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FINAL REPORT 6/90-12/91

PROJECT CSE16C - CONTROL OF *HELICOVERPA* USING MONOCLONAL ANTIBODIES

This project was a feasibility study undertaken to determine if feeding of *Helicoverpa* larvae could be inhibited by polyclonal antibodies raised against gut tissues.

Gut, peritrophic membrane and salivary gland tissues dissected from late third instar *Helicoverpa punctigera* were injected into rabbits to raise polyclonal antibodies to each tissue type. *In vitro* assays with gut tissue and peritrophic membrane confirmed that the respective antibodies recognised and bound to these tissues.

Antibodies were purified from the antisera, mixed with artifical diet and fed to first instar *H. punctigera* larvae; control larvae were fed on antibodies purified from serum taken from the rabbits prior to injection with the *H. punctigera* tissues. The larvae were maintained on this diet for seven days and then weighed. No significant differences between treatment and control larvae for any of the *H. punctigera* antibodies were detected.

Four possibilities for the failure of the polyclonal antibodies to inhibit feeding were considered. It is unlikely that the antibody titre was too low because sectioned peritrophic membrane was strongly labelled by a 1:2000 dilution of the antibody. Initial trials utilised a once only feed of antiserum with a high titre of antibody. When this failed to elicit an inhibition the bioassay was modified so that the insects fed on for 7 days on a diet containing 75% antiserum.

The possibility of proteolytic degradation of antibodies in the diet was rejected after testing by Western blotting. There was no appreciable decline in antibody concentration in the medium over 7 days in the presence or absence of insects.

The rejection of these hypotheses left the posssibilites that the antibodies were unable to reach the target tissues or that having bound, they had no effect on feeding. Larvae were fed on diet containing 75% midgut epithelium antiserum and then fixed, embedded and sectioned. Examination of the tissues by electron microscopy using labelled anti-antibodies showed that the antibodies did not bind to gut tissues and consequently were unable to affect feeding behaviour.

It was concluded that it would be useful to determine the mechanism that prevented the antibodies reaching the gut tissues. Protease inhibitors could be used to determine whether the antibodies were degraded in

the gut and inert molecular size markers to determine if the antibodies could pass through the peritrophic membrane to reach the gut. However, it was not possible to conduct these experiments within the limits of the grant.

An extension of this project was not sought at this time because its likelihood of success was judged to be too uncertain.

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