RAPID PERMANENT MOUNTS OF MOSQUITO LARVAE WITH CREOSOTE-ALCOHOL, PHENOL-ALCOHOL, LACTOPHENOL AND POLYVINYL ALCOHOL

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While engaged in malaria control activities in Liberia, West Africa, the writer has found it necessary to experiment with shorter and less involved methods of mounting mosquito larvae and other stages of mosquitoes, so that local technicians being trained could prepare slides with a minimum of handling and in the shortest possible time.

In a previous article (1953) the use of beechwood creosote as a clearing agent, and euparal, diaphane, Piccolyte, Clarite, Permount, and Canada balsam as mounting media, were discussed. Briefly, the procedure involved killing the larvae in hot water, dehydrating with 70 per cent alcohol and 95 per cent alcohol, clearing with beechwood creosote, and then mounting in one of the media listed above.

Experiments were undertaken to attempt to combine some of these steps so as to shorten the procedure and still obtain the slides desired. Trials were also made with lactophenol and polyvinyl alcohol, two substances not previously mentioned. Each method is discussed separately.

CREOSOTE-ALCOHOL AND PHENOL-ALCOHOL METHOD. In this procedure, either beechwood creosote or pure liquid phenol, U.S.P., may be used, as the results are identical. Seventy cc. of pure ethyl alcohol are mixed with 25 cc. of either beechwood creosote or phenol. The alcohol acts as the dehydrating agent, and the creosote or phenol as the clearing agent, both actions taking place simultaneously. The living larva is dropped into this mixture and dies immediately in an extended condition. It may also be transferred fron 70 per cent ethyl alcohol, or may be kille first in lactophenol. At the end of tw hours the specimen will have been suff ciently dehydrated and partially clearec It is then transferred to a Syracuse watc glass containing pure beechwood creosot where it will become fully cleared in on or two minutes, occasionally longer. It i now ready for mounting.

The larva is lifted up with a sma spatula or fine loop of wire, and is tran ferred to the center of a clean slide. Se eral drops of euparal or diaphane ar placed upon it with a medicine droppe In culicine larvae and in certain ar opheline larvae the posterior portion i severed with a razor blade, and the later aspect is brought into view. In culicine the siphon should be flopped over on it right side. In anophelines either the righ or left pecten may be viewed. Car should be taken to see that the head i not upside down, especially in anophelin larvae. Additional drops of euparal o diaphane are then applied in a squar pattern the same size as the cover glass If a round cover glass is used, th mountant should be shaped circularly.

If the cover glass is placed at an angl against one edge of the mountant, th moving edge of the mountant may swee the severed posterior portion out o position. This is avoided or minimize by holding the cover glass directly ove the specimen and parallel to the slide, an then dropping it vertically upon th larva. The severed portion will either re main in position, or may move to one sid slightly.

When transferring the larva fron water to the dehydrating-clearing mixture no water should accompany the specimen

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'his is accomplished simply by picking up ıy number of larvae with a wide-opening ıedicine dropper, and pipetting them on ɔ a sheet of toilet tissue or several layers f gauze. The water is absorbed rapidly, randing the larvae. The tissue or gauze then turned upside down and is touched ɔ the surface of the mixture, thus trans- ɛrring the larvae with a very minimum f water. When this is done rapidly no ɛtae will be detached. If a Syracuse ʼatch glass is used for the initial action, ıe mixture may be removed with a ipette after two hours and the pure eechwood creosote introduced. This voids physical transfer of the specimen.

If the atmosphere is very humid, diffi- ılty may be experienced with a whitish recipitate which forms at the outer edge f the mounted cover glass and proceeds ıward toward the specimen. A precipi- ıte may also form when the euparal or iaphane has been diluted too much. If ıis precipitate is allowed to remain, the ıount will be spoiled. It may be removed t the same time that the slide is dried ıpidly in an oven.

