

Dispersal and Survival of *Aedes albopictus* (Diptera: Culicidae) Males in Italian Urban Areas and Significance for Sterile Insect Technique Application

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ABSTRACT The dispersal and survival of laboratory-reared *Aedes albopictus* Skuse males were investigated during the summer of 2007 in three Northern Italy urban localities by mark-release-recapture techniques. Two marking methods were compared: one group of males was dusted with fluorescent pigments on the body (FP), and the other group was obtained from a strain whose natural infection of *Wolbachia* had been removed (WB0). FP- and WB0-marked males were released as adults and pupae, respectively, in one fixed station at each locality. Recaptures were performed by skilled technicians, within a radius of 350 m from the release site, on days 4, 5, and 7 after the release, and the males were collected while flying around the technician's body or in swarms. Recapture rates ranged from 0.63 to 4.72% for FP males and from 2.39 to 11.05% for WB0 males. The mean distance traveled for WB0 males was significantly higher than for FP males; no difference was observed between the dispersal distance measured for the males recaptured on human host versus males recaptured while swarming. No further increase of the dispersal occurred during the postrelease period investigated (from day 4 to day 7 after release). The mean survival rate at the release was 0.51 for FP-marked males and 0.81 for WB0 males. The data obtained are discussed for their significance in planning sterile insect technique programs against *Ae. albopictus*.

KEY WORDS sterile insect technique, fluorescent pigment, *Wolbachia*, mark-release-recapture, dispersal and survival

Aedes albopictus (Skuse) is an Asian mosquito species, which in the last decades invaded wide regions of North and South America, Africa, and Europe (Hawley 1988, Lounibos 2002, Benedict et al. 2007). Its spread was mainly the result of the international shipping trade of secondhand tires, which provides an ideal habitat for immature stages' passive dispersion (Reiter and Sprenger 1987, Knudsen 1995).

In the Italian peninsula, the colonization process started at the beginning of the 1990s (Sabatini et al. 1990). The species is currently found in most of the Italian regions, including the main islands, and has become the most important pest and vector mosquito species in urban and suburban areas (Romi 2001). The

recent outbreak of Chikungunya virus in Northern Italy (Angelini et al. 2007), which occurred in the summer 2007, showed that even recently colonized temperate areas are exposed to vector disease transmission, and sanitary authorities are facing a scenario that was not predictable just a few years ago (Gratz 2004). Besides the vector role, *Ae. albopictus* causes serious problems because of its high anthropophily and painful bite, with a strong impact on everyday outdoor activities during the summer months (from May to October).

Conventional methods, including larval control in public drains and continuous information campaigns, gained only partial or unsatisfactory results (Carrieri et al. 2006). The main reason for the failure of conventional control practices is the peculiar ecobiology of the species, whose reproductive habitat requirements can be satisfied by a variety of water-collecting containers placed in private gardens, backyards, and vegetated areas. The public institutions that are in charge of the mosquito control programs found it extremely challenging to get these breeding sites under control because of the high costs of the monitoring and treatment activities required for such widely diffused larval microhabitats and because of the insuffi-

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cient awareness of the private residents. The campaigns for community involvement, even when regularly conducted with professional methods, rarely achieve a level of active participation that can be considered sufficient for an adequate sustainment of the mosquito control programs (Morrison et al. 2008). At the same time, the demand to implement the use of adulticide treatments in private and in public areas is increasing, with negative environmental and sanitary side effects as a result of exposure to toxic products.

Starting from the scenario described above, a research project for the development of a sterile insect technique (SIT) program for the suppression of *Ae. albopictus* in Italy started in 1999 (Bellini et al. 2007). The SIT strategy consists of mass rearing, sterilization, and repeated release of sterile insects, to progressively reduce the reproductive rate of the target species (Alphay et al. 2010). In the case of mosquito species, it is necessary to release only males, as the release of biting females is not acceptable.

Some biological and ecological features make *Ae. albopictus* a good candidate for the application of SIT. Mass rearing of *Aedes* species is relatively easy when compared with other mosquito species (*Anopheles*), and a pilot model system to rear *Ae. albopictus* has already been set up at our facility in Crevalcore, Italy (Bellini et al. 2007). The genetic differentiation observed among the Italian populations showed at the regional geographic scale the existence of structured populations with restricted gene exchange among them (Urbanelli et al. 2000); the active dispersal of the species is recognized to be poor (Hawley 1988, Rai 1991, Niebylski and Craig 1994, Takagi et al. 1995, Honório et al. 2003).

Knowledge about male survival and dispersal capacity in the field is of fundamental importance to develop SIT programs. Being hematophagous, the vector, mark-release-recapture experiments have been used largely to study mosquito female dispersal and survival in different ecological conditions (Bonnet and Worcester 1946, Mori 1979, Niebylski and Craig 1994, Takagi et al. 1995, Lacroix et al. 2007). On the contrary, very few studies were targeted on mosquito males (Ferguson et al. 2005), and little information is available on male biology, ecology, and behavior (Trips and Hauserman 1986, Niebylski and Craig 1994, Muir and Kay 1998, Lacroix et al. 2007).

