A Simple Agar Gel Electrophoretic Method to Investigate Esterase Inhibition in Certain Stored Grain Insects by Malathion and its Oxygen Analogue[1]

by A. M. KADOUM[2] and S. W. SAE[3]
Department of Entomology, Kansas State University
Manhattan, Kansas

A simple starch-gel electrophoretic method has been reported by Kadoum (3) for separating insect blood proteins during investigations of interactions occurring between common organic insecticides and enzymes. The method describes a simple zone electrophoresis procedure, using agarose as a convenient support medium. This method provides good resolution of esterases in the lesser rice weevil Sitophilus oryzae (L.) red flour beetle, Tribolium castaneum (Herbst); and lesser grain borer, Rhyzopertha dominica (Fab.) as well as a means of demonstrating the effects of malathion and malaoxon on their esterases. Malathion, the principal insecticide used as grain protectant because of its low mammalian toxicity and its effectiveness against storage pests, was chosen.

Materials and Methods

Reagents and Equipment

Agarose. SeaKem agarose manufactured by Marine Colloid, Inc. was obtained through Bausch and Lomb, Rochester, N. Y.

---

[1] Contribution No. 1002, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan, Kansas, U. S. A. Research, supported in part by Regional Research Projects NCM-37 and NC-85, and Kansas projects 687 and 481. Appreciation is expressed to Mr. Charles Anderson III for his technical assistance and to Mrs. Kusuma Reddy for rearing the insects.

[2] Assistant Professor of Entomology.

[3] Assistant Professor of Chemistry, Eastern New Mexico University, Portales, New Mexico 88130.

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 3, 1970, published by Springer-Verlag New York Inc.

Apparatus. The electrophoresis apparatus and gel cutter were identical to those reported by Kadoum (3). A series of slot-formers (200u thick) was made from polyethylene terephalate (Fig. 1).

Stains. 2-Naphthyl acetate was obtained from Eastman Organic Chemicals (Rochester, N. Y.) and diazo blue B (du Pont Naphthanil Diazo Blue B) from Nutritional Biochemical Corp. (Cleveland, Ohio).

Insects. The lesser rice weevil, red flour beetle and lesser grain borer were reared in a rearing room of approximately 80° F and 70% RH.

A suspension of 5.4 g SeaKem agarose in 90 ml of distilled water was prepared and was autoclaved for 10 minutes at 15 psi at 121° C. The melted agarose then was mixed with 90 ml hot 0.05M tris (hydroxymethyl) aminomethanehydrochloride (Tris-HCl) buffer of pH 8.0 and poured into the electrophoresis tray. Excess gel was scraped off quickly to remove bubbles and give even gel thickness. The sample slot-formers were placed in the middle of the gel strips. The gels were used after they cooled to room temperature.

Enzymes were prepared by grinding separately 3 g of seven-day-old adult insects in a cold mortar with cold 3 ml 0.05M Tris-HCl of pH 8.0 and a small amount of 60-mesh alundum. The homogenates were centrifuged at 10,000xG for 30 minutes. The supernatant solution was filtered through glass wool before addition of malathion and malaoxon.

Malathion solutions having concentrations of 1.7, 3.3, 5 and 10 ug in 1 ul of acetone were added to 0.2 ml aliquots of the centrifuged homogenate. Malaoxon (10 ug per ul of acetone) was also used. The control which contained 1 ul acetone, as well as the treated homogenate, was incubated for 10 minutes at 30° C before being applied to the gel.

After the slot-formers were removed, 15 ul of the sample were added to each strip of gel located in the slot, using a Hamilton syringe. The procedure was performed quickly to minimize diffusion of samples into the gel before electrophoresis.

The tray buffer was 0.025M Tris-HCl of pH 8.0. All trials were operated at 80 volts and about 9 ma. per strip for 1 1/2 hours.

