Pilot Field Trials With *Aedes albopictus* Irradiated Sterile Males in Italian Urban Areas

R. BELLINI, A. MEDICI, A. PUGGIOLI, F. BALESTRINO, AND M. CARRIERI


**ABSTRACT** The pilot field studies here presented are part of a long-term research program aimed to develop a cost-effective sterile insect technique (SIT) methodology to suppress *Aedes albopictus* (Skuse) populations. *Aedes albopictus* is a mosquito species mainly developing in man-made containers and with an island-like urban and suburban distribution. These two features make the application of the sterile insect technique a possible control strategy. Five trials have been performed in three small towns from 2005 to 2009 (Emilia-Romagna region, northern Italy). Reared male pupae, sexed by a sieving technique allowing the recovery of ~26–29% of males, were exposed to gamma rays and immediately released in the field. Adult population density was estimated based on a weekly monitoring of egg density in the ovitraps, whereas induced sterility was estimated by measuring the hatching percentage of weekly collected eggs in SIT and control areas. Results showed that sterile males released at the rate of 596–1,590 males/ha/wk induced a significant sterility level in the local population. In addition, when the sterility level achieved values in the range of 70–80%, a similar reduction also was found for the egg density in the ovitraps. We could estimate that the minimum egg sterility value of 81% should be maintained to obtain suppression of the local population. Immigration of mated females was not a main issue in the small villages where trials have been run.

**KEY WORDS** *Aedes albopictus*, sterile insect technique, gamma-rays, induced sterility, population density.

*Aedes albopictus* (Skuse) is an invasive species rapidly spreading and establishing in a number of countries with different climates (Benedict et al. 2007) and it is known to be an efficient vector of many arboviruses causing human diseases, including yellow fever (*family Flaviridae*, genus *Flavivirus*, YFV), dengue (*family Flaviviridae*, genus *Flavivirus*, DENV), and Chikungunya (*family Togaviridae*, genus *Alphavirus*, CHIKV), in tropical as well as in temperate regions (Carrieri et al. 2011a). Moreover, *Ae. albopictus* can transmit indigenous arboviruses in newly colonized areas (Shroyer 1986), as well as filariasis (*Wuchereria bancrofti* and *D. repens* Railleiet and Henry) (Cancrini et al. 1992, Mitchell 1995). The species shows an urban–suburban distribution, mainly exploiting man-made containers, and catch drains have been found as the main breeding sites in Italy (Carrieri et al. 2011a). Conventional control methods cannot achieve satisfactory results (Carrieri et al. 2011b), but its island-like distribution (Urbanelli et al. 2000) makes it possible to consider as convenient the application of genetic control methods aimed to suppress or eliminate the species.

The sterile insect technique (SIT) approach on mosquito control suppression and elimination has been tested in the past against several species. In 1959–1962, a field release study was conducted in Florida against *Anopheles quadrinaculatus* Say with unsatisfactory results because of the poor quality of the sterile males released (Weidhaas et al. 1962, Dame et al. 1964). An ambitious SIT program was started in El Salvador in the 1970s against *An. albimanus* Wiedemann, primary vector of malaria. A mass rearing facility with a production capacity of one million males per day was built. In a pilot field trial in 1972, the daily sterile male releases were actually in the 40,000 range, and a total of 4.3 million sterile males were released during a 5-mo period in an isolated area close to the Apatenque Lake. Sterilization was achieved using a chemosterilant (bisazir) administrated at the pupal stage, observing an induced sterility of ~99% in the local population (Lofgren et al. 1974). An important project was funded by the World Health Organization (WHO) and by the United States Public Health Service (USPHS) in cooperation with the Indian Council of Medical Research (ICMR), targeted firstly against *Cx. pipiens quinquefasciatus* Say and secondly against *Ae. aegypti* L. and *An. stephensi* Liston in India. The mass rearing facility of New Delhi achieved a production of one million males per day at the cost of US$40 per million. The sterilization was obtained by treating the pupae in the water with the chemosterilant thiopeta, inducing 99% sterility (Pal and LaChance 1974). The project was stopped in 1975 for political contro-
verses (Anonymous 1975). In the 1970s, a project was organized in Kenya to replace the wild Ae. aegypti population by using translocation homozygote strains. It was observed that the field fitness was somewhat poor in terms of fertility, larval development time, larval and adult survival when compared with the natural population. Consequently, the introduced strains rapidly disappeared (Lorimer et al. 1976). Successively, during the period 1977–1983, a project was launched against Cx. tarsalis Coquillett in California. In this case, the radiation doses used to obtain male sterility only slightly affected their mating capacity with wild females in cage trials (Zalom et al. 1981), whereas the impact of colonization was more prominent (Reisen et al. 1982). Researches on a Cx. tritaeniorhynchus Giles strain in Pakistan showed that the genetically sterilized male strain (tsl) were highly competitive for reared females, but much less for wild females. This finding was confirmed by an ad hoc experiment using another mutant strain (In[3]T[2,3]l) (Reisen et al. 1980).