Our study was designed to investigate, through mark-release-recapture trials, the dispersal pattern and survival in urban areas of *Ae. albopictus* males. Two methods were used to mark the reared males before the release. The first one involved the use of fluorescent pigments, and was already applied by several authors to a number of mosquito species in different habitats and with different dispersal behavior (Service 1997, Vlach et al. 2006, Bogojević et al. 2007).

The second marking method involved the use of an aposymbiotic strain, whose *Wolbachia* infection had been removed. To our knowledge, this is the first time this approach has been used in mark-recapture-release trials. *Wolbachia* are maternally transmitted rickettsia-like bacteria, estimated to

infect as many as 16–22% of all insects (Werren et al. 1995, West et al. 1998, Werren and Windsor 2000). *Ae. albopictus* is reported to be uniformly superinfected with two *Wolbachia* strains (*wAlb A* and *wAlb B*) throughout its geographical area of distribution, and the occurrence of noninfected males has never been reported (Zhou et al. 1998; Dobson et al. 2001; M.C., unpublished data). Aposymbiotic strains may be produced by providing adult mosquitoes with tetracycline, according to the protocol of Dobson and Rattanadechakul (2001). Previous research found that uninfected females, obtained with the antibiotic treatment, showed a decrease in their fitness, compared with females normally infected with *Wolbachia* (Dobson et al. 2004). In contrast, no difference was observed in longevity and mating parameters of uninfected males in comparison with the natural infected ones (Calvitti et al. 2009). Consequently, we assumed as feasible the use of an aposymbiotic strain for mark-release-recapture studies, focused on *Ae. albopictus* males. The release of *Wolbachia*-free *Ae. albopictus* males does not pose any environmental risk, because the aposymbiotic status can be only maternally inherited to progeny.

Materials and Methods

Study Area. Mark-release-recapture experiments were conducted in three urban localities, all situated in the Po plain (Northern Italy, Bologna province; Fig. 1): Castel Maggiore (44°34' 40"N, 11°21' 42"E), with 13,769 inhabitants and an average density of 6,551 inhabitants/km² and 1,222 houses within an area of 2.1 km²; Altedo (44°40' 4"N, 11°29' 30"E), with 3,512 inhabitants, an average density of 2,319 inhabitants/km², and encompassed 1,090 houses on an area of 1.5 km²; and Castello d'Argile (44°40' 52", 11°17' 48"E), with a population of ~2,964 inhabitants, an average density of 2,298 inhabitants/km², and encompassed 709 houses on an area of nearly 1 km². The percentages of land covered by vegetation within a radius of 350 m from the center of the investigation areas were 37% for Castel Maggiore, 37% for Altedo, and 46% for Castello d'Argile. Each locality was surrounded by rural areas and included usually two-storied houses, separated by narrow lanes, with many private and some public gardens. These landscape features are representative of most of the small towns in Northern Italy. The presence of *Ae. albopictus* populations in these areas had been proven by monitoring activities conducted since 2003 (R.B., unpublished data).

Mosquito Rearing and Marking Procedure. All released males were obtained from the mass rearing pilot system of the Laboratory of the Medical and Veterinary Department of the Environmental and Agriculture Centre "G. Nicoli" in Crevalcore (Bologna, Italy). Standard rearing conditions were 27 ± 1°C, 85% RH, 15- to 9-h light-dark photoperiod. Adults were kept in Plexiglas cages (50 × 50 × 50 cm) supplied with a 10% sucrose solution. Females were blood fed with fresh mechanically defibrinated bovine blood, by means of

