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A STANDARDIZED PROCEDURE FOR THE QUANTITATIVE SURVEILLANCE OF CERTAIN *CULEX* MOSQUITOES BY EGG RAFT COLLECTION

PAUL REITER[1, 2]

Mosquitoes of five genera, *Coquillettidia*, *Culex*, *Culiseta*, *Sabethes* and *Uranotaenia*, lay their eggs in the form of compact floating rafts. The size, shape, and appearance of these eggs rafts is so distinctive that they are readily visible and easy to collect. Although their morphologic characteristics are often insufficient for species identification, hatching normally occurs within hours or days of oviposition, and the resulting larvae can be readily identified in the first instar (Dodge 1966, Haeger and O'Meara 1983). Egg raft collections, coupled with larval taxonomy, can therefore be useful for monitoring population levels and oviposition activity (Service 1976, Madder et al. 1980, Leiser and Beier 1982).

Among the *Culex* species that are important vectors, many share a preference for oviposition sites with a high level of microbial activity. This feature can be exploited by placing hay infusions or other live cultures in artificial oviposition sites to collect their egg rafts (Service 1976). A difficulty with this approach is that the microbial flora of such attractants is constantly changing, so that rapid changes in the attractance of the oviposition medium are inevitable (Ikeshoji et al. 1975; Kramer and Mulla 1979). An ideal method might be to identify the attractant substances and then use them in measured dosages in the field, but this has yet to be achieved. An alternative is to develop a strict routine for producing and using the attractant. The procedure described in this note was developed in Memphis, Tennessee for monitoring the activity of *Culex restuans* Theobald and *Culex pipiens* s.l. and has been in routine use for 4 years.

The basis of the method was suggested by Mr. J. Haeger of the Florida Medical Entomology Laboratory, Vero Beach. An oviposition attractant, produced by steeping 0.5 kg of grass-hay and 5 gm each of lactalbumen powder and dried brewer's yeast in 114 liters of water for 6 days, is left overnight in a large black pan at the required site and egg rafts collected on the following day. The rafts are hatched in the laboratory and identified to species in the first instar.

Figure 1 shows a convenient arrangement for producing the attractant. It consists of two 120-liter garbage cans, one placed inside the other. The outer can is mounted on a 4-wheel dolly and has a spigot installed near its base. The base of the inner can is perforated with a

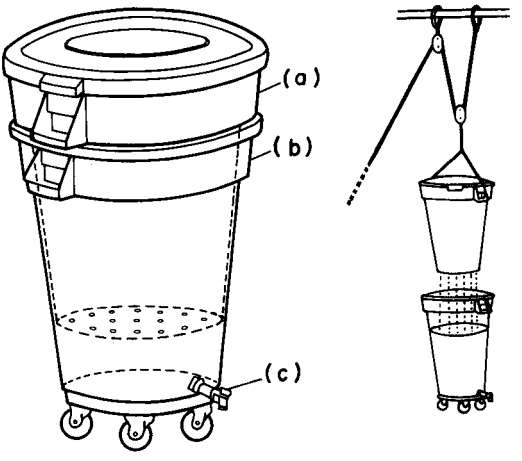

Fig. 1. Arrangement for production of attractant. (a) Inner can, (b) Outer can, (c) Spigot.

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[2] The use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

large number of 0.6 cm holes. The hay and nutrient powders are placed in the inner can and 114 liters of tap water added. When incubation is complete, the inner can is hoisted out of the other by a rope-and-pulley arrangement. The filtered attractant can now be rolled into a pick-up truck for transportation to the collection sites.

The oviposition pan is a black plastic "tote-box," 47.0 × 35.6 × 16.5 cm (Tablecraft Products (No. 1537), Chicago, IL), available from suppliers of restaurant equipment. Pans containing 4 liters of attractant are set out in the afternoon, preferably at least 2 hr before sunset, at sites sheltered from direct sunlight. New attractant of identical age is used for each collection, even when these occur on successive days. Individual sites are always visited at the same time of day; the egg rafts are collected on the following day. Although collections are occasionally lost through heavy rainfall, no protection against rain is used because open pans are much more attractive to mosquitoes.

A maximum of 24 rafts are collected from each site. When more rafts are present, the surplus is counted and discarded. Egg rafts are transferred to 24-well tissue culture plates (Flow Laboratories Inc., McLean, VA 22102), one raft per well, and one plate per collecting site. The individual rafts are conveniently transferred from the oviposition pan by dipping one corner of the lid of the culture plate under the raft and transferring it with enough water to half fill a well (Fig. 2a).

In warm weather, culture plates are stacked in a cooled container during transport. At the laboratory, they are laid out on the bench, and their lids displaced so that one end is propped up by the end of the plate (Fig. 2b). This prevents condensed moisture from forming a seal between the lid and the body of the plates which can suffocate the eggs. Once this moisture has evaporated, the lids can safely be replaced and the plates stacked at room temperature until hatching occurs.

It is not necessary to feed the larvae if they are only to be used for identification. Without feeding they quickly die, but not before the head capsule and other diagnostic characters are fully developed. If examination of later instars is required, larvae can be conveniently reared in 6-well tissue culture plates.

Plates containing unfed dead larvae can be rapidly scanned under the binocular dissecting microscope. Several larvae must be examined because individual eggs occasionally transfer between adjacent egg rafts in the oviposition pan before collection, or because more than one egg raft may have been placed in a well. If larvae are alive or many food particles are present, then it is preferable to transfer a sample from each well to a white porcelain tile with depressions in the same 6 × 4-array as the culture plate.

