Enhanced production of aggregation pheromones in four stored-product coleopterans feeding on methoprene-treated oats[1]

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Summary. Production of aggregation pheromones by male *Oryzaephilus surinamensis, O. mercator, Cryptolestes ferrugineus,* and *Tribolium castaneum* was enhanced by feeding on methoprene-treated oats, implicating juvenile hormone in control of pheromone production. Methoprene application to control insects in stored food products may cause enhanced pheromone production by these insects, thus drawing additional beetles into the treated product.

Key words. Methoprene; aggregation pheromones; pheromone production; stored-product coleopterans; *Oryzaephilus surinamensis; Oryzaephilus mercator; Cryptolestes ferrugineus; Tribolium castaneum;* insect growth regulator; juvenile hormone.

Methoprene [isopropyl (2*E*, 4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate], an analogue of the natural juvenile hormones (JHs), is a potent insect growth regulator that has recently been registered for use against insect pests of stored products. Hedin et al.[3] reported that JH III [methyl (2*E*, 6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate] when incorporated at a concentration of 1 ppm in the diet of the adult male boll weevil, *Anthonomus grandis* Boh., increased production of each of its four monoterpene pheromones by approximately threefold. JH I [methyl (2*E*, 6*E*, 10*Z*)-3,11-dimethyl-10,11-epoxy-7-ethyl-2,6-dodecadienoate], however, had no effect upon boll weevil pheromone production. This and earlier observations that JH is involved in the control of aggregation pheromone production in some forest coleoptera[4,5] suggest that exposure to methoprene might influence production of aggregation pheromones by coleopteran pests of stored products. Male-produced aggregation pheromones have recently been identified in two economically important families of long-lived stored-product Coleoptera, the Cucujidae[6-9] and the Tenebrionidae[10,11]. We report that production of aggregation pheromones in three species of cucujids and one species of tenebrionid is enhanced by feeding on methoprene-coated rolled oats.

Materials and methods. Methoprene (technical Altosid® IGR, Zoëcon Corporation, Palo Alto, California 94303) was dissolved in purified pentane (1 mg/ml) immediately before use. For coating of oat flakes, 3 ml of methoprene stock solution was added to 300 ml of purified pentane in a 1-l round-bottomed flask that had a longitudinal indentation (approximately 0.5 cm deep). Large-flake rolled oats (150 g) were slowly poured into the flask and the solvent carefully removed on a rotary evaporator at low temperature (25–40 °C). Residual solvent was removed by vacuum pumping for 0.5 h. Preliminary tests using butter yellow (*p*-dimethylaminoazobenzene) showed that the coating procedure resulted in dyed oats of a uniform color, with no dye left on the flask wall. For control media, 150 g large-flake rolled oats were treated with pentane in the same manner but without methoprene.

Beetles were reared on large-flake rolled oats and brewer's yeast (95:5, wt:wt) at 28–29 °C and 50–70% relative humidity. The species used were *Oryzaephilus mercator* (Fauvel), *O. surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens), (Cucujidae) and *Tribolium castaneum* (Herbst) (Tenebrionidae).

Beetle-produced volatiles were obtained by aerating adults in vertically-oriented, cylindrical glass chambers containing 150 g of treated large-flake rolled oats[8]. Charcoal-filtered, humidified air was drawn at 1.65 l/min by aspiration through a culture (held at 23–24 °C in darkness) and then through a glass trap (14 mm OD × 150 mm) filled with conditioned Porapak Q (50/80 mesh). At intervals of 7 days, the culture was removed from the aeration chamber, the beetles separated from the oats and returned to a clean chamber with a fresh 150 g portion of treated oats, and the aeration continued using a fresh Porapak Q trap. Two aeration cultures (pentane-treated control and methoprene-treated experimental) were set up simultaneously for each species using 12,000, 15,000, 20,000 and 4,000 mixed-sex adults per aeration for *O. mercator, O. surinamensis, C. ferrugineus,* and *T. castaneum,* respectively. For each species, the beetles for both aeration cultures came from the same batch and had an approximate sex ratio of 1:1. Each group of beetles was aerated for a total of 21 consecutive days. Pheromone-production data (table) were recorded for days 15–21 of the 21-day treatment period.

Volatiles were recovered by extraction of the Porapak Q with purified pentane in a Soxhlet extractor for 24 h, and the extract was concentrated by distilling the pentane through a Dufton column. The concentration of aggregation pheromones in a pentane extract was determined on a Hewlett-Packard 5830A gas chromatograph using a Superox 4 capillary column (50 m × 0.5 mm ID) with either methyl myristate or methyl laurate as an internal standard.

Results and discussion. Production of aggregation pheromones was enhanced for males of all four species feeding on methoprene-treated rolled oats (table). The total enhancement was greatest for *O. surinamensis* for which the comparatively low control values reflect the negative effect of crowding on pheromone production[7]. The magnitude of the enhancement factors for pheromone production for the four species are quite similar to that found for male boll weevils maintained on a JH III-treated food source[3]. In contrast to the equal enhancement of the four boll weevil pheromones, however, methoprene treatment caused an unequal increase of the individual pheromone components of *Oryzaephilus* spp. and *C. ferrugineus* (table). Although we have not examined the effects of JHs on pheromone production in the four species of stored-product beetles, our results implicate JH as a regulatory factor in aggregation pheromone production in males of these species. Moreover, our results suggest that pheromone production in these species may be manipulated by use of methoprene or other synthetic chemicals.