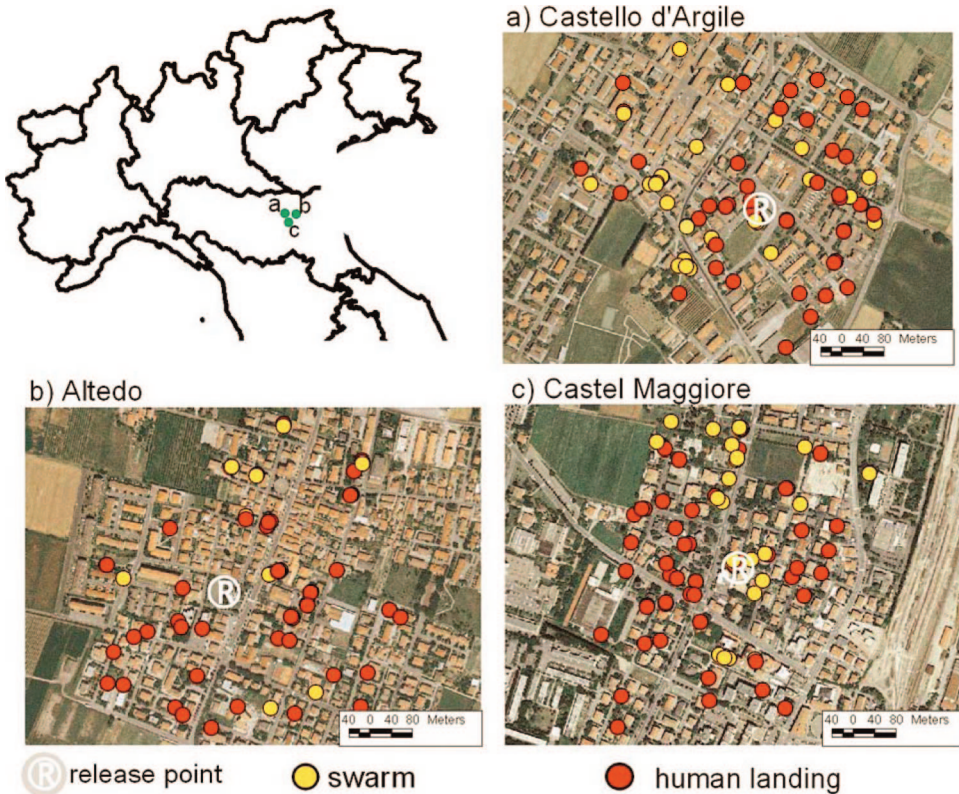


Fig. 1. Top left: the green dots and letters indicate the position of the three localities within the Po' Valley (Emilia-Romagna region, Northern Italy). Top right and bottom: maps of Castel Maggiore (a), Altedo (b), and Castello d'Argile (c), showing the sites where marked *Ae. albopictus* males were released and the sites where swarming and human landing *Ae. albopictus* males were collected during the three recapture sessions (4, 5, and 7 d after release). Yellow dots indicate the swarming males' capture points; red points indicate the human landing males' capture points. In case of superimposition, dots position was slightly changed to make all sampling points visible on the maps. (Online figure in color).

a special thermostatically controlled heating apparatus, and eggs were laid on filter paper. Larvae were reared in white plastic trays (41 × 31 × 11 h cm) containing 3 liters of dechlorinated water provided with air insufflators. Larvae were provided with a diet of 2.1 mg/larva FriskiesAdults dry cat food + 0.38 mg/larva of yeast + 0.15 mg/larva Tetramin (Bellini et al. 2007). Sexing was performed during the pupal stage by using the sieving technique (Bellini et al. 2002, 2007).

The males to be marked with fluorescent dust originated from three strains reared according to the method described above (Rimini F₂₀, Matera F₄, Rimini field collected). The fluorescent pigment RADGLO JST44 RED ORANGE dust (Day Glo, Cleveland, OH) was applied to adult males in 50 × 50 × 50-cm cages with manual insufflators just before the release.

The males of the aposymbiotic (WB0) strain were first produced at the Laboratory of Biological Control and Insect Biotechnology of Italian National Agency for New Technologies Energy and Sustainable Economic Development (ENEA)-Casaccia Research Center (Rome, Italy), starting from eggs collected in

the same area, through a process of selection of isofemale uninfected lines, performed during four generations. *Wolbachia* infection was removed by tetracycline treatment of adults, according to the method of Dobson and Rattanadachakul (2001). A sample of ≈500 eggs of the F₁₀ generation was then moved to Crevalcore to be mass reared, as described above.

Mosquito Release and Recapture. The study was undertaken from 19 July to 6 September 2007. One release of males marked with fluorescent pigment (FP) and one of aposymbiotic males (WB0) were performed in each locality (Castel Maggiore, Castello d'Argile, and Altedo) for a total of six release sessions. The release sites were chosen with the aid of Google Earth maps by identifying a green area in the central zone of each locality.

The dusted males were released as young adults (24–48 h) by placing and opening the cages in a shaded area. The cages were gently shaken for 30 min, to induce the males to exit. The males that remained in the cage after 30 min were considered dead.

The aposymbiotic males were released as pupae of 24–40 h in the same sites used for the release of the dusted males by simply positioning a black plastic

container in a shadow, covered by a metallic grid to protect its contents. Dead pupae were counted on day 3 of postrelease.

After each of the six male releases, three recapture sessions were conducted on days 4, 5, and 7 postrelease (in case of adverse weather conditions, the captures were performed on the following day) by a team of four to five 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 area with the help of red, green, and blue (RGB) Orthophoto maps, to identify suitable resting and mating sites, within a radius of 350 m from the release site. All the most favorable sites were sampled at least once (often more than once) during the three recapture sessions.

To verify the rate of replenishment of the spermathecae, females were collected while landing on the human host (the technician).

Release and recapture data were geo-referenced using a Global Positioning System (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 recapture sites.

