Active Cyanogenesis – in Zygaenids and Other Lepidoptera

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The liberation of hydrocyanic acid by intact not damaged living insects is described as "active cyanogenesis". This phenomenon – as in contrast to "passive cyanogenesis" observed in plants, where enzyme and substrate get in contact only after tissue damage – is reported from the Zygaenidae. Quantitative data demonstrate that the Zygaenidae can increase the amount of emitted HCN upon irritation. The significance of this phenomenon as a mean of defense is discussed.

Introduction

As defined by Conn 1981 [1] the term cyanogenesis denotes the ability of living organisms to produce hydrocyanic acid. It is a common phenomenon in bacteria, fungi, algae, plants and animals [2]. In plants cyanogenesis occurs when the cyanogenic tissues are crushed and the respective cyanogenic compounds (glucosides, lipides or alkaloids) come into contact with the HCN-liberating enzymes. Thus, substrate and enzyme are restricted to certain compartments, usually in different tissue layers or in different cellular organelles. In animals few cases have been described of arthropods, certain Myriapoda [3] and a Chrysomelid species [4] actively liberating hydrocyanic acid. However, in Myriapoda [5, 6], α-hydroxynitrile-lyase is stored in the cells of a specialized reaction chamber as in plants [3, 7, 8]. Access of the cyanogenic nitrile to the reaction chamber is controlled through a specialized muscle attached to the connecting tube. The defensive secretion of the larvae of the chrysomelid *Paropsis atomaria* releases HCN [4]. The sources of cyanide are the unstable mandelonitrile and prunasin [9]. The release of HCN may be due to hydrolysis.

In the Lepidoptera, amongst a few other taxa, Zygaenid moths are well known for their cyanogenic properties [10]: release of hydrocyanic acid occurs when their tissues are crushed. The sources of cyanide are the cyanoglycosides linamarin and lotaustralin [11], which are stored in the haemolymph and in specialized cuticular cavities [12]. There are high hydrocyanic acid liberating enzyme-activities in the haemolymph of the Zygaenid moths [13] and they also have high detoxification capacities for hydrocyanic acid: cyanide is transferred into β-cyano-L-alanine, which seems to be widely distributed within the Lepidoptera [14].

Zygaena larvae produce a cyanogenic defensive secretion which is stored in regularily arranged cuticular cavities. The latter possess highly specialized opening mechanisms which are used to release the defensive secretion when the larvae are irritated or mechanically stimulated [12].

Materials and Methods

Living larvae of various Zygaenid species were obtained from their original habitat, as follows:

Pryeria sinica	Kyoto (Japan)
Orna subdiaphana	Kubusie Forest, Eastern Cape Province (South Africa)
Neurosymploca caffra auct.	Cape Town (South Africa)
Zygaena trifolii	Valencia (Spain)
Gymnogramma rufiventris	McLeodstown (South Africa)
Rhagades pruni	Bielefeld (Germany)

The larvae were kept on their natural foodplants.

We have tested the capacity of *Zygaena* moths and larvae to release hydrocyanic acid actively. The principle of HCN-determination involves the transfer of hydrocyanic acid liberated from the insects in a closed glass apparatus into a trap with 0.1 M NaOH.

* 53rd contribution to the study of the genus *Zygaena* F. and related taxa (Insecta, Lepidoptera) (52: Z. Paläontologie: submitted).

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In our experiments a Wissing apparatus [15] has been modified as follows (see Fig. 1):

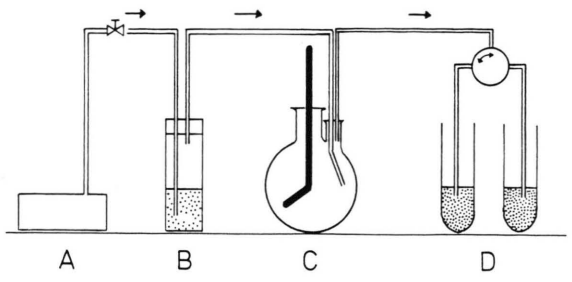

Fig. 1. Apparatus used for the detection of HCN released from living insects. A, air pump; B, washing flask; C, two-necked flask with gas inlet and outlet rod stick for irritating the insects; D, trap with 0.1 M NaOH.

A flow of air is produced by an air-pump (WISA 130) and regulated with a dosing valve to 20 ml/min. Greater rates of flow cause incorrect results because a part of the hydrocyanic acid is then transported too fast through the trap so that it cannot react with the hydroxide. In order to clean the gas-stream it is led through a washing-flask with 1 M NaOH from where it proceeds into a 100 ml two-necked flask. This flask contains up to ten insects (imagines or larvae). A rubber fitting with a gas inlet and outlet closes one neck. A septum with a thin rod (glass or metal) for mechanical irritation of the insects closes the other neck. After passing the two-necked flask, the air is transported through a three-way valve into the test tubes with 1 ml 0.1 M NaOH, where the cyanide is collected in defined intervals.

The total volume of the apparatus is about 150 ml. With a flow rate of 20 ml/min the total gas volume will be exchanged within approximately 10 min. The quantitative determination of the cyanide in the trap was done following the method described by Nahrstedt [16].

