First evidence of resistance to pyrethroid insecticides in Italian *Aedes albopictus* populations after 26 years since invasion

Running title: **Pyrethroid-resistance in Italian *Aedes albopictus* populations**

Verena Pichler[1], Romeo Bellini[2], Rodolfo Veronesi[2], Daniele Arnoldi[3], Annapaola Rizzoli[3], Riccardo Paolo Lia[4], Domenico Otranto[4], Fabrizio Montarsi[5], Sara Carlin[5], Marco Ballardini[6], Elisa Antognini7, Marco Salvemini[8], Emanuele Brianti[9], Gabriella Gaglio[9], Mattia Manica[1,3], Pietro Cobre[1], Paola Serini[1], Enkelejda Velo[10], John Vontas[11,12], Ilias Kioulos [11], Joao Pinto[13], Alessandra della Torre[1], Beniamino Caputo[1§]

1 Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
2 Department Medical and Veterinary Entomology, Centro Agricoltura Ambiente "G.Nicoli", Crevalcore, Italy
3 Fondazione Edmund Mach, San Michele all'Adige (TN), Italy.
4 Department of Veterinary Medicine, University of Bari, Valenzano (BA), Italy.
5 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy.
6 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy
7 Istituto Zooprofilattico Sperimentale Umbria e Marche, Ancona, Italy
8 Department of Biology, University of Naples Federico II, Naples, Italy
9 Dipartimento di Scienze Veterinarie, Polo Universitario dell'Annunziata, Messina, Italy
10 Institute of Public Health, Tirana, Albania
11 Agricultural University of Athens, 11855, Greece
12.Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 70013, Greece
13 Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.

ABSTRACT

BACKGROUND. *Aedes albopictus* has spread during the last decades all over the world. This has increased significantly the risk of exotic arbovirus transmission (e.g. Chikungunya, Dengue, and Zika) also in temperate areas, as testified by the Chikungunya 2007- and 2017-outbreaks in north-east and central Italy. Insecticides represent a main tool for limiting the circulation of these mosquito-borne viruses. The aim of the present study is to start filling the current gap of knowledge on pyrethroid insecticide resistance of European *Ae. albopictus* populations focusing on populations from Italy, Albania and Greece.

RESULTS. Bioassays for resistance to permethrin (0.75%), α-cypermethrin (0.05%) or deltamethrin (0.05%) were performed according to WHO protocols and showed reduced susceptibility (<90% mortality) of some Italian populations to permethrin and α-cypermethrin, but not to deltamethrin.

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CONCLUSION. This study reports the first evidence of resistance to pyrethroids in adult Italian *Ae. albopictus* populations. Results refer to the season preceding the Chikungunya 2017-outbreak in central Italy and highlight the need to increase efforts to monitor insecticide resistance spreading and the need to develop strategies limiting the spread of insecticide resistance, particularly in areas where extensive treatments have been carried out to contain disease outbreaks.

Keywords: insecticide resistance, *Aedes albopictus*, pyrethroids, Chikungunya vector, vector control

1. INTRODUCTION

More than half of human population is at risk of contracting *Aedes* borne-viruses (e.g Dengue, yellow fever, Chikungunya and Zika virus), which cause thousands of deaths/year and potentially millions of debilitating and economically damaging illnesses (1). Although these arboviral diseases are mostly endemic in the tropics, outbreaks have already occurred in temperate regions in the past, mainly mediated by *Aedes aegypti*, a major tropical vector species, which was reported repeatedly in Mediterranean countries before 1950 and only sporadically afterwards (e.g. in northern coast of the Black Sea since 2008; (2,3).

The risk for exotic arbovirus transmission in Europe, however, has significantly increased during the last decades due to the rapid spread of the Asian tiger mosquito *Aedes albopictus* (4–6). This species is classified as one of the 100 most invasive species (Global Invasive Species Database, http://www.issg.org/database/) and, during the last 40 years, has spread from its native range in Southeast Asia all over the world including temperate regions, thanks to the production of cold-hardy and long-lived eggs, as well as to the capacity to exploit anthropogenic water containers (e.g. tires, flowerpot saucer, and water storage containers) as breeding sites (7,8). Even though *Ae. albopictus* is a less efficient vector for most arboviruses than the more anthropophilic *Ae. aegypti*, in 2005-06 it has been responsible for large Chikungunya epidemics in Indian Ocean islands and has also been recognized as competent vector for Dengue and several other arboviruses, including Zika (9,10). In Europe autochthonous cases of Dengue, vectored by *Ae. albopictus* have been recorded in France (11) and in Croatia (12) during the last decade. Moreover two autochthonous Chikungunya outbreaks occurred in Italy (in 2007 in north-east Italy (13,14) and in in 2017 in central-Italy (15)). In addition , due to its opportunistic feeding behaviour (16,17) *Ae. albopictus* has the potential to act as ‘‘bridge vector’’ of zoonotic pathogens (e.g. canine dirofilariosis by *Dirofilaria* spp.) to humans (18–20).