Recently, under the stimulus of new genetic and technological tools, the SIT strategy is back into the game (Franz and Robinson 2011). Oxitec Ltd. (Abingdon, United Kingdom) has developed a transgenic approach termed RIDL (release of insects carrying a dominant lethal genetic system) based on the development of engineered Ae. aegypti and Ae. albopictus strains (http://www.oxitec.com/ridl-sit-and-dengue-fever). These strains carry a lethal genetic system repressed by the administration of tetracycline in the laboratory rearing, whereas the field offspring cannot survive to adulthood in the absence of the repressor (Harris et al. 2011).

The use of the maternally-inherited intracellular bacterium Wolbachia seems to be another promising technique. The wMel Wolbachia infection, introduced into Ae. aegypti from Drosophila melanogaster Meigen block the transmission of the dengue virus and influence key life history traits of the mosquito such as lifespan. Thus, this technique can be used to replace mosquito natural populations with noncompetent strains (Hoffmann et al. 2011).

In Italy, since 2004, researches have been conducted to investigate the feasibility of introducing the SIT strategy in the control programs of Ae. albopictus (Bellini et al. 2007). A small scale mass rearing unit for Ae. albopictus has been established at the Centro Agricoltura Ambiente laboratory (CAA) in Crevalcore (BO). Several important issues related to the application of the SIT strategy have been investigated, such as: gamma ray dosimetry (Balestrino et al. 2010), male dispersal capacity (Bellini et al. 2010), and sterile male competitiveness under large enclosures (Bellini et al. 2012). We also developed a reliable ovitrap-based quantitative monitoring system (Albieri et al. 2010; Carrieri et al. 2011a,b), and we are now able to present a comprehensive measurement of the efficacy of the field sterile male releases in different Italian urban localities.

### Materials and Methods

#### Mass Rearing and Strains

The mosquito strains used in the trials were started from field materials collected in different Italian localities and maintained for several generations under laboratory conditions (Medici et al. 2011). Adult mosquitoes were reared at standard conditions (28 ± 1°C, 85% RH, 12-h scotophase) in Plexiglas cages (40 by 40 by 40 cm) and fed on a 10% sucrose solution, to which they had constant access. Females were offered fresh blood every day through a thermostat-controlled device. Eggs laid on wet filter paper were removed from the adult cages and placed in closed plastic boxes to maintain relative humidity close to 100%. This method kept eggs alive for some months. When needed, the filter papers with the eggs were dried in climate chamber for 1 d before submersion and eggs were counted through ImageJ software. The desired number of eggs then was put in a sealed container with nutrient broth solution for hatching. After hatching, young F1 larvae were transferred a plastic tray at the density of one larva per ml (2,500 larvae into 2.5 liters of dechlorinated water), and fed on crushed dry cat food (Friskies; Nestlé Purina Pet-care Company, St. Louis, MO).

#### Sexing and Transportation

Male pupae were collected by using metal sieves with square holes of a width of 1,400 μm, at 24–30 h from beginning of pupation (Bellini et al. 2007, Medici et al. 2011) and transferred in petri dishes (12 cm in diameter) with some water. They then were transported to the Medical Physics Department of the St. Anna Hospital (Ferrara, Italy) for irradiation (≈1 h driving) and afterwards to the release stations (≈1–2 h). During transportation they were maintained in thermal insulated plastic containers (Balestrino et al. 2010).

