The presence of white eggs in the monitoring of *Aedes albopictus (*Diptera: Culicidae) by ovitraps

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ABSTRACT: Using international trading and passive transportation routes, the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), has colonized Europe. While the most common tool to monitor the presence of the mosquito is the ovitrap, other kinds of eggs are occasionally found in the traps as well. Most of the eggs are easy to distinguish, however, some white-yellow eggs have a similar shape and size to those of the tiger mosquito and are often falsely identified as freshly laid tiger mosquito eggs. We have shown that these eggs had been laid by *Psychoda alternata* Say, 1824, and the misinterpretation may cause large errors in calculating density and patterns of *Ae. albopictus.* To avoid mistakes, a microscopic observation should be done at least 48 h after collecting the sample to permit *Ae. albopictus* eggs to darken. ***Journal of Vector Ecology* 38 (2): 326-329. 2013.**

Keyword Index: Asian tiger mosquito, *Psychoda alternate*, eggs, false positives, evaluation infestation, *Aedes albopictus.*

INTRODUCTION

Since its first establishment in Italy in the city of Genoa (Sabatini et al. 1990) and the Padua Province (Dalla Pozza and Majori 1992) originating from the importation of used tires from the United States of America (Dalla Pozza et al. 1994, Roiz et al. 2011), the Asian tiger mosquito (*Aedes albopictus* Skuse, 1894) has quickly spread all along the Italian peninsula (Romi 1996). Using international trading and passive transportation routes, this mosquito is now colonizing Europe. In the new colonized areas, the tiger mosquito has quickly became an important pest mosquito due to its aggressive behavior that makes it very annoying to the population, in addition to causing an increase of hypersensitivity reactions (Romi 2001). Nevertheless, in the endemic areas *Ae. albopictus* can transmit many arboviruses, such as dengue and chikungunya (Gratz 2004). Up until a few years ago, these diseases have been a potential risk within Italy and generally within Europe, but an epidemic of chikungunya in the Ravenna province in the 2007 with 204 cases (Angelini et al. 2007) and some autochthonous cases of dengue in France (La Ruche et al. 2010) and Croatia (Gienero-Margan et al. 2011) have switched concern to a concrete risk.

A frequently used tool to monitor the presence of the tiger mosquito is the ovitrap: a small dark plastic container with water and a wooden stick where *Ae. albopictus* can lay its eggs. The determination of the eggs is quite easy even if there is another mosquito, *Ae. geniculatus* (Olivier, 1791), laying eggs in the same way. These eggs are very similar to *Ae. albopictus* eggs and only an expert eye can recognize the difference. The eggs of *Ae. geniculatus* are a little bit different in shape and in the chorion's structure, while the size is almost the same (Zamburlini 2003). There are other treehole breeding mosquitoes that can lay eggs on the sticks, in particular *Ae. berlandi* (Seguy 1921) and *Ae. echinus* (Edwards 1920). Together, these species can cause errors in evaluating the density and presence of *Ae. albopictus*, but usually their density is low. Therefore, at least where tiger mosquitoes are already present, over-counting is not very significant.

Since the arrival of the Asian tiger mosquito, other kinds of eggs have occasionally been found on the sticks during the monitoring activity. Most of them are easy to distinguish (because of their shape and size) with the exception of some white eggs, up to now unidentified, that are commonly found in Veneto Region but are also present at other sites in Italy. These eggs have the same shape and size of the ones laid by *Ae. albopictus* and they were previously defined as tiger mosquito eggs that were recently oviposited and not yet pigmented (they had no time to darken). Often, the number of these eggs is very high and is found mixed with dark eggs, so the counting of these yellowish eggs as belonging to the tiger mosquito can heavily affect the results. The explanation that the lighter color is due to the young age of the eggs is not convincing because it has been empirically observed that when the white eggs are collected, they do not become dark with time, and also when they are dried, they stay yellowish in color (Drago, personal observation).

Here, we report the evidence that these eggs are indeed not those of *Ae. albopictus* but rather the results of ovipositing activity of Psychodidae of the species *Psychoda alternata* Say, 1824, a non-hematophagous dipteran very common in urban and suburban environments in southern Europe.

