

GUT CONTENT ANALYSIS OF THREE SPECIES OF SAC SPIDERS BY ELECTROPHORESIS

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Predation by spiders under field conditions is inherently difficult to study; however, the existence of specific predation wounds produced by three species of sac spiders on citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, could make it possible to quantify their predation. One drawback in this system is that one of the predation marks made by the sac spiders on CLM larvae is somewhat similar to the feeding mark made by *Pnigalio minio* (Walker), an eulophid ectoparasitoid of CLM. A way to overcome this predation assessment problem is through analysis of predator gut contents. Preliminary observations on the analysis of the prey remnants inside the gut of the spiders are presented and discussed. The main purpose of this study is to develop a method to detect prey remnants in the gut of spiders, which will lead to better assessment of the efficiency of spiders on CLM control in nurseries and orchards.

The prey remnants inside the gut of the three species of sac spiders, *Chiracanthium inclusum* Hentz, *Hibana velox* (Becker), and *Trachelas volutus* (Gertsch), were detected by polyacrylamide gel electrophoresis (PAGE). This method is based on the detection of prey enzymes in homogenates of the predator after PAGE and staining for esterase activity (Van Der Geest & Overmeer 1985, Murray & Solomon 1978, Solomon et al. 1985). Esterase was selected as the indicator protein because its detection employs an enzymic reaction with substrate yielding a stain with a high extinction coefficient as shown from the previous study on analysing diets of invertebrate predators by electrophoresis (Murray & Solomon 1978) which allow detection of very small quantities of enzyme by staining for extended periods.

Spiderlings of *C. inclusum*, *H. velox*, and *T. volutus* were obtained from laboratory cultures. Spiders fed with an artificial diet were individually reared in laboratory glass vials (15 mm diameter × 60 mm long). The artificial diet consisted of a mixture of soybean liquid, homogenized milk, and egg yolk (Amalin et al. 1999). Spiders fed with CLM larvae were reared individually in plastic petri dishes (10 cm diameter × 1 cm high). Samples of CLM larvae were gathered from field collections. Homogenates were obtained from fourth-nymphal spiderlings fed for 2 days with a total of 5 second larval instars of CLM, spiders fed with artificial diet, and second larval instars of CLM. Spiders were placed in a Perspex® plate and squashed individually with a glass rod in 5-10 µl of maceration fluid (1X TBE buffer [0.09 M Tris-borate + 0.002 M EDTA] with 0.2% Triton X-100 and 10% sucrose). A similar maceration procedure was used for CLM larvae except that the numbers of larvae varied from 1, 2, 5, to 10 CLM in different homogenate samples. This range of larval densities was used in order to determine the difference in the intensity of esterase bands with varying numbers of CLM larvae. For each sample, 20 µl homogenate was dispensed with the aid of a loading tip to the sample holders that were positioned on top of the gel.

Electrophoresis was carried out as described by Murray & Solomon (1978) and by Solomon et al. (1985) with some modifications. Polyacrylamide slab gels with a total gradient concentration of 5-28% and a cross-link gradient of 2.5-6.2% (Margolis &

Wrigley 1975) were prepared between two glass plates using a gel gradient maker. A 1X TBE buffer pH 8.3, to which 0.2% w/v Triton X-100 was added, was used as the gel buffer. The running buffer was also 1X TBE without Triton X-100. The samples were run to endpoint for 20 h at 200 volts.

After electrophoresis, gels were incubated in a medium containing 30 mM 1-naphthyl acetate and 0.2% Fast Blue RR Salt in 0.2 M phosphate buffer, pH 6.0, in order to stain proteins with esterase activity. The gel separated from the glass plate was submerged in the medium and kept inside a dark container and shaken on a shaker (Gio Gyrotory®) at a speed of 28 RPM for 24 hours or until the bands appeared. Conclusions about the identity of prey remnants inside the gut of predators were drawn by visual comparison of esterase patterns of the artificially fed spiders and CLM-fed spiders.

