Increased detection of Aedes albopictus in Belgium: no overwintering yet, but an intervention strategy is still lacking

Article *in* Parasitology Research · June 2015

DOI: 10.1007/s00436-015-4575-z · Source: PubMed

6 authors, including:

Isra Deblauwe
Institute of Tropical Medicine
50 PUBLICATIONS **931** CITATIONS

Adam Hendy
University of Texas Medical Branch at Galveston
28 PUBLICATIONS **337** CITATIONS

Charlotte Sohier
Sciensano
14 PUBLICATIONS **92** CITATIONS

M. Madder
University of Pretoria
261 PUBLICATIONS **2,454** CITATIONS

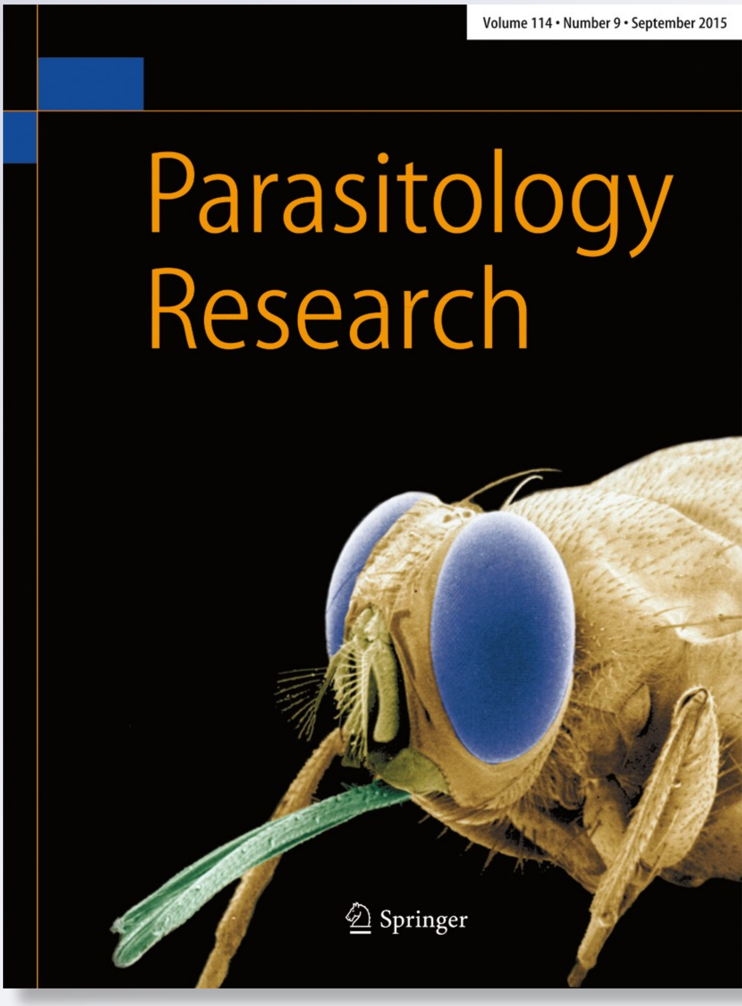
Increased detection of Aedes albopictus *in Belgium: no overwintering yet, but an intervention strategy is still lacking*

Isra Deblauwe, Julie Demeulemeester, Jacobus De Witte, Adam Hendy, Charlotte Sohier & Maxime Madder

Parasitology Research
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113
Volume 114
Number 9

Parasitol Res (2015) 114:3469-3477
DOI 10.1007/s00436-015-4575-z



Parasitol Res (2015) 114:3469–3477
DOI 10.1007/s00436-015-4575-z

ORIGINAL PAPER

Increased detection of *Aedes albopictus* in Belgium: no overwintering yet, but an intervention strategy is still lacking

Isra Deblauwe[1] · Julie Demeulemeester[1] · Jacobus De Witte[1] · Adam Hendy[1] · Charlotte Sohier[1] · Maxime Madder[1,2]

Received: 23 April 2015 / Accepted: 10 June 2015 / Published online: 26 June 2015