#### Irradiation Treatments

Irradiation treatments were performed by using an IBL 437 irradiator (CIS Bio International, Bagnols-sur-Ceze, France) equipped with a 50.9 TBq Cs-137 linear source with central dose rate of 2.4 ± 3.5% Gy/min in 2005, 2.3 ± 3.5% Gy/min in 2008, and 2.2 ± 3.5% Gy/min in 2009. The prefixed gamma ray doses were achieved by exposing pupae to the irradiation treatment for the time needed to reach them. Based on the results of the dosimetry studies (Balestrino et al. 2010), the dose of gamma rays was progressively reduced: from 85 Gy in 2005 to 30–40 Gy in 2008, and to 30 Gy in 2009.

#### SIT and Control Localities, Dose and Release Intervals

Four localities were chosen for sterile male releases (SIT areas) and four as control areas.

#### Santamonica 2005, 85 Gy

Santamonica was chosen as the SIT area (coordinates 43°57′22″ N, 12°41′18″ E; urban area of ≈45 ha), whereas Rimini was chosen as the control area (44°03′38″ N, 12°33′59″ E; urban area of ≈3,000 ha). Sterile male pupae were released on a weekly basis in the period 14 June–20 September, for a total of 13 releases (Table 1).

#### Boschi 2008, 40–30 Gy

Boschi was chosen as the SIT area (coordinates 44°38′51″ N, 11°32′7″ E; urban area of 16 ha), whereas Baricella was the control area (44°38′44″ N, 11°32′13″ E; urban area of 16 ha). Males
irradiated at 40 Gy were released from 29 April to 9 August, and from 6 September to 2 October, whereas males irradiated at 30 Gy were released from 13 August to 2 September. Release intervals were 6–8 d from 29 April to 8 July, and from 10 September to 2 October; shorter release intervals of 4–5 d were applied from 8 April to 8 July; and in total, 25 releases were performed (Table 1).

**Budrio di Correggio 2008, 40 Gy.** Budrio (coordinates 44° 45′11″ N, 10° 4′36″ E; urban area of 17 ha) was chosen as the SIT area, whereas Lemizzzone acted as control area (44° 43′59″ N, 10° 45′09″ E; urban area of 12 ha). Sterile male releases occurred with a 6–7-d interval in the period 30 April–8 August, and in total 18 releases were performed (Table 1).

**Boschi and Caselline 2009, 30 Gy.** In Boschi (see above for SIT area details) 18 releases were performed in the period 14 May–10 September, based on a weekly interval (Table 1). In Caselline di Albinea (SIT area, coordinates 44° 37′0″ N, 10° 36′0″ E; urban area of 18 ha) 18 releases in the period 21 May–29 September, were performed at a 6–7-d interval. Baricella was chosen as control areas for both (see above for details) (Table 1).

**Wild Male Population Estimate.** In all localities *Ae. albopictus* has become well established (Albieri et al. 2010). Wild male population density was estimated on the basis of the model developed by Carrieri et al. (2011b) in similar environments to calculate the ratio between the number of females and the average number of eggs per ovitramp per hectare, which was 1.57 ± 0.77, based on a female survival rate of 0.91. Male survival rate in northern Italy urban areas is lower with respect to female survival rate (from 0.52 to 0.97, Bellini et al. 2010); and considering a mean male survival rate of 0.81 ± 0.25, the ratio between the number of males and the average number eggs per ovitramp per hectare was 1.42 ± 0.72 (Carrieri et al. 2011b). To calculate the initial number of males per hectare in the study localities, we multiplied this value for the mean number of eggs per ovitramp registered at the first sterile male release: Santamonica 2005, 98.96 ± 61.56; Boschi 2008, 43.32 ± 37.38; Budrio 2008, 59.49 ± 58.99; Boschi 2009, 31.10 ± 27.71; Caselline di Albinea, 118.28 ± 104.98.

**Other Mosquito Species.** Apart from *Ae. albopictus*, in northern Italy urban areas *Ae. geniculatus* is the only other species of the same genus occasionally laying eggs in the ovitramps. Based on the results of previous monitoring activities, we knew that *Ae. geniculatus* was usually absent in the study areas, or it had been detected at very low population density (R. B., unpublished data). The species has one generation per year, and adults are present mainly in May–June, as verified by adult monitoring activities (R. B., unpublished data). For these reasons, the number of *Ae. geniculatus* eggs in the ovitramps has been considered negligible, therefore we judged it unnecessary to discriminate between the two species.

