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Bioanalogues of juvenile hormones and intestines mycoflora of some insects

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The subject of this paper is the influence of JH bioanalogues on the mycoflora of intestines of three species of inscets: *Dysdereus cingulatus*, *Pyrrhocoris apterus* and *Tenebrio molitor*. The results are presented in tables.

Hormones undoubtedly belong to the most important biological-active compounds exhibiting high selectivity of action. Their action, controlling almost all reactions proceeding in a living organism, determines the corectness of its development. On the other hand, factors disturbing the hormonal administration cause stable irreversible changes.

A new direction of research concerning the insect juvenile hormones has developed during recent years creating possibilities of their application as "3rd generation insecticides" (Williams 1956; 1967). Synthetic bioanalogs of juvenile hormones exhibit an activity analogous to that of natural compounds produced in an insect body by so-called corpora allata. During the postembryonal growth of insects, changes in the endogeneous level of natural JH, regulating essentially the efficient course of metamorphosis, are observed (Slama 1971; Sehnal 1971). The introduction of a synthetic bioanalog of JH on the body surface or to the intestines during the suitable development stage of an insect results in morphogenetic changes manifesting themselves in the formation of intermediate forms, so-called adultoids, and of additional larval stages, so-called superlarvae, incapable of further development and reproduction (Slama 1974).

Juvenile hormones and their bioanalogs are characterized by the high

selectivity of action, thus man has gained a new weapon in the fight against economically harmful species of insects — the factor acting on exactly one definite species and apparently not disturbing the balance of nature.

The first compound from the juvenoids group which in obtained 1975 the permission of the Agency of Environment Protection of USA for sale the was isopropyl ester of ZE, 4E-11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienic acid called Metopren (D j e r a s s i 1974-75). It was applied in preparations of the Altosid type for exterminating larvae of mosquitoes and other *Diptera,* including the housefly.

There are many methods for determinating the biological activity of JH bioanalogs. They are similar to methods used in investigations of hormonal effects in living cells. Thus, the method of estimation of ED_{50} (effective dose 50%), ID_{50} (inhibition dose 50%), ID_{50}-Ovic. (inhibition dose 50% Ovicidal) and many others are applied. The action of juvenoids is estimated in all cases on the basis of external morphogenetic effects on the insect body caused by them. The field experiments of application of new compounds of the juvenoid type have shown that insects from the natural environment react to these compounds in a different manner than insects raised in a laboratory for years. Contrary to the standard laboratory conditions, the population of insects whose growth proceeds in the natural environment is more or less heterogeneous. Various conditions in which the populations of definite species of insects grow in the natural environment can essentially influence the effectiveness of the action of juvenoids. Thus, the relationships between laboratory and field effectiveness of these compounds action of various stabilitie should be considered (S l a m a 1974).

There are hypotheses that insects owe the resistance to the action of juvenoids to symbiotic microorganisms living inside or on the surface of their bodies (W i g g l e s w o r t h 1974, S l a m a et al. 1971). Their metabolic products, gathering in the insect body, can affect the metabolism of its own cells which can next control the reactions of the organism on the external impulse, the action of bioanalogs of juvenile hormones. This thesis should, of course, be confirmed by experiments which are the subject of this work. They were limited to the study of mycoflora of three insect species tested for the action of juvenoids. The work presents a preliminary analysis of flora of fungi existing in the intestines of these insects. The studies should give answers to a series of questions: has this flora any definite character, is it specific for each tested species, and does it change under action of the definite JH bioanalogs? The known phenomena of the stable biological relationships between insects and fungi allow us to consider the problem in such

a way. For example, the fungal species from the genus *Termitomyces* lives exclusively inside the heaps of termites (Heim 1942). The mycelium of a representative of the *Septobasidiaceae* family forms the specific environment for some insect species from the family *Coccideae* (Couch 1938). Fungi from the order *Laboulbeniales*, living on the surface of wing-sheaths, are also closely bound with the life of insects. These fungi are, in the opinion of many specialists (Gäumann 1964 and others), ectoparasites decomposing chitin and they exhibit more rarely a tendency to the parasitism of internal organs of insects. Gams (1971) mentioned a series of saprophytic species, living on media rich in chitin, in the monography of fungi sporulating according to the *Cephalosporium* type. They are fungi in which the boundary between the saprophytic and parasitic phase is labile. They exist on the body surfaces of arthropods and some of them become typical parasites. Then their mycelium overgrows the tissues of larvae, pupae, or imago stage. The species from the genus *Verticillium* and *Aphanocladium* are most often mentioned in the literature. These fungi especially prefer the chitin insect bodies for their development or they live in the intestines of insects as saprophytic mycoflora.

