EFFECTS OF COLLECTION AND HANDLING TECHNIQUES ON RICELAND MOSQUITOES USED IN LABORATORY AND FIELD INSECTICIDE SUSCEPTIBILITY TESTS[1, 2]

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ABSTRACT. A modified CDC miniature light trap and a hand-held battery powered aspirator were compared for collection of *Psorophora columbiae* for use in laboratory and field tests. No significant differences were found in mosquito mortality between the collection methods at 1, 12 or 24 hr posttreatment in the laboratory test or at 1 hr posttreatment in the field tests. Both methods proved viable alternatives for collection of *Ps. columbiae*.

Posttreatment handling techniques in field tests were investigated to determine if significant differences in mortality existed between mosquitoes transferred 15–20 min posttreatment to clean holding cages and mosquitoes which remained in the treatment cages after insecticide exposure. Synthetic pyrethroid, carbamate and organophosphorus insecticides were used against *Anopheles quadrimaculatus*. No significant differences in mortality existed between mosquitoes handled by the 2 techniques when treated with resmethrin. Significant differences were found in mosquito mortality between the 2 techniques when the treatments were benidiocarb and malathion. It is important to consider handling of the mosquitoes after treatment when comparing results of field tests involving caged mosquitoes, as different handling techniques influence levels of mortality.

INTRODUCTION

Collection methods for adult mosquitoes to be used in laboratory and field tests are numerous and varied with respect to equipment and handling techniques. Light traps and battery powered aspirators are commonly used in mosquito research for collection of adult mosquitoes for identification, disease surveillance, and other purposes. Many of these techniques are well documented and quite functional; however, when mosquitoes are to be kept alive for subsequent testing little procedural documentation exists.

Of the light traps used for mosquito collection, the CDC miniature light trap (Sudia and Chamberlain 1962) is very practical as it is easily transported and maintained. One of the potential problems associated with CDC miniature light trap collections is injury to the mosquitoes caused by passing through the blades of the light trap fan. This paper reports a change in the design of the CDC miniature light trap body to eliminate this possible problem.

In addition to the CDC miniature light trap, hand-held battery powered aspirators are commonly used for collecting mosquitoes from resting stations or various warm blooded hosts (Magnarelli 1975, Coombes et al. 1977, Roberts et al. 1980). Each collection technique has inherent advantages and disadvantages, depending upon the purpose for which the collection is being made. The light trap offers ease of collection and requires little manpower to make collections of large numbers of mosquitoes; however, collections must obviously be done after dark. The hand-held battery powered aspirator enables diurnal collection which conforms to normal working hours. Homogeneous collections can be accomplished with the aspirator whereas light trap collections are heterogeneous. More manpower is required for aspirator than for light trap collections.

The first objective of this study was to determine if significant differences existed in mortality observed in laboratory and field insecticide susceptibility tests using mosquitoes collected either with modified CDC miniature light traps or with hand-held battery powered aspirators. The second objective was to determine whether mortality was significantly affected by transfer of treated mosquitoes from exposure cages to untreated holding cages 15–20 min posttreatment.

Psorophora columbiae (Dyar and Knab) was chosen for the collection method study as this species is readily collected in large numbers using light traps baited with CO_2 as well as with hand-held battery powered aspirators. *Anopheles quadrimaculatus* Say was selected for the posttreatment handling study. This mosquito is rather cage tolerant, particularly when compared with *Ps. columbiae* which becomes

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hyperactive when caged. Also, it is not readily attracted to light traps.

MATERIALS AND METHODS

COLLECTION METHODS—WIND TUNNEL EXPOSURES. Adult *Ps. columbiae* were collected using modified CDC miniature light traps of our own design (Fig. 1). With this modification, mosquitoes do not pass through the fan blades but enter portals located below the blades, and are subsequently blown into the trap bag. This modification was accomplished by reversing the orientation of trap motor/fan/light assembly in the trap body. The top of the trap body was closed off by aluminum window screening so mosquitoes could enter only through 4 evenly spaced portals (5 cm diam) that were cut near the base of the trap housing. A 2 cm wide, funnel-shaped baffle (made from a section of a plastic funnel) was positioned inside the trap housing just above the entry portals in order to guide the airflow directly into the trap bag and away from the portals. Trap wiring was reversed so that the air would blow downward.

The light traps were placed along wooded areas bordering rice fields and suspended ca. 2 m off the ground. The traps were activated at dusk and were returned to the laboratory 1 hr later. Each light trap was augmented by suspending an 8 cm^3 block of dry ice in a perforated paper bag above the trap hood. The trap bags were covered with damp cheese cloth during transportation to maintain high humidity. Upon returning to the laboratory, the mosquitoes were aspirated from the trap bags using hand-held battery powered aspirators, anesthetized with CO_2, and placed in cylindrical (5.2 × 8.6 cm) unwaxed cardboard treatment cages (25/cage) with 16 mesh screen wire ends.

