abundance of them, they are completely absent in *Goniotrichum*[11]. The further addition of lanosol in the highest concentration, 2×10^{-4} *M*, could thus produce an inhibiting surplus in *Polysiphonia*, while *Goniotrichum*, having no pool of bromophenols, was stimulated by all concentrations tested.

As some simple phenolic compounds influence growth and/or morphology in *Goniotrichum*, they are presumed to play a fundamental role in metabolism, either as such or as lower steps in the formation of some growth regulators. In many other red algae, bromophenols are stored either as sugar derivatives[13] or as sulphates. It might be possible that aromatic compounds are mobilized from this pool when required in the metabolism of these species, while *Goniotrichum* has to be supplied by exudates from other organisms. Lanosol is as yet the only naturally occurring bromophenol tested, and since the role of bromine in this connection is completely unknown, no further conclusions can be drawn. Further work is now in progress to elucidate these problems.

Zusammenfassung. Wachstumstimulation von 2,3-Dibromo-4,5-Dihydroxybenzylalkohol (Lanosol) in Konzentrationen von 4×10^{-5}–4×10^{-6} wurde in axenischen Kulturen der Rotalgen *Goniotrichum alsidii* und *Polysiphonia urceolata* festgestellt. Während das Disulfatdikaliumsalz nicht toxisch und von niedrigerer Aktivität war, hatte 2×10^{-4} *M* Lanosol eine Hemmwirkung auf *Polysiphonia*.

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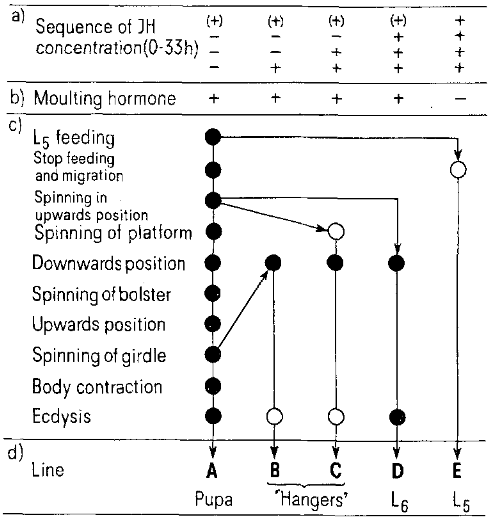
[13] S. RÖNNERSTRAND Bot. Marina *11*, 107 (1968).

Reversal of Spinning Behaviour in Last Instar Larvae of *Pieris brassicae* Treated with Juvenile Hormone Derivatives[1]

More than 20 years ago PIEPHO[2] demonstrated that the presence or absence of active corpora allata influences the spinning behaviour of last instar larvae of *Galleria mellonella*. When NOVAK[3] discussed this work, he claimed that the juvenile hormone (JH) would influence the instinct of spinning indirectly by inhibiting the morphogenesis of the pupal or imaginal brain. Since morphogenesis in insects is supposed to be a one-way process, the theory of NOVAK would imply that no reversal of behaviour is possible. However, reversible JH-dependent behaviour has been demonstrated in the adult colorado potato beetle[4]. The following observations on last instar larvae of the cabbage butterfly *Pieris brassicae* treated with JH derivatives (JHD) show that reversal of JH dependent behaviour is also possible in an insect which has not finished development.



Influence of juvenile hormone (JH) on pattern of behaviour of last instar larvae of *P. brassicae:* a) Hypothetical sequence of JH concentrations in larvae at 0–33 h after the L_4/L_5 moult (JHD present = +, absent = – ; hypothetical presence of small amounts of natural JH = (+)). b) Production of moulting hormone. c) Sequences of actions, i.e. patterns of behaviour according to pattern of JH concentrations and presence or absence of moulting hormone; the sequence of line A represents the natural pattern of behaviour leading to the pupal moult; all degressions from this line have been induced by treatment with JHDs. Full circles, action completed; open circles only part of action exhibited. d) Developmental result (L_6 = supernummerary larval stage; L_5 = 'dauer-larva').

