EVALUATION OF INSECT PREDATOR-PREY RELATIONSHIPS BY PRECIPITIN TEST STUDIES [1]

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The control of noxious insects by chemical methods has received new impetus in recent years with the advent of the chlorinated hydrocarbons and organic phosphates. It has become evident, however, that these newer insecticides do not furnish panaceas. The development of resistant strains of insects and the destruction not only of the noxious insect but its parasites and predators as well by the use of these insecticides are two recognized conditions.

These developments have served to focus attention on the importance of biological control, although it is generally recognized that natural control agents themselves do not give an absolute form of permanent control.

Entomophagous insects constitute one of the major groups of natural control agents. The assessment of predator effectiveness is frequently difficult. Laboratory trials or field observations may give inconclusive information. New methods of evaluation are being sought continually. Debach (2) has suggested a method for checking the efficacy of entomophagous insects by using a DDT sprayed plot as a check and an untreated plot as the test area. This method obviously would be applicable only in certain environments and requires that the DDT eliminates the predators or parasites while having practically no effect on the host species. Radioisotopes offer an exact means of checking predator feeding habits. However, a limitation is imposed by the necessity of first making the host population radioactive.

The preliminary investigations of Brooke and Proske (1) who used the precipitin test as a method for determining the predators of mosquito larvae and pupae suggested another approach which has been followed in this laboratory. The precipitin test offers a method by which it can be determined what proportion of a sample of predators has fed on a particular host species.

The precipitin test is a well-established serological procedure widely used in many fields of biology and medicine. Its use in entomology has been discussed by West (8). It is commonly used in determining the source of blood-meals of biting flies (Eligh, 3) and systematic serology has been used in the study of certain taxonomic groups such as Orthoptera (Leone, 6, 7). Brooke and Proske (1) furnish the only published reference dealing with the use of the precipitin test for the determination of insect predator-prey relationships. These authors described experiments in which observed or suspected predators of immature stages of mosquitos were macerated in physiological saline and the extract titrated against anti-mosquito sera.

In studies of blood-meals of biting flies it is the usual practice to smear blood-engorged flies on pieces of filter paper. These smears are dried and at a later time are extracted with saline and the extract titrated against suspected host antisera. In the present work this filter paper smear technique has been adapted to the study of insect predator hosts.

MATERIALS AND METHODS. It is not the purpose of this paper to discuss in detail the technical aspects of the precipitin test as used in these studies. During the past

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three years much of the work has been of a developmental nature since the precipitin test is not applicable as a routine procedure. These technical details which have been reported on in part by Hall (5) will be discussed in a later publication. The present paper is concerned with demonstrating the effectiveness of the precipitin test when applied to the study of predator-prey relationships. Methods of study of a reduviid predator (*Zelus exsanguis* (Stahl)) of the forest tent caterpillar (*Malacosoma disstria* Hbn.) are described as an example of the technique used.

Preparation of antigens. The preparation of a cell-free extract used as an antigen was patterned on the procedure described by Leone (6, 7). Forest tent caterpillar larvae, approximately half-grown, were collected and starved for 48 hours to permit emptying of the gut, thus reducing the possibility of contamination from the gut contents. Extraneous material was removed by washing in several changes of distilled water following which the larvae were dried by gently rolling them between paper towels. The larvae were then macerated using a mortar and pestle with the addition of buffered physiological saline (Evans, 4) during grinding. The volume of saline added was equal to the volume of unmacerated larvae. The saline-insect mixture was placed in bottles and refrigerated at 8° C. for 48 hours. At the end of this period the extraction mixture was filtered through a Buchner funnel using negative pressure and "Celite" as aids. The clear filtrate was sterilized by passage through a Seitz filter and was bottled in 20 ml. serum vials. Merthiolate preservative was added to give a final concentration of 1:10,000 and the antigen was stored at 8° C. until required for use.

Production of antisera. Three rabbits were used for the production of antisera against any one antigen. The antigen injection series consisted of an initial dose of 1/4 ml. given intravenously followed on successive days by 1/2, 1 and 2 ml. given subcutaneously. Trial bleedings were made at intervals after 7 days. With the forest tent caterpillar antigen one rabbit showed a titre of 1:12,500 on the fourteenth day and was bled for a supply of antiserum. By administering booster shots at subsequent intervals an adequate supply of anti-forest tent caterpillar larva serum was collected. The titre was determined as the highest dilution of the antigen which gave a positive ring test with a constant dilution of antiserum. Experience showed that titres of at least 1:5,000, using undiluted antiserum, were desirable for predator studies. Antisera were Seitz-filtered and stored in a frozen state at —25° C. until required for use.