MATERIALS AND METHODS

The studies were carried out on three insect species: *Dysdercus cingulatus* L. and *Pyrrhocoris apterus* L. from the family *Pyrrhocorideae*, and *Tenebrio molitor* L. from the family *Tenebrionideae*. The two former species were raised in glass jars at ±24°C and at a relative humidity of 70-75%. *D. cingulatus* were fed with cotton seeds, *P. apterus* — with *Tilia* seeds. The cultivation of *Tenebrio molitor* was carried out at ±27°C and at 70-80% of relative humidity. Their diet contained bran, yeasts, flour, and flaked oats.

The normally developed insects in the imago stage, the fifth larval stage, pupae (for *T. molitor*), and adultoids, resulted from the topical application of 1 µl of acetonic solution of juvenoid on the cuticle of a freshly moulted larva or pupa (0-20 hrs after moulting for *D. cingulatus* and *P. apterus*, 1-24 hrs — for *T. molitor*), which were used for isolation. Fungi were isolated from individual insects after their superficial desinfection (washing with a 40% alcohol solution) under sterile conditions. Skeletonized fragments of the intestines of an insect were laid out in Petri-dishes with the agar medium. The appearing colonies of fungi were put on the agar slants and then purified by the method of dilution into one-spore cultures. The growth and development of the fungal colonies were carried out at about 26-28°C. The pure cultures

Table 1

Isolated species of fungi

Developmental stages of insects species and theirs intermediate forms after treatment of JH analogues		Number of intestines isolates	Number of fungi isolates	Isolated species of fungi and number of isolates														
				Aphanocladium album Gams	*Aspergillus flavus* Link	*Aspergillus wentii* Wehmer	*Mucor circinelloides* v. Tiegh.	*Mucor heterosporus* Fisch.	*Mucor spinosus* v. Tiegh.	*Mortierella mutabilis* Linn.	*Mortierella* sp.	*Penicillium citrinum* Thom	*Penicillium diversum* var. *aureum* Rap. et Fenn.	*Penicillium janthinellum* Biourge	*Penicillium variabile* f. A	*Penicillium* sp. 1	*Penicillium* sp. 2	*Rhizopus nigricans* Ehr.	*Spicaria divaricata* Gilman et Abbott
Dysdercus cingulatus L.	imago	60	59		2	2							5	15	27				8
	V instar	42	31		6										23		2		
	intermediate forms (adultoids)	12	2												2				
	JH-III 80 µg/spec.																		
	JH-III 20 µg/spec.	6	2												2				
	JH-III 1 µg/spec.	6	5												5				
	KD-215 80 µg/spec.	12	—																
	MW-178 4 µg/spec.	12	8												8				
	MW-178 16 µg/spec.	12	8												8				
	WP-146 80 µg/spec.	6	4												4				
	MW-178 8 µg/spec.	6	—																

Pyrrhocoris apterus L.	imago	48	44				5		12	6		6			10	5				
	V instar	6	3					1			1	1								
	intermediate forms (adultoids)	6	—																	
	KD-215 8 µg/spec.																			
	JH-III 80 µg/spec.	6	4												4					
	KD-215 80 µg/spec.	6	4									4								
Tenebrio molitor L.	imago	60	50	18			12			8								12		
	pupa	12	—																	
	intermediate forms (adultoids)																			
	KD-215 80 µg/spec.	24	5	5																
	WGU-108 80 µg/spec.	30	4															4		
	JH-III 20 µg/spec.	6	4																	
	JSz-152 8 µg/spec.	6	—																	

Table 2

Comparison colony *Penicillium* from series of *Purpurogenum*

Species	Diameter (after 14 days)		Surface	Colour		Odour	Drops of exudate liquid	Colour of Čapek agar		Sterigmata	Conidia
	Čapek	Malto		Čapek	Malto			reverse	medium		
P. purpurogenum	2,5-3,5	2,5-3,5	velvety or floccose	pale yellow-green, lily green to dull greenish black	olive-green, yellow green	slightly moldy	orange-red shades	deep red, blood-red	light red	10-12× ×2-2,5 tapered	3-3,5× ×2,5-3 rough or almost smooth
P. rubrum	1,0-2,0	6,0-6,5	zonate velvety	yellow to grey-green nonsporulate areas orange-red	olive-green gray-green	indistined	limited bright red	cherry-red	lighter tinst of the same shades	8-12×2-2,5 tapered	2,2-3,5× ×2-2,5 smooth
P. aculeatum	2-2,5	5,0-6,0	wrinkled velvety	yellow-green, artemisia green with pinkish cast	dark yellow green to dusky olive-green	almost lacking	abundant uncolored to vinaceous	purplish-red	not strongly discoloring	7-9×3 somewhat swollen	3-3,5 heavy echinulate

