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SUPSI

Final Report

Project title:	Pilot project for the application of the sterile insect technique on <i>Aedes albopictus</i> in Canton Ticino (2022-2024)
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1. General considerations

Aedes albopictus, also called tiger mosquito, is an invasive allochthonous species that is established in most of the territory of the Canton Ticino since almost 20 years and is also recently expanding in other regions of Switzerland (Invasive Mosquitoes – Swiss mosquito network, <u>https://www.zanzaresvizzera.ch/</u>). This species of mosquito is considered one of the most dangerous invasive species in the world, for its ability to spread and to transmit exotic viral diseases such as dengue, chikungunya and Zika. In fact, worldwide the resurgence of such *Aedes*-borne disease outbreaks underscores the limitations of conventional vector control programs, which are heavily focused on insecticide application and the elimination of larval breeding sites. Challenges include the development of insecticide resistance, the presence of cryptic breeding sites, insufficient infrastructure or government support and high costs. The tiger mosquito is also of tremendous nuisance to citizens, who unfortunately may react by applying biocides in an uncontrolled manner, thus causing health and environmental risks. Thus, there is a pressing need for innovative, sustainable and cost-effective control strategies targeting *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, the two major vectors of arboviruses that together are responsible for more than 99% of arbovirus transmission within human populations.

In Canton Ticino, *Ae. albopictus* is currently managed with satisfactory results, through larval control and community participation. So far, there have never been any cases of local transmission of the above-mentioned diseases, thanks also to an action plan prepared in collaboration with the World Health Organization (WHO) and coordinated by the office of the cantonal doctor. Nevertheless, the remaining population densities are estimated to be high enough to cause a potential risk of transmission of exotic diseases. It is difficult to keep the dedication the population consistent and, it must be said, there is an objective difficulty in identifying the totality of tiger mosquito breeding sites, since many of them are cryptic.

TDR (the Special Programme for Research and Training in Tropical Diseases co-sponsored by UNICEF, UNDP, the World Bank and WHO), together with the International Atomic Energy Agency (IAEA), strongly encourage the application of the Sterile Insect Technique (SIT) to mosquitoes, because traditional biocides are losing effectiveness worldwide due to resistance and SIT is a targeted control system with no known environmental or health impact. Indeed, SIT is a genetic control method that has been largely applied to prevent, suppress or eradicate key insect pest of agricultural, sanitary and veterinary importance over the past 60 years. In this technique, it is the mosquitoes that go looking for mosquitoes.

In Europe, nowadays resistance genes to some adulticides are rapidly spreading. Adulticide biocides are not used in Switzerland in regular control activities but are needed when it is necessary to carry

out extraordinary control measures once the risk of disease transmission occurs. If the public does not use this kind of biocides, more and more citizens are installing automatic devices that apply this kind of products, increasing this way the risk of the resurgence of resistance. The SIT technique could be a useful tool to decrease this risk. The SIT is still expensive, but mass production facilities are improving constantly, and the costs are reducing every year.

<u>The purpose of this project</u>, carried out under WHO's solicitation, is to test whether the application of sterile male tiger mosquitoes in the already applied integrated control system in Canton Ticino could lead to a further decrease in the presence of the insect, and to assess what significance it has both in terms of annoyance to citizens and risk of disease transmission. To this end, it is worthwhile to conduct these assessment release trials and obtain initial data and improve this methodology with the aim to applicate it in Switzerland. Indeed, this pilot project provides the first detailed application in Switzerland with the SIT technique. Once the efficacy will be proved, it might be appropriate to have local production facilities that will facilitate access to sterile males to the cantons, regions or municipalities that will request them as an integrated control tool. Canton Basel City and Canton Grisons as well as some municipalities of Canton Ticino have already shown interest in that sense.

The project is a collaborative effort of the Institute of Microbiology, SUPSI (Dr Eleonora Flacio, Vector Ecology Group), the Centre Agriculture and Environment "Giorgio Nicoli" (CAA, an IAEA Collaborating Centre, Crevalcore, Italy) (Dr Romeo Bellini), whereas TDR WHO (Dr Florence Fouque) and IAEA (Dr Jérémy Bouyer) have assured scientific and technical support to the project. The experiments were carried out over two years, 2023 and 2024, to obtain more solid data. In 2022 a single release had been already performed, not yet with the purpose to demonstrate the decrease of tiger mosquitoes in the territory, but to gain mastery and useful field parameters to best carry out the full pilot experiment supported by this contract.

