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INTEGRATED CONTROL OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) IN ALLOTMENT GARDENS, RESIDENTIALS, AND SEWERAGE SYSTEMS

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Abstract The mosquito species *Aedes albopictus* (Skuse, 1895) has spread rapidly in Europe over the last 40 years and has been established as an invasive species in Germany for the last 15 years. In 2019, individual specimens were found for the first time in the allotments "SÜD I", "SÜD II" and the adjacent "Kalbssiedlung" in Fürth, Germany, and were identified as an overwintering and established population by the monitoring of the company Biogents AG in 2020. Due to the massive extent, regular comprehensive control measures were carried out by the pest control company APC AG from September 2020 to October 2021. The control concept included 1.) the information of the citizens with the call for active cooperation, 2.) the monitoring measures to identify the hotspots and 3.) the actual control actions in all gardens and in the public sewerage with *Bacillus thuringiensis israelensis* (Bti) as well as passive traps. Parallel to the control, external monitoring with active and passive traps was carried out by the Biogents company to control the measures and to determine the further spread of *Ae. albopictus*. Due to the control procedures, the tiger mosquito population in Fürth was strongly decimated. Monitoring data from the company APC showed a decrease of 86% in the allotment "SÜD I" in 2021 compared to the previous year. The data of the company Biogens recorded a decline of 67% in the entire control area, but also a small expansion of the population into adjacent areas. For sustainable containment of the tiger mosquito population in Fürth, control measures and external monitoring must be continued in subsequent years by professional staff. Individual measures can be taken over by trained janitors or allotment gardeners with regular control.

Key words Tiger mosquito, invasive species, vector control, active and passive traps

INTRODUCTION

Increasing globalization and the associated increase in travel and trade are promoting the introduction of non-native species. Facilitated by global warming, many of these exotic species can easily establish themselves in the new environments and continue to spread. When they cause severe environmental, economic, and health damage, these species are called invasive (Keller et al., 2011). Among the mosquito species that are invasive in Europe, *Aedes albopictus* (Asian tiger mosquito), *Aedes aegypti* (yellow fever mosquito), and *Aedes japonicus* (Asian bush mosquito) play an important role because they have already established themselves in some European regions and are vectors of various human and animal pathogenic diseases (Medlock et al., 2012). Autochthonous transmissions of chikungunya, dengue, and Zika viruses have been consistently recorded in Mediterranean regions (Angelini et al., 2007; Lindh et al., 2018; Barzon et al., 2021). The geographical occurrence of these invasive mosquitoes is therefore closely monitored by the European Centre for Disease Prevention and Control (ECDC) (ECDC, 2021).

The Asian tiger mosquito originates from Southeast Asia but has spread rapidly in Europe in recent decades (Benedict et al., 2007, Bellini et al., 2020, Sabatini et al., 1990). Within a few years, this mosquito species also arrived in Germany. The first finding of four *Ae. albopictus* eggs was detected in 2007 at a motorway service station on the A5, a truck route from Italy (Pluskota et al., 2008). The first local mass development of *Ae. albopictus* was documented in 2015 at an allotment site in Freiburg, southwestern Germany, and has since been controlled and closely monitored (Becker et al., 2017). In summer 2019, several *Ae. albopictus* were detected for the first time via the Citizen Science project "Mosquito Atlas" in the Franconian city of Fürth. Monitoring conducted the following year revealed an already very large and established population of tiger mosquitoes in the allotments and adjacent settlements (Göttler et al., 2021). In order to control the tiger mosquito population there, the pest control company APC AG was commissioned to draw up a comprehensive control concept and carry out the control measures.

MATERIAL AND METHODS

The control concept and measures were developed and implemented in close cooperation with the Office for Environment, Public Order and Consumer Protection of the City of Fürth and the company Biogents. The control measures started in 2020 with the area "SÜD I" and "SÜD II" and "Kalbssiedlung" (Figure 1; black marker) with approx. 40 ha. "SÜD I" includes 189 and "SÜD II" 157 allotments. The adjacent "Kalbssiedlung" includes 43 three-story apartment blocks with 666 units and 222 gardens with some additional gardens for tenants and owners between the apartment blocks. In addition, the garden of 11 single-family houses were included. In 2021, three more allotment sites (A1 with 56, A3 with 33, and A4 with 44 allotments) and 36 more blocks with 612 housing units and 204 gardens were added to the area (85 ha; Figure 1; red marker; extended area 1 and 2). In cooperation with the local urban drainage authority, all sewers (manholes, gullies, drains, street drains) were mapped and treated from 2021, as infestations were detected during random inspections (total control area: 150 ha; Figure 1; blue marker). The control measures started in September 2020 and were carried out at approximately four-week intervals. From week 26 in 2021, the interval in "SÜD I" and "SÜD II", the "Kalbssiedlung", the extended area 1 and the sewerage was shortened to approximately two weeks (Table 1).