**Release Protocol.** Sterilized male pupae were released within 1–2 hr from irradiation, by placing plastic containers on the ground in green and shaded areas. The planned dose of 1,000 sterile males/ha/wk (from now on sterile males/ha/wk) was adopted for the study. Mortality of male pupae in the containers was checked 2–3 d after release when adult emergence was completed. Precautionary high densities of sterile male release stations (0.44–0.67 stations/ha) were set to allow the overlapping of sterile male dispersal range, previously estimated in ~150 m (Bellini et al. 2010).

**Efficacy Measurement.** Efficacy of irradiated males in inducing sterility into the local population has been assessed 1) by estimating egg density in the ovitramps, 2) its decrease in SIT versus control areas (Carrieri et al. 2011b), and 3) by calculating the percentage of sterility of eggs collected in the ovitramps placed in SIT versus control areas. An ovitramp consists of a black plastic cylinder filled with water and equipped with a wooden stick for oviposition (Carrieri et al. 2009, Albieri et al. 2010).

In 2005, 40 ovitramps were positioned and weekly managed in Santamonica (SIT area), whereas 90 ovitramps were managed in Rimini (control area), from 7 June to 22 September.

In 2008, in Boschi and Budrio (SIT areas) 15 standard ovitramps were checked from 6 May to 21 October, whereas 10 ovitramps were managed in each of the two control areas (Baricella and Lemizzzone) from 7 May to 29 September.

In 2009, 15 ovitramps were managed in Boschi and Caselline di Albinea (SIT areas) from 18 May to 25 September, whereas 10 ovitramps were managed in Baricella (control area) from 21 May to 10 October.

**Egg Density and Egg Density Decrease.** Ovitramps were checked weekly and eggs were collected,
brought to the lab, and counted by stereomicroscope. Mean egg density was calculated as the mean number of eggs per ovitrap per week or per month, according to the intended analyses. The percentage of decrease of egg density (D) was calculated by means of the following equation:

\[ D = \frac{(E_{\text{SIT}} - E_{\text{Control}})}{E_{\text{Control}}} \]  

[1]

were \( E_{\text{SIT}} \) and \( E_{\text{Control}} \) are the mean number eggs per ovitrap per week in SIT and in control area, respectively.

The percentage of egg sterility for SIT and control areas was estimated by calculating the hatching rate obtained through standard procedures, as reported in Bellini et al. (2007).

**Induced Sterility.** The percentage of induced egg sterility was calculated by means of the Abbott’s equation:

\[ S = \left[ 1 - \left( \frac{(E_{\text{SIT}}/E_{\text{SIT}}) \times (E_{\text{Control}}/E_{\text{Control}})}{1} \right) \right] \times 100 \]  

[2]

were \( S \) is the percent egg sterility, \( E_{\text{SIT}} \) and \( E_{\text{Control}} \) are the number of hatched egg in the SIT and in the control area, respectively.

**Influence of Weather Variables.** The main weather variables, RH (average daily mean), air temperature at 2 m from the ground (daily mean), and daily rainfall (millimeters per day), were measured by three meteorological stations of the Emilia-Romagna Region: the first one was located 3 km from Budrio and 10 km from Boschi, the second one was located 11 km from Caseline, and the third one was 13 km far from Santamonica (CLINUR net, wwwarpa.emr.it). Their impact on the percentage of the monthly and seasonal egg sterility and on the mean egg density was assessed.

**Statistical Analyses.** Data have been presented as mean ± SD.

Block analysis of variance (ANOVA) has been used to analyze differences in egg density between SIT and control areas, considering the area and the week as the main variables. Percentage data were submitted previously to angular transformation before the ANOVA. The Newman–Keuls multiple comparison test was used for mean separation.

Correlations between the monthly averages of percent egg sterility, egg density, and meteorological parameters (air temperature, RH, and rain) were performed by means of the Pearson product number. Regression analyses were used to verify the correlation level between monthly averages of percent egg sterility and egg density. The parallelism and elevation tests were used to determine parallelism and coincidence between the data obtained by the 2008 and 2009 trials with males irradiated at 30 and 40 Gy.