2. 2022 preliminary test: single release

2.1 Purpose

With the 2022 single release, we wanted to assess whether there were any difficulties in implementing the SIT regarding different parameters (e.g., transportation, customs, public acceptance). We also evaluated survival and dispersal of released males, as well as their residual fertility. Also important was the consolidation of collaboration with CAA, the producers of the sterile mosquito males.

2.2 Materials and methods

2.2.1 Production of sterile males and shipment

CAA produced sterile males from Canton Ticino's *Ae. albopictus* strains (11 generations). Pupae (20-40 hours old) were irradiated with 55 Gy (Radgil2). 22,500 sterile adult males (kept at 8-10°C for 30 min) were tagged with fluorescent powder (FAO/IAEA 2020), refrigerated, packed in stacked plastic cups at 10-12°C and transported by car (3 hours) to the field site in Morcote.

2.2.2 Release area and field experiment

A permit was obtained from the Federal Office for the Environment (FOEN), according to the Ordinance on the Handling of Organisms in the Environment (No. 814.911), to release sterile male tiger mosquitoes in Morcote in 2022 (BAFU-217.23-64633/7). The same permit was also valid for the releases carried out in 2023. On the 2nd of August at 18:00 local time, 22,550 marked (pink) sterile males, contained in cups (1,500 males/cup), were released in the municipality of Morcote (**Fig. 1** yellow star). Mortality and female presence were checked after 1 hour. In the following days (3rd-9th August) Mark Release Recaptures (MRRs) were performed between 18:00 and 19:30 by Human Landing Collections (HLCs) with manual battery aspirators and black boxes, 5 min/collection point. Four field technicians were daily rotating in 32 collection points (8 collection points/technician) (**Fig. 1** red dots). The MRR area was divided into four concentric circles 50 m apart in which HLC points were evenly distributed (distance between contiguous points 50-100 m). HLCs were carried out in areas ideal for mosquitoes to rest (shaded and close to vegetation). No HLC was carried out in windy and stormy days. Collected mosquitoes (males and females) were killed by freezing and brought to the laboratory for checking the presence of the coloured marking using a stereomicroscope.



Figure 1. A) Release point (yellow star) and HLC points (red dots) in Morcote. B) Marked sterile males. Map created in https://map.geo.admin.ch/.

2.2.3 Sterile male residual fertility

Radiation reduces mosquito viability, so the dosage is adjusted so that mosquitoes are still able to mate. The residual fertility of the released males was tested in the laboratory by mating 200 irradiated males with 200 non irradiated females. An equal number of non-irradiated males and females were used as control. The egg hatching protocol of IAEA was applied and residual fertility checked.

2.2.4 Information to citizens

The media were invited to the release and several interviews were given in the following days.

2.3 Results

No transportation problems occurred.

Among the 22,500 sterile males, 20,085 were successfully released and 2,415 mosquitoes (10.73%) were not able to fly, of which 10 were females. Therefore, the residual presence of females was assumed to be about 0.41%.

The 6th and 7th August stormy wind was present, so no HLC recaptures were performed.

Only *Ae. albopictus* were collected with MRR. Based on the rate of sterile male recaptures (0.21%), the daily survival rate of sterile males was SR=0.59 while the average life expectancy (ALE) was 1.89 days. Relative humidity during the days 2nd-6th August ranged 56-59%, which is low for the survival of *Ae. albopictus*, while during the days 7th-9th August it ranged 67-80%, which is ok.

The mean distance travelled by the sterilized males was 90.95 m and the maximum distance was 175 m.

The non-irradiated-male control carried out at SUPSI's laboratory suggested a natural egg fertility of 87.6%, while the test with irradiated males and wild females showed a residual fertility of 1.5%. The sterility level agreed with expectations.

