Spatial distribution of immature stages of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in flower pots in a Spanish cemetery and field evaluation of metallic copper as a control agent

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Abstract: Cemeteries have been shown to permit the development of the invasive mosquito *Aedes albopictus* in Southern Europe and this has facilitated its establishment on account of the huge quantity of flower pots which are adequate breeding sites for this container-adapted species. A control technique consisting of the use of pieces of metallic copper has been repeatedly proposed as a control solution for mosquito larvae in these pots. Although theoretically promising, this technique has not been used at an operative level, as there has been a lack of information on the best adapted copper formulation as well as on the mosquito larval population dynamics. The present study was planned to characterise the spatial larval distribution of *Aedes albopictus* in flower pots in a wall-niche burial system, and to test the cost-effectiveness of metallic copper application. We found that the preliminary distribution of larvae and pupae in the cemetery was not related to height from the ground but varied between buildings. During the 8 weeks of the trial, application of copper in the form of thin electric wire resulted in a season-accumulated reduction of 90.95% in the production of larvae and 97.06% of the pupae. Application costs and social interactions are discussed as other control methodologies would be more cost-efficient in this specific context. Copper application could be suitable for domestic environments, if application is performed adequately. ***Journal of the European Mosquito Control Association*** **35: 13-17, 2017**

Keywords: *Aedes albopictus*, larvae, cemetery, distribution, copper, control

Introduction

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an aggressive biting species causing a high degree of biting nuisance to humans and an efficient vector for viruses such as chikungunya in Europe (Angelini *et al*., 2007). The species was first found in Spain in 2004 (Aranda *et al.*, 2006). Its distribution range expanded very rapidly in the Barcelona Metropolitan Area and by the end of 2015, at least 453 municipalities were colonised with more than 6 million inhabitants.

The Baix Llobregat region includes 30 municipalities, all of which have confirmed as colonised by *Ae. albopictus* (Eritja *et al.*, 2008). As a Mosquito Control Service (hereafter MCS) existed there since 1983, integrated control (hereafter ICM) programmes targeting *Ae. albopictus* started as early as 2005, when the species first established locally. The aim of these programmes is mosquito control through community-based actions to raise social awareness, combined with extensive larviciding, adulticiding and source management measures.

It is well known that cemeteries are among the most problematic areas for *Ae. albopictus* control, as this species is a container breeder that takes advantage of the flower pots in these facilities (O'Meara *et al*., 1992a). Cemeteries also offer shelter, high vegetation coverage and low building levels, human visitors for bloodmeals, and flowers for carbohydrates (Vezzani, 2007). Two additional factors contribute to the problem. Firstly, due to urban growth many cemeteries in the Baix Llobregat are now embedded in urban downtown areas, facilitating mosquito transfer to the street. Secondly, the common burial system in wall niches of up to 4-5 levels makes for a high vertical density of flower pots. Cemeteries are a perfect mosquito habitat as up to 31 species of Culicidae have been reported in flower pots (Vezzani, 2007).

Cemeteries as ecosystems have been considered by some authors as places to relax and enjoy a natural environment (Laske, 1994 [in Vezzani, 2007]) or as turf-covered, polluted artificial locations hosting invasive plant and animal species (Stowe *et al*., 2001 [in Vezzani 2007]). Social considerations must also be taken into account as cemeteries are symbolic places inspiring fear, sorrow, mourning and respect. These emotional issues overlap with the nuisance caused by *Ae. albopictus* to visitors, turning these places into very sensitive spots and forcing MCS to take into account social issues in any control operations performed there. Specific enforcement policies have been proposed worldwide to prevent water accumulation in flower pots, such as drilling drainage holes or refilling with sand. Whereas preventing this could appear as nonsense, in the real world waterless pots should not be a problem nowadays since most flowers are made of plastic. However, administrative and social issues prevent drilling, so that only temporary control solutions can be planned at present.

