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SUPSI

Report

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Project title: **Funding request for improving data collection and analysis under the sterile male technique project on Aedes albopictus in Canton Ticino**

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1. General considerations

Aedes albopictus, also called tiger mosquito, is an invasive allochthonous species that is now established in most of the territory of the Canton of Ticino for almost 20 years and is also recently expanding in other Swiss regions (Invasive Mosquitoes - Swiss mosquito network (zanzare-svizzera.ch)). This species of mosquito is considered one of the most dangerous invasive species in the world, both for its ability to spread and to transmit exotic diseases such as dengue, chikungunya and Zika. This 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. In Canton Ticino *Aedes albopictus* is currently managed with satisfactory results (Presentazione - ICM (DSS) - Repubblica e Cantone Ticino; SUPSI - Istituto microbiologia - Zanzare), through larval control and community participation. However, a limit of effectiveness is thought to have been reached somewhat due to, for instance, the presence, both in public and private spaces, of cryptic and/or non-accessible breeding sites, the lack of control in specific locations (e.g., building sites, secondary houses, and abandoned sites), or the difficulty in consistently keeping the dedication of citizens alive. The remaining mosquito population densities are estimated to be sufficient to cause a potential risk of transmission of exotic diseases. Thus, there is still a need to improve the control methods to overcome these limitations.

The Sterile Insect Technique (SIT) could provide a sustainable solution. The SIT uses irradiation to sterilize male mosquitoes, which are then released in large numbers in target areas to mate with wild females, thus preventing wild female eggs from hatching and leading, over time and successive releases, to a decline in the wild population. Its mode of action is therefore different from traditional control methods that focus on detecting and removing/treating breeding sites. In the SIT, it is the mosquitoes that go looking for mosquitoes, which makes it an interesting method to integrate into the existing control measures. The SIT is considered without any hazards for the public and animal health as well as for the environment.

The Vector ecology (ECOVET) group of the Microbiology Institute of SUPSI considers fundamental to test this technique in Ticino to see whether it could be integrated into the tiger mosquito control measures already in place. In partnership with the Centre Agriculture and Environment “Giorgio Nicoli” (CAA) in Bologna, a European leader in the production of sterile males, we carried out a feasibility study initially supported financially by ECOVET itself, the Kantonales Laboratorium of the Canton Basel City and the ‘Amt für Natur und Umwelt’ of Canton Grisons. Proven the implementation, in 2023 it was possible to launch a pilot study which was in addition financially supported by the Swiss Expert Committee for Biosafety (SECB), the Federal Office for the Environment, the municipality of Morcote and ISIDORe.

The studies are part of a global network of SIT trials taking place simultaneously in various parts of the world under the auspices of the WHO's Special Programme for Research and Training in Tropical Diseases (TDR) and the International Atomic Energy Agency (IAEA), who provide scientific and technical support. The plan of action follows the phased conditional approach (PCA) recommended by FAO/IAEA Insect Pest Control Subprogramme, in which advancement to the next phase depends on completion of activities in the previous one.

The results for the 2023 and 2024 pilot trials were very encouraging: an overall decrease in mosquitoes was seen in both eggs (55%) and adults (65%) was observed with the most important decrease during the seasonal peak. This despite the fact that the induced sterility was low (18%). It was also observed that 80% of the sterile males remained within 100 m of their release point, which shows the safety of the experiment, and that their average survival was around 3.5 days, which justifies biweekly releases.

Thanks to recent involvement in these SIT trials of a professional consultant in statistics of the Zurich Data Scientists (ZDS), which already collaborate for other projects with SUPSI, it was possible to realize that many studies with this kind of technique are based on poorly constructed experimental designs, inadequate statistical methods, and misleading assumptions even if used as references by the scientific community. For example, if a significant reduction in mosquitoes in the treated area is observed, compared to the control, the difference could be due to other factors related to the control area not considered yet in the analysis. Instead of comparing the treatment area with the control area, it makes more sense to look at the dynamic of egg density (fertility) in the treatment area, using appropriate models.

