

TWO-YEAR POPULATION SURVEY OF MOSQUITOES (DIPTERA: CULICIDAE) IN MILAN, ITALY

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Abstract Surveys of mosquitoes were carried out in the area of Milan in 2006 and in 2007. Nineteen species were collected. The most important were: *Culex pipiens*, *C. modestus* and *C. hortensis*, two *Ochlerotatus caspius* and *O. detritus* and three *Aedes vexans*, *A. geniculatus* and *A. cinereus*. *Stegomyia albopictus* is very harmful from June to the first week of October. The dynamic of the populations of mosquitoes, the larvicide and adulticide treatments are referred, with some considerations on the possibility of control of these pests.

Key Words Mosquito management, *Culex*, *Ochlerotatus*, *Stegomyia albopictus*

INTRODUCTION

For many years, the issue of the mosquitoes control has been managed in different ways in Milan. In 2005, the Municipality entrusted the treatments two companies of pest control, through a three-year-contract (2005-2007). Another company was responsible for controlling the rice-fields surrounding the town with aerial treatments (in this case the contract had a length of a single year). In 2006, the Institute of Agricultural Entomology of Milan was asked to give a scientific and technical collaboration for the control against these pests.

The different interventions managed by the Municipality are referred to about 30% of the total area of territory (182 km²), since 70% of territory is owned by private properties or other Administrations (Urban Transport Company, Hospitals, State Railways); for these areas, the Municipality can only issue instructions, that every Organization has to follow as guidelines. The program of technical and scientific assistance had different goals, in particular: 1) detecting species; 2) their monitoring with CO₂ traps; 3) supporting larvicide treatments in rice-fields; 4) supporting adulticide treatments in the city; testing the efficacy of larvicide treatments in the manholes; 5) monitoring oviposition of *Stegomyia (Aedes) albopictus*; 6) evaluating the possibility of use of GPS on pick-up used for treatments with insecticides; 7) giving information to people.

MATERIAL AND METHODS

Different methods were used, because the problem concerning the control of mosquitoes includes several aspects. In particular: 1) monitoring of the species, 9 different environments, with carbon dioxide traps, once a week, from June to September in the year 2006 (14 samplings) and from May to September in the year 2007 (20 samplings); 2) treatments in rice-fields with helicopter, using Bti; 3) monitoring of *St. albopictus* with 72 black jar oviposition traps, placed in 9 points of the city, checked once a week; 4) adulticide treatments: with pyrethroids; 5) larvicide treatments with pyriproxyfen or temephos (in case of polluted water) in the year 2006; with pyriproxyfen alternated with diflubenzuron in the year 2007; 6) monitoring the efficacy of the treatments in the manholes using sticky traps before and after treatments, on different kinds of manholes; 7) information for citizens with a manifesto, 8 posters and leaflets.

RESULTS AND DISCUSSION

Monitoring of Species

In 2006, 12 different species were detected, in particular: *Culex pipiens pipiens* L., *Culex modestus* Ficalbi, *Culex hortensis* Ficalbi, *Culex territans* Walker, *Aedes vexans* (Meigen), *Aedes cinereus* Meigen, *Stegomyia (Aedes) albopictus* (Skuse), *Ochlerotatus caspius* (Pallas), *Ochlerotatus detritus* s.l., *Ochlerotatus geniculatus* (Olivier), *Anopheles claviger* (Meigen), *Anopheles maculipennis* s.l.. In addition three other species were found in 2007: *Culex torrentium* Martini, *Culiseta subochrea* (Edwards) and *Culiseta longiareolata* (Macquart). The classification of adults has been done according to Becker et al. (2003); the classification of the larvae according to Romi et al. (1997).

In the area of Milan *Culex pipiens pipiens*, *Ochlerotatus caspius*, *Ochl. detritus* and *Stegomyia albopictus* resulted the most important mosquitoes. *C. pipiens* lives in micro water-depot, also temporary, and in manholes, while *Ochl. caspius* and *Ochl. detritus* are very spread in rice-fields surrounding the city, although they can be located also in water-depot, temporary or permanent, inside the city. *Stegomyia albopictus* finds favourable conditions in the urban areas of Italy; the spread of the Asian tiger mosquito in Milan has been facilitated by the presence of a wide variety of artificial water containers, in particular manholes, tires for recycling and pots for flowers in the cemeteries. *C. pipiens* is active from April to September; it overwinters as adult in repaired areas (canteens, garages, underground tunnels, etc.). *Ochl. caspius*, present from April to October, decreases its activity at about the end of July and it overwinters as egg. In 2006, *Ochl. detritus* was present from June to September while in 2007 the first catches took place only in the first week of July. In 2006, 41% of the total catches of mosquitoes in Milan concerned *Culex* spp., while in 2007 they were 35%. As far as *Ochlerotatus* spp. is concerned, the total catches of these mosquitoes in 2006 were 37%, while in 2007 they were 29% (Figures 1-2).