Past studies showed that cemetery liners made of copper or bronze prevented the development of mosquito larvae (O'Meara *et al*., 1992b). The use of pieces of metallic copper in the water has been demonstrated in the laboratory to inhibit larval development because of the release of copper ions, which are toxic for algae and for mosquito larvae (Bellini *et al*., 1998). Field-oriented tests also showed that concentrations of ca. 1,000 ppb (parts per billion) of ionic copper completely inhibited larval development, and that it was possible to reach this level by applying 20 grams of metal copper per litre (Romi *et al.*, 2000). Therefore, a number of municipal regulations in Italy used to enforce the application of metallic copper to small receptacles at this dosage (see e.g. Anon., 2005).

In Spain, some cemetery managers performed self-designed copper application programmes as early as 2005, since this type of application did not need any official permit. However, the efficacy of this technique depends not only on dosage, but also on the nature of the raw material. Copper release rate being a function of the metal-to-water contact surface (Bellini *et al*., 1998), so the surface/volume ratio must be maximised by using small, thin metal elements. Failing to take into account the form factor of the copper led to the failure of many of these control attempts. Anecdotally, cases have been verbally reported of uninformed people throwing large pieces of copper into the water, such as sections of waterpipe, enamelled chunks of metal and even coins.

It is normally assumed that copper wires obtained by stripping common electric cable are the best form available. This is a widely available material, which additionally is not enamelled so that an efficient delivery rate of copper ions should be expected.

Considering the scarcity of information available to the public, the existing interest in this control method and the special conditions found in local cemeteries, a test was designed to assess the cost and the control efficiency that could be achieved using metallic copper, in order to issue a guideline for further applications.

However, as a first methodological step, it was necessary to determine the distribution of mosquito larvae in relationship to the location of flower pots. Although some studies on *Ae. aegypti* showed differences in selection of breeding places in relation to sunlight (Vezzani & Schweigmann, 2002), no information was available on *Ae. albopictus* larval densities in pots relative to their orientation, position or height from the ground. This knowledge was needed to minimise any sampling deviation in test design and would also be important for control treatments, as significant labour time and product savings could be obtained by only applying copper or any other control agent to the more productive flower pots.

Materials and Methods

The cemetery of Molins de Rei (Baix Llobregat, Catalonia, Spain; 41°25'13.66"N; 2°1'7.63"E) was selected as this municipality was the first one in the region to be colonised by *Ae. albopictus*, and the facility was heavily infested. This is an area of ca. 2,745 sq m completely delimited by walls and with no external breeding sites.

Only the modern section of the cemetery was included to the study, including nine blocks of different shapes and orientations so that the amount of shading varied (Fig. 1). The vertical distribution of niches was identical for all blocks across a height of 4 rows of 30 to 60 units depending on the block, individual niche stones measuring 1.2 square metres each. Both faces were used in the case of two-sided blocks. Vertical levels were numbered as row 1 (ground level) to row 4, which was at 4.5 metres from the ground. Thus, the test area consisted of a grid of 4 rows per several hundred columns, distributed across 9 buildings. Each niche stone had two steel liners attached via two metallic arms. By their nature these metal liners couldn’t collect water as they were intended as a holder for plastic flower pots which measured 17 cm high, 4 cm diameter at the base and 7.5 cm diameter at the opening. Their mean approximate capacity was measured at 350 ml.

The initial sampling was performed in August 2007 to assess the larval distribution in the flower pots according to factors such as the building block, shade, orientation, and height above ground level. A total of 116 water-filled pots were randomly selected from the flowerless pots at that time; all immature stages were counted and left back in the water.

As an analysis of the data revealed no significant differences related to the row (see Results), the test itself was restricted for convenience to the two lowest rows, making it easier to sample since a ladder was not needed.

The test took place in 60 flower pots randomly selected by a mechanical draw from the two lowest rows from the preliminary set, and attributed to the treated (30) and untreated (30) groups. The higher rows were not included in the study, and due to the random selection not all blocks had the same number of pots studied. As the mean larval and pupal densities were already known, it was statistically confirmed before starting the trial that there were no differences in the initial populations between these two subgroups (ANOVA, $P<0.01$; analysis details not shown).

Interactions with visitors had to be considered because the facility remained open to the public across the test period. Each pot was labelled with a unique code for tracking in case people shifted it to another location or removed the water, the copper material, or both. As already noted, only flowerless pots were selected in an effort to minimise these interactions assuming those pots were less likely to be removed or otherwise influenced by human handling.

The dosage of copper was set at 20 grams per litre as per Romi *et al.* (2000). Unbranded electric multi-wire cable of 1.5 sq mm section, 48 wires weighing at ca. 192 mg/cm was purchased at a general store. At the 20 g/l dosage, the weight of metallic copper necessary for this pot size was contained in a length of 36 cm. Pieces of cable of that length were cut and stripped from the external plastic insulation, and the wires were twisted at one tip to keep them together. The accuracy of the metal dosage was verified by measuring 10 randomly selected units.

The wires were placed in the pots on 30 August 2007 and the pots were sampled weekly from 6 September to 25 October 2007, resulting in 8 data collections for a theoretical target of 480 samples, from which casualties caused by visitors were removed. Larvae (L2-L4) and pupae were counted in the pot itself, or (in the case of large numbers) transferred by gentle pipetting to trays with clean water, counted there and put back in the pot. Previous experience from the operators as well as final mortality in the control, allowed the operation to be considered as harmless for the immatures. Pupae were not checked for emergence success. It was decided to leave the individuals in the pots because of the slow action of the ionic copper, which also makes it difficult to verify mortality by bringing samples to the laboratory.

The water in the study was consistently clear as it originated from rainfall and tap water used by visitors. No visible amount of organic material was detected in any of the samples that could have biased the results by interacting with the ionic copper release. To keep the level at 350 ml the pots were refilled with tap water after each sampling, if needed. The water was obtained from the same sources available to visitors. Neither the final concentration of ions in water nor the water chemistry were assessed.

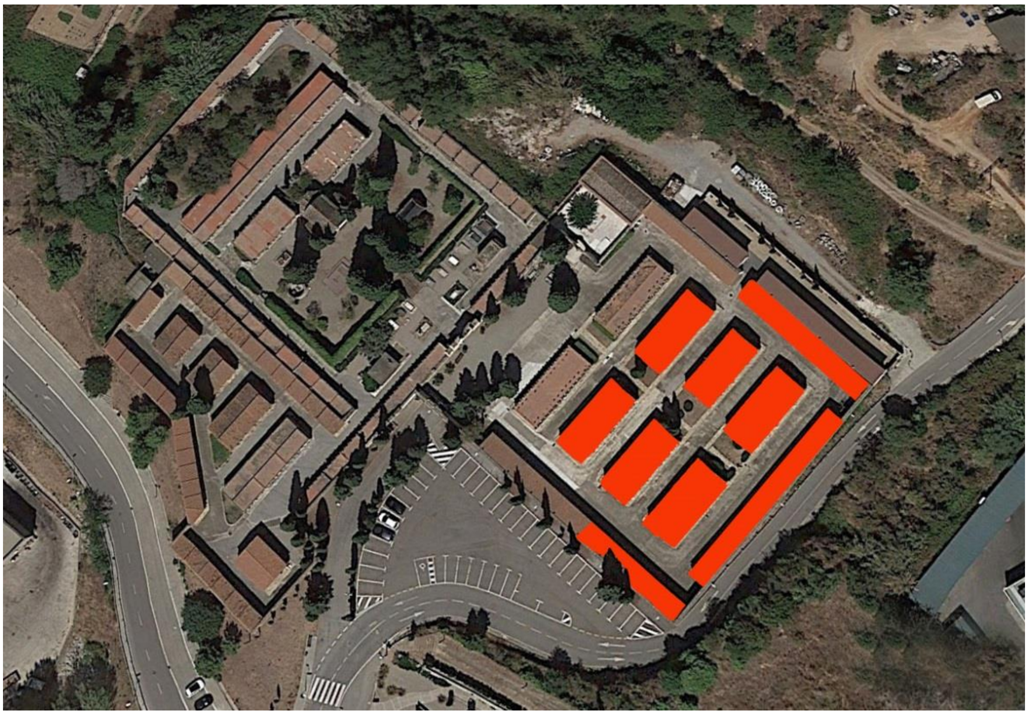

Figure 1: Aerial view of the cemetery of Molins de Rei (Barcelona, Spain) showing (in red) the studied blocks. Image credit: Google Earth, Google inc.

Although *Ae. albopictus* larvae can be found as late as mid-November in this area, the test ended by 25 October to avoid All Saints' Day (November 1st) and the Day of the Dead (the following day) which bring a number of visitors replacing the water in the pots, supplying fresh flowers and getting rid of the copper wire. All pots were additionally reviewed on 31 October and finally on 7 November to assess the final percentage of copper loss for a cost-benefit evaluation of the technique as a long-term solution.

The larval and pupal count variables were normalised by the arcsine transformation of their relative frequencies. Then, univariate ANOVA tests were performed on data from the initial situation and from the 8 weeks' follow up. Results were confirmed by non-parametric tests on the original data.

An ovitrap was placed in an adequate plot at the cemetery's main entrance to obtain an estimate of the local reproductive mosquito population. Only one ovitrap was used because the studied cemetery surface was small; assuming a closed universe the sampling intensity was equivalent to 3.67 ovitraps per hectare.

The trap was a black plastic beaker 12 cm high, with a 6 cm diameter at its base and 8.5 cm at the opening, and a maximum capacity of 275 ml. The oviposition bait surface was a 2 x 12 x 0.2 cm piece of Mansonite^{TM} wooden board which was sampled weekly to count all eggs, including those on edge surfaces. Water was added to keep the standard level. All collected eggs were initially determined as belonging to *Ae. albopictus* due to their shape, as well as the absence of other Aedine treehole breeding species in the area. This was confirmed later by breeding these eggs in the laboratory, which resulted in 100% *Ae. albopictus* larvae.

The relationship between egg counts in the ovitrap and the larval and pupal population sample in the untreated flower pots was calculated via Spearman's correlation analysis, both parametric and non-parametric. All tests were carried out using SPSS 15.0 statistical package.

Results

The accuracy of the treatment dosage was assessed by measuring 10 randomly selected copper wire units resulting in a mean length of 36.06 cm (the target being 36.00) and a standard deviation of 0.21, so that the accuracy of the application dosage was deemed satisfactory.

Table 1: Analysis of variance for the larval density in the initial situation.

Source	Sum of Squares	DF	Quad mean	F	Sig
Model	2.228	29	0.077	1.604	0.049
ROW	0.146	3	0.049	1.018	0.389
BLOCK	1.259	8	0.157	3.286	0.003
ROW*BLOCK	0.849	18	0.047	0.985	0.485
Error	4.119	86	0.048		
Total	9.383	116			

In the analysis of the initial larval densities in the cemetery, there were no differences between the 4 row levels ($P<0.05$, Table 1). Significant differences were found between blocks, as mosquito populations were more abundant in certain buildings. However, there were no interactions with the height levels. The analysis of pupae yielded similar results ($P<0.05$, Table 2).

Table 2: Analysis of variance for the pupal densities in the initial situation.

Source	Sum of Squares	DF	Quad mean	F	Sig
Model	2.917	29	0.101	1.652	0.039
ROW	0.066	3	0.022	0.359	0.783
BLOCK	1.636	8	0.204	3.358	0.002
ROW*BLOCK	1.033	18	0.057	0.943	0.532
Error	5.236	86	0.061		
Total	9.714	116			

Several flower pots were affected during the test by visitor activities such as removal of the copper. As data of these pots were discarded from the whole trial, the total number of valid samples was 412 (210 in control flower pots and 202 in the copper application condition). This made for 14.1% missing cases up to October 25. On the post-test count on October 31, the percent of missing pots and copper pieces was 80%, and in the final verification survey on 7 November, after the Day of the Dead, the figure was 96% of missing pots.

