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ESTERASE ISOZYMES IN *TRIBOLIUM CASTANEUM* (COLEOPTERA, TENEBRIONXDAE) : POTENTIAL USES IN ETHOLOGICAL AND ECOLOGICAL STUDIES OF STORED-PRODUCT INSECTS

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SUMMARY. — An esterase polymorphism in *Tribolium castaneum* has been detected and studied during ontogenesis. The presence of a biochemical marker throughout development offers many possibilities to follow the development of pest infestations in storage systems and to analyze flow of genes conferring resistance to insecticides into this closed ecosystem.

RÉSUMÉ. — *Polymorphisme des esrérases chez* Tribolium castaneum *(Coleoptera, Tenebrionidae) : possibilité d'utilisation en éthologie et en écologie des insectes des denrées entreposées.* — Un polymorphisme au niveau des estérases chez *Tribolium castaneum a* été mis en évidence et a. été suivi au cours de l'ontogénèse. La présence d'un marqueur biochimique à tous les stades de développement de *T. casraneum* offre de nombreuses possibilités pour comprendre la migration et le mouvement d'insectes dans la filière de stockage ainsi que 1' impact de ces phénomènes sur l'évolution de la résistance aux insecticides chez les ravageurs des denrées entreposées.

INTRODUCTION

Resistance to the stored-product protectants is a worldwide phenomenon.. Malathion resistance in the *flour* beetle (*Tribolium castaneum*) is virtually universal and has been recorded in at least 70 countries (CHAMP & DYTE 1976). CHAMP & CAMPBELL-BROWN (1970) found that resistance to malarhion in T. *castaneum* was govemed by a single autosomal semidominant gene.

T. castaneum is a cosmopolitan pest of cereal mills and generally of any places where flour is stored. Its region of origin is unknown as are its natural habitats. In addition to its economic importance, this insect is largely studied because it is easy and. cheap to rear in the laboratory. The developmental time is fairly short (25 days at 32° C and 70% RH) and reproduction takes place the whole year round without diapause.

As a result of the development of many biochemical techniques, some biochemical markers can now be utilized for studying the biology and ethology of insects. In resolving problems associated with insect pests in agriculture, bio-

chemical characters have proved to be very successfull to reveal and to monitor resistance to insecticides in *Myzus persicae* (Wo)moptera, Aphididae) (DEVONSHIRE 1977) and *Culex pipiens* (Diptera, Culicidae) (PASTEUR et *al.* 1981) as well as to unravel the reproductive behaviour of *Diabrotica virgifera* (Coleoptera, Chrysomelidae) (RUUD *et al.* 1988). Detection of esterase polymorphism in *T. castaneum* offers many possibilities to understand the development of pest infestations in storage systems and to study the malathion resistant gene flow into this closed ecosystem.

MATERIAL AND METHODS

Insects. Seven strains of the flour beetle were used in this study. Two strains specifically resistant to malathion were obtained from the Natural Resource Institute in Chatham, England. They have been collected in storage systems in The Philippines and Botswana and kept in the labaratory of this Institute for several generations. One malathion non-specific resistant strain (CTCl2) was obtnined from the cultures of the Department of Zoology, George S. WISE Faculty of Life Sciences. Tel Aviv University. Tel Aviv. Israel. Finally four populations susceptible to malathion have been collected in storage places by the authors at Jamagne (Belgium), Bordeaux (France), Abidjan (Ivory Coax) and Nioro (Senegal). The insecrs were reared wirh whole wheat flour enriched with brewer yeast and kept in the darkness at 30° C and 60% of relative humidity.

Enzyme preparation. 100 adult insects of each strain were respectively ground in a mortar containing 1 ml of phosphate buffer (0.05 M, pH 7.2) with 10mM EDTA. The mixture was filtered through glass wool; the filtrate was stored in Eppendorf tubes at 4° C. and centrifuged at 20.000 g. 4° C for 15 minutes. Finally a sample of 20 µl was poured in a gel well.

Gel electrophoresis preparorion. Non denaturing discontinuaus polyacrylamide gel electrophoresis (PAGE) was used to examine various esterase isozymes. The gels have a thickness of 0.75 mm. The separation gel (7.5% acrylamide) was firstly prepared with 3.8 ml of acrylamide stock solution (containing 29.1% acrylamide and 0.9% N,N'-methylene-bis-acrylamide). 8 ml of distilled water. 3 ml of Tris-HCl buffer (1.875 M, pH 8.8). 10 µl of N,N,N',N'-tetramethylethylenediamine (TEMED), 50 µl of 10% ammonium persulphate and 74 µl of 0.5% Triton X-100. This first gel was left 60 minutes for polymerization and then the concentration gel (5% acrylamide) was prepared with the following ingredients : 0.8 ml of acrylamide stock solution, 3.6 ml of distilled water, OS mi of Tris-HCl buffer (1.25 M, pH 6.8), 7 µl of TEMED and 15 µl of ammonium persulphate.