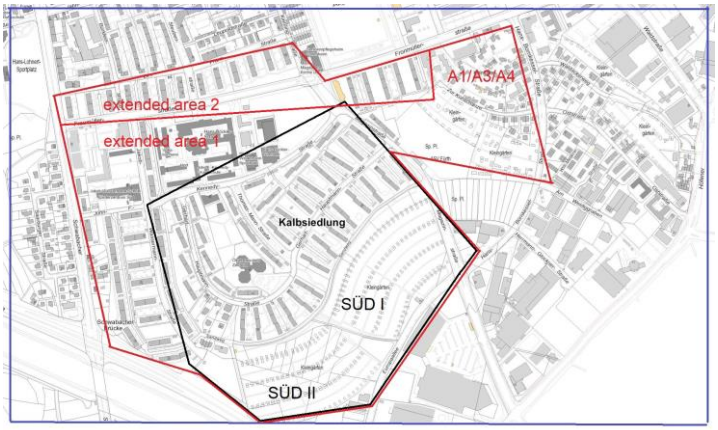

Figure 1 Control area Fürth. The allotment sites "SÜD I" and "SÜD II" and the adjacent "Kalbssiedlung" represent the hotspot (black marker). In the extended area 1 and 2 and in the allotment sites A1/A3/A4 (red marker) clearly less tiger mosquitoes were caught. In the entire area (blue marker), the public sewerage was treated.

	2020		2021								
SÜD I / II	36	41	20	26	29	31	33	36	38	40	42
Kalbssiedlung	39	-	21	26	29	31	33	35	37	39	41
Extended area 1	-	-	21	26	29	31	33	35	37	39	41
Extended area 2			21	26	29	-	33	35	38	-	42
A1/A3/A4	-	-	21	26	29	-	-	35	38	-	42
Sewer system	-	-	21	26	29	31	33	36	38	40	43

Table 1 Overview of the time points of the control measures in Fürth in calendar weeks. "SÜD I", "SÜD II" and "A1/A3/A4" (allotments).

Cooperation of citizens. Success can only be achieved through comprehensive information of the citizens and their active cooperation. To this end, several information letters were sent to the residents of the "Kalbssiedlung" and the tenants of the allotment gardens. Flyers with contact details of APC and Biogents were posted in the corridors of the "Kalbssiedlung" and in the showcases of the allotment sites. A permanent contact person was always on site who managed the control measures and answered all questions.

The active cooperation of the citizens included the following: (1) allowing access to the gardens; (2) emptying, inverted dry storage and, if necessary, disposal of all containers where water can accumulate (buckets, watering cans, flower pots, toys, wheelbarrows, vases, coasters); (3) weekly emptying and cleaning of other breeding sites (bird feeders, gutters); (4) filling coasters with sand or gravel; (5) covering rain barrels, cisterns and catch basins with tight mesh netting or lids. Implementation of these items was checked by APC during regular inspection activities. In addition, a checklist was prepared for allotment tenants ("SÜD I / II") that was distributed at the end of the 2021 treatment season. The assessments allowed tenants to see where there was room for improvement in their garden.

Internal monitoring. To determine the population density and distribution and/or hotspots of *Ae. albopictus* over time, the entire control area was mapped and all potential breeding sites (rain barrels, cisterns, etc.) were listed. In addition, 510 passive BG-GAT traps (gravid aedes trap; BG-GAT Neighborhood Bundles; Biogents) were regularly distributed and their locations documented. The passive BG-GAT traps specifically attract female tiger mosquitoes in search of a suitable oviposition site. The traps are specifically designed for tiger mosquitoes, which are primarily container breeders and frequent artificial small water bodies. In the trap, the mosquitoes remain attached to a sticky surface of a sticky card. The number of female *Ae. albopictus* on the BG-GAT trap sticky cards was determined and noted at each control round based on the white line on the head and chest.

