STUDIES ON *CULICOIDES FURENS* (POEY) AT VERO BEACH

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LARVAL COLLECTION TECHNIQUE. Salt and Hollick (1944) described a method of separating Elateridae from the soil using a concentrated $MgSO_4$ solution. This was first adapted to recovering sand fly larvae by Cameron (1948) in Scotland. R. L. Goulding and co-workers developed a somewhat similar technique during the existence of the Co-operative Sand Fly Research Unit at North Miami, Florida, that has not yet, apparently, been published. As this technique was extensively used by this Unit, it is described briefly below.

The field sample was placed in a topless one-quart oil can so that the can was nearly filled. It was then flooded, thoroughly stirred to eliminate water and air pockets, and allowed to stand overnight. During the night most of the larvae will make their way to the surface of the soil. The water and the top 1½ inches of the sample were then washed through a coarse (8 to 14 mesh) screen and then through a 100-mesh screen. The material remaining on the 100-mesh screen was placed in a small six-inch dish and flooded with a saturated $MgSO_4$ solution. Because of the high specific gravity of the solution, many of the larvae may be seen swimming on the surface of the liquid, where they can be removed with a pipette. After all visible larvae have been removed, the material is stirred and re-examined until three consecutive negatives occur. If no larvae were found upon the first examination, six consecutive negatives would be required in order to record the sample as negative. According to the unpublished reports of the Co-operative Sand fly Research Unit, two men could handle from 200 to 300 samples daily.

This technique was used as a standard for comparison as other techniques were investigated, although a comparable speed in processing the samples was never attained at this laboratory. The most time-consuming part of the technique was that of examining the salt solution for and removing the larvae.

It was thought, since most of the larvae concentrate in the surface layer of the soil after the sample has been allowed to stand, that perhaps they would enter other materials that might be placed above the field sample. One of the materials tried was "Diluex A," a fuller's earth commonly used as a diluent for insecticides. A 1½ inch layer of a soft consistency was poured into the container on top of the sample and flooded. The next day the "Diluex A" was scooped out and washed through a 140-mesh screen. The screen was then inverted and the residue washed into a black photographic developing tray, about 12 inches by 10 inches by 2 inches deep. The whitish larvae are easily seen as they swim across the dark bottom of the tray. They were removed with a rubber siphon hose which had a glass capillary tube at the upper end to reduce the flow of water.

Usually, the tray would contain very little sediment, most of it coming from the inside of the can which had become soiled at the time the field sample was collected. Some field samples will contain annelids, fiddler crabs or other crustaceans and these will sometimes carry a certain amount of marsh soil with them as they also burrow upward to the surface.

As the "Diluex A" had to be mixed with water before using it, ordinary builders' sand, such as is used for masonry work, was substituted. About 2 inches of dry sand was poured into the container on top of the field sample and flooded with water. The following day the sand was removed, placed in a dishpan and washed, the wash-water being poured through the 140-mesh screen. The residue on the screen is then washed into the black tray and the larvae removed.

The three techniques were compared by placing a known number of larvae in marsh soil in the bottom of a can and attempting to recover them. A series of replicates showed the salt technique to recover an average of 74 percent of the larvae, the sand 70 percent and the "Diluex A" 67 percent. To process samples containing 100 larvae, took from 10 to 12 minutes each, using either the sand or "Diluex A" techniques. The salt method would take about twice as long. At present, the use of building sand appears to be the most rapid and convenient, and will be used in the future for processing field samples .

RESULTS FROM COLLECTING FIELD SAMPLES. Field samples have been collected for one year to become familiar with the distribution of sand fly larvae in the local salt-marshes. Samples were taken along the edges of ditches and ponds and also along transects across the surface of the marsh. Samples taken from along shorelines contained, on the average, more than 10 times as many larvae as those taken on transects which extended across *Batis* and *Avicennia* areas. Moreover, when larvae were found along the transects, they would disappear with the next drought, although they would sometimes reappear when the marsh was reflooded. It has not been determined what becomes of these larvae as the marsh dries: larvae have been found as deep as 24 inches, but crab-holes may provide the means of descent. When holes were dug to a depth of 24″ into the dry marsh and sealed, sand fly larvae were found in the bottom of some of the holes three weeks later.

The largest numbers of larvae per sample were found along the larger drainage ditches. Because of their depth, these ditches fluctuated daily with the tide, and the larvae prefer the zone between the high and low tide marks. The soil in this zone is usually covered with a layer of sediment that remains soft and wet, as it is not exposed to the air sufficiently to become compacted. These ditches are usually shaded for most of their length by mangroves, and as a rule, more larvae can be found in the shady sections. One ditch, which had a very low larval population, was fully exposed to the sun, but the erection of artificial shade at intervals along its banks did not increase the numbers of sand fly larvae.

The field-collected samples were also grouped according to soil type. The soils were merely classified as peat, sand, clay, and mixtures of these. Clay soils appear to be least suitable for sand fly larvae, probably because of their compactness. Clay samples had the lowest average number of larvae per sample and the largest proportion of negative samples. Other types of soil and their mixtures were about equally suitable when comparisons of the average number of larvae per sample were made. There was no indication that organic soils were favored.

Samples of sandy soils were surprisingly productive, with a smaller percentage of negative samples than any other. These sands were not the wave-washed beach sands which may be found scattered along the Indian River. The samples were collected along drainage ditches traversing the upland part of the marsh, and the sandy soil contained a considerable admixture of fine silt.

EMERGENCE AND PUPATION IN THE LABORATORY. Some observations were made upon the emergence and pupation of sand flies in the laboratory. "Diluex A" is a cream-colored clay-like material that was frequently used in these studies as a substrate for sand fly larvae. The larvae were observed to pupate above the water-line where the dark pupal cases were easily seen embedded in the surface of the clay. When these pupae were flooded, they would free themselves from the bottom after a number of hours, and float to the surface. Emergence would then continue successfully.

Two groups of mature larvae were placed in jars and fed with brewers' yeast and a commercial dried tropical fish food. The jars of one group were tilted so that the substrate was partially exposed above the water level. The other group was kept flooded beneath about ½ inch of water. This test was replicated three times, but no emergence occurred in the flooded jars while the emergence rate in the tilted jars was 70 percent. Larvae in the flooded jar were observed to leave the soil occasionally and swim around the surface.

In another series, mature larvae were placed in jars of water containing 6 to 8 pencil-sized *Batis* twigs. The larvae in the jars would make their way above the water-line and pupate in crevices in the bark. The emergence rate for these jars was 71 percent.

It appears that the larvae of *C. furens* must be above the water in order to pupate, although emergence is not affected if flooding occurs after pupation has been completed.

SUMMARY. New techniques were tested to improve existing methods of collecting sand fly larvae. The results of larval sampling in the field are reported, as well as some laboratory observations on pupation.

References

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