Preparation of smears. The predator smear technique used is similar to that used by Eligh (3) and others in studies of blood meals of biting flies. This method permits the collecting and smearing on filter paper of the entire predator or the gut contents of the predator. The smear is placed in an envelope on which pertinent data are recorded. These smears can be stored for an indefinite period. Some of the tests described in this paper were performed with smears which had been stored for two years. Smears of host material for use as positive controls are prepared in the same manner.

Performance of tests. For testing, the smear areas were cut from the filter papers and placed individually in 5 ml. beakers. Two ml. of buffered physiological saline were added to each beaker and the smears were allowed to soak for 4 to 6 hours at 21° C. It is probable that a shorter extraction time would be satisfactory. Following this extraction 0.2 ml. of the extracts were pipetted into serological test-tubes. Two-tenths ml. of anti-forest tent caterpillar serum, whose titre had been checked after thawing, were underlayered in each tube. The tubes were incubated at 37° C. and were read at one and two hours. A positive test was indicated by the appearance of a precipitin "ring" at the interface. In these initial studies all tests were read independently by three observers and any cases of disagreement were called negative. In these particular studies the antiserum was diluted 1:2 with glycerine-saline.

EXPERIMENTAL PROCEDURE AND RESULTS. To demonstrate the effectiveness of the precipitin test, series of "known-feeding" smears were prepared. Adult male and female predators (*Zelus exsanguis,* Reduviidae) were collected in the field and were placed individually in shell vials in the laboratory. Each predator was supplied with a tent caterpillar larva and the feeding time was recorded as closely as possible. Series of smears of predators which were known to have fed on a tent caterpillar were prepared immediately after feeding and at approximately 24-hour intervals over a period of several days. One series of predators was held at room temperature which varied between 18 and 25° C. and a second series was held in an incubator at a constant temperature of 25° C. For negative controls smears were prepared of field collected predators which apparently had not fed recently and were then starved for several days, of predators which had fed on larvae of the linden looper (*Erannis tiliaria* Harr.) and of larvae of the linden looper itself. For positive controls smears of forest tent caterpillar larvae and of the eastern tent caterpillar (*Malacosoma americanum* F.) were prepared.

A number of *Z. exsanguis* collected in an area where the forest tent caterpillar was extremely abundant and where feeding on this host had been observed were smeared as "unknowns."

After an interval of two years these smears were tested using an antiserum which had been stored in a frozen state for approximately 10 months. The results of these tests are summarized as follows:

1. The anti-forest tent caterpillar larva serum gave precipitin rings with extracts of smears of various larval stages of both the forest and eastern tent caterpillars.

2. This antiserum did not react with extracts of smears of linden looper larvae, of smears of reduviids which have been starved at 25° C. for 57 hours or at room temperature for five days, of smears of reduviids which had fed on the linden looper or of a smear of a reduviid which had fed on a scarab beetle.

3. The reactions with "known-feeding" smears are summarized in Table 1. Larger numbers of smears were prepared for the shorter holding periods but were not tested because of the consistently positive results obtained for these shorter periods.

4. Sixty of 118 smears of field collected reduviids prepared as "unknowns" gave precipitin rings. Included in this series were two nymphs, one of which gave a positive reaction. There was no difference between sexes as to proportion of positive tests.

These results suggest that the reduviid

TABLE 1.—Results of precipitin tests on smears of a reduviid predator fed on forest tent caterpillar larvae and held for various intervals

Predators held at 25° C.			Predators held at room temperature		
Holding time after feeding	Precipitin reaction		Holding time after feeding	Precipitin reaction	
	Positive	Negative		Positive	Negative
Not held	2	0	24 hr.	2	0
24–32 hr.	3	0	36 hr.	1	0
48–60 hr.	2	0	48 hr.	2	0
72–76 hr.	2	0	72 hr.	2	0
72–81 hr.	2	0	84 hr.	2	0
72–84 hr.	2	0	96 hr.	2	0
96–108 hr.	8	0	108 hr.	2	0
			132 hr.	2	0
			144 hr.	8	2
			168 hr.	5	1

Z. exsanguis commonly feeds on forest tent caterpillar larvae at least when that host is excessively abundant. Since occasional individuals of the eastern tent caterpillar were present in the area it is possible that some predators had fed on that species although this feeding was never observed in the field. Serologically the forest and eastern tent caterpillar larvae could not be separated since the anti-forest tent caterpillar larva serum reacted to the limit of its titre with an eastern tent caterpillar larva antigen. Such a cross-reaction is to be expected within a genus but could possibly be eliminated if necessary by absorption of antisera.