**Information to Authorities and Citizens.** The work was funded largely by the Public Health Department of the Emilia-Romagna Region and by the Municipalities, which were exhaustively informed about the scientific content of the program. Before starting with the program, local authorities as well as citizens leaving in the localities where trials had been planned were informed about the research program and its working modalities through public assemblies and technical meetings with municipality’s operators. In particular, both health officials and general public were acknowledged that irradiated mosquito pupae would have been released in several stations of their town and that irradiated mosquitoes are not genetically modified organisms as defined in the International Standards for Phytosanitary Measures (see https://www.ippc.int/index.php?id=ispms&nocache=1&L=0). For releases and traps placed on private property, owner was given the opportunity to decline participation in the study. Information to citizens was done also through regional and local newspapers. Specialized CAA personnel were at disposal through a phone number for any query.

**Results**

**Information to Authorities and Citizens.** The SIT mosquito control program obtained the full support from the Local and Regional Public Health authorities. We never received complaints, and all citizens which were asked to host release stations or ovitraps in their property accepted and collaborated to the program.

**Rearing Productivity.** In total, about two million sterile males have been produced and released during the 3-yr study. The relevant parameters describing the productivity of the rearing system are reported in Table 1. By using the sieving system to separate males from females, the percent of recovered males was in the range of 26.0–29.0%, which corresponds to the 13.0–14.5% of the initial number of L1 larvae, (in consideration of the 1:1 female:male ratio), with a mean residual presence of 1.21 ± 1.22% females on the total number of released pupae. No statistically significant difference was observed in the male pupa production among the 3 yr (\( F = 0.75, df = 2, 94, P = 0.48 \)), with a cumulative mean of 13.93 ± 3.47%. Similarly, no statistically significant difference in the female/male ratio was observed in the 3 yr (\( F = 0.31; df = 2.56; P = 0.73 \)).

**Mortality.** Highly significant differences were found among the cumulative mortality data (occurred during irradiation, transport to release station and emergence) comparing the different gamma ray doses (30, 40, 85 Gy) (one way ANOVA, \( F = 69.96, df = 2, 94, P < 0.0001 \)). Male pupa mortality was much higher at the radiation dose of 85 Gy with respect to the others, and decreased when doses of 30–40 Gy were used (Table 1). In 2009, when the 30 Gy dose was used and particular caution was paid to all procedures by skilled personnel, percent mortality was very low.

**SIT Efficacy by Locality.** For each trial, the details about the release program (release area, number of releases, number of SIT stations, number of males released per hectare and total number of males released) are reported in Table 1. The ratio between released sterile males and wild males (Table 1) showed a seasonal trend, depicted in Fig. 1. The sterile/wild male ratio was higher at the beginning and at the end of the favorable season than in the middle,
when the peak of density of the wild mosquito population occurred. When considering only data collected in June, July, and August, the block ANOVA did not show any significant difference in the sterile/wild male ratio among the different trials.

Mean seasonal egg sterility induced by sterile males, egg density in the ovitraps, and percent egg density decrease (see equation 1) in each SIT locality in comparison to controls are reported in Table 2.

**Santamonica 2005, 85 Gy.** In the course of the season, 896.0 ± 306.0 pupae/ha/wk were released. Egg density in SIT and control areas are reported in Fig. 2A, whereas percent egg hatching in control and SIT areas are reported in Fig. 2B. Mean egg density in the ovitraps were not significantly different in the SIT area versus the control area ($F = 0.0012$, df = 1.30, $P = 0.9727$). The mean seasonal percentage of induced sterility in the SIT area was only 18.72 ± 13.90% with respect to control area (Table 2).

**Boschi 2008, 40–30 Gy.** The mean seasonal rate of released sterile males was 1,590.9 ± 317.2 pupae/ha/wk. Egg density in SIT and control areas are reported in Fig. 2C, whereas percent egg hatching in control and SIT areas are reported in Fig. 2D. The mean seasonal number of eggs was significantly lower in SIT area versus control area (28.81 ± 26.11 versus 58.40 ± 60.45 eggs/ovitrap/wk, respectively) ($F = 4.87; \text{df} = 1.47; P = 0.03$). The mean monthly Abbott corrected egg sterility was 28.81 ± 40.74% in May, 65.16 ± 13.46% in June, 58.59 ± 22.02% in July, 58.27 ± 16.46% in August, 76.43 ± 15.95% in September, and 97.29 ± 3.83% in October. The mean seasonal induced sterility in the SIT area was 64.03 ± 123.80% (Table 2).

