Case histories of 5 adult *M. rosenbergii* males that underwent autotomy

Animal	1	2	3	4	5
2 days after molting	2.2	1.9	1.4	2.5	2.4
Mid cycle (intermolt)	3.8	1.5	2.7	2.3	1.6
4 days prior to molting	13.2	4.5	4.1	9.5	8.9
2 days prior to molting	56.0	61.3	72.0	68.0	63.4

Results expressed in picograms of 20-hydroxyecdysone equivalent per µl hemolymph.

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Effect of the juvenoid methoprene on the hemolymph composition of the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae)

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Summary. Treatment of post-feeding larvae of the cabbage maggot *Delia radicum* with methoprene did not affect the capacity of the insect to pupate, but suppressed eclosion to the adult stage. The concentration of hemolymph trehalose was significantly decreased by methoprene treatment, although hemolymph protein and amino nitrogen levels were unaffected.
Key words. Amino nitrogen; *Delia radicum;* eclosion; hemolymph; methoprene; protein; trehalose.

A variety of compounds with juvenile hormone activity offer considerable promise as a replacement for or adjunct to existing methods for controlling insects[2]. Under laboratory conditions, synthetic cecropia juvenile hormone and a juvenile hormone analog possessed ovicidal activity against eggs of the cabbage maggot *Delia radicum* and prevented adult emergence from puparia[3]. Field trials, using the juvenoid, showed promising control potential[4]. More recently, it was found that eggs of *D. radicum* hatched subsequent to treatment with the juvenoid methoprene (isopropyl (2E,4E)-11-methoxy-3,7-11-trimethyl-2,4-dodecadienoate), but pupae that eventually developed failed to undergo eclosion to adults[5]. No information is available concerning the physiological effects of juvenoids on *D. radicum,* although such a knowledge would provide insight into their mode of action and prove helpful in identifying side effects on non-target invertebrates[6]. In view of the potential usefulness of insect growth regulators for controlling populations of *D. radicum,* a major pest of root and stem crucifers in North America and Europe, this study was done to determine the effect(s) of methoprene treatment on the hemolymph composition of post-feeding larvae.

Delia radicum larvae were obtained from a stock colony of the insect, maintained in the greenhouse[5]. To demonstrate that the post-feeding larval stage was sensitive to methoprene, insects were removed with forceps from the soil in which infested rutabagas had been grown, within 24 h after they had left the host plant, rinsed in distilled water and placed on filter paper until needed. Larvae were held anteriorly with forceps and, using a micropipet, topically treated over the entire thoracic and abdominal regions with 1 µg methoprene in 1 µl dimethylsulfoxide (DMSO), or with 1 µl of DMSO (controls). Treated larvae (three replicates of 10 insects per treatment) were reared (20 °C; 16-h photoperiod) using a small-scale petri dish rearing system[5] and percent pupation and adult eclosion recorded.

For experiments on hemolymph composition, methoprene or DMSO-treated larvae were maintained (36 h) in pupation units made from 9-cm-diameter petri dish bases[5], prior to hemolymph extraction. Hemolymph was not collected from larvae that pupated during the 36-h incubation period. Surface-dried larvae were held with forceps under a stereomicroscope and punctured on the ventral surface, immediately posterior to the larval mouth hooks, with a fine insect pin. The fluid which exuded was drawn to fill a 1-µl micropipet, then applied to the base-line of an activated thin-layer chromatography plate for carbohydrate analysis, or blown into 5 ml of trichloracetic acid (TCA) for amino nitrogen and protein quantification. A total of 5 µl of pooled hemolymph was required for each carbohydrate determination and 10 µl for each amino nitrogen/protein replicate. Since only a very small volume of clear hemolymph (1–2 µl) could be obtained from each cabbage maggot, each carbohydrate value was based on hemolymph taken from 3–5 insects, while each amino nitrogen/protein determination involved hemolymph taken from 5–10 larvae. Carbohydrates were separated by TLC and fractions detected using an α-naphthol-sulfuric acid reagent spray[7]. To quantify separated carbohydrates, plates were developed containing hemolymph samples spotted alongside standards, but only the standards were subsequently sprayed with the detection reagent. Areas of the unsprayed plate coating (2 × 2.5 cm) corresponding in R_f values to the standards were scraped into centrifuge tubes. Anthrone reagent (3 ml) was added to each sample, which was colorimetrically assayed for carbohydrate[8], values being calculated by reference to a calibration curve prepared from the requisite standard. Hemolymph aliquots blown into TCA were assayed colorimetrically for amino nitrogen[9] and protein[10] concentration. Amino nitrogen concentration of the hemolymph was calculated by reference to a glycine calibration curve and protein concentration was expressed relative to a bovine serum albumin standard. Three replicates of

