Gossypol: Antagonistic Effect on Toxicity of Insecticides to *Spodoptera littoralis*

Cottonseed meal has been an important source of protein in ruminant diet for many years. However, its utilization by monogastric animals has been limited because of the unfavorable effect of gossypol[1]. Gossypol toxicity has been attributed to the uncoupling of respiratory chain-linked phosphorylation[2]. Gossypol occurs in the pigment glands of the seed, leaf, stem, laproof bark and roots of the cotton plant[3]. An ideal solution to this problem is the production of gossypol-free cotton, and such a variety *Gossypium barbadense*, was developed by irradiation[4]. The glandless charcater was dominant and simply inherited[5]. It differed in this respect from the glandless variety of *G. hirsutum*[6]. However, the potential of gossypol in cotton as a source of resistance to the bollworm, *Heliothis zea* (Boddie), and to the tobacco budworm, *H. virescens* (F.), was demonstrated[7,8]. Because of the possibility that low gossypol content might make cotton more susceptible to feeding by insect pests, we investigated the effect of gossypol on the toxicity of some insecticides to *Spodoptera littoralis* (Boisduval), the results of which are reported below.

Materials and methods. Purified gossypol (1, 1′, 6, 6′, 7, 7′-hexahydroxy-3, 3′-dimethyl-5, 5′-diisopropyl-2, 2′-binaphthyl-8, 8′-dialdehyde) was obtained from the Southern Utilization Research Development Division, USDA, New Orlean, Louisiana, USA. Leptophos (0-(4-bromo-2, 5-dichlorophenyl) 0-methyl phenylphosphonothioate), Cyolane (2-(diethoxyphosphenyl)-1, 3-dithiolane), Zectran (4-N, N-dimethylamino)-3, 5-dimethylphenyl N-methylcarbamate, and endrin (1, 2, 3, 4, 10, 10′-Hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo, endo-5, 8, dimethanonaphthalene) were obtained from their manufacturers. Specimens of *S. littoralis* were from a laboratory colony that had been reared for 15 generations on the semi-synthetic diet of LEVINSON and NAVON[9] as modified by BAKERY et al.[10]. In one experiment, unanesthetized 4th instars weighing about 80 mg each were topically treated on the dorsal region with acetone solutions of gossypol and the 4 insecticides using a micropipet syringe fitted to a micrometer (4 replicates, 25 each). Mortality counts were recorded 24 h after treatment and LD_{50} values were determined[11]. In a 2nd experiment the larvae were topically treated with a single subtoxic dose of gossypol (100 µg/g). 24 h after treatment with gossypol no mortality had occurred in the treated larvae which were then topically treated with different levels of test insecticides and the LD_{50} values determined as before.

Results and discussion. The Table gives the LD_{50} of gossypol and 4 insecticides against normal and gossypol-pretreated larvae. The results show that gossypol was toxic to *S. littoralis*. The toxicity of gossypol to *S. littoralis* is in agreement with that to *H. zea* and *H. virescens*[7,8] and to the pink bollworm, *Pectinophora gossypiella* (Saunders)[12]. The LD_{50} for each compound including gossypol was significantly increased by pretreating the larvae with gossypol. The increase in LD_{50} was not the same for all insecticides however. The increase was highest in the chlorinated insecticide, endrin (200%) followed by the organophosphate insecticide, Cyolane (154%) and the phosphorothioate insecticide, leptophos (123%). The increase in the LD_{50} of Zectran the only tested carbamate insecticide was 87%, while the increase in the LD_{50} of gossypol was the lowest (26%).

In the present investigation the insecticides used represented 3 different groups of insecticides: chlorinated, organophosphorus and carbamates. All organic insecticide chemicals, to a varying degree, metabolize in the living organisms. The extent and nature of the biotransformations vary with species and the chemical. The biotransformations mentioned are the result of various enzyme systems. In mammals, these systems are frequently localized in the microsomal fraction of the liver, while in insects the comparable system resides mainly in the fat body[13]. As for the mode of action of the antagonism of insecticide toxicity by gossypol two mechanisms are conceivable: 1. gossypol may reduce penetration of the insecticide entering the body; 2. gossypol may enhance detoxification of the absorbed insecticides. The 2nd assumption is in harmony with the finding that in gossypol fed rats the liver microsomal oxidases increased in activity[14].

Since gossypol decreased the toxicity of the tested insecticides, the effect of gossypol may be considered as an adaptive detoxification system. It seems that insects would be more sensitive to insecticide toxicity when fed gossypol-free cotton than glanded cotton. The results indicate that chemical control would be more effective on gossypol-free cotton.

Effect of gossypol on the LD_{50} of some insecticides against *S. littoralis*[a]

Compound	LD_{50} (µg/g)		Increase in LD_{50}
	Normal	Gossypol[b]	(%)
Leptophos	15	34	123
Cyolane	22	56	154
Zectran	160	300	87
Endrin	40	120	200
Gossypol	310	374	26

[a] Each larvae (4th instar) weighed 80 mg. [b] Each larvae was topically treated with 100 µg/g body weight of gossypol 24 h before determination of LD_{50}.

