whole and, in this respect, Silkie fowl melanoblasts do not differ from those of the quail. At this point, the question of the role played by the tissue itself arises. As those Silkie fowl organs that are not pigmented in situ were not invaded by quail pigment cells, it appears that the homing of melanoblasts is determined by environmental cues created in organs.

As for the environmental cues which regulate seeding of melanoblasts, one can ask the question whether they are permissive or attractive. The heavy accumulation of melanocytes in the depth but also at the periphery of some grafts, strongly suggests that they are not only permissive but also attractive for prepigment cells. Is the definite localization of pigment cells regulated by a chemo-attractant? The hypothesis of the production of a chemo-attractant by the primary lymphoid organs (thymus and bursa of Fabricius in birds) regulating the seeding of hemopoietic cells was proposed by Le Douarin[16]. This author also asks the question whether the colonization of epidermis by melanoblasts in bird embryos could be regulated by such a mechanism[2]. In any case, the chemo-attractant would be widely distributed in the Silkie fowl embryo. The question also arises whether fibronectin, which plays a major role in neural crest cell migration[4,16], is implicated in homing. As in quail embryo, neural crest derived cells, including prepigment ones, do not enter tissues even though they are fibronectin-rich[4]; the presence of fibronectin could be a prerequisite factor but not a sufficient one to ensure melanoblast seeding. Work is in progress showing that, by the stage of melanoblast homing, all the colonized organs are fibronectin-rich.

The broad but selective localization of melanoblasts in Silkie fowl could be of some help in studying the factor(s) playing a role in the seeding process and in the definite localization of neural crest cells, which remains a particularly challenging problem[2].

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Effects of 2-β-D-glucosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one on *Schizaphis graminum* (Rondani) (Insecta, Aphididae) feeding on artificial diets

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Summary. 2-β-Glucosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc), the main hydroxamic acid from maize and wheat, and its aglucone, decreased survival of *Schizaphis graminum* reared on artificial diets. Both compounds were toxic for aphids and acted as feeding deterrents, at concentrations as low as 1 mM. The natural concentrations of glucosides of hydroxamic acids in wheat leaves reach up to 6 mmoles/kg fresh weight, thus falling within the range in which DIMBOA-Glc causes deleterious effects to diet-fed aphids.
Key words. Hydroxamic acid; cereals; maize; wheat; aphids; greenbug.

Hydroxamic acids from Poaceae (Gramineae) seem to play a role in plant resistance to *Ostrinia nubilalis*[2], *Rhopalosiphum maidis*[3], *Metopolophium dirhodum*[4] and *Schizaphis graminum*[5]. DIMBOA, the main hydroxamic acid from maize and wheat extracts, decreases feeding, survival and reproduction rate of aphids reared on artificial diets[6,7]. However, DIMBOA is present in tissues as a glucoside (DIMBOA-Glc). The purpose of this work is to describe the effects on aphids of DIMBOA-Glc and to compare them with those of DIMBOA.

Experimental. Isolation of DIMBOA-Glc. Seedlings (*Zea mays* L. cv T129s) were grown in a greenhouse under permanent light at 30 °C. 7-day-old seedlings (950 g) were slowly added to 1000 ml of boiling water, keeping the temperature above 90 °C. After 15 min the seedlings were cooled down, homogenized and filtered through cheesecloth. The pH of the extracts was adjusted to 10 and the sample centrifuged at 10,000 × g for 10 min. The supernatant fluid was acidified (pH 3, HCl) and washed three times with diethylether (2:1 v/v ether:extract). The volume of the aqueous phase was reduced to 25 ml and added to 600 ml of methanol. The resulting suspension was filtered and the solid (22.9 g) discarded. The volume of the filtrate was reduced to 20 ml to which 100 ml of methanol were added. The solid formed (1.2 g) was removed by filtration and discarded. Acetone (300 ml) was added to the solution and the precipitate formed (17.6 g) was also removed by filtration. The solution was evaporated to dryness and dissolved in 50 ml of water:methanol 3:1 v/v.

