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NOTA CORTA / SHORT NOTE

SEQUESTRATION OF TROPANE ALKALOIDS FROM *Brugmansia suaveolens* (SOLANACEAE): NEW RECORDS FOR ORTHOPTERA AND COLEOPTERA

Secuestro de alcaloides tropánicos de *Brugmansia suaveolens* (Solanaceae): nuevos registros en Orthoptera y Coleoptera

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ABSTRACT

Tropane alkaloids (TAs) are the main secondary metabolites found in Datureae (Solanaceae). These compounds are neurotoxic to many organisms. However, some insect species can sequester and accumulate TAs in their bodies. *Brugmansia suaveolens* (Humb. and Bonpl. ex Willd.) Bercht. and J.Presl, a Neotropical shrub, is known to produce TAs. In this study, we report the sequestration of TAs from *B. suaveolens* by *Chromacris speciosa* (Thunberg, 1824) (Orthoptera: Rhomaleidae) and *Lema daturaphila* Kogan & Goeden (Coleoptera: Chrysomelidae). Both of these insects have been observed feeding on leaves from various species of Solanaceae in the study site. We analyzed extracts from insects and the host plant using gas chromatography and mass spectrometry (GC-MS). In *B. suaveolens*, we identified five TAs, while in the insect bodies, we found four. Scopolamine was the most abundant alkaloid in all the samples. This is the first report of TAs in these insects. While TAS may play a role in defense against generalist herbivores, further investigation is needed to understand the association between TAs and predators since both insects exhibit moderately aposematic features.

Keywords: atropine, herbivory, scopolamine, secondary metabolites

RESUMEN

Alcaloides tropánicos (ATs) son los principales metabolitos secudarios de plantas de la tribu Datureae (Solanaceae). Esos compuestos son neurotóxicos para insectos, sin embargo algunas espécies pueden secuestrar y acumular ATs en sus cuerpos. *Brugmansia suaveolens* (Humb. and Bonpl. ex Willd.) Bercht. and J.Presl es un arbusto neotropical que produce ATs. En este trabajo reportamos el secuestro de ATs de *B. suaveolens* por *Chromacris speciosa* (Thunberg, 1824) (Orthoptera: Rhomaleidae) y *Lema daturaphila* Kogan & Goeden (Coleoptera: Chrysomelidae). Extractos de los insectos y de la planta huesped fueron analizados por CG-MS. Fueron identificados cinco alcaloides en *B. suaveolens* y cuatro en los insectos, siendo escopolamina el alcaloide más abundante. Esta es la primera vez que estos insectos son registrados atacando *B. suaveolens*. El papel de los ATs en insectos puede estar relacionado a la defensa contra herbívoros generalistas, sin embargo, la asociación entre TAs y depredadores necesita ser investigada, ya que ambos insectos son moderadamente aposemáticos.

Palabras clave: atropina, escopolamina, herbivoría, metabolitos secundarios

Tropane alkaloids (TAs) constitute the main secondary metabolites in six tribes of Solanaceae: Datureae, Solandreae, Solaneae, Mandragoreae, Hyoscyaneae, and Physaleae. Scopolamine and atropine are the most distinctive TAs, and they are implicated in the resistance against various herbivore insects (Griffin-W and Lin-G 2000; Wink-M 2003).

Brugmansia suaveolens (Humb. & Bonpl. Ex Willd.) Bercht. & Presl. (Solanaceae: Datureae) is a Neotropical shrub widely distributed around the world as an ornamental plant. The leaves and flowers of *B. suaveolens* contain several secondary metabolites (Petricevich-V et al. 2020), with TAs being the most abundant (Freitas-A et al., 1996). In Southeastern Brazil, the leaves of *B. suaveolens* are primarily consumed by the larvae of the TA specialist *Placidina euryanassa* (Felder & Felder, 1860) (Lepidoptera: Nymphalidae). While TAs are effective against generalist herbivores, *P. euryanassa* incorporates ingested TAs into their cuticles (Freitas-A et al. 1996). Nonetheless, there is limited research on the interaction between TAs and other insects.

