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Peptidase Polymorphism in Natural Populations of the Cocoa Pest, *Helopeltis theobromae* Miller

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ABSTRAK

Helopeltis theobromae *yang ditangkap daripada tanaman koko di Kuala Selangor dan daripada tanaman* ***Acalypha*** *di Serdang, Selangor, Malaysia telah disiasat dengan teknik elektroforesis untuk sembilan penanda biokimia iaitu: peptidase, α-gliserofosfat dehidrogenase, 6-fosfoglukonat dehidrogenase, xanthina dehidrogenase, aldehid oksidase, fosfoglukosa isomerase, isositrat dehidrogenase, glutamat oksaloasetat transaminase dan adenilat kinase. Hanya peptidase-2 menunjukkan polimorfisma yang dapat dibincang dengan mudah.*

ABSTRACT

Helopeltis theobromae *caught off cocoa plants in Kuala Selangor and off* ***Acalypha*** *plants in Serdang, Selangor, Malaysia were investigated electrophoretically for nine biochemical markers: peptidase, α-glycerophosphate dehydrogenase, 6-phosphogluconate dehydrogenase, xanthine dehydrogenase, aldehyde oxidase, phosphoglucose isomerase, isocitrate dehydrogenase, glutamate transaminase and adenylate kinase. Only peptidase-2 polymorphism could be easily interpreted.*

INTRODUCTION

Helopeltis theobromae Miller (Hemiptera: Miridae) or the mosquito bug is a serious pest of cocoa in Malaysia. The adults and nymphs attack both the pods and shoots of cocoa. This leads to severe plant damage even when the insects are low in numbers (Ibrahim, 1983). The pods are attacked at almost every stage of development but young developing pods are preferred by this pest and significant crop losses occur when heavy infestation is unchecked (Taylor *et al.*, 1982). It has been observed that *H. theobramae* also attacks other crops such as cashew, mango and the ornamental plant *Acalypha* spp. in Malaysia.

No previous work on enzymic polymorphisms of this insect has been done in this country. We now report our screening of this insect pest for the following biochemical markers: peptidase (PEP, E.C. 3.4.1.1), α-glycerophosphate dehydrogenase (α-GPDH, E.C. 1.1.1.8), 6-phosphogluconate dehydrogenase (6 PGD, E.C. 1.1.1.4.4), xanthine dehydrogenase (XDH, E.C. 1.2.1.37), aldehyde oxidase (ALDOX, E.C. 1.2.3.1), phosphoglucose isamerase (PGI, E.C. 5.3.1.9), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1) and adenylate kinase (AK, E.C. 2.7.4.3).

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MATERIAL AND METHODS

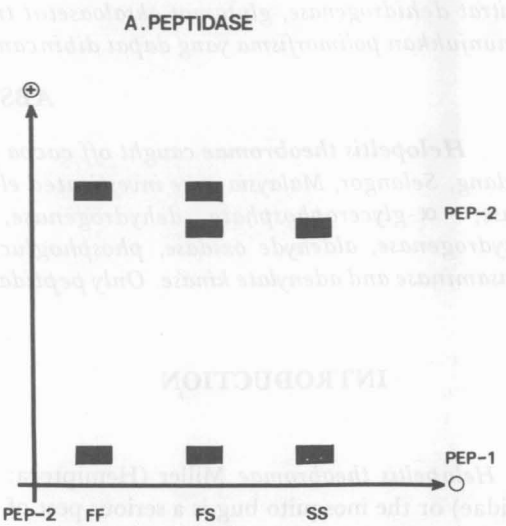
Insects were collected off *Acalypha* plants in the campus of Universiti Pertanian Malaysia (UPM) Serdang, Selangor and off cocoa trees in Kuala Selangor (KS), Selangor, Malaysia. They were stored in a deep freezer set at −70°C until used for electrophoresis.

Single adult insect of both sexes was homogenised in a drop of cold distilled water immediately before use. The homogenate was then absorbed onto a piece of 2 × 6 mm. Whatman 3 filter paper wick for insertion into the appropriate electrophoretic gels.

A seven percent polyacrylamide gel system (Tan and Teng, 1979) was used together with the CA-7 buffer of Steiner and Joslyn (1979) for PEP, GOT, PGI, α-GPDH, IDH and AK and together with TEMM buffer (Spencer *et al.*, 1964) for 6PGD, ALDOX and XDH. All electrophoretic runs were made in a refrigerator set at 4°C, 0.5% bromophenol blue in 1% acetic acid was used as the tracker dye and a constant current of 30 mA was applied to each gel. The run was terminated when the tracker dye had migrated about 8.5 cm from the origin. This took about 2 to 3 hours. Staining procedures for the various enzymes were according to Steiner and Joslyn (1979) except that 0.1 M Tris-HCl pH = 8.0 was used as the staining buffer for all stains, $MgCl_2$ was used in place of $MnCl_2$ as a co-factor in IDH staining and that PEP was stained according to Loxdale *et al* (1983) with L-leucyl-L-alanine as the substrate, but using the above staining buffer.

