SOME TECHNIQUES USED IN THE STUDY OF *AEDES* EGGS IN IRRIGATED PASTURES IN CALIFORNIA

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Ecological investigations into the problems pertaining to mosquitoes produced by irrigated pastures have been in progress in California for several seasons. As these investigations developed it became apparent that more information was needed pertaining to the more complex aspects of mosquito ecology, and especially about that often neglected portion of the life history of mosquitoes, the egg stage. For this reason, special emphasis was placed upon the study of *Aedes* eggs during the 1951 season. In order to accomplish this work it was necessary to develop improved techniques for the rapid and accurate measurement of egg distribution and densities. Previous experiences with this problem have indicated several experimental approaches to the subject.

Many workers in the field of mosquito ecology have developed improved methods of sampling areas for the presence of mosquito eggs. Gjullin (1938) gave a method of egg separation that used a mechanical seed cleaner with special screens installed for that purpose. Gjullin, *et al.* (1941), Filsinger (1940), Connell (1941), Abdel-Malek (1948), Horsfall (1949), and Bodman and Gannon (1950), devised methods of investigating egg distribution or showed the relationship of distribution to factors which influence egg hatch, such as temperature, dissolved oxygen, and plant hormones. Although this work was basic to approach the study of *Aedes* eggs associated with irrigated pastures, none of the techniques proved to be completely applicable to the conditions and species encountered in pastures.

Aedes nigromaculis (Ludlow) constitutes the major species present in irrigated pastures in California and produces a brood of adults with each irrigation. Eggs are produced within three to four days after the mass emergence of a brood. After these eggs mature, hatching can occur within fifteen minutes after water is applied to the field, and if the eggs are not conditioned by environmental factors to prevent hatching, a majority of the eggs will hatch with each irrigation. A measurement of relative egg densities can be obtained by flooding samples of the pasture, but several unknowns, such as the influence of temperature or seasonal variations, make this procedure questionable. Actual densities can therefore be measured best by actual counts of eggs present, and this can only be accomplished by the removal of a portion of the soil surface for screening and examination.

Methods used by previous workers required that a sample of soil be removed from the area of study by cutting to a predetermined depth and removing the sod for flooding or for drying and screening. The best samples that could be obtained by this method were generally from one to two inches thick, and since the surface of a pasture is relatively stable and because eggs could not be deposited below this surface to a very great depth, a thick sample would contain much soil that would mask the eggs present because of the dilution factor of the soil. Therefore, in order to overcome this problem which produced an excess of soil and destroyed the plants in the area sampled, a method was devised which used a tank type vacuum cleaner to remove the sample surface. A portable generator mounted in a jeep made the unit mobile so that samples could be taken from the field under most conditions. The cleaner was modified by substituting a hose with a smooth lining for the regular extension tube, and samples were collected within the cleaner in small cloth bags which prevented abrasion and damaging agitation of the eggs. Tests