In many cases with other predators, the specific esterase activity patterns allow the identification of prey remnants inside the predator gut (Van Der Geest & Overmeer 1985). After electrophoresis and staining for esterase activity, the CLM larvae and prepupae showed only one esterase band (Figs. 1 and 2). There was a difference in the intensity of the esterase bands on the different numbers of CLM included in each macerated sample. The intensity increased as the number of individuals per sample increased (Figs. 1 and 2). This difference in intensity could possibly be used to quantify the number of prey consumed by the predator. No esterase was obtained from the *C. inclusum* and *T. volutus* fed with artificial medium (Fig. 1, lanes 3 to 5); however, *H. velox* that fed on the artificial medium gave one esterase band with a higher molecular weight than the CLM esterase (Fig. 2, lanes 2 to 4). The esterase obtained from *C. inclusum* fed with CLM larvae in the laboratory was somewhat similar to the esterase of CLM (Fig. 1, lane 2). Similarly, a single esterase band was obtained from *H. velox* that fed on CLM, and this band also appears to be identical to the CLM esterase (Fig. 2, lane 9). The result of this experiment is similar to that of Murray & Solomon (1978) on the single esterase pattern of *Panonychus ulmi* (Koch), the European red spider mite, and differs from the results obtained by Dicke & DeJong (1988) who obtained several esterases also on *P. ulmi*. During the current test, the CLM esterase appears to have been expressed only in spiders that fed on CLM except for *T. volutus*. No esterase activity was obtained from *T. volutus* that fed on CLM larvae. This could be attributed to the retention time of the CLM esterase in the gut of *T. volutus*. Probably, the lifetime of CLM esterase in the gut of *T. volutus* is shorter compared to that of *C. inclusum* and *H. velox*. It is then worthwhile investigating the retention time of CLM esterase in the gut of the three species of sac spiders.

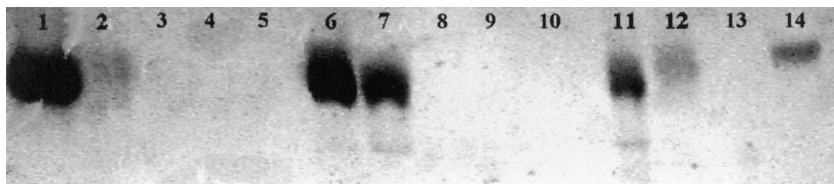


Fig. 1. Esterase patterns of CLM, *C. inclusum*, and *T. volutus*. The identity of the different lanes are as follows: 1—standard, 2—two *C. inclusum* fed with CLM in the laboratory, 3 to 5—two *C. inclusum* fed with artificial diet, 6—ten CLM prepupae, 7-five CLM prepupae, 8 to 9—two *T. volutus* fed with CLM in the laboratory, 10—two *T. volutus* fed with artificial diet, 11—ten CLM larvae, 12—five CLM larvae, 13—blank, 14—two CLM larvae.

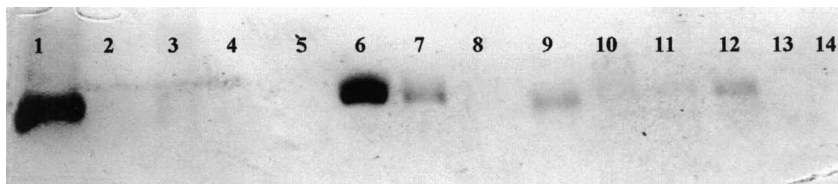


Fig. 2. Esterase patterns of CLM and *H. velox*. The identity of the different lanes are as follows: 1—Standard, 2 to 4—two *H. velox* fed with artificial diet, 5—blank, 6—ten CLM prepupae, 7—five CLM prepupae, 8—blank, 9—two *H. velox* fed with CLM in the laboratory, 10—ten CLM larvae, 11—five CLM larvae, 12—two CLM larvae, 13—one CLM larva, 14—blank.

More electrophoresis runs should be done including field-collected individuals. However, using field-collected spiders, a difficulty may arise in the identification of prey because spiders in the field are generally feeding on multiple prey species. Moreover, not all esterases of a prey may be found in the gut of the spider. Certain esterases may be localized in tissues that are not ingested, complicating the identification of the prey for field-collected specimens. Therefore, it is necessary to co-electrophorese all suspected prey species with the spiders on the same gel. The use of other enzymes (i.e. fumarate hydratase, glucose-6 dehydrogenase, hexokinase, and other enzymes) must also be considered in order to look for a more stable enzyme that will be used for gut content analysis.

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SUMMARY

Gut content analysis using polyacrylamide gel electrophoresis (PAGE) was performed on three sac spider species. Results from the electrophoresis showed that *H. velox* fed on artificial medium gave one esterase band; whereas, no esterase was obtained from the *C. inclusum* and *T. volutus* fed on artificial medium. The esterase obtained from *C. inclusum* and *H. velox* fed with citrus leafminer (CLM) larvae in the laboratory seems to be similar to the CLM esterase. No esterase activity was obtained from *T. volutus* that fed on CLM larvae. The preliminary result of the gut content analysis using PAGE showed the potential of this method in studying the predatory spider - CLM relationship in lime orchards.

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