This solution was added to a column (300 × 35 mm i.d.) of SP-Sephadex C-25 (Pharmacia) in the Fe(III) form and equilibrated with water:methanol 3:1 v/v. This column binds hydroxamic acids, turning a deep blue[8]. The column was washed with 1700 ml of the same solvent (33 ml/h). The sample was then eluted with 500 ml of water:methanol 3:1 v/v saturated with NaCl. The blue fractions were collected (158 ml). The iron was displaced from its hydroxamic acid complex by addition of 70 ml of 0.83 M EDTA pH 8.5. The precipitate formed was removed by filtration. The solution was neutralized with NaOH, concentrated and filtered. Aliquots of 6.5 ml were added to a Sephadex G-10 column (720 × 45 mm i.d.) previously equilibrated with $CHCl_3$-saturated water and eluted with the same solvent as described[9], with a flow rate of 13 ml/h, collecting 2-ml fractions.

Elution profiles were made by measuring the absorbance of the fractions at 260 nm, by treating the fractions with $FeCl_3$ and

measuring the absorbance at 590 nm, and by treating the samples with anthrone reagent for sugars and measuring the absorbance at 620 nm[10]. Only one peak which contained both hydroxamic acid and sugar was observed. This peak had an elution volume of 615 ml. Peaks with elution volumes of 424 and 475 ml co-chromatographed with Fe-EDTA complexes. The $FeCl_3$ and anthrone positive fractions were pooled and evaporated to dryness. The compound obtained (m.p. 250–251 °C) had equimolar amounts of hydroxamic acid and sugar, as determined by the $FeCl_3$ and anthrone tests, respectively. The compound was identified as DIMBOA-Glc by its UV, NMR and mass spectra, which were the same as those published[11,12]. The yield of DIMBOA-Glc was 1.3 g/kg of fresh tissue. DIMBOA was isolated as described[13].

Aphid assays. Individuals of *Schizaphis graminum* (Rondani) were collected from naturally-infested barley near Santiago and allowed to reproduce on plants of *Hordeum distichum* L. cv Fola Union kept under continuous light at room temperature. Feeding assays were made with diets placed between two layers of Parafilm M[14]. The diet was as described[15], plus DIMBOA-Glc or DIMBOA. When young aphids were used for experiments, they were 3rd and 4th instar nymphs. All experiments were performed at 28 °C under continuous light. SE were less than 10% and were omitted from the figures for simplicity.

Results. Survival and reproduction of aphids. DIMBOA-Glc and DIMBOA were offered separately to nymphs of *S. graminum* in diets (fig. 1). Both compounds decreased survival of aphids, DIMBOA being more lethal than DIMBOA-Glc. The concentrations necessary to produce 50% mortality were 4.0 and 1.2 mM DIMBOA-Glc and DIMBOA, respectively.

Adults of *S. graminum* were fed DIMBOA-Glc or DIMBOA and reproduction rates were measured (fig. 2). Both compounds decreased reproduction rate at concentrations as low as 0.25 mM.

Toxicity and feeding deterrence. Aphid nymphs of *S. graminum* were exposed to diets containing DIMBOA-Glc or DIMBOA and the fraction of aphids that were feeding on the diets was determined. Both compounds showed appreciable feeding deterrent activity even at the lowest concentration tested (0.5 mM).

Cohorts of nymphs of *S. graminum* were offered diets containing DIMBOA-Glc or DIMBOA and then transferred to diets without hydroxamic acids. Aphid survival was determined (fig. 3). The lowest survivals were observed with 6 mM DIMBOA-Glc and 4 mM DIMBOA. At higher concentrations both DIMBOA-Glc and DIMBOA have a strong feeding deterrent activity. Hence, ingestion of the diets with the compounds was limited and survival was higher. At intermediate concentrations more compound was ingested causing a lower survival of the aphids. Thus, DIMBOA-Glc and DIMBOA have both feeding deterrent and toxic activities on *S. graminum* feeding on artificial diets.

