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ENLARGING KNOWLEDGE ON CANTHARIDIN IN BLISTER BEETLES (Coleoptera: Meloidae)

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Introduction

Cantharidin (CA, Fig. 1) is a toxic terpenoid produced by blister beetles (Coleoptera, Meloidae). Meloids use to exudate it along with haemolymph (autohaemorraging) (Fig. 2) with a defensive purpose since it is highly venomous and vescicating (Fig. 3).

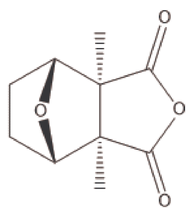
CA could also have a role in courtship and copulation, being transferred from males to females in order to protect the eggs from predators and fungal attacks.

Notwithstanding its toxicity, CA has been sold and used as a sexual stimulator (Fig. 4). Novel medical applications involve anti cancer agents and tattoo removal.

Despite its traditional use, scarce information are available on CA body content in meloid species,

methods are often outdated as well as the experiments run so far which are very ancient. Furthermore, exuded haemolymph has never been investigated for its CA content so far!

Chemical Formula: $C_{10}H_{12}O_4$



3a, 7a - dimethylhexahydro - 4,7 - epoxyisobenzofuran - 1,3 - dione

Fig. 1

Fig. 2

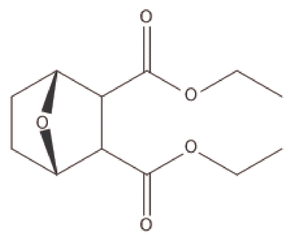
Fig. 3

Fig. 4

Fig. 5

Fig. 6

Chemical Formula: $C_{12}H_{18}O_5$



(1,4)-diethyl 7- oxabicyclo heptane - 2,3 - dicarboxylate

Fig. 7

Materials and methods

Samples were collected from May to November 2016 in 10 different Italian locations, transferred

Prof. Marco A. Bologna, and then reared for a maximum of 45-50 days in fauna boxes (Fig. 5). Exudated haemolymph from every individual was collected every two days using a capillary tube (Fig. 6) and then stored at -70 °C. Bodies as well were stored at -70 °C after death in order to avoid degradation.

Freeze-dried bodies were smashed and then

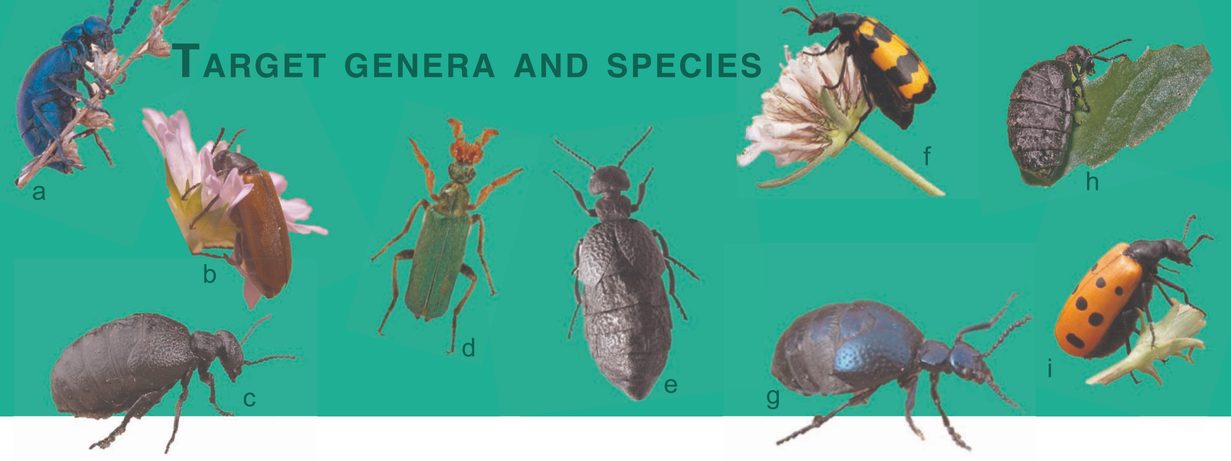
acetate for 48 hours in order to extract cantharidin from the tissues. The exudated haemolymph was treated exactly in the same way.

The solutions obtained, divided by sex and species, were injected in GC-MS using the diethylic ester of norcantharidin (Fig. 7) as an internal standard in order to quantify CA content both in the bodies and in the exudated haemolymph.

Goal of the research

The objective of this study was to quantify the CA content in the exuded hemolymph (to be compared with CA quantity in body tissues) of eight species never investigated so far, belonging to *Mylabris*, *Lydus*, *Meloe* and *Cerocoma* genera.

