

PROTOCOL FOR MARK-RECAPTURE STUDY AEDES ALBOPICTUS

Released *Aedes albopictus* males were obtained from the mass rearing pilot system of the Laboratory of the Medical and Veterinary Department of the Centre "G. Nicoli" in Crevalcore (Bologna, Italy).

Timing of delivery was previously tested by sending a batch of eggs on August 26 and resulted 46 hours (from pick up at CAA lab to Univ. of Montenegro, Podgorica).

Marking and release method

L₄ larvae were sent by express courier from Crevalcore to Podgorica.

Sexing was performed in Montenegro by using the sieving technique on pupae in the water (Bellini et al. 2002, 2007).

The male pupae were positioned in cages..... to let the adults emerge and to be marked with fluorescent dust (RADGLO JST44 RED ORANGE dust (Day Glo, Cleveland, OH) just prior the field release. A paper strap with 10% sugar solution was positioned in the cage. Marking was performed with manual insufflators.

The study was undertaken from

The release of males marked with fluorescent pigment was performed in Radovici (see attached map).

The dusted males were released as adults aged 48-72 h by placing and opening the cages in a shaded area. The cages were gently shaken for 10 min, to induce the males to exit. The males that remained in the cage after 10 min were considered dead.

Recapture method

Following the male releases, three recapture sessions were conducted on days 2, 4 and 7 post-release (in case of adverse weather conditions, the captures were performed on the following day) by a team of three skilled technicians using manual aspirators to catch the males flying around the human host (the technician) and sweeping nets for those flying in mating swarms, for 3 h per day during the male peak of activity (from 4:30 p.m. to 7:30 p.m.). The team walked randomly within the study area with the help of Orthophoto maps, to identify suitable resting and mating sites, within a radius of 250-350 m from the release site. The most favorable sites were sampled at least once (often more than once) during the three recapture sessions. Density of recapture station was kept homogeneous following the scheme:

Basically the idea is to keep homogeneous density of stations inside each circle such as:

< 50 m = 7850 sqm = N. stations 2

50-100 m = 23550 sqm = N. stations 6

100-150 m = 47100 sqm = N. stations 12

150-200 m = 78500 sqm = N. stations 20

200-250 m = N. stations 30

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Release and recapture data were geo-referenced using a Global Positioning System device (Holux GR-230 bluetooth GPS Receiver; Holux Technology, Hsinchu, Taiwan). All coordinates were entered into a Geographical Information System (ESRI ArcView 3.3), which calculated the distances between release and each recapture sites.

Weather parameters were recorded throughout the course of the study (air temperature, relative humidity, wind speed and direction, rainfall) by a weather stations situated ina few kilometers from the three study localities.

Marked Male Discrimination. Male individuals were placed at 20°C soon after the collection and screened the following morning. FP males were recognized by visual inspection using a stereomicroscope.