In Europe, *Ae. albopictus* was first reported in 1979 in Albania (21), in 1990 in Italy (22) and nowadays it has established in 12 countries. In Italy, where in the last 30 years urban and peri-urban areas have been widely colonized by *Ae. albopictus* (22,23), national guidelines (24), in agreement with ECDC ones (4) recommend to prioritize larval over adult control, because of the higher expected impact and the lower environmental costs of larvicidal interventions. Adulticide interventions instead, are recommended only when infected human travellers coming from endemic countries are detected, in order to prevent autochthonous disease transmission, or in the case of extremely intense nuisance. Nevertheless, private citizens and some public administrations implement adulticidal control measures in order to achieve immediate and tangible, even though short-termed, effects on mosquito nuisance (25–27).

Pyrethroids are the only chemicals allowed for mosquito adulticiding in Europe (28–30). The most commonly used pyrethroids are α-cypermethrin, permethrin and deltamethrin, sometimes in combination with the synergist piperonyl butoxide (PBO). These compounds are also extensively used to control adult abundance of major tropical vector species (as *Ae. aegypti, Culex quinquefasciatus* and *Anopheles* vectors of malaria) and are the only ones recommended by the World Health Organisation for the treatments of bed-nets. In regions where these species represent major public health problems and are thus target of extensive control activities, the high selective pressure exerted by the pyrethroid-based interventions in public health and/or agriculture has led to increasing levels of insecticide resistance (IR) with the risk to reduce efficacy of these major vector control tools (31,32). To prevent this, WHO has drafted guidelines to monitor IR in major vector species and to avoid its insurgence and spread (33–35).

In contrast with the extensive knowledge on IR in major tropical mosquito vector species, knowledge on IR in *Ae. albopictus* is still fragmented, as pointed out by Moyes et al. (32) and Vontas et al (36). Available data documenting IR is highly clustered, making comparisons difficult, since different methods were used to generate results. So far, resistance to pyrethroids has been reported in the last years in adult populations from South-East Asia, the native range of *Ae. albopictus*, (37–40), as well as from the Indian subcontinent (41–43) and Africa (44,45). Almost no reports came instead from temperate areas, with the exception of those from Richards et al. (46), who recorded reduced susceptibility to permethrin in the USA, and Bengoa et al. (47) who revealed first signs of resistance of Spanish *Ae. albopictus* populations to cypermethrin and possible resistance to deltamethrin and permethrin. In Italy, no resistance to pyrethroids was found in early 2000 in adult *Ae. albopictus* populations from Rome and other sites across the country (48). Later on, in 2009, also Vontas et al. (36) observed full susceptibility in one population from Rome (Italy) and one from Athens (Greece).

The need of a better understanding of IR in invasive mosquito species and of coordinated strategies for early detection and management of IR was recognized during the first International Workshop on "Insecticide resistance in vectors of emerging arboviruses: Challenge and prospects for vector control" (Rio de Janeiro, Brazil, December 2016) organized by the Worldwide Insecticide Resistance Network (32,49). Herein, we report the first evidence of resistance to permethrin and α-cypermethrin in adult *Ae. albopictus* populations primarily from Italy, which should serve as a warning for all Europe and encourage further efforts in monitoring this phenomenon.

2. MATERIALS & METHODS

2.1.Mosquito collections and rearing

Ovitrap collections of *Ae. albopictus* eggs were carried out by local entomology teams from May to October 2016 (except for the untreated population from Rome, RM-NT, which has been sampled and tested in September 2015) in 16 sites across Italy, as well as in two sites from Albania and one from Greece (Table S1, Figure 1). Collections at each sampling site were conducted with ≥ 5 ovitraps to avoid oversampling of siblings, and, whenever possible, in a site where adulticide treatments using pyrethroids were known to have been performed during the sampling season (labelled with TR in site acronyms), as well as in a second untreated site in the same area (labelled with NT) (Table S1). Besides field-collected populations, also a lab-colony from Athens, Greece, selected for resistance to temephos, was included in the study to evaluate a possible cross-resistance between organophosphates and pyrethroids. The carboxyl esterase amplifications found in this colony enhances resistance to temephos (50) and has been detected also in field-collected specimens from Greece, coherent with reduced susceptibility to this organophosphate detected in Greek populations in previous studies (36). Egg samples sealed in plastic bags were sent by express courier to the Department of Public Health and Infectious Diseases (DPHID) at Sapienza University of Rome.