The dynamic of population of the main species in 2006 and 2007 is shown in Figures 3-8. The low catches on 11.07.06, 08.08.06 and 09.08.07 20.09.07 are the consequence of meteorological events during the monitoring such as rain, heavy wind, which prevented mosquitoes flight. It was noticed that in 2006 mosquitoes were less than in 2007 (Fig. 9). The winter 2006-2007 was very mild, this fact helped the survival of those species overwintering as adults (*Culex* in particular). As a consequence the monitoring started in May 2007, a month before what happened in 2006. Another aspect differentiating the two years is in 2007 the high presence of mosquitoes which generally live in rice-fields (*Ochlerotatus*, *Aedes* and *Anopheles*), which was due to the delay in starting larvicide treatments with Bti by helicopter.

The comparison among groups of species which typically live in an urban environment and those living around the city is not very different in the two years, but the total density of population is significantly higher in 2007.

Treatments in Rice Fields

In the Municipality of Milan there are approximately 680 hectares of rice fields from the half of April to the end of August. In several cases the irrigation by flooding is made with 2 or 3 periods of drying. The technique of cultivating dry rice, with weekly irrigations starting from June, is more and more used. This helps the development of *Ochl. caspius* and other floodwater mosquitoes which lay their eggs not on water but into the moist soil or on grasses. Eggs hatch during irrigation, while with this technique the natural enemies of mosquitoes larvae are absent. This agronomical

practice, which needs an increasing number of drying and irrigation periods, is mainly responsible for a higher reproduction rate of these species. Migration of *Ochl. caspius*, *Ochl. detritus* and *Aedes vexans*, is approximately ten or more kilometres starting from the areas where they were; this makes it possible their arrival in the city.

In order to control mosquitoes living in rice fields - which in years before 2006 reached a very high density in the city of Milan - treatments with Bti have been made by helicopters. The strategy of intervention required a regular monitoring of the flooded rice fields (even twice per week). The insecticide was distributed as soon as the first larvae appeared, without considering any threshold. That's why many rice fields were treated 5-6 times, while other ones were treated less. Treatments were not made with a fixed timetable, but according to the needs resulting from the monitoring and it finished at the end of July. In fact, carrying on with treatments by helicopter using Bti in August, when the rice is already developed, was useless. In 2007 a test of seed treatment was made using diflubenzuron before planting, according to tests referred by Marciano (2007). The results were excellent, with an absence of larvae for at least 15 days. This method was proposed for a general application in 2008, because it affords a sure lowering of the first generations of mosquito larvae; this is guarantee for the success of the global control.

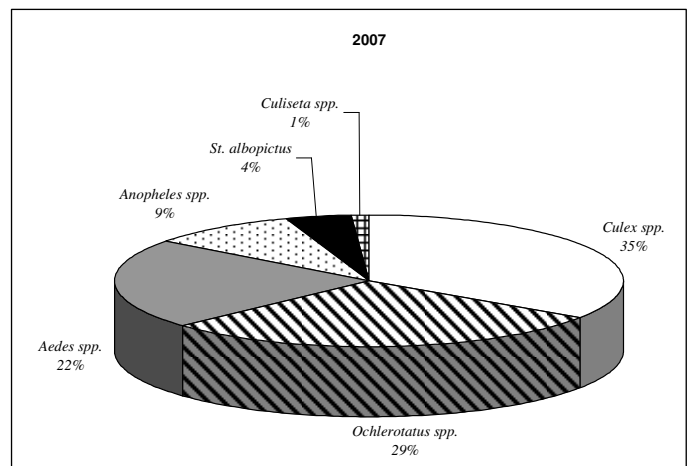
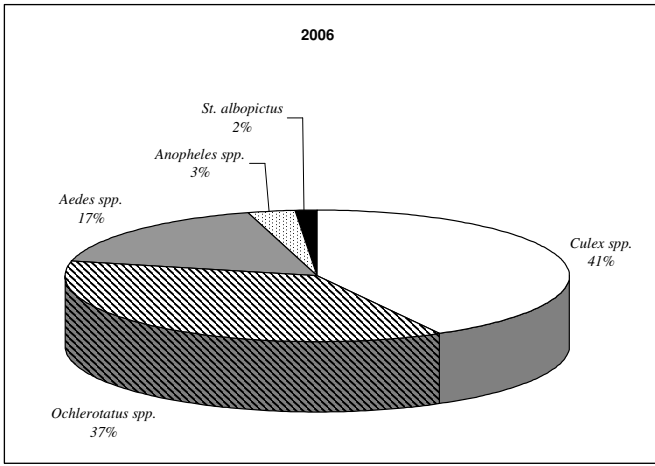
Monitoring *Stegomyia albopictus*