The newly prepared slide should be laced in a drying oven and kept for 30 ıinutes at 100 degrees F., or longer at a ɔwer temperature. At the end of this me the precipitate will have been re- ıoved by the heat, and the mountant will e sufficiently thickened so as to permit nmediate study of the specimen without anger of moving the cover glass. If de- red, this procedure may be repeated the ɔllowing day at the same temperature, ut only for 15 minutes. The heat sually removes bubbles, providing there re not too many of them.

If the whitish precipitate originates ɔm the specimen itself instead of from ıe edge of the mountant, this is an indi- ıtion that the specimen has been insuffi- ɪently dehydrated, and should be replaced ı the dehydrating-clearing mixture.

Only euparal or diaphane can be used in ıis method of mounting. It will not ʼork with any of the xylene-soluble media ıentioned above, as the contact of alcohol ʼith xylene results in a precipitate within the specimen. The larva may be left indefinitely in either the mixture or in the pure beechwood creosote. The latter may be used many times over. Mosquito eggs and pupae may also be mounted with this procedure. Egg rafts are easy to handle, but individual eggs must be transferred with care. A simple method of removing eggs from the water surface is to lower a small square of toilet tissue or lens tissue directly upon the eggs, which will stick to the paper. The tissue is then touched to the slide, and the eggs will adhere to its surface right side up. A cover glass may be used instead of a paper square for this purpose.

LACTOPHENOL AND POLYVINYL ALCOHOL METHOD. Mounting experiments were carried out with lactophenol and polyvinyl alcohol in order to verify their usefulness, and claims for permanence of the latter (1943). Lactophenol is prepared by mixing the following chemicals:

Phenol (absolute)	20 cc.
Lactic acid	40 cc.
Glycerine	40 cc.
Water	20 cc.

When larvae collected in the field are placed directly into lactophenol, they die immediately. This mixture, being somewhat viscous, prevents setae from being detached. Specimens may be stored indefinitely in lactophenol. A minimum amount of water should be added when transferring the larvae to the lactophenol, so as to prevent excessive dilution of the latter.

If it is not desired to carry lactophenol into the field, the specimens may be brought to the laboratory in water and transferred to lactophenol later. Larvae preserved in 70 per cent ethyl alcohol may also be transferred to lactophenol. When first placed into lactophenol the larvae sometimes shrink or contract, but after a short time they regain their original condition and become cleared. Lactophenol may also be used as a substitute for hot water when using other methods of mounting, as larvae which are killed in lactophenol do not turn dark.

The refractive index of lactophenol is such that the most minute structures are rendered visible with unusual clarity. For this reason, some entomologists have adopted the procedure of mounting larvae in lactophenol, and then ringing the cover glass with a mixture of dry Canada balsam and a paraffin wax of high melting point. Such mounts may be considered to be "indefinite" rather than permanent, as the lactophenol remains in a liquid state; however, if the seal is not broken these mounts will last for many years. Some writers designate lactophenol as a mountant instead of a clearing agent as such.

Polyvinyl alcohol mountant is prepared by mixing the following chemicals:

Stock polyvinyl alcohol ...	56 cc.
Phenol (absolute)	22 cc.
Lactic acid	22 cc.

Polyvinyl alcohol may be purchased as a white odorless powder or in granular form. To prepare the stock solution, add 100 cc. of water to 15 grams of polyvinyl alcohol while stirring and heating at 80 degrees C. in a water bath. When it becomes as viscous as molasses, filter through several layers of gauze.

Living larvae may be mounted directly in polyvinyl alcohol mountant from water; however, many larvae thus mounted tend to contract, especially in the abdominal region, so that the body is shortened. The head also tends to recede into the prothoracic region. This contraction may be lessened by placing the living larva in a Syracuse watch glass containing polyvinyl alcohol mountant, prior to actual mounting on the slide. After one hour, the larva will have relaxed sufficiently so that the actual mounting may be initiated. Living larvae may be transferred in groups by following the procedure outlined in the previous section.

If the larva is mounted directly in polyvinyl alcohol on a slide, and the posterior portion is severed, there is a tendency for the viscera to emerge through the cut end because of telescoping and contraction of the abdominal segments. This is avoided to some extent by waiting 10 minutes before severing the posteric portion on the slide, or, as already su; gested, by allowing the larva to remai in the mountant for one hour prior t mounting on the slide.