Larvae were reared at larval density of 0.05 larvae/ml in the insectary of DPHID at T=26 ±1 °C, RH=60 ± 5% and at 14:10h light:dark photoperiod and fed with artificial dry cat-food. Pupae were collected daily and transferred into 40 cm-cubic cages. Emerged adults were identified as *Ae.*

albopictus using morphological keys (51) and kept at the same temperature and humidity as larvae until used for the bioassays. When samples from field collected eggs were not sufficient to complete the experiments, adults were blood-fed and the progeny (F1) was used for bioassays (Table 1).

2.2. Insecticide susceptibility bioassays

Bioassays were performed according to WHO protocols (33,34) in WHO test tubes lined with filter papers impregnated with one of the following insecticides: Permethrin (0.75%), α-cypermethrin (0.05%) or deltamethrin (0.05%) (Vector Control Research Unit, School of Biological Sciences, 11800 Minden, Penang, Malaysia). Insecticide concentrations were selected based on the dosages most frequently used for *Ae. albopictus* in order to allow comparison of results with previous studies (40–43,45,52,53). The 0.05% concentration for deltamethrin was chosen based on data available on a candidate *Ae. albopictus* susceptible reference strain (54). Insecticide impregnated (and control) papers were discarded after being used in 6 bioassays.

Bioassays were performed in the insectary at the same conditions of mosquito rearing (see above) by using ~25 unfed *Ae. albopictus* females (3 to 5-day old), either directly emerged from field collected eggs/larvae (F0), or from their progenies (F1) (Table 1). Mosquitoes were exposed to insecticides for 1 hour and the number of knocked down mosquitoes (i.e. mosquitoes unable to stand or fly in a coordinated way; (34)) was recorded every 10 minutes during exposure time; after 1 hour of exposure, the mosquitoes were transferred into tubes with untreated papers and allowed a 24 h recovery period after which mortality was recorded. Depending on mosquito availability, 3-4 replicates/population/insecticide were performed and for each population/insecticide also a control tube (i.e. lined with filter papers impregnated only with the insecticide excipient but without the active ingredient) was set up and manipulated as the test tubes.

Mean values of mortality were computed for each population. When mortality in control cages exceeded 5%, Abbott's correction for natural mortality was applied. According to WHO guidelines (34) populations were considered "susceptible" if mortality at 24 hours after exposure was ≥ 98%, "possibly resistant" if mortality ranged between 90% and 97% and "resistant" if mortality was ≤ 90%.

For knock-down assessment, a log time-probit statistical model was applied to compute KD-curves for each population and to calculate 50% (KDT50) and 95% (KDT95) knockdown times. A binomial Generalized Linear Model (GLM) was carried out to test the effect of insecticide control activities on mosquitoes and to evaluate if there is any significant difference between KD-curves of populations from treated and untreated sites. Pearson's correlation coefficient was computed to evaluate correlation between KDT values and percentage mortality. All analysis were carried out using R software version 3.3.3 (55). The R-script used for computation can be provided by the authors upon request.

3. RESULTS

Susceptibility to permethrin, α-cypermethrin and deltamethrin as well as KDTs were assessed in 20, 14 and 10 *Ae. albopictus* populations respectively (Figures 1 and 2, Table 1). Mortality in control tubes was always <5%, except for the permethrin bioassay of the Greek field-population from Athens (mortality = 8%), for which Abbott-corrected values are reported. No knock-down was observed in control tubes during the one-hour exposure to insecticides.

3.1. Permethrin. Bioassays suggested resistance to permethrin only in the treated populations (i.e. populations for which insecticide applications have been reported during the sampling season) from Ferrara province in Emilia-Romagna (mortality: FE-TR1 =81.3%, FE-TR2 =68.9%) and from Bari province in Puglia (BA-TR mortality=89.6%), while the field-population from Athens (Greece) appeared to be possibly resistant (GR-NT, mortality= 93.5). Correlation between KDT50, KDT95 and percentage mortality was significant ($r_{KDT50/mortality}$ -0.71; $r_{KDT95/mortality}$ = -0.85) with populations from Ferrara and Bari province showing the highest KDT50 and KDT95 values. A large variability of KDT50 and KDT95 values was observed across Italy (KDT50: 13'-43'; KDT95: 23'-154'; see Figure 2), with significantly higher values in populations from treated sites in Veneto and Puglia, when compared to populations from neighboring untreated sites ($p<0.05$; Figure S1).