External monitoring. In order to control the effectiveness of the measures and to investigate a further spread, an independent monitoring (=external monitoring) was commissioned by the city of Fürth. For this purpose, Biogents distributed 38 passive BG-GAT traps and 8 active BG-Pro traps (BG-Pro All-In-One; Biogents). The active BG-Pro traps specifically attract all types of female mosquitoes looking for a blood meal. The BG-Pro traps were activated at two-week intervals with CO_2 (Biogon® C E290 2 kg short; Unterbichler Gase GmbH) and an attractant (BG-Sweetscent; Biogents) for 24 hours using a power bank. The trapped mosquitoes were collected the next day, frozen, and determined macro- and microscopically. The BG-GAT traps were permanently in place and the sticky cards were replaced and checked for mosquitoes every two weeks. The locations of the traps were based on what was found in 2019 and 2020, as well as the control area (red marker Figure 1). The outer limits of monitoring were set within an approximately one-hundred-meter radius of the control area to determine possible expansion of *Ae. albopictus*. For the area of expansion, only passive BG-GAT traps were used. Within the area of control, passive and active traps were used. By using both traps, area-wide monitoring can be ensured to detect all mosquito species and determine their population dynamics.

Control. In each control round, after prior announcement by ringing the front doorbell or calling loudly, every accessible garden was entered and the following measures were carried out. (1) all small containers that could collect water were emptied, inverted, and stored away from the rain if possible; (2) all water containers that could not be emptied (rain barrels, cisterns, catch basins) were treated with Bti (1. 000 ITU/mg; Culinex Tab plus, registration no. DE-0003009-18) in H_20 at a concentration of 2.75 mg/ml by spraying with a pressure spray pump (Gloria; special pressure sprayer; type 89; 1 liter; PPS GmbH). 200 ml was used in 200l rain barrels / underground cisterns, 600 ml in large water catchment basins and 1000l cisterns, and between 100-600 ml in ponds without fish. Ponds with fish or other insectivorous animals were not treated. In addition, 2 Bti Tabs undiluted were used as a depot in full rain barrels / water catch basins. Heavy coasters, umbrella stands, handle pumps, and the manholes under downspouts (leaf catch baskets were emptied prior to treatment) were treated with circa 10-200 ml of Bti solution and ½-1 Bti Tabs, depending on the surface area. Random checks were made circa ½ h after treatment for dead mosquito larvae. The presence and location of live larvae was documented during each control round; (3) In addition to monitoring the passive BG-GAT traps simultaneously serve as an effective control measure by catching egg-laying females in large numbers. The number of *Ae. albopictus* trapped was regularly documented as part of the monitoring; (4) 600 rain barrel nets (GartenMeister rain barrel net, 95 cm, black; EAN 04041908123367; Westfalia) were distributed to cover the 200l rain barrels. Despite nets, the rain barrels were regularly treated with Bti.

Sewer manholes in private and public areas were treated with the product Vector BAC WG (3.000 IU/mg; DE-0011520-18) in water by spraying. Here, a stock solution of 0.9 mg/ml in water was filled into 25l canisters and 200 ml of each was sprayed with the pressure spray pump and a lance directly into the water or through the openings of the hanging baskets. Bti Tabs were not added additionally.

RESULTS

Cooperation of the citizens. The percentage of accessible gardens in the “Kalbssiedlung” ranged from 75.2±12.8 to 93.4±6.5 (mean±SD) per treatment in 2021. Nearly all gardens in the “Kalbssiedlung” were accessible at least once during the course of the interventions, with approximately ten residents behaving uncooperatively at times and denying access to their gardens. The majority of residents in the “Kalbssiedlung” fully implemented the recommended measures. Almost all allotments of “SÜD I” and “SÜD II” were permanently accessible or could be entered through neighboring gardens after agreement of the garden boards. More than 60% of the allotments of “SÜD I” and “SÜD II” were in optimal condition at the end of the 2021 treatment season, with partial implementation of measures in up to 20% of gardens and inadequate to no implementation in up to 20% of gardens.

