OPERATIONAL AND SCIENTIFIC NOTES

COLONIZATION OF *Uranotaenia lowii* THEOBALD (DIPTERA:CULICIDAE)[1]

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Of the three species and one subspecies of *Uranotaenia* (*U. anhydor anhydor* Dyar, *U. anhydor syntheta* Dyar and Shannon, *U. lowii* Theobald, and *U. sapphirina* (Osten Sacken)) that occur in the United States, only *U. a. anhydor* has previously been colonized (Chapman, 1964). Later Chapman and Barr (1969) reported that the colony was maintained for about a year before it was terminated. The colonization of *U. lowii* is reported here.

Initially, about 50 pupae of *U. lowii* collected as larvae in the field by personnel of the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory were placed in a cage. Several days later, coupled adults were observed on the sides of the pupal container. Since *U. lowii* exhibited this stenogamic trait, a toad, raisins, and an oviposition container were placed in the cage. All subsequent egg rafts were viable.

Egg rafts of *U. lowii* are small compared with those from our laboratory colonies of *Culex* and *Culiseta* spp. and are bow shaped, heavily sculptured, especially at the apex of each egg, and devoid of apical droplets. The number of eggs per raft among 30 rafts ranged from 32 to 116 with a mean of 77. At a room temperature of 27° C., the eggs hatch in 2 days, and the larval and pupal stages are completed in a minimum of 7 and 2 days, respectively. Larvae can be grown easily by using our normal rearing procedures; they are fed ground rabbit chow, and the pans are aerated continuously. Adults are maintained in a cage (10 x 10 x 10 in.) in the adult insectary which is held at a temperature of 27° C. and a relative humidity of 75–85 percent. They have continuous access to raisins, a toad (*Bufo valliceps* Wieymann), and an oviposition container. The adults couple shortly after emergence, feed the third day after emergence, and have a preoviposition period of 3 days. Egg rafts are always deposited on the surface of water and never on the paper toweling that extends above the water. *U. lowii* has now been reared through 20 generations and apparently has readily adapted to our handling.

Researchers studying diseases of poikilothermic animals have been interested in our colonies of *Culex peccator* Dyar and Knab and *C. territans* Walker because these species feed almost exclusively on cold blooded vertebrates. The availability of colonized *U. lowii* may be of interest to them. The colony also provides us with another genus for some of our biological control studies.

References

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A METHOD OF EVALUATING MOSQUITO LARVICIDES UTILIZING THE ULTRA-LOW-VOLUME TECHNIQUE ON SMALL PLOTS

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The ultra-low-volume (ULV) technique of applying insecticides by aircraft for mosquito control has been widely accepted in California. Investigations concerned with improving formulations, evaluating new materials, or assessing the effect of varying volumes have required the use of aircraft until recently. These tests have required fairly large plots, relatively large amounts of technical material, and the utilization of expensive pilot and aircraft time. We have found that data obtained from small plot tests with portable ground equipment have proved to be useful in indicating what might be expected in the way of performance when the same type of formulation is applied by aircraft.

Small plot tests offer advantages in providing a method to evaluate materials in the field with much less preparation time required than when aircraft must be used, and more materials or formulations can be evaluated in a reduced time interval. It must be emphasized, however, that this type of test does not involve all conditions of an aircraft test. Portable ground equipment is available that produces ULV droplet sizes well within the range suitable for larvicide applica-

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tions. Experience has indicated that coverage and distribution of insecticide in small scale plots with ground equipment tended to be very uniform.

We have used ⅛ acre plots to obtain results indicative of what might be expected from ULV airplane applications. Specially formulated insecticides designed for ultra-low-volume application have been evaluated by means of a portable unit called the Mity Moe Sr. (Fig. 1).[2] In order to obtain good pressure the one liter tank must contain at least 500 to 600 ml. of insecticide. Pacing, wind velocity, and plot width are variables which must be considered with care if meaningful results are to be obtained. A rectangular plot, 136 by 40 ft. (approximately ⅛ acre), provides a convenient pattern for use of this equipment in experimental tests. The delivery of insecticide to the minispin nozzle is adjusted so that the operator can walk along the upwind side of the plot, covering the 136 ft. distance in one minute, to deliver the desired volume to the entire plot. A 2 to 5 m.p.h. breeze will carry the droplets across the 40 ft. width.

To confirm this, a dispersion and distribution test was conducted. Aluminum pie plates (8 inch diameter) were placed 25 ft. and 50 ft. downwind from the discharge nozzle, each distance replicated 5 times with the plates spaced at 20 ft. intervals. At the time of the test the air temperature was 21° C. and the wind speed was approximately 5 m.p.h. The insecticide applied was fenthion 7 lb./gal. emulsifiable concentrate, extended with polypropylene glycol at 1.8 parts insecticide to 4.2 parts polypropylene glycol. The needle valve was adjusted to deliver 6 fluid oz./acre. Results indicated that at 25 ft. droplets averaged 30/sq. inch, and at 50 ft. 10/sq. inch.

In another test to determine droplet size, a series of seven magnesium oxide coated microscope slides were placed along a 136 ft. course at 27-ft. intervals. The Mity Moe insecticide tank was filled to capacity with 2 lb./gal. Dursban® and extended by adding 1 part of formulated insecticide to 1 part of polypropylene glycol. The needle valve was set to deliver 6 fluid oz./acre. The machine was held 3 ft. above ground level and the application was made at right angles to the line of slides with a 3–5 m.p.h. breeze. Discharge from the nozzle was maintained at 25 ft. from the slides. The range of droplet sizes and total number from the seven slides were as follows:

FIG. 1.—Portable ULV unit in operation. Photograph by T. D. Mulhern.

