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Influence of Previous Feeding Regimes and Ambient Temperatures on Degradation and Storage of Pyrrolizidine Alkaloids in the Moth Species Creatonotos transiens (Lepidoptera: Arctiidae)

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VON NICKISCH-ROSENEGK E & WINK M [Inst Pharm Biol, Univ, D-69210 Heidelberg]:**Influence of Previous Feeding Regimes and Ambient Temperatures on Degradation and Storage of Pyrrolizidine Alkaloids in the Moth Species Creatonotos transiens (Lepidoptera: Arctiidae).**- Entomol Gener 19(3): 157-170; Stuttgart 1995. --- [Article].

Larvae of the arctiid moth species *Creatonotos transiens* (Walker 1855) take up dietary pyrrolizidine alkaloids (PA) and store them predominantly in their integument. A substantial part of the ingested alkaloids is not stored but degraded into non-alkaloidal compounds which are excreted with the faeces. If larvae had previous feeding experience with dietary alkaloids, their capacity to store PA in the last larval stage (L_7) is markedly reduced. After eclosion, part of the stored alkaloids is redistributed: In MM, PA are either transferred to the spermatophore (PA serve as a "nuptial gift") or are metabolized into the pheromone 7R-hydroxydanaidal. In FF, PA are translocated into the ovaries and subsequently to the eggs, where they could function as chemical defence compounds. Transfer rates and translocation were optimal at ambient T of 21° C, but were reduced at 4° C or 30° C. Thus, nutritional conditioning and ambient T in addition to varying PA supplies by food plants influence PA storage and PA utilization in larvae and imagines of *C. transiens*.

Keywords: *Creatonotos transiens* (Walker 1855) - chemical defence - alkaloid degradation - alkaloid storage - integument - pheromone.

VON NICKISCH-ROSENEGK E & WINK M [Inst Pharm Biol, Univ, D-6900 Heidelberg]: **Einfluß der Ernährungsvorgeschichte und der Umgebungstemperatur auf Metabolisierungsraten und Speicherungsraten von Pyrrolizidin-Alkaloiden bei der Bärenspinner-Art Creatonotos transiens (Lepidoptera: Arctiidae).**- Entomol Gener 19(3): 157-170; Stuttgart 1995. --- [Abhandlung]

Larven des Bärenspinners *Creatonotos transiens* (Walker 1855) speichern die Pyrrolizidin-Alkaloide ihrer Futterpflanzen und deponieren sie zum größten Teil im Integument. Ein beträchtlicher Teil applizierter Alkaloide wird jedoch metabolisiert und mit den Exkrementen als Abbauprodukte ausgeschieden. Hatten die Larven bereits vor einer gezielten Fütterung im letz-

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ten Larvalstadium (L_7) Pyrrolizidin-Alkaloide in ihrer Nahrung kennengelernt, reduziert dies die Aufnahme- und Speicherrate. Nach dem Schlupf der Falter werden die gespeicherten Alkaloide umverteilt: Bei **MM** werden sie in der Spermatophore angereichert und so an die Nachkommenschaft (Fraßschutz) weitergegeben oder zum Pheromon (7R-Hydroxydanaidal) metabolisiert. **FF** transportieren Alkaloide aus dem Integument in die Ovarien und Eier. Die Transportraten und die Umverteilung verlaufen optimal bei T von 21° C, bei 4° C und 30° C sind sie herabgesetzt. Demnach hängen Alkaloid-Speicherung und -Metabolisierung auch von der Ernährungsvorgeschichte und der Umgebungstemperatur und nicht nur vom absoluten Anteil der Pyrrolizidin-Alkaloide in der Diät ab.

1 Introduction

Several insects are known which utilize the defence chemicals of their host plants [HARBORNE 1988]. Mainly, the dietary allelochemicals serve as acquired chemical protectants. In few instances even more elaborate functions have been detected recently. In the arctiid moth, *Creatonotos transiens* (Walker 1855), dietary pyrrolizidine alkaloids (PA) are resorbed by aid of an intestinal carrier-mediated process [WINK & SCHNEIDER 1988]. Following transfer to the haemolymph, PA are stored in the larval integument, where they remain throughout the following life stages. Their main function is understood as chemical defence against predators [EGELHAAF et al 1989, WINK et al 1990, VON NICKISCH-ROSENEGK et al 1990].