Discussion. Most of the knowledge about the activity of hydroxamic acids from maize and wheat on insects has been obtained by testing the effects of DIMBOA[2–7]. Since DIMBOA is

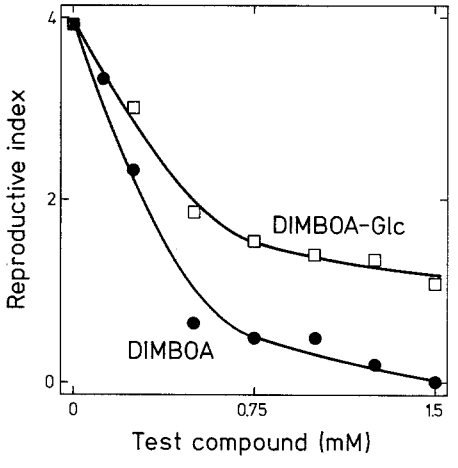

Figure 2. Effect of DIMBOA-Glc and DIMBOA on reproduction of adults of *S. graminum* reared on artificial diets. Reproductive index (number of nymphs born/number of adults alive) was determined after feeding adults for 50 h. Each point is the mean of five samples of five adults each.

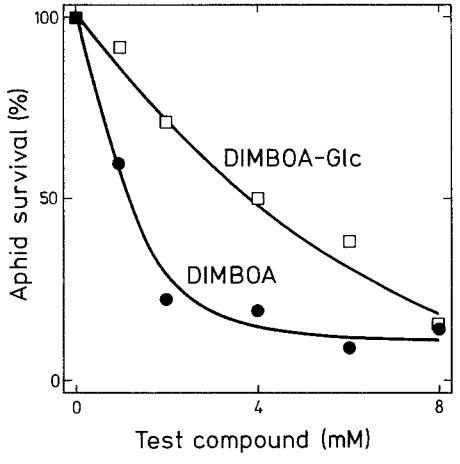

Figure 1. Effect of DIMBOA-Glc and DIMBOA on survival of nymphs of *S. graminum* reared on artificial diets. Survival, expressed as percent of initial individuals, was determined after 24 h of feeding. Each point is the average of three samples of 10 aphids each.

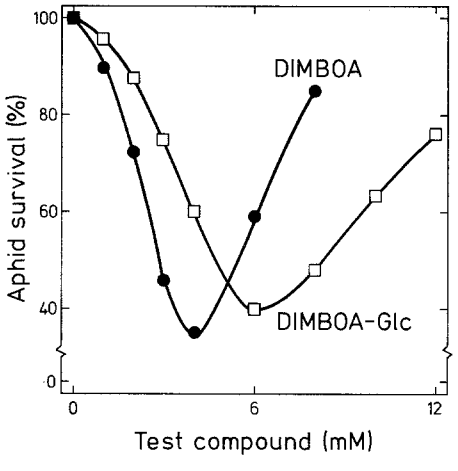

Figure 3. Effect on aphid survival of exposure for a limited time to DIMBOA-Glc or DIMBOA. Nymphs of *S. graminum* were exposed to diets with DIMBOA-Glc or DIMBOA for 12 h and then transferred to control diets without hydroxamic acids. Survival was determined 24 h later as a function of hydroxamic acid concentration in the initial diets. Each point is the average of three samples initially of 10 aphids each.

not found in intact tissues, the properties of the naturally-present DIMBOA-Glc may be important in plant-insect interactions. Although DIMBOA was always a more active molecule, DIMBOA-Glc also showed feeding deterrent and toxic activities on *S. graminum*. Since DIMBOA-Glc may be hydrolyzed to DIMBOA upon disruption of cells during insect feeding[11], the toxic and feeding deterrent properties of both compounds may be relevant to plant protection. The effects of both compounds on aphids fed on artificial diets were observed at a concentration range similar to that found in wheat leaves (0.1 to 6.3 mmoles/kg of fresh weight). Since an inverse correlation between hydroxamic acid content of leaves and aphid infestation level has been found[4,5], it is possible that varieties resistant to aphids may be obtained by selecting those with higher hydroxamic acid content. It is difficult to predict the concentration of hydroxamic acid necessary to achieve plant protection against *S. graminum*. However, it has been found that plants with more than 4 mmoles/kg of fresh weight in their leaves are not susceptible to *S. graminum* and *Metopolophium dirhodum*[4,6].

