EVALUATION OF A *WOLBACHIA* TRANSINFECTED *AEDES ALBOPICTUS* STRAIN AS A VECTOR POPULATION SUPPRESSION AGENT

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INTRODUCTION

ARwP *Aedes albopictus* line was developed thanks to the artificial infection with a heterologous *Wolbachia* strain, resulting in a bidirectional incompatibility pattern with wild-type *Ae. albopictus*. ARwP was tested for its suitability to support intensive rearing conditions as required for mass production and field release for SIT purposes. In this study, we compared AR*w*P and wild-type *Ae. albopictus* strains, both reared under Standard Operating Procedures (SOPs), evaluating the following parameters: i) male and female pupation dynamics; ii) efficiency in mechanical sexing; iii) male mating competitiveness in comparison with laboratory irradiated and wild-type males.

MATERIALS AND METHODS

Mosquito strains: Rimini F_{53} (RN) is a strain of *Ae. albopictus* colonized in CAA starting from eggs collected by means of ovitraps in the city of Rimini (northern Italy) and reared for 61 generations under SOPs; Wild F_0 (W) is a strain originated from eggs collected in Crevalcore (Bologna, northern Italy) by means of ovitraps. ARwP F_{81} (ARwP) is a CI-inducing strain, established at ENEA-Casaccia Research Center (Rome) [1].

SOPs for larval rearing and pupal sexing: Larval rearing trays were set up starting from 2,000 first instar larvae in 1,000 ml of deionized water (larval density of 2 larvae/ml), at a temperature of 28°C, 80% RH and a photoperiod of 14:10 (L:D). Larvae were fed for four days with increasing doses (0.2, 0.4, 0.6 and 0.8 mg/larva/day respectively) of a liquid diet consisting of 50% tuna meal, 36% bovine liver powder, 14% brewer's yeast and 0.2% w:v Vitamin Mix (IAEA-BY diet [2].

Pupae were sexed mechanically when aged 24 h using a metal sieve (mesh 1400 µm) and applying a three minutes treatment at 34°C to favour ascent [3,4]. After the sieving, pupae that passed through the mesh were expected to be mostly males [2] but, in the case of experiments requiring certainty of no female contamination, pupae were manually checked and sexed under the stereomicroscope on a cold plate used for their immobilization.

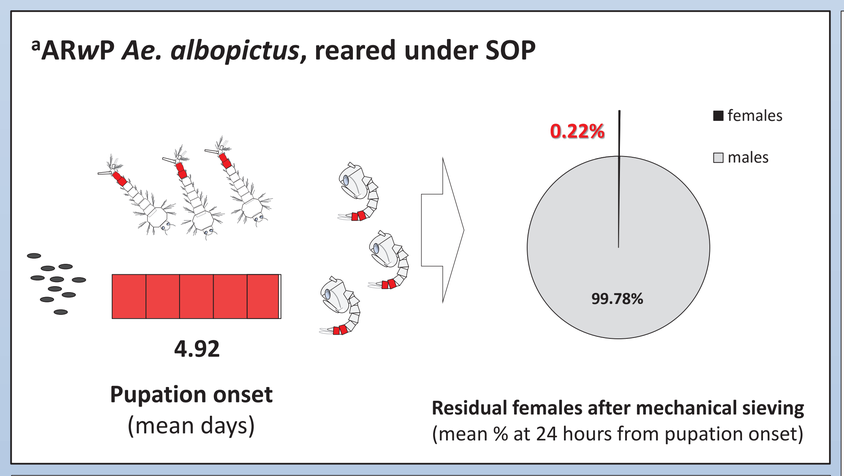
Pupation dynamics and mechanical sex separation efficiency in ARwP and RN Ae. albopictus*:* A first experiment was performed to assess the strain-specific time of pupation onset (i.e., the time needed to observe the first pupae) by checking each larval rearing tray daily (at 10:00 am, 2:00 and 6:00 pm). Pupal and male production (i.e. the number of produced pupae and males, respectively, out of the total number of L1) were measured at 24, 48, and 72 h after pupation onset by counting and sexing (under the microscope) the individuals reaching pupal stage. Four replicates (=larval trays as specified above) were performed for each treatment.
A second experiment was performed to compare ARwP and RN strains in terms of sex separation efficiency by applying a mechanical sieving treatment at 24 hours after pupation onset. The pupal production after the sieving procedure and the percentage of "residual" females out of the pupae passing through the metal sieve (expected to be males) were measured. Five replicates were set up for each treatment.