In **FF**, part of the stored PA is transferred to the ovaries and subsequently to the eggs which then gain chemical protection. Also the **MM** can contribute to the chemical fitness of the clutch, in that PA are transferred through the spermatophore as a nuptial gift [VON NICKISCH-ROSENEGK et al 1990], similar to the situation in *Utetheisa* moths [DUSSOURD et al 1988, CONNER et al 1981]. In **MM**, PA have additional functions: PA serve as chemical precursor for the pheromone hydroxydanaidal [BELL , MEINWALD 1986, BOPPRÉ & SCHNEIDER 1985, WINK et al 1988]. PA also serve as a morphogen for the development of the abdominal coremata, which dissipate the PA-derived pheromone [SCHNEIDER et al 1982, BOPPRÉ & SCHNEIDER 1985, SCHMITZ et al 1989, EGELHAAF et al 1992].

The size of the coremata directly corresponds to the amount of PA ingested during the larval stages [BOPPRÉ & SCHNEIDER 1985]. It is assumed that **FF** select their mates according to their pheromone content [WUNDERER et al 1986]. Since pheromone content and alkaloid storage are correlated [BOPPRÉ & SCHNEIDER 1985, VON NICKISCH-ROSENEGK et al 1990], it is thus likely, that a **F** which selects an alkaloid-rich mate, contributes to the fitness of its offspring, since it gains additional chemical protection via the **M** spermatophore [VON NICKISCH-ROSENEGK et al 1990].

Accordingly, the capacity to accumulate maximal amounts of PA should be of prior importance for both **MM** and **FF** of the moth species *Creatonotus transiens*. In this communication, a number of factors are to be analyzed which might contribute to the storage of PA by larvae and imagines, such as food selection, degradation of PA, influence of previous dietary experience and ambient temperature.

2 Materials and Methods

2.1 Growing conditions, feeding procedures

Imagines from the Philippines were used for starting a laboratory culture of *Creatonotos transiens* (Walker 1855). (Animals from the Philippines have also been described as *Creatonotos wilemani* Rothschild 1933). Only for the experiments described in Fig 1 E-H, animals from

Sumatra were used (3-5 animals per mean value). Larvae were reared on a semiartificial diet [BERGOMAZ & BOPPRÉ 1986] without preservatives (except sorbic acid) and antibiotics, to avoid a possible interference with digestion processes. L_5-L_7 instar larvae were kept singly in Petri dishes and supplied in L_7 with a defined amounts of 7S-heliotrine (Chemasea, Manuf. PTY Ltd., Sidney, Australia; alkaloid solution adjusted to pH 7.0) or senecionine (kindly provided by R. Molyneux, USDA Albany) on leaf discs (*Senecio jacobaea*, 1.0 cm x 1.0 cm) or pieces of diet (0.5 cm x 0.5 cm). 48 h after consumption of the alkaloid animals were dissected in gut (including gut content), haemolymph, fatbody and integument. Faeces were collected in time intervals of 6 h. Samples were frozen immediately and stored at -20 °C until alkaloid extraction.

Radiolabelled ^{14}C-senecionine was obtained by feeding ^{14}C-1,4-putrescine (NEN, Dreieich) to root cultures of *Senecio vulgaris* according to HARTMANN et al [1988]. Root cultures were established from seedlings in RS-Medium [UFFELIE 1951]. After feeding labelled senecionine (specific radioactivity 2 mCi/mmol) to the larvae, they were dissected, tissues were ground in a suitable amount of 0.5 M HCl. 200 ml aliquots of these homogenates were transfered into 5 ml scintillation cocktail and their radioactivity was counted in a liquid scintillation counter (Rackbeta LSC, LKB).

2.2 Alkaloid extraction and gas-liquid chromatography (GLC)

Techniques were carried out as described in VON NICKISCH-ROSENEGK et al [1990]. Animals/tissues were ground in a mortar in 5-10 ml 0.5 M HCl. Zink powder was added to the homogenates to reduce PA-N-oxides, which were left standing for at least 5 h at room T, occasionally mixed by shaking. Samples were made alkaline with 4 M NaOH and poured onto Chem elut columns (Analytichem, ICT, Frankfurt) for solid phase extraction with CH_2Cl_2 as an eluent. The eluate was evaporated *in vacuo*. Crude pyrrolizidine extracts thus obtained were taken up in MeOH and analyzed by capillary GLC. The instrument used was a Varian 3300 instrument equipped with a nitrogen specific detector. Column: DB1, 30 m x 0.3 mm, film thickness 1 mm. GLC conditions: Oven: 150-300° C with 20° C/min, then 5 min isothermal. Injector: 250° C, split injection 1 : 20; detector: 300° C; carrier gas: helium, 90 kPa; make up gas: nitrogen. 7S-heliotrine or senecionine were used as external standards for quantification (Spectra Physics Integrator SP 4270). 7S-heliotrine and its metabolite 7R-heliotrine have been identified in previous studies [WINK et al 1988, EGELHAAF et al 1989] by GLC-mass spectrometry.

