MARKING ADULT MOSQUITOES USING AN AERIALLY APPLIED FLUORESCENT PIGMENT[1,2]

C. L. MEEK,[3] B. B. BROUSSARD[4] AND M. D. ANDIS[3]

Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

ABSTRACT. A water soluble, fluorescent pigment was aerially applied to caged *Culex quinquefasciatus* adults in a south Louisiana marshland pasture. Mosquitoes held in cages on 1 m stakes were >90% marked. This number was significantly greater ($P < 0.01$) than the number of marked mosquitoes held in cages that were placed in dense vegetation (≧0.5 m high) near the ground surface (70% marked). In a second aerial test with caged *Aedes sollicitans* in an open, grassy area of the marshland pasture, the pigment marked 100% of the adult mosquitoes held in cages 1 m above the ground and 98% of the caged mosquitoes on the ground surface. Greater than 96% of the adults collected from an emerging population of *Ae. sollicitans* within the test area were marked as well as 100% of wild caught deer fly adults, *Chrysops flavidus* complex, in the test area.

INTRODUCTION

A fundamental component in studying the dispersal of flying insects is the ability to determine, as accurately as possible, the point of origin of individuals collected. Service (1976) reviewed mark-recapture techniques and the research on dispersal for adult mosquitoes. He noted that most studies involving the marking of adult mosquitoes have been used to measure dispersal, and the knowledge of their flight range was of great importance to personnel involved in mosquito control efforts. However, attempts to mark and recapture mosquitoes, have been characterized by low numbers being marked in proportion to the total number of individuals in the natural population, and this in turn has led to disappointingly few marked individuals being recaptured.

The basic hypothesis of this study was to determine if an aerial application of a fluorescent pigment could effectively mark exposed adult mosquitoes while they are swarming and/or dispersing. This study was conducted to evaluate concurrently the efficacy of a fluorescent pigment to mark a natural population of adult mosquitoes and assess its application through a high volume, aerial application spray system. If successful, the technique will be incorporated into a dispersal study in southern Louisiana using riceland and/or marshland mosquitoes as research models.

MATERIALS AND METHODS

A Grumann AgCat® aircraft was used on two occasions to aerially apply a water soluble fluorescent pigment[5,6] to caged, adult mosquitoes in a marshland pasture in southcentral Vermilion Parish, Louisiana. Prior to each application, 18.9 liters of a 50% water suspension pigment were mixed with a hydroxy ethyl cellulose stabilizer (Cellosize®)[7] at a rate of 0.05% by weight, after being hydrated. The stabilizer was hydrated by adding a sufficient amount of isopropyl alcohol to 100 g of the stabilizer to make a thin slurry. The stabilizer inhibited the pigment particles from settling in the water suspension prior to application.

One liter of tapwater was heated to 26°C and adjusted to pH 8.0 with KOH. A surfactant, such as a household detergent, was added to the alkaline water at a rate of 0.05 ml/liter of alkaline water. The stabilizer was then slowly added while stirring. This procedure tended to inhibit the formation of large coagulants that could eventually clog the aircraft spray system. After the mixture became clear and viscid following 30–45 min of stirring, it was added to the pigment suspension. One hundred milliliters of for-

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[3] Department of Entomology, Louisiana Agricultural Experiment Station, LSU Agricultural Center, Louisiana State University, Baton Rouge, LA 70803.

[4] Vermilion Parish Mosquito Control District, P. O. Box 209, Abbeville, LA 70510.

[5] Mention of a commercial product does not constitute a recommendation for use or endorsement for sale by the Louisiana State University Agricultural Center.

[6] Fluorescent pigment No. 15 (Blaze Orange) manufactured by DAY-GLO Color Corp., 4515 St. Clair Ave., Cleveland, OH 44103.

[7] A commercial product by Union Carbide Corporation.

malin were then added to the suspension to prevent bacterial degradation of the stabilizer.