PAGE Electrophoresis. Vertical electrophoresis was performed at 4° C under 20 mA and 120 V for approximatively 2 hours until the tracking dye (bromophenol blue) has migrated to the end of the gel.

Esterase staining. After completion of electrophoresis. the gel was equilibrated for 10 minutes in the dark, in a Tris-HCl buffer solution (0.1M, pH 7) containing 10 mM EDTA. Then the buffer was replaced by a solution containing 200 mg of 1-naphtyl acetate, 200 mg of 2-naphtyl acetate, 20 ml of acetone and 80 ml of Tris-HC1 buffer (0.1M, pH 7). This mixture was agitated for 5 minutes in the dark. Then 100 mg of Fast Garnett RR salt was added and agitation was continued for 30 minutes. Finally, the gel is transierred to 7% acetic acid for 24 hours as a fixation step.

RESULTS

Adults of six out of the seven populations possess two distinct isozyme groups labelled 1 and 6 on figure 1 : insects of Belgium (lane 1), Israël (lane 2). Botswana (lane 3), The Philippines (lane 4), Senegal (lane 5) and Ivory Coast (lane 6). However the two strains specifically resistant to malathion that are located at the third and fourth fanes. display a weaker activity for the esterases of the group 1 than the samples of lanes 1, 2. 5 and 6. (figure 1).

The French population (lane 7) which is susceptible to malathion, has a different esterase isozyme pattern. A highly stained group of bands appears at the position 4 (figure 1).

The activity of the esterase isozymes has also been analyzed throughout rhe developmental stages of *T. castaneum* of the Philippines and rhe French breeds (figure 2). For insects from The Philippines, it appears that the activity of the esterases 1, 3 and 4 is very low during the egg instar but increases during the larval stage. In pupae the esterases are again weakly stained while they reappear strongly in adults. The French insecrs have a different pattern : esterases 1 and 3 are hardly visible during all stages of devel-

opment but esterase 4 is very active from egg to adult. As a consequence, the latter esterase is a specific biochemical marker for the population fro m Bordeaux.

DISCUSSION

PAGE electrophoresis enables rapid resolution of the different groups of isozymes as well as direct detection of esterase polymorphism of *T. castaneum*.

The seven populations of *T. castaneum* that have been examined in this study are sorted out in two groups in respect of their esterase isozymes. On the one hand there are two groups of isozyme in the insects from Belgium, Israël, Botswana, The Philippines, Senegal and Ivory Coast . On the *orher* hand the French breed is characterized by an other pattern of bands (figure 1). COHEN *et al.* (1977) and SVERDLOV *et al.* (1976) have also observed two different esterase patterns with four groups of isozymes. More recently COHEN (1952) has made an electrophoretic analysis of 19 strains of *T. castaneum* and he did not detect any orher esterase polymorphism in this species.

It is also obvious that the first group of six populations is not homogeneous regarding the activity of the isozyme group number 1 : thc esterase of the two populations specifically resistant to mnlathion are less stained than the corresponding enzyme in the flour bectle from Belgium, Israël, Senegal and Ivory Coast. BEEMAN & SCHMIDT (1982) have observed the same results for malathion specific resistant strains of *Plodia interpunctella* (Lepidoptera, Pyralidae). They attributed this phenomenon to a mutation at an esterase gene. The mutant esterase has an aitered substrate specificity including increased activity toward malathion.

Studies of biochemical mutaants that have been performed on *T. castaneum* arevery limited : YEH & SCHEINBERG (1972) have observed an alcohol dehydrogenase polymorphism ; BREMMER *et al.* (1980) have reported on amylase polymorphism.

Insects and mites are responsible for adulteration of alimentary productsand they cause yearly losses estimated at about 30% of 1800 millions tons of stored grain (BULOT 1990). Moreover intensive control of theses pests with pesticides such as phosphin and malathion triggered the development of resistant strains. As trade of cereals in the world implies circulation of freight from one storage place to another it allows dispersion of pest from different geographic areas and results sometirne in the introduction of susceptible strain in silos already contaminated with insects with single locus mechanism resistance to pesticides. Theoretically such introduction will increases the frequency of sensible alleles in the population. However heterozygotes for the resistance characrer will also appear that growth faster than resistant homozygotes (GEORGHIOU & TAYLOR 1977, COMINS 1977). Beyond these general statements, dispersion of pest has attracted very little attention and it is therefore difficult to predict what could be the influence of such transfers on the development of resistancr in pests of stored products. The French breed that is biochemicaly marked by the esterase isozyme 4 throughout all the life stages will be a valuable material to improve our knowledge of population ecology of the pests living in storcd products. Experimental designs combining the French strain with a second breed biochemically different from the first will probably dissipate some clouds over the population ecology of *T. castaneum* in silos of cereals.

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