3 Results

3.1 Selection of PA-rich food

It has been reported that larvae actively search for PA-containing food and ingest even filter-paper that was soaked with PA [BOPPRÉ 1990]. Here, these findings have been confirmed in a number of choice experiments, in which larvae had the choice between a PA-free diet and diets with different amount of heliotrine or senecionine. As can be seen from Fig 1A-D, PA-rich diet is clearly preferred over PA-free diet, even in low concentrations. Larvae were also able to select the diet with the highest amount of alkaloid, when given the choice between 2 different concentrations (Fig 1E-H).

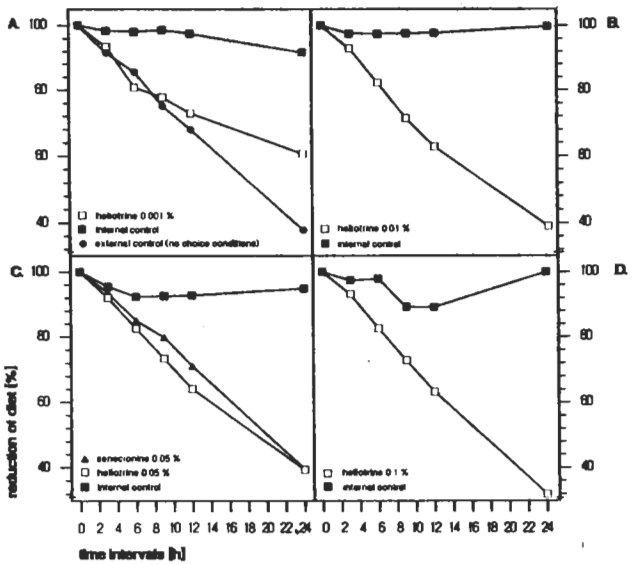


Fig 1: A-D Time dependent food selection of PA-free and PA-containing diet in choice-experiments with larvae (L_7) of *Creatonotos transiens* (Walker 1855) [Lepidoptera: Arctiidae]. Larvae (animals from the Philippines) were placed singly in Petri dishes, containing 4 pieces of diet (0.7 cm x 0.7 cm). 2 pieces were free of alkaloid (=internal control), the others contained the alkaloid (heliotrine/senecionine) in different amounts: **A** 0.001 % (= 10 µg/g diet) and as an external control the uptake of PA-free diet under normal conditions without choice conditions, **B** 0.01 % (= 100 µg/g diet), **C** 0.05 % (0.5 mg/g diet), **D** 0.1 % (= 1 mg/g diet). At the time intervals given the remaining pieces of diet were weighed; these values (data points = means of 5 animals) were corrected by the loss of water in the diet.

3.2 Detoxification of PA

Previous experiments showed that a proportion of 30-50 % of the ingested pyrrolizidine alkaloids could not be recovered either from the body tissues of *Creatonotos* or the faeces of the larvae [VON NICKISCH-ROSENEGK et al 1990]. It was suggested that the missing alkaloids had been degraded to non-alkaloidal compounds, which escaped the evaluation procedure. The missing alkaloids were considered to be degraded. In order to confirm this assumption we fed ^{14}C-senecionine (in which the necinbase was labelled) to the larvae and followed its fate in detail [cf HARTMANN et al 1990].