The pigment mixture was poured into the aircraft hopper that had been filled previously with 397.5 liters of tapwater, resulting in an approximate mixture ratio of 21:1 (water: pigment). The aircraft was equipped with 42 adjustable D2 MulteeJet® nozzles.[8] The test area consisted of a 1.13 ha plot in the marshland pasture. The first of two aerial applications was made at noon on May 9, 1984 with a wind speed of <4.8 km/hr and an ambient temperature of 27°C. A second aerial test was conducted at mid-morning on August 29, 1984 with the wind speed ranging between 9.7 and 12.9 km/hr and an ambient temperature of 31°C. The aircraft speed during both tests was 160.9 km/hr. At an altitude of 15.2 m, a swath width of 15.2 m was obtained to insure maximum coverage of the pigment in the test plot.

The first test consisted of two plots designated A and B. Each plot had 16 stakes (1 m high) arranged in a 4 x 4 pattern. At least 15.2 m separated each stake for a total of 32 stakes in the two plots. Thirty-two screened (16 mesh), 15.2-cm-long cylindrical cages, each containing 10 laboratory-reared adults of *Culex quinquefasciatus* Say, were attached to the tops of the stakes in plots A and B. Both plots were located in an area of the marshland pasture that had 0.5 to 1.0 m-high vegetation. To further challenge the aerial marking technique, 16 other cages of similar size and also containing 10 adult mosquitoes each (hereafter referred to as Plot C) were placed at or near the ground surface amid the vegetation throughout plots A and B.

The second test consisted of 32 staked cages and 8 ground cages, each with 10 adults of *Aedes sollicitans* (Walker). These cages were of the same dimensions and construction as those used in the first test. Sixteen, 1 m stakes were arranged in a 4 x 4 pattern with 2 cages attached to the top of each stake. This test site was located in a more open, grassy area of the same marshland pasture used in the first test. The vegetation was approximately 15 cm high and was more typical of a larval mosquito habitat in an upland marsh pasture. This test also took advantage of a newly-emergent *Ae. sollicitans* adult population resting in the vegetation. Following the aerial application of the marker, 428 *Ae. sollicitans* adults were collected from the vegetation using an aspirator described by Meek et al. (1985). Care was taken not to contact the vegetation with the aspirator in order to prevent contamination by the collection tube of the aspirator. Twenty-three adult specimens from a native deer fly population, *Chrysops flavidus* Wiedemann complex (Diptera: Tabanidae), also were collected in the vicinity of the test plot after the aerial application.

To evaluate the dispersal of the pigment spray within the test plots, eight 0.24 liter paraffin-coated paper cups were inverted and placed on the ground throughout each of the plots in both field tests. The number of pigment spots per 3 cm^2 on the bottom of each cup was recorded in the laboratory following the aerial application. The spots ranged in size from 0.1 to 1.25 mm in diameter with an average diameter of 0.5 mm and were enhanced for counting by using a long wave, ultra violet (UV) light source and a stereoscope.

It was possible that the adult mosquitoes were being marked by the fluorescent pigments attached to the screened cages that were involved in the tests. Ten adult mosquitoes, previously unexposed, were placed in each of 10 cages retrieved from the field tests. After 3 hr the specimens were removed and observed under the UV light source for the presence of the pigment. An individual mosquito was considered marked if there was at least one pigment spot on the body. No increased value was given an individual if there were ≧2 spots on the specimen.

Personnel handling the field cages and conducting the experiments in the laboratory were instructed to exercise appropriate care to avoid contaminating test mosquitoes and deer flies. Prior to the processing of the test specimens and the recording of the number of marked individuals, all personnel washed their hands and periodically checked under the microscope and UV light source for contamination. Hand-held instruments, used to manipulate specimens while under observation, also were checked periodically for pigment spots.