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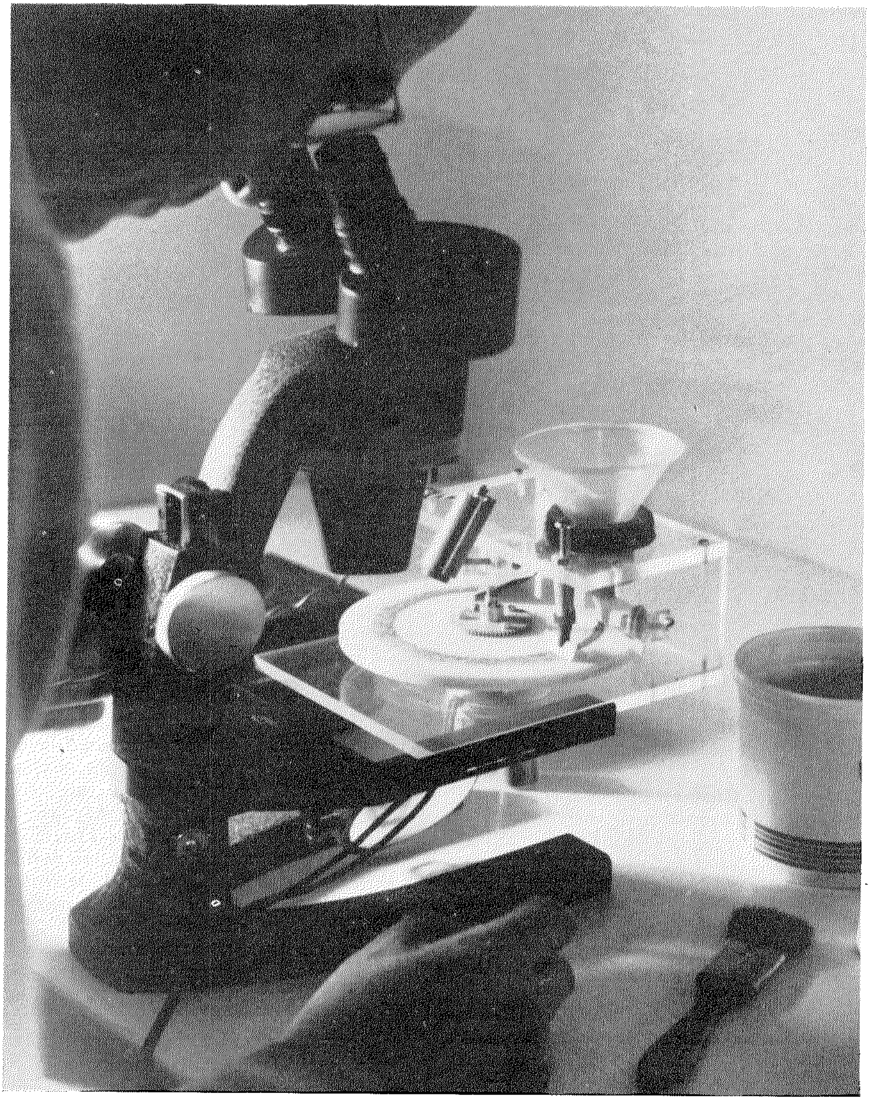

FIGURE 1. Rotary Stage used in the examination of soil samples for the presence of *Aedes* eggs.
Photo T. D. Mulhern

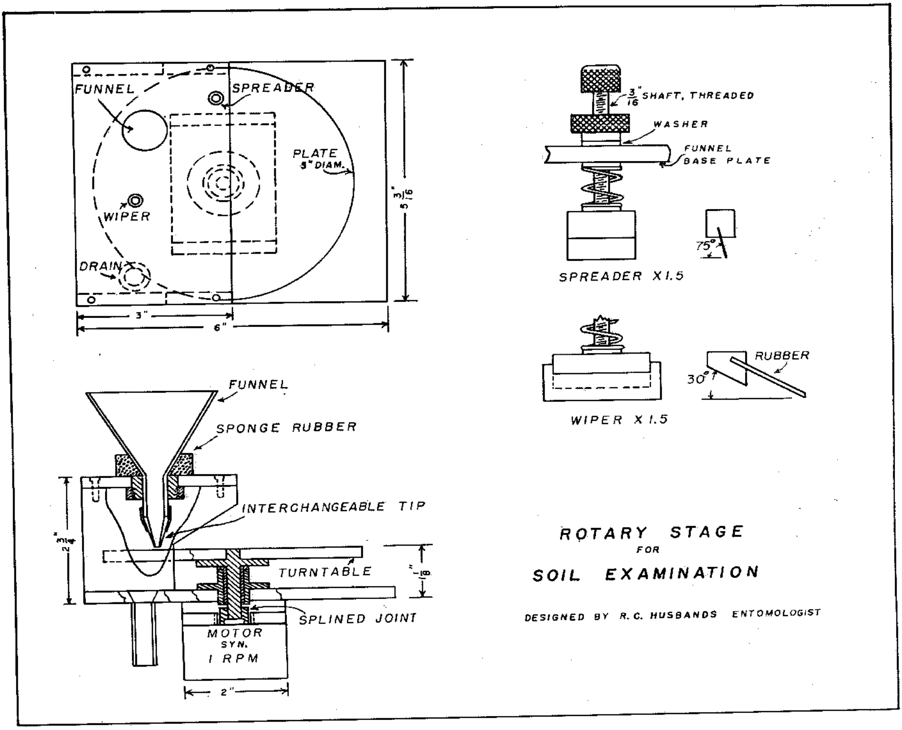

FIGURE 2. Diagram for the construction of the Rotary Microscope Stage for soil examination.

showed that less than 0.1 per cent of the eggs collected by this method were destroyed by vacuum collection. In order to take a sample the covering grass was clipped short over the area to be sampled, and the soil surface was raked loose to a shallow depth before vacuuming. The samples taken by this method could be limited to very narrow layers without disturbing the root growth of the plants within the area of the sample, and the volume of soil taken by this method seldom exceeded a pint of soil per square foot of surface examined. After drying the sample to a consistency suitable for screening (not excessive drying) the sample was easily hand-screened because the soil particles were separated during vacuuming which allowed the eggs and soil to pass freely through the screens. *Aedes nigromaculis* and *Aedes dorsalis* (Meigen) eggs will pass through a 60-mesh screen and will be retained by an 80-mesh screen.