With this additional statistician support we want to improve the analysis and representation of the 2023 results, as well as the fine-tuning design of the experiment and analysis in 2024. Furthermore, a plan with adjusted design for the futures seasons is prepared to achieve a comprehensive approach to implement the SIT in Switzerland as an integrated control measure for *Ae. albopictus* populations. Here we present the statistical methods used to analyze the data collected in 2023 and 2024 and the plan with adjusted design for the future seasons.

2. Analysis of the SIT data collected in 2023 and 2024

2.1 Modelling number of eggs

2.1.1 Generalised Additive Mixed-Effects Model (GAMM)

All statistical analyses were conducted using R. We modelled the response variable, "total number of *Ae. albopictus* eggs" (a count variable), using a Generalized Additive Model (GAM) with a negative binomial family to address overdispersion. The model included a smooth effect for "sampling date", represented as the day of the year (a numeric variable), which interacts with municipality (municipality.fac). municipality.fac is a categorical variable, consisting of two levels: Caslano and Morcote. Its effect was modelled as a fixed effect. We controlled for the non-independence of observations by including trap ID (a categorical variable) as a random effect, with data from the distinct traps. Model complexity was evaluated, and the best-fitting model was selected using a Chi-square test and AIC and BIC criteria. The significance level was set at 5%.

2.1.2 Spatial Generalised Additive Model (spatial GAM)

We fitted two separate models to analyse the response variable, total.albopictus.eggs, using a Generalized Additive Model (GAM) with a negative binomial family to account for overdispersion. One model was fitted for the municipality of Morcote, and the other for Caslano. Both models included a smooth effect for the “sampling date”, represented as day of the year (yday), and a combined smooth effect for geographic coordinates to capture spatial variability within each municipality.

2.2 Modelling percentage of hatched eggs

2.2.1 Generalised Additive Mixed-Effects Model (GAMM)

We modelled the percentage of hatched *Ae. albopictus* eggs (combining the variables hatched.albo.eggs.after.proc and non.hatched.albo.eggs.after.proc to a binomial response variable), using a Generalized Additive Model (GAM) with a quasi binomial family to address overdispersion. The model included a smooth effect for “sampling date”, represented as the day of the year (yday, a numeric variable), which interacts with municipality (municipality.fac). municipality.fac is a categorical variable, consisting of 3 levels: Caslano, Morcote, Vicomorcote; its effect was modelled as a fixed effect. We controlled for the non-independence of observations by including trap ID (unique.ID, a categorical variable) as a random effect, with data from 62 distinct traps. Model complexity was evaluated, and the best-fitting model was selected using a Chi-square test and AIC and BIC criteria. The significance level was set at 5%.

2.2.2 Spatial Generalised Additive Model (spatial GAM)

We fitted two separate models to analyse the percentage of hatched *Ae. albopictus* eggs (combining the variables hatched.albo.eggs.after.proc and non.hatched.albo.eggs.after.proc to a binomial response variable), using a Generalized Additive Model (GAM) with a quasi binomial family to account for overdispersion. One model was fitted for the municipalities of Morcote and Vico Morcote, and the other for Caslano. Both models included a smooth effect for the “sampling date”, represented as day of the year (yday), and a combined smooth effect for geographic coordinates to capture spatial variability within each municipality. We selected the best-fitting models by evaluating AIC and BIC criteria. The significance level was set at 5%.

2.3 *Modelling number of adults*

2.3.1 Generalised Additive Mixed-Effects Model – Females (GAMM)

We modelled the response variable, “number of *Ae. albopictus* females” (Ae.albopictus.female, a count variable), using a Generalized Additive Model (GAM) with a negative binomial family to address overdispersion. The model includes a smooth effect for sampling date, represented as the day of the year (yday), a numeric variable, which interacts with municipality (municipality.fac). The municipality variable consists of 2 levels (Caslano, Morcote), and its effect was modelled as a fixed effect. We further controlled for the non-independence of observations by including trap ID (ID_BG_trap.fac) as a random effect, with data from 33 distinct traps. Model complexity was evaluated, and the best-fitting model was selected using AIC and BIC criteria.