3.2. α-cypermethrin. resistance to α-cypermethrin was suggested for the treated populations from Ferrara province (FE-TR1, mortality = 64.8%) Venezia province (VE-TR, mortality = 85.3%) and Rome (RM-TR1, mortality = 89.2%). These populations showed also the highest KDT50 and KDT95 values.

Results, suggestive of possible resistance were obtained for several other tested populations, (see Figure 1), while full susceptibility was observed only for 4 Italian populations (mortality: TN-NT1=98.7%; TN-NT2 =100%; AN-NT=100%; RM-TR2=100%), and one population from Vlore-county in Albania (AL-TR, mortality =98.6%). Correlation between KDT50, KDT95 and percentage mortality was significant ($r_{KDT50/mortality}$ -0.96; $r_{KDT95/mortality}$ = -0.96) and large variability for knockdown times was observed across Italy (KDT50: 22'-62'; KDT95: 40'-186'). Anyway, no significant differences were detected among populations in neighboring treated vs untreated sites.

3.3. Deltamethrin. All the 8 Italian populations tested, as well as the Albanian one, were fully susceptible to deltamethrin while resistance was observed only in the Greek laboratory colony (mortality = 89.0%). KDT50 and KDT95 were highest in RM-TR1, but no significant differences were observed among treated and untreated sites and no strong correlation between KDT values and mortality was detected ($r_{KDT50/mortality}$ -0.62; $r_{KDT95/mortality}$ = -0.62).

4. DISCUSSION

We report the first evidence of resistance to permethrin and α-cypermethrin in adult *Ae. albopictus* populations from Italy. The lowest mortality rates (<70%) were detected in populations from two sites along the Adriatic coast in Comacchio area (Emilia-Romagna region, North-East-Italy). No detailed data on adulticide usage in Italy are available, but it is relevant to note that the two sites are highly touristic and insecticide spraying is extensively conducted since 1991 during the summer seasons to reduce nuisance mostly due to *Aedes caspius* and *Culex pipiens* (56). In fact, preliminary results on sympatric *Cx. pipiens* showed mortality rates <20% after exposure to 0.75% permethrin (FE-TR2, data not shown), confirming that mosquito populations in that area are likely to be exposed to high selective pressure by adulticides. It would be interesting to test the susceptibility to pyrethroids of *Ae. albopictus* populations collected in neighbouring localities where no or scattered adulticide treatments are conducted.

Mortality rates suggestive for resistance (<90%) were obtained also for populations from Puglia (BA-TR) when exposed to permethrin, and Veneto (VE-TR) and Lazio (RM-TR1) when exposed to α-cypermethrin. Four additional populations from Italy (from treated as well as untreated sites) showed

mortality rates indicative of possible resistance to α-cypermethrin (mortality <98%). Further tests on larger sample sizes are needed to confirm these preliminary results.

Evidence of lower susceptibility to both pyrethroids is also provided from the significant increase in the time to knockdown observed in some populations. The large variability observed across Italy for KDT50 and KDT95 values likely reflects differential adulticide usage. Nevertheless, both values showed a good correlation with mortality and confirm the presence of some populations with strongly reduced susceptibility to permethrin and α-cypermethrin. In the case of permethrin, also significant differences between treated and untreated sites were found: Populations collected in treated sites in Veneto and Puglia showed higher KDT50 and KDT95 values than populations collected in the same region in neighboring but untreated sites, suggesting that adulticide spraying carried out at high frequency during the whole season in these sites lowered the species' susceptibility. This appeared not to be the case in Lazio and Sicilia possibly due to less effective or more recent adulticide treatments.

Differently from what observed for permethrin and α-cypermethrin, all Italian populations were susceptible to deltamethrin. Similar results were obtained in Greece (36), Spain (47) and the US (54). This result is consistent with the hypothesis of a lower usage of this insecticide in Italy but could also indicate that the deltamethrin dosage used was inappropriate (too high) for *Ae. albopictus*.

Mechanisms producing the permethrin/α-cypermethrin resistance phenotype in Italian populations will be evaluated in future studies. While target-site-resistance mechanisms, which typically induce cross-resistance between pyrethroids (57–60), are widespread and well-known in anophelines (31), far less information is available for *Ae. albopictus*. Several target site mutations have been identified in this species but their association with IR is still unclear (32,60) and appears to be less strong compared to other mosquito species. Also, the lack of cross-resistance to different pyrethroids in the Comacchio population suggests that multiple/other resistance mechanisms, possibly including detoxification pathways (61,62) may be involved.