Abstract In 2013 and 2014, routine surveillance for invasive mosquito species was implemented in Belgium at 13 potential points of entry. Following the introduction of *Aedes* (*Stegomyia*) *albopictus* (Skuse 1895) to Belgium via a used tyre import company (Vrasene, Province of East Flanders) in July 2013, one female and 17 larvae were collected outdoors during a period of intensive surveillance in summer and autumn 2013, but no control measures were implemented. Although climatic conditions were suitable during the winter of 2013–2014, this reproducing population did not overwinter. Lack of genetic variation, incomplete diapause adaptation and egg desiccation due to long dry periods during diapause or competition with endemic species are possible reasons. More studies on the diapause/longevity of *Ae. albopictus* eggs in northern temperate climatic conditions and on the competition with endemic species in western and central Europe are warranted to assess the potential for this invasive mosquito to overwinter. Furthermore, following the detection of four *Ae. albopictus* larvae in a shipment of lucky bamboo at the port of Antwerp in August 2014, one female, one male, 11 pupae and six larvae were collected at the destined lucky bamboo company (Lochristi, Province of East Flanders) in autumn 2014. In this case, immediate control measures were successfully implemented at the nursery. Because of increasing threats and the absence of an invasive mosquito species control policy in Belgium, the need for a permanent vector surveillance and control plan has never been so high.

Keywords Tiger mosquito · Overwintering · Surveillance · Control · Tyres · Lucky bamboo

Electronic supplementary material The online version of this article (doi:10.1007/s00436-015-4575-z) contains supplementary material, which is available to authorized users.

✉ Isra Deblauwe
ideblauwe@itg.be

1 Veterinary Entomology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine (ITM), Nationalestraat 155, 2000 Antwerp, Belgium

2 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa

Introduction

Mosquitoes and mosquito-borne diseases pose an increasing threat to animal and human health in temperate regions. Important drivers for their emergence and spread include climatic and environmental changes and a significant increase in international trade, tourism and travel (Harrus and Baneth 2005; Becker 2008). One of the most invasive species in the world is the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse 1895) (*Stegomyia albopicta* sensu Reinert et al. 2004). During the last three to four decades, this container-breeding mosquito has expanded its range because of the international trade of used tyres, lucky bamboo shipments and transport on major traffic routes (Medlock et al. 2012). In Europe, it has become established in the Mediterranean region with the most northern distribution range being Rhône-Alpes in France, Ticino in Switzerland and Trentino-Alto Adige in Italy (ECDC 2015). However, a breeding population, not in the vicinity of tyre storage facilities, horticultural companies and motorways, was recently found much more north in Freiburg, southern Germany (Werner and Kampen 2015).

The settling of *Ae. albopictus* in southern Europe is responsible for the recent localised transmission of chikungunya and dengue viruses (Gould et al. 2010; Rezza et al. 2007;

Schaffner and Mathis 2014; Schaffner et al. 2014). Repeated introductions of *Ae. albopictus* occurred in Germany (Becker et al. 2013; Kampen et al. 2013) and the Netherlands (Scholte et al. 2008, 2012), and the species is increasing its distribution northwards (ECDC 2015; Werner and Kampen 2015). Although the colonisation of western and central Europe by *Ae. albopictus* is unlikely to be prevented, the process can be slowed down by avoiding introduction and controlling vectors in existing habitats. Mosquito surveillance and sustainable integrated mosquito control is therefore needed (Schaffner and Mathis 2014).

Ae. albopictus was first reported in Belgium on 31 October 2000 at a used tyre import company in Vrasene (Province of East Flanders) during an extension of a French surveillance programme (Schaffner et al. 2004). Only one pupa and one larva were found. After further inspections and short-term surveillance projects between 2001 and 2012, it became clear that the species did not establish in the area (Deblauwe et al. 2014; Versteirt et al. 2012; Schaffner unpublished data). An invasive mosquito species (IMS) surveillance programme was set up by the Institute of Tropical Medicine (ITM) in Belgium in 2012 to evaluate the guidelines of the European Centre for Disease Prevention and Control (ECDC) (Deblauwe et al. 2014) and is still ongoing at 12 potential points of entry (PoEs). During routine surveillance by ITM in 2013, the University of Liège also sampled mosquitoes at the same tyre company as in 2000 for an endosymbiont study and collected one male tiger mosquito in July (Boukraa et al. 2013). ITM immediately intensified surveillance at this company and continued to investigate possible overwintering in 2014. Also in 2013, a single, live *Ae. albopictus* larva was detected in a shipment of hygroscopic gel-transported lucky bamboo in the port of Antwerp (Demeulemeester et al. 2014). Here, we report results of the routine and intensified IMS surveillances in 2013 and 2014 and of the subsequent efforts to control *Ae. albopictus* populations.

Materials and methods

Routine surveillance

Potential PoEs (Fig. 1) were surveyed for IMS in Belgium from mid-April until mid-November 2013 and from mid-April until the beginning of October 2014. Potential PoEs included four used tyre import companies; three shelters/nurseries for imported cