

## ENTOMOLOGY

Oviposition on Dry Surfaces by *Anopheles (Cellia) gambiae* Giles

4.5-5.5. To stop further activity after incubation, one drop of 10 per cent trichloroacetic acid was added to both sets of disks, which were then extracted in a little water, centrifuged and the supernatants analysed for inorganic phosphate by a modification<sup>2</sup> of the method of Dickman and Bray, using an EEL colorimeter with red filter OR1. Table 1 gives the results obtained for a number of organisms.

Table 1. PHYTASE ACTIVITY OF MICRO-ORGANISMS

Organism	Inorganic phosphorus in control disk ( $\mu\text{g}$ )	Inorganic phosphorus in experimental disk ( $\mu\text{g}$ )	Percentage hydrolysis of sodium phytate
<i>Aerobacter aerogenes</i>	5.0	15.0	50
<i>Rhodotorula</i> sp.	1.25	4.75	17.5
<i>Streptomyces</i> sp.	7.00	10.5	17.5
<i>Bacillus</i> sp.	15.0	25.0	50
Unidentified short Gram-negative rod	8.0	28.0	100
Unidentified Gram-negative pleomorphic rod	9.75	17.25	37.5

The reproducibility of the method was checked using a strain of *Aerobacter aerogenes* and the following results, expressed as  $\mu\text{g}$  phosphorus released, were obtained for five replicates disks: 10.0, 9.5, 9.80, 10.0, 10.5.

This method has shown that 30-50 per cent of the organisms in soil and on plant roots possess an enzyme capable of hydrolysing sodium phytate with consequent release of inorganic phosphate. Further details of this work will be published elsewhere.

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<sup>1</sup> Stewart, D. J., *J. App. Bact.*, 26, 75 (1963).

<sup>2</sup> Mehta, N. C., Legg, J. O., Goring, C. A. I., and Black, C. A., *Proc. Soil Sci. Soc. Amer.*, 18, 443 (1954).

## Facilitation of the Removal of the Total Surface Growth of Bacteria from Solid Media

To make a suspension of bacterial colonies by removing them from the surface of culture media, using a wire loop, is often difficult. In using a wire loop for this purpose the growth generally slides over the wire of the loop, and the complete removal of all surface growth is thus rendered both difficult and time-consuming.

It has been found that if a wire loop is first charged with a film of liquid before attempting the removal of surface growth and then run across the bacterial colonies growing on the plate, the film of liquid allows the colonies to collect in the loop.

In practice the technique used is to sterilize the loop (2.5 mm diam.), cool in the suspending liquid, remove the loop being careful not to break the liquid film, touch off the excess liquid, run the side of the loop across the plate with the loop at right angles to the surface of the agar, continue running the loop across the bacteria until completely full, shake off into the suspending liquid and start the cycle again.

By using this technique the whole growth from a standard Petri dish can be removed in ten such operations. The use of this procedure reduces the risk of contamination associated with the more usual techniques utilized for this purpose.

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UNTIL now, it has been held that, before any species of mosquito will oviposit, water must be present as surface moisture or as a collection<sup>1</sup>. Some forty-two years ago, however, Wesenberg-Lund<sup>2</sup> recorded *Aedes (Ochlerotatus) communis* (De Geer) ovipositing among superficially dry leaves on a dried lake bed and under leaves in the laboratory, and concluded that this mosquito probably lays its eggs on dry earth. The observations recorded here have shown definitely that *Anopheles (Cellia) gambiae* Giles will oviposit on absolutely dry surfaces (at least in the laboratory), if the gravid female ready to lay its eggs is denied access to water. The eggs so oviposited, however, fail to turn black and are not viable. A survey of the literature locally available has revealed no definite previous record of oviposition on dry surfaces by mosquitoes.

Fully gravid *A. gambiae* females were put in 8 in.  $\times$  8 in.  $\times$  8 in. and 11 in.  $\times$  11 in.  $\times$  10 in. cages, in lots of 50 and 100-200 per cage, respectively. Each cage had a sheet of dry white blotting paper covering the entire floor area. No water was provided for oviposition. The mosquitoes were maintained at 25° C and 85 per cent relative humidity, and were subjected to 12 h light and 12 h dark in each 24 h.

After a day or two, several of the females in a cage were observed to have flat abdomens (that is, in Sella's stage I<sup>3</sup>). At first, this was puzzling, since, at a casual glance, the familiar black eggs of mosquitoes could not be seen on the blotting paper. It was only later that thorough examination with a magnifying glass revealed numerous off-white eggs on the blotting paper, indicating that oviposition was taking place in the absence of water.

It was assumed that since mosquito eggs on a dry surface are subjected to maximum exposure to oxygen, this should facilitate tanning and melanin formation. Yet eggs oviposited on dry surfaces failed to darken. It was then thought that perhaps a gravid mosquito denied access to water, when it is ready to oviposit, might undergo some physiological change affecting the eggs; so that when and wherever these are laid, darkening would be inhibited anyway. This hypothesis was tested using the arrangement shown in Fig. 1. Those eggs which were trapped into the funnel and succeeded in reaching the water below, through a very tiny hole at the apex of the filter-paper cone, became black and were viable; those which fell on to the dry blotting paper on the floor or remained on the dry surface of the filter-paper cone retained their pale coloration and were not viable. This indicates that eggs oviposited on dry surfaces are basically normal. With regard to coloration, it might well be that eggs laid on a dry surface would lose water from the chorion so rapidly that this would inhibit darkening (Prof. V. B. Wigglesworth, personal communication), and it is known that if the eggs of anopheline mosquitoes are removed from contact with water immediately after being laid, they retain their pale coloration<sup>4</sup>.

Whether oviposition on dry surfaces occurs under natural conditions is at present unknown; but the presence, during the dry season, of *A. gambiae* females with well-developed eggs and ready to oviposit as soon as a certain degree of mean relative humidity is reached has been reported by a number of workers<sup>5</sup>. A comprehensive study of the age composition and oviposition habits of dry-season populations of this mosquito might prove to be of more than academic interest; for if it can be shown that oviposition on dry surfaces does occur in the field (with the result that further blood-taking is not held up), this would contribute immensely to our understanding