The examination of screened soil samples by the usual method of pouring thin streams of soil upon a white card for examination under the microscope proved to be slow and inaccurate. In order to overcome this difficulty a rotary microscope stage was developed which mechanically applied a thin layer of soil to a white disk which in turn moved the sample into the field of vision in a continuous stream (Figure 1). This stream of moving particles can be examined carefully and can be stopped when needed to remove eggs or

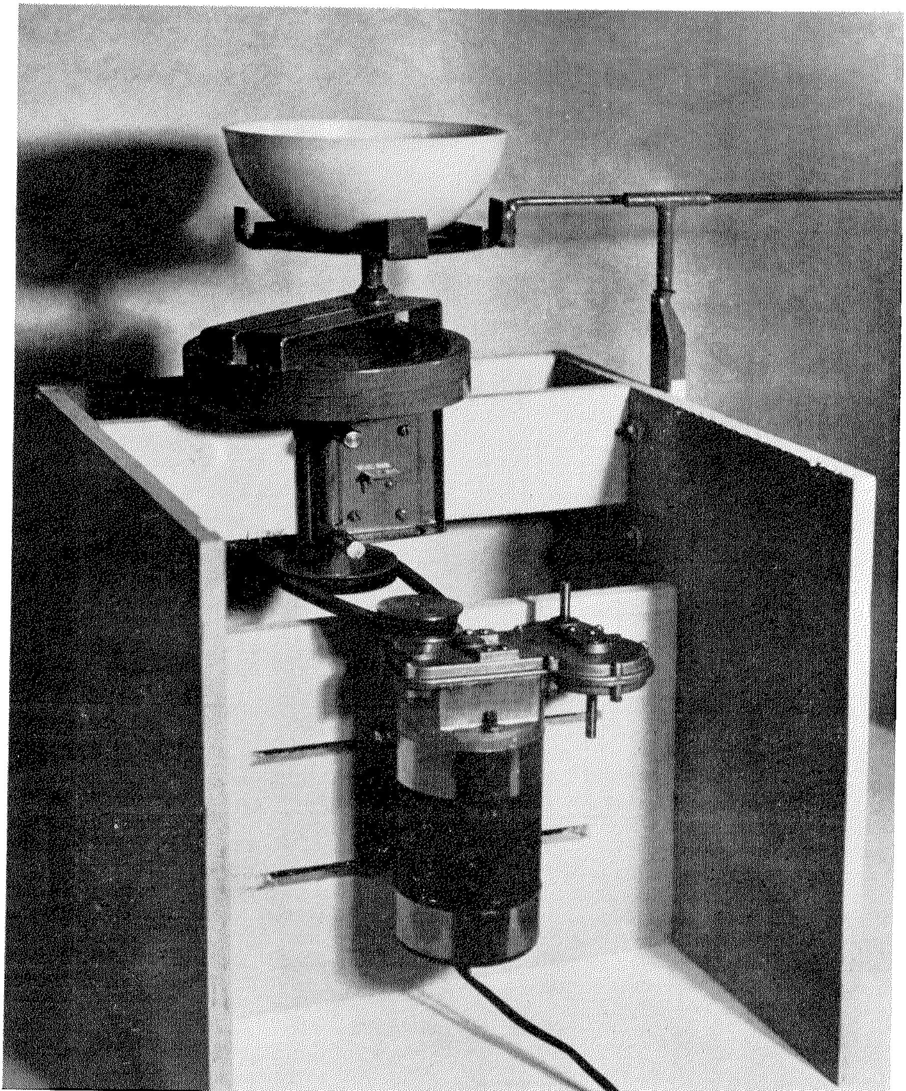

FIGURE 3. Flotation Separator used in the recovery of *Aedes* eggs from laying media when the eggs are laid below the surface.

Photo T. D. Mulhern

for more careful examination of questionable material. The re-examination of soil which was handled by the non-mechanical method showed that eggs were overlooked in the original process, and such samples generally contained eggs when given the more accurate technique of examination. Two-thirds of the samples examined by the rotary stage method contained whole or hatched eggs. The construction of a rotary stage for this type of work is simple and is illustrated in figure 2.