The pooled number of immature stages by week is described in Table 3. A total of 1,173 larvae and 212 pupae were counted in the control samples during the 8-week test, whereas only 102 larvae and 6 pupae showed up in the copper-treated replicates. This represents a difference of 90.95% for larvae and 97.06% for pupae when the respective mean values for the whole period are compared.

Table 3: Weekly immature stages sampled in both experimental conditions.

	Larvae (L2 to L4)		*Pupae*		*N*	
	UTC	Copper	UTC	Copper	*UTC*	*Copper*
Sep 6	43	63	8	3	*28*	*29*
Sep 13	144	20	10	0	*28*	*28*
Sep 20	243	9	29	1	*28*	*29*
Sep 27	200	6	63	1	*28*	*29*
Oct 4	189	3	36	1	*28*	*29*
Oct 11	157	0	29	0	*28*	*29*
Oct 18	113	0	19	0	*27*	*29*
Oct 25	84	1	18	0	*23*	*25*
TOTAL	1,173	102	212	6	*218*	*227*

Table 4: Analysis of variance of the larval densities for the test period.

Source	Sum of Squares	DF	Quad mean	F	Sig
Model	1.993	22	0.091	8.596	0.000
ROW	0.000	1	0.000	0.045	0.832
COPPER	1.106	1	1.106	104.935	0.000
BLOCK	0.046	8	0.006	0.542	0.824
ROW*COPPER	0.040	1	0.040	3.839	0.051
ROW*BLOCK	0.200	5	0.040	3.786	0.002
COPPER*BLOCK	0.074	5	0.015	1.396	0.225
ROW*COPPER *BLOCK	0.000	0			
Error	4.100	389	0.011		
Total	8.832	412			

An inference analysis was also performed on the pooled data from all samplings, excluding the time factor (Tables 4 and 5 for larvae and pupae, respectively). Univariate ANOVA showed that during the test there were no significant differences within buildings or rows ($P<0.05$), but a highly significant difference between the copper treatment and the control.

Interactions were found between the rows and the blocks, both for larvae and pupae, and between the copper application and block in the case of pupae. Both interactions suggested that specific height levels could lead to variations in mortality evolution over time, depending on the amount of shade in a block, and that the effect of the application on pupae was not the same for all blocks. Such observations were expected for a real situation in heterogeneous environments, as already suggested by the initial variations in the densities of immature stages across blocks.

Table 5: Analysis of variance of the pupal densities for the test period.

Source	Sum of Squares	DF	Quad mean	F	Sig
Model	1.875	22	0.085	6.439	0.000
ROW	0.008	1	0.08	0.600	0.439
COPPER	0.905	1	0.905	68.384	0.000
BLOCK	0.087	8	0.011	0.818	0.587
ROW*COPPER	0.031	1	0.031	2.377	0.124
ROW*BLOCK	0.286	5	0.057	4.324	0.001
COPPER*BLOCK	0.159	5	0.032	2.409	0.036
ROW*COPPER *BLOCK	0.000	0			
Error	5.163	390	0.013		
Total	7.038	412			

The ovitrap sampling was positive for 5 weeks between 3 September and 4 October, and was then negative until the end of October. The figures ranged between 67 and 138 eggs, reaching an accumulated total of 500 eggs and a mean of 62.5 eggs per positive sampling. This is considerably higher than the routine samplings by the Mosquito Control Service using the same kind of ovitraps in the region (mean: 26.6, N=283), but is consistent with the historical sampling mean of that cemetery from 2006 to 2011 in the same ovitrap location (mean: 48.25 eggs/sampling, N=48; all unpublished data collected from the same test equivalent period).

