

## Low-melting point paraffin used to close puncture wounds improves success of ecdysis triggered by ecdysone injection

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**Summary.** The injection of ecdysone into pupae of Sphingidae (Lepidoptera) is more successful when the puncture wound is closed with low-melting point paraffin instead of soluble collodion, the common approach in this field. As a result of this change in procedure, the rate of flawless eclosion increased from 1:3 (Loeliger & Karrer, 1996) to more than 9:10 ( $p = 0.005$ ). Glue and polish were found to be even more noxious than collodion. Mastering the paraffin closure technique requires no more than a little practice. Ecdysone-induced synchronisation of the eclosion of adults which under mid-European climatic conditions only haphazardly enter metamorphosis, a notorious example being *Hyles centralasiae siehei*, will easily be obtained with this new technique for closure of the puncture wound.

**Zusammenfassung.** Die Erfolgsrate der Injektion von Ecdyson in Puppen von Schwärmern (Sphingidae, Lepidoptera) ist höher, wenn die Einstichwunde mit niedrigschmelzendem Paraffin verschlossen wird anstelle von gelöstem Kollodium, wie es gemeinhin üblich ist. Als Ergebnis dieses geänderten Vorgehens stieg die Rate fehlerlos geschlüpfter Tiere von 1:3 (Loeliger & Karrer, 1996) auf mehr als 9:10 ( $p = 0.005$ ). Klebstoff und Lack erwiesen sich als noch schädlicher als Kollodium. Die Paraffin-Verschlusstechnik erfordert nur wenig Übung. Durch Ecdyson lässt sich die Synchronisierung des Schlupfs der Imagines mancher Arten induzieren, die unter mitteleuropäischen Klimabedingungen nur sporadisch metamorphosieren; ein notorisches Beispiel ist *Hyles centralasiae siehei*. Mit der neuen Technik zum Verschluss der Injektionswunde wird dies leicht zu erzielen sein.

**Résumé.** L'injection d'ecdysone en des chrysalides de Sphingidae (Lepidoptera) est mieux couronnée de succès quand la blessure causée par celle-ci est fermée au moyen de paraffine fusible à basses températures, plutôt qu'avec du collodion soluble, l'approche habituelle en ce domaine. Suite à ce changement de procédure, la proportion d'éclosions parfaites augmenta de 1:3 (Loeliger & Karrer, 1996) à plus de 9:10 ( $p = 0.005$ ). La colle et la laque s'avèrent encore plus nocives que le collodion. La maîtrise de la technique de la fermeture à la paraffine ne demande qu'un peu de pratique. La synchronisation de l'éclosion des adultes, qui n'entrent qu'accidentellement en métamorphose sous les conditions climatiques d'Europe centrale, est rendue possible par l'ecdysone, un exemple notoire en étant fourni par *Hyles centralasiae siehei*.

**Key words:** Lepidoptera, Sphingidae, metamorphosis, ecdysone.

## Introduction

Shortly after the publication on the induction of metamorphosis of Lepidoptera by means of the injection of ecdysone or 20-hydroxy-ecdysone (Loeliger & Karrer, 1996), Spanish researchers pointed out by letter their experience with the ecdysone-induced metamorphosis of *Graellsia isabellae* (Graëlls, 1849) four years earlier (Ylla & Bellés, 1992). These authors had treated their pupae without anesthesia and without wound coverage. The pupae had been immobilized by exposure to ice for 10–15 minutes. The eclosing moths were flawless. Herewith, the authors indirectly suggested that collodion coverage of the wound might be toxic and therefore responsible for the low rate of flawless eclosion. Unfortunately, exposure to ice as applied by the Spanish researchers only insufficiently immobilizes pupae of Sphingidae and large amounts of lymph may be lost already before the injection site can be closed, resulting in insufficient unfolding of the wings upon eclosion. Nevertheless, the excellent results as obtained by the Spanish authors were an incentive to look for expert advice. We turned to professor Gilbert of the Biology Department of the University of North Carolina at Chapel Hill, a well-known expert in the field of endocrine control of moulting (Gilbert, 1989). By letter, he explained that “The procedure for closing a wound elicited by an injection is very simple. First, you make sure that the surface of the wound area is completely dry and then add a very small drop of a low-melting point paraffin that has been heated on a needle”; and: “I am sure that with very little practice you will master the technique”. Hereupon, we decided to undertake further experimentations.

