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AEDES ALBOPICTUS RESTISTANCE STATUS AND POPULATION DYNAMICS ACROSS THE SWISS-ITALIAN BORDER

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Abstract *Aedes albopictus* is a vector for over 20 viruses, including chikungunya and dengue and where present may pose a threat to human health. Since 2003 *A. albopictus* is also present in Southern Switzerland in the Canton of Ticino and has since then been continuously surveyed and controlled, mainly by larviciding and larval source reduction. The Italian communities just across the border, however, lack such a surveillance and intervention programme. Here, we examined the seasonal and spatial abundance of *A. albopictus* in forest and urban areas across the Swiss-Italian border during the mosquito's active season in 2012 and 2013, and measured the susceptibility of the local mosquito population against larvicides in use. We found that mosquito densities peaked between end of August and early September and that they were higher in Italy and in the urban areas. Laboratory bioassays showed that the local mosquito population is still susceptible to the applied larvicide, *Bacilus thuringiensis* var. *israelensis (Bti)*. Together the results support the hypothesis that the intervention programme in Ticino successfully lowers the local *A. albopictus* population.
Key words Culicidae, Asian tiger mosquito, vector control, *Bacilus thuringiensis* var. *israelensis*

INTRODUCTION

The Asian tiger mosquito *Aedes (Stegomyia) albopictus* (Family Culicidae; Skuse, 1894) originates from Southeast Asia. Over the recent decades *A. albopictus* has spread to the United States of America, Europe, Latin America and Africa, primarily by transport of dormant eggs in used tyres and trade of "lucky bamboo", *Dracaena sanderiana* (Scholte et al., 2007). At regional level, ground transport (i.e. cars and lorries) further added to the passive dispersal of the mosquito. Once the vector is established in a new area, *A. albopictus* may then also disperse actively to suitable habitats (Straetemans, 2008).

A. albopictus is a competent vector for at least 22 arboviruses, notably dengue, chikungunya, yellow fever, West Nile fever and *Dirofilaria* (Gratz, 2004). Establishment of this mosquito species has led to autochthonous cases of chikungunya in Ravenna, Italy with over 200 confirmed cases in 2007 (Angelini et al., 2008; Sambri et al., 2008) and dengue in Croatia and metropolitan France in 2010 (La Ruche et al., 2010; Gjenero-Margan et al., 2011).

In Switzerland, the first *A. albopictus* mosquitoes were detected in the Canton of Ticino in 2003 (Flacio et al., 2004) and was since then sporadically re-introduced to Ticino until the situation changed significantly in 2007 when in Chiasso, at the border to Italy, the monitoring network detected a dramatic increase in positive mosquito traps, indicating that a local mosquito population had then been established (Wymann et al., 2008). In response to this increase the monitoring network in Ticino has been expanded and interventions including larviciding were implemented. Today, over 1,200 ovitraps (traps collecting the eggs of container breeding *Aedes* mosquitoes) are used for surveillance in Ticino. In contrast to Ticino no such systematic programme exists across the border in Northern Italy.

In the present study, we set out to investigate whether we find evidence that the Ticino model may have a an impact on the local mosquito population and whether *A. albopictus* would be more present in human settlements as compared to the forested surroundings. In addition, the susceptibility of the local *A. albopictus* population to *Bti* was studied in order to assess the efficacy of the microbial larvicide that is part of the overall intervention strategy.

MATERIALS AND METHODS

Spatial and Temporal Distribution of *A. albopictus*

Surveys of relative mosquito abundance across the Swiss-Italian border were carried out in July – November 2012 and May - November 2013. For the surveys an area of 118 km^2 has been chosen from each of the adjacent side of the Swiss-Italian border (65 km^2 on the Italian side and 53 km^2 on the Swiss side). Using the ArcGIS version 10.0 (ESRI Inc., USA) geographic information system (GIS) software the study area was divided into grid squares, each measuring 250 m by 250 m. The grid squares were then stratified into "urban" and "forest" areas. A grid square was classified as forest area if 50% or more of the area within that square was covered with trees, and vice versa. From the total grid 70 squares (35 urban area squares and 35 forest area squares) were randomly selected in each country. Within a selected grid square relative *A. albopictus* density was then sampled by placing two ovitraps at a distance of at least 50 m between them to avoid interference. All traps were geo-referenced.

Ovitraps mimic breeding sites, attracting gravid females to deposit their eggs. Our traps consisted of 1.5 litre, black plastic flower pots, filled with tap water. The eggs were then collected on a wooden paddle that was partially submerged and partially stuck out of the water. In order to prevent the ovitraps from becoming potential breeding sites we added larvicide granules of *Bti.* The paddles were replaced biweekly and eggs, where present, counted with the aid of a stereo microscope.

Larval Susceptibility Assays

For the larvicide susceptibility bioassays, colonies were established from eggs collected in ovitraps and reared at the insectarium of the Department of Entomology-CPqAM at 26±1 °C temperature, 70±5 % relative humidity and a light:dark photoperiod of 12:12 hours. Larvae were kept in trays filled with tap water and fed with cat chow (Friskies – Seafood Sensations, Nestlé Purina Pet Care). Adults were fed with 10% sucrose solution and females given chicken blood.