Aedes albopictus populations from Albania were found fully susceptible to all pyrethroids tested, with relatively low KDTs, despite being sampled in insecticide treated sites. On the other hand, the field population from Athens (which was shown to be susceptible to deltamethrin in 2009, (36)) did not show full susceptibility to permethrin and exhibited KDT95 values higher than all other tested populations, except those from Comacchio. Surprisingly, however, no public pyrethroid space-spraying has been carried out in Athens since 2007, although a selective pressure by intensive treatments performed by private citizens cannot be excluded. The lower susceptibility of the field-collected population from Greece to permethrin could be explained by a different origin of the Greek population compared to the Italian and Albanian ones, as supported by population genetic data (63,64), but also by cross-resistance between organophosphates and pyrethroids, as already reported for other mosquito species (65,66). In fact the same amplified carboxyl esterase genes (CCEs) responsible for the temephos-resistance of the laboratory colony have been observed, as explained above, also in Greek field-populations (50) and could be associated with a reduced susceptibility to permethrin which can be hydrolysed by CCEs as shown in other insect species (67).

Data herein presented need to be interpreted with caution considering some limitations inherent to the study design and sampling efforts. First, WHO provides specific diagnostic dosages based on data available only for *Ae. aegypti*, *Cx. quinquefasciatus* and anopheline mosquitoes. The dosages used in this study were higher than those recently recommended as tentative for *Aedes* mosquitoes (68), and this choice was made in order to obtain comparable results with previous studies (see Materials and

Methods). This implies that our results certainly do not overestimate resistance levels, but may underestimate them. Further studies on a susceptible reference colony are needed to more precisely assess diagnostic dosages, the lack of which strongly limits the possibility to compare and interpret results across studies (36). It should also be noted that, although the use of a reference colony is also recommended to ensure the quality of insecticide impregnated filter papers, the presence in our experiments of field populations showing 100% mortality to all the 3 insecticides confirms the effectiveness of the WHO filter papers used. Second, we chose to perform bioassays with F0 females or, when not possible, F1 progenies, in order to avoid loss of selective pressure and inbreeding under laboratory conditions. This choice, however, implied that in some cases we did not have the possibility to have a minimum of 100 females tested per insecticide, as recommended by WHO (34) to confirm resistance. Third, the classification of "treated site" in the study is heterogeneous as it reflects different mosquito control activities carried out in Italy, Albania and Greece, including differences in the used pyrethroid compounds, dosages, spraying methods, protocols and time-schedules as well as different histories of pyrethroid applications in agriculture. Nevertheless, it is notable that only the populations from Trentino subjected to occasional adulticide spraying (Rizzoli A.P., personal communication), together with populations from Marche (Ancona province; AN-NT), showed complete susceptibility to all the tested insecticides, while highest resistance was observed in Comacchio sites, where very intensive control activities following a well-defined monitoring plan have been implemented even before the *Ae. albopictus* invasion to reduce nuisance due to *Ae. caspius* (a very aggressive autochthonous species). In most other sites adulticide treatments were adopted only after the colonization of the areas by invasive *Ae. albopictus*.

Overall, this first assessment of resistance to permethrin and α-cypermethrin in adult *Ae. albopictus* populations from Italy represents a first step to fill a gap of knowledge on resistance to pyrethroids in invasive populations now fully established in Europe, where the species is becoming an increasing health threat. The results show that resistance to the most commonly used pyrethroids (i.e. permethrin and α-cypermethrin in Italy) is arising in areas where the species has been well established for several years, reaches high densities and has been cause of high nuisance. Coupled with possible resistance observed recently in Spain (47) and the high levels of resistance found in the only west European *Ae. aegypti* population from Madeira island (69), the results should serve as a warning for Europe and encourage further efforts in monitoring this phenomenon and in standardizing protocols for IR detection and guidelines for IR management in temperate areas. Studies of this typology are in fact highly needed to support local public health authorities in managing and planning effective control measures and to maintain insecticide-based vector control options effective. The large Chikungunya outbreak (15) that occurred in central Italy in summer 2017 clearly highlights the urgency of more extensive studies to better understand and monitor the spread of resistance phenotypes with a larger spatial and temporal coverage particularly in areas at risk of autochthonous arbovirus transmission (32,70–72), as well as the implementation of synergic and coordinated actions aimed at controlling the mosquito population abundance at the larval stage .