Each gel strip was then sliced horizontally into three layers. The middle layer was stained for esterase activity, using the histological staining method of Gomori (2) with the following modifications: twenty milligrams of 2-naphthyl acetate were dissolved in 4 ml methanol. This solution was

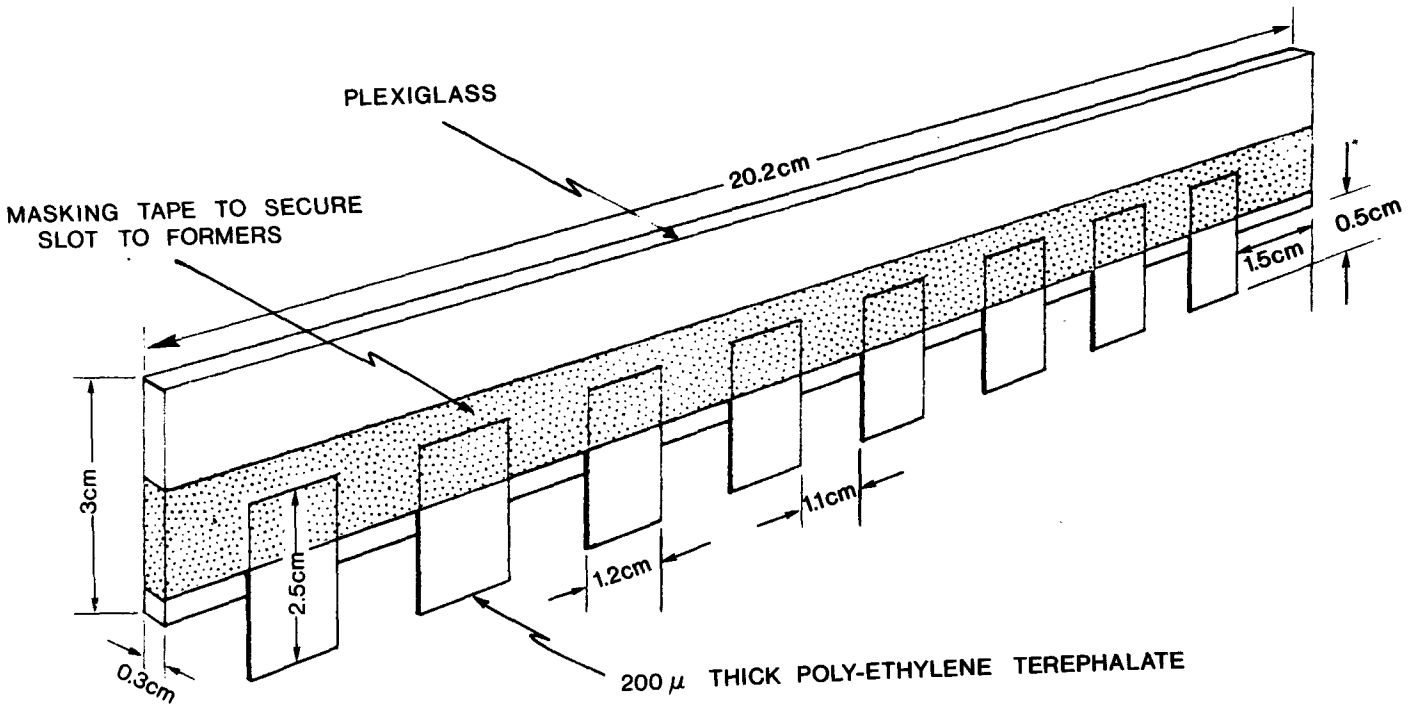

Fig. 1. Slot-former bar for starch-gel apparatus.

then added to 100 ml 0.05M phosphate buffer of pH 8.0, followed by the addition of 100 mg of diazo blue B which was prepared fresh before use. The gels were immersed in this solution for 10-15 minutes, the solution then was decanted, and the gels rinsed thoroughly with tap water before being photographed.

Results and Discussion

Organophosphorus compounds have been found to cause strong inhibition of several esterases in a number of insects (1, 5). Using agarose gel electrophoresis, this experiment demonstrates the effect of malathion and malaoxon on esterase activities of the lesser rice weevil (Fig. 2), red flour beetle (Fig. 3) and lesser grain borer (Fig. 4). Malathion of various concentrations did not show marked inhibition of esterases from the red flour beetle and the lesser grain borer; only two minor bands (2 and 3) of the esterase zymogram of the lesser rice weevil showed susceptibility to malathion. Malaoxon, however, strongly inhibited band 5 of the lesser rice weevil (Fig. 2), bands 3 and 5 of the red flour beetle (Fig. 3) and band 3 of the lesser grain borer (Fig. 4). It also inhibited slightly bands 1 of the lesser rice weevil and red flour beetle and band 2 of the lesser grain borer. Bands 2 and 4 of the red flour beetle and band 1 of the lesser grain borer were hardly affected. These observations indicate the relative susceptibility of these insect esterases toward malaoxon, and may reflect their susceptibility to malathion *in vivo*. King et al. (4) indicated that malathion was more effective in control of rice weevil than red flour beetle. However, the ability for converting malathion to malaoxon may differ among these insects and the conditions of treatment used, making direct correlation difficult. Therefore, such studies may be facilitated by the agarose gel electrophoresis technique described herein.