Since its introduction in Italy, the diffusion of *St. albopictus* has been gradual and unstoppable, (Sabatini et al., 1990; Romi, 1994; Romi, 1996; Romi, 2001; Toma et al., 2002). In Milan the Asian tiger mosquito has been present since 2001 and in the following years there has been a rapid spread in the city; since 2005 *St. albopictus* has been present everywhere, particularly in the month of August and September. In Milan and other urban areas, its appearance has already been noticed in the third week of June instead of August not only in parks and gardens but also in homes.

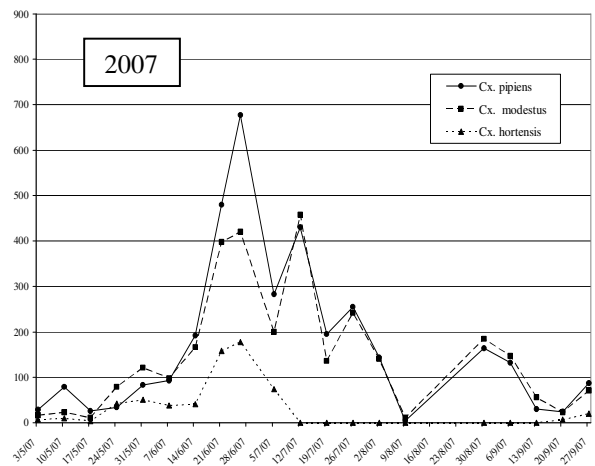
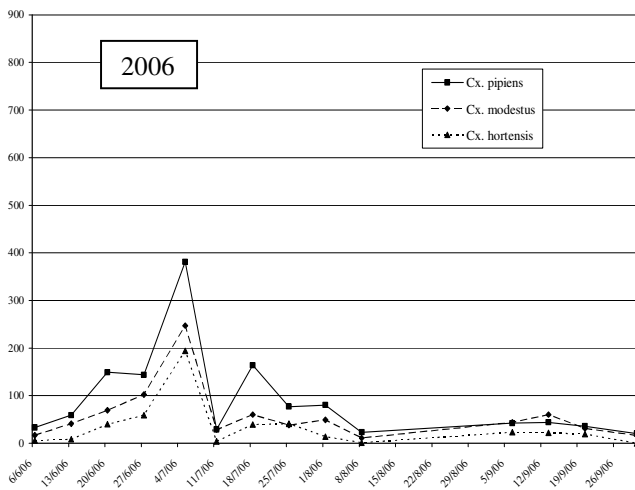
In 2007 the monitoring was made from July to October using oviposition traps, placed in several points of Milan. Some data are reported in Table 1. In the park near the city, where adulticide and larvicide treatments have not been made, the presence of *St. albopictus* was higher than in the public park in the city where treatments have been made. The data collected in two streets, taken as example, highlight a steady presence of *St. albopictus* eggs, caused by the colonization of manholes. To control the activity of the Asian tiger mosquito treatments against adults and larvae have been made on public parks, cemeteries, hedges, vegetable gardens. The Municipality issued an ordinance for the private administrations, concerning the way to make treatments against adults and larvae in all areas of own authority. At the same time larvicide treatments have been planned in all manholes of the city.