## Materials and methods

*Collodion*: 3% dinitrocellulose in ethanol/diethylether 20/77.

*Glue*: marketed under the trade name *UHU Alleskleber* in Germany and Switzerland, produced by UHU G.m.b.H., Bühl (D).

*Polish*: marketed under the trade name *Bourgeois, Formule aux Protéines*, Paris (F).

Both glue and polish were applied by means of thin paint brushes, in one or two rapidly drying layers.

**Paraffin:** Paraffinum solidum 48–51. Pharmacopoeia Nederlandica VI.

**Injection technique:** as described earlier (Loeliger & Karrer, 1996). For the site of injection, we randomly chose the traditional apex of the head of the pupa as well as the abdomen, i.e., segment 5 between the anterior midline and the right-hand spiracle.

**Application of paraffin:** the head of an ordinary pin (synthetic material, 3.5 mm diameter) was immersed in liquid paraffin heated to the boiling point and, after a few seconds, abruptly removed; as a rule a substantial droplet hung from the head of the pin. After rapid transfer to the puncture wound in the cuticle of the pupa, the drop coagulated almost instantaneously.

**Live stock:** *Hyles centralasiae siehei* pupae obtained from larvae collected in 1995 in two different parts of Turkey, kindly placed at our disposal by F. Renner, Erbach Ringingen (D), and purchased by one of us (FK), respectively. From some of the latter parasitoids emerged. One pupa of each of the two series displayed spontaneous metamorphosis in the spring of 1997, the emerging adults being crippled, however.

*Hyles euphorbiae mauretanica*: non-inbred eggs kindly supplied by H. Harbich, Salz (D). Loss-free breeding (EAL) of larvae resulted in flawless pupae.

*Hyles euphorbiae dahlii*: apparently healthy pupae obtained from mature larvae collected just before pupation in Sardinia (I), purchased by one of us (FK).

## Results

Table 1 summarizes the results obtained with three different materials used for wound closure after uncomplicated ecdysone injection into non-hybrid pupae of two Sphingidae species. Males and females are not shown separately as there was no difference in eclosion rates between the two sexes.

Of the *Hyles euphorbiae* pupae, those belonging to the subspecies *mauretanica* apparently were the healthiest: the 14 pupae treated with paraffin all displayed flawless eclosion irrespective of whether the ecdysone had been injected via the apex of the head deep into the thorax or via the abdominal segment into the abdomen. Accordingly, the vitality and mating behaviour of the imagines were normal with abundant progeny, the number

Table 1. Results of uncomplicated ecdysone injection into non-hybrid pupae of Sphingidae (Lepidoptera)

	Number head	injected abdomen	Wound glue	closure polish	with paraffin	none	Flaws slight	severe
<i>H. centralasiae</i> <i>siehei</i>	5	1			6	5		1
<i>H. euphorbiae</i> <i>dahlui</i>	3 2 2	4 3 2	7	5	4	2	1 4 2	6 1
<i>H. euphorbiae</i> <i>mauretanica</i>	10	4			14	14		
Total:	22	14	7	5	24	21	7	8

Note. Of the paraffin-treated pupae, only one adult did not eclose and develop normally; of the 12 glue and polish-treated pupae, 5 produced slightly and 7 severely defective imagines ( $p \chi^2 < 0.005$  if none and slight flaws are taken together). Injection via head resulted in a success rate similar to that via abdomen ( $p \chi^2 > 0.25$ ).



of eggs deposited by one of the females on *Euphorbia* exceeding 300.