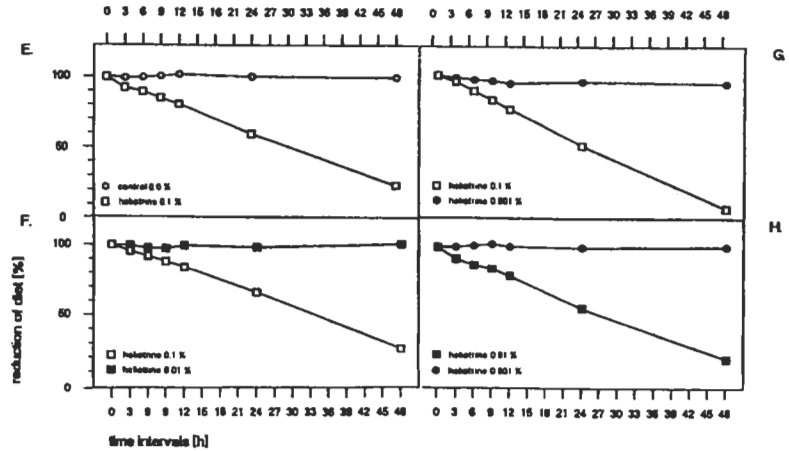


Fig 1: E-H Time dependent food selection of PA-free and PA-containing diet (different amounts of alkaloid) in choice-experiments with larvae (L_7) of *Creatonotos transiens* (Walker 1855) [Lepidoptera: Arcti-idae]. Larvae (animals from Suma-tra) were placed singly in Petri dishes, containing 4 pieces of diet (0.7 cm x 0.7 cm). **E** 2 pieces were free of alkaloid, the others contained 0.1 % of heliotrine, **F, G** and **H** different concentrations (2 pieces of diet per concentration) of heliotrine (0.001 %, 0.01 % and 0.1 %) in 3 different combinations. At the time intervals given the remaining pieces of diet were weighed; all values (datapoints = means of 5 animals) were corrected by the loss of water in the diet.

M and **F** Lv obtained 2 mg of pure senecionine (Fig 2A, 2B upper half; experiment A.), others were fed with 10,000 cpm ^{14}C-senecionine (Fig. 2A, 2B lower half, white columns = experiment B.). As a third variation, larvae were fed with the same amount of radiolabelled senecionine plus 2 mg of the unlabelled substance in order to reduce its specific activity (Fig 2A, 2B lower half, black colums = experiment C.). 48 h after ingestion of the samples larvae were dissected and the amount of alkaloids or radioactivity per tissue were determined in A. by capillary GLC and in experiment B. and C. by liquid scintillation counting. In experiment A. the major part of senecionine was found in the integument as reported previously [VON NICKISCH-ROSENEGK et al 1990]. The proportion of alkaloids which could not be recovered was up 30 % in **F'**Lv and about 48 % in **M'**Lv. The faeces which were collected during the 48 h duration of the experiments contained <10 % of PA. All other tissues contained only trace amounts of alkaloids.

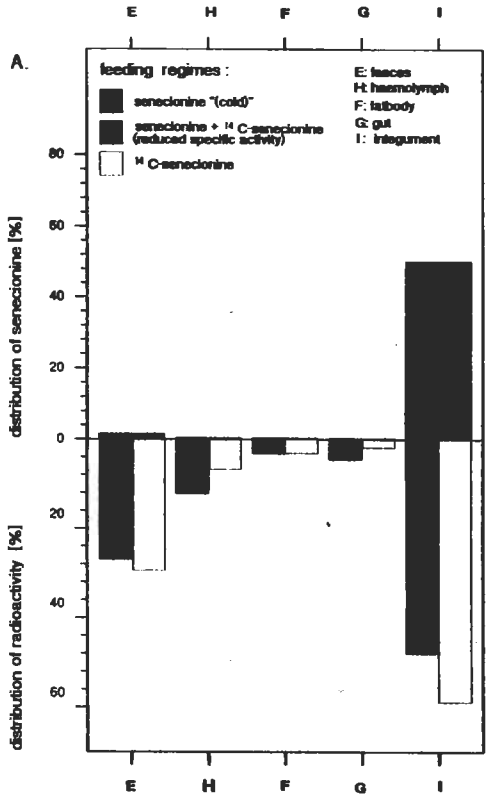
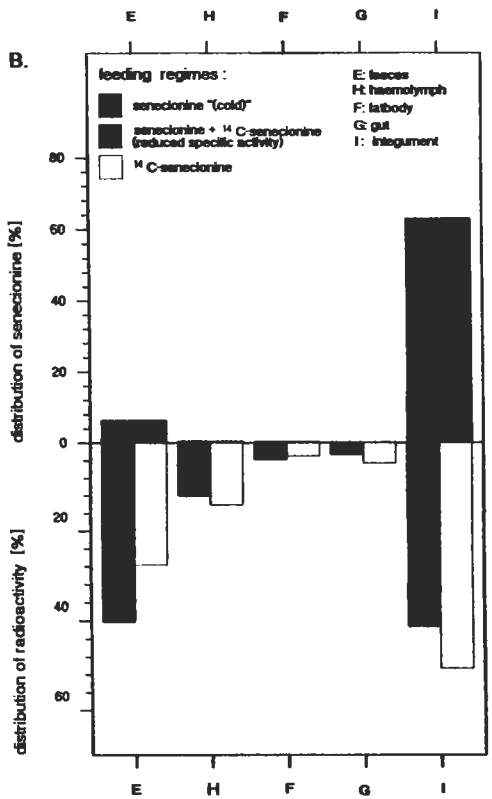