Pheromone production (pg/bh) by four species of stored-product beetles 15–21 days after initial placement on methoprene- or pentane-treated oats

Species	Pheromone	Pheromone production, (pg/bh)		
		Pentane control	Methoprene 20 ppm	Enhancement factor
O. mercator	I	770	1190	1.5
	II	1200	5280	4.4
O. surinamensis	II	10	35	3.5
	III	25	110	4.4
	IV	28	130	4.6
C. ferrugineus	I	980	1700	1.7
	V	1630	3500	2.1
T. castaneum	4,8-DMD	440	900	2.0

Production values were obtained from beetles 1-2 months posteclosion; pg/bh = the amount of pheromone in picograms produced by a male beetle aerated for 1 h. I = (*Z*)-3-dodecen-11-olide; II = (*Z*,*Z*)-3,6-dodecadien-11-olide; III = (*Z*,*Z*)-3,6-dodecadienolide; IV = (*Z*,*Z*)-5,8-tetradecadien-13-olide; V = 4,8-dimethyl-(*E*,*E*)-4,8-decadienolide; 4,8-DMD = 4,8-dimethyldecanal.

At concentrations comparable to that used in our pheromone-production experiments, methoprene hindered survival of immature stages and prevented emergence of F_1 progeny[12,13,14], but did not affect survivorship of reproductively mature adults[12] of these target species. In the present series of experiments, we did not observe any mortality of adult beetles with methoprene treatment.

Dose-response curves obtained in laboratory bioassays with *C. ferrugineus*[6] and *Oryzaephilus* spp.[7,9] indicate that a two-to four-fold increase in pheromone dose above the lower threshold for activity could significantly increase attraction of these species. In preliminary field tests, small traps baited with macrolide pheromones and 4,8-dimethyldecanal were effective in recapturing released *C. ferrugineus* and *T. castaneum*[15]. Addition of methoprene to such attractant baits in a pest-monitoring system could result in trapped living adults functioning as enhanced pheromone sources. If food baits are used[16], prior treatment of the baits with methoprene might enhance species-specific attractiveness, with concomitant inhibition of reproduction of the aggregated populations. Finally, enhanced pheromone production by beetles feeding on methoprene-treated stored products may concentrate populations in the treated product, thereby improving the effectiveness of methoprene as an insect control agent.

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Effect of various doses of catecholestrogens on uterine eosinophilia in the immature rat[1]

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Summary. This paper describes the induction of uterine eosinophilia as well as of deep endometrial edema and increase of uterine wet weight in the immature rat by the catecholestrogens 2-OH-estradiol and 4-OH-estradiol. These effects are thought to be mediated by eosinophils via a specific eosinophil receptor system. 4-OH-estradiol was equipotent with estradiol, whereas the effect of 2-OH-estradiol was significantly weaker.
Key words. Catecholestrogens; uterus; estrogenic responses; uterine eosinophilia.

It has been previously shown that estrogens exert their action in the uterus by at least two independent mechanisms: 1) by activation of the genome via the cytosol-nucleus receptor system, leading to induction of several genomic responses (RNA and protein synthesis, morphologic and functional differentiation of target cells)[2], and 2) by an attraction of eosinophil leucocytes to the uterus via the eosinophil-estrogen receptor system postulated by Tchernitchin[3,4], and an induction of several non-genomic responses (edema, increase in vascular permeability and release of histamine in the uterus) under the action of enzymes released in the organ by the eosinophils[4,5]. The hypothesis of multiple and independent mechanisms of estrogen action mediating separate groups of responses is supported by the dissociation of these groups of estrogenic responses, by a number of agents or conditions that selectively interfere with the mechanisms of hormone action involved[4].

The existence of estrogens which exhibit weak estrogenic activity for the induction of some responses but strong for others led us to investigate the estrogenic properties of the 4-OH- and 2-OH-metabolites of estradiol-17-β (E2-17β), whose physiological role is still uncertain. Of these two compounds, called catecholestrogens (CE), 4-OH-estradiol (4-OH-E2) is currently considered to be a relatively strong estrogen with 45% relative binding affinity (RBA) for the cytosol-nucleus receptor, whereas 2-OH-estradiol (2-OH-E2) is thought to be a relatively weak agonist with only 24% RBA and possible partial antagonist properties[6]. The present study reports on the effects of these compounds on uterine eosinophilia, on the eosinophil-mediated edema and on cytosol-nucleus receptor mediated genomic responses.

Materials and methods. 24-day-old female Wistar rats, 45 ± 5 g b.wt, were used. Under ether anesthesia, the rats were i.v. injected with 1, 3, 10 or 30 µg/100 g b.wt. of E2-17β (Sigma Chemical Co., ST. Louis, USA), 2-OH-E2 or 4-OH-E2[7], dissolved in 10% ethanol-saline containing 0.1% ascorbic acid. The control group received the vehicle alone. 6 h after injection the rats were sacrificed, and both uterine horns were excised. After length and wet weight determination, one uterine horn was kept in 0.25 M saccharose for the determination of DNA[8], RNA[8] and protein[9]; the other uterine horn was fixed in 10% neutral formalin for at least 24 h and subsequently histologically processed for the determination of the total number of eosinophils and the number of endometrial and myometrial eosinophils[10,11], and also for evaluation of tissue eosinophil degranulation and deep endometrial edema[12].