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Defensive steroids from a carrion beetle (*Silpha americana*)[1]

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Summary. The defensive anal effluent discharged by *Silpha americana* in response to disturbance contains a mixture of steroids stemming from a glandular annex of the rectum. The compounds have been characterized as 15β-hydroxyprogesterone (**1**, principal component), 5β-pregnan-15β-ol-3,20-dione (**2**), 5β-pregnan-3α,15β-diol-20-one (**3**), 5β-pregnan-7β, 15β-diol-3,20-dione (**4**), 5β-pregnan-3α, 7β, 15β-triol-20-one (**5**), 5β-pregnan-16α-ol-3,20-dione (**6**), and 5β-pregnan-3α, 16α-diol-20-one (**7**), none previously found in insects. Bioassays with jumping spiders showed compounds **1** and **6** to be feeding deterrents at the 1 μg level.
Key words. Coleoptera; chemical defense; steroids; pregnanes; isoprenoids.

Carrion beetles of the genus *Silpha* (family Silphidae) characteristically emit a malodorous ooze from the anus when disturbed. The effluent is strongly alkaline, rich in ammonia[4], and presumed to be defensive. We found that in *Silpha americana* the fluid also contains a mixture of pregnanes, derived from a glandular annex of the hindgut. We here report on the structure of these compounds, and on their feeding deterrency to an arthropod predator.

Beetles were collected by the hundreds at baits (dead fish and chickens) near Ithaca, New York, and Lake Placid, Florida. The rectal gland, first noted by Leydig[5], was revealed by dissection. It consists of a blind sac, opening directly into the rectum, and an elaborately subcompartmented diverticulum of the sac. The entire gland is lined with membranous cuticle, continuous with the lining of the rectum itself. Treatment of beetles with aqueous potassium hydroxide dissolves away tissues and results in isolation of the lining. The relationship of gland to rectum, and particularly the subcompartmentalization of the diverticulum, is clearly evidenced in such preparation (fig. 1). In the untreated gland the diverticulum is contiguous with the glandular sac, and its subcompartments are tightly appressed.

Several hundred glands were dissected from beetles freshly killed by freezing. They were extracted whole with a 1:1 dichloromethane-methanol mixture (ca. 15 μl per gland). Direct analysis of the extract by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) proved impractical, since the chief components were of low volatility, and appeared to suffer thermal degradation. Trimethylsilylation of the extract also gave disappointingly complex results as a consequence of variable degreees of silylation, as well as of the thermal instability of some of the products. Thin-layer chromatography (TLC) of the extract avoided these difficulties, and revealed the presence of one component with strong UV absorption, along with about a half dozen additional components. A completely satisfactory TLC separation, however, was difficult to achieve. Finally, reverse phase high performance liquid chromatography (HPLC), using a C-18 column and 45% acetonitrile–55% water as solvent, permitted the preparative separation of seven pure compounds. Each of these components was isolated by lyophilization of the liquid phase, and each was then subjected to spectroscopic analysis (electron impact, chemical ionization, and high resolution mass spectrometry, ^{1}H NMR and UV spectroscopy).

The major component in this mixture was the one exhibiting strong UV absorption (λ) EtOH/max 240 nm). High resolution MS established its molecular formula ($C_{21}H_{30}O_2$), and the low resolution MS data suggested that this compound might be a 15- or 16-hydroxylated progesterone[6]. Direct comparison of this material (MS, ^{1}H NMR, HPLC) with an authentic sample