At the Atlantic Forest Reserve of the Serra do Japi, located in the Jundiaí Municipality, São Paulo State, Brazil (23°13'S, 46°58'W), leaves of *B. suaveolens* serve as a food source for various insects. These include, *P. euryanassa*, the grasshopper *Chromacris speciosa* (Thunberg, 1824) (Orthoptera: Rhomaleidae) as well as both the larvae and adults of the leaf-beetle *Lema daturaphila* Kogan & Goeden (Coleoptera: Chrysomelidae). Insects were collected and kept in plastic boxes and supplied with fresh *B. suaveolens* leaves under controlled conditions (25° C; 60 % relative humidity). Before chemical analysis, insects were kept starved for 24 h in order to eliminate TAs contained in the food and feces. Rooted cuttings of 10 *B. suaveolens* genotypes were grown in individual 5 L pots filled with soil and kept in a greenhouse under natural temperature and photoperiod conditions. TAs from *B. suaveolens* and insect samples were extracted in EtOH. The solution was acidified with 1N H_2SO_4 and extracted three times with CH_2Cl. The aqueous solution was reduced with Zn dust for 3 h, alkalinized with NH_4OH and eluted ten times with 5 ml CH_2Cl. The residue represented the total alkaloids as free bases. TAs were identified by gas chromatography coupled with mass spectrometry (GC-MS). Before both chemical analyses, samples were derivatized with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (Acros Organics™) to prevent the alkaloid pyrolysis. TAs were quantified by gas chromatography coupled with flame ionization detection (GC-FID) using sparteine (Acros Organics™) as standard.

In this research, we report two new records of insects sequestering TAs from *B. suaveolens*: the grasshopper *C. speciosa* and the larvae of the leaf-beetle *L. daturaphila*. Both insect species are known to feed on the leaves of *B. suaveolens* and other solanaceous plants (Kogan-M and Goeden-R 1970; Turk-S and Barrera-M 1977; Schmitt 1988) (Fig. 1a). *B. suaveolens* contain five TAs being scopolamine the most abundant (Fig. 1c) and (Table 1). We identified four TAs in the extracts of *C. speciosa* and larvae of *L. daturaphila*, indicating these insects sequester these alkaloids (Table 1). Total concentration of TAs was 16.34 ± 13.20 mg/g dry weight for *B. suaveolens*, 11.54 ± 18.21 mg/g dry for *L. daturaphila* larvae and 16.42 ± 12.06 mg/g dry for *C. speciosa*. We did not detect TAs in *L. daturaphila* adults.

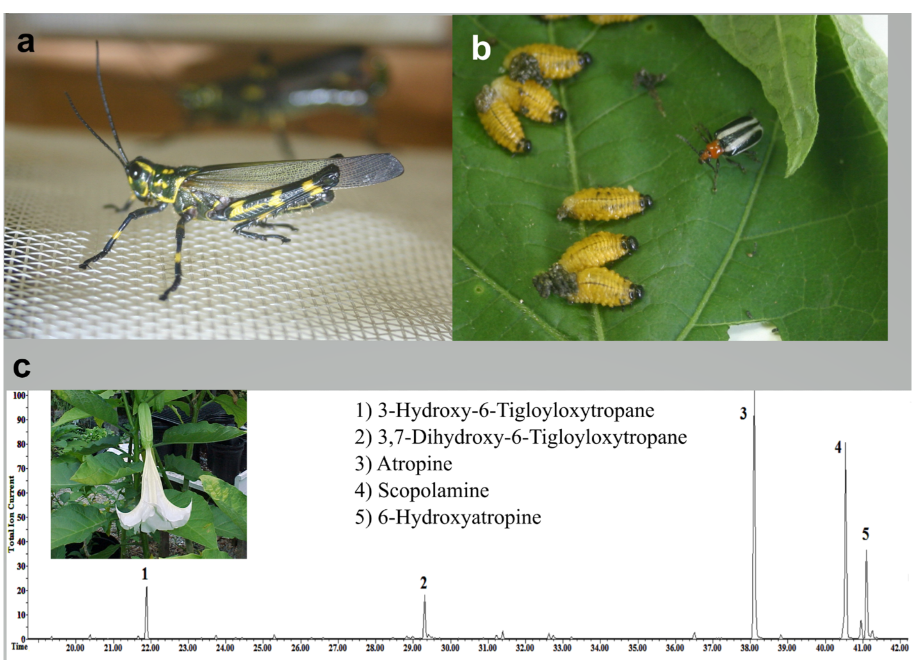

Figure 1. a) Detail of an adult of *Chromacris speciosa*. b) Larvae and an adult of *Lema daturaphila*. c) GC chromatogram of the TAs profile of *Brugmansia suaveolens* leaves