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TABLES AND FIGURES

Table 1 – Results of WHO tube bioassays performed on *Aedes albopictus* populations from Italy, Albania and Greece. Generation and number of female mosquitoes tested for pyrethroid resistance (i.e. permethrin 0.75%, α-cypermethrin 0.05% and deltamethrin 0.05%) are reported, as well as mortality (%) at 24 hours after 1h exposure and times to knock-down (KDT) of 50% and 95% of population (95% confidence intervals). Sites for which adulticide treatments have been reported during the sampling season are labelled with –TR. Sites in which adulticide treatments were not carried out during the sampling season are labelled with –NT. GR-LAB= laboratory-selected temephos resistant colony. Results indicating resistance or possible resistance according to WHO (34,68) are highlighted in bold.

	region/country	site-code	treatment	tested generation	N tested	Mortality % (95% CI)	KDT50 (95% CI)	KDT95 (95% CI)
0.75% Permethrin	Trentino	TN-NT1	N	F0	74	100	12.9 (11.9- 14.0)	26.5 (23.9 - 31.8)
		TN-NT2	Y	F0	82	100	27.9 (26.3 - 29.5)	55.1 (50.7 - 62.4)
	Veneto	PD-NT	N	F0	74	100	15.5 (14.7- 16.3)	22.7 (21.2 – 26.4)
		VE-TR	Y	F0	108	99.0	22.1 (21.1- 23.2)	38.7 (36.1 - 42.8)
	Emilia Romagna	FE-TR1	Y	F0	74	**81.3**	42.8 (38.4- 47.6)	154.2 (121.8 – 260.9)
		FE-TR2	Y	F0	75	**68.9**	36.4 (33.2 - 39.9)	119.2 (98.2 – 172.1)
	Liguria	IM-NT	N	F1	100	99.0	23.5 (22.2- 24.8)	47.1 (43.5 - 52.9)
	Marche	AN-NT	N	F0	75	100	19.5 (18.4- 20.7)	33.8 (31.2 - 39)
	Lazio	RM-NT	N	F0	122	100	21.1 (20.1 - 22.1)	39.3 (36.7 - 43.3)
		RM-TR1	Y	F0	96	99.0	25.2 (23.9- 26.6)	48.9 (45.4 – 54.6)
		RM-TR2	Y	F1	77	100	21.5 (20.4-22.7)	35.5 (33 -40.6)
	Campania	NA-TR	N	F0	99	100	18.6 (17.9- 19.4)	26.4 (24.9 – 30.1)
	Puglia	BA-NT	N	F0	75	100	23.1 (21.9- 24.3)	36.6 (34.1– 41.6)
		BA-TR	Y	F1	77	**89.6**	31.3 (29.4- 33.3)	66.7 (60.4 - 78.3)
	Sicily	ME-NT	N	F0	50	100	18.6 (17.5- 19.8)	29 (26.5 – 37.1)
		ME-TR	Y	F0	75	100	18.5 (17.5- 19.6)	30.9 (28.5 – 35.8)
	ALBANIA	AL-TR	Y	F0	77	100	22 (20.9- 23.1)	33.5 (31.3– 38.3)
		AL-NT	Y	F1	74	100	21.3 (20.2- 22.4)	33.6 (31.2– 38.5)
