J. Biol. Control, 9(2):123 - 124, 1995

Disc gel electrophoresis in evaluating spiders for their predatory role in sugarcane ecosystem

S. VENNILA* and S. EASWARAMOORTHY
Sugarcane Breeding Institute, Coimbatore - 641 007, India

ABSTRACT : Use of disc gel electrophoresis towards evaluating spiders for their predatory role in sugarcane system was tested under laboratory conditions. Specific protein bands for different pest types and different species of spiders could be distinctly found. Spider species having fed upon a particular prey type gave a banding pattern that comprised the protein band of both. Method of sample preparation for the electrophoresis, precautions to be taken and further applicability of the method in quantitative assessment of field predation by spiders are discussed.

KEY WORDS : Gel electrophoresis, predatory role, protein bands, spiders, sugarcane pests

In recent times, there is growing awareness of the importance of biological control by native predators in Integrated Pest Management (IPM). The greatest concern lies with the practical evaluation of the role of native natural enemies, which may comprise in any one crop a heterogeneous group numbering a few to hundreds of species but about the ecology of which little may be known at the beginning of a proposed IPM programme (Putman and Wratten, 1984).

Nearly sixty species of spiders have been reported from sugarcane fields of the Sugarcane Breeding Institute, Coimbatore, and cultural practices such as weeding and irrigation have been found to influence their population (Anon., 1992). However, there is no information on their predatory role. Conservation methods can be implemented only, after a thorough research has demonstrated their role as predators. The present study used one of the analytical approaches, *viz.* disc gel electrophoresis to assess the potential importance of spiders as predators of sugarcane pests.

Disc gel electrophoresis was performed under non dissociating multiphasic buffer system with polyacrylamide gel set in 12 cm long and 0.6 cm diameter glass tubes to provide 10 cm high 'small pore' resolving gel and 1 cm 'large pore' stack gel, following Davies and Ornstein (1961).

Pests of sugarcane, *viz.* early shoot borer (ESB), *Chilo infuscatellus* Snellen; internode borer (IB), *Chilo sacchariphagus indicus* (Kapur); top borer(TB), *Scirpophaga excerptalis* (Walker); mealybug (MB) *Saccharicoccus sacchari* (Cockerell) and scales (S) *Melanaspis glomerata* (Green), and spider species *viz* *Argiope aemula* Walckenaer, *Clubiona ludhianensi* Tikader, *Cyrtophora cicatrosa* Stoliczka, *Hippasa greenaliae* Blackwall and *H. pisaurina* Blackwall constituted the prey and predatory test samples, respectively.

Each prey sample for electrophoresis was prepared from an entire prey (ESB, IB and TB) or a number of prey (MB and S) and in case of spiders, legs and pedipalpi removed before the sample preparation. Spiders starved for 24 h and then fed with known prey types were used to compare the specific prey proteins with the prey in the gut of the spiders.

Samples were prepared in 1/4th dilution of the stack gel buffer (Tris HCl pH 6.8) (1:1 W/V) with 10% sucrose and 0.0002% tracking dye (Bromophenol blue). Sample loading was done with 50ul l of preparations. Interval between sample maceration and application of the gel was usually less than 24 h. During this period samples were stored at 0-4°C. Tris-glycine (pH 8.3) was used as reservoir buffer. A current of 3 mA / tube was applied during the

Present Address: Central Institute of Cotton Research, P.B. No.225. G.P.O., Nagpur 440001

experiment. Protein bands were detected by staining gel rods with 0.1% amido black in 7% acetic acid and destained with 7% acetic acid. Zymograms of protein bands from the prey, starved and prey fed spiders were prepared.