During the process of attempting to develop better techniques for recovering eggs from soil samples a flotation process was investigated. This process proved to be successful but time-consuming when applied to soil containing finely divided organic material or clay particles. When applied to the recovery of newly laid eggs which were deposited in white sand the flotation process proved highly successful. Caged *Aedes nigromaculis* females will deposit their eggs in moist white sand and most of the eggs are visible although they generally may be deposited just below the surface. In making counts of eggs laid in sand it was found that in many cases a small percentage of the eggs were deposited below the surface to a depth that made them invisible from above. By placing the laying media in the flotation separator (Figure 3), such eggs were quickly brought to the surface and accurate counts were obtained. The accuracy of this process could be demonstrated by placing a single egg below the surface of clean sand in the flotation separator. One minute after the rotation process was started the egg was visible on the surface of the sand.

The flotation separator for the recovery of mosquito eggs from soil is based upon the principle that the continuous motion of soil particles in the presence of a liquid medium (water) and at a speed which will produce a stratification of the particles will cause a segregation of the particles dependent upon their specific gravity and shape. At the proper speed of action the light particles will work to the top or surface. Mosquito eggs will be found on the upper surface under such conditions, since they are much lighter than most soil particles.

In order to obtain this separation a special rotary mechanism was designed[1] which moved a quart-sized bowl in a rotary path at about 60 rpm. The motion of the bowl can best be described as similar to that used in panning for gold. A mechanical separator is not necessary since the same motion can be obtained by holding the bowl in one hand and rotating the bowl at approximately the proper speed. The soil or sand in the bowl is covered with about one inch of water. Hatching can be prevented by holding the sample of soil at 33° F. for ten days and by using very cold water to cover the eggs.

As mentioned before, separation in clean sand is more satisfactory than in soil. When soil is used the eggs will be hidden under a layer of slower settling residue. This layer with the eggs can be removed with a pipette and filtered to recover the eggs. This process is time consuming and not accurate, and therefore it is recommended that, except in special cases, the examination of soil for eggs be carried out by means of the rotary stage technique.

Summary and Conclusions

1) A portable tank type of vacuum cleaner can be used successfully for collecting soil samples for the study of *Aedes* eggs.

2) A great increase in the accuracy of examining screened soil samples for *Aedes* eggs can be obtained by the use of a mechanically rotated microscope stage to which a very thin layer of the soil is continuously applied.

3) A flotation separator can be used to facilitate the accurate counting of eggs laid in artificial media such as clean white sand.

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THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE'S CONTRIBUTION TO RESEARCH ON MOSQUITOES DURING 1951

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The research on mosquitoes carried on by the Bureau of Entomology and Plant Quarantine during 1951 is briefly summarized under the headings "Taxonomy" and "Biology and Control" as in my report for last year. Again this information has been made available to me by Alan Stone, taxonomist in Washington, by A. W. Lindquist, in charge of our Corvallis, Oregon, laboratory, and by W. C. McDuffie, in charge of our Orlando, Florida, laboratory. W. V. King, who was formerly in charge at Orlando, has been assigned new duties. They consist of analyzing data, revising and bringing up to date old publications, and preparing new manuscripts giving data on the results of research conducted at that station. As reported last year, the taxonomic investigations and those conducted at Corvallis are financed by regular appropriations from Congress, whereas those at Orlando are financed by funds allotted by the Department of Defense.

TAXONOMY

Research at the United States National Museum. Alan Stone described a new species of *Aedes* from Tahiti early in 1952. Kathryn Sommerman was engaged to prepare pictorial keys to mosquitoes of medical importance in several overseas areas for use by the Army. Her work is financed by funds allotted the Bureau by the Department of the Army. A number of outside workers have been aided in their taxonomic studies, the most important being H. R. Foote in his study of the larvae of *Culex* (*Melanoconion*) species, E. F. Cooke in his study of the *Chaoborinae*, Marion Smith in her study of a new species of *Aedes* (*pseudodiantaeus*), G. W. Lassman in his description of a new *Psorophora,* and Elizabeth L. Marks in her study of *Aedes scutellaris* and its relatives. Several entomologists studied the mosquito collections at the United States National Museum before going to overseas assignments in the Orient and in Africa.

BIOLOGY AND CONTROL

Research at the Corvallis, Oregon, Laboratory. *Mosquito resistance to insecticides.*—As far as can be determined, insecticides are satisfactorily controlling *Aedes* mos-