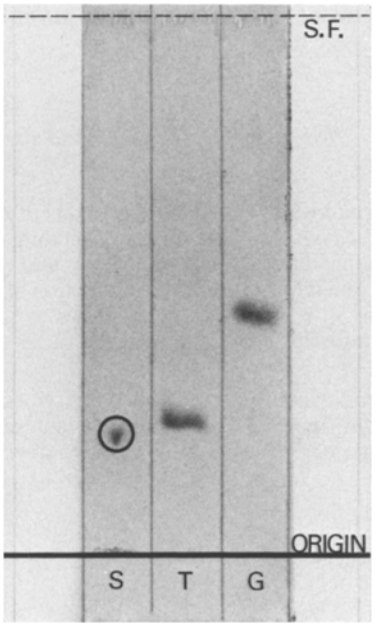

Figure 1. Portion of a TLC plate after spraying with detection reagent. Positions of origin and solvent front (S.F.) are shown. Glucose (G) and trehalose (T) standards were spotted alongside a hemolymph sample (S). Circled is the carbohydrate (trehalose) that separated from S.

pooled hemolymph were assayed for carbohydrate, protein and amino nitrogen concentration. Data were tested for statistical significance using a 'single classification analysis of variance'[11].

Methoprene treatment did not affect the capacity of larvae to pupate. By 48 h after treatment, all (100%) control and treated insects had pupated. However, eclosion to the adult stage was completely suppressed in insects treated with the juvenoid. Almost 8 weeks (52 days) after treatment, none (0%) of the methoprene-treated insects had eclosed, whereas adults had emerged from 70.0 (± 5.8)% of the controls. The effect of methoprene on pupal-adult development, when applied to the post-feeding larval stage, was identical to that observed when the egg stage of *D. radicum* was treated with this compound, i.e. uneclosed pupae contained pupal-adult intermediates, with pupal abdomens and heads/thoraces comprising a mixed assortment of pupal and adult characters[5]. The concentration of amino nitrogen and protein in the hemolymph of *D. radicum* larvae was unaffected by methoprene treatment. Measured 36 h after treatment, the amino nitrogen concentration of methoprene-treated and control insects was 91.0 (± 24.0) and 78.0 ± 12.3 mg amino N/100 ml hemolymph, respectively. The protein concentration of methoprene-treated larvae was 4.1 (± 0.2), and of controls 4.1 (± 0.1) g equivalents bovine serum albumin/100 ml hemolymph. Thin layer chromatography showed trehalose to be the only carbohydrate detectable in the hemolymph of *D. radicum* post-feeding larvae (fig.). Methoprene treatment caused a significant ($p < 0.05$) reduction in the hemolymph trehalose concentration, from 1.62 ± 0.15 (controls) to 1.16 ± 0.02 (treated) g trehalose/100 ml hemolymph.

The effect of methoprene on the hemolymph of *D. radicum* larvae differs in detail from that reported for the larval (fourth-instar) mosquito *Aedes aegypti,* which showed an elevation in carbohydrate and a decrease in protein concentration of the hemolymph after exposure to the juvenoid. In the latter species, however, the hemolymph carbohydrate concentration of treated insects decreased significantly (cf. controls) subsequent to pupation, as glycogenolysis by the fat body was impaired due to diminished activity of glycogen phosphorylase[12]. Fat body glycogenolysis and hemolymph trehalose levels are controlled by the hyperglycemic factor(s) secreted by the corpora cardiaca[13]. Thus, the reduction in the hemolymph trehalose concentration of *D. radicum* caused by methoprene treatment is consistent with the proposition that juvenoids function, at least in part, by disrupting the normal pattern of neuroendocrine activity in the insect[6,12,14]. Studies are in progress to verify such an indirect mode of action in *D. radicum.*

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