In each set of experiments 10 last instar larvae (or imagines) have been tested at one time in order to minimize technical problems. First, the potential of hydrocyanic acid issued by resting larvae has been recorded over an extended period of time (see Fig. 2). Next, the larvae were irritated by movement of the rod. This disturbance lasted for approximately one minute. The larvae were thus induced to exude their defensive secretion, but they had not been injured during the experiment, neither internally nor externally. The larvae were then allowed to settle down and the increase in the release of HCN was recorded for up to 200 min. The results are given cumulatively for three species of Zygaeninae in Fig. 2.

Results

As can be seen from the graph (Fig. 2) all three species of Zygaeninae tested have a low resting potential of hydrocyanic acid, which is practically identical in all three species. In contrast the concentration of HCN built up around disturbed larvae differs considerably from the resting potential and from

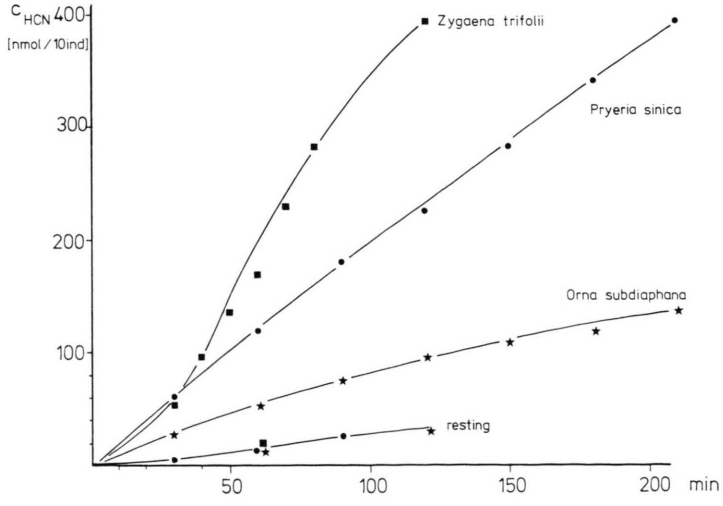

Fig. 2. HCN release of Zygaenini larvae ($n = 10$, last instar, cumulative plot). ■ *Zygaena trifolii*, ● *Pryeria sinica*, ★ *Orna subdiaphana*.

species to species. The release of HCN takes place very quickly and the increase of HCN can be observed immediately. In the graph the increase in HCN output appears retarded because of the volume and the slowness of the gas exchange in the apparatus. High amounts of HCN are released over a period of 2–3 h.

The amount of HCN is highest in *Zygaena trifolii* (28 nmol/ind. h), a species in which both the larva and imago have characteristic aposematic patterns. The situation is similar in *Pryeria sinica* (12 nmol/ind. h) in which the larvae, at least, are aposematic. The lowest level was observed in *Orna subdiaphana* (4.7 nmol/ind. h) in which the larvae have a cryptic pattern. Velocity and amount of HCN release may indicate that the gas emission is due to enzymatic liberation.

In addition to those discussed above, some other species of Zygaeninae were also tested for HCN-release. A time-dependent release could not be recorded because of the small amounts of HCN of the larvae tested (*N. caffra* auct., *G. rufiventris*) or because of the high activity of the imagines, which prevented a controlled release. Therefore the maximum amounts of HCN released are recorded in Table I, including the data represented in Fig. 2.

Table I. Release of maximum amounts of hydrocyanic acid (HCN) by some Zygaenoidea.

Zygaena trifolii	larva 28 nmol/ind. h imago 10 nmol/ind. h.
Neurosymploca caffra auct.	larva 1.5 nmol/ind. h
Orna subdiaphana	larva 4.7 nmol/ind. h imago not detected
Pryeria sinica	larva 12 nmol/ind. h imago not detected
Rhagades pruni	imago 0.5 nmol/ind. h
Gymnogramma rufiventris	larva 1.3 nmol/ind. h

Discussion

Our data demonstrate that an actively increased release of HCN occurs in all Zygaenid species tested, at least in the larval instars. All these species are known to be cyanogenic when their tissues are crushed [14]. Also the larva of the South African Yponomeutid moth *Gymnogramma rufiventris* has not only cyanogenic properties but also actively releases HCN. This species belongs to a group of genera which show close relations to the Zygaenoidea and should be excluded from the Yponomeutidae [17].

The biological significance of this specialized form of cyanogenesis is still obscure. It is not connected to tissue damage or to neuronally controlled dissolving of a separate localization of the enzyme and substrate as in the milliped *Harpaphe*. The substrate and high enzyme activities are localized in the haemolymph. A sudden release of HCN might bear some important information to potential predators, like carabid or staphylinid beetles or to certain parasitoids, although it is still unknown whether insects have receptor cells for hydrocyanic acid. With respect to the extended release of HCN observed in the larvae, two preconditions should be fulfilled if it is to be regarded as part of the insects defensive system: firstly the predator should possess specialized HCN-detecting receptors, and secondly such a system would only be operational as long as the HCN field around a larva is not dispersed or diluted by air movement. The latter condition might be true in the natural habitat of *Zygaena* and *Pryeria* larvae, which prefer lower parts of the herbaceous vegetation, where air movement may be considered low.

The release of hydrogen cyanide in response to external stimulation is here described as "active cyanogenesis" when the tissues are not crushed and the emission is regulated by the insect. This is in contrast to "passive cyanogenesis" which is characteristic for plants and possibly other arthropods. Active cyanogenesis may represent a derived character of the Zygaenidae but we suppose that it also occurs in the Nymphalidae (*Heliconius* and *Acraea*), where it must have evolved independently.

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