

LABELLING OF ADULTS OF AN INSECT PARASITE *BRACON GELECHIAE* ASHMEAD WITH RADIOACTIVE PHOSPHORUS (P^{32})

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Received July 26, 1958

INTRODUCTION

WITH a view to study the distribution of insects under field conditions, their flight range, the capacity of the parasites to locate their hosts, etc., the insects were used to be labelled with coloured dyes. The use of radioactive isotopes revolutionised the method of labelling insects. A number of methods of labelling insects with radioactive isotopes have been described by some workers within recent years which to some extent have surpassed in efficiency the earlier methods of labelling insects with coloured dyes. Roth and Hoffman (1952) made flies and mosquitoes radioactive by dipping them individually for ten seconds in P^{32} labelled phosphoric acid solution after immobilising them with carbon dioxide. This method is quite a complicated and laborious one, especially when quite a large number of labelled insects are required for mass release in the field. Furthermore, conventional methods of labelling insects with radioactive isotopes by physical application to the body of the insect are equally impracticable in the case of tiny and delicate parasitic insects. Lindquist, Yates, Hoffman and Butts (1951) demonstrated that the adults of *Musca domestica* Linn., *Lucilia sericata* Meig., and *Phormia regina* Meig., could be made radioactive by confining them with water containing radioactive phosphoric acid. A slightly modified version of this method, *i.e.*, glucose solution made radioactive with a solution of P^{32} labelled phosphoric acid was used and it was found that it did not work well with the adults of the parasite, *Bracon gelechia* Ashmead, as they were unable to pick up enough radioactivity. Yates, Gjullin, Lindquist and Butts (1951) produced radioactive adults of *Aedes* sp. by allowing them to feed on a rat that had been given previously an intraperitoneal injection of radioactive phosphoric acid. Jensen and Fay (1951) made adults of *Musca domestica*, *Callitroga macellaria* and *Phanicia pallescens* radioactive by rearing them in a medium containing radioactive phosphoric acid. The latter method appears to be promising which was modified in this laboratory to

suit the requirements of *Bracon gelechiæ*, a beneficial larval parasite of a serious pest of potatoes *Gnorimoschema operculella* Zell.

MATERIALS AND METHODS

Phosphoric acid (H_3PO_4) labelled with P^{32} having a specific activity of 0.5 mc. per c.c. which was supplied by The Radiochemical Centre, Amer-sham, England, was used. The experiments were conducted with the radio-active phosphoric acid after making the necessary dilutions with distilled water. The actual details of the procedure are given along with the experi-ments.

The measurements on the activity of the adult parasites and their host caterpillars were taken with the help of a halogen quenched, end-window mica, G. M. tube and a scaling unit supplied by Philips India Ltd. The mica thickness of the G.M. tube window was 2-3 mg./cm.² To reduce the error in counting rates due to the background counts, the G.M. tube as well as the sample were housed in a convenient box prepared out of 3/16 inch thick perpex. Under these shielding conditions the background count rate was 30 ± 5 counts per minute. During all the measurements on activity of the insects, a fixed geometry was maintained. The adult parasites as well as their host caterpillars were kept singly in one inch diameter aluminium flat planchet and positioned in the G.M. tube assembly so that the adults were 3 cm. away from the G.M. tube window.

B. gelechiæ and *Corcyra cephalonica* Stainton used in these experiments were obtained from the cultures maintained in the divisional laboratory. The eggs of *C. cephalonica* were obtained by keeping gravid females in tin oviposition cages having glass top and wiregauge bottom. These containers were placed in trays that were to receive the eggs laid by the moths. The females oviposited through the wiregauge and the eggs dropped in the trays below were collected. The larvæ hatching from these eggs were kept in glass jars covered with muslin at the open end and they were fed on crushed maize. *B. gelechiæ* was conveniently reared as follows: The males and females were kept in 3 × 2 inches glass dishes containing cotton pads soaked in 10 per cent. glucose solution and covered on one end with muslin, the full-grown host caterpillars of *C. cephalonica* being kept on the muslin and covered over by the glass plate. The female parasites paralysed the host caterpillars and laid eggs on their body which hatched and the young grubs fed on the tissue of the host. Pupation took place within 48-72 hours after hatching and the adults emerged about a week thereafter. All these insects were reared at $25 \pm 2^\circ C.$ and 70 ± 4 per cent. relative humidity.

