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USING THE OVIPOSITION TRAPS FOR INVASIVE MOSQUITOES SURVEILLANCE - CASE OF *STEGOMYIA ALBOPICTA* SKUSE, 1894 (DIPTERA: CULICIDAE)

SUMMARY

The Asian tiger mosquito, *Stegomyia albopicta*, is originally an oriental species that has in the last couple of decades invaded and established itself in the USA, Central and South America, Africa and Europe. Since this species is an important vector of dengue and more than 20 other arboviruses (**ar**thropode **bo**rn **viruses**) in countries throughout the world, its rapid spread in Europe is viewed as a matter of great public health concern in all countries.

In project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" during 2012. on Montenegro coast 41 black plastic oviposition traps were used. Ovitraps were placed in green, shaded, easily accessible places, on the ground with a free space above of at least 1 meter. The ovitraps were placed at used tire storage places (4), urban (11), semiurban (13), main resting places (12) and one border crossing. The trap stations were kept fixed for the whole season and inspected every 10th day, from 07st May to 30th October. All strips from the traps were dried before counting the eggs and after counting they were placed in glass pots with the same collected water from the traps, to grow the larvae and adults. Mosquitoes were identified morphologically by using the diagnostic keys. After breeding and the mosquitoes determination, glass pots with content: water, strips, larvae and adults; were kept in freezer 48 hours, than defrosted and spilled to prevent contamination.

Using this kind of oviposition traps 80.582 eggs were counted from 671 positive traps, from total 738 just 67 traps were negative.

Keywords: *Stegomyia albopicta*, oviposition traps, surveillance.

INTRODUCTION

Stegomyia albopicta (*Aedes (Stegomyia) albopictus*) (Skuse 1894) is an insect from order Diptera, family Culicidae (mosquitoes) known as "Asian Tiger Mosquito". This species originates from tropical forests in South Eastern Asia;

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New Guinea - South; Hawaii - East; Beijing area in China - North and Madagascar - West (Novak, 1992) (Figure 1).

It has started spreading all over the world at the end of seventies of 20th century. Nowadays is obvious that spread of the Asian Tiger Mosquito has been hastened by inter state shipments of used tires, primarily by eggs. The species is very capable to utilize artificial water containers, vases, dishes, tins, pots, flower pot plates, bottles, cemetery urns, urban water drainage systems and especially used tires for deposition of eggs. Used tires are usually stored outdoor, and after each rainfall inside the tires there is some water, this amount of water is enaff for *St. albopicta* to complet it's life cycle (Reiter & Sprenger, 1987; Reiter, 1998; Lyon & Berry, 1991). It's ability to colonize used tires combined by intensive world wide scrap automobile and truck tires trade is the basis of rapid establishment of Asian tiger mosquito in new geographical areas – the drought-resistant eggs deposited in used tires can remain viable for a long period of time.

It's global distribution is enhanced by capability to overwinter in the egg-stage and therefore to permanently colonize temperate areas. January isotherm of 0°C, July 20°C and annual rainfall of 500 mm in more than 60 rainy days delineate the areas of risk for *St. albopicta* establishment (Knudsen, 1995).

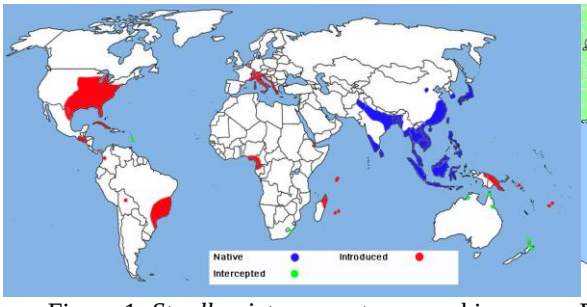


Figure 1: *St. albopicta* current geographic occurrence in the World [2]

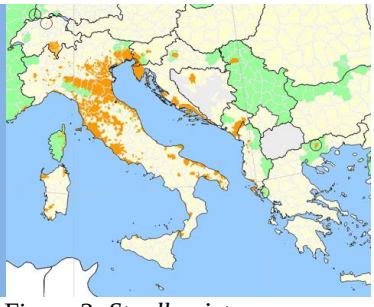


Figure 2: *St. albopicta* occurrence in South-East Europe [2]