MATERIALS AND METHODS

During the periods from May to October, 2010 and June to October, 2012, 20 ovitraps (from the end of June, 2012 the number of ovitraps was 21) were used to monitor

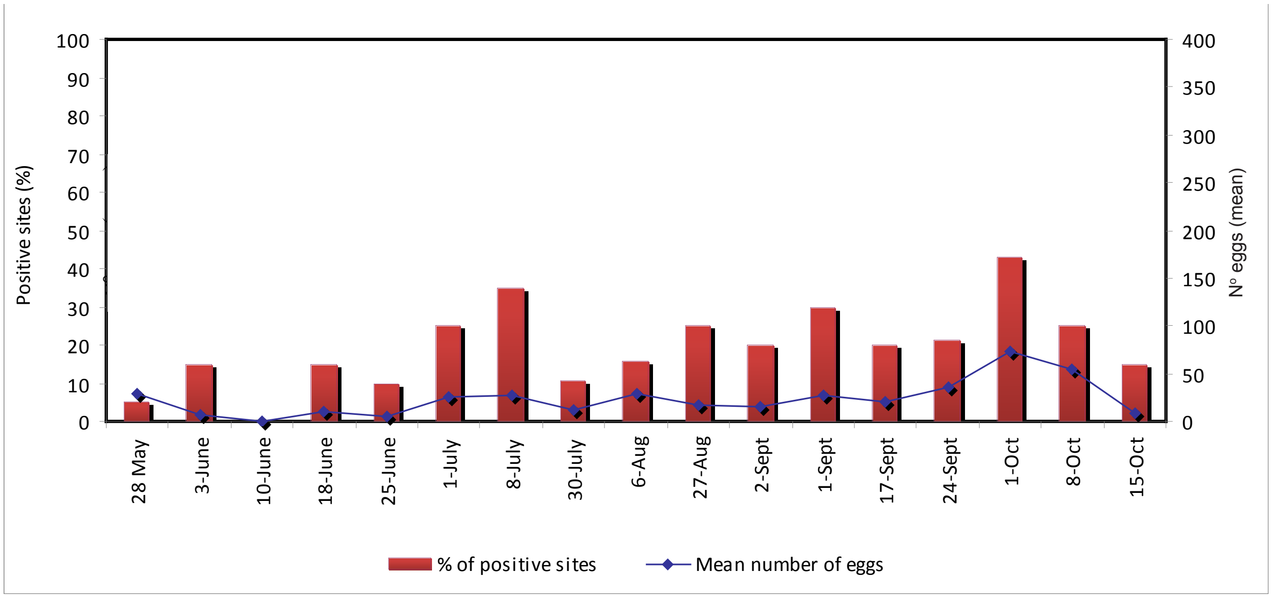

Figure 1. Population trend of *Psychoda alternata* in 2010.

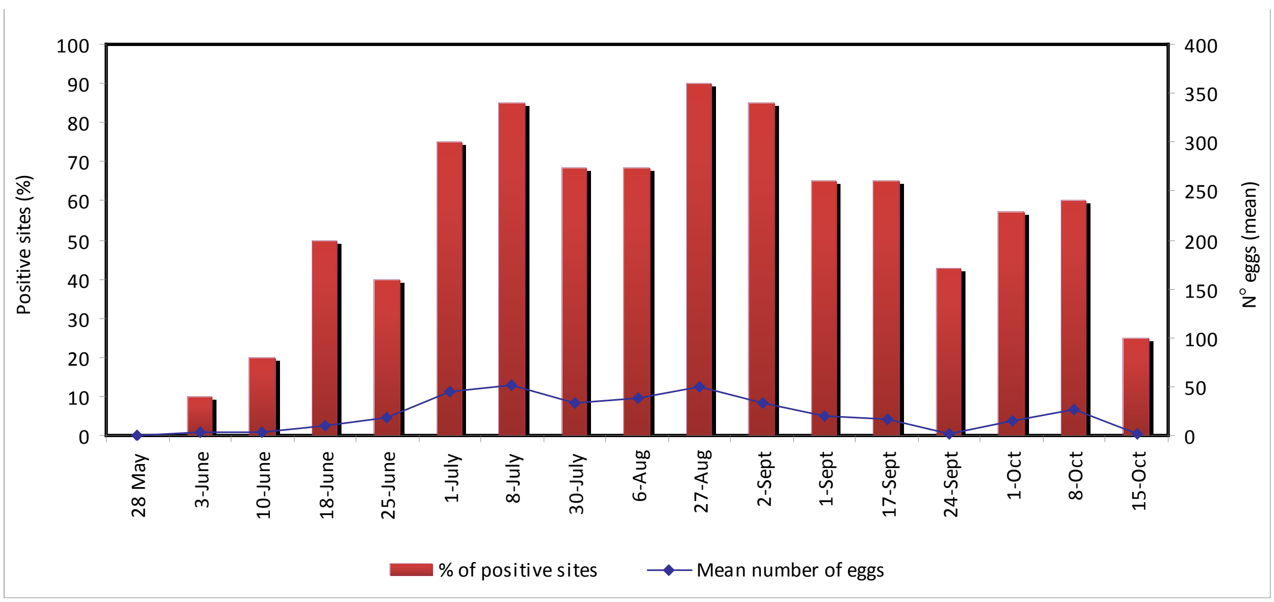

Figure 2. Population trends of *Aedes albopictus* in 2010.

