

not penetrate deeply into several layers of tissue, as is possible with the osmium salt. It fixes the outer cell layers very well but it appears to have been mostly decomposed before cells in the interior of a structure can become fixed. Methods to overcome this difficulty are being studied.

To familiarize the reader with the use of the fixative, the following general procedure is given—(1) Smear anthers between two slides, (2) immediately drop diluted fixative onto slides and leave for 3 minutes, (3) pour off fixative and replace with one drop of Linder's medium (glycerine 40, lactic acid 20, phenol 20 and water 20 per cent.), (4) cover and seal. The fixative should render the material distinctly gray (but not black) during the fixation process when viewed against a white background. Under the microscope the chromonemata appear dark against the gray protoplasm. If staining is desired, a small amount of carmine may be added to Linder's medium or the slides may be dehydrated after fixation and stained by other methods. Treatment with  $H_2O_2$  was found to be distinctly detrimental to maintaining the fixed cell structures.

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#### A METHOD FOR LOCATING THE LARVAE OF THE MOSQUITO *MANSONIA*

ENTOMOLOGISTS and sanitary engineers engaged in mosquito work often experience difficulty in locating the breeding places of *Mansonia perturbans* Walk. even though the abundance of this mosquito at the particular time and place is such as to make it a very serious pest. The writer has been engaged in investigations of the biology of *Mansonia* in central Florida for the past two years and has developed the method here described for collecting larvae of this insect. Of several methods tried, this has proven the most satisfactory in locating breeding grounds of *Mansonia*.

The larvae of *Mansonia* differ from those of other mosquitoes in that, with the exception of the first few days of larval life, the larval and pupal periods are spent at the bottom of the ponds and marshes where they breed. Peculiar adaptations of the larval air tube and of the pupal breathing trumpets enable them

to pierce submerged roots and stems of plants and obtain air therefrom. Difficulty in locating the breeding grounds has undoubtedly arisen on account of the fact that the larvae quickly detach from stems and roots when disturbed and bury themselves in the debris at the bottom of the pond. Thus very rarely are they found by merely examining submerged stems and roots which have been pulled out of the water.

Actually to determine whether or not a marsh is breeding *Mansonia*, the plants over a small area (in practice about one square yard) should first be pulled up, thus disengaging any larvae that may be attached thereto; immediately after which the debris from the bottom of this area, in which if present the larvae are hidden, should be scooped out to a depth of about one inch. This may be done by means of a vessel having a screened bottom. A regular water bucket, the bottom replaced by twenty mesh screen wire, has proven satisfactory for this operation. As each scoop of debris is collected it is placed in a twenty mesh screen wire basket which is held partly submerged and holds in captivity any larvae thus collected. By keeping this basket partly submerged and by occasional shaking a large quantity of mud and minute trash is washed out, thereby lessening the quantity of debris to be examined later. This wire basket may be of any shape, but one recommended on account of ease of construction is conical, having a diameter at the mouth of eighteen inches and a depth of twenty-four inches.

The basket with its contents is next carried ashore for examination. The procedure usually followed in examining the debris is to place a small handful of it in a white enameled laboratory pan, adding about one quart of clear water, and then carefully to search the pan for larvae. If present, the larvae, which are whitish and very active, will be found at the bottom of the pan. Often, however, some individuals, usually those which have been injured by rough handling, are found at the surface of the water.

The number of these examinations necessary to determine whether or not a given area is infested with *Mansonia* will, of course, depend on the size of the marsh and on the number of different types of environment present therein.

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## SPECIAL ARTICLES

### ON ATMOSPHERIC ELECTRICITY

ACCOUNTS of certain remarkable effects of atmospheric electricity on the growth of plants have been related by enthusiasts both in this country and

abroad. Yield increases of more than thirty per cent. have been reported from fairly definite and systematic yield tests. The results of other investigations have been negative, no significant yield increases having

been obtained through the use of the electrical charge as secured from the air.

In order to check some of these results, experiments were instituted at the Agronomy Farm of Iowa State College by the departments of farm crops and soils and of physics, during the spring and summer of 1930.

No significant differences in yields were obtained as a result of the first year's trials. However, these tests were not taken as very conclusive as the summer was very dry and the amounts of charge obtained throughout the period were exceedingly small. The tests are to be continued for the coming season.