Weather parameters were recorded throughout the course of the study (air temperature, relative humidity, wind speed and direction, rainfall) by two weather stations situated in Bologna and S. Pietro Capofiume (Molinella) (Dexter data; Regional Agency for Environmental Protection [ARPA] Emilia Romagna Region) a 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. Potential WB0 males were individually put into Eppendorf with 75% ethanol until molecular analysis was performed. DNA was extracted from individual mosquitoes using the cetyltrimethyl ammonium bromide (CTAB) procedure, as described by Collins et al. (1987). Assays for *Wolbachia* infection were performed by polymerase chain reaction (PCR) amplification of the *Wolbachia* surface protein (*wsp*) gene using the diagnostic primers 81 F and 691R (Braig et al. 1998). PCR cycling procedure was as follows: 95°C for 5 min, followed by 33 cycles of 93°C for 1 min, 52°C for 45 s, 72°C for 1 min 30 s, and a single final step at 72°C for 10 min. Amplified fragments were electrophoresed on 2% agarose gel, stained with ethidium bromide (1 $\mu\text{g}/\text{ml}$), and visualized with ultraviolet light. DNA template quality was assessed by successful amplification of a fragment of insect mitochondrial cytochrome oxidase I DNA by using the primers CI-J-1632 (5'-TGATCAAATTTATAAT-3') and CI-N-2191 (5'-GGTAAAATTTAAAATATAACTTC-3') (Kambhampati and Smith 1995). In every DNA extraction and PCR reaction, positive and negative controls were included.

Data Analysis. Two-way analysis of variance (ANOVA) was performed to assess differences in mortality and recapture rate between FP and WBO males, as well as to assess differences between males recaptured on humans and in swarms, for each marking method.

The dispersal pattern was summarized by the mean distance traveled (MDT), the maximum distance traveled (MAX), and the flight range (FR) for each urban locality and for each marking method. Dispersal distance of *Ae. albopictus* males was measured by drawing annuli 50 m apart around the release site and applying a correction factor to accommodate unequal catch densities in the calculation (Lillie et al. 1981, White et al. 1985, Morris et al. 1991).

Two-way ANOVAs were performed to evaluate differences in MDT and MAX, as follows: 1) among the three study areas for each marking method; 2) among the three sampling days for each marking method and each locality; and 3) between the FP and WBO marking method.

The FR was estimated through the linear regression of the cumulative estimated recaptures performed within each annulus (x -axis) on the \log_{10} (annulus median distance + 1). The FR_{50} and FR_{90} indicate the distance that comprehends the maximum flight distance reached by 50 and 90% of the individuals. These parameters were calculated from the equation of regression as the value of y at 50 and 90% of the largest value of x , respectively.

The direction of dispersion was analyzed by means of circular statistics (Zar 1999). The mean angle of dispersion from the release point (a) was calculated for FP- and for WB0-marked males for each locality of release. For each mean angle, the length of the mean vector (r) was calculated. The value of r is a measure of concentration of the dispersal directions, and varies from 0 (nondirectional dispersion) to 1 (unidirectional dispersion). To determine whether the dispersal direction differed significantly from nondirectional uniformity, Rayleigh's test (Zar 1999) was applied. To compare the angle of dispersion between FP and WB0 males in each locality, the Watson-Williams test was used (Zar 1999).

Circular statistic methods were applied also to the data on wind direction during the sampling sessions to determine the presence of predominant wind direction (r_{wind}).

The linear corrected method was used to estimate the survival rate (Harrington et al. 2001, Buonaccorsi et al. 2003). The recapture and survival rates were estimated as follows:

$$\theta = e^a / (N + e^a)$$

$$s = e^b (1 - \theta)^{1/d}$$

where a and b were the regression coefficients of the linear regression of the log-transformed captures as a function of time, N is the number of individuals released, θ is the recapture rate, d is the number of days after release, and s the survival rate.

A multiple regression analysis was performed among the dispersal parameters and the survival rate, the vegetation covering, and the weather conditions in the course of the study.

Results

Recapture Rates. In the three localities of Castel Maggione, Altedo, and Castello d'Argile, 1,700, 3,600, and 2,000 *Ae. albopictus* FP males, and 920, 1,600, and 2,100 WB0 males were released, respectively. The time actually spent in recapture activity was estimated in 40–45 technicians/h for each session.

The percentage of mortality of FP males registered at the release (dead individuals plus individuals unable to leave the release cage) ranged from 6.90 to 15.90; for the WB0 males, the mortality calculated 3 d after the release as the number of dead pupae out of the total number of released ones ranged from 1.10 to 4.40%. The statistical analyses showed that the mortality rates at the release were significantly higher for FP males when compared with WB0 males ($F = 8.67, P < 0.04$).

The percentages of recapture varied from 0.63 to 4.72 for FP males ($2.30 \pm 2.15\%$, mean \pm SD), and from 2.39 to 11.05 for WB0 males ($5.43 \pm 4.87\%$, mean \pm SD) (Table 1). Even if the average recapture ratio was higher for WB0 males than for FP males, at the statistical analysis no significant difference was found ($F = 1.04, P = 0.36$).

The daily ratio, males-collected-in-swarm/males-collected-on-human-host, was not significantly different among the FP males (0.43 ± 0.38), the WB0 males (1.1 ± 0.77), and the wild males (0.78 ± 0.72) ($F = 2.11, P = 0.14$).