All four *Hyles euphorbiae dahlia* pupae treated with paraffin emerged, although two of them displayed some defects. In sharp contrast, none of the 12 pupae treated with either glue or polish resulted in normal imagines, 7 being severely defective. However, also those treated with paraffin had low vitality. Mating did not occur in spite of conditions similar to those prevailing for *Hyles euphorbiae mauretanica* moths. Dissection of the females revealed a strikingly low number of apparently mature eggs.

For *Hyles centralasiae siehei*, the results were as follows. The two spontaneously developing pupae both produced abnormal adults. One female emerging from an injected pupa displayed obstruction of the cloaca, pointing to some kind of disturbed intestinal function of the caterpillar at the time of pupation. However, five of the six pupae injected, the injection wound being closed with paraffin, appeared to be suitable for mating and oviposition, although the conspicuous restlessness known for moths of this species hampers their feeding and causes wing damage such that flying is no longer possible after one week. Three of these five, one female and two males, emerged on July 2nd and were placed together in a gauze-covered circular cage 65 cm across and 80 cm high, in a large well-ventilated and daylight-illuminated loft. Mating took place within the first 24 hours after eclosion, the night being cold (lowest temperature 10°C) due to brightness of the sky. There was no moonlight illumination as new moon occurred on 3/4 July. Copulation, observed when the second male was still flying, lasted from 22:00 till 04:00, when the position of the pair had changed from vertical to horizontal. Room temperature had decreased from 19°C to 17°C. Oviposition could be observed from the second day after copulation on. Deposition of the eggs occurred exclusively on the gauze of the cage in the centre of which a bouquet of abundantly flowering *Kniphofia* (Liliaceae) had been placed, flowers which had been accepted for oviposition in earlier experiments. The female died eight days after mating, after having deposited 14 eggs, all sterile, however. On dissection only 85 more of apparently mature oocytes were counted.

## Discussion and conclusion

As pointed out in the Introduction above, toxicity of collodion was suggested indirectly by Spanish researchers, who observed a 100% flawless eclosion rate after injection of ecdysone into pupae, the puncture wound of which not being covered with this rapidly drying material. Collodion consists of solid dinitrocellulose. Of its dissolvent, an ethanol/diethyl ether mixture, the alcohol component is unlikely to be toxic as it is also used to dissolve ecdysone. Ether, however, is a well-known neurotoxic substance, which indeed might be responsible for the rather high failure rate observed in our earlier experiments with ecdysone injections and coverage of the puncture site with collodion (Loeliger & Karrer, 1996).

The procedure as suggested by Gilbert for closing the wound elicited by the ecdysone injection appeared easy to apply and haemolymph leakage no longer ever occurred. More important, in contrast to what we had observed for glue, polish and collodion, paraffin obviously causes no toxic damage despite the fact that it enters the pupa in small amounts, as demonstrated microscopically. (Sucking in of the material is the result of the negative intrapupal pressure which develops during the cooling down period when CO<sub>2</sub> vapour emerges from the dry ice crystals and surrounds the pupa).

It is perhaps astonishing that no difference in eclosion success was observed between pupae injected via the apex of the head deep into the thorax and those injected intra-abdominally. But the small amount of injected fluid obviously does not disturb the loose anatomical structures of the thorax and abdomen.

Lastly we warn not to rely on too short a dissolution time for the crystalline ecdysone, the dissolution rate of which is directly proportional to its surface area. Larger crystals take up to half an hour at room temperature to dissolve.

In conclusion, low-melting point paraffin appears to be the material of choice for non-toxic and safe closure of the wound made by the injection needle during ecdysone treatment of pupae of Sphingidae. After injection of appropriate amounts of the hormone, normal development can be expected for healthy pupae, resulting in adults which easily mate and produce normal amounts of fertile eggs. Synchronisation of eclosion and breeding

of even the most difficult species, such as *Hyles centralasiae siehei*, which as far as we know has never been successfully bred in captivity, should now be feasible.

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