the population of tiger mosquitoes in Chioggia, Cona and Cavarzere municipalities in northeastern Italy. The traps were modelled after those used by Carrieri et al. (2011) and managed by changing the sticks every week. The sticks containing white-yellowish eggs were carefully observed at 10X magnification to identify any other kinds of eggs. After 48 h, they were withdrawn and put into basins with rain water, kept at 24-25° C, and a small quantity (± 2 g) of fish food. The emerged adults were identified through the morphological characteristics proposed by Severini et al. (2009). The percentage of positive sites was calculated for each date as follows:

$$\% \text{ positive sites} = \frac{\text{N of positive sites} * 100}{\text{N of active sites}}$$

The number of active sites was obtained by subtracting those sites whose data were missing (e.g., because of a missing stick) from the total number of observation sites. On the obtained percentages, mean, standard deviation and standard error of the mean were calculated.

RESULTS

Figures 1-4 show the egg-laying pattern of *Ae. albopictus* and of *P. alternata*. Numbers refer to the average number of eggs laid at the places that were monitored. The total number of sites for the year 2010 was 20, while for the year 2012 it was 20 until June 20, when it increased to 21 for all the remaining dates.

While analyzing the data, a few differences between the

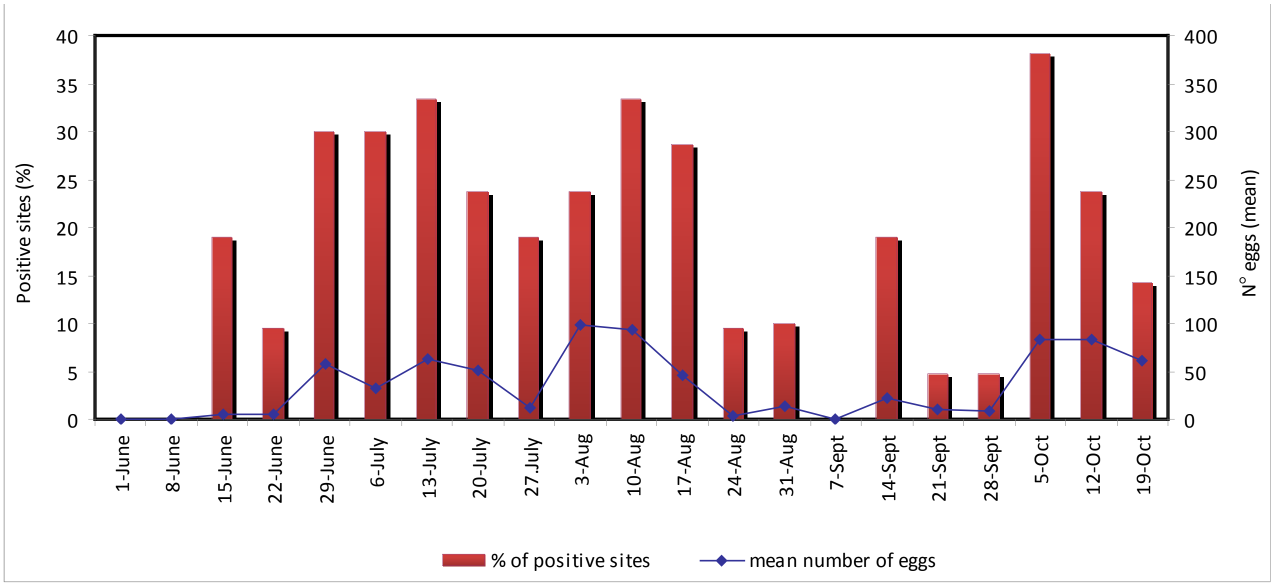

Figure 3. Population trends of *Psychoda alternata* in 2012.