Fig 2: Illustration of the PA metabolization in larvae of *Creato-notos transiens* (Walker 1855) in L_7-stage (**A M'** larvae (left side), **B F'** larvae (right side); n = 3). In the upper half of the graph the distribution per tissue of unlabelled senecionine between faeces and the different larval tissues is shown. The alkaloid dosis fed (= 2 mg) was set 100 %, to evaluate metabolic degradation of senecionine. The alkaloid was quantified by GLC. The lower half of the graph shows the distribu-tion of radioactivity after feeding of ^{14}C-senecionine (10,000 cpm) plus 2 mg of unlabelled senecionine, to reduce specific radioactivity (black columns), measured by liquid scintillation counting. The white columns give the distribution of labelled senecionine without added "cold" senecionine. The comparison of the distribution of labelled and unlabelled compounds show, that at least in the faeces and to a minor degree in other tissues, senecionine must have been degraded into non-alkaloidal compounds (not detectable-by GLC).

The experiments with radioactive labelled senecionine help to explain the metabolism of PA by *C. transiens* larvae. Almost the total radioactivity that was applied could be recovered. Similar to the results of experiment A, a major part of radioactivity was detected in the integument (experiment B. and C.; B: 60 % **MM**; 50 % **FF**. C: 48 % **MM**, 42 % **FF**) and represents the intact senecionine.



However, in contrast to experiment A, radioactivity was high in the faeces, which was 28-32 % for **M'** and 40-30 % for **F'** Lv. The radioactivity of the remaining tissue fractions was below 10 %. Because only a minor % of PA were detected in the larval faeces in experiment A, the high radioactivity of the faeces determined in experiments B or C must be due to non-alkaloidal degradation products of PA. A similar conclusion was reached for the other tissues examined (haemolymph, gut, fatbody). These results imply that part of the PAs ingested are metabolized by the larvae to products, which could not be detected by GLC as PA any longer and must be non-alkaloidal metabolites.

3.3 PA degradation in relation to previous feeding experience

In a second set of experiments, the influence of previous PA feeding experience on PA-degradation by *C. transiens* larvae was determined. When larvae were reared on PA-rich food-plants, such as *Senecio jacobaea*, the application of 2 mg heliotrine to L_7 larvae resulted in a much higher degradation of heliotrine into non-alkaloidal compounds as compared to larvae, which were reared on a PA-free artificial diet (Fig 3).

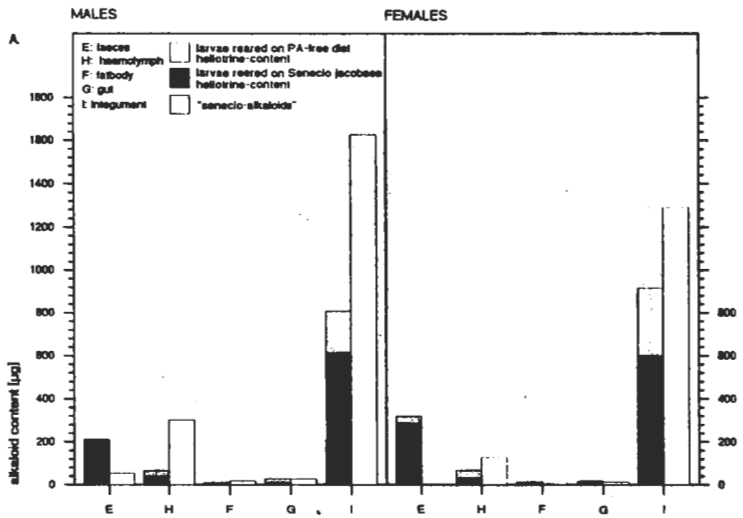


Fig 3: Influence of previous alkaloid experience on the alkaloid storage rate in larvae of *Creatonotos transiens* (Walker 1855). Larvae (**A MM; B FF**; n = 3) were kept on semi-artificial PA-free diet or on leaves of *Senecio jacobaea* through all larval stages. In L_7 they were fed with 2 mg of heliotrine which was consumed within 15 min; larvae were dissected 48 h later. White columns represent the storage rate of animals which were reared on artificial diet from L_1- L_7, black columns mark the amount of heliotrine in larvae kept on *Senecio* plant material. Dotted areas indicate the amount of Senecio-alkaloids obtained from the foodplant.

In order to evaluate the quantitative influence of previous PA-ingestion defined amounts of the pyrrolizidine alkaloid heliotrine were added to the semiartificial diet in concentrations of 0.001 %, 0.01 % and 0.1 % (fresh weight). These values correspond to the amounts of alkaloids which were found in different PA-plants of about 0.1 to 0.2 % of dryweight [DANNINGER et al 1983], or i e 0.005-0.02 % fresh weight in annual species of *Senecio* [HARTMANN & ZIMMER 1986].