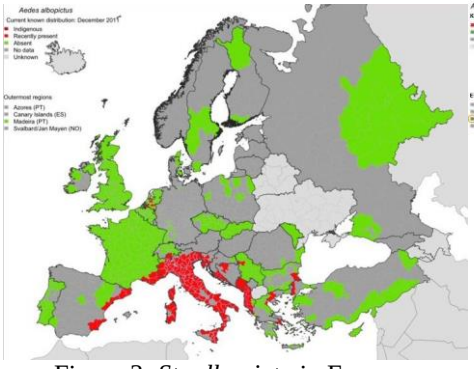


Figure 3: *St. albopicta* in Europe December 2011. (http://ecdc.europa.eu)

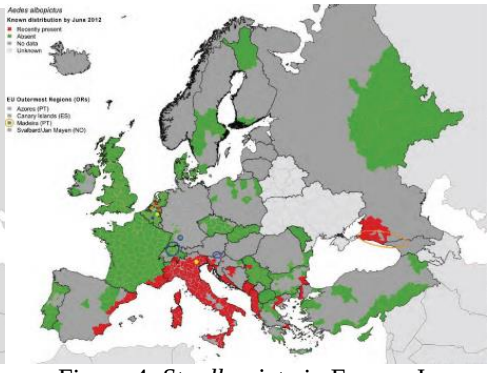


Figure 4: *St. albopicta* in Europe June 2012. (http://ecdc.europa.eu)

[2] *http://ecdc.europa.eu/en/publications/ter-mosquito-surveillance-guidelines.pdf*

So, main way of dispersion of this species throught the world is connected with combination of it's adaptability and antropogenic influence.

Until 1962 the scientific opinion was that this species is not able to compete successfully with other related species. But the species successfully and rapidly spread from Indo Malayan region to and from Japan due to the worldwide trade of used tires; in 1985 it appeared in the USA.

In Europe species was first reported in Albania at 1979 (Adhami, Murati, 1987; Adhami, Reiter, 1998). Than it is introduced in Italy 1990 in Genoa by used tires imported from USA (Sabatini et al, 1990; Dalla Pozza, Majore, 1992; Romi, 1995). Than it was found in northern France (Normandy) in 1999, also imported with used tires from USA (Schaffner, Karsh, 1999). The species was reported also for Belgium in 2000 (Schaffner et al, 2004). In the year 2001 tiger was found in the state of Montenegro (Petrić et al, 2001); than in Switzerland, probably Hungary in 2003, but no confirmation to date (Scholte & Schaffner, 2007), and in Croatia and Spain in the year 2004 (Klobučar et al, 2006; Aranda et al, 2006 loc. cit. in Kampen & Schaffner in Bonnefoy et al, 2008). In the year 2005 species was found in Bosnia and Herzegovina (Banja Luka), Greece, the Nederlands and coastal Slovenia (sea resort Portoroz) (Petrić et al, 2006.; Scholte et al, 2007 lok cit. Kampen & Schaffner in Bonnefoy et al, 2008). From the year 2005 we can conclude that all Continental Mediterranean European countries are infested with *St. albopicta* (Figures 2-4).

In order to raise official and public awareness on *St. albopicta* European Mosquito Control Association (EMCA) has pointed out the importance of the creation of a "continent widespread surveillance network as no country is safe from invasion of this species…". Due to its immense invasive capacity, it is listed in the inventory of "100 of the World's Worst Invasive Alien Species".

First specimen of *St. albopicta* in former Federal Republic of Yugoslavia was registered in Podgorica (42°28′12″N longitude; 19°16′48″E latitude; 44m above sea level) on August 21st 2001. One last instar larva was found in rural area of Zeta - Golubovci in water collected from used tires produced in France and imported from Germany. It was developed to a male adult in laboratory (Petrić et al, 2001; Becker et al, 2003; Petrić et al, 2012b). It is interesting that in bordering Albania, species was found in the Skutari neighbour municipality to Podgorica, but there is no clear evidence of introduction from neighboring Albania or from Italy.

During period 2002-2011 species is found in Podgorica (Petrić et al, 2003), Bar, Ulcinj, Tivat, Andrijevica, Budva, Herceg Novi (Petrić et al, 2006), Kotor and around Skadar Lake (Pajović et al, 2012, Petrić et al, 2012a).