P. variabile	2,5-3,0	3,0-3,5	radially furrowec velvety granular	sporulate areas white to yellow shades: sage green to storm gray, non sporulate areas cream yellow to orange buff shades	dull yellow-green to gray-green shades	not pro nounced	lacking or limited, clear	yellow to orange-brown	not co-lored	10-12×2,2 lanceolate	3,0-5,0× ×2-2,5 elliptical smooth
P. variabile f.A	3,5-4,0	2,0-3,0	radially furrowed velvety or floccose	sporulate areas: blue green to storm green: non sporulate areas: cream yellow with pinkish cast	gray green with white or dulle yellow margin	strong honey-sweet	aboundant orange-red to vina-ceous	brown-red	pinkish	8-10×2-3 tapered	3-4,5× ×2-2,5 smooth

were included in definite genus or species using corresponding keys or treatises of the monography type. The following compounds, designated with conventional symbols in Fig. 1, were used as the bioanalogs of juvenile hormones.

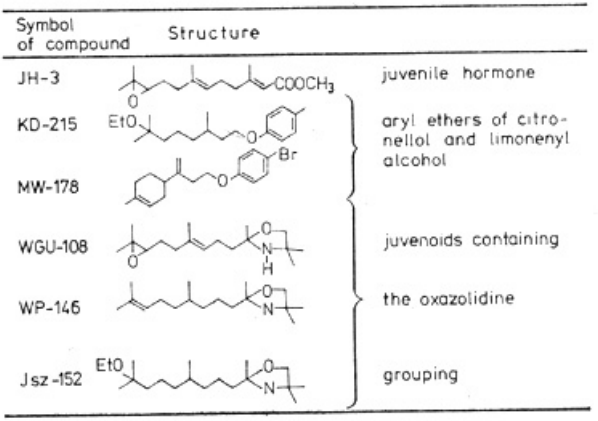

Fig. 1

Media used for the cultivation of fungi

Agar malto — 25 g of maltose extract, 1000 ml of distille water, 15 g of agar.

Čapek's agar — 1000 ml of distilled water, 3.0 g of $NaNO_3$; 1.0 g of K_2HPO_4; 0.5 g of $MgSO_4 \cdot 7H_2O$; 0.5 g of KCl; 0.01 g of $FeSO_4 \cdot 7H_2O$; 30.0 g of saccharose; 20.0 g of agar.

RESULTS OF EXPERIMENTS

396 isolations of fragments of intestines of three insect species under investigation were carried out (186 for *Dysdercus cingulatus*, 72 for *Pyrrhocoris apterus*, and 138 for *Tenebrio molitor*); 243 incula, belonging to 16 species of fungi, were received from them (Table 1). The species from the genus *Penicillium* occurred most of ten among them (6 species). The species designated provisionally as *Penicillium variabile* f. A was isolated predominantly. Comparison of this form with other species of the series *Purpurogenum* is presented in Table 2. The species from the family *Mucoraceae* (*Mucor circinelloides*, *M. spinosus*, *Rhizopus nigricans*) and *Aphanocladium album* producing spores according to the *Cephalosporium* type were isolated in small numbers.

The results obtained allow some conjectures to be made:

1. Certain specificity of fungal species living inside intestines of

definite insects can be observed. Among seven species isolated from intestines of *Dysdercus cingulatus*, only two are common to those living in *Pyrrhocoris apterus*, not one, however, occurred in the isolates from *Tenebrio molitor*.

2. A distinct difference occurs in the number of fungal species isolated from the imago and adultoid stage. In the last case the fungal flora is markedly poorer or even nonexistent. In the case of *Dysdercus cingulatus*, only one species (*Penicillinum variabile* f. A) was isolated from adultoids. From the adultoids of Pyrrhocoris apart from this species *P. citrinum* was obtained additionally. Adultoids of *Tenebrio* had two fungi (*Rhizopus niger* and *Aphanocladium album*) in intestines.

3. While the intestines of two species, *Pyrrhocoris apterus* and *Dysdercus cingulatus* belonging to the same family *Pyrrhocorideae*, were a suitable environment for the development of the ubiquitous species *Penicillium*, these fungi were not found in *Tenebrio molitor*. The greatest number of isolates from this species belonged to *Aphanocladium album*, the fungus mentioned in the literature (G a m s) as a chitinophilous species.

4. The imagoes and larvae of *Pyrrhocoris apterus* had the most differentiated composition of fungi (9 species), the imagoes of *Tenebrio molitor* — the poorest one (4 species).

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