Discussion

Aedes albopictus is usually described as a low-flying mosquito that has a relationship with the vegetation levels that are nearer to the ground (Bohart 1957). This has strong implications for the residual adulticiding operations as most pesticide applications are usually performed below the 1.5 metre height. This study, however, showed uniformity in the vertical distribution of immature stages up to more than 4 m. Differences in sun exposure (shading) of each block probably influenced the larval densities but no interactions were found with their vertical distribution. Such widespread larval presence across flower pots is not good news for control operations as it shows that –oppositely to adulticiding on vegetation- larvicidal applications in pots might need to be performed at all levels from the ground to more than 4 metres. High adult densities of *Ae. albopictus* might yield a wider oviposition coverage reaching even less suitable places. Although to be considered as indicative only due to the low number of capture locations, the 62.5 mean egg count is considered high for the region also taking into account the presence of flower pots as competitive oviposition places.

The copper application in the tested conditions and dosage was found to be highly efficient, though this was indirectly assessed as no direct mortality was recorded. Individuals were put back in the flower pots once accounted, for a better fit to the long-term toxicological characteristics of the copper. Adult emergence rate from the pupae was not assessed and larval cadavers were impossible to track in the scheduled sampling. Although first-instar larvae were not counted, they couldn't complete their cycle as no corresponding pupal density was ever recorded.

Slow copper intoxication prevents larvae from reaching the pupal stage, which highlights the need to assess pupal densities when testing any type of long-acting biocide. Della Torre *et al.* (1993) also emphasised that copper must be present from the first instars to prevent larvae reaching stage II, whereas mortality was much lower if the metal was introduced 4 days after egg hatching. Therefore, the conclusion of the study is that the metallic copper used at this dosage and form is highly efficient in reducing the L2-L4 larval and pupal densities (ca. 91% and 97%, respectively) of a real *Ae. albopictus* population breeding in cemetery flower vases over a period of 8 weeks. The pattern of larval reduction during the first weeks (Table 3) was quite progressive and might be consistent with a slow initial release of the fresh metal.

It is worth noting the relevance of the form factor of the copper in order to obtain the required delivery surface by using the minimum amount of metal. The raw copper material used in this test was reduced to 6.9 grams per pot thanks to optimising a delivery surface evaluated at 10,824 sq mm, computed by the sum of each of the 48 individual filament threads considered as cylinders. The water-to-metal contact surface would have been only ca. 450 sq mm if that amount of copper had been used in one solid, spherical block. This can easily lead to failure of applications performed using the same weight of copper under varying shapes.

Copper application could probably provide extremely long effectiveness but is influenced by human actions, as the Day of the Dead celebrations on November 1st resulted in more than 95% of the copper being lost due to cleaning and refilling of pots. The amount of metal filament needed to achieve 20 g/l is bulky enough to make it very visible in the water, which increases the likelihood of being removed from the pot. Whereas specific education campaigns could prevent this to some extent by informing citizenship, possible losses would probably make the technique financially unsuitable because copper is expensive and preparation work is needed to strip and separate the electric wire. The cost of the copper material was evaluated at 0.15 euro per flower pot.

One of the alternatives to copper are Pyriproxyfen-based chemicals such as Sumilarv™ 0.5WG (Sumitomo Corp), which is currently used in Spain for *Ae. albopictus* control at 100 g a.i./Ha (Schaeffer et al. 1988). Thus, 0.016 mg of granular formulation should be added to each flower pot, with a cost of 0.0011 euro per unit which would ensure a control of ca. 5 to 7 weeks (our own observations). Oppositely to copper, significant losses should not be expected from human activity as the formulation consists in sandy granules remaining at the bottom and cannot be seen. Manpower used for the application would be the same as for the copper.