The alkaloid content of the adult moth which had obtained heliotrine from L_4-L_7 or from L_4-L_7 was determined by GLC. In both groups, only trace amounts of alkaloid were discovered in the body of both sexes in the 0.001 % feeding regime. When offering 0.01 % heliotrine throughout all larval stages, about 15 µg of alkaloid were detected in both sexes. In contrast, 50 µg (**M**) or 90 µg (**F**) were detected in animals which obtained the PA-diet only from L_4-L_7. This trend was also evident in the 0.1 % feeding regime. PA-experience during L_1-L_3 reduced PA-accumulation in later larval stages by more than 50 % in both sexes (Fig 4).

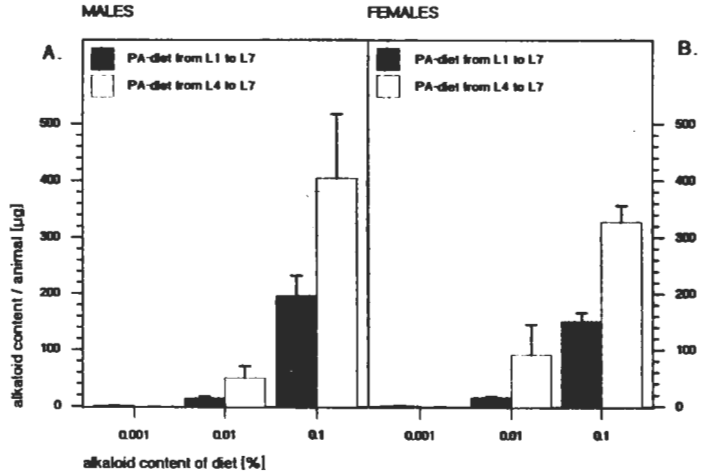


Fig 4: Heliotrine storage in the body of adult *Creatonotos transiens* (Walker 1855) in relation to previous (larval) feeding experience and absolute PA-amounts (**A MM, B FF**; n = 3). Semiartificial diet contained defined concentrations of alkaloids (0.001 %, 0.01 % and 0.1 % heliotrine). These diets were offered to the larvae from L_1- L_7 (black columns) or from L_4-L_7 (open columns). Imagines were analyzed immediately after eclosion. Bars represent SD.

3.4 Influence of ambient temperatures on PA degradation and PA distribution

As mentioned above, in **MM**, PA serve as a precursor for the biosynthesis of their pheromone (synthesis starts at the time of eclosion) and as a nuptial gift for **FF** provided with PA-rich spermatophores. In **FF**, a part of the stored alkaloid is translocated to the ovaries and is subsequently deposited in the clutches. If both partners obtain an equal amout of PA in the larval stage, 75 % of the alkaloid content of the eggs were donated from the **F**, whereas **MM** contribute about 25 % [VON NICKISCH-ROSENEGK et al 1990].

One can ask whether PA degradation and PA distribution within the imagines might be influenced by ambient T. In this set of experiments, L_7-Lv had obtained 3 mg of heliotrine; they were reared at 21° C and could pupate undisturbed. After eclosion, imagines were kept at T of either 30° C, 21° C and at 4° C. After the time intervals given **FF** were dissected into ovaries (with eggs) and the body fraction (Fig 5B). From **M'** moths the pheromone bearing coremata were removed with scissors. Alkaloid content of the body and the amount of the pheromone hydroxydanaidal were determined by GLC (Fig 5A). At 4° C, pheromone production in **MM** was low and PA transfer into the ovaries was equally reduced in **MM**. Both sexes retain their alkaloid in the body, mainly the integument. Maximal pheromone production and PA-transfer to the ovaries was observed at 21° C, whereas higher T (30° C) resulted in significant lower values. At 21° C, about 70 % or 50 % of the alkaloid stored in the integuments, were mobilized in **MM** and **FF**, respectively.

When animals, kept at 4° C for 48 h, were transferred back to 21° C for 24 h, the pheromone production in **MM** rose from 9 % to 31 %. In **FF**, the rate of alkaloids transported into the ovaries measured 15-38 %.

