Wild-type (*Oregon R*) and *st* mutant strains were grown, according to KELLER and GLASSMAN[11], on different concentrations of HPP: 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08 and 0.10 g%. Adult flies were taken 2–3 days after they emerged and placed in boiling water for 1 min; their heads were dissected and squashed on Whatman No. 1 filter paper. The chromatograms, developed by ascending chromatography in *n*-propanol/2% ammonium acetate (1:1) in water, according to VISCONTINI et al.[12], were observed in the visible light and under an UV-lamp, emitting mainly at 365 nm. An arbitrary visual grading system (from '0' to '4' units) was applied to quantitate the results.

The concentration of HPP required to obtain 50% of adult flies with phenocopy eye colours was about 0.08% in the case of *Oregon R* and about 0.03% for *st*.

As the most evident modification produced by HPP, the chromatograms revealed a partial loss of the red eye pigments roughly proportionate to the concentration of the inhibitor. However, the relative amounts of individual drosopterins were not affected to the same degree: neodrosopterin appeared to be the most susceptible component, i.e. its loss was much more prominent than the loss of drosopterin and isodrosopterin, so that the latter substances accounted for a larger proportion of the total red pigments in the flies raised on effective concentrations of HPP as compared to the normal flies. Moreover, no observable change of the yellow eye pigments (sepia- and isosepiapterin) accompanied the loss of the drosopterins.

It seems, therefore, that the phenocopy eye colours, produced by HPP, can be ascribed to the marked drop of the reddest neodrosopterin and to the relative prevalence of the orange coloured drosopterin and isodrosopterin and of the yellow sepia- and isosepiapterins.

Riassunto. Esemplari di *D. melanogaster* di tipo selvaggio o del mutante *st*, allevati su terreno contenente l'inibitore della xantino-deidrogenasi 4-idrossipirazolo-(3,4 d)pirimidina (HPP), presentano l'occhio colorato in arancione scuro o in giallo. Cromatograficamente è stato possibile precisare che siffatte modificazioni dipendono dalla diminuzione dei pigmenti rossi contenuti nell'occhio, diminuzione che risulta molto più accentuata nel caso della neodrosopterina, il cui colore proprio è più rosso di quello degli altri pigmenti.

P. BONI, B. DE LERMA and G. PARISI[14]

Istituto di Biologia e Genetica dell'Università di Napoli (Italy), 10th November 1966.

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Fungistatic Action of Aflatoxin B_1

BURMEISTER and HESSELTINE[1] demonstrated that growth of some bacteria is inhibited by aflatoxin. The toxicity of aflatoxin towards microorganisms has been investigated further by examination of the effect of aflatoxin B_1 on *Flavobacterium aurantiacum*[2]. Although the molecular site of inhibitory action has not been established, it has been shown that aflatoxin contains a coumarin nucleus[3]. KNYPL's[4] report that coumarin inhibits germination and growth of certain fungal spores prompted experiments designed to measure the fungistatic capacity of aflatoxin B_1 towards certain organisms.

2 nutrient media were employed. One (A) a modified Czapek's broth containing glucose, 45 g; $NaNO_3$, 4.5 g; KH_2PO_4, 3.0 g; KCl, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; $FeSO_4$, 0.02 g; tap water to 1 l. The other (B) contained the same constituents as (A) except that 5.0 g/l of yeast extract was substituted for the $NaNO_3$.

The fungi were grown by inoculating $2.5 \cdot 10^6$ fungal spores into 25 ml of culture medium A or B in 300 ml Erlenmeyer flasks. Incubation was carried out for a specific time on a rotary shaker at 30 °C. Dry weights were measured by harvesting the flask contents and drying them for 12 h at 110 °C.

Growth of several species of *Aspergillus* and *Penicillium* was inhibited by aflatoxin B_1 (Table) when grown in medium A. It is noteworthy that mycelial growth of strains of *A. flavus* was inhibited by B_1, since these organisms produce aflatoxin. Strain NRRL 3239 of *A. flavus* is a poor aflatoxin producer whereas strains NRRL 3000, NRRL 2999, and NRRL 3240 elaborate significant quantities of the toxin. Since growth of both the poor aflatoxin-producing strain and the actively producing strains is inhibited by B_1, it appears that capacity for toxin production is not directly linked to aflatoxin sensitivity.

Growth of the following organisms was not significantly inhibited by aflatoxin B_1: *A. terreus* NRRL 1967, *A. ochraceus* NRRL 408, *A. niger* NRRL 3, *A. clavatus* NRRL 1.