**Budrio di Correggio 2008, 40 Gy.** The mean seasonal rate of released sterile males was 1,340.8 ± 374.9 pupae/ha/wk. Egg density in SIT and control areas are reported in Fig. 2E, whereas percent egg hatching in control and SIT areas are reported in Fig. 2F. A non-statistically significant mean decrease of 10.26% in the SIT treated area with respect to the control area was registered ($F = 2.87$, $\text{df} = 1, 2$, $P = 0.11$). The mean increase in egg sterility in the SIT area with respect to the control area was 48.91 ± 21.97% until August 18, and dramatically dropped down to 2.75 ± 5.23% from 25 August to 22 September, after the end of the release of sterile male pupae. The mean seasonal induced sterility in the SIT area was 37.37 ± 27.97% (Table 2).

**Boschi 2009, 30 Gy.** A mean seasonal rate of 1,048.9 ± 143.4 sterile male pupae/ha/wk was used. Egg density in SIT and control areas are reported in Fig. 2G, whereas percent egg hatching in control and SIT areas are reported in Fig. 2H. The mean egg density in the SIT area (24.16 ± 19.23 eggs/ovitrap/wk) was 72.36% lower than in the control area (87.40 ± 53.39 eggs/ovitrap/wk), with a highly significant difference ($F = 25.60; \text{df} = 1, 36; P < 0.0001$). The mean seasonal induced egg sterility in SIT area was 68.46 ± 20.48%.

**Caselline di Albinea 2009, 30 Gy.** A mean seasonal rate of 997.1 ± 217.5 sterile male pupae/ha/wk was released. Egg density in SIT and control areas are reported in Fig. 2I, whereas percent egg hatching in control and SIT areas are reported in Fig. 2J. A slight, nonstatistically significant, difference was registered in the mean number of eggs collected in SIT area (83.30 ± 73.93 eggs/ovitrap/wk) versus the control area (87.40 ± 53.39 eggs/ovitrap/wk) ($F = 0.07; \text{df} = 1.18; P = 0.79$). The mean seasonal induced egg sterility in the SIT area was 46.67 ± 25.99%.

**Correlations Between Induced Sterility and Egg Density.** A statistically significant negative correlation between the monthly means of induced egg sterility and egg density was observed in both 2008 and 2009, whereas a positive correlation was found between induced sterility and percent decrease of mean egg density (calculated by means of equation 1 for both years (Table 3).

**Table 2.** Mean induced sterility, mean egg density in SIT and control areas, and percent egg density decrease

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Induced egg sterility in SIT area (%)</th>
<th>Mean no. eggs/ovitrap/week in SIT area</th>
<th>Mean no. eggs/ovitrap/week in control area</th>
<th>Egg density decrease in SIT versus control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santamonica 2005</td>
<td>85</td>
<td>18.72 ± 13.90</td>
<td>65.64 ± 85.04</td>
<td>65.43 ± 88.40</td>
</tr>
<tr>
<td>Boschi 2008</td>
<td>30–40</td>
<td>64.03 ± 23.80</td>
<td>27.66 ± 50.34</td>
<td>37.71 ± 92.46</td>
</tr>
<tr>
<td>Budrio 2008</td>
<td>40</td>
<td>37.37 ± 27.97</td>
<td>81.16 ± 141.36</td>
<td>93.46 ± 151.26</td>
</tr>
<tr>
<td>Boschi 2009</td>
<td>30</td>
<td>68.46 ± 20.48</td>
<td>24.02 ± 46.36</td>
<td>86.46 ± 88.03</td>
</tr>
<tr>
<td>Caselline 2009</td>
<td>30</td>
<td>46.67 ± 25.99</td>
<td>83.89 ± 105.53</td>
<td>86.46 ± 117.37</td>
</tr>
</tbody>
</table>

Statistically significant differences * $\text{df} = 1, 24 F = 13.43, P < 0.01$ ** $\text{df} = 1, 18, F = 40.44, P < 0.0001$. 