The susceptibility of two *A. albopictus* colonies and the *A. aegypti* Rockefeller, an insecticide susceptible reference colony, were assessed against the *Bti* standard IPS-82 reference strain from the Institute Pasteur, Paris (containing a mixture of crystals and spores). The two *A. albopictus* colonies were the "RecAlb_Lab" colony, a laboratory reared *A. albopictus* colony founded from mosquitoes collected in Recife, Brazil and "Switzerland", a colony established from field-caught *A. albopictus* of the 2012 field survey in Switzerland.

Larval bioassays were conducted following the guidelines for larval susceptibility tests of the World Health Organization (WHO) Pesticide Scheme (WHO, 2005). Data were reported as the lethal concentration that would kill 50% or 90% of the larvae (i.e. LC_{50} and LC_{90}, respectively).

RESULTS AND DISCUSSION

Spatial and Temporal Distribution of *A. albopictus*

In total, 6,440 paddles containing 225,120 eggs of *A. albopictus* were recovered during a trapping period of 48 weeks (20 weeks in 2012 and 28 weeks in 2013). Number of eggs per trap ranged between 0 and 1,537 eggs. Egg counts reached a maximum around end of August, beginning of September in both years (Figure 1), in accordance with previous studies (Carrieri et al., 2011; GLZ, 2013).

Throughout the whole season egg densities were lower in Ticino as compared to Italy and lower in the forest than in the urban areas (Figure 1). At the peak, the ratio of the total egg counts between the Italian and Swiss urban was 2.2 and 2.6 in 2012 and 2013, respectively. The ratio in peak egg counts between urban and forest areas was also rather similar across the two countries and years, being 3.6 (2012) and 2.3 (2013) in Switzerland and 3.2 (2012) and 2.3 (2013) in Italy.

The results are consistent with the hypothesis that the intervention programme in Ticino has an impact on mosquito densities although other reasons such as potential differences in breeding site quality, mosquito species competition etc. might also explain the differences observed in this study.

Susceptibility to *Bti*

All colonies tested showed LC_{50} and LC_{90} values typical for *Bti* susceptible mosquitoes (Kamgang et al., 2011; Liu et al., 2004) albeit the Switzerland colony was slightly less susceptible than the Rockefeller *A. aegypti* reference strain and the RecAlb_Lab laboratory colony (Table 1).

Table 1. Susceptibility in third instar larvae of *Aedes* spp. against *Bacillus thuringiensis* var. *israelensis*.

Species	Colony	No. larvae exposed	LC_{50} (95% CI) [mg/l]	LC_{90} (95% CI) [mg/l]
Aedes aegypti	Rockefeller	1,620	0.008 (0.007-0.009)	0.026 (0.021-0.036)
Aedes albopictus	RecAlb_Lab	1,080	0.009 (0.008-0.011)	0.028 (0.023-0.037)
	Switzerland	1,440	0.015 (0.012-0.018)	0.036 (0.030-0.060)

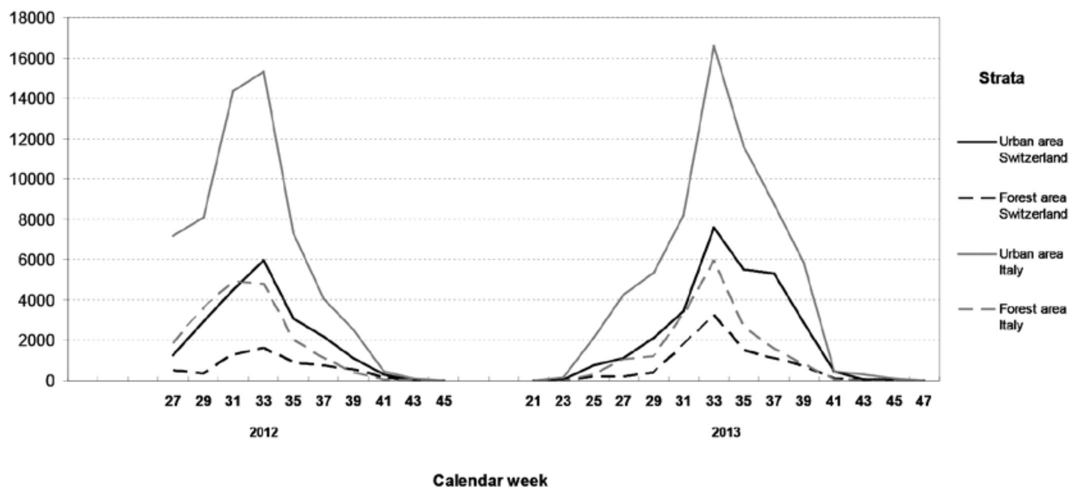

Figure 1. Temporal and spatial distribution of *Aedes albopictus* across the Swiss-Italian border. Numbers show total egg counts for each strata and time point.

CONCLUSIONS

The larvicide, *Bti* currently in use against *A. albopictus* in Ticino, Switzerland is still effective and, although not conclusively, the data support the hypothesis that the current intervention programme in place significantly reduces mosquito densities in the control area.

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