EXPERIMENTAL DETAILS AND OBSERVATIONS

The adults of *B. gelechiæ* were labelled by feeding them on 10 per cent. glucose solution containing different activities of radioactive phosphoric acid. Dilutions of the radioactive phosphoric acid were made with 10 per cent. glucose solution in a manner that gave 25, 50 and 100 microcuries of the radioactivity per c.c. Small cotton pads were soaked with 1 c.c. of different dilutions of the radioactive material and kept in separate rearing jars along with the single mated female parasite, the mouth of the jar being covered with a fine muslin cloth held in position by Indian rubber bands. To prevent external radioactive contamination of adult insects from direct contact with the cotton pads, the latter were covered with a wiregauge of fine mesh. Simultaneously a control with 10 per cent. glucose solution only as feed was also kept. All the cages were kept in a chamber at 72–92° F., with 62–87 per cent. relative humidity. Each experiment along with the control was replicated four times. After feeding the adults for 24 hours both the living and the dead ones were removed from the cages and assayed for radioactivity. The dead ones were assayed as such, whereas the living ones were anæsthetised with chloroform and then assayed. Observations on their activity measurements are given in Table I.

TABLE I

Treatment	Average activity in c.p.m. of the adult parasites	Percentage mortality after 48 hours
25 μ c. of P ³²	30	100
50 μ c. of P ³²	70	100
100 μ c. of P ³²	120	100
Control	nil	50

It is evident that there is very heavy mortality after 48 hours among the parasites fed on radioactive phosphoric acid of 25–100 μ c. per c.c. of 10 per cent. glucose solution. Moreover, in view of the very low activities shown by the adults fed on the lowest dose of the active material in these treatments it is not practicable to get adults of still lower activity with further reduced dosages of radioactive substance in the food, which could be easily assayed.

The main idea in these experiments is to get labelled adult parasites which are sufficiently radioactive and which could survive for a considerable time with enough detectable radioactivity so that they could be tried for mass release under field conditions. The main purpose is not served by this method of labelling the parasites. Consequently the technique of Jensen and Fay (1951) was modified to suit the parasite. The technique is described below:

The parasites were reared on host caterpillars which were made radioactive by allowing them to feed on food mixed with radioactive phosphoric acid. Radioactive phosphoric acid of 500 and 750 microcuries activity was mixed with 30 grams of crushed maize and to facilitate uniform mixing of the active substance a little quantity of distilled water was added. The ingredients were thoroughly mixed and dried under an infra-red lamp. Thirty, fifth and sixth instar caterpillars of *C. cephalonica* which were previously starved for 48 hours were released in each lot of maize and allowed to feed for 48 hours. The caterpillars were then washed thoroughly with water to remove radioactive contamination from external parts of their body. The repeated washing of these caterpillars showed that a minimum of four thorough washings were sufficient to decontaminate the body surface completely. The caterpillars were then dried over filter-papers and assayed in shallow aluminium planchet with the same geometry and under the same G.M. tube as was used in the previous experiment. While assaying, the caterpillars were made to remain stationary in the planchet by covering it with a thin cellophane paper. The absorption of radiations by the cellophane covering was negligible. The active caterpillars were separated in three activity ranges (*viz.*, 100-250 c.p.m., 251-500 c.p.m. and 501-1000 c.p.m.) and were given to the mated females of *B. gelechiæ* for parasitisation. The parasitised caterpillars along with the parasite eggs on them were removed and kept in 4 inches diameter petri dishes for further development at 72-92° F. and 62-87 per cent. relative humidity. The parasite grubs after hatching continued feeding on the host caterpillars, they later pupated and eventually emerged as adults. Observations on the developmental period from the egg to the adult on radioactive host caterpillars and the longevity of the emerging adults are given in Table II. Some of these adults were collected separately in test-tubes, anaesthetised with chloroform and assayed for radioactivity. The measurements on the radioactivity of the adult parasites are given in Table II.