Internal monitoring. The results of the internal monitoring show a significant decrease in the average number of adult *Ae. albopictus* in "SÜD I" and "SÜD II" in 2021 compared to the same period in 2020 and an overall low activity in the whole treatment season in 2021 (Figure 2). While 14.3±9.98 and 7.2±6.3 (mean±SD) tiger mosquitoes per trap were counted in "SÜD I" and "SÜD II" in calendar weeks 36-41 in 2020, the numbers were 2.0±1.3 and 1.6±1.0 (pooled data from calendar weeks 36-42) in 2021, representing a decrease of 86.1% for "SÜD I" and 77.8% for "SÜD II". In 2020, only one treatment was conducted in the “Kalbssiedlung”, so BG-GAT trap catches in 2020 were not evaluated and no comparative data are available for the “Kalbssiedlung” for 2020.

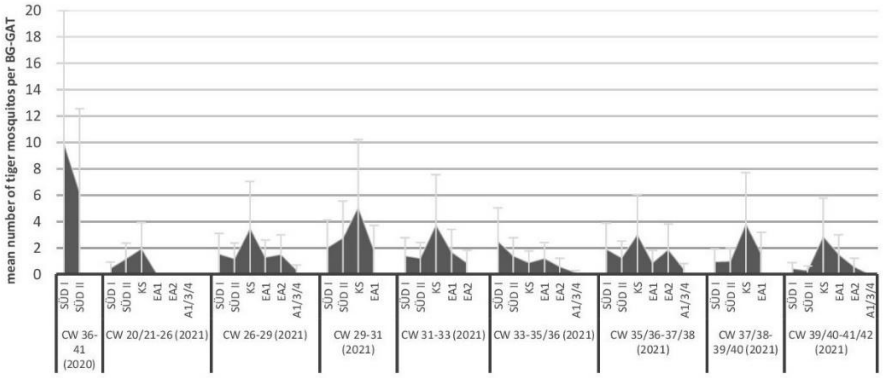

Figure 2 Mean number of female tiger mosquitoes captured per BG-GAT trap (n=510) per calendar week in 2020 and 2021. “SÜD I” and “SÜD II” (allotments); “KS” (Kalbssiedlung); “EA1” (extended area 1); “EA2” (extended area 2); “A1/3/4” (allotments A1, A3, and A4); “CW” (calendar week). Internal monitoring by APC.

External monitoring. The results of the monitoring programs clearly show that the measures taken to control tiger mosquitoes in Fürth were successful. For example, the evaluation of the BG-Pro traps showed that in 2021 only half the previous year's quantity of tiger mosquitoes were caught. The evaluation of the BG-GAT traps even shows a reduction of 75% (Table 2). Figure 3 shows the average female tiger mosquitoes captured per BG-GAT trap per calendar week in 2020 (grey line) and 2021 (blue line). In 2020, the tiger mosquito population increased very sharply from week 26 and then remained at a very high level for 3 months, which was about 10 times higher than the following year. In 2021, the number of tiger mosquitoes captured also increased from week 27, but only very slightly. Thereafter, tiger mosquito capture rates remained at a low level throughout the season. At the end of July 2021, the first *Ae. albopictus* appeared in the BG-GAT traps 200 meters outside the control area. At the sites where specimens were repeatedly found, it could be assumed that they were already reproducing.

Traptype	*Year*	*Housemosquitoes* (*Culex pipiens, Culiseta annulata*)	*Floodwatermosquitoes* (*Ae. vexans, Ae. sticticus, Ae. cinereus*)	*Tigermosquitoes* (*Ae. albopictus*)
BG-Pro	2020	941	57	488
	2021	1264	782	280
BG-GAT	2020			1192
	2021			287

Table 2 Total number of determined female mosquito species from external monitoring by Biogents.

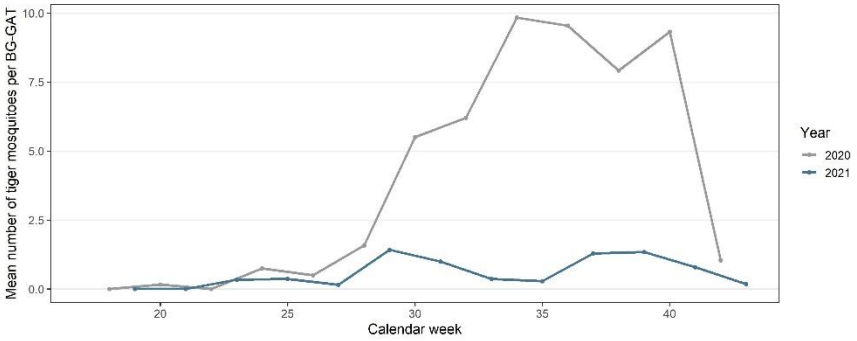

Figure 3 Mean number of female tiger mosquitoes captured per BG-GAT trap per calendar week in 2020 and 2021. External monitoring by Biogents.