During the course of the experiments some effects of a purely electrical nature appeared which it seemed worth while to investigate further. It has been found by several investigators that there is a continual current of electricity flowing between the earth and the air. The work of C. T. R. Wilson (*Phil. Trans.*, 221: 73, 1921) (*Proc. Roy. Soc.*, A vol. 80, p. 539, 1908), Schonland (*Proc. Roy. Soc.*, A vol. 118, p. 229, 1927), Swan, (*I. C. T.* VI, 442), and others indicate that the direction of the positive current is from the air to the earth during fair weather, *i.e.*, the earth is losing negative charge, and that during storm periods, the current is more often in the other direction. According to Wilson (*Proc. Roy. Soc.*, A vol. 80, p. 539, 1908), and Gerdien (*Physikal. Zeit.*, p. 647, Jahr. 6, 1905), this fair-weather current for the whole earth amounts to about 1,000 amperes.

The plot of ground under investigation in our experiments was one hundred and fifty feet square. This was wired about twelve inches under ground with copper radio cable, the wires extending entirely across the plot north and south, being spaced thirty feet apart east and west. The apparatus used for collecting the charge from the atmosphere consisted of one original Christofleau apparatus (*Bull. of the Etablissement J. Christofleau Industriel*) and four variations, three of the Vincent type and one of our own. These were essentially brushes of wires on an iron standard, the brushes containing from eighteen to twenty-seven wires. The lengths of the wires varied from approximately six inches to eighteen inches and they were so arranged on the standard that the tops of the wires were about even. The diameters of the brushes varied from about eight inches to fourteen inches. Our design was slightly different, consisting of six brass bars eighteen inches long, radiating from a central plate and carrying several copper wires projecting upward. These "heads" were mounted on 4" x 4" poles twenty feet high, bolted permanently to posts set solidly in the ground. The heads were insulated from the poles by a resistance composition of slag oil and clay made

by the Vulrox Co., of St. Louis. The connecting wires leading down the poles were mounted on glazed insulators. These were connected through a series of switches to one plate of a high grade mica condenser, the other plate being connected to the ground wires. A high sensitivity Leeds and Northrup ballistic galvanometer was used to measure the charges collected. Another Leeds and Northrup R type high sensitivity galvanometer was installed to measure the current directly, but it was useless during the summer except during showers when the currents became sufficiently large to produce appreciable deflections.

The poles carrying the collector heads were erected along the south side of the plot under investigation, and about two feet inside a woven wire fence which inclosed the south and west sides of the plot. There were several tall trees to the north of the plot but as none of these were within 160 feet of our apparatus, it was not thought that they would materially affect the results. A cinder road ran along the south side of the plot and on account of the very dry weather of the last summer, was usually quite dusty. Clover and small grain were grown across the road and corn to the east of the plot. The plot itself contained corn, soy beans, turnips, garden beans, and swiss chard. The measuring instruments were housed in a sheet-iron shack at the southwest corner of the plot.

From June 25 to July 10 the currents were upward, *i.e.*, the ground was gaining negative charge. These currents were small, being of the order of  $5 \times 10^{-9}$  ampere. This is about one hundred times the average fair-weather current as given by Wilson but as points were used here as dischargers, larger results were to be expected. The last rain of the season of any considerable quantity occurred on July 4 so that by July 10 the ground was quite dry and remained so until about the middle of September. The summer was very hot, the temperature on several days reaching 98° F. or higher during the afternoon. During the interval from July 11 to September 6, when readings at this location were discontinued, the prevailing direction of the currents (except during light showers) was downward. The currents during this period were very small being of the order of  $5 \times 10^{-10}$  ampere.

Late in October, the apparatus was dismantled and three of the heads mounted on the top of the physics buildings. The height of the heads above the ground was about thirty feet. The mounting was similar to that used previously except bakelite replaced the slag oil composition as insulator and a ground plate replaced the long ground wires. Several small rains had fallen in the interval between the last readings taken at the farm and the installation of the instru-

ments on the building. The currents during most of the time since have been large enough to measure directly with the type R galvanometer, or of the order of  $5 \times 10^{-9}$  ampere. These are about the same as those of June, 1930, and in the same direction, *i.e.*, upward currents.