As a follow-up to the experiment, there were more than 20 media runs throughout Switzerland (television, radio, newspapers). All interested in the experiment. There were no negative comments. Some municipalities expected the technique to be adopted already in the regular surveillance and control system in Ticino Canton in 2023.

3 2023 and 2024 pilot tests

3.1 Materials and methods

3.1.1 Study area

The study was conducted in the central region of Canton Ticino, in two municipalities on the shores of Lake Ceresio. Both municipalities are part of the integrated surveillance and control system against invasive mosquitoes in place in Canton Ticino since 2009. Control measures consist of public catch basins treatments with VectoMax[®] FG (every 8 weeks between May and September), while private areas should be managed by citizens, who are invited, through an information campaign (<u>www.supsi.ch/go/zanzare</u>), to use VectoBac[®] G weekly between May and September and to remove larval breeding sites. The releases were carried out in the municipality of Morcote, in an urbanized area of about 45 hectares (**Fig. 2**). This area is bordered by woods and the lake and could only be affected by introductions of wild female and male mosquitoes on the south-eastern side, which borders the town of Vico Morcote. The municipality of Caslano, about 5 km away in a straight line from the test area and with similar climatic and housing characteristic, was chosen as control (**Fig. 2**). To reduce the workload, the entire municipal area of Caslano was not used, but only 14 hectares was used as a control, which corresponds to about 30% of the test area. No additional control measures other than those already part of the surveillance and control system were carried out during the study period.





3.1.2 Study design

In 2023 and 2024, regular releases of unmarked sterile males of *Ae. albopictus* were carried out in the municipality of Morcote (**Fig. 3**) during the mosquito reproductive season (May to end of September). Mosquito populations in the release (Morcote) and control (Caslano) areas were monitored weekly from mid-April to mid-October with egg traps. Egg density and fertility was determined. In parallel to the releases, adults were collected by traps in both the test and control areas to assess the reduction in the density of adult females and adult competitiveness. Three MRRs were also conducted in Morcote, releasing sterile males at the same location where the preliminary MRR test was conducted in 2022 (**Fig. 1**). These tests were conducted from May to September and involved the release of tagged sterile males at a single location, followed by their recapture at monitoring stations with adult traps evenly distributed within a 200-m radius of the release point.



Figure 3. Outline of the activities carried out in 2023 and 2024 for the SIT pilot tests.

3.1.3 Production and shipment of sterile males

Sterile males of *Ae. albopictus*, whose strain came from eggs collected in Canton Ticino, were routinely produced by CAA by X-ray radiation (40 Gy in 2023 and 35 Gy in 2024) of 2-48 hours old pupae in water (Balestrino et al. 2014). The males were two to four days old at the time of release and were powder-marked (according to IAEA protocol) only in case of MRR testing. The main transport box for the sterile males consisted of a polystyrene box with cardboard tubes inside, which contained the cups (12 cm diameter) with the mosquitoes. The temperature in the shipping packages was maintained at 8-12°C and 80% RH (verified by data loggers inserted in the packages) to keep the sterile males in a state of dormancy during the journey. The polystyrene box was surrounded by cooling packs and bubble wrap to maintain temperature and airtightness. Tests were also conducted with other types of transport containers, such as the box recommended by the IAEA with plastic carrying cases, as well as with a refrigerated suitcase and bag for transportation by train.

In May, a shipment of sterile males was sent to verify the correct distribution through courier services. With these mosquitoes, a wake-up test was conducted to understand how the duration of stay in the packages affected male mortality. We observed that 1) males that move within the release cups after one hour but are not able to fly cannot be considered alive; 2) even with the package completely sealed and isolated, after 24 hours the mortality increases by 17%. The sterile males were transported to destination by express courier (estimated 18 hours) or directly by CAA or SUPSI operators via train/car (3 hours). The second option was implemented to reduce transport time, because excessive mortality rates were observed with courier transportation.

Radiation reduces mosquito viability. Therefore, the radiation dose is calibrated to ensure that mosquitoes retain the ability to mate. To monitor the quality of the released sterile males, a random assessment of their residual fertility was performed three times during the experiment in the receiving laboratory. A random sample of about 200 sterile males was placed in a small cage together with about 200 virgin females. The mosquitoes were fed with sugar solution and the females were fed blood twice on days 3 and 4 from introduction into the cage. The eggs produced were allowed to mature for one week and then subjected to the IAEA hatching protocol. A similar procedure was adopted with non-irradiated males as an untreated control.