The JHDs used were mixtures of isomeres of either methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-trideca-dienoate or 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)-phenoxy]-2-nonene. Last instar larvae of *P. brassicae* of different age were treated topically with 2 times 1 µl of acetone for controls or acetone solutions with different concentrations or one of the JHDs, applied dorsally on the thorax and the last third of the abdomen. Time of treatment is indicated as hours after the L_4/L_5 moult.

Our observations show that spinning behaviour of normal premoulting larvae of *P. brassicae* proceeds along one of two lines, depending on whether (at a relatively high level of JH) a larval/larval or (at a very low level of JH) a pupal moult is induced.

Untreated joung larvae of *P. brassicae*, when ready for a larval moult, spin a net of silk threads and take on a position with the head pointing downwards. They keep this position until the old skin ruptures on the back of the thorax and is shed. After a rest of about 2 h the larva resumes feeding. This behaviour corresponds approximatively with line D in the Figure.

Untreated or acetone treated last instar larvae ready for a pupal moult leave the food and, as a rule, move to a vertical wall where they construct a dense silk platform with a bolster of loose silk threads at the lower end of it. Each larva then positions itself on the platform with the head pointing upwards and the hind end lying on the bolster. It bends the head backwards and, moving it from one side of the platform over the back of the second abdominal segment to the other side of the platform, spins a girdle of silk threads which later on will fix the anterior part of the pupa to the platform. After some time the larva contracts to form the prepupa or pharate pupa. Within 12 h the larval skin ruptures on the back of the thorax and the old skin is shed. The hooks of the last

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[1] The author thanks Hoffmann-La Roche Ltd., Basel for samples of the substances.

[2] H. PIEPHO, Z. Tierpsychol. *7*, 424 (1950).

[3] V. J. A. NOVAK, *Insect Hormones* (Methuen, London 1966), p. 104.

[4] J. DE WILDE and J. A. DE BOER, J. Insect Physiol. *15*, 661 (1969).

abdominal segment of the pupa become entangled in the threads of the bolster which, together with the girdle, holds the pupa in a vertical position. This behaviour corresponds to line A in the Figure.

The great majority of the JHD treated larvae attempted moulting. Larvae treated at 33 h or later prepared usually for a pupal moult, whereas those who had been treated earlier prepared for a supernumerary larval moult (L_6). The former behaved exactly like normal controls ready to pupate. The larvae preparing for an L_6 moult moved to the walls of the rearing boxes, where, after more or less intense spinning, they took on a position with the head pointing downwards. The spinning of these insects was, as a rule, more intense than that of L_4. The insects treated with JHD at 0–9 h usually spun light webs only and, as a rule, were able to complete moulting to L_6 (Figure, line D) or, in a few cases, never moulted again and became 'dauer-larvae' (Figure, line E).

On the other hand, L_5 treated at 9–33 h with the second JHD spun denser nets (sometimes as densely woven as the platform of a prepupa) and had moulting difficulties. They rarely succeeded in completing the moulting process, because they were not able to get rid of the old head capsule and/or the exocuticula of the body. Except for the last abdominal segments the majority of these larvae could not loosen the old skin from the new integument. This was partly due to the fact that the dorsal rupture in the old exocuticle of the thorax had not taken place. The loosened exocuticle of the hind end became extensively stretched, by the weight of the larval body. After a day or two of unsuccessful moulting movements the insects became quiet, but stayed alive for another 2–3 days. The appearance of these larvae was very characteristic and since they were fixed to the wall of the rearing vessel by the cast skin of the prolegs of the last abdominal segment only, they will be called 'hangers' (Figure, line C).

