IDENTIFICATION OF WEEVIL EGG MASSES (COLEOPTERA: CURCULIONIDAE) BY ENZYME ELECTROPHORESIS

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In recent years, electrophoresis has been applied to insect proteins as a method to distinguish between immature stages of related species which cannot be readily identified by conventional methods (Berlocher 1980). Egg masses of several weevil species attacking citrus roots and other hosts in Florida are difficult to distinguish from one another. In instances where an egg mass might be mistaken for *Diaprepes abbreviatus* (L.), a regulated species, identification must be absolute to satisfy quarantine requirements. Three species, *Pachneus litus* (Germar), *Artipus floridanus* Horn, and *Diaprepes abbreviatus* (L.), were examined for enzyme differences after polyacrylamide disc-gel electrophoresis.

Egg masses stored at —40°C were homogenized in a 5 ml Potter-Elvenhjem homogenizer at 4°C in a sample buffer (0.013 M H_3PO_4, 0.024 M TRIS, 0.01 M β-mercaptoethanol, 0.0005% bromophenol blue, and 10% glycerol at pH 6.9). Proteins in sample buffer were applied to the gels at about 10-15 μg in 100 μl/gel. Electrophoretic enzyme separations were performed in the Ornstein-Davis standard (7.5% acrylamide, w/w) system (Davis 1964, and Ornstein 1964). Enzyme-staining recipes were adapted from Bush and Huettel (1975), and Harris and Hopkins (1976). All stains were used at room temperature (25°C) for 12 h.

Enzyme-specific staining was obtained for 11 of 16 enzymes tested. Adenylate kinase, alkaline phosphatase, α-glycerophosphate dehydrogenase, hexokinase, isocitrate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase (NADPH requiring) staining produced faint or no banding patterns. Alcohol dehydrogenase, hexanol dehydrogenase, and 6-phosphogluconate dehydrogenase staining resulted in banding patterns which were not obviously different for the 3 species. Five enzymes (malate dehydrogenase (NAD requiring) (MDH)), glucose 6-phosphate dehydrogenase, esterase (α, β, 5), phosphoglucomutase (PGM), and phosphoglucose isomerase produced strong staining and clear differences in electromorph mobilities between the species. Representations of the results for PGM and MDH staining are presented in Fig. 1. For the populations tested, eggs of any of these 3 species can be identified by enzyme electrophoresis when compared to eggs of known origin of the same 3 species.

Individual intraspecific genetic variation for the populations tested was not a factor in these studies, because whole egg masses comprising 70-100 individuals were used in interspecific comparisons. Our data do not consti-

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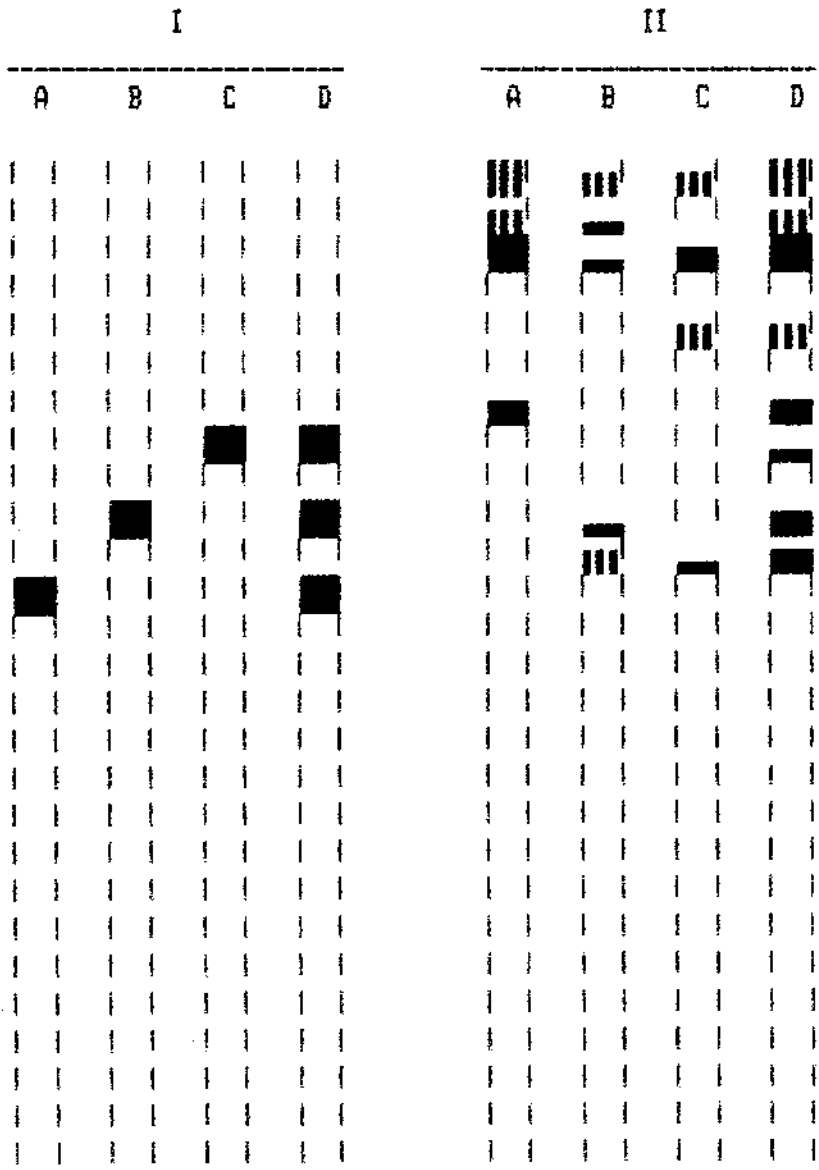

Fig. 1. Electrophoresis of egg proteins of *Diaprepes abbreviatus* (A), *Pachneus litus* (B), *Artipus floridanus* (C), and the 3 species combined (D). Specific staining for the enzymes phosphoglucomutase (I) and malate dehydrogenase (II) is represented. Dense and diffuse staining are represented by a solid band or an interrupted band, respectively.

tute an electrophoretic key, but do demonstrate that sufficient genetic differences in enzyme expression in the 3 weevil species tested exist to construct such a key. Mention of a trademark, warranty, propietary product, or vendor does not constitute a guarantee by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable. We would like to acknowledge the technical assistance of S. Lovestrand, B. Hewitt, and the advice of R. Huettel and M. Huettel. Florida Agricultural Experiment Station Journal Series No. 5268.

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CHEMICALS TESTED AS INTERNAL DYE MARKERS FOR THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA* (LOEW) (DIPTERA: TEPHRITIDAE)

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A study was undertaken to find an internal dye marker for the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), that would color adults if the material were mixed into the larval diet. An acceptable marker should not affect fly behavior or biology and would be detectable for 1 to 2 weeks after eclosion.

Anastrepha suspensa eggs (1/4 ml or 3841 eggs) were surface sterilized with 0.3% solution of sodium benzoate and pipetted onto white filter paper; they were incubated then for 48 h. Next the paper and eggs were placed