2.3.2 Spatial Generalised Additive Model – Females (spatial GAM)

We fitted two separate models to analyse the response variable, “number of *Ae. albopictus* females” (Ae.albopictus.female, a count variable ranging from 0 to 20), using Generalized Additive Models (GAM) with a negative binomial family to account for overdispersion. One model was fitted for the municipality of Morcote, and the other for Caslano. Both models included a smooth effect for the sampling date, represented as day of the year (yday), and a combined smooth effect for geographic coordinates to capture spatial variability within each municipality. This allowed us to assess the temporal trends and spatial distribution of egg counts separately for Morcote and Caslano. We selected the best-fitting models by evaluating AIC and BIC criteria.

2.3.3 Spatial Generalised Additive Model – Males (spatial GAM)

The same model as for section Spatial Generalised Additive Model – Females (spatial GAM) was fitted, only for males.

3. Plan for future seasons

The objective is to update the methods used to test the efficacy of SIT, from the design of the experiments to the data collection and analysis. Using Ticino as a testing field, we want to advance various aspects of SIT implementation and research in the field. The comprehensive new protocol design will be used to understand the main factors affecting the success of SIT in typical *Ae. albopictus* habitats to assess whether where and how the application of this technique is of interest (costs/benefits). Consideration will also be given to whether SIT can lower the risk of disease transmission.

3.1 Design of the experiment

The SIT field study should be run over three years (for instance, 2025, 2026 and 2027) during the mosquito active season, from May to October. The study is to be carried out in 21 sites distributed in the four districts of Ticino (i.e., Mendrisio, Lugano, Bellinzona and Locarno) with highest densities of *Ae. albopictus*. The sites cover several types of habitats typically related to *Ae. albopictus* infestation in Ticino such as urban city centre, village core, residential area, campsite, hotel and surrounding. Each site has a central area of approximately 12 ha (**Fig. 1**). The central area is represented as a circle with a radius of 200 m based on the maximal distance travelled by *Ae. albopictus*. The measure of 100-200 m of autonomous tiger mosquito displacement is the one adopted by the European systems of Italy, France, Spain and Switzerland in the case of adulticidal intervention against the mosquito in case of arbovirus introduction. The size of the area helps to ensure absence of mosquito's natural entry all the way to the center of the experiment. In SIT treated sites (**Fig. 1**, red circle), sterile males are released two times per week over the whole area by means of 18 evenly distributed release stations (red dots in the red circle). Egg density and fertility are assessed both in SIT and control sites every two weeks by means of ovitraps (1 ovitrap/ha; blue dots). Adult density and sex ratio are assessed with the same frequency by means of adult traps (green dots) distributed over a transect. The border effects of SIT are assessed by collecting egg and adult data in a randomly selected border area (the green sector) of 12 ha (extending approx. 300 m from the border of the central area).