Polyvinyl alcohol is a clearing agent a well as a mounting medium, so that spec mens will become cleared while the mour is hardening.

There is a tendency for minute bubble to form when the polyvinyl alcoho mountant is applied to the slide wit either a glass rod or swab stick. Thi results in an unsightly mount. Th bubbles often arrange themselves alon the sides of the larva. Such bubbles ar prevented by using a medicine dropper a an applicator. Fill the dropper halfwa with the mountant; then, holding th dropper against the slide, press the bul very slowly. The mountant will ooze ot without bubbles. Avoid dropping th mountant on the slide, as bubbles will the form.

Another hazard is too rapid lowering c the cover glass, resulting in minute bul bles along the sides of the larva. Th cover glass should be lowered very gentl preferably vertically upon the specimei after the mountant has been spread to th size and shape of the cover glass. If th amount of mountant has been underest mated, additional drops may be adde from the upper edge of the cover glas using a medicine dropper.

A considerable convenience is the eas removal of the mountant from slides an cover glasses. Merely dip slides or cove glasses in water and wipe dry.

Egg rafts, individual eggs, and pupa may also be mounted directly in polyviny alcohol mountant as described for larva It is not recommended for mounts c adults, however, as this mountant cause scales to come loose from the body an wings.

In humid areas, polyvinyl alcoho hardens very slowly if left to harden i air. An oven should be used to shorte the hardening time. Warm the slide i an oven for 30 minutes at 100 degrees F or longer at lower temperatures. A 10(

watt bulb within a small wooden or metal box will usually provide sufficient heat for drying the mount. It is not recommended that a heating table or hot plate be used, as they tend to cause bubble formation. The heat should come from all directions, rather than just from below.

Summary. Rapid mounting of mosquito larvae has been described. One technique involves 2 hours storage of the larva in a dehydrating-clearing mixture consisting of either 70 cc. of beechwood creosote and 25 cc. of 95 per cent pure ethyl alcohol, or 70 cc. of absolute phenol and 25 cc. of 95 per cent pure ethyl alcohol. Then the larva is cleared fully in pure beechwood creosote for several minutes, and mounted in either euparal or diaphane. The second technique involves the use of lactophenol as a clearing agent or temporary mountant, and polyvinyl alcohol as a clearing agent and permanent mountant. In every case living larvae may be used directly without prior killing. Eggs, egg rafts, and pupae may be mounted also with these procedures. Heating of the finished slide in a drying or cooking oven at 100 degrees F. for 30 minutes assists materially in rapid drying of the mount.

Literature Cited

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SIMPLIFIED TECHNIQUES FOR THE CONTINUOUS REARING OF *CULEX TARSALIS* WITH ADDITIONAL NOTES AND OBSERVATIONS [1]

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Since the preliminary report on the colonization of *Culex tarsalis* by Brennan and Harwood (1953), we have been able to refine somewhat the methods earlier used in maintenance of the Rocky Mountain Laboratory's stock colony. A subcolony has been successfully established in a small cage in a window-equipped room of the insectary without controlled lighting, and a considerable amount of miscellaneous biological information has been accumulated. The report cited above is a necessary adjunct to this paper.

Main Colony. The stock colony is still housed in the large walk-in cage. Although there are strong indications that our strain of *C. tarsalis* is now well adapted to conventional rearing techniques, we are still continuing with a simplified conditioning process as a precaution against the loss of the colony. Temperature and humidity are maintained at about 70° F. and 70 per cent, respectively. Light is provided by overhead fluorescent tubes and by a rheostat-controlled 300-watt lamp now directed toward an upper corner of the room. A twilight period is initiated by extinguishing the ceiling lights, giving an exposure meter (Weston Master II) reading in the cage of approximately 6.5. After about 15 minutes, voltage to the rheostat-controlled lamp is rapidly reduced from 110

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