It will be noted in this connection that our results, except during the very driest part of the last summer, are in direct contradiction to the fair weather results of the investigators mentioned above. Our results indicate that in this vicinity the earth is nearly always gaining negative charge. Our results check the others for the prevailing direction of the current during a rain, *i.e.*, that the earth is gaining negative charge. It was also noted that, during a thunderstorm, the direction of change of field during a lightning stroke, was predominately in one direction.

Most of the changes noted occurred when the storm was overhead so that the distance to the points of discharge were comparatively short. All the changes in field—with two exceptions—were positive, *i.e.*, the fields set up by the discharges were downward. One of these two exceptions occurred as the storm was approaching and was quite small. The other occurred while the storm cloud was overhead and was comparatively strong. These results check those of Schonland and Craig for near discharges. (*Proc. Roy. Soc.*, A vol. 114, p. 229, 1927). As there was only one observer and he was continually occupied with the measuring instruments and as the storm was approaching from the opposite side of the building, there was no opportunity to note the character of the discharges or to make any actual estimates of the distances to the points of discharge.

Thinking that there might possibly be some error in our method of determining the direction of the current, an electrometer was set up and the direction again determined. Both galvanometers and the electrometer gave the same results. Frequent tests were also made to determine whether the direction of the currents noted might not be due to small e.m.f.'s developed in the switches but no evidence to this effect was found.

While the apparatus was set up in the field, it was noted that whenever an auto passed stirring up a cloud of dust which drifted across the apparatus, there was an added current set up. This was always in one direction and was interpreted as being due to tribo-electricity generated between the rubber tires of the car and the cinders on the road. Without exception, however, this was followed at an interval of about thirty seconds by a reverse current. This reverse current has not been explained.

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## USE OF PROTOZOA IN MEASURING THE NEUTRALIZING VALUE OF COBRA ANTI-SERUM<sup>1</sup>

IN previous studies of the effect of toxins and venoms on protozoa<sup>2</sup> it has been shown that paramecia may be used in measuring the potency of venoms. Attempts were made to use paramecia in measuring the neutralizing value of the anti-serum of *Crotalus* (rattlesnake) venom, but results were not wholly satisfactory. More recently the study has been extended to the use of paramecia in measuring the strength of cobra anti-serum. In this the results have been more satisfactory. It is the purpose of this paper to describe the methods used and the results obtained in ten different titrations where paramecia were used in measuring the neutralizing value of a sample of cobra anti-serum.

The method which was used in measuring the strength of the cobra anti-serum consisted in placing paramecia (in each case four animals in 1 cc) in various mixtures of cobra venom and anti-serum and thus determining the least amount of anti-serum required to protect the animals from a given amount (0.000005 gram) of venom. The method and results of ten different titrations are shown in the accompanying table.

The venom which was used in these titrations was supplied in desiccated form by the Pasteur Institute. A stock solution of this venom was made by dissolving 0.05 gram of the dry venom in 9 cc of distilled water to which 1 cc of glycerine had been added. This solution was brought to a pH of 7.0 by the addition of  $\text{Na}_2\text{HPO}_4$ . This stock solution did not deteriorate in strength during the period of the investigation and was used in making all dilutions of venom indicated in the accompanying table. The solution was kept at from 5 to 10° C. Repeated titrations showed that the least concentration (minimal lethal concentration) of this venom required to kill paramecia (*P. multimucleatum*) was 0.000002 gram per cc and that the maximum tolerance of this species of paramecia for the venom was 0.0000016 gram per cc of medium.

The anti-serum used in these titrations was a sample supplied in desiccated form by the Pasteur Institute. A fresh stock solution of the desiccated anti-serum was made up for each titration shown in the accompanying table. This, in each case, was made by weighing 0.05 gram of the desiccated anti-serum (the equivalent in this case of 0.5 cc of liquid anti-serum) and dissolving in 10 cc of neutral distilled water. From such stock solution all dilutions of the anti-serum shown in the table were made.

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<sup>2</sup> C. H. Philpott, *Jour. Exp. Zool.*, vol. 56, No. 2; *Jour. Morph. and Physiol.*, vol. 46, No. 1; *Proc. Soc. Exp. Biol. and Med.*, vol. 26; *Biol. Bull.*, vol. 60, No. 1.