Toxicon 131 (2017) 16–19

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Feeding on toxic prey. The praying mantis (Mantodea) as predator of poisonous butterfly and moth (Lepidoptera) caterpillars

Dietrich Mebs*, Cora Wunder, Werner Pogoda, Stefan W. Toennes

Institute of Legal Medicine, University of Frankfurt, Kennedyallee 104, D-60956 Frankfurt, Germany

A R T I C L E I N F O

Article history:
Received 9 January 2017
Received in revised form
6 March 2017
Accepted 9 March 2017
Available online 11 March 2017

Keywords:
Mantids
Mantodea
Toxic caterpillars
Lepidoptera
Cardenolides
Atropine

A B S T R A C T

Caterpillars of the monarch butterfly, *Danaus plexippus*, feed on milkweed plants, *Asclepias* spp. (Apocynaceae), and sequester their toxic cardenolides aimed at deterring predators. Nevertheless, Chinese praying mantids, *Tenodera sinensis*, consume these caterpillars after removing the midgut ("gutting") including its plant content. In the present study, monarch caterpillars raised on *A. curassavica*, and those of the death's-head hawkmoth, *Acherontia atropos*, raised on *Atropa belladonna* containing atropine, were fed to mantids, *Hierodula membranacea*, which removed the gut of both species discarding about 59% of cardenolides and more than 90% of atropine, respectively. The ingestion of these compounds produced no apparent ill effects in the mantids and both were excreted with faeces. On the other hand, when mantids were fed with larvae of two moth species, *Amata mogadorensis* and *Brahmaea certia*, raised on non-poisonous host plants, the mantids showed the same gutting behaviour, thereby discarding indigestible plant material. As polar compounds, e.g. cardenolides and atropine, are not absorbed from the mantids midgut and do not pass the gut membrane, this enables the mantids to feed on toxic prey.

1. Introduction

Chemical defence strategies are widespread among insects (Laurent et al., 2005; Dettner, 2015). A wide array of natural products renders them noxious, unpalatable and even toxic to potential predators. Various taxa either synthesize these compounds or sequester them from external sources such as from their food plants. Consequently, phytophagous insects evolved physiological adaptations to tolerate and resist these often toxic plant products (Duffey, 1980; Opitz and Müller, 2009; Dobler et al., 2012; Petschenka and Agrawal, 2016; Erb and Robert, 2016).

Larvae of the monarch butterfly, *Danaus plexippus*, preferably feed on host plants of the genus *Asclepias* (Apocynaceae), which contain toxic cardenolides. These compounds are sequestered in their body providing effective defence against predators (Nishida, 2002; Agrawal et al., 2012). Despite that, assassin bugs (Zalucki and Kitching, 1982) and predatory wasps (Rayor, 2004; Rayor et al., 2007) prey upon monarch caterpillars.

Praying mantids are generalist predators and feed on a variety of prey including numerous invertebrate species and eventually overwhelm even small vertebrates (Reitze and Nentwig, 1991) such as a red-spotted newt, *Notophthalmus viridescens* (Mebs et al., 2016). These newts are known to contain tetrodotoxin, a specific blocker of voltage gated sodium channels (Narahashi et al., 1964; Kao, 1966). When this toxin was orally administered to four adult mantis species (*Tenodora sinensis, Sphodromantis viridis, Hierodula membranacea, Mantis caffra*), they survived even high doses up to 30 µg/g body mass without ill effects (Mebs et al., 2016). It was shown that the toxin passes the midgut unabsorbed and is gradually excreted with faeces enabling the mantis to feed on toxic prey without risking poisoning.

Rafter et al. (2013) observed late-instar Chinese mantids, *Tenodera chinensis*, capture and consume larvae of the monarch butterfly, *Danaus plexippus*, by chewing open the integument, removing ("gutting") the midgut filled with plant material, eating only the remains. About 40% of the prey biomass was discarded. In contrast, non-toxic larvae of the European corn-borer, *Ostrinia nubialis*, and of the greater wax-moth, *Galleria mellonella*, were wholly consumed by the mantids, suggesting that cardenolide-rich tissue of the monarch caterpillar is discarded by the gutting-behaviour to reduce exposure to the prey's toxicity. However, it was found that consumed as well as discarded tissue from the caterpillar had equal cardenolide concentrations. On the other hand, the behaviour of the mantids may also reflect their

* Corresponding author.
E-mail address: mebs@em.uni-frankfurt.de (D. Mebs).

http://dx.doi.org/10.1016/j.toxicon.2017.03.010

preference for high nitrogen-containing tissue (Rafter et al., 2013).

In the present study, praying mantids (*Hierodula membranacea*) were fed with caterpillars of butterflies and moths, such as *D. plexippus* larvae reared on cardenolide-rich *A. curassavica* plants, of the death's-head hawkmoth, *Acherontia atropos,* reared on atropine-rich deadly nightshade, *Atropa belladonna* (Solanaceae), of the tiger moth, *Amata mogadorensis*, reared on non-toxic dandelion, *Taraxacum officinale* (Asteraceae), and of the Sino-Korean owl moth, *Brahmaea certia*, reared on lilac, *Syringa vulgaris* (Oleaceae). These experiments were performed to find out whether plant toxins trigger the gutting behaviour of the mantids.

