Santoro et al. (2004) demonstrated that A. mellifera was also susceptible to tannins of Stryphnodendron spp. with mortality rates increasing on day 3 at all concentrations tested (1.25, 2.5 and 3.75%). These two studies showed that A. mellifera seems to be sensitive and have a rapid response to the toxins of native plant species, while the same did not occur with S. postica native bee species.

We note that for the above studies, bees were exposed to toxic treatments with extracts in laboratory experiments, whilehere we report that *T. recursa* spontaneously consumed *C. micans*. However, in more similar circumstances, Del Lama and Peruquetti (2006) observed that the consumption of the toxic plant *Caesalpinia peltophoroides* in a natural setting caused a large number of mortalities in bees, with 273 indi-

viduals of 20 different species dying after visiting the plant. Toxicity seemed to vary by time and individual tree. Although the long term effects could not be observed, most bees dropped to the ground in narcosis and died immediately

Species of the orders Lepidoptera, Coleoptera, Diptera, and Orthoptera are known to sequester pyrrolizidine alkaloids, including those found in *Crotalaria*, for purposes of defense against predators and as precursors to pheromones (Boppré, 1990). Notably, studies have shown that the moth species Utetheisa ornatrix obtains the toxins from plants during its larval stage, retaining the compounds as an adult and then passing it on to their eggs (Eisner & Eisner, 1991). Laboratory and field experiments have confirmed that the consumption of pyrolizidine alkaloids protects individuals from predation (Dussourd et al., 1988; Boppré, 1990; Eisner & Eisner, 1991).

In this case, there was no immediately apparent negative effect on *T. recursa* from consuming *C. micans*. Although we cannot be sure of the longer-term effects on the bees' fitness, it is possible that *T. recursa* benefits from the protection from monocrotaline. The toxins could also have a longer-term negative effect or intoxicate the bees after several days, but we were unable to confirm or rule out any of these possibilities.

We also note that this is the first record of *T. recursa* in the state of Mato Grosso do Sul. The species has been previously recorded in the neighboring states of São Paulo, Goiás, and Mato Grosso (Camargo & Pedro, 2012).

Acknowledgements

We would like to thank Gabriel Melo for assisting in insect identification, Thales Henrique Dias Leandro for plant identification, and the Fulbright Program for the support of Julie Shapiro. We thank two anonymous reviewers for valuable comments that improved this article.

References

Alfonso, H.A., Sanchez, L.M., Figeurdo, M.A. & Gomez, B.C. (1993). Intoxication due to *Crotalaria retusa* and *C. spectabilis* in chickens and geese. Vet. Hum. Toxicol., 35(6): 539.

Boppré, M. (1990).Lepidoptera and pyrrolizidine alkaloids. Exemplification of complexity in chemical ecology.J. Chem. Ecol., 16(1): 165–185.

Camargo, J.M.F.& Pedro, S.R.M. (2012). Meliponini Lepeletier, 1836. In Moure, J. S., Urban, D. &Melo, G. A. R. (Orgs). Catalogue of bees (Hymenoptera, Apoidea) in the neotropical region - online version. Available at http://www.moure.cria.org.br/catalogue. Accessed 1 March 2013.

Del Lama, M.A. & Peruquetti, R.C. (2006). Mortalidade de abelhas visitantes de flores de *Caesalpinia peltophoroi*-

des Benth (Leguminosae) no estado de São Paulo, Brasil. Rev. Bras. Entomol., 50(4): 547–549. doi: 10.1590/S0085-56262006000400017

Eisner, T. & Eisner, M. (1991). Unpalatability of the pyrrolizidine alkaloid-containing moth, Utetheisaornatrix, and its larva, to wolf spiders. Psyche, 98: 111–118. doi: 10.1155/1991/95350

Jacobi, C.M., Ramalho, M. & Silva, M. (2005). Pollination Biology of the Exotic Rattleweed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. Biotropica, 37(3):357–363. doi: 10-111/j.1744-7429.2005.00047.x

Jarau, S., Hrncir, M., Schmidt, V.M., Zucchi, R. & Barth, F.G.(2003). Effectiveness of recruitment behavior in stingless bees (Apidae, Meliponini). Insectes Soc., 50(4): 365–374. doi: 10.1007/s00040-003-0684-2

Kissmann, K.G.& Groth, D. (1999). Plantas infestantes e nocivas, Tomo II (2°ed.). São Paulo: Basf.