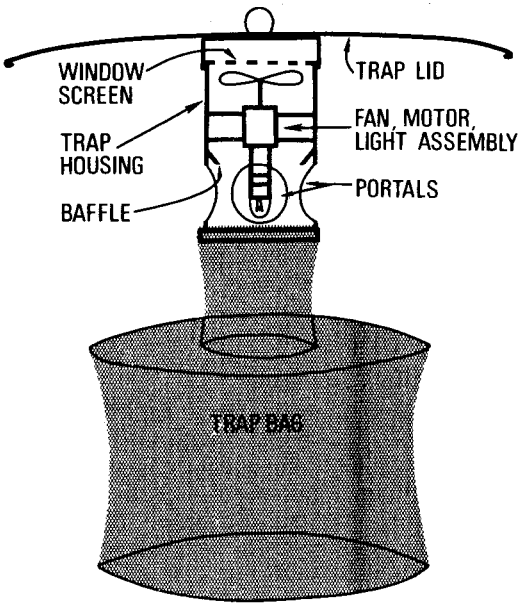

Fig. 1. Modified CDC minature light trap.

Psorophora columbiae were aspirated using rechargeable Black and Decker MOD 4 Spot VACs® modified so that aspirator tubes measuring 12.5 × 5.5 cm fit over the vacuum intake. Adult female *Ps. columbiae* were aspirated from human attractants at the same time and vicinity of the light trap operation. Aspirator tubes each containing ca. 25 female *Ps. columbiae* were returned to the laboratory in insulated chests with damp paper towelling to maintain high humidity. The mosquitoes were anesthetized with CO_2 and placed in treatment cages as per the light trap collected mosquitoes. The caged mosquitoes were then treated with acetone (manner in which controls are normally handled in wind tunnel testing) in a wind tunnel as described by Mount et al. (1976). An automatic pipette was used to deliver 0.25 ml aliquots of acetone into the wind tunnel nozzle. After treatment, the mosquitoes were anesthetized with CO_2, transferred to clean holding cages and furnished with water-soaked raisins (1/cage). Mortality was recorded at 1, 12 and 24 hr after exposure in the windtunnel. This test was designed to determine if significant differences existed in mortality due to collection method.

Mortality data were transformed to arc sine and subjected to analysis of variance. Means were separated based on least significant difference ($P = 0.05$).

COLLECTION METHODS—FIELD EXPOSURE. Field tests were conducted with mosquitoes which were collected and anesthetized as per the laboratory wind tunnel testing. The mosquitoes were then placed in 16 mesh galvanized screen wire treatment cages (25/cage) measuring 20 × 8 cm. The caged mosquitoes (1 cage of each collection method) were suspended on steel rods 1.3 m above the ground in a soybean field 15.2, 30.5, 61.0 and 91.4 m perpendicular and downwind from the spray route. The soybean plants were ca. 0.5 m high. Each test plot was comprised of 4 rows, spaced 15.2 m apart. Treatments were applied with a truck-mounted Heavy Duty LECO® cold aerosol generator with nozzle pressure at 20.7 kPs. Ground speed for all tests was 16 kph. Treatments were resmethrin (7.84 g/ha) and 3 dosages of resmethrin/piperonyl butoxide (1:3); 3.92/11.76, 1.96/5.88 and 0.98/2.94 g/ha. Each collection method was replicated 4 times at each distance. Untreated controls from each collection method were handled identically and placed 500 m outside

and upwind of the treatment zone. The wind during testing varied between 8 and 11 kph and temperature was between 28 to 30°C. At 10–15 min posttreatment, the cages were transported to the laboratory, maintained in an environment of high humidity and the mosquitoes furnished with water-soaked raisins (1/cage). Mortality was checked at 1 hr posttreatment. All tests were conducted within 2 hr after dusk.

Mortality data were corrected for control mortality using Abbott's formula, transformed to arc sine and subjected to analysis of variance. Means of percent mortality were separated by Duncan's multiple range test (P = 0.05).