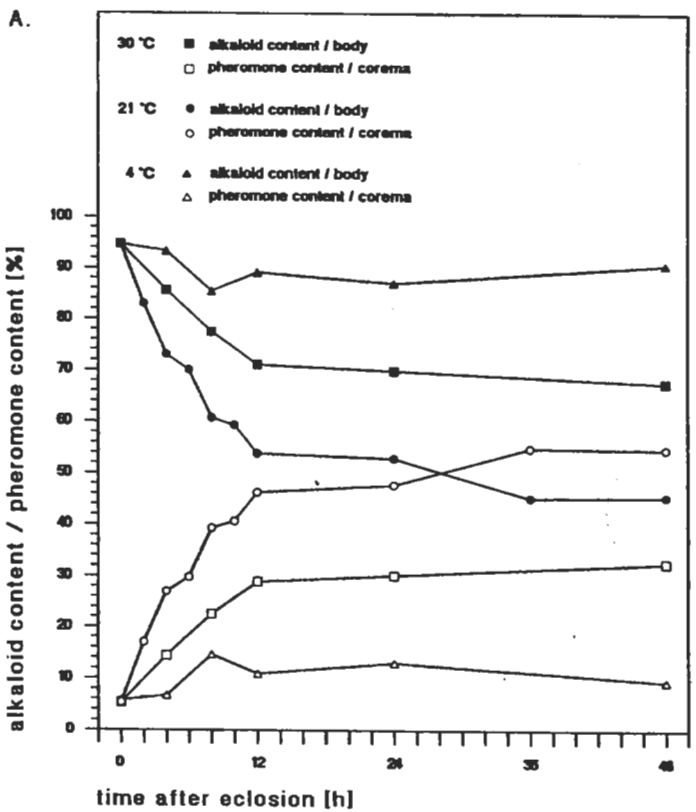
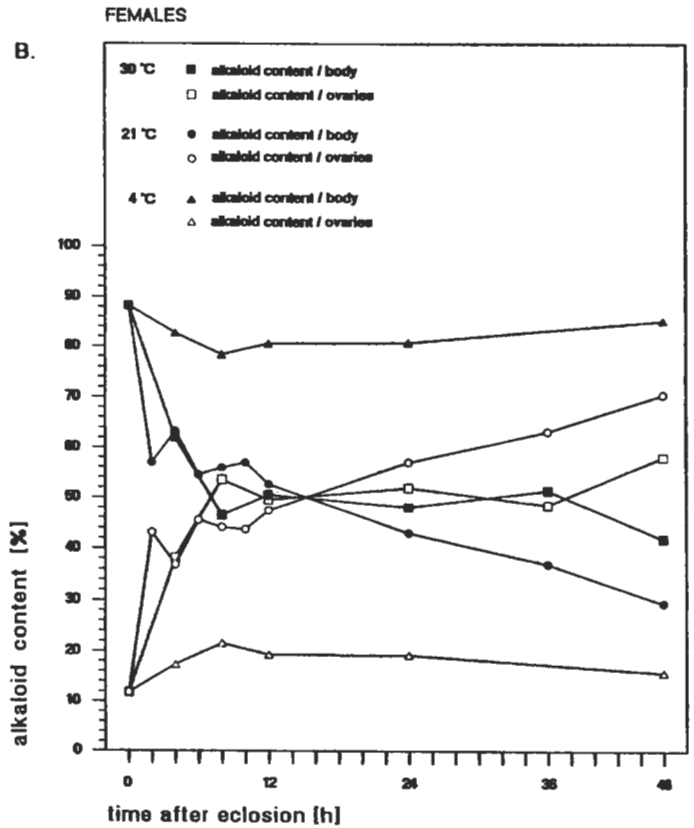


Fig 5: Temperature-dependent distribution of alkaloid in imagines of *Creatonotos transiens* (Walker 1855); **A MM, B FF.**- Larvae were fed in the last larval stage (L_7) with 3 mg of the PA-monoester heliotrine. Larvae and pupae were kept at 21 °C. Immediately after eclosion the imagines were transferred to different ambient temperatures: 30 °C, 21 °C and 4 °C. At the time intervals given the animals were dissected, alkaloid and pheromone (hydroxydanaidal) contents were determined after extraction by capillary gas liquid chromatography (GLC). The total alkaloid content per animal was set 100 %. To calculate PA contents of **MM**, the different molecular weights of heliotrine (MW 311) and hydroxydanaidal (MW 151) were taken into account. Values represent means of 3 analyses per datapoint.



4 Discussion

In the arctiid moth, *Creatonotos transiens*, the accumulation and storage of high amounts of PA should be of prime importance, since reproduction and fitness seem to be strongly influenced by these compounds. As a first prerequisite, larvae of this moth should be able to select foodplants, rich in PA. At least in laboratory experiments, a clear preference of PA-rich food can be observed (Fig 1 A-H) [BOPPRÉ 1986, 1990]. However, under natural conditions only food plants, which do not produce PA, have been reported [BOPPRÉ & SCHNEIDER 1990]. A thorough investigation of the food choice of this species in the wild is thus certainly necessary to explain this discrepancy.