Droplet size (μ)	10	15	20	25	50	75	100	200	250	300	400	500
Total of 7 slides	13	3	10	6	14	3	53	8	12	8	2	1

The preponderance of 100 μ. diameter droplets appears to be well suited to obtaining good results in larviciding tests. For example, during the 1969 emergency control of *Culex tarsalis*, the small plot technique was used to determine whether fenthion, 7 lb./gal. emulsion concentrate, could be extended with polypropylene glycol for ULV application by helicopter. A rapid answer was obtained with ⅛ acre plot applications on *C. tarsalis* larvae in Kings Mosquito Abatement District.[3] Two plots were treated at the rate of 6 fluid oz./acre of finished insecticide (1.8 fluid oz. of fenthion formulation to 4.2 fluid oz. of polypropylene glycol). Twenty-four hour counts showed 93 and 100 percent mortality, indicating that this formulation was satisfactory for use in the emergency program. This was verified by highly successful helicopter applications throughout the season.

[2] Buffalo Turbine, Inc., Gowanda, New York. Mention of this equipment is for purposes of identification and does not imply endorsement.

[3] The cooperation of Richard F. Frolli, Manager, Kings Mosquito Abatement District, in arranging for these tests is sincerely appreciated.

This same equipment was used to evaluate other ULV formulations during the summer, but sufficient data have not been obtained to present the results at this time.

Experience gained during 1969 with this equipment in making experimental application of ULV mosquito larvicides has convinced us that this technique can be utilized to achieve a significant savings of time and funds in obtaining answers to pressing field problems.

A GRAVID INTERSEX OF *Culicoides obsoletus* [1]

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Biological anomalies possessing both male and female characteristics are generally termed gynandromorphs or intersexes. Intersexes, unlike genetically abnormal gynandromorphs, are genetically normal individuals (presumably males) that have undergone some form of stress resulting in a developmental modification so that their potential sexual characteristics do not develop as they would have otherwise (Smith and Perry, 1967). The occurrence of intersexual forms in *Culicoides* has been reported in 11 of approximately 800 described species (Callot, 1959; Dzhafarov, 1960; Callot and Kremer, 1963; Smith, 1966; Smith and Perry, 1967; and Navai and Mesghali, 1969). Although Smith and Perry (1967) reported high percentages of intersexes in three soil-dwelling (larvae) species collected in Florida, the occurrence of intermediate forms within the genus is rare.

The only report of an intersex of *C. obsoletus* (4 ♀♀ and 1 ♂) was made by Callot and Kremer (1963). Their description of a single male specimen of this species is similar to that of other species (Smith, 1966; and Navai and Mesghali, 1969). Typically, the head, antennae, and wings of a "male" intersex resemble those structures of the female; the intersex, however, retains the male terminalia. In some instances, the intersex has disoriented, atypical parameres, and the aedeagus may also be intermediate to some degree. Primarily, the secondary sex characteristics are altered in intersexes; although, according to Smith and Perry (1967), some primary sex characteristics may also be of an intermediate nature.

Nothing in the literature indicates to what extent a *Culicoides* intersex "functions" as either a male or female, or if it is sterile. There is evidence that mermithid nematodes produce sterility in some nematocerous Diptera.

A particularly interesting intersex of *C. obsoletus* was collected in a light trap at Malvern, Iowa. The specimen, one of 895 *C. obsoletus* collected during the summer of 1969, is an obvious intersex. Its head, antennae, and wings are typically female, but it has well-developed conspicuous external male terminalia. Moreover, in spite of the intermediate nature of the organism, the abdomen is replete with well-developed eggs. Figure 1 is a photograph of the tip of the abdomen of this "functional" intersex, and Figure 2 is an artist's illustration showing details of the same structure. The basistyles and dististyles are fully developed and near normal; however, both the ventral and dorsal roots of the basistyles are absent. The 9th tergite of the male is atypical, being intermediate between male and female. In a normal male *C. obsoletus,* the 9th sternite has a deep posterior emargination. The 9th sternite of this intersex is not cleft. The aedeagus and parameres are absent. Examination of the specimen does not reveal the presence of either of the two spermathecae normal for female *C. obsoletus.* An oval, lightly sclerotized structure appears near the base of each basistyle (Figures 1 and 2). A distinct oviduct is associated with the dense amorphous structure located near the anterior margin of the terminalia; a second amorphous mass at the tip of the terminalia is not identifiable and is seemingly an artifact.

Parasitic nematodes associated with larval forms of *Culicoides* are known to produce intermediate forms, at least in some instances (Callot and Kremer, 1963; Smith, 1966; and Smith and Perry, 1967). Evidently, parasitic nematodes alter maleness more drastically than femaleness. Beck (1958) noted no changes in parasitized females, and Smith and Perry (1967) had to dissect female *Culicoides* to determine the incidence of parasitism. Callot and Kremer (1963), however, reported some changes in parasitized females of three species of *Culicoides.* These changes were much less drastic than those observed in parasitized males. Indeed, it may be impossible to recognize an intersex that was a genetically intended female, especially if the changes are only slight variations in the already female secondary sex characteristics.

The formidable male terminalia of the intersex described here did not prevent the individual from functioning reproductively as a "normal" female. Such ability indicates the presence of a full complement of primary and, to some extent, secondary female sex characteristics. One can speculate about the difficulties an intersex of this type might encounter during the mating process and even at the time of oviposition.

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