Köhler, A., Pirk, W.W.C. & Nicolson, W.S. (2012). Honeybees and nectar nicotine: Deterrence and reduced survival versus potential health benefits. J. Insect Physiol., 58:286–292. doi: 10.1016/j.bbr.2011.03.031

Lorenzon, M.C.A.I. & Matrangolo, C.A.R. (2005). Foraging on some nonfloral resources by stingless bees (Hymenoptera, Meliponini) in a caatinga region. J. Biol., 65(2):291–298. doi: 10.1590/S1519-69842005000200013

Neto, R.T.J., Leite, D.T., Maracajá, P.B., Pereira-Filho, R.R. & Silva, O.S.D.(2011). Toxicidade de flores de Jatropha gossypiifolia L. a abelha africanizada em condições controladas. Rev. Verde, 6 (2): 64–68.

Noll, F.B., Zucchi, R., Jorge, J.A. & Mateus, S. (1996). Food collection and maturation in the necrophagous stingless bee, *Trigona hypogea* (Hymenoptera: Meliponinae). J. Kans. Entomol. Soc., 69(4): 287–293.

Oliveira, F.P.M., Absy, M.L. & Miranda, I.S. (2009). Recurso polínico coletado por abelhas sem ferrão (Apidae, Meliponinae) em um fragment de floresta na região de Manaus-Amazonas. Acta Amazon., 39(3): 505–518.

Pitanga, S.P.B., Silva, A.D.V., Souza, S.C., Junqueira, H.A., Fragomeni, N.O.B., Nascimento, P.R., Silva, R.A., Costa, D.F.M., El-Bacha, R.S. & Costa, S.L. (2011). Assessment of neurotoxicity of monocrotaline, an alkaloid extracted from *Crotalaria retusa* in astrocyte/neuron co-culture system. NeuroToxicology, 32: 776–784. doi: 10.1016/j.neuro.2011.07.002

Rebêlo, M.M.J., Rêgo, M.M.C. & Albuquerque, C.M.P. (2003). Abelhas (Hymenoptera, Apoidea) da região setentrional do Estado do Maranhão, Brasil. In G.A.R. Melo& I. Alves-dos-Santos (Eds.), Apoidea Neotropica: Homenagem aos 90 Anos de Jesus Santiago Moure (pp. 265–278).

Criciúma: Editora UNESC.

Rose, A.L., Gardner, C.A., McConnell, J.D. & Bull, L.B. (1957). Field and experimental investigation of "walkabout" disease of horses (Kimberley horse disease) in northern Australia: Orota-Laria Poisoning in horses. Part II. Aust. Vet. J., 33(3): 49–62. doi: 10.1111/j.1751-0813.1957. tb08270.x

Rother, C.D., Souza, F.T., Malaspina, O., Bueno, C.O., Silva, F.G.F.M., Vieira, C.P. & Fernandes, J.B.(2009). Suscetibilidade de operárias e larvas de abelhas sociais em relação à ricinina. Iheringia, 9(1): 61–65. doi: 10.1590/S0073-47212009000100009

Santoro, K.R., Vieira, M.E.Q., Queiroz M.L., Queiroz M.C. & Barbosa S.B.P. (2004). Efeito do tanino de *Stryphnodendron* spp.sobre a longevidade de abelhas *Apis mellifera* L. (abelhas africanizadas). Arch. Zootec., 53: 281–291.

Silva-Neto, J.P., Barreto, R.A., Pitanga, B.P.S., Souza, C.S., Silva, V.D., Silva, A.R., Velozo, E.S., Cunha, S.D., Batatinha, M.J.M, Tardy, M., Ribeiro, C.S.O., Costa, M.F.D., El-Bachá, R.S. & Costa, S.L. (2010). Genotoxicity and morphological changes induced by the alkaloid monocrotaline, extracted from *Crotalaria retusa*, in a model of glial cells. Toxicon, 55 (1): 105–117. doi: 10.1016/j. toxicon.2009.07.007.