The adult parasites reared on the radioactive host caterpillars of *C. cephalonica* contained enough detectable radioactivity. Further the

TABLE II

Range of activity in c.p.m. of host caterpillars	Average activity in c.p.m. of the host caterpillars exposed for parasitisation	Temperature °F.	Per cent. relative humidity	Time required to complete development of parasite in days	Longevity of adult parasites in days	Average activity in c.p.m. of freshly emerged adult parasites	
						Male	Female
100-250	192	72.92	62.87	10.12	6.9	60	75
251-500	408	72.92	62.87	10.12	6.9	98	157
501-1000	677	72.92	62.87	10.12	4.9	176	287
Control	Without any activity	72.92	62.87	10.12	6.8

radioactive adults lived for a period which is sufficient for the purposes of mass release with a view to study population density, flight range, etc. Moreover, it may be mentioned that the developmental period and the longevity of the adults were not adversely affected by these levels of radioactivity. The labelling of the adults of *B. gelechiæ* by rearing them on radioactive host caterpillars appears to be a more effective method than allowing the adult parasites to feed on glucose solution containing radioactive phosphoric acid. It appears that the rearing of parasites on host caterpillars having an average activity of 677 c.p.m. is most suitable to obtain the labelled adult individuals. Incidentally the adult female parasite reared in this way show more radioactivity than the males. This is an important point as it is the female which after fertilisation has to go in search of the host for parasitisation. In view of this there is greater necessity of finding out the flight range, dispersal, population density, etc., of the female and consequently if they are labelled with tolerably higher radioactivity it is easier to detect them even after a week of the initial mass release when the activity of the P^{32} in their body after going through some radioactive decay and physiological elimination is still enough for the purposes of easy detection.

SUMMARY

Labelling of the adults of *Bracon gelechiæ* Ashmead by allowing them to feed on 10 per cent. glucose solution containing phosphoric acid (H_3PO_4) with P^{32} is not practicable as the adult parasites are unable to pick up enough radioactivity. On the other hand labelling of the adults by rearing them on host caterpillars of *Corcyra cephalonica* Stainton, the latter being fed on crushed maize mixed with radioactive phosphoric acid is quite a convenient and satisfactory method for the purpose of mass release of the parasite under the natural conditions, as such individuals show tolerably high degree of radioactivity and are therefore easy to detect. Moreover, the developmental period of the parasite by rearing it on radioactive host caterpillars is not adversely affected and the adults remain alive for a considerable period.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. B. P. Pal, Director, Indian Agricultural Research Institute, for the keen interest he has taken in this piece of research work. Their thanks are also due to the staff of the Parasitology Laboratory for the supply of the host and the parasites used in this investigation.

REFERENCES

1. Hoffman, R. A., Lindquist, A. W. and Butts, J. S. "Studies on treatment of flies with radioactive phosphorus," *J. econ. Ent.*, 1951, 44 (4), 471-73.
2. Jensen, Jens, A. and Fay Richard, W. "Tagging of adult house flies and flesh flies with radioactive phosphorus," *Amer. J. trop. Med.*, 1951, 31 (4), 523.
3. Lindquist, Arthur, W., Yates, W. W., Hoffman, R. A. and Butts, J. S. "Studies on the flight habits of three species of flies tagged with radioactive phosphorus," *J. econ. Ent.*, 1951, 44 (3), 397-400.
4. Roth, A. R. and Hoffman, R. A. "A new method of tagging insects with P³²," *Ibid.*, 1952, 45 (6), 1091.
5. Yates, W. W., Gjullin, C. M., Lindquist, A. W. and Butts, J. S. "Treatment of mosquito larvæ and adults with radioactive phosphorus," *Ibid.*, 1951, 44 (1), 34-36.