The convenience of considering both the mating modalities (in swarm or onto the host), to maximize the male recapture, was confirmed by the investigation conducted on the status of the spermathecae of the females, collected while landing on the human host in the different urban localities and hours. Our investigation demonstrated that 16.5% of the biting females were still virgin and a similar percentage of females had only one replenished spermatheca. Therefore, males flying around the hosts could have some chance of an effective mating.

Flying males were already active at the beginning of the collection period, and still were so at the end (4:30–7:30 p.m.), but swarming started only at approximately 6:00 p.m. Considering the entire period of flying activity, wild male collections were much higher for the recaptures performed on the host than in the swarms (Table 1). However, if we considered the time interval 6:00–7:30 p.m., when the swarms occurred, the number of collected males was similar for the two recapture modalities, and no statistically significant difference was observed in the daily ratio between the recaptures of males performing the two mating behaviors (males-collected-in-swarm/males-collected-on-human-host; $F = 2.11, P = 0.14$).

Dispersion Pattern and Survival Rate. The MDT, the MAX, and FR₉₀ and FR₅₀ for each locality and

Table 1. Number of released males, mortality at release, and number of captured mosquito males for each locality and marking method

| Locality | Date of release | Marking method | Strain | No. released males | Mortality at release (%) | Recapture percentage ^a | No. marked males and total no. captured males (day postrelease) ^b | | | | | | | |
|-------------------|-----------------|----------------|----------------------|--------------------|--------------------------|-----------------------------------|--|----------|---------|----------|--|-----------|----------|--|
| | | | | | | | No. marked males and total no. captured males per session | | | | No. marked males and total no. captured males on host or in swarm ^b | | | |
| | | | | | | | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | On host | In swarm | |
| Castel Maggione | 9 Aug. | FP | BO-RN-F ₁ | 1,700 | 15.90 | 1.54 | 7 (301) | 15 (325) | — | 0 (297) | — | 13 (631) | 9 (292) | |
| | 19 July | WB0 | APO | 920 | 1.10 | 2.86 | 18 (74) | — | 7 (97) | 1 (113) | — | 6 (124) | 20 (160) | |
| Altedo | 6 July | FP | RN-F ₂₀ | 3,600 | 6.90 | 0.63 | 12 (36) | — | 7 (369) | 2 (269) | 0 (342) | 17 (737) | 4 (279) | |
| | 30 Aug. | WB0 | APO | 2,100 | 2.50 | 2.39 | 19 (291) | 10 (145) | — | 20 (339) | — | 20 (324) | 29 (451) | |
| Castello d'Argile | 3 Aug. | FP | MT-F ₄ | 2,000 | 10.00 | 4.72 | 62 (425) | 21 (257) | — | 2 (130) | — | 71 (621) | 14 (191) | |
| | 23 Aug. | WB0 | APO | 1,600 | 4.40 | 11.05 | 80 (552) | 30 (330) | — | 59 (476) | — | 106 (897) | 63 (461) | |

BO-RN, Bologna-Rimini locality; FP, fluorescent pigment; MT, Matera locality; WB0, aposymbiotic males.

^a Percentage of recaptured males out of the number of alive males released.

^b Numbers between brackets stand for the total number of captured males during the sampling session, performed at fixed time intervals (from day 4 to day 8 after the release).

Table 2. Distances (m) between release point and recapture sites registered for FP- and WB0-marked males caught at the three localities, either on host or in swarm

| Dispersal parameters | Locality and strain | | | | | |
|---|---------------------|---------|---------|---------|-------------------|-----------------------------|
| | Castel Maggiore | | Altedo | | Castello d'Argile | |
| | FP | WB0 | FP | WB0 | FP | WB0 |
| MDT | 148.69 | 97.60 | 115.10 | 203.61 | 109.46 | 212.52 |
| FR ₅₀ | 125.36 | 74.00 | 71.15 | 183.55 | 82.37 | 194.30 |
| FR ₉₀ | 198.11 | 162.39 | 174.70 | 308.97 | 176.27 | 307.17 |
| MAX | 217.9 | 196.4 | 235.5 | 322.5 | 237.9 | 311.9 |
| Mean compass direction (dispersal angle) (<i>a</i>) | 55.54° | 65.55° | 240.15° | 35.83° | 273.44° | 90.65° |
| Length of the mean vector (<i>r</i>) | 0.33 | 0.73 | 0.63 | 0.23 | 0.50 | 0.24 |
| Z value (Rayleigh's test) | 2.34 | 13.75** | 8.26** | 2.65 | 21.41** | N.A. (bimodal distribution) |
| WW value (Watson-Williams test) | | 69.07** | | 55.00** | | 155.27** |

FP adult males marked by fluorescent pigment; WB0 adult males marked by removing *Wolbachia*. FR₅₀ and FR₉₀, flight range (distance within which the maximum flight distance of 50 and 90% of the released males was encompassed); MAX, max registered distance from release point to site of recapture; MDT, mean distance traveled from release to recapture site; Z, WW, see the data analysis paragraph. *, *P* < 0.05; **, *P* < 0.01.

marking method are shown in Table 2. In all the release experiments, the males dispersed during the first 3–4 d, as we observed that the dispersal distance did not increase anymore throughout the period of observation (days 4, 5, and 7 after release), and no difference was found among the dispersal distances measured (*F* = 1.87, *P* = 0.20).