RESULTS AND DISCUSSION

During the first field test, the aerial application technique was effective in that >90% of the caged *Cx. quinquefasciatus* adults located 1 m above the ground were marked with the fluorescent pigment. Plot A had 91.6% (132 of 144) of the caged adults with at least one pigment spot per mosquito. Plot B had 90.3% (131 of 145) of the adults marked in cages positioned on stakes. These data indicate that the 16-mesh screened cages did not inhibit the pigment appreciably from penetrating the cage to the adult mosquitoes. More importantly, the data support the hypothesis that aerially exposed adults (i.e., swarming and dispersing mosquitoes) can be effectively marked.

There was no significant difference ($P > 0.01$) in the number of marked individuals between

[8] Spraying Systems Co., Wheaton, IL 60187.

plots A and B. However, there was a significant difference ($P < 0.01$) between the number of marked adults in plot C and those in the other two plots where only 69.7% (108 of 155) of the adults became marked with the pigment.

It appears that tall vegetation inhibits, to some degree, the availability of the pigment near the ground surface. A significant difference ($P < 0.01$) was recorded between the number of pigment spots per square centimeter on paper cups within the tall vegetation of plot C of the first test (31 spots/cm^2) versus the number of pigment spots on cups within the shorter grass of the second aerial test (104 spots/cm^2).

There are no available data to indicate the residual pigment on the vegetation. Treated vegetation may mark future populations of mosquitoes as they emerge from the larval habitat. In addition, it may serve to mark emigrant adult mosquito adults using the pigment treated area as a resting site prior to subsequent dispersion.

Laboratory experiments indicate that nontreated mosquitoes can become marked with the fluorescent pigment when exposed to treated screened cages. When untreated *Cx. quinquefasciatus* adults were introduced into screened cages recently removed from the field test, approximately 20% of the adults acquired at least one pigment particle during 3 hr of exposure. These data indicate that marked vegetation within a sprayed area may mark a portion of the emerging or emigrant mosquito adults as they rest or move about upon the vegetation.

In the second field application of the fluorescent pigment there was no significant difference between the number of marked *Ae. sollicitans* held in cages 1 m above the ground and in cages located on the ground. Mosquitoes held in 1 m cages were 100% marked (N = 208) and those adults in ground cages were 98.1% marked (209 of 218). These data suggest that in pastures or fallow fields where the predominant vegetation is relatively short (i.e., ≦15 cm), one can expect a very high percentage of the emerging or resting adults to become marked with the pigment. In treatment areas where the vegetation is taller and has a greater number of plants per square meter, the percentage of marked adults can be expected to be proportionately smaller.

The technique was successful in marking an emergent population of *Ae. sollicitans* adults within the treatment area. Four hundred and twenty-eight mosquitoes were collected and approximately 95% (412 of 428) of the *Ae. sollicitans* were marked with the pigment (confidence interval of 0.0198, Steel and Torrie 1980). In addition, 23 adult deer flies were captured in the treatment area, transferred to clean cages and returned to the laboratory where all were found to be marked. It is not known whether the flies were marked while resting on vegetation or flying through the treatment area. In any case, this information demonstrates another benefit of the aerial application technique of the fluorescent pigment—that is, other flying, haematophagous insects can be marked successfully and their dispersal behavior studied.

Before this technique can be implemented fully, there are several informational gaps still remaining. A study is needed to determine the impact the pigment has on mortality, flight performance and other behavioral aspects of the treated adults. Another aspect of the technique in need of study is the drift factor inherent to each aerial application of the pigment. Ideally, the pigment should be confined to the target area where eclosing adults are found. There is some evidence that the pigment may be carried >500 m from the target area if >16 kph winds are present at the time of aerial application. This is in spite of the large droplet size and high volume application rate required to insure maximum and intensive coverage of the pigment in the target area.

In summary, the aerial application technique was effective for marking adult mosquitoes with a fluorescent pigment under field conditions. It was also effective in marking other flying insects that ventured into the treatment area. The pigment spotting of vegetation provided a potential advantage by marking emergent and/or emigrant adult mosquitoes using the treatment area as a resting site before continuing their dispersal.

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