3.1.4 Releases

Upon arrival to the receiving facility, sterile males were immediately transferred to the release site in Morcote. Approximately 3,000 sterile males per hectare per week were released by means of 75 predefined release stations spaced 50-80 m apart, with 2,000 males per week released at each station evenly distributed throughout Morcote (**Fig. 4**).



Figure 4. Morcote aerial map with the 75 sterile male release points (blue) and 45 ovitraps (red). A and B: cups tested for release. A: plastic ice cream cup. B: cup made from ovitraps with mosquito walking surface. Map created in https://map.geo.admin.ch/.

The most suitable method for the release of sterile males in this trial involved the creation of 75 release stations that contained 8 cm x 12 cm plastic containers (like those used for ice cream) with a sanded interior or the cup part of an ovitrap with white card inside. These containers were hung on a string and coated with Vaseline to prevent the entry of ants (**Fig. 4**). This system is highly effective as it allows for a quick and efficient release of sterile males in many areas.

Once the males were released, their mortality was checked by counting the number of adults that did not fly out of the container after one hour in a sample of 5 randomly selected release stations. A high mortality rate was found during the experiment, so it was decided to decrease the density of males per cup by placing only 1,000 males and then arrange 2 cups per release point.

Also, during the hottest period of the season and following the MRRs' survivorship results, two weekly releases of 75,000 males each were made to ensure the activity of sterile males throughout the week. In summary, in May, June and from the second half of September (cooler period) males were released once per week. From July to mid-September (warmer period: minimum temperature above 20°C and maximum temperature above 30°C), males were released two times per week (on Tuesday and on Friday). In total, 34 releases were carried out and 3,450,000 sterile males were released during the season, with 3,000 sterile males released per hectare per week.

3.1.5 Monitoring of mosquito population: egg density and viability

Population density and fertility were monitored in the release and control areas using ovitraps, which consisted of black plastic jars (Ramona Ø13/H12, Luwasa[®] Interhydro AG, Allmendingen, Switzerland) containing each a steamed beechwood slat (200 x 25 x 5 mm) that served as oviposition support. The jars, which were provided with a top border efflux hole, were filled with tap water to act as a breeding site. In 2023, 45 ovitraps in Morcote and 14 in Caslano (one ovitrap per ha, **Fig. 5**). In 2024, 33 ovitraps were added in Morcote to better monitor the buffer area around the release sites and six ovitraps were added in Caslano to cover more completely the study area. Ovitraps were monitored, weekly in 2023 and every two weeks in 2024, throughout the season starting as early as 2 weeks before releases (mid-April) and ending 2 weeks later (mid-October). The wooden slats were replaced with new ones and taken to the laboratory. *Aedes albopictus* eggs were differentiated from the other species via optical species determination (Anicic et al. 2023) and counted.

Fertility of the eggs was checked performing hatching (**Fig. 5**) and then counting the eggs again using the same methodology as described above. Due to workload issues, fertility was checked every two ovitrap control rounds.

To check the possible border effect of sterile males from the trial area, ovitraps located in Vico Morcote, the municipality bordering Morcote, which are part of the normal *Ae. albopictus* surveillance system in the Ticino region, were checked for eggs densities and residual fertility every two weeks, with the same protocol.



Figure 4. Ovitrap locations (red points): trial area (A); control area (B).