In the course of the 7 d after the release, FP- and WB0-marked males could disperse to a maximum of 237.9 m (registered in Castello d'Argile) and of 322.5 m (registered in Altedo). The two-way ANOVA did not find statistically significant differences in the MDT for the two marking methods among the three study localities (*F* = 0.79, *P* = 0.48; Table 2).

On the contrary, statistically significant differences were found in the MDT between the FP- and WB0-marked males (*F* = 10.61, *P* < 0.01), which ranged from 109.5 to 148.7 m for FP males and from 97.6 to 212.5 m for WB0 males (Table 2). On average, the MDT was of 124.42 ± 21.21 m for the FP males and of 171.24 ± 63.93 m for the WB0 males.

For FP-marked males, the distance that 50% (FR₅₀) and 90% (FR₉₀) of the released males traveled ranged from 71.1 to 125.4 m and from 174.7 to 198.1 m, respectively. The same estimates ranged from 74.0 to 194.3 m (FR₅₀) and from 162.4 to 308.9 m (FR₉₀) for WB0-marked males (Table 2).

We compared the MDT for males collected in swarm and males collected on human host for the two marking methods. Block ANOVA showed no statistically significant difference (*F* = 0.16, *P* = 0.76) in MDT for the two recapture methods (Fig. 2). The correlation between the MDT values and the recapture rates was found to be weak (*R*² = 0.29).

We found some evidence of statistically significant preferential dispersion directions in all of the localities, at least for one male group: FP males in Castello d'Argile and Altedo and WB0 males in Castel Maggiore. In Castello d'Argile, the WB0 males showed a bidirectional dispersal (diametrically bimodal distributions). In two cases (FP males in Castel Maggiore and WB0 males in Altedo), the dispersal was uniform

(Table 2). A statistically significant difference was observed in the dispersal direction when comparing the two marking methods for each locality (W-W test; Table 2).

In our conditions, the two methods (FP versus WB0) resulted in different survival rates. According to the linear corrected method, the global survival rates were 0.52 ± 0.04 for FP males and 0.81 ± 0.25 for WB0 males (Table 3). The regression analyses of the log transformed + 1 number of marked males collected against the time after release did not fit significantly in the regression models for both marking methods (Table 3).

The weather conditions during the course of the trials, from July to September 2007, are shown in Fig. 3. The regression analysis performed to put in evidence possible correlations among key weather parameters and the dispersal patterns showed that the MDT was positively influenced by RH, whereas solar radiation intensity negatively influenced the dispersal rate (Table 4). The homogeneity of the direction of male dispersal (*r*; i.e., the existence of a predominant dispersal direction) was negatively cor-

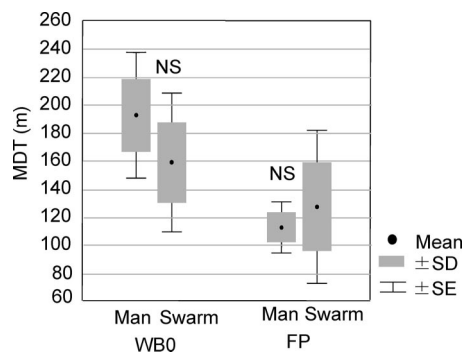


Fig. 2. MDT by recaptured WB0- and FP-marked *Ae. albopictus* males on humans and in swarm. WB0, males marked for the absence of *Wolbachia*. FP, males marked by means of fluorescent pigment.

Table 3. Statistics summary for survival rate of marked-released-recaptured *Aedes albopictus* males at the three localities

| Statistics | Locality and strain (*) | | | | | |
|------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Castel Maggiore | | Altedo | | Castello d'Argile | |
| | FP | WB0 | FP | WB0 | FP | WB0 |
| Survival rate | 0.48 | 0.52 | 0.54 | 0.94 | 0.56 | 0.97 |
| R ² | 0.70 | 0.90 | 0.88 | 0.06 | 0.35 | 0.1 |
| Linear regression parameters | F(1,1) = 2.37 and P < 0.37 | F(1,1) = 8.92 and P < 0.21 | F(1,2) = 15.23 and P < 0.06 | F(1,1) = 0.07 and P < 0.84 | F(1,1) = 0.55 and P < 0.59 | F(1,1) = 0.01 and P < 0.92 |

FP adult males marked by fluorescent pigment; WB0 adult males marked by removing *Wolbachia*.

related to the RH and positively correlated to the temperature and solar radiation intensity (Table 4). In the course of the study, the wind did not attain high intensity (maximum speed registered: 2.04–3.01 m/s at 10 m height, i.e., much less at the ground level in urban conditions) and no precise dominant direction was registered (r_{wind} in the range 0.12–0.33), thus resulting in a weak correlation with the male flying behavior (Table 4).