RESULTS AND DISCUSSION

Peptidase showed two strong regions of enzyme activity *(Fig. 1A)*. PEP-1 which did not migrate much anodally from the origin was monomorphic showing one band per sample in 32 samples from UPM and 52 samples from KS. The more anodal migrating PEP-2 region was polymorphic showing three phenotypes, FF which had one fast band, SS which had one slow band and FS which showed both the fast and slow bands. We propose that the PEP-2 region is coded for by one locus which two codaminant alleles, $PEP\text{-}2^F$ which codes for the fast band and $PEP\text{-}2^S$ which codes for the slow band. Heterozygous individuals had both the fast and slow bands. Population data is presented in Table 1. This population data is a pool of both male and female samples since all three phenotypes were present in both sexes thus ruling out sex linkage for PEP-2. Both the UPM and KS populations were in Hardy-Weinberg equilibrium for PEP-2 with their allelic frequencies close to one another. Since the heterozygote FS showed two bands, PEP-2 is most probably a monomeric protein. As the PEP-1 banding pattern was unaffected by variation in the PEP-2 pattern, it is likely that PEP-2 is coded for by a different locus. Family studies should be done in the future to confirm the mode of inheritance of PEP-2.

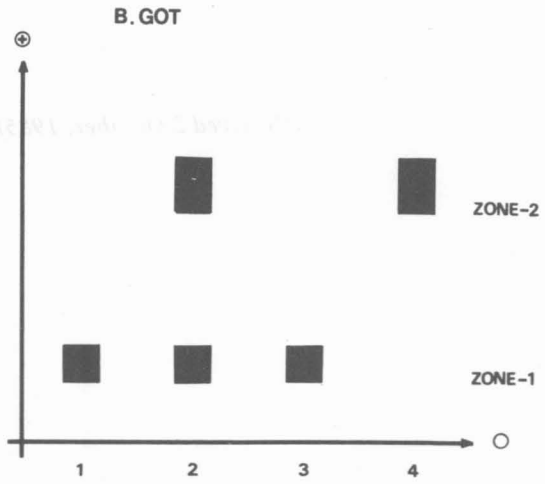


All the 61 KS insects caught off cocoa that were typed for GOT *(Fig. 1B)* showed one band per sample (zone 1). However, for insects caught off *Acalypha* plants in UPM, 27 samples showed only the single band at zone 1, while 26 samples showed the band at zone 1 and another at zone 2. When an aqueous extract of *Acalypha* leaf was run on the gel for GOT, a single band of GOT activity corresponding to zone 2 was observed. However, no GOT activity was observed when an aqueous extract of cocoa pod was similarly analysed. Since adult insects can feed on either cocoa pod sap or *Acalypha* leaf sap, it

TABLE 1

PEP-2 phenotypic distribution and gene frequencies in *Helopeltis theobromae*. Within brackets are the expected numbers assuming Hardy-Weinberg equilibrium

Population	No. Tested	Phenotypes FF	FS	SS	Gene frequencies $PEP\text{-}2^F$	$PEP\text{-}2^S$
UPM	32	16 (15.13)	12 (13.75)	4 (3.13)	0.688 ± 0.058 $\chi^2_1 = 0.52$	0.312 ± 0.058 $P > 0.20$
KS	52	18 (20.94)	30 (24.12)	4 (6.94)	0.635 ± 0.047 $\chi^2_1 = 3.10$	0.365 ± 0.047 $P > 0.05$



Legend to Figure 1.

was likely that in those insects caught off *Acalypha* plants which showed both zones 1 and 2, zone 1 represented insect GOT while zone 2 represented GOT from freshly sucked *Acalypha* leaf sap.

PGI showed a complex pattern with each sample showing 4 to 6 bands which migrated close to one another making interpretation difficult in the 155 KS and 97 UPM samples that were typed.

All the other enzymes that were screened were monomorphic and showed similiar bands for both the UPM and KS samples. AK showed two major bands per sample in 57 UPM and 107 KS insects. Sometimes, the staining for the more anodal of the two AK bands was interferred with by the appearance of a colourless band of superoxide dismutase at almost the same position. 6PGD (155 UPM and 91 KS) showed one major and one more anodal minor band per sample. XDH (122 UPM, 75 KS) and α-GPDH (103 UPM, 98 KS) showed one band per sample. ALDOX (122 UPM, 85 KS) usually showed one major band sample. Some samples however showed, in addition, one or two more cathodal minor bands which could represent breakdown products of the major Aldox band.

Since both alleles $PEP\text{-}2^F$ and $PEP\text{-}2^S$ were common in the two populations of *H. theobromae* we investigated, PEP-2 is a very polymorphic enzymatic marker which could be useful for future studies of this agriculturally important cocoa pest. For example electrophoretic data could provide information about the population structure of intraspecific populations from different geographical areas as well as reveal the existence of genetically distinct species in sympatric populations of morphologically similar insects (Berlocher, 1984). Such information have both theoretical as well as practical applications and are essential for formulating effective pest control programmes. In *H. theobromae* it would be important to know whether insects that attack cashew and mango belong to the same species as those that attack cocoa and *Acalypha* or whether they belong to genetically distinct but morphologically-cryptic species.

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