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Examination of the inhibitory capacity of coumarin had shown that substituting yeast extract for sodium nitrate as the nitrogen source reversed the retardation of growth[4]. In our experiments, growth inhibition due to B_1 observed in medium A could be reversed by substituting the yeast extract medium B. Figure 1 shows the growth pattern of *A. flavus* NRRL 3240 at various levels of aflatoxin B_1 cultured in medium A; Figure 2 demonstrates growth of the same organism under identical conditions but with culture medium B. A 10 ppm level of aflatoxin in medium B gave results similar to the concentrations in Figure 2. The B_1 appears to extend the lag period (Figure 1) in medium A followed by a subsequent recovery of developmental activity. Thus, it seems that the sensitive organisms are capable of adapting to the B_1. However, mycelial growth in yeast extract medium B was not affected by aflatoxin B_1.

Aflatoxin B_1 has been implicated as an in vivo inhibitor of both nucleic acid metabolism and protein synthesis[5–7]. Therefore, it appears likely that the yeast extract component responsible for reversal of inhibition is related to one of these 2 functions. This question remains to be investigated.

Effect of aflatoxin B_1 on fungal growth

Organism	Aflatoxin B_1 (ppm)	Dry weight[a] (mg)	Inhibition (%)
Aspergillus flavus	0	80	
NRRL 3000	20	35	56
Aspergillus flavus	0	96	
NRRL 2999	20	36	62
Aspergillus flavus	0	74	
NRRL 3240	20	35	53
Aspergillus flavus	0	90	
NRRL 3239	20	26	71
Aspergillus awamori	0	156	
NRRL 2042	20	10	93
Penicillium chrysogenum	0	80	
NRRL Q-176	20	50	38
Penicillium duclauxi	0	112	
NRRL 2020	20	29	74

[a] Dry weights were determined after 48 h of growth in 25 ml of culture medium A in 300 ml Erlenmeyer flasks on a rotary shaker at 30 °C. Dry weight values are the mean of 3 runs and are significantly different at the 95% confidence level.

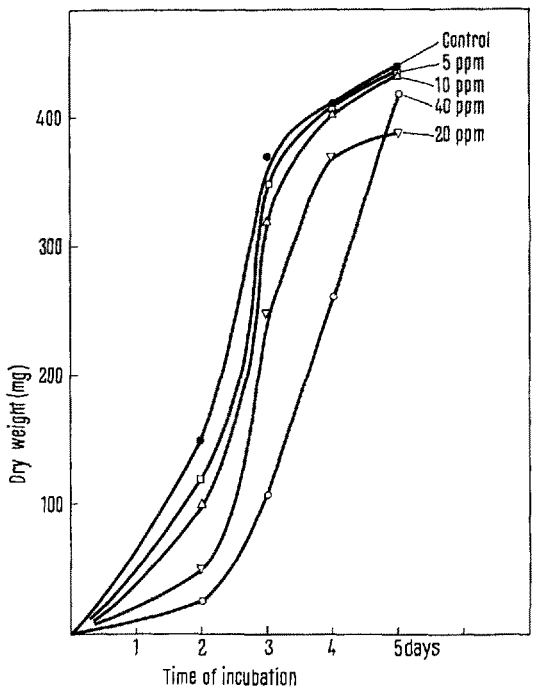

Fig. 1. Effect of aflatoxin B_1 on growth of *Aspergillus flavus* NRRL 3240 cultured in medium A. 25 ml of medium inoculated with $0.5 \cdot 10^8$ spores. Entire flask contents harvested for dry weight determinations at the designated times.

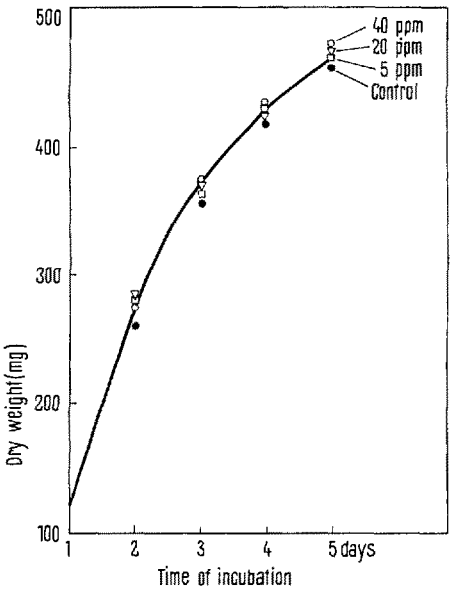

Fig. 2. Effect of aflatoxin B_1 on growth of *Aspergillus flavus* NRRL 3240 cultured in medium B. 25 ml of medium inoculated with $0.5 \cdot 10^8$ spores. Entire flask contents harvested for dry weight determinations at the designated times.

Résumé. La croissance des plusieurs espèces d'*Aspergillus* et de *Penicillium* dans un milieu modifié de Czapek a été inhibitée par l'aflatoxin B_1. Cet arrêt de croissance pourrait être annulé en substituant au nitrate de sodium un extrait de levure comme source d'azote.

E. B. LILLEHOJ, A. CIEGLER and H. H. HALL

Northern Regional Research Laboratory[8], *Peoria (Illinois 61604, USA), 26th August 1966.*

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