	GREECE	GR-NT	N	F1	100	**93.5**	43.8 (41.2 - 46.5)	95.9 (85 -119)
		GR-LAB	lab	F1	100	100	27.9 (26.5 - 29.5)	57.1 (52.7 - 64.4)
0.05% α - Cypermethrin	Trentino	TN-NT1	N	F1	78	98.7	25 (23.7-26.3)	39.9 (37.3-44.8)
		TN-NT2	Y	F1	90	100	22.2 (20.9 - 23.7)	53.5 (48.5– 61.4)
	Veneto	PD-NT	N	NA	NA	NA	NA	NA
		VE-TR	Y	F0	75	**85.3**	40 (37.7- 42.3)	76.2 (68.9 – 91.5)
	Emilia Romagna	FE-TR1	Y	F1	73	**64.8**	62.3 (54.2-71.6)	186.3 (142.7 - NA)
		FE-TR2	Y	NA	NA	NA	NA	NA
	Liguria	IM-NT	N	F1	100	**95.0**	23.4 (21.8-25.2)	69 (60.9 - 82.7)
	Marche	AN-NT	N	F0	75	100	28.5 (26.9- 30.2)	53.6 (49.3 - 61)
	Lazio	RM-NT	N	NA	NA	NA	NA	NA
		RM-TR1	Y	F1	74	**89.2**	39.1 (36.5 - 41.9)	89.8 (78.7 – 114.3)
		RM-TR2	Y	F1	78	100	26.6 (25.1-28.2)	51.2 (47.1 - 58.3)
	Campania	NA-TR	N	NA	NA	NA	NA	NA
	Puglia	BA-NT	N	F0	76	**96.1**	31.2(29.6- 32.8)	50.9 (47.5 – 56.8)
		BA-TR	Y	NA	NA	NA	NA	NA
	Sicily	ME-NT	N	F1	76	**96.7**	32.3 (30.6-34.1)	57.5 (53.2 - 65.1)
		ME-TR	Y	F1	75	**94.7**	33.7 (31.8-35.7)	64.9 (59.4 - 75)
	ALBANIA	AL-TR	Y	F0	72	98.6	26.1 (24.7 - 27.6)	45.5 (42.2 – 51.3)
		AL-NT	Y	F1	75	**97.3**	30.3 (28.2 - 32.5)	73.6 (65.3 – 89.4)
	GREECE	GR-NT	N	NA	NA	NA	NA	NA
		GR-LAB	lab	F1	100	**93.0**	32.7 (30.8 - 34.7)	76.3 (68.8 - 89.5)
0.05% Deltamethrin	Trentino	TN-NT1	N	F1	78	100	15.7 (14.7 - 16.8)	30.7 (27.9 - 35.9)
		TN-NT2	Y	F1	75	100	18.3 (17.3 - 19.4)	30.3 (27.9 – 35.1)
	Veneto	PD-NT	N	NA	NA	NA	NA	NA
		VE-TR	Y	F1	77	98.7	18.3 (17.1 -19.6)	39 (35.4 - 45.4)
	Emilia Romagna	FE-TR1	Y	F1	78	100	20.2 (19 - 21.5)	39 (35.7 - 44.8)
		FE-TR2	Y	NA	NA	NA	NA	NA
	Liguria	IM-NT	N	F1	100	98.0	20.4 (19.3 - 21.6)	39.9 (36.8 - 44.8)
	Marche	AN-NT	N	F1	77	98.7	19.2 (18.1-20.4)	35.8 (32.9 - 41.3)
	Lazio	RM-NT	N	NA	NA	NA	NA	NA
		RM-TR1	Y	F1	74	100	25 (23.5 - 26.5)	46.4 (42.8 - 52.9)
		RM-TR2	Y	NA	NA	NA	NA	NA
	Campania	NA-TR	N	NA	NA	NA	NA	NA
	Puglia	BA-NT	N	F0	77	100	17.8 (16.7 - 18.9)	32.5 (29.8 - 37.7)
		BA-TR	Y	NA	NA	NA	NA	NA
	Sicily	ME-NT	N	NA	NA	NA	NA	NA
		ME-TR	Y	NA	NA	NA	NA	NA
	ALBANIA	AL-TR	Y	NA	NA	NA	NA	NA
		AL-NT	Y	F1	78	100	20.4 (19.2 - 21.6)	36.1 (33.3 - 41.3)
	GREECE	GR-NT	N	NA	NA	NA	NA	NA
		GR-LAB	lab	F1	100	**89 .0**	25.8 (24.5 - 27.1)	47.8 (44.5 - 53.1)