Egg collection	Step 1. Collect the oviposition substrates and keep them wet (use wet kitchen paper to wrap them). IMPORTANT NOTE: eggs should not dry during the trip from the field to the laboratory (avoid leaving the eggs in the car, under the sun).
orage	Step 2. Oviposition substrates should be left in the wet paper under standard laboratory conditions ($25\pm1^{\circ}$ C, 80% RH, 14:10 L) for 1 day to dry.
Egg st	Step 3. The next day the oviposition substrates are placed in a sealed plastic container at 23°C to embryonate for one week.
Hatching solution	Step 4. Preparation of hatching solution. The maternal solution is prepared using 12.5 g broth (Nutrient Broth OXOID) + 2.5 g yeast powder/100 ml of deionized water. IMPORTANT NOTE: The hatching solution must be used immediately and cannot be stored.
Hatching	Step 5. For egg hatching, 1 L volume glass jars with caps are used. Use 700 ml of deionized water and 2 ml of the hatching solution in each pot. Put 5 oviposition substrates in one glass jar. The jars must be hermetically closed at 28°C and opened 20-24 hours later (not before 16 hours). Count the hatched and unwatched eggs under the stereomicroscope.

Figure 5. Egg hatching protocol.

3.1.6 Monitoring of mosquito population: adult density

Adult mosquitoes were collected in the study areas using the modular adult trap Biogents BG-Pro in sentinel mode baited with CO_2 . (**Fig. 6**). This type of trap is powered by a power bank and can be conveniently transported and installed. We chose to produce CO_2 on site through yeast fermentation in concealable blue bags (https://eu.biogents.com/bg-co2-generator/) because dry ice was difficult get hold of on a regular basis, and the CO_2 canisters on hand were too conspicuous and might have caused alarm to the citizens.



Figure 6. BG-Pro trap in sentinel more, baited with CO₂ (blue bag on the right).

Adult density was monitored from the beginning of May until the end of September. Morcote had 25 evenly distributed sampling sites, each consisting of one BG-Pro (1 trap / 2 hectares), while Caslano had 8 sampling sites composed in the same way as Morcote.



Figure 7. BG-Pro traps (orange dots) test area (A); control area (B) (1 trap / 2 ha).

Traps were displayed every two weeks, 24 hours after the release, and checked after 40-48 hours. This monitoring was useful to determine parameters such as sex ratio, competition, population density of females, reduction in female density etc. Samples collected were labeled and frozen in the laboratory for later identification. The identification was be done using a stereomicroscope and the *Ae. albopictus* individuals were counted by sex.

3.2 Results

3.2.1 Production of sterile males and shipment

There were no issues with the production of sterile males by CAA, both in 2023 and in 2024.

In 2023, delivery through courier lasted around 18-19 hours, except for one instance when the package arrived with a delay of two days. Sterile male mortality reached high rates (51%) early in the season. The rates could be decreased to 22.7% by reducing the mosquito density in the transportation cups and improving adult sugar intake. Mosquito mortality was also decreased by reducing the transportation time to 3 hours by switching from courier transportation to direct transportation of the package via train/car by CAA or SUPSI operators, although this was done only at the end of the season (i.e., in September 2023). In 2024, the same problems with duration of transportation by courier were observed: mortality rose above 43% when courier transportation time exceeded 24 hours (July 2024), while reducing transport time to three hours (by direct transportation via car) lowered the mortality rate to 2.1%.

3.2.2 Quality of males: sterile male residual fertility

In 2023 and 2024, the natural fertility tested in the laboratory with non-irradiated males was on average 96.2%. In 2023, the sterile male residual fertility tested in the laboratory with males irradiated at 40 Gy was on average 0.03%. In 2024, the males to be released were irradiated with a lower dose (i.e., 35 Gy) to increase the survival of released males. Consequently, the residual fertility was also higher (0.3%). According to previous tests, the residual fertility of the released males should be limited according to the population basic offspring number and anyway below 2% to achieve an effective control of *Ae. albopictus* populations in a tropical area using the SIT as part of an integrated pest management approach (Aronna and Dumont 2020). The CAA has observed in release studies in Italy that the use of radiation doses between 30 and 40 Gy effectively induce sterility into the natural population with an acceptable residual fertility lower than 2% (Balestrino et al., 2010; Bellini et al., 2021). Therefore, the sterility level of irradiated males that were released in Morcote is considered in line with expectations.

3.2.3 Releases of sterile males

We conducted 34 sterile releases per season with a weekly (May, June, and the second half of Sept) or bi-weekly frequency (July, August, and the first half of Sept). In total, about 3.5 mio sterile males were released per season. The release time took approximately 3 hours with the involvement of two technical operators by car to cover the test area of 45 hectares.