2. Materials and methods

2.1. Animal experiments

Adult specimens of the Indian praying mantis, *Hierodula membranacea,* were obtained from a private breeder. They were maintained individually in 10 l plastic containers at ambient temperature and were fed with semi-adult crickets (*Acheta domesticus*). After starving for three days, 10 mantids were offered on three consecutive days one fourth instar caterpillar of *Danaus plexippus* each (three in total), which had been reared on *A. curassavica*. Larvae were raised from eggs purchased from private butterfly breeders (Spain origin). Non-consumed material and faeces from each mantis were collected over a period of 8 days and placed in 80% methanol for cardenolide extraction. At the end of the experiment, the mantids were killed and extracted accordingly.

In another experiment, one third instar caterpillar each of *Acherontia atropos*, raised from eggs (obtained from private breeders) and reared on *Atropa belladonna,* was fed to four mantids. Likewise, discarded material, faeces and the body of the mantids were extracted for alkaloid analysis using 80% methanol.

In a series of behaviour studies, mantids (n = 5) were fed with caterpillars of *Amata mogadorensis* raised from eggs (obtained from private breeders), reared on *Taraxacum officinale*, and of *Brahmaea certhia* reared on *Syringa vulgaris* leaves.

2.2. Analytical procedures

For the analysis of cardenolides by liquid chromatography linked to time-of-flight mass spectrometry (LC/TOF-MS), the samples were evaporated to dryness at 25 °C in a stream of air and were dissolved in 100 μl acetonitrile:water (50:50, v/v) containing 0.1% formic acid. Of each sample, 1 μl were injected onto a Polaris-C18-Ether column (100 × 2.0 mm, 3 μm particle size) from Varian (Darmstadt, Germany) using the Agilent 1100 Series HPLC system. Gradient elution was performed using 0.1% formic acid solution containing 5 mM ammonium formate (A) and acetonitrile containing 0.1% formic acid (B) at a flow rate of 0.5 ml/min. The system was interfaced to an Agilent 1100 Series-TOF system (Waldbronn, Germany) operated in positive electrospray ionization mode (ESI) (Fig. 2). As no reference substances of the two major cardenolides, calotropin and calactin, are available for quantitative analysis, their concentrations were calculated based on values for digitoxin used as standard compound and are expressed as digitoxin equivalents.

Atropine and scopolamine in the methanolic extracts were analysed by LC/MS (1290 Infinity liquid chromatograph coupled to a G6460A Triple Quadrupol mass spectrometer via JetStream electrospray interface, Agilent Technologies, Waldbronn, Germany). With an injection volume of 1 μl chromatographic separation was achieved using a Kinetex XB-C18 column (100 × 2.1 mm, 2.6 μm 100 Å particles) from Phenomenex (Aschaffenburg, Germany) equipped with the corresponding guard column. Gradient

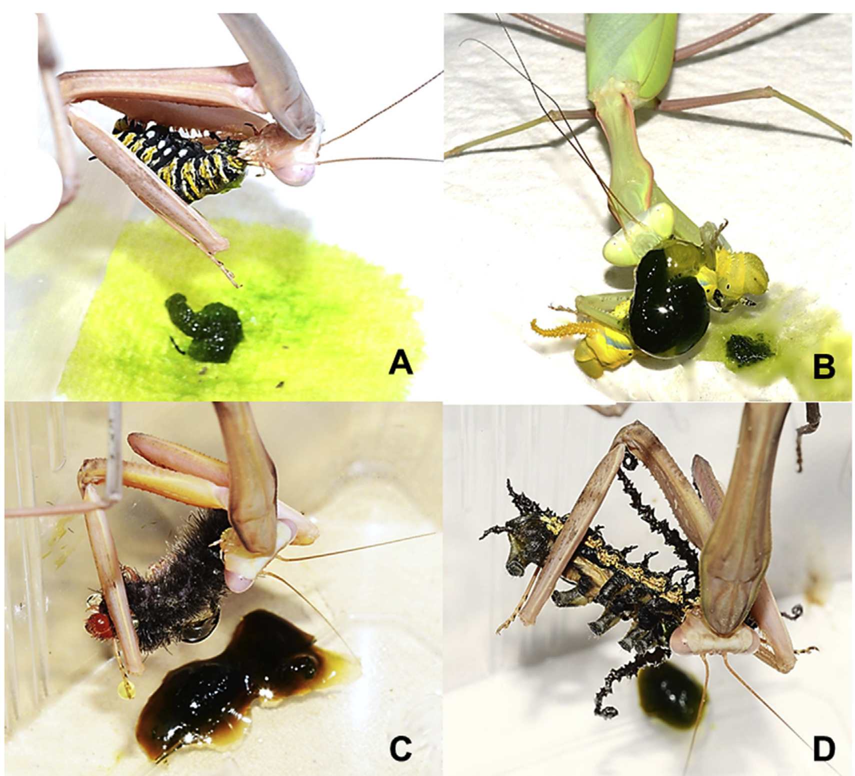

Fig. 1. Adult mantids (*Hierodula membranacea*) removing the midgut ("gutting") while feeding on (A) *Danaus plexippus,* (B) *Acherontia atropos,* (C) *Amata mogadorensis* and (D) *Brahmaea certhia* caterpillars showing the same behaviour independent of toxic (cardenolides, A, atropine, B) or non-toxic (C, D) prey.