Discussion

Mark-release-recapture experiments have been undertaken to study the dispersion and survival of *Ae. albopictus* males in three urban localities in Northern Italy. The mean recapture percentages we obtained, despite the variability observed between localities and sessions (e.g., weather conditions; see below), may be considered within the range usually found in mark-release-recapture studies (Service 1993).

The convenience of considering both the mating modalities (in swarm or onto the host), to maximize the males recapture, was supported by the data from the dissection of the female's spermathecae, which showed that a portion of the host-seeking females was still virgin, and confirmed that the male sampling on the host can be an informative way to obtain data on sexually active males.

Even if some differences were observed between the two marking methods applied, FP and *Wolbachia*-free strain (WB0), the results are congruent in de-

scribing the general dispersion pattern of *Ae. albopictus* males in the urban areas studied. The area of dispersion was defined by the maximum observed distances traveled and by the flight range. After the release, males could disperse in 7 d as far as 200–300 m.

As past mark-release studies on *Ae. albopictus* focused mainly on females, little data are available to compare with our results. Niebylski and Craig (1994) observed that MAX for *Ae. albopictus* males marked with fluorescent dye was 225 m in a scrap tire yard in Missouri. In the study of Takagi et al. (1995), conducted in a grassy and scrub area in the campus of Nagasaki University School of Medicine (Nagasaki, Japan), fluorescent dye-marked males were collected <36 m far from the release point. Recently, Lacroix et al. (2007), studying the dispersal of *Ae. albopictus* males in La Réunion, obtained value of MDT ranging from 29 to 46 m, but they referred to a radius of recapture of 50–130 m, whereas we investigated an area of up to 350 m away from the release site.

In all of the release experiments we performed, the distance traveled by the males did not significantly increase in the course of the 3 d of recapture, showing that the behavioral pattern of dispersion includes an active dispersal phase during the first days after the release, then the males become sedentary, or disperse randomly, in the following period. This kind of early dispersal behavior has been described for the females of several mosquito species (Bidlingmayer 1985, Service 1997).

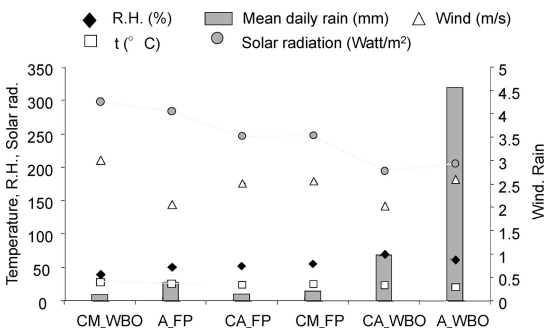


Fig. 3. Weather parameters measured at the three localities on the day of release of the two marked *Ae. albopictus* males' group. WB0, males marked for the absence of *Wolbachia*. FP, males marked by means of fluorescent pigment. CM, Castelmaggiore. CA, Castello d'Argile. A, Altedo.

Table 4. Coefficients of correlation R calculated among survival rate, weather parameters, vegetation covering (as influencing variables), the mean distance travelled, and dispersion direction vector r (observational behavioral data)

| Influencing variables | R for observational data | |
|------------------------------------|--------------------------|---------------------------------|
| | MDT | Dispersion direction vector (r) |
| Survival rate | 0.904* | - |
| R.H. | 0.929** | -0.94** |
| t (°C) | -0.694 | 0.80* |
| Rain (mean mm/h/d) | 0.676 | -0.58 |
| Wind speed (mean m/s) | -0.393 | 0.35 |
| Wind direction (degrees) | 0.468 | -0.67 |
| Visible radiation (mean/h/Watt/m²) | -0.927** | 0.95** |
| Atmospheric pressure (Ettopascal) | 0.692 | -0.47 |
| % vegetation covering | -0.383 | 0.31 |

*, P < 0.05; **, P < 0.01.

Statistically significant differences emerged in the mean angle of dispersion of the males of the two groups and of the three localities, without a consistent preference for any direction (Table 2). Differences in the dispersal direction between the FP and WB0 males were statistically significant for all of the three localities. Dispersal ability of a given species may depend on the weather condition during the study period, as well as on the characteristics of the study locality. In urban areas, we may consider as important factors the vegetation type, its abundance and distribution, the shape and position of buildings, squares, and main roads (Beier et al. 1982, Muir and Kay 1998, Reisen et al. 2003, Russell et al. 2005). All these factors could have been involved in generating the differences among the dispersal patterns that we observed in the course of the study.