Larvicide in Manholes

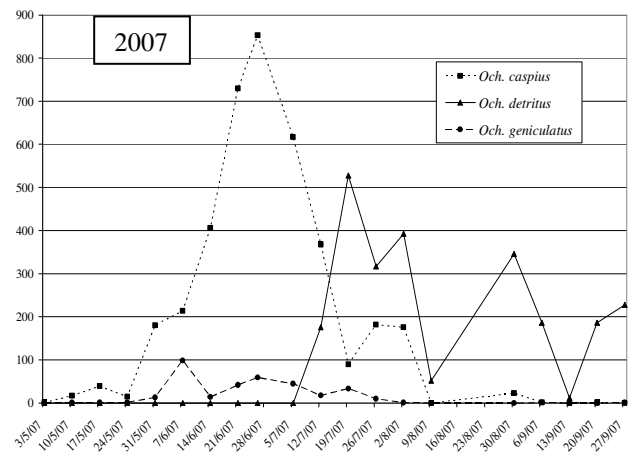
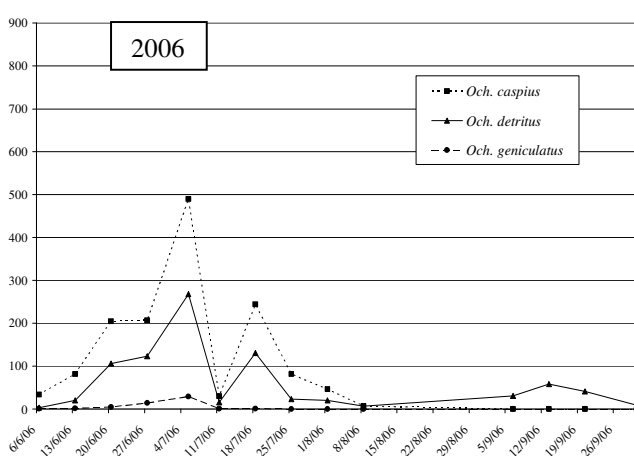
This complex aspect in the control against mosquitoes should be improved. In Milan, there are indeed about 120.000 manholes, of different kinds. Previous studies (data not published) show that only a part of manholes is colonized by mosquitoes, even if it's impossible to establish a connection between the kind of manhole and the presence of mosquitoes, with the exception of grating manholes present in green public areas. The contract of 2005 foresaw 4 treatments per year for all the manholes in the streets. The most critical aspect is that every single cycle of treatments is completed after 30-40 days: in some manholes the treatment is prompt, while in many other manholes it is made too late. This delay causes the development of several mosquito generations.



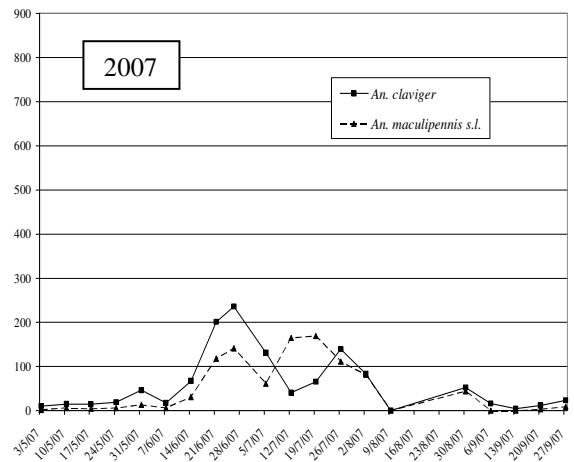
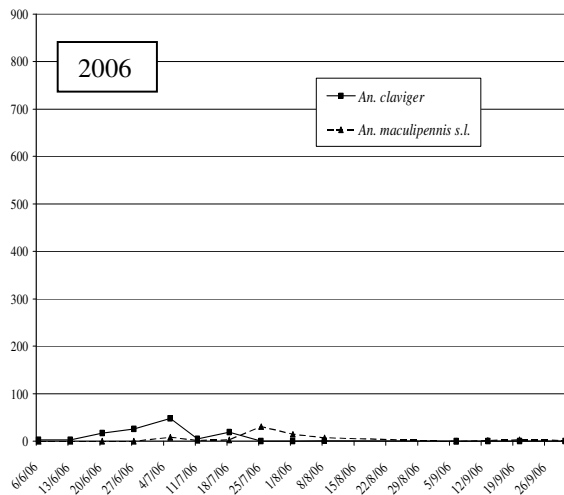
Figs. 1-2. Percentage of different genera of mosquitoes, detected with CO₂ traps in Milan, in 2006 and 2007.



Figs. 3-4. Population dynamics of *Culex* spp. in Milan.



Figs. 5-6. Population dynamics of *Ochlerotatus* spp. in Milan.