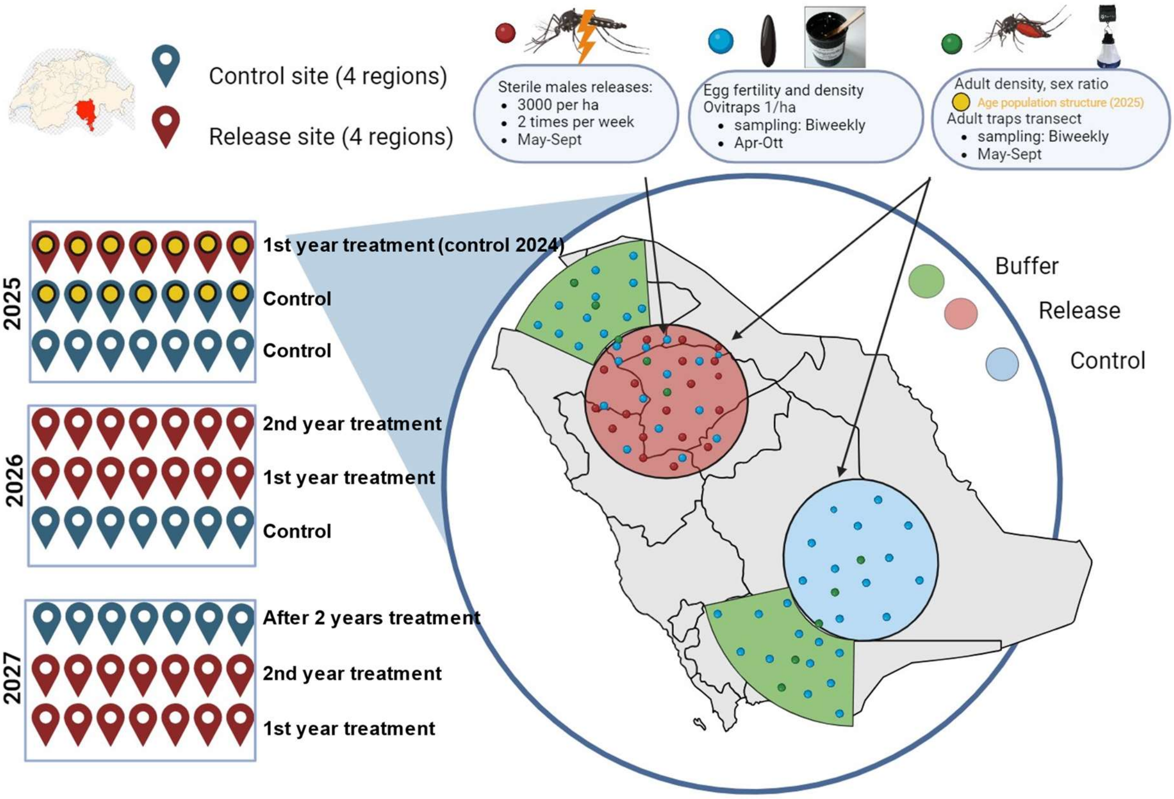

Figure 1. Experimental design. Image created with BioRender.com.

The sites enter the SIT treatment progressively (**Fig. 1**, on the left). In 2025, seven sites are treated while 14 sites act as a control. In 2026, seven of the sites that were used as control in 2025 are treated bringing the total number of treated sites to 14. In 2027, the seven sites used as control in 2025 and 2026 are treated, while the seven sites treated in 2025 and 2026 are not treated but still monitored. The idea of the design is that each site is firstly available as control and then as treated. For the seven sites that are treated in 2025, the first year of the study, control data are being collected in 2024. This implies that each site can act as its own control. This is relevant as we know from other studies in Ticino that the variability among sites and among ovitraps, used to measure the density of eggs in one site, can be important. Another advantage of this design is that it enables us to disentangle the effect of "calendar year" (2025, 2026 and 2027) from the effect of "year of treatment" (i.e. first and second). Indeed, if we were to follow a different design treating say half of the sites in 2025 and keeping them treated in 2026 it would be difficult to know whether a very good effect in 2026, the second year of treatment, is due to an adverse year for tiger mosquitos and thus the treatment is more effective or whether in the second year of treatment the effect is truly very strong. So, this design also enables us to better assess what the year-to-year variation actually is. Lastly, seven sites are treated in 2025 and 2026, but not in 2027, allowing us to observe what happens when a site is not treated after two consecutive years of treatment.

Fixed elements of the design

The frequency of treatment (two times per week) is held constant, as well as the density of the releases (3,000 sterile males per ha per week; suitable dose estimated based on the local mosquito population density; a ratio between sterile to wild males in the range of 5:1 to 10:1 must be maintained along the season to see a substantial impact). This is required to keep the design simple and feasible. The frequency of data sampling (every two weeks) is consistent with the surveillance system currently in place and has been proven to be adequate for modelling.