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Zusammenfassung. Nachweis, dass Vorbehandlung der Larven von *Spodoptera littoralis* (Boisduval) mit subtoxischen Dosen von Gossypol die Toxizität der lokal applizierten Insektizide Leptophos, Cyolan, Zectran und Endrin reduziert. Es wird angenommen, dass Gossypol die Toxizität dieser Insektizide neutralisiert, indem es deren Detoxifizierung durch die Larven stimuliert.

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Immunological Evidence for the Presence of Smooth Muscle-Type Contractile Fibres in Mouse Macrophages[1]

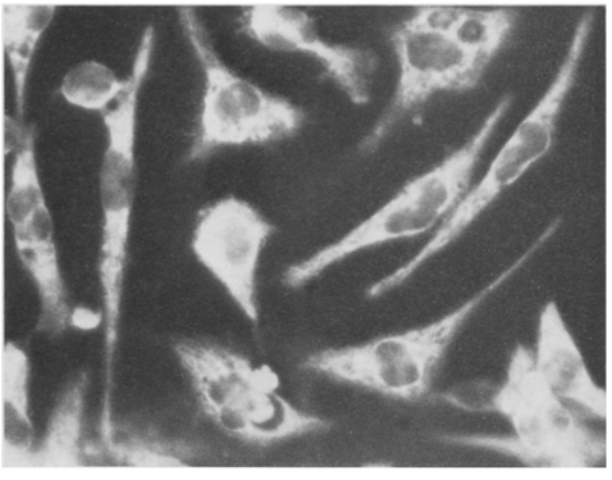
Proteins with properties similar to smooth muscle actomyosin, myosin and actin have been isolated from non-muscular cells such as rat sarcoma cells[2], blood platelets[3], leukocytes[4], and fibroblasts[5,6]. The presence of microfibrils in monocytes with dimensions similar to actin fibrils was first demonstrated by de PETRIS et al.[7], and it was later suggested that these are contractile[8].

It was recently shown by the senior author that antibodies prepared against actomyosins from smooth muscle, although species non-specific, are muscle-type specific and are capable of reacting with contractile elements from non-muscular cells[9,10]. We assumed that the use of such antibodies tagged with fluorescein might assist in localization of contractile fibrils in macrophages.

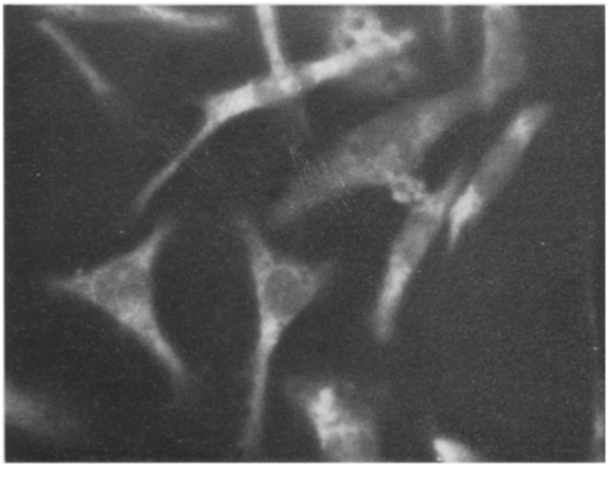
Mouse peritoneal macrophages were harvested and allowed to attach to glass slides according to a modification of GILL and COLE's method[11]. After 16 h of incubation (37 °C, 5% CO_2 in air) the cells were fixed for 30 sec in icecold methanol, and then incubated with the γ-globulin enriched fraction of fluorescein-tagged anti-chicken gizzard (smooth muscle) myosin, anti-chicken cardiac (striated) muscle myosin and anti-chicken actin. (The latter was previously shown not to be muscle-type specific[12].) Bright cytoplasmic staining, excluding the nuclear region, was observed when antisera against smooth muscle myosin or actin were applied (Figure 1a). Antibody against striated muscle myosin did not stain beyond the usual background obtained with non-immune control globulin (Figure 1b).

When isolated and washed macrophages were incubated in the presence of fluoresceinated antibodies against smooth and striated muscle myosins or non-specific γ-globulin, the cells were attached and showed no signs of toxicity after 16 h. Diffuse cytoplasmic fluorescent staining was not observed with any of the three antisera; however, many of the cells showed fluorescent staining of small vesicles near the surface of the cell (Figure 2).

a)



b)



← Fig. 1. Methanol-fixed mouse macrophages stained with fluoresceinated antiserum against: a) chicken actin: diffuse cytoplasmic fluorescence. ×1,000. b) chicken striated cardiac muscle myosin: background fluorescence only. ×1,000.

[1] Dedicated to Prof. H. SCHWALM, Würzburg, in honor of his 70th birthday.

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