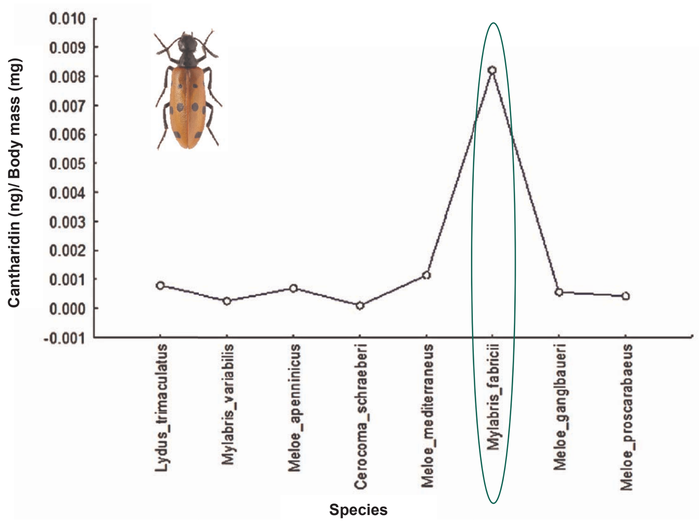
TARGET GENERA AND SPECIES



a. *Meloe proscarabaeus* Linnaeus, 1758
b. *Lydus trimaculatus* (Fabricius, 1775)
c. *Meloe apenninicus* Bologna, 1988
d. *Cerocoma schreberi* Fabricius, 1781
e. *Meloe mediterraneus* G. Müller, 1925
f. *Mylabris variabilis* (Pallas, 1781)
g. *Meloe autumnalis* Olivier, 1797
h. *Meloe ganglbaueri* Apfelbeck, 1905
i. *Mylabris fabricii* Sumakov, 1924

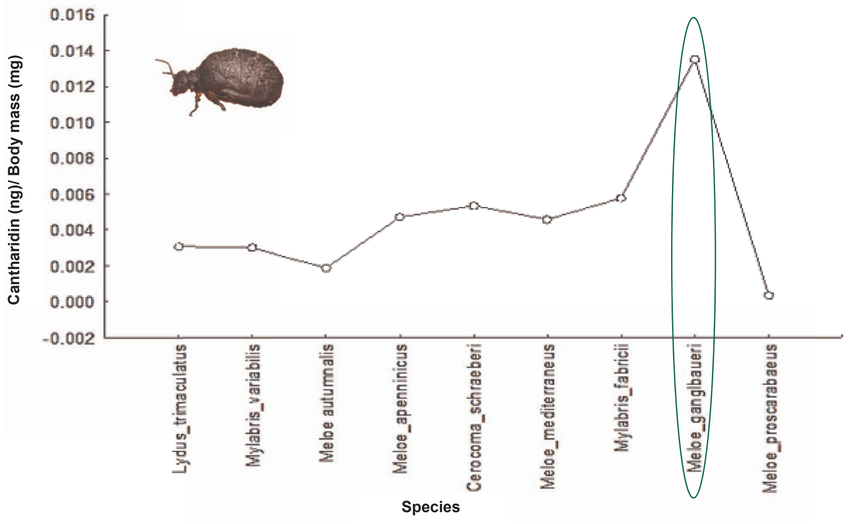
Results

The CA content in the exuded hemolymph (Tab. 1) is highly variable among meloid species, even among members of the same genus.



Tab. 1. Haemolymphatic CA content for the target species.

The CA content in body tissues among species (Tab. 2) is highly variable as well.

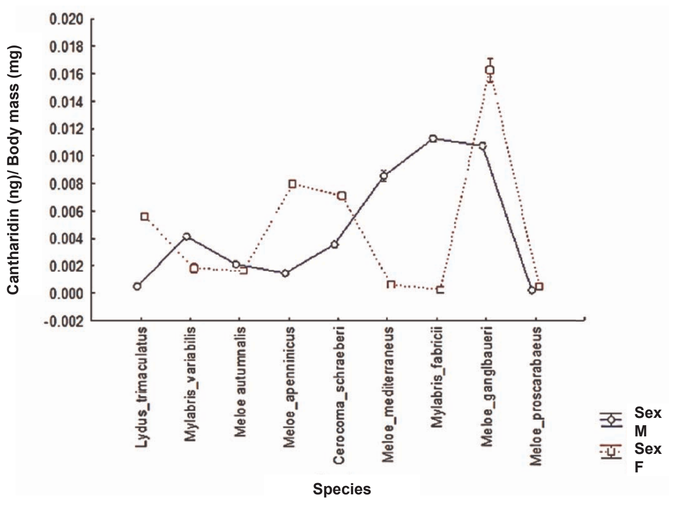


Tab. 2. Body CA content for the target species.

AND

Then, unexpectedly, sexes (Tab. 3) do not show

tissues.



Tab. 3. CA body content: a comparison between species and sexes.

Conclusions

CA content is highly variable among species (and between sexes) of blister beetles, but appears less concentrated in the exuded hemolymph than in the body: the latter probably acts as a massive reservoir for a prompt use in defense mechanisms.

Apart from the defensive role, novel approaches are required to better understand CA modulation and role in reproduction of blister beetles. Despite

understand, for istance, if the CA content varies during the adult stage due to different mating phases: pre-mating, post-mating and availability of partners.

Novel experimental activities are on the way to give an answer to a number of open questions on CA ecological role.

Males and females do not have statistically relevant differences: is CA really overproduced only by males?

Does it really have a role during courtship and copulation?

Is CA content so different between species within the same genera also considering more specimens?

Is the environment involved in the CA production level? And how?

Why is CA more diluited in the haemolymph?

To be continued …

References: Beuregard, 1890; Bologna & Pinto, 2002; Mehdinia et al., 2011; Nikbakhtzadeh et al., 2007