Control. Regular interventions in all gardens and in private and public sewerage have greatly reduced infestations, as evidenced by internal and external monitoring. In addition, BG-GAT traps from APC and Biogents have already intercepted at least 3498 egg-laying *Ae. albopictus* females in 2020 and prevented up to 1 million offspring, assuming that a female can lay 150-300 eggs during its lifetime (Delatte, 2009; Hawley, 1988).

DISCUSSION

According to Becker et al. (2017), mass development of *Ae. albopictus* is typically defined when biting tiger mosquitoes are observed and more than 1,000 *Ae. albopictus* in various developmental stages are present in an area of about 3 ha. Between September and October 2020, we already captured more than 3,500 adult female tiger mosquitoes ready to lay eggs within a 40-ha radius. In addition, we were regularly attacked and tracked by the tiger mosquitoes, so that mass development was already present in 2020. During external monitoring in 2020, the first specimens of *Ae. albopictus* were detected as early as early May. Due to the Corona pandemic, travel and freight were severely restricted, making a reintroduction in spring 2020 unlikely. Moreover, the captured animals were in very good morphological condition. The early occurrence and good conservation status of the animals indicate that this is an already established and reproducing population.

It was recommended by Pluskota et al. (2018) to use 8,000-9,000 ITU/liter Bti for the initial treatment of the 200l rain barrels and 5,000-6,000 ITU/liter for the post-treatments. Since a majority of the rain barrels and water catchments were organically contaminated, we used a total of 8,250 ITU/liter permanently for the 200l rain barrels. The tablets used in addition to the spray treatment were to serve as a depot during heavy rains, as they dissolve with a slight time delay. Due to the persistent heavy rainfalls in summer 2021, it was not sufficient to treat the breeding sites with Bti every four weeks, as described for other cities (Kroeger et al., 1995; Becker et al., 2022). During visual inspections, we saw larvae in the first larval stage after only two weeks of treatment and larvae in the last larval stage after four weeks. After the five-week treatment break, the increase was also clearly evident in monitoring (Figure 2; increase week 29 and Figure 3, increase week 27). Therefore, we shortened the treatment interval in the most heavily infested areas to two weeks.

The main breeding sites in the allotments were rain barrels, cisterns, and water catch basins, which were easily treatable. Irregular hotspots in the allotments could mostly be assigned to breeding sites that could not be treated during the last pass (due to closed doors, filled wading pools, etc.). In the “Kalbssiedlung”, occurring hotspots could not always be assigned to breeding sites. The hotspots were increasingly located near “SÜD I” in shady bushes of the “Kalbssiedlung”. Since tiger mosquitoes have been shown to have a flight radius of up to 250 m (Marini et al., 2010), it is possible that they hatched in the allotments and flew into the bushes of the “Kalbssiedlung” 50 to 200 m away. Other possible breeding sites for these hotspots include roofs of trash can houses, flat roofs of the kindergarten and school, clogged gutters, or the flower pot coasters and planter boxes on balconies, which will be objects of further investigations in 2022.

A special feature in the “Kalbssiedlung” are the handle pumps placed in each garden with access to underground cisterns. Because these cisterns were obstructed in most gardens and could not be opened, all openings of the handle pumps and cistern lids and all accessible supply lines to the system were treated with Bti. For the next treatment season in 2022, active BG Mosquitaire CO_2 traps (Biogents) will be placed in the hotspot areas of the “Kalbssiedlung” to trap adult mosquitoes, in addition to the passive BG-GAT traps (Degener et. al., 2019). The use of sand and gravel in flowerpot coasters should be more heavily controlled and professionally implemented. Measures should be expanded into surrounding areas following tiger mosquito findings from external monitoring, and additionally, balconies should be included in measures. Furthermore, the information event planned for 2020/2021 for the allotment gardeners, respectively the residents of the calf settlement should be made up for and a training of the janitors for the independent control of the breeding sites should be carried out.

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