The second most abundant lepidopterous host in the area was the linden looper. It is demonstrated that no cross-reaction occurs between looper antigen and tent caterpillar antiserum.

It is recognized that in the course of digestion of the tent caterpillar material by the predator the caterpillar antigens would eventually be altered or destroyed and precipitin reactions would no longer occur. The rate at which this alteration or destruction occurs would be a function of temperature. The "known-feeding" tests show that the antiserum can detect the presence of antigens for at least four days at 25° C. or for six to seven days at room temperature. These studies were conducted in a field laboratory where room temperatures differed but little from outdoor temperatures. During the present season studies with individual predators have shown that with few exceptions the predator will feed on a tent caterpillar at least every fourth day and rarely only after five days at room temperature. Thus it is concluded that the serological technique gives an accurate assessment of the proportion of predators which are feeding on the tent caterpillar.

OTHER STUDIES. The precipitin test has been applied to the study of several other predator-prey associations. Brief mention of some of these will serve to demonstrate further the possibilities of this technique.

One of the initial "known-feeding" studies was conducted with a species of megalopteran larva as predator and chironomid larvae as hosts. It was shown that an anti-chironomid serum could detect chironomid antigen in the gut of the predator for as long as four days when the predator larvae were held at water temperatures which varied between 16 and 24° C.

The possible value of cross-reactions was also demonstrated in this study. When the supply of anti-chironomid serum had been exhausted it was found that precipitin reactions would occur between extracts of the majority of the megalopteran predator smears and an anti-mosquito larva serum. It was known that the predators had not fed on mosquito larvae. This anti-serum had a homologous titre of 1:10,000 and a heterologous titre of 1:1,500 with chironomid antigen. Had it been desirable it would have been possible to dilute out this cross-reaction which would be expected to occur because of the close taxonomic relationship of the Culicidae and the Chironomidae. Ordinarily a heterologous combination would not be used if avoidable since except for very close relationships a lower degree of sensitivity would exist.

As part of an extensive study of biting fly biology in the Canadian north [1] investigations in biological control have been conducted. Again it has been possible to demonstrate the possible usefulness of the precipitin test. Smears of known or suspected predators of mosquito larvae have been titrated against an anti-mosquito larva serum. No specific study has yet been conducted but positive reactions have been obtained with extracts of smears of Dytiscid larvae and adults and of at least one species of caddice fly larva. No posi-

[1] These studies have been sponsored by the Defence Research Board of Canada and have been under the direction of the Division of Entomology, Canada Department of Agriculture. The writers are indebted to Dr. C. R. Twinn, Chief, Veterinary and Medical Entomology Unit, and to the Superintendent, Defence Research Northern Laboratory, Ft. Churchill, Manitoba, for cooperation.

tive reactions have been obtained with extracts of smears of snails.

Several species of chaoborines are among the most commonly observed predators of mosquito larvae in the vicinity of Churchill, Manitoba. However, the anti-mosquito larva serum was of no value in assessing smears of chaoborines because of the close taxonomic relationship of the chaoborines and culicines. Extracts of smears of starved chaoborines commonly gave precipitin reactions with the anti-mosquito larva serum. Theoretically a tribe specific antiserum could be prepared by absorption.

A further demonstration of the possible usefulness of the precipitin test in studying predator-prey relationships has been made in connection with studies of the predators of the codling moth (*Carpocapsa pomonella* L.) in Nova Scotia apple orchards. This study is complicated by the fact that in addition to the codling moth (family Tortricidae) a number of species of this and the closely related family Olethreutidae occur. The ugly-nest caterpillar (*Archips cerasivorana* Fitch) is commonly an abundant form. A cross-reaction between anti-codling moth larva serum and ugly-nest caterpillar antigen and the reciprocal cross-reaction have been demonstrated in the laboratory. In the future it is hoped to demonstrate that these cross-reactions can be eliminated by absorption.

Positive reactions have been obtained in "known-feeding" tests using both codling moth and ugly-nest antisera to titrate extracts of smears of four species of coccinellids, one pentatomid, one nabid and two arachnids which had been fed on ugly-nest caterpillars. Positive reactions have also been obtained using an anti-codling moth larva serum to titrate extracts of smears of one species of an ostomid, one pentatomid, one reduviid, one chrysopid and one nabid which had fed on codling moth larvae.