"Fig. 1. Seasonal trend of the ratio sterile to wild males."
Fig. 2. A-J. Graphics on the left: egg density in SIT and control areas. Graphics on the right: percent egg hatching in SIT and in control areas. Data are presented per week of sampling, therefore points do not correspond exactly to the dates and to the total number of releases. A-B: 2005, 85 Gy, SIT area in Santamonica, control area in Rimini. Sterile male pupae were released from 14 June to 20 September. C-D, 2008, 40–30 Gy, SIT area in Boschi, control area in Baricella. Male pupae irradiated at 40 Gy were released from 29 April to 9 August, and from 6 September to 2 October; male pupae irradiated at 30 Gy were released from 13 August to 2 September. E-F: 2008, 40 Gy, SIT area in Budrio, control area in Lemizzone. Sterile male pupae were released from 30 April to 8 August. G-H: 2009, 30 Gy, SIT area in Caselline, control area in Baricella. Sterile male pupae were released from 21 May to 29 September. I-J: 2009, 30 Gy, SIT area in Boschi and control area in Baricella. Sterile male pupae were released from 14 May to 10 September.
The regression lines between mean percent induced sterility and egg density calculated in the trials with 30 Gy and 40 Gy were parallel ($t = 0.0018, df = 12, P = 0.9986$) and coincident ($t = 0.2637, df = 13, P = 0.7961$). We thus merged the data of two trials and performed the regression analysis between the mean percent induced sterility and the mean egg density in the ovitraps. A significant negative correlation was found, as reported in Fig. 3 ($R^2 = 0.53; F = 15.58; df = 11,4; P < 0.002$).

Similarly, the regression lines calculated for the trials with 30 and 40 Gy between the mean percent induced sterility and the mean percent egg density decrease in treated areas were parallel ($t = 0.5541, df = 12, P = 0.5897$) and coincident ($t = 0.0216, df = 13, P = 0.9831$). Being the regression analysis results not dependent on the radiation dose, we merged the data and the regression depicted in Fig. 4 showed a positive correlation between percent egg density decrease and percent induced sterility ($R^2 = 0.55; df = 11,4; F = 19.21$ and $P < 0.001$).

**Influence of Weather Parameters.** No statistically significant correlation was found between the monthly mean of percent egg sterility and the measured weather parameters, such as RH, mean temperature, and mean rainfall (Table 3).

**Discussion**

The sieving technique exploits the size difference between sexes to collect males at the pupal stage. The low male recovery efficacy of the sieving technique at the pupal stage may be considered not suitable for a large scale mass rearing, as in our study this method allowed the recovery of $<30\%$ of the reared males, with a residual presence of females around 1.2%. These females, when irradiated with a dose $\geq 20$ Gy, became permanently sterilized and produced no eggs; therefore, they had no direct influence on the population density (Balestrino et al. 2010). Nevertheless, as females may divert sterile male mating activity from wild females, and still maintain their biting activity, their number must be kept as low as possible, especially in disease endemic areas.

Moreover, the collected and released males constitute the dimensionally smaller part of the stock. The importance of size in male mating success is still a controversial issue (Yuval et al. 1993, Charlwood et al. 2002), but evidences that small mosquito males are less performing in mating competition than larger males recently have been found (Gary et al. 2009, Ponlawat and Harrington 2009, Helinski and Harrington 2011). Regardless of the regular elimination of small males from the rearing (to be sterilized), the male productivity of our mass rearing system has remained stable during the 4-yr study, and no evidence of selection for large-sized individuals in the strain under rearing has been found. Nevertheless, there is a critical need to develop more efficient sexing systems for *Ae. albopictus* based on genetic methods or exploiting other features than body size, such as development time (proterandry).