Table 1. Tropane alkaloids (TAs) concentration (mg/g dry weight) in the bodies of *Lema daturaphila* (Coleoptera), *Chromacris speciosa* (Orhtoptera) and leaves of *Brugmansia suaveolens* (Solanaceae)

TAs Mass spectra m/z (% relative intensity)	**Mean concentration ± SD (% abundance)**		
	B. suaveolens	*L. daturaphila*	*C. speciosa*
3-Hydroxy-6-Tygloiloxytropane [M]+ 239 (12), 122 (12), 114 (11), **113 (100)**, 112 (17), 96 (39), 94 (33), 82 (14), 55 (14), 42 (13)	5.98 ± 6.32 (26.8)	1.36 ± 1.16 (8.3)	5.39 ± 3.01 (30.1)
3,7-Dihydroxy-6-Tigloiloxytropane [M]+ 255 (1), 156 (27), 155 (20), 138 (11), 127 (27), 114 (10), **113 (100)**, 112 (36), 110 (17), 96 (57), 95 (10), 94 (41), 86 (12), 84 (13), 83 (29)	1.56 ± 1.54 (3.9)	-	-
Scopolamine [M]+ 303 (34), 154 (50), 139 (13), 138 (100), 137 (26), 136 (71), 121 (19), 120 (22), 110 (21), 109 (16), 108 (94), 103 (41), 97 (42), 96 (15), **94 (100)**, 93 (14), 91 (31), 90 (14), 89 (10), 84 (5), 82 (21), 81 (30), 79 (18), 77 (28), 68 (18), 67 (14), 65 (11), 57 (11), 42 (44), 41 (15)	8.75 ± 6.10 (60.7)	3.92 ± 6.43 (34.8)	7.24 ± 6.43 (45.2)
Atropine [M]+ 289 (7), 125 (11), **124 (100)**, 94 (21), 83 (15), 82 (20), 67 (10)	0.73 ± 0.76 (4.4)	3.19 ± 5.38 (28.9)	2.04 ± 2.08 (14.1)
6-Hydroxyatropine [M]+ 305 (2), 140 (33), 96 (11), 95 (69), **94 (100)**, 91 (12)	0.89±0.84 (4.2)	3.07±5.23 (28.0)	1.75±1.27 (10.6)

Characteristic ions of mass spectra with parent peak in bold. TAs profile of *B. suaveolens* corresponds to one genotype. Only larvae of *L. daturaphila* contained TAs

This is the first report of *L. daturaphila* and *C. speciosa* sequestering TAs from *B. suaveolens*. While TAs are potentially neurotoxic to insects, specialist herbivores have developed tolerance to these compounds. Consequently, the selective advantage of TAs on plant fitness appears to be more significant when plants are attacked by generalist herbivores (Arab-A and Trigo-J 2011; Griffin-W and Lin-D 2000). In many insects, a substantial portion of accumulated toxic plant secondary metabolites is excreted. However, specialist insects have developed adaptations to sequester these compounds from their host plants and employ them as a defense against their own natural enemies (Ode-P 2006). Notably, the adults of *L. daturaphila* did not sequester TAs from the host plant. This discrepancy might be since immature insect stages are more vulnerable to predators than adults. Therefore, the higher accumulation of TAs in their bodies could provide an advantage during this developmental stage.

Other alkaloids sequestered by insects have been found to be effective against natural enemies (Bernays-E et al. 1977; Hartmann-T 1999). For instance, TAs sequestered by *P. euryanassa* have been observed to protect the larvae of this butterfly from avian predators (Arab, A, personal observation). Both the larvae of *L. daturaphila* and the adults of *C. speciosa* exhibit moderately aposematic coloration and behavior (Fig. 1a-1b), which could be linked to their unpalatability (Pasteels et al. 1988; Turk-S and Barrera-M 1977). Nevertheless, further research is needed to elucidate the specific defensive role of TAs in these insects.

AUTHOR'S PARTICIPATION

The authors contributed equally to field sampling, laboratory and greenhouse experiments, statistical analysis, and manuscript preparation.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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