Part of the hanger-syndroms is the failure to tear the old tracheal cuticle fully out of the spiracles. Therefore, wherever the old cuticle becomes loose, white bands of tracheae run backwards from the spiracles. It is probable that diffusion of air into tracheae is handicapped by the old tracheal strands. This may cause hypoxia in the hangers and hasten their death.

The intense spinning of larvae that will become 'hangers' indicates that these insects, although preparing for a larval moult, partly behave like larvae in the prepupal phase. According to NOVAK's theory this would show that between the L_4/L_5 moult and the time of application of the JHD the nervous system had already developped pupal characters and that this process could not be reversed by the JHDs applied.

However, the most interesting behaviour has been shown by a number of larvae which first spun a complete prepupal silk platform with a bolster of loose silk threads at the lower end and a girdle around the body, but then moved head and thorax out of the girdle, took on a position with the head pointing downwards, and eventually died as 'hangers' (Figure, line B). These insects first showed the typical prepupal behaviour of larvae ready for the pupal moult, but afterwards changed their behaviour to one related with larval/larval moulting. However, it may be noteworthy that JHDs, when applied at 60 h or later, could never induce such a reversal of behaviour.

The results show that the spinning behaviour of *P. brassicae*, like that of *G. mellonella*[2], is strongly influenced by the titer of the JH. Initiation of migration and spinning behaviour of normal larvae of *P. brassicae* seems to be induced by the presence of the moulting hormone (MH) and/or a reduced titer of JH. Thus some larvae treated with an JHD during or right after the L_4/L_5 moult will become 'dauer-larvae' which never again spin and moult (Figure, line E) or, after some time of feeding, will migrate and behave like younger larvae preparing for a larval/larval moult (Figure, line D). It is probable that in the former an extremely high titer of JH inhibits the synthesis of the MH, whereas in the latter a short period of a reduced titer of JH allowed MH production.

The development of typical 'hangers' (Figure, line C) seems to indicate that differentiation of the nervous system in the pupal direction begins already at about 9 h after the last larval moult. In agreement with NOVAK's theory the morphogenetic changes in the nervous system cannot be reversed by the application of JH, with the result that prepupal behaviour (intense spinning) is followed by larval behaviour, i.e. taking on a position with the head pointing downwards before the silk platform is finished.

However, contrary to the predictions of NOVAK's theory, a direct influence of JH on behaviour can also be demonstrated in those individuals which show the normal sequence of prepupal behaviour to the point when prepupal contraction of the body and a pupal moult should occur, but which instead return to larval behaviour (Figure, line B). These individuals show that true reversal of JH dependent behaviour from an ontogenetic later to an ontogenetic earlier pattern is possible, if the morphogenetic processes of nervous differentiation have not proceeded too far.

Zusammenfassung. Werden Raupen des Kohlweisslings *Pieris brassicae* im letzten Stadium mit synthetischen Juvenilhormonderivaten behandelt, so zeigen sie vor der nächsten Häutung, je nach Hormonspiegel, entweder das für eine Puppen- oder für eine Larvenhäutung charakteristische Verhalten. In Insekten, deren Nervensystem bereits teilweise präpupal determiniert ist, kann ein hoher Hormonspiegel eine Umkehr vom ontogenetisch späteren Präpupalverhalten zur ontogenetisch früheren Stufe des typischen Larvalverhaltens induzieren.

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Mise en évidence d'une émission d'isoxanthoptérine et de riboflavine par différentes espèces d'Ascidies

GOODBODY[1], en mettant en évidence une apparition d'ammoniaque dans l'eau où avaient séjourné des Ascidies, avait montré que l'excrétion par accumulation ne pouvait être considérée comme la forme exclusive de l'élimination de l'azote par les espèces de ce groupe. Nous décrivons ici des expériences analogues portant sur 9 espèces différentes, qui nous ont permis d'identifier 2 composés organiques fluorescents riches en azote: la riboflavine et l'isoxanthoptérine, parmi d'autres métabolites externes. La présence des ptérines[2] parmi les