From 2012 new project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" was launched, allowing systematic approach aimed to depict both distribution and numerosity of *St. albopicta* across Montenegro in next three years. First year surveillance is focused to Montenegro coast, and until 31st of August Asian tiger mosquito is detected on 41 out of 41 sampling sites.

MATERIAL AND METHODS

As mosquito larvae occur in a wide-range of natural aquatic habitats including ditches, ponds, tree holes and bromeliad plants. Besides natural, also artificial containers with stagnant water such as used tires, cemetery vases and buckets are known to be a suitable habitat. Used tires are usually abandoned, dumped, recapped or stored for trading and eventually re-used. Abandoned or outdoor stored tires are ideal mosquito incubators, exposed to the elements tires accumulate persistent water and organic material (Beeuwkes et al, 2011). In such circumstances the best way to monitor the mosquito population is using the traps.

In the survey programs carried out in Asia, America and Europe, *St. albopicta* is mainly monitored by means of ovitraps - rounded plastic dishes deep 20 cm, and 10 cm in diameter (Schaffner et al, 2012a).

The oviposition traps can be metal, plastic and glass made. The metal trap results are less sensitive and their use is not recommended, especially in low population density conditions. Since the differences between the plastic and glass traps with regard to sensitivity and conformity are not evident, the choice must be made based on their utility in the field. Evaluation characteristics are unit cost, shock resistance, the possibility of making a hole which, should it rain, will allow water to drain and consequently maintain constant water levels, and possibility of being stacked during transport (Bellini et al, 1996).

RESULTS AND DISCUSSION

In "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" project used oviposition traps consisted a black plastic container (total height: 15 cm, height of water gauge: 10 cm, diameter: 11.5 cm) filled with rainwater and with plywood strip (15x2.5 cm) in it, as an oviposition substrate (Figures 5-8).

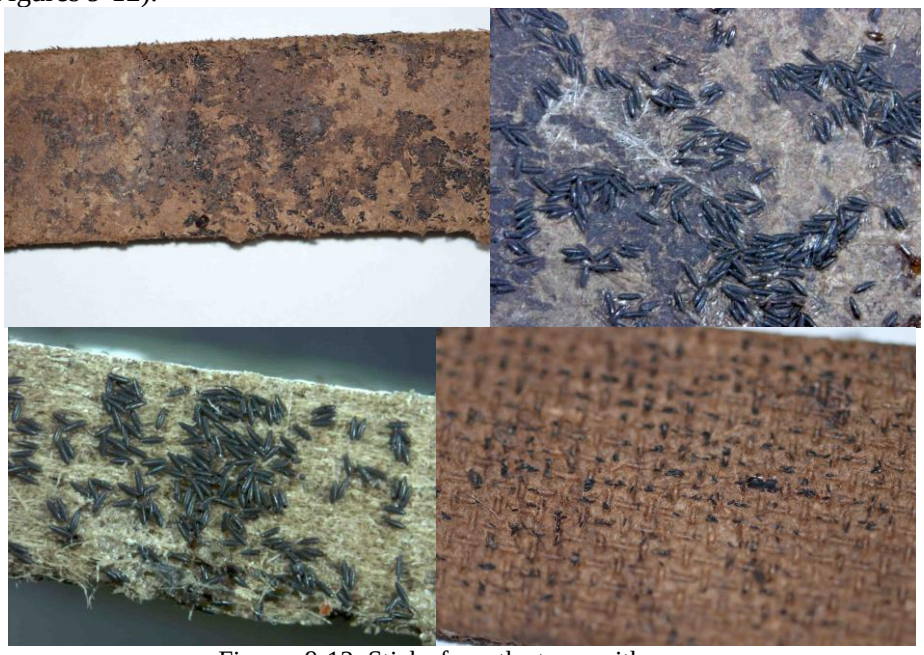
Figures 5-6: Ovitraps used in monitoring of *St. albopicta* in Montenegro 2012.

41 ovitraps were placed in green, shaded and easily accessible areas on Montenegro coast. They were positioned on the ground, with a free space above of at least 1 meter (Carrieri et al, 2012). The ovitraps were placed at used tire storage places (4), urban (11), semiurban (13), main resting places (12) and one

border crossing. The trap stations were kept fixed for the whole season and inspected every 10th day, from 07st May to 30th October. Each time the strips and water was collected and replaced.