Our results indicate that a good control level could be expected from a single copper application for a whole season of 24 weeks. Whereas this is 5X longer than the effectiveness of a single Pyriproxyfen application, the product cost would be about 155 times higher and manpower costs from the 4 additional Pyriproxyfen applications do not probably compensate this difference. Copper use could only be considered if the metal was reasonably expected to stay in the pots until the next year. Otherwise, other techniques or biocides should be given priority for larval control in cemeteries.

Other environments with more controlled human interactions would be more suited to this application technique. For example, people could use copper wires in their homes, which would enhance social acceptation and awareness while reducing risks from the use of household chemicals. However, to achieve good results it is crucial to provide operators and public with information on the specific raw materials to be used, their form and the necessary pre-processing.

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References

Angelini, R., Finarelli, A.C., Angelini, P., Petropulacos, K. & Macini, P. (2007) An outbreak of Chikungunya Fever in the province of Ravenna, Italy. *Eurosurveillance* 7:319-327

Anonymous (2005) *Provvedimenti per disinfestazione della Aedes albopictus "zanzara tigre" nel territorio del Comune di Roma* – Ordinanza del Sindaco N. 6 del 30 Marzo 2005, Prot. 452, 23pgs

Aranda, C., Eritja, R. & Roiz, D. (2006) First record and establishment of the mosquito *Aedes albopictus* in Spain. *Medical and Veterinary Entomology* 20: 150-152

Bellini, R., Carrieri, M., Bacchi, M., Fonti, P. & Celli, G. (1998) Possible utilization of metallic copper to inhibit *Aedes albopictus* (Skuse) larval development. *Journal of the American Mosquito Control Association* 14(4): 451-456

Bohart, R.M. (1957) Diptera: Culicidae. *B. P. Bishop Museum, Honolulu, Hawaii. Insects of Micronesia 12 (1): 1-85*

Della Torre, A., Raineri, V. & Cancrini, G. (1993) Effetto del rame metallico sullo sviluppo larvale di *Aedes albopictus*: primi dati di laboratorio. *Parassitologia* 35: 51-53

Eritja, R., Roiz, D., Ruiz, J., Rojo, M., Torrell, A., Escosa, R., Marquès, E., Ruiz, S., Melero, R., Molina, R. & Lucientes, J. (2008) Evolution of the distribution of *Aedes albopictus* in Spain. Sixteenth E-SOVE meeting. 25-28 March 2008, Cambridge, UK: 64, Abstract number 6.11

O'Meara, G.F., Gettman, A.D., Evans Jr, L.F. & Scheel, F.D. (1992a) Invasion of cemeteries in Florida by *Aedes albopictus*. *Journal of the American Mosquito Control Association* 8(1):1-10

O'Meara, G.F., Evans Jr, L.F. & Gettman, A.D. (1992b) Reduced mosquito production in cemetery vases with copper liners. *Journal of the American Mosquito Control Association* 8(4): 419-420

Romi, R., Di Luca, M., Raineri, W., Pesce, M., Rey, A., Giovannangeli, S., Zanasi, F. & Bella, A. (2000) Laboratory and field evaluation of metallic Copper on *Aedes albopictus* (Diptera: Culicidae) larval development. *Journal of Medical Entomology* 37(2): 281-285

Vezzani, D. & Schweigmann, N. (2002) Suitability of containers from different sources as breeding sites of *Aedes aegypti* (L.) in a cemetery of Buenos Aires city, Argentina. *Memorias Instituto Oswaldo Cruz*, Rio de Janeiro, 97(6):789-792

Vezzani, D. (2007) Review: Artificial container-breeding mosquitoes and cemeteries: a perfect match. *Tropical Medicine and International Health* 12(2): 299-313

Zhang, J., Kobert, K., Flouri, T. & Stamatakis, A. (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, **30**(5), 614-620.

Zhou, X., Li, Y., Liu, S., Yang, Q., Su, X., Zhou, L., Tang, M., Fu, R., Li, J. & Huang, Q. (2013) Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. *GigaScience*, **2**(1), e4.