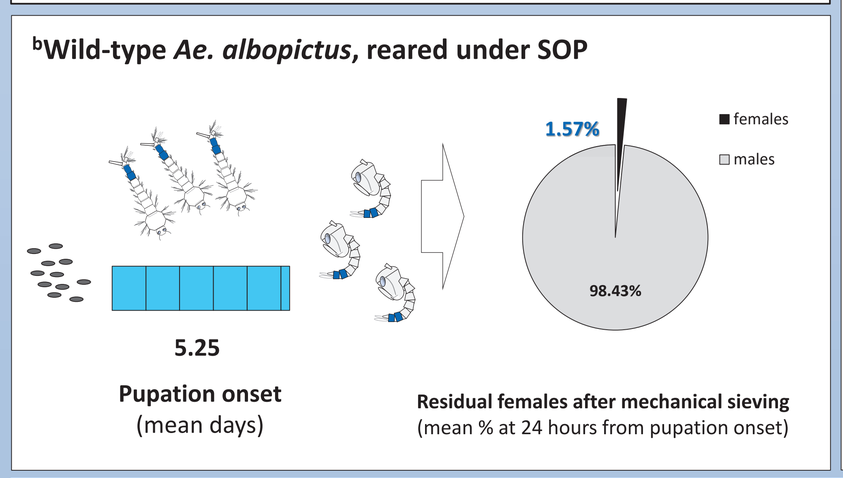
Male mating competitiveness under large enclosures: Competitiveness trials were run to compare CI-inducing (AR*w*P) and gamma ray irradiated RN males (RNi) with fertile (W) males in combinations of 100:100:100 sterile:fertile W males:virgin W females. Control enclosures were provided with 200 fertile male pupae and 100 virgin adult females. The experiments were carried out in large enclosures (8x5x2.8 m) built in a suburban, vegetated area in Crevalcore (Bologna, Italy) and constituted by a metal framework supporting an insect-proof net-screened (40 mesh) Arrigoni Biorete equipped with Arrigoni Ombraverde shading net (70% shadow).

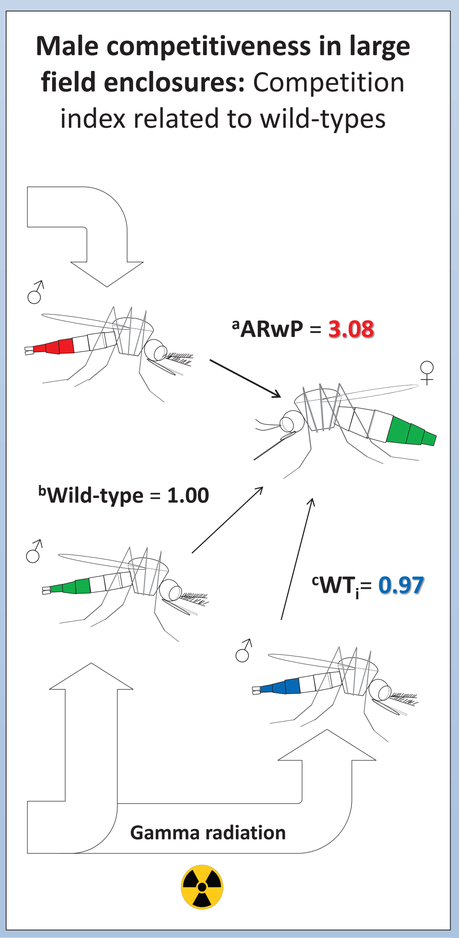
RESULTS

Pupation dynamics and mechanical sex separation efficiency: Under SOPs, AR*w*P and RN pupation onset took about 5 days. Four days and 22 hours were needed by ARwP pupae to appear in all of the rearing trays while at least 8 additional hours were necessary to observe pupae in all the RN trays.
At the tested intervals, ARwP and RN showed significantly different values of developed pupae. At 24 h after pupation onset, the pupal production of ARwP was significantly higher than that of RN ($t = 2.4905$, $df = 6$, $P = 0.04713$).
The highest male production was obtained by AR*w*P at 24 h ($t = 2.7482$, $df = 6$, p-value = 0.03337). Under conditions of mechanical sexing, ARwP and RN did not show significantly different results in terms of pupal production ($t = 0.15913$, $df = 8$, p-value = 0.8775), however a significantly lower percentage of females was found when sorting ARwP pupae (Kruskal-Wallis chi-squared = 4.9304, df = 1, p-value = 0.02639).

Male mating competitiveness: A multiple comparison test evidenced statistically significant differences between treatments ($F_{(2,12)} = 45.79$, $P < 0.001$). Interestingly, the mean egg fertility observed in enclosures treated with ARwP males was significantly lower than in enclosures with irradiated males ($F_{(1,8)} = 7.74$, $P = 0.02$). Thus, the CIS Index related to the ARwP males, was significantly higher in comparison with that computed for the RNi males ($t = 2.526$, $df = 8$, p-value = 0.036).







[a]Artificially infected (*w*Pip *Wolbachia*); [b]Naturally infected (*w*AlbA-*w*AlbB *Wolbachia*); [c]Irradiated (30 Gy) wild-type males

CONCLUSIONS

ARwP strain showed a higher production rate of male pupae in the 24 hours after the pupation onset and a lower percentage of residual contaminant females when applying mechanical sexing procedures in comparison with RN strain.

ARwP males were more efficient than wild-type irradiated males in competing for wild-type females in large enclosures, thus inducing a level of sterility significantly higher than that expected for an equal mating competitiveness.

These results encourage the use of AR*w*P *Ae. albopictus* as suppression tool against *Ae. albopictus* by using SIT approaches both exploiting Cytoplasmic Incompatibility alone or in combination with irradiation treatments aiming at sterilizing the residual females.

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