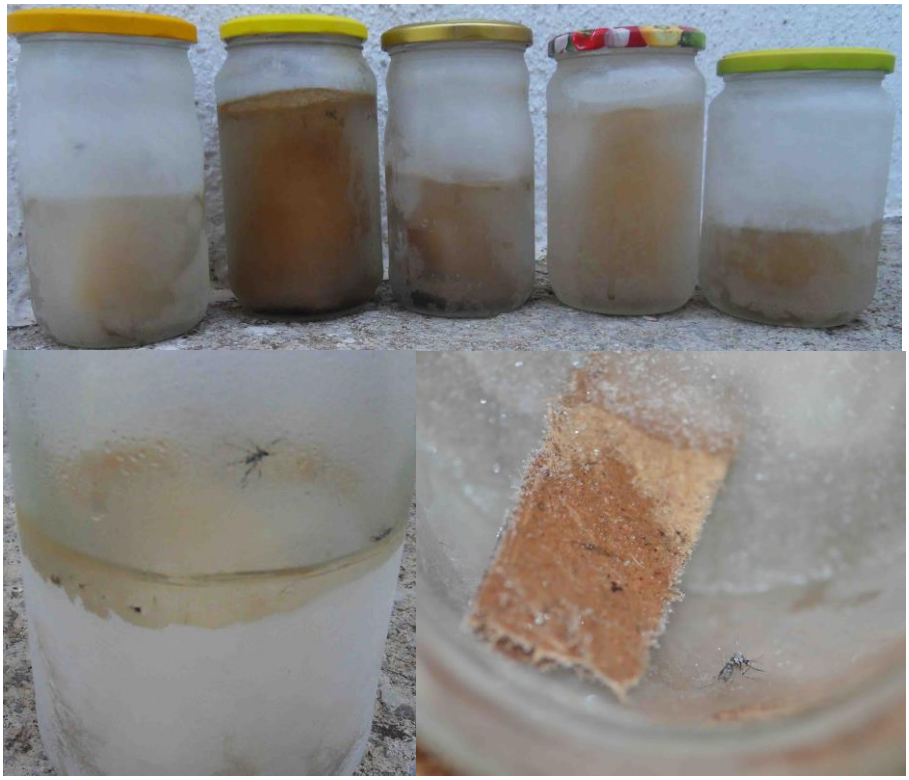
Figures 7-8: Ovitraps used in monitoring of *St. albopicta* in Montenegro 2012.

All samples were labeled and brought to the Laboratory in Biotechnical institute for identification. The number of eggs was counted by observing the oviposition strips under a microscope. Quite important is to count the eggs from all sides of strips, from frontal, back as well as from the sides part of the strips (Figures 9-12).



Figures 9-12: Sticks from the traps with eggs.

Figures 13-14: Brreading larvaes up to the adults in laboratory.



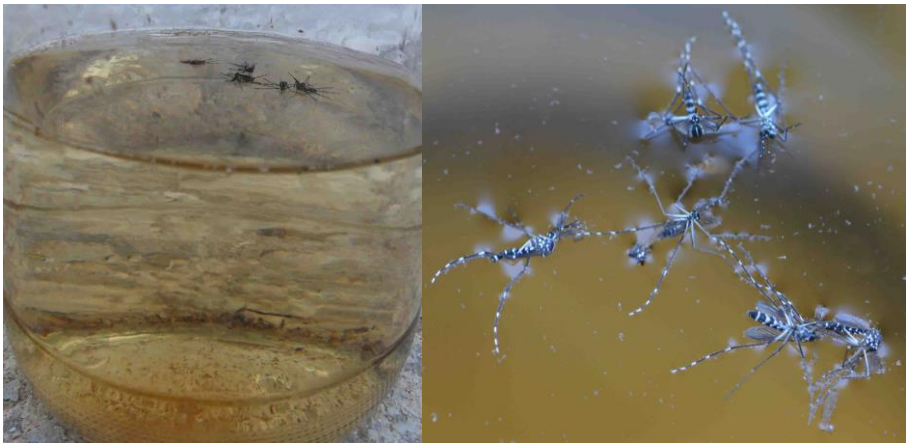
Figures 15-17: Freezing the sample content - killing the mosquitoes adults.