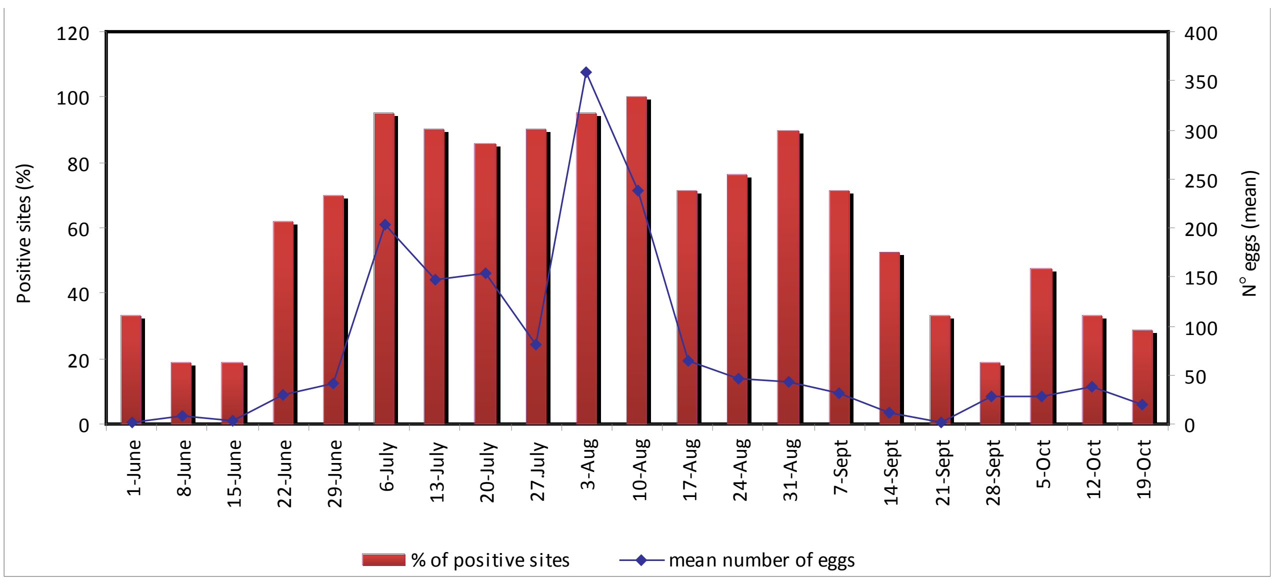

Figure 4. Population trends of *Aedes albopictus* in 2012.

eggs of *Ae. albopictus* and those of *P. alternata* emerged. In 2010, the percentage of positive sites for *P. alternata* eggs was much lower than the percentage of sites positive for *Ae. albopictus*: 53.3± 6.5% of the sites was found to be positive for the eggs of *Ae. albopictus* during the entire observation period, whereas 19.4± 2.6% was only found positive for *P. alternata* eggs. In 2010, the mean number of *P. alternata* eggs did not differ compared to that of *Ae. albopictus* (425.9±71.8 and 422.8±84.3, respectively). On the other hand in 2012, the mean number of *P. alternata* eggs was found to be much less than that of *A. albopictus* (746.1±156.9 and 1570.7±429.1, repectively).

The data obtained in 2010 show that *P. alternata* tend to concentrate in fewer sites than *Ae. albopictus*, even though they are more widespread in the environment. This is emphasized by the fact that the mean numbers of eggs for both species are not much different, whereas a sharp difference can be observed in the percentage of positive sites which is much lower for *P. alternata* and *Ae. albopictus* (19.4± 2.6% and 61.1± 6.3%, respectively). In 2012, this tendency is less remarkable because of the difference in the mean number of eggs between *Ae. albopictus* and *P. alternata* (1570.7± 429.1 and 746.1± 156.9, respectively).

DISCUSSION

This work clarifies that the insect laying these yellowish eggs on the ovitraps used for *Ae. albopictus* monitoring is *P. alternata*. Many field operators still continue to consider these yellowish eggs as freshly laid *Ae. albopictus* eggs, causing mistakes in the evaluation of the relative density of the species. In fact, our results showed how important such an error in the counting can be by misunderstanding the egg identification: the double amount of the actual number.

It is also possible to see how the pattern of the two populations differs (Figures 1-4): a typically bell-shaped pattern for the tiger mosquito, while *P. alternata* has been observed to have a pattern that continuously increases until a sudden drop. Including this aspect of population dynamics by merging the presence of *P. alternata* and *Ae. albopictus* can cause mistakes in estimating mosquito densities that can lead to an incorrect planning of control interventions as well as to a false evaluation (false positive presence) of the risk of arbovirus transmission. To reduce false positives as much as possible, the sample should be kept for at least 48 h at 22-26° C in a dark place before observation. In any case, the white eggs with a similar shape and chorion structure like tiger mosquito eggs (Romi et al. 1994) should be left to hatch to confirm the identification.

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