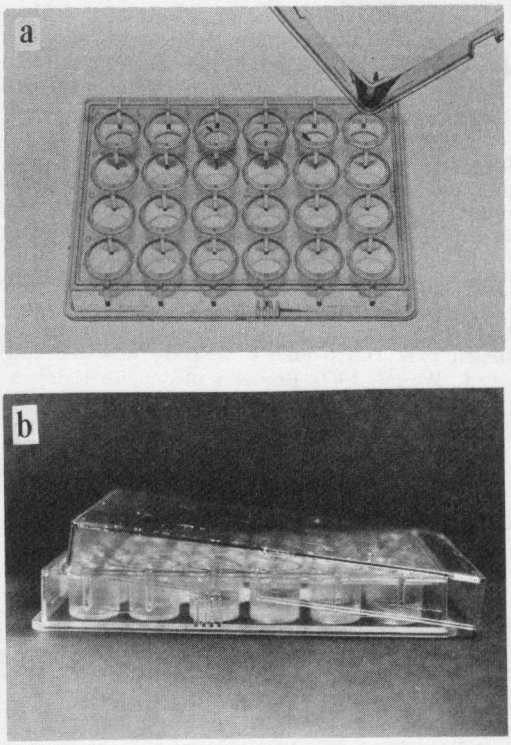

Fig. 2. Transfer of egg rafts tissue culture plates (a) and arrangement of plates on arrival at the laboratory (b).

The key to first-instar larvae (Table 1) is principally derived from the work by Dodge (1966), but is restricted to species which are likely to be collected as egg rafts on a hay infusion in the eastern U. S. and Canada. It is designed to facilitate the rapid processing of large amounts of material rather than to serve as a dichotomous key. All the characters used are readily visible at 30× magnification without any preparation.

The tissue culture plates are robust and can be washed and reused repeatedly. Live larvae can be shipped by mail if the wells are sealed. The manufacturer supplies ready-cut adhesive film for this purpose, but cellulose tape is also effective. Pinholes must be punched over each well to allow for pressure changes. When air transport is involved, plates must be dispatched in a pressure-tight container unless it can be assured that they will not be stored upside-down during flight.

Table 1. Key for the rapid determination of first-instar larvae from egg rafts of mosquitoes likely to be collected with hay infusion in the eastern U.S. and Canada (mainly after Dodge 1966).

1.	Egg breaker preceded by completely clear (transparent) "window" area	2
	Egg breaker not preceded by transparent "window" area	4
2(1).	Transparent "window" in front of egg breaket with larger diameter than width of egg breaker	3
	Transparent "window" in front of egg breaker same width as egg breaker; siphon short, with 0.2 sclerotization;[1] antennal setae trifid; terminal spines of antenna shorter than shaft	*Culex (Culex) tarsalis*
3(2).	Siphon of medium length (3:1),[2] approx. 0.33 sclerotized with definite bulge near base; antennal seta bifid; head setae (C-5,6,7) in straight transverse row	*Culex (Culex) restuans*
	Siphon long (4:1) with dark sclerotization 0.5, antennal seta single and long	*Culiseta (Climacura) melanura*
4(1).	Terminal antennal spines equal to or shorter than shaft of antenna	5
	Terminal antennal spines longer than shaft of antenna	7
5(4).	Siphon stout, less than 3:1; apex of siphon and head both black; antennal seta single; sclerotization of siphon 0.5	*Culiseta (Culiseta) inornata*
	Siphon (3:1) or longer	6
6(5).	Siphon long (4:1) with sclerotization 0.5; head setae (C-5,6) longer than antennal shaft	*Culex (Culex) nigripalpus*
	Siphon medium (3:1), approx. 0.33 sclerotized; antennal setae and terminal spines 0.5–0.75 of shaft	*Culex (Culex) pipiens* s.l.
7(4).	Siphon lightly sclerotized almost to base, long (at least 4:1) and tapered; antennal seta triple or quadruple	*Culex (Culex) salinarius*
	Siphon heavily sclerotized 0.5 with distal half parallel-sided; egg breaker bordered on either side by parenthesis-shaped mark; prothoracic seta 3 is 0.66 of setae 1 and 2	*Culex (Neoculex) territans*

[1] Proportion of total length of siphon which is sclerotized.
[2] Ratio of length of siphon to width at base.

More than 60,000 egg rafts, mostly either *Culex restuans* or *Culex pipiens* s.l., were collected and processed by this method in Memphis in 1982–84. From May through mid-September, mean egg rafts per pan generally ranged from 20 to 80 per night, with counts of over 200 per pan in some cases. One operator serviced 20–30 sites per day without difficulty. A major advantage of the method is that the catch is limited to species of interest, which can be identified without difficulty, whereas collections of adult insects often contain a wide range of species in variable condition.

In addition to providing a practical approach for the large-scale monitoring of seasonal activity and population levels of St. Louis encephalitis vectors, the procedure has proved valuable in pinpointing major urban breeding sites, and for assessing the efficacy of adulticiding methods in the urban habitat (Reiter, unpublished data). In these operations, the 24-well plates enable large quantities of material to be collected and stored until it is convenient to begin examination. The plates have also been used to ship live larvae to other laboratories for virus testing, insecticide resistance studies and other research purposes.

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