All strips from the ovitraps were dried before counting the eggs, and after counting they were placed in glass pots with the same collected water from the traps, to grow the larvae and adults (Figure 13-14).

Mosquitoes were identified morphologically by using the diagnostic keys (Becker et al, 2003, Schaffner et al, 2012b).

Quite important part of the procedure was freezing the sample content, or killing the mosquito's adults. After breeding the mosquitoes identification and determination, glass pots with content: water, strips, larvae and adults; were kept

in freezer 48 hours (Figures 15-17), than defrosted (Figures 18-19) and spilled to prevent contamination.



Figures 18-19: Freezing the sample content - killing the mosquitoes adults.

CONCLUSIONS

41 oviposition traps were used during May-October on Montenegrin coast.

Used oviposition traps consisted a black plastic container total height: 15 cm, height of water gauge: 10 cm, diameter: 11.5 cm. They were filled with rainwater. And as oviposition substrate were used plywood strip 15x2.5 cm.

Ovitraps were placed in green, shaded and easily accessible, they were positioned on the ground, with a free space above of at least 1 meter. The ovitraps were placed at used tire storage places (4), urban (11), semiurban (13), main resting places (12) and one border crossing. The trap stations were kept fixed for the whole season and inspected every 10^{th} day when the strips and water were collected and replaced. The number of eggs was counted by observing the oviposition strips under a microscope. All sides of the dried strip were checked counting the eggs.

After counting the eggs, strips were placed in glass pots with the same collected water from the traps, to grow the larvae and adults. Mosquitoes were identified morphologically by using the diagnostic keys. After breeding and the determination, glass pots with content were kept in freezer 48 hours, than defrosted and spilled to prevent contamination of *St. albopicta* free areas.

Using this kind of oviposition traps 80.582 eggs were counted from 671 positive traps, from total 738 - 67 traps were negative (9%).

ACKNOWLEDGEMENTS

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KORIŠĆENJE OVIPOZICIONIH KLOPKI ZA PRAĆENJE INVAZIVNIH KOMARACA - PRIMJER *STEGOMYIA ALBOPICTA* SKUSE, 1894 (DIPTERA: CULICIDAE)

SAŽETAK

Azijski tigrasti komarac, *Stegomyia albopicta*, je porijeklom orjentalna vrsta koja se u poslednjim dekadama proširila i nastanila u SAD, Centralnoj i Južnoj Americi, Africi i Evropi. Kako je vrsta prirodni vektor denga virusa i dvadesetak drugih ar-bo virusa njeno širenje u Evropi je shavćeno kao jako velika prijetnja javnom zdravlju.

Tokom 2012. prilikom rada na projektu "Utvrđivanje rasprostranjenja i brojnosti invazivne vrste invertebrata *St. albopicta* u Crnoj Gori" 41 crna plastična ovipoziciona klopka je korišćena na crnogorskom Primorju. Klopke su postavljane na zemlji u zelenilu, hladovini i na lako pristupačnim pozicijama, sa slobodnim prostorom iznad klopke od minimum jednog metra. Ovipozicione klopke su postavljene na četiri mjesta na kojima se čuvaju polovne automobilske gume, 11 u urbanim mjestima, 13 u semiurbanim, 12 lokacija u trurističkim mjestima i na jednom državnom graničnom prelazu. Pozicija klopki cijele sezone nije mijenjana, a uziman je sadržaj klopki svakih deset dana od sedmog maja do tridesetog oktobra. Pločice iz klopki su sušene, zatim su brojana jaja koja su položena na njih, a nakon toga su vraćane u istu vodu sakupljenu iz klopke u cilju gajenja larvi, odnosno dobijanja adulta. Komarci su identifikovani morfološki pomoću ključeva za determinaciju. Poslije dobijanja adulta, odnosno njihove determinacije sadržaj klopke (voda, pločica, larve i adulti) su držani u zamrzivaču 48 sati, zatim razmrzavani, pa tek onda bacani da bi se spriječila kontaminacija.

Koristeći ovaj tip klopki za ovipoziciju 80582 jaje je prebrojano iz 671 pozitivne klopke, od ukupno 738 pregledanih klopki samo 67 je bilo negativno.

Ključne riječi: *Stegomyia albopicta*, ovipozicione klopke, praćenje.