The basis of using electrophoretic approach is that specific protein bands for different prey types and predators could be found when each of them is electrophoresed separately and that predators having fed upon a particular prey type would give a banding pattern comprising protein bands of both. The present study has shown distinct differences in the number and/or pattern and/or relative positions of protein bands among the pests and spiders, tested (Fig. 1). In case of spider species fed with a known prey, it has been possible to identify additional protein bands that

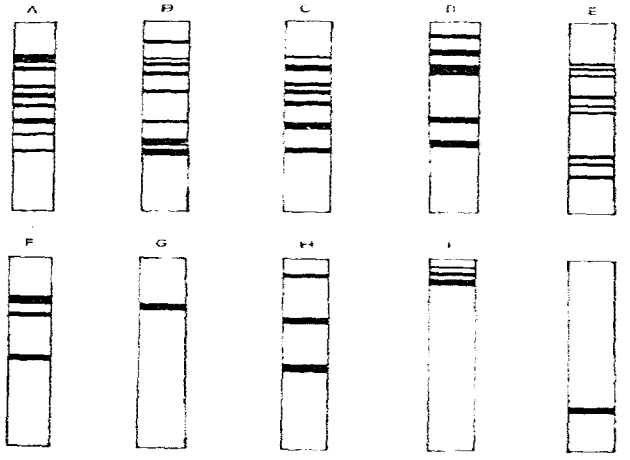

Fig 1. Zymograms for different species of spiders (A-E) and sugarcane pests (F-J)
(A) *Argiope aemula*, (B) *Clubiona ludhianensis*, (C) *Cyrtophora cicatrosa*, (D) *Hippasa greenaliae*, (E) *H. pisaurina*, (F) *Chilo infuscatellus*, (G) *C. sacchariphagus indicus*, (H) *Scirpophaga excerptalis*, (I) *Saccharicoccus sacchari*, (J) *Melanaspis glomerata*

ire related to that seen with the prey themselves (Fig. ?). The results indicate that through recognition of pecies specific qualitative differences among and etween prey and predators, through comparison of lectrophoretic bands, it is possible to identify the pider-sugarcane pest interactions and in turn to assess ie extent of predation by spiders on sugarcane pests. lowever, this analytical electrophoretic approach equires careful control of experimental conditions ich as gel size and current applied.

By examining the field sampled spiders for the presence of prey proteins in their diet based on the electrophoretic banding pattern, it is possible to tell the quantitative predation occurring at a particular time and place. Thus it is proved that the technique of gel electrophoresis is an useful experimental approach for evaluating spiders' role under field conditions.

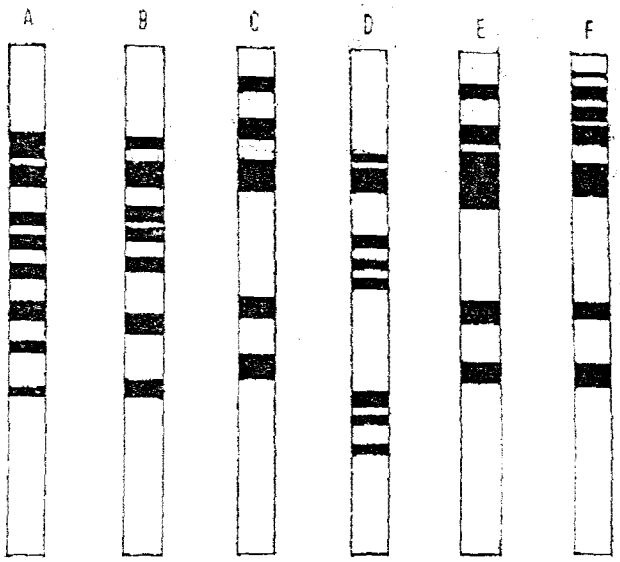

Fig 2. Zymograms for spiders fed with sugarcane pests. (A) *A. aemula*, (B) *C. cicatrosa*, (C) *H. greenaliae* & (D) *H. pisaurina* fed on the internode borer, and *H. greenaliae* fed on the early shoot borer (E) & mealy bugs (F)

ACKNOWLEDGEMENTS

We are thankful to Dr. K. Mohan Naidu, Director and Dr. K. Alexander, Head, Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore for providing the facilities.

REFERENCES

ANONYMOUS, 1992. Annual Report, 1991-1992. Sugarcane Breeding Institute, Coimbatore. 84pp.

DAVIES, R. and ORNSTEIN, L. 1961. Disc Gel Electrophoresis. Pre-printed by Canelco and Distillation Products Industries. 146pp.

PUTMAN, R. J. and WRATTEN, S. D. 1984. Principles of Ecology. Croom Helm, London. 388pp.