3.2.4 Monitoring of mosquito population: egg abundances and viability

The average egg density in the SIT area was systematically lower than in the control area, both in 2023 and 2024 (**Fig. 8**). Overall, in the SIT area the egg density was reduced by 54.8% throughout the entire activity period of the mosquitoes.





The induced sterility remained consistent across years but had values lower than expected, around 18% less in the release area. Mean egg hatching in the SIT area was 76.4%, compared to 93.1% in the control area.

3.2.5 Monitoring of mosquito population: adult abundances

The adult traps displayed for the test worked correctly in both areas. The data indicated an overall decrease in the density of adult females throughout the 2023 and 2024 mosquitoes' entire activity period by 64.9%. The seasonal peak was confirmed at the end of August (2023) or beginning of September (2024) and was notably higher in the control area. In the release area, the typical exponential growth in number of females did not occur at all in 2023 and was very light in 2024 (**Fig. 9**).



Figure 9. Fortnight average adult female density in the SIT area and in the control area in 2023 and 2024.

3.2.6 Difference between areas in the release municipality

Independent analysis of the areas within the sterile male release zone shows notable differences regarding the reduction of egg number, number of adult females, and induced infertility (**Fig. 10**). The SIT seems to have worked best in the most isolated zone, namely, the western zone. This zone borders only with the lake and the forest and is characterized by homogeneous building of family houses with gardens (mostly secondary dwellings). The second-best SIT area was the old town, where the village core, characterized by stacked old houses, very close together and narrow streets is located. The SIT was less effective in the East area, which is less isolated because it borders the neighboring municipality of Vico Morcote. This area is characterized by different types of construction, single-family houses, multi-family, core, the church and several orchards. This area is very touristy, in fact there are numerous restaurants, stores and parking lots. The Border area is very close to the town hall of Vico Morcote, where no sterile males' releases were performed. Therefore, this area can easily be affected by fertile females' introductions, as reflected by the results.



D (reduction in egg density)		FD (reduction in female density)		S (induced sterility)		
Area	2023	2024	2023	2024	2023	2024
WEST	73,3	60,7	72,1	68,5	25	20
OLD TOWN	33,4	38,4	82,9	75,4	18	18
EAST	19,2	28,9	64,3	57,0	16	16
BORDER	11,5	17,0	51,1	47,8	15	14



3.2.7 Mark, release, recaptures of sterile males

During 2023, three MRR were performed: 1 at the beginning (13th of June 10,000 males), 1 in the middle (11th of July 20,000 males) and 1 at the end of the season (22nd of August 20,000 males). Among the totally 50,000 sterile males released, 19,835 (39.67%) were not able to fly after one hour from the release. Therefore, we may assume that about 30,165 sterile males were successfully released. In 2023, both mortality and sex of mosquitoes were counted in some cups: among a total of 6,867 dead adults 24 were female, therefore it can be assumed a 0.35% residual presence of female.

In 2024, the rate of sterile male recaptures was 5.44%, higher than 2023 (0.61%), the daily survival rate of sterile males was SR=0.63 (0.63 in 2023) while the average life expectancy (ALE) was 2.18 days (3.5 days in 2023; **Tab. 1**). The mean distance travelled by the sterilized males was 69 m (97.3 in 2023) and the maximum distance was 115.8 m (184.4 m in 2023). According to the date we can estimate that 80% of released males covered a surface of 100 m and only 18% could go over this 100 m to 200 m.

Table 1. MRR results in the trial area.

	2023	2024
Average recapture rate of marked males	0.61%	5.44%
Daily survival rate	0.63	0.54
Average life expectancy [days]	3.5	2.18
Average distance travelled [m]	97.3	69
Maximum distance travelled [m]	184.4	115.8

3.2.8 Communication to citizens

As a follow-up to the experiment, there were numerous media coverage events throughout Switzerland (television, radio, newspapers) and internationally (Italy, Germany), public events organized by SUPSI, etc. All of them showed interest in the experiment about the technique and the preliminary results. There were no negative comments. Some municipalities even expected the technique to be integrated into the regular surveillance and control system in the Ticino Canton in 2023.