Use of agarose gel as a supporting medium has the advantage over starch-gel of ease of handling, although choice of supporting medium for separation of certain proteins remains quite empirical. The agarose gel strips can be stained for protein by fixing them in 10% sulfosalicylic acid for 10 minutes followed by staining in 2% aqueous Coomasie brilliant blue for 30 minutes. They are then washed in tap water for 16-20 hours to destain. Nigrosin staining as described by Kadoum (3) may also be utilized by preparing the nigrosin solution in 5% acetic acid.

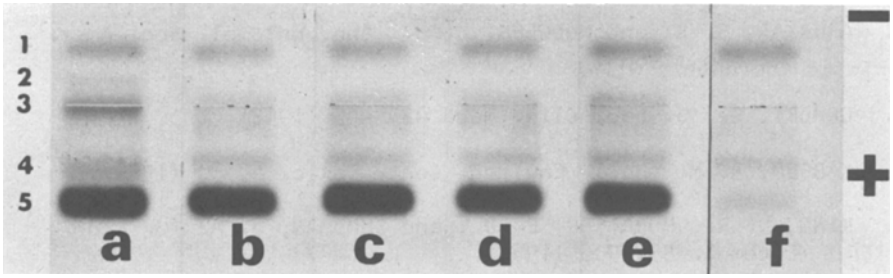

Fig. 2 Zymograms of lesser rice weevil esterases *

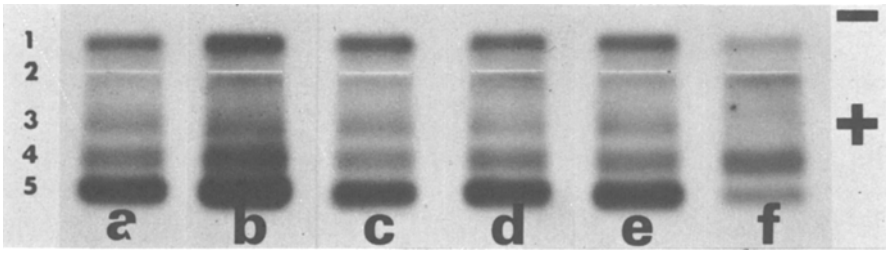

Fig. 3. Zymograms of red flour beetle esterases *

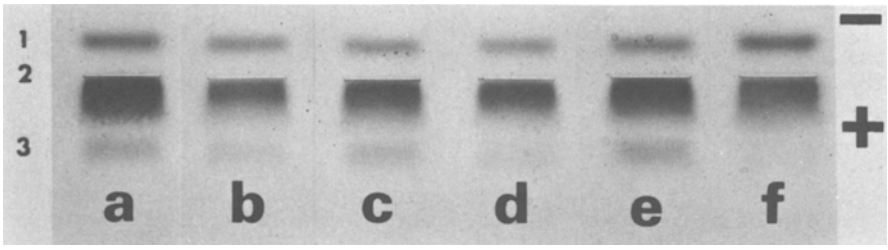

Fig. 4. Zymograms of Lesser grain borer esterases *

* A. Control
B. With 10 ug malathion
C. With 5 ug malathion
D. With 3.3 ug malathion
E. With 1.7 ug malathion
F. With 10 ug Malaoxon
each in 0.2 ml of 10,000 X G supernatant of the insect homogenate
Stain: 2-Naphthol acetate - diazo blue B.

References

1. ARURKAR, S. K. and KNOWLES, C. O. Ann. Entomol. Soc. Amer. 61, 686 (1968).

2. GOMORI, G. J. Lab. Clin. Med. 42, 445 (1953).

3. KADOUM, A. M. Bull. Environ. Cont. Toxic. 4, 80 (1969).

4. KING, D. R., MORRISON, E. O., and SUNDMAN, J. A. J. Econ. Entomol. 55, 506 (1962).

5. VELTHUS, H. H. W., and VAN ASPEREN, K. Ent. Exp. Appl. 6, 79 (1963).