As demonstrated by means of the regression analyses, low relative humidity, high temperatures, and intense solar radiation negatively influenced the MTD and reduced the dispersion homogeneity (Table 4). In the hot and dry summer weather conditions, the mosquito males seemed to reduce their dispersal capacity and follow specific directions, possibly toward the shaded corridors. More specific investigations must be planned to understand this behavior.

Data on *Ae. albopictus* male longevity in nature are reported by Hawley (1988), who analyzed by regression analysis the row data presented in the mark-release-recapture study of Mori (1979). Daily survival rate was estimated to be 0.86 and 0.88 (6.6–7.8 d), respectively, for males reared under high and low larval density (Mori 1979, Hawley 1988). In our conditions, the survival rate was slightly lower for WB0 males (0.81) and even much lower for FP males (0.52) (Table 3). As discussed in the next section, the WB0-marking method, which allowed the release of the males in the pupal stage, appeared to be less stressful and more reliable with respect to FP, which involves the release of adult males.

Comparison of the FP- and WB0-Marking Methods. An optimal marker, in insect marking release-recapture experiments, must be persistent enough in/on the animal, and its application has not to affect the normal dispersal behavior nor decrease its longevity (Hagler and Jackson 2001).

We may compare the two marking methods in terms of mortality, as a result of the marking release procedure, and in terms of MDT. ANOVA showed that the percent mortality was lower for the WB0 males with respect to the FP males (Table 3), and MDT of the WB0 males was significantly higher than that of FP males.

We may consider that the better performances of the WB0-marked males, enlightened by our data, could be because of the higher resistance of the WB0 male pupae to the hurts and damages caused by the manipulation, transport, and release practices with respect to the adults, and to the younger age of the WB0 males versus FP males (adult emergence from the WB0 pupae in the release container was gradual and took up to 48 h).

The release of male pupae better simulates the male emergence as it occurs under natural conditions, and is the preferred release method adopted in case of SIT programs based on pupal irradiation. The field observations confirm the laboratory evidences regarding the negligible incidence of negative side effects of *Wolbachia* removal on the fitness of *Ae. albopictus* males (Dobson et al. 2004, Calvitti et al. 2009), although for a more correct comparison, the dispersal of the WB0 males should be compared with that one of the normal males. The use of an aposymbiotic strain, at our knowledge applied for the first time in mark-recapture-release trials, could be of interest for studying other insect species having a favorable *Wolbachia* profile in nature. The only disadvantage of the WB0 method is the high costs of the PCR analyses for the thousands of recaptured individuals.

Considerations for SIT Application. It is likely that both the mark-release methods have some influence on the fitness of *Ae. albopictus* males, including dispersal capacity, survival rate, and mating behavior. These influences must not necessarily be considered as negative a priori, but it is likely that cumulatively they may reduce the male performances. The data we obtained may therefore be considered as a useful tool in the planning of SIT pilot field studies. However, it must be considered that the males used in these experiments had not been irradiated.

The distance between sterile male release sites and the timing of the releases are two crucial factors in planning SIT programs. The density of the release sites is important for the complete covering, by sterile males, of the treated area and must be planned on the basis of the dispersal behavior of the released males in the urban environment. Therefore, information about the dispersal pattern is important to define the optimal distances between the release points. In addition to this, information on survival is useful to assess the best cost/benefit frequency of the releases. This study was planned to address these two main issues. The mean distances traveled as well as the flight range observed suggest that distances between release sites in the range 150–200 m could be optimal in Northern Italian urban areas.

When considering the survival rates, a possible flexible strategy could be designed on the basis of the weather parameters expected in the course of the season (e.g., larger periodicity in early and late season and higher frequency in midsummer). The global mean survival rate we observed in our study for WB0 males was 0.81. Assuming the survival rate to be constant in the course of the year, with a stable natural male population density of 1,000 males/ha, if the aim is to achieve a sterile/wild males ratio of 20:1, fixing the periodicity of the releases to once per week, one would need to release 87,500 sterile males/ha even at the beginning of the reproductive season. Luckily, this is not likely to be the scenario in the temperate areas, like in Northern Italy, where the high yearly winter egg mortality causes a collapse of the population density, with adult population densities very low at the beginning of spring (our unpublished data).

We must consider that this experiment was carried out using nonsterilized reared males, and therefore, an analogous study using WB0-irradiated pupae should be planned, to investigate the possible effect of radiation on male mating behavior, dispersal, and survival.

The effect of rearing was not taken into consideration in this study, and more detailed studies should be planned to assess the cost/benefit ratio of different rearing schemes in terms of fitness of the males. Mori (1979) observed that *Ae. albopictus* females produced by rearing at higher larval density disperse more than females developed under lower larval density conditions. This possible influence must be considered in planning mass rearing and sterile males' release.

In summer, the dry conditions and the high temperature, amplified by the cement and asphalt surfaces, make the Italian urban areas an unfavorable environment for *Ae. albopictus* males, also because of the scarcity of water and sugar sources. The possible convenience of developing a sterile male release device designed with the aim of furnishing a sugar source to the newly emerged males should therefore be considered.

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