As a next step, PAs are actively resorbed from the diet with aid of a carrier-mediated process [WINK & SCHNEIDER 1988], which does not discriminate the various PA-esters, but seems to

recognize the heliotridine/retronecine moiety of the PA-molecules. PAs with different necine bases, such as otonecine are not taken up [VON NICKISCH-ROSENEGK 1991]. Shortly after resorption, PAs such as 7S-heliotrine are modified in 2 ways: The free PA-base is completely converted into its N-oxide [VON NICKISCH-ROSENEGK et al 1990]. Furthermore, a stereochemical inversion is observed at the hydroxyl group in position C7, thus yielding 7R-heliotrine, which has then the correct conformation for the biosynthesis of the pheromone 7R-hydroxydanaidal [VON NICKISCH-ROSENEGK et al 1990, WINK et al 1988].

PA only shortly stay in the haemolymph. They are quickly transferred and stored in the integument. There, they can be found during all subsequent developmental stages, i e pupae and adult imagines. PA are not lost during ecdysis [VON NICKISCH-ROSENEGK et al 1990]. It may be assumed that the storage of PA in the integument may serve as a means for chemical protection against predators [VON NICKISCH-ROSENEGK et al 1990, BOPPRÉ 1990] since PA are toxic to many animals. They bind to ACh-receptors and form covalent bonds with DNA or proteins after enzymatic activation in the vertebrate liver [WINK 1993]. In addition, this chemical defence principle can be transferred to the offspring: FF translocate up to 70 % (Fig 5) of their PA into the ovaries and pass them to the eggs [WINK et al 1990, VON NICKISCH-ROSENEGK et al 1990]. Furthermore, also the M contributes to the chemical protection of the clutch: Upon eclosion, a substantial part of the stored PA is transferred to the spermatophore and thus passed to the F and eventually to the eggs. Up to 50 % of the M' PA is converted into the pheromone hydroxydanaidal, which is dissipated by the inflatable scent organ, the corema [SCHNEIDER et al 1982]. Since a high pheromone content is correlated with high PA-storage, FF should select hydroxydanaidal-rich MM, which could provide a substantial amount of PA as a nuptial gift [VON NICKISCH-ROSENEGK et al 1990].

For all these purposes, an efficient uptake and storage system for PA should be present. Uptake was in fact almost complete. PA türned up in the faeces only, when amounts higher than 3 mg were applied [WINK et al 1989, VON NICKISCH-ROSENEGK et al 1990]. Since PA concentrations are usually below 2 mg/g fresh weight, the uptake system seems to be sufficient under natural conditons.

In nature, the amounts of alkaloids and other secondary compounds vary between plant organs and developmental stages [WINK 1992]. In *Senecio*, the highest amount of PA could be found in the vegetation point of young leaves, flowers and fruits whereas stems and older leaves accumulate smaller amounts [HARTMANN & ZIMMER 1986]. Therefore larvae are likely to take up varying amounts of PA while feeding on the same plant. On the first view, the relative high degree of PA-degradation which can be seen in Fig 2-4, seems to be contradictory and contraproductive. How can it be explained? It could be argued that PA degradation is a common feature of herbivorous insects as in *Melanoplus sanguinipes* [EHMKE et al 1989] and still active in a PA-exploiting insect, such as *Creatonotos transiens*.

Since PA serve as chemical defence compounds, it should be important that the compounds do not loose their intrinsic activity. Because a random desintegration of the PA-molecule at the site of storage cannot be prevented, the only way for an organism to maintain a certain level of active compounds would be a steady turnover, a phenomenon known for many enzymes, hormones or other vital metabolites. Under natural conditions, i e a larvae living on a PA-plant, a steady supply would be provided, thus allowing a steady turnover without problems. However, the later argumention does not explain the obvious induction of PA-degradation by previous PA-exposition (Fig 3-4). In could be better interpreted as a means to prevent intoxication by PA, or perhaps as an evolutionary relict from PA-non-utilizing insects.

Despite of PA-degradation, *Creatonotos transiens* accumulates sufficient quantities of PA, to exploit them as defence compounds for both sexes and even the eggs, as pheromone precursors and morphogen. *C. transiens* thus seems highly adapted to an allelochemical which was orginally derived as a defence chemical by the producing plants [BOPPRÉ 1990, VON NICKISCH-ROSENEGK & WINK 1992).

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This manual is a basic guide for those involved in any aspect of pest and vector control. It provides essential facts and useful information, as well as a firm foundation for further study. It may be used as a supplement to a recognized system of pest control or as a reminder of safe and efficient practice. Colour and monochrome illustrations enhance the sections dealing with epidemiology and pest identification. A series of specially-develloped line drawings illustrate the external and internal anatomy of insects and other pests.