Non-fixed, but potentially controlled elements of the design

The sterile male quality may vary over time and is expected to improve during the study. Due to the sequential nature of the design, the male quality would not interfere in the estimation of the "year of treatment effect", but rather with "calendar year" effect. This is good, as we want to best estimate the "year of treatment effect" while "calendar year" is rather a variable we want to control for. Note, however, that sterile male quality could be quantified over time (i.e. with flying, survival or mating tests) and this information may be incorporated in the analyses.

Border effects and minimal treatment size

A very important element of the design is that in each site there will be some ovitraps placed in a border area (the green sector in **Fig. 1**) outside the treated area. These ovitraps are essential to estimate how the effectiveness of the treatment drops when going further away from the treated area. In practice, we will define a polygon of the treated area, and use this to estimate the distance to treated area from the treated area for each ovitrap laying outside the treated area. We expect the effect of the treatment to drop the further away from the treated area it is. The drop of effectiveness will be estimated as a smooth function of “distance to treated area”. One may say that border effects are rather inwards effects where at the border although an ovitrap is in a treated area show less reduction than in the centre of the treated area. This is why this analysis will also be conducted inwards, meaning that “distance from border of treated area can also take negative values”. Greater negative values here imply being closer to the centre of the treated area. This analysis may also provide us with information about what is the minimal area size that can be treated. This analysis can be extended to exploratively test whether these distances to border have the same effects in different landscapes. The total area treated may have an effect on the border effects themselves. It may occur that being at 20 m of a small, treated area border is very different from being at 20 m of a large, treated area. These effects can also be assessed.

3.2 Expected Results

Strong and robust results: The proposed design allows us to estimate well the effect of “calendar year” and “year of treatment”. As mentioned above, there will be more observations for the “first year of treatment” and the estimate of the effect will be more precise and robust than for the “second” year. However, the sample size is more than enough to estimate these parameters. In addition, this design allows us to estimate the variation among year of the treatment effects. So, we may be able to say something like “The first year of treatment effect was -10% in 2025, -15% in 2026 and -8% in 2027. So, there are non-negligible differences among years”. Furthermore, we will also be able to quantify to what extent these effects vary across sites. So, we may be able to say something like “On average the first year of treatment effect is -15%, however, variability among sites is large. Some sites showed as -1% while other showed extremes of -70% in the first year”.

Feasible analyses: One may want to address the question whether the treatment effect is constant within a given year. “So, is this -15 % true from May to September?”. By extending the initial model with e.g. some smoothers, we can investigate the question whether the effect changes within a given year. These analyses are weak in the periods where there are little eggs or adults, that is, at the beginning and at the end of the season. These analyses may nevertheless be used to generate hypotheses on whether the frequency of treatment should be changed within a season. So, useful information for a potential follow-up study.

The distance to border or centroid analyses are expected to provide with insights on the spatial effectiveness of the treatment as well as with hints on the minimal size to be treated. Given the large variability within sites, it is not clear to what extend these results will be clear and robust.

Exploratory analyses: With exploratory analyses, one can ask whether the landscape has a great influence on the effectiveness of the treatment. This is particularly relevant to be able to provide targeted recommendations to municipalities. To inspect landscape effects, we will use the geographic information (e.g., land cover information). One interesting advantage of these analyses is that land cover is available for the whole Switzerland territory, meaning that municipality-level predictions could be done. These analyses are to consider rather exploratory as i) it is not clear which elements play a role, ii) at what spatial level and iii) the design is not maximised to inspect this aspect. Alternatively, we could also run a “crude” analysis where landscape is classified in a few classes (e.g., urban, peri-urban and village core). The distance to border or centroid analyses should also provide us with information on whether landscape can heavily influence border effects.

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