Figs. 7-8. Population dynamics of *Anopheles* spp. in Milan

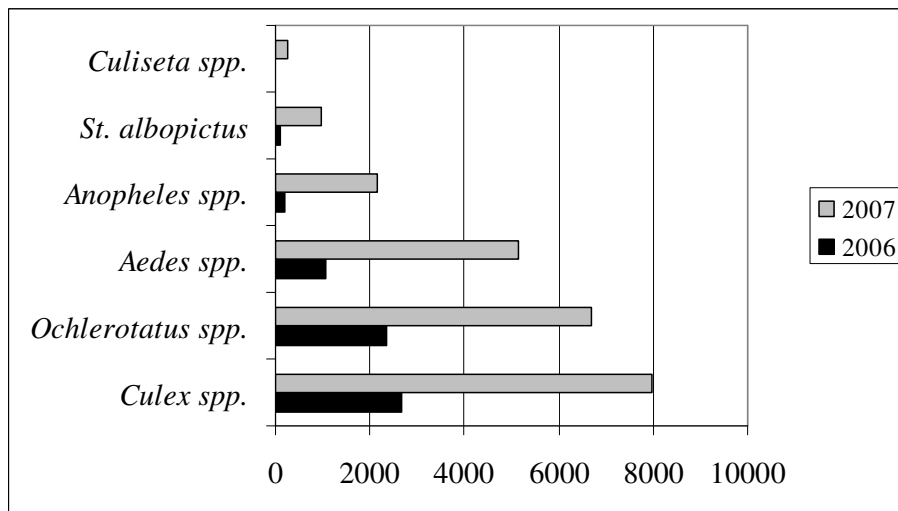


Fig. 9. Total number of mosquitoes detected with 9 CO₂ traps in Milan in 2006 and 2007.

Table 1 - Average eggs number of *St. albopictus* collected using oviposition traps in 2007.

	Average eggs number/trap 1st week July	Average eggs number/trap 1st week October	Weekly average eggs number/trap from July to October
Park near the city	200	18	110
Park in the city	0*	0**	29
Street 1	9	50	52
Street 2	31	5	38

*first eggs collected in the 3rd week of July (106 eggs/trap); **last eggs collected in the 4th week of September (56 eggs/trap).

In 2006 treatments were done with pyriproxyfen, using temephos only in the manholes where water was very rich of organic substance. In 2007 pyriproxyfen and diflubenzuron were used because temephos was no more saleable. Before and after the treatments, several manholes were examined to test the presence of mosquitoes larvae and the treatment efficacy. Monitoring different streets, it has been observed that 10% of manholes were dry, 32% were infested and 58% were not infested. It was observed that treatments, if prompt, gave positive results for two weeks. The interval of time between two treatments decreased the global efficacy of this operation.

Adulticide

In 2006 a Global Positioning System was installed on vehicles to verify the trips, the operative car speed, the eventual periods without activity during the treatments and the respect of the program. This system was abandoned in 2007, because it was noticed that it was very difficult to obtain good data; in fact, the treatments were carried out in streets between high buildings, or in public parks, with dense vegetation. In 2007 there were 45 localised adulticide treatments with pyrethroids, mostly throughout the night, in public parks, on bushes, rows of plants in streets, cemeteries, dams of streams, uncultivated areas, hedges of orchards, factory areas. Other treatments have been done in consequence of specific request from citizens.

Information to Citizens

Eight posters were provided, with the biology and ethology of the species and the possibility of control, with advices to the people for the prevention in the gardens and at home; this information is very important because it's necessary a personal involvement of the citizens, to avoid the development of the micro foci of the larvae in the private areas. A specific web site was organised with weekly news about the collected data of the monitoring and the date of the larvicide or adulticide treatments in the streets. In 2006 an information point was installed also in the city centre; several meetings with distribution of leaflets were organised in public parks and near the entrance of the cemeteries, because there was a high infestation of Asian tiger mosquito in the pots of flowers. The same method was managed in 2007, but the information point in the centre of Milan was no longer available for logistic problems.

CONCLUSIONS

The activity of monitoring and information requested the engagement of 12 technicians, from Mars to the end of October. The results were concrete: the presence of mosquitoes in Milan was remarkably reduced if compared with the years before 2006; complains expressed by the citizens were very sporadic. In the past years, on the contrary, complains were frequently reported by newspapers. It should be possible to improve the situation if in the future some measures, coming from the experience got in 2006 and 2007, were adopted.

In particular it is necessary: 1) to sensitize in a more effective way citizens, because the presence of appropriate places for egg-laying of mosquitoes is localized mostly in private environments not managed by Public Administration; 2) to control against overwintering adults of *C. pipiens* in man-made habitats, underground tunnels and manholes; 3) to make larvicide treatments in manholes with the frequency of 15 days after having managed a census of manholes (this needs, obviously, a number of teams greater than nowadays); in this way it should be possible to exclude those that, for specific localization or model, aren't suitable for larval development; 4) to force

farmers who want to cultivate rice to treat seeds with a larvicide; 5) to manage larvicide treatments in rice fields prompt so that it's possible to control the first generations; 6) to manage larval control in irrigation canals when water doesn't flow because rice fields are dry.

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