POSTTREATMENT HANDLING STUDY. *Anopheles quadrimaculatus* adults were collected with the hand-held battery powered aspirators previously described in the collection methods study from a barn which served as a resting station for engorged females. The mosquitoes were transported, anesthetized and placed in treatment cages as in the field tests of the collection methods study. Insecticides representing 3 different classes applicable to mosquito adulticides were chosen for this study in an attempt to determine if class of insecticide had an influence on the results. Resmethrin, bendiocarb and malathion represented the synthetic pyrethroid, carbamate and organophosphorus classes, respectively. One cage of mosquitoes for each handling method was suspended on the steel rods of the test plot and treated. The test plot was the same as described for the field tests of the collection methods study, and ground speed of the treatment vehicle was the same; however, nozzle pressure varied among treatments. Treatments were applied as in the field tests of the collection methods study with nozzle pressure at 20.7, 34.5 and 34.5 kPs for the resmethrin, malathion and bendiocarb dosages, respectively. Treatments were resmethrin (7.84 g/ha) and 3 dosages of resmethrin/piperonyl butoxide (1:3); 3.92/11.76, 1.96/5.88 and 9.8/2.94 g/ha. Other treatments were malathion (57.9 g/ha) and bendiocarb (1.5 g/ha). Each handling technique was replicated 4 times at each distance. Other test parameters, wind speed, temperature and controls were consistent with those of the collection methods study. At 10–15 min posttreatment the cages were transported to the laboratory, where the transfer group of caged mosquitoes were anesthetized with CO_2 and transferred to clean unwaxed cardboard holding cages with 16 mesh screen wire in the lid. Both groups of caged mosquitoes were offered water-soaked raisins (1/cage). Mortality was observed at 1 and 24 hr posttreatment.

Mortality data were corrected for control mortality using Abbott's formula, transformed to arc sine, and subjected to analysis of variance. Means of percent mortality were separated by Duncan's multiple range test (P = 0.05). Results of tests where control mortality exceeded 20% were not included.

RESULTS AND DISCUSSION

EFFECT OF COLLECTION METHODS. Laboratory and field tests were conducted to determine if significant differences existed in mortality between mosquitoes collected by modified CDC miniature light trap and a hand-held battery powered aspirator. No significant differences in mortality were found between means of the collection methods at 1, 12 or 24 hr after exposure in the wind tunnel (P = 0.05). Significant differences did exist between mortality means of hours posttreatment. Both collection methods appeared to be equally well suited for collection of *Ps. columbiae*.

Field tests confirmed the findings of the laboratory wind tunnel tests as no significant differences in mortality were found between collection methods at 1 hr posttreatment for the various dosages tested (P = 0.05). The additional stress of an insecticide treatment in the field tests could make differences in mortality between the 2 collection methods more apparent than in the wind tunnel tests.

These data confirm the findings of the laboratory wind tunnel test and suggest that for collection of *Ps. columbiae*, both the modified CDC miniature light trap and the hand-held battery powered aspirator are viable alternatives for collection, and results of tests may be compared although the collection methods differ. It was observed that the modified CDC miniature light trap bag could not be allowed to become markedly overcrowded as this increased control mortality. Leaving the test insects in the trap bags for prolonged periods of time also tended to exacerbate control mortality, especially under crowded conditions. Care must be exercised when using the hand-held battery powered aspirator to not hold the mosquitoes under suction for prolonged periods of time. This becomes very difficult when only small numbers of mosquitoes are available for collection. Prolonged suction and overcrowding of the aspirator tubes seemed to exaggerate mortality.

EFFECT OF POSTTREATMENT HANDLING. Field tests were conducted to determine if significant differences existed in mortality between mosquitoes transferred 15–20 min after treatment to clean holding cages and mosquitoes which remained in the treatment cages after exposure. Results of these tests indicate that no significant differences occurred when resmethrin

alone and the 2 higher dosages of resmethrin: piperonyl butoxide were used. Mosquitoes exposed to resmethrin: piperonyl butoxide at the lowest dosage (0.98/2.94 g/ha) showed increased mortality when transferred. It is a possibility that the mosquitoes were stressed immediately after treatment due to the fast mode of action, and when transferred to the holding cages this additional handling and anesthetization exacerbated mortality.

Significant differences were found between bendiocarb and malathion treatments. The corrected mortality rates of bendiocarb for transferred and nontransferred mosquitoes were at 1 hr posttreatment 16.2 and 22.8%, and at 24 hr 38.0 and 83.0%. Respective mortality rates for transferred and nontransferred mosquitoes exposed to malathion were at 1 hr posttreatment 8.0 and 25.8%.

The general trend in these results indicated that mosquitoes in the nontransfer group received longer exposure to the insecticide and exhibited higher mortality than transferred mosquitoes which received only 15–20 min insecticide exposure. This was particularly exemplified by the results of the bendiocarb test. The quick knockdown capability of resmethrin alone and the higher dosages of resmethrin: piperonyl butoxide was such that mosquitoes encountered a lethal dosage prior to transfer, therefore negating any detectable differences between the 2 handling techniques.

In conclusion, results of these tests indicate that for fast acting compounds such as resmethrin and/or resmethrin:piperonyl butoxide mixtures, transferring the mosquitoes after treatment is less of a concern than for slower acting compounds.

With slower acting compunds, such as bendiocarb and malathion, it would seem advisable to consider transferring mosquitoes after treatment in field tests. Mortality may be exaggerated in treatments where mosquitoes are not transferred shortly after treatment. The results of these tests indicate that in data presentation involving cage tests, it is important to state whether or not the mosquitoes were transferred from exposed treatment cages.

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