PLANT FEEDING HABITS OF NORTHERN MOSQUITOES STUDIED WITH RADIOISOTOPES [1]

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Flower visitations and apparent feeding by arctic and subarctic mosquitoes have been observed by a number of investigators. In addition to direct observations of visitation, several mosquito species have been collected with the pollinia of orchids of the genus *Habenaria* adhering to their eyes. (Twinn *et al.*, 1948).

Particular interest centers on the problem as to whether some species of northern mosquitoes may be able to produce viable eggs without a blood meal. The similarity of fractions of vertebrate blood to plant juices strongly suggests the possibility that some species might be able to reproduce on adult food of either blood or plant juices. The relative commonness of flower visitations and the apparent scarcity of mammalian or avian hosts has led some observers to suggest that plant food may well be a normal diet. Trembley (1947) has shown that *Aedes atropalpus* (Coq.) does not require a blood meal in order to lay viable eggs, although the species is a vicious biter when offered a blood meal under laboratory conditions.

As part of a more extensive study of the adult feeding habits of northern mosquitoes, it was considered necessary to first demonstrate that mosquitoes actually ingest plant food, probably nectar, when visiting flowers. Radioactive isotopes offer an admirable tool for such problems. Studies were undertaken during the summers of 1949 and 1950 at Churchill, Manitoba. Twenty-five species of tundra flowers were collected and their stems cut carefully under water. The stems were put into a solution of radiophosphorus for about 24 hours until the flowers had become radioactive. The flowers were then put into field and laboratory cages with *Aedes communis* (DeG.) mosquitoes which had been reared from larvae in the field.

P^{32} was selected for the experiments because it is taken up and accumulated by plants and insects, is readily available and relatively safe to handle, emits only beta rays which are easily measured with field instruments, and has a 2 week half life which is suitable for these experiments. The P^{32} was supplied as $KH_2P^{32}O_4$ from the Atomic Energy Project, Chalk River, Ontario. Counts of radioactivities of the flowers and insects were made with Marconi and Beckman MX5 beta-gamma survey meters. All counts of radioactivity are at 1 cm. from the Geiger-Muller tube, and have been corrected for background and coincidental counting. Background averaged 38 counts per minute (cpm) on the basis of several hundred measurements.

RESULTS

Data are presented from three experiments attempting to show that the mosquitoes actually ingest plant juices or nectars.

During the summer of 1949, West put activated flowers into field cages measuring

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6 ft. on a side and 8 ft. high. The cages contained large populations of *Aedes communis* adults which had been reared from the larval stage. At no time was a significant proportion of the caged population observed to visit any of the 25 species of flowers tested. Nearly one-third of the recorded visits were made by females. More than 200 mosquitoes, observed to have visited and to have actively probed in flowers, were measured for radioactivity. Only three individuals, one female and two males, which had visited pistillate flowers of *Salix arctophila* showed significant activity (three to four times background count). It was concluded that experimental conditions were unsatisfactory for the objective in view. Flower activities of 200–800 cpm (individual florets) were possibly too low for a significant activation of mosquitoes. It is possibly significant that *Salix* catkins registered the greatest activity (up to 3000 cpm) per catkin. No significance was attached to the relative attractiveness of the various flowers under caged conditions.

To further determine the amount of P^{32} required to activate flowers and mosquitoes, three nasturtium flowers and one dandelion were put in water containing 0.5 microcuries of P^{32}/ml. This experiment was undertaken in the laboratory at Army Chemical Center, Md. Fifty male and fifty female *Culex pipiens* L. were put in a cage with the flowers and after two days 20 males showed a radioactivity of 120 cpm and 20 females 150 cpm (background average 30 cpm). The nasturtium flowers averaged 450 cpm and the dandelion 240 cpm. These results are significant but indicated that a higher concentration of P^{32} is required for larger scale testing.

The following experiment was carried out by the authors during the summer of 1950 at Churchill, after completing the above preliminary tests.