The mortality level cumulatively registered during transportation, irradiation, and release progressively decreased in the course of the study, mainly because

---

**Table 3.** Pearson product number (R) between the monthly mean of percent egg sterility and egg density, percentages of decrease of egg density (calculated by equation 1), and weather parameters (RH, mean temperature, and mean rainfall)

<table>
<thead>
<tr>
<th>G-rays dose and year</th>
<th>Egg density (no. eggs/ovitrap/wk)</th>
<th>% decrease of egg density</th>
<th>RH</th>
<th>Mean temperature</th>
<th>Mean rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 Gy - 2008</td>
<td>$R = -0.669$</td>
<td>$R = 0.774$</td>
<td>$R = -0.204$</td>
<td>$R = 0.277$</td>
<td>$R = 0.205$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.070$</td>
<td>$P = 0.024$</td>
<td>$P = 0.628$</td>
<td>$P = 0.507$</td>
<td>$P = 0.626$</td>
</tr>
<tr>
<td>30 Gy - 2009</td>
<td>$R = -0.776$</td>
<td>$R = 0.748$</td>
<td>$R = 0.279$</td>
<td>$R = -0.2430$</td>
<td>$R = 0.024$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.024$</td>
<td>$P = 0.033$</td>
<td>$P = 0.563$</td>
<td>$P = 0.562$</td>
<td>$P = 0.956$</td>
</tr>
<tr>
<td>Global analysis</td>
<td>$R = -0.726$</td>
<td>$R = 0.761$</td>
<td>$R = -0.188$</td>
<td>$R = 0.111$</td>
<td>$R = 0.079$</td>
</tr>
<tr>
<td></td>
<td>$P = 0.001$</td>
<td>$P = 0.001$</td>
<td>$P = 0.456$</td>
<td>$P = 0.652$</td>
<td>$P = 0.773$</td>
</tr>
</tbody>
</table>
of the reduction of the gamma ray dose. A significant decrease in mortality was obtained not only by decreasing the gamma ray dose from the initial 85 to 40 Gy, but also by further decreasing it from 40 to 30 Gy (Table 1). Also, the step up of procedures and methods, and the increase of technician skillfulness were crucial issues to achieve the acceptable level of \( \approx 1\% \) in the last trial.

The mass rearing capacity of the CAA facilities, the densities of sterile male release stations (0.44–0.67 stations/ha) and the periodicity of the releases were high enough to achieve the planned density of sterile males per hectare per week. As a result of the combination of these parameters, the sterile male release rate was in the range 896–1,590 males/ha/wk. The urban conditions of the four villages hosting the SIT trials were similar, but anyhow differences existed among them in mosquito carrying capacity, as shown by the egg density dynamic monitored by means of ovitraps. This monitoring method can provide a good estimation of \textit{Ae. albopictus} population density in Italian urban areas, as demonstrated by Carrieri et al. (2011b). In 2005, in Santamonica, releases were started later than usual, when the wild population was already well developed and this may have affected the level of efficacy of the release. In 2008, in Boschi, the high ratio of sterile to wild males obtained in September was consistent with the level of induced sterility, and this may explain the low population density registered at the beginning of the 2009 season, as showed in Fig. 1. Despite the occurrence of a large variability, as expected in such a kind of field trials, we put in evidence the importance of the wild population density in determining the level of achievable induced sterility (Table 2). This finding corroborates one of the theoretical bases of the SIT strategy, regarding the convenience to release sterile males when the density of the wild target population has been adequately reduced by nature or conventional control methods.

The correlation between mean percent induced sterility and mean egg density in treated areas was not dependent on the radiation dose, as shown by the parallelism test on data obtained by releasing males irradiated at 30 and 40 Gy. In addition, the correlations we observed between the monthly mean percent egg sterility and the monthly mean percent egg density reduction in SIT areas (in comparison to control areas) indicate the absence of any clear effect of reduction of the adult population density when induced sterility was below 50%. On the contrary, when sterility levels increased over this value, a significant impact on adult density reduction was obtained, and an almost directly proportional correlation was achieved between the two variables when induced sterility reached 70–80% (Fig. 3). Based on our data, we may estimate that to obtain a complete suppression of the local population in our conditions it would be necessary to achieve a minimum egg sterility level of \( \approx 81\% \), as the percent induced egg sterility necessary to obtain 50 and 90% population reduction is \( \text{SL}_{0.5} = 63.26\% \) and \( \text{SL}_{0.9} = 77.96\% \), respectively.


Received 5 March 2012; accepted 23 November 2012.