Figure 1 – Distribution of *Aedes albopictus* tested populations and mortality (%) after 1h exposure to pyrethroids. Permethrin 0.75%: blu; α-cypermethrin 0.05%: red; deltamethrin 0.05%: green. Red vertical lines indicate 90% and 98% mortality thresholds (34,68). Sites for which adulticide treatments have been reported during the sampling season are labelled with –TR. Sites in which adulticide treatments were not carried out during the sampling season are labelled with –NT. GR-LAB= laboratory-selected temephos resistant colony.

Figure 2 - Knock down times and 95% confidence intervals of 50% (KDT50, blu) and 95% (KDT95, yellow) of *Aedes albopictus* exposed to pyrethroids (permethrin 0.75%, α-cypermethrin 0.05% and deltamethrin 0.05%). Sites for which adulticide treatments have been reported during the sampling season are labelled with –TR. Sites in which adulticide treatments were not carried out during the sampling season are labelled with –NT. GR-LAB= laboratory-selected temephos resistant colony.

SUPPLEMENTARY MATERIAL

Table S1 - *Aedes albopictus* sampling sites and performed insecticide resistance bioassays. P=0.75% permethrin; C=0.05% α-cypermethrin; D=0.05% deltamethrin. Sites for which adulticide treatments have been reported during the sampling season are labelled with –TR. Sites in which adulticide treatment were not carried out during the sampling season are labelled with –NT. GR-LAB= laboratory-selected temephos resistant colony.

Figure S1- Comparison of knock-down curves obtained for *Aedes albopictus* exposed to permethrin 0.75%. Dotted lines show 95% confidence intervals, dots observed data.

a) Populations from Veneto; Blue= VE-TR; Black= PD-NT
b) Populations from Puglia; Blue= BA-TR; Black= BA-NT

Below the graphs the summary statistics obtained for a binomial Generalized Linear Model (GLM) carried out to test the effect of insecticide control activities on mosquitoes. The interaction term (LogTime*treated) allows to test if the relationship between the proportion of dead mosquitoes and logtime is statistically different between treatment statuses.

Figure 1

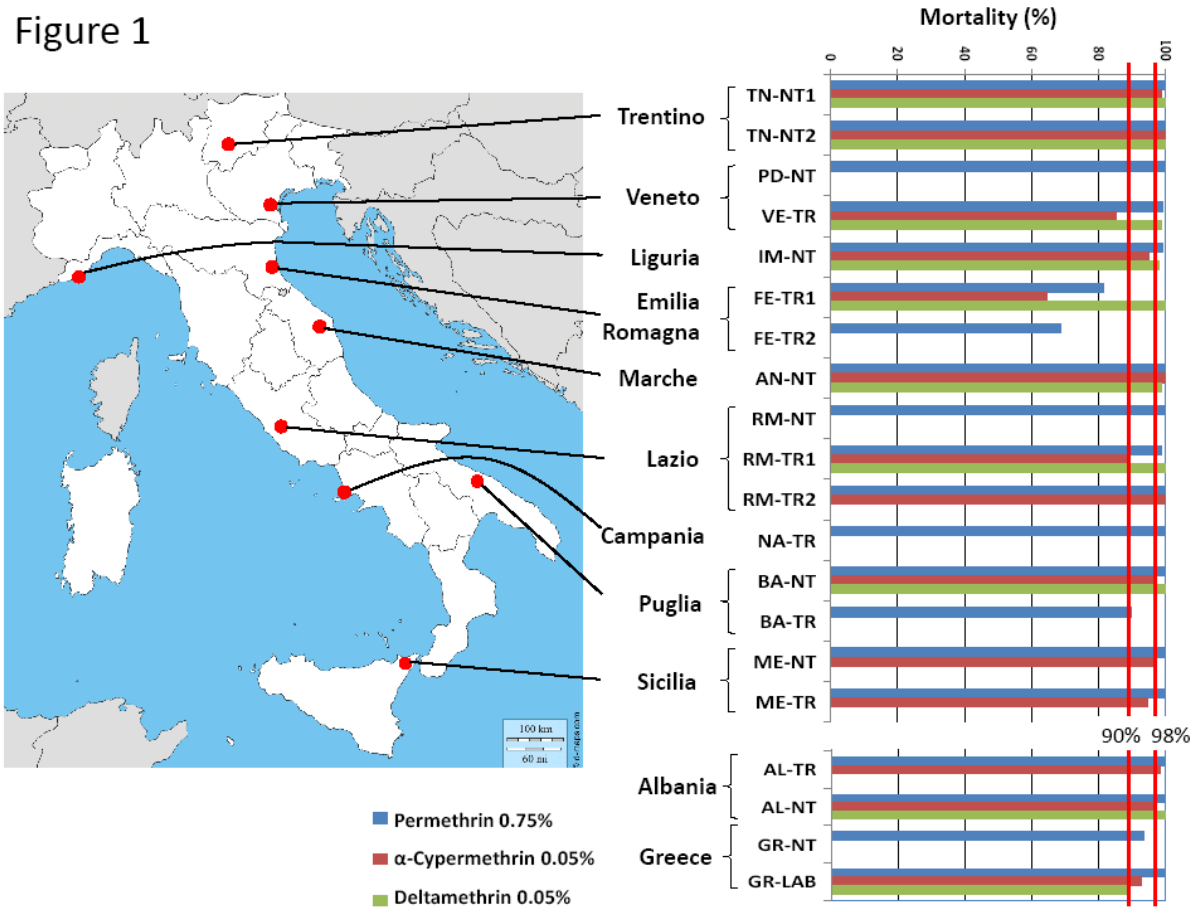

Figure 2