3.3 Discussion

Thanks to the two seasons of continuous releases, SUPSI has acquired the technical and scientific capabilities to carry out the experiments.

The results are encouraging. An overall decrease in mosquitoes was seen in both eggs (54.8%) and adults (64.9%). The decrease was especially important during the seasonal peak, despite the fact that the induced sterility was low (17.8%). It will be necessary to investigate the reason for this figure, probably due to introductions from the transit of people to the municipality of Morcote, which is very touristy. It is also interesting to see that the data are different according to the area in which they are collected: more isolated residential area, core, border area. In the future, it will be necessary to better investigate the effectiveness as a function of spatial structure.

It was interesting to confirm the data from the ordinary surveillance system and to see that infertile males do not seem to have had an influence on the neighboring municipality of Vico Morcote. The experiment was therefore quite isolated, as we were promised. By means of MRR tests, it was seen that 80% of the sterile males remained within a distance of 100 m and the average survival of these is around 2 to 3.5 days, which justifies biweekly releases and shows the safety of the experiment along with the residual fertility datum of 0.03 to 0.3% of the sterilized males and the presence of 0.35% of released females.

At the beginning of the 2023 season, we had to do numerous trials to reduce the mortality of the sterile males to be released, so we came to the conclusion that by providing an additional meal of sugar in production and decreasing both the densities per cup and the travel time of the pack we

can arrive at an acceptable mortality to carry out the experiment. Transportation of the sterile males by courier remains at this time a point to be improved.

Acceptance by the public in Ticino and elsewhere has been extraordinary. The SIT experiment attracted great interest from the public with more than 70 coverage in the media, and all were supportive of the technique. Citizens organized a fundraiser to support the project, and surveys and public activities consistently showed no criticism. Many municipalities and cantons would already like to apply this technique, but further scientific evidence of effectiveness in different territorial situations is needed as well as a biofactory that can support such a production.

Doing a bit of rough math with the studies already carried out in Canton Ticino, we have shown that the integrated control measures in place allow to decrease the presence of tiger mosquito by 74% (Ravasi et al. 2021) and the estimated cost per citizen is 1.50 francs. To these measures can be added an additional contribution from citizens if they worked individually optimally by 68% (data collected during pandemic years) for a cost per citizen of about 11 francs (the 50g sachet of VectoBac[®] G). The same is true for volunteer citizen groups in Canton Ticino that take care of their neighborhood with an 'additional 66% effectiveness at the baseline system. Doing door to door can lead to exceptional results, but in places where there are no continuous introductions and over limited areas, such a strategy is unimaginable on a large scale because of the prohibitive cost and difficulty of accessing every home. Thus, the SIT technique has an efficacy, at the moment and in the area where the test took place, comparable to the citizens' contribution by having a cost per citizen (current costs) of 9.50 francs if there is a scientific data analysis facility, while it would cost 4 francs per citizen if only sterile males were to be released. Another advantage of this technique is that it has a different mode of action than the current ones, i.e., one does not have to go looking for breeding sites, which is not always obvious, but it is the mosquitoes that look for mosquitoes all over the territory both public and private.

We plan to expand the trial starting in 2025 over much of the territory considering the following points:

- Improvement of the quality of the males (production, sterilization, packaging and transport).
- Response of the technique with different ground structures
- Cost-benefit analysis (timing and scale of releases)
- Impact on nuisance and risk of disease transmission
- Evaluation of public response to this new technique.

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Abbreviations

- ALE: Average life expectancy CAA: Centre Agriculture and Environment "Giorgio Nicoli", Crevalcore, Italy
- ECOVET: Vector Ecology Group, SUPSI

SUPSI

FOEN:	Federal Office for the Environment
HLC:	Human Landing Collections
IAEA:	International Atomic Energy Agency
MRR:	Mark release recaptures
SIT:	Sterile Insect Technique
SR:	Survival rate
SUPSI:	University of Applied Sciences and Arts of Southern Switzerland
TDR:	Special Programme for Research and Training in Tropical Diseases co-sponsored by UNICEF, UNDP, the World Bank and WHO
WHO:	World Health Organization

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