Larvae of *Aedes communis* were collected from pools and reared to the adult stage in enamel pans in a large outdoor cage. For experimental purposes adults approximately one week to 10 days old were removed to small laboratory cages (ca. 12" × 12" × 18"). The species of flowers tested were selected on the basis of importance for mosquito feeding as observed in the field and included *Ledum decumbens, Rhododendron lapponicum, Dryas integrifolia, Rubus chamaemorus, Habenaria obtusata.* Freshly picked flowers were brought into the laboratory and the stems were cut short under water. Bunches of flowers were placed in small vials so that the ends of the stems were in the P^{32} solution. Each vial contained 8 ml. of P^{32} solution having a concentration of 9.1 microcuries per ml. The stems were inserted so that no mosquito could come in direct contact with the P^{32}.

The vials were set in small beakers and immediately placed in cages containing both male and female *Aedes communis.* During an exposure period of two to three days, numerous mosquitoes were observed feeding in the flowers. At the end of the exposure period the mosquitoes were killed and measurements of radioactivity were taken. Some mosquitoes had died during the experiment, but they were included with live ones for measuring. Since the objective was to find out if plant juices and nectars were ingested, variable numbers and ages of flowers and mosquitoes were permissible.

It was found that a significant proportion of the mosquitoes became radioactive from ingesting plant material from flowers which gave counts ranging from 31,000 to 800,000 per flower. Counts on individual mosquitoes ranged from 20 to 6970. Averages of separate counts of males and females caged with the several species of flowers ranged from 315 to 1210. The percentage of radioactive individuals ranged from 9 to 93. No greatly engorged adults were observed during the course of the experiments, and none were tested for egg laying or viability. No harmful effect on egg laying would be expected from the amount of radiation accumulated in these mosquitoes, according to the results of Hassett and Jenkins (1951).

SUMMARY

1. Various species of northern flowering plants take up and retain radiophosphorus when their stems are put in a solution of this radioisotope.
2. Under laboratory conditions *Aedes communis* males and females visit flowers and ingest plant juices and nectars as shown by their accumulation of P^{32} from the activated flowering plants.
3. Northern mosquitoes have frequently been observed to visit and probe several species of arctic and subarctic flowers. Proof in the present studies that the mosquitoes ingest plant juices lends indirect support to the hypothesis that some arctic mosquitoes may be able to produce viable eggs without a blood meal from a mammalian or avian host.

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TAGGING TECHNIQUE FOR USE IN FLIGHT RANGE STUDIES OF THE *HIPPELATES* EYE GNAT

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A special technique had to be worked out in order to tag *Hippelates* with a dye. The regular method of tagging and detecting mosquitoes in flight range studies has many pitfalls when it is applied to gnats. The first attempt at tagging was done with a mixture of fluorescein dye and gum arabic applied as a dust to the gnats. Then the dusted gnats were subjected to a very high humidity in order to make the dye-arabic mixture stick. The result was that the gnats stuck to each other. Very few gnats recovered. Next an aqueous solution of the fluorescein dye was sprayed directly on the gnats, but as in the first case, very few gnats recovered from this operation. Next a plain dust of fluorescein dye was applied to the gnats. About 90% of the gnats recovered from the operation, but the fluorescent light would not activate the dye unless it was in contact with water, or had been in contact with water. It was discovered that when the dead gnats were put in water even the smallest particle of dye would go into solution and was detectable with the fluorescent light.

To test this technique about five hundred gnats were dusted with the powdered fluorescein dye and released in the field one hundred feet from a Tinkham Gnat Trap. The next day the catch from the trap was brought into the laboratory, killed and tested. Only a few gnats at a time were put into the water and observed under the mineral light. It was found that some of the gnats had ingested the dye and by crushing them the dye could be detected. By crushing the gnats it was also discovered that some of the very small dye particles were caught in the sutures of the insect. Apparently, the surface tension of the water prevented the wetting of the dye in the sutures. It is planned to try a wetting agent in the water, such as Triton X-100, in order to reach the small dye particles without crushing the gnats.

From the first test out of the hundreds of gnats caught each day 35 gnats were recovered on the first day, the second day no gnats were recovered, the third and fourth day (a week-end) one gnat was recovered, the fifth day one gnat was recovered, the sixth day no gnats were recovered and on the seventh day no gnats were recovered.

Further tests are in progress to determine other phases in the flight range of the *Hippelates* gnat.