A series of smears of suspected predators of eggs of tortricids and olethreutids were titrated with an anti-codling moth pupa serum. Positive reactions were recorded for all species of predators which included three mirids, one anthocorid and two phloeothripids. The sensitivity of the precipitin test is emphasized by the latter observation that the antigen of the codling moth egg or the egg of another tortricid or olethreutid can be detected in insects as small as predatory thrips using an antiserum prepared by injecting a rabbit with codling moth pupal antigen.

SUMMARY

The studies in this laboratory to date have been mainly developmental and have been concerned with demonstrating the usefulness and limitations of the precipitin test for evaluating insect predator-prey relationships. Considerably more developmental work remains to be done before extensive applied studies can be undertaken.

The advantages and limitations of this technique can be summarized as follows:

1. Considerable difficulty has been experienced in producing regularly satisfactory antisera of high titre and avidity. This problem is one of the main concerns at the present time.

2. The occurrence of cross-reactions constitutes a limitation which may be of importance depending upon the complexity of the particular problem being studied. The elimination of cross-reactions by absorption and dilution processes is being investigated at the present time.

3. The precipitin technique is highly sensitive. It offers a means of determining quantitatively to what extent a predator feeds upon a particular host. At the present time the technique is more applicable to determining the predators of a particular host rather than all the hosts of a particular predator. The latter would require the preparation of antisera for all possible hosts.

4. It is felt that the studies reported in this paper indicate that the technique shows considerable promise and offers a valuable additional method of study of insect predator-prey relationships. The development of such techniques should aid

eventually in a better understanding of the rôle and importance of natural control agents.

ACKNOWLEDGMENTS

The writers are indebted to Dr. G. B. Reed and Dr. J. H. Orr of the Department of Bacteriology, Queen's University, for providing animal house facilities and for valuable suggestions. Particular thanks are due to Dr. R. J. Defalco, Department of Zoology, Rutgers University, for frequent advice and criticism.

References

1. BROOKE, M. M. and PROSKE, H. O. 1946. Precipitin test for determining natural insect predators of immature mosquitoes. J. Nat. Malaria Soc. 5:45–56.
2. DEBACH, P. 1946. An insecticidal check method for measuring the efficacy of entomophagous insects. J. Econ. Ent. 39:695–697.
3. ELIGH, G. S. 1951. The precipitin test for mosquito blood meal determination. M.A. Thesis. Queen's University, Kingston, Ontario, Canada.
4. EVANS, A. C. 1922. A buffered physiological salt solution. J. Infect. Dis. 30:95–98.
5. HALL, R. R. 1952. The precipitin test for insect predator-prey relationship studies. M.A. Thesis. Queen's University, Kingston, Ontario, Canada.
6. LEONE, C. A. 1947. A serological study of some Orthoptera. Ann. Ent. Soc. Amer. 40:417–433.
7. LEONE, C. A. 1947. Systematic serology among certain insect species. Biol. Bull. 93:64–71.
8. WEST, A. S. The precipitin test as an entomological tool. Can. Ent. 82;12:241–244.

ARTICLES

CONSTRUCTION AND USE OF A SIMPLIFIED WINDOW TRAP FOR INSECTS

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In the course of studies on the nocturnal activities of anopheline mosquitoes in the Western Ghats of Mysore State, India, it became desirable to construct a number of window traps. The device here described was designed in the course of studies performed by the Mysore State Health Department in cooperation with the Division of Medicine and Public Health of The Rockefeller Foundation. Because of budgetary limitations, shortage of personnel, and the large number of proposed experiments in distantly separated villages, it was necessary that the traps be sturdy, cheaply and quickly made, easily operated, conveniently transported, and adaptable to any sort of window or other aperture of houses and cattle sheds. After numerous models had been made and tested, the design described below was adopted as a standard.

1. MATERIALS REQUIRED FOR ONE TRAP.—(a) A sheet of thin transparent celluloid, 24″ x 24″. (b) A sheet of light plywood, 3′ x 6′. (c) A small quantity of any sort of 1″ boards that can be cut and drilled. (d) A dozen binding staples, 1½″ long. (e) Six-inch Barraud cage frames. (f) Barraud cage nets.

Barraud cages may be of various sizes, but most commonly they are cubes of 6″ or 12″. They are easily made by soldering or welding metal rods or stiff wires at the eight corners of an imaginary cube, so that a framework representing the 12 edges of the cube results. A net is then prepared by sewing together eight square pieces of 26-mesh bobbinet, each piece having the dimensions of one face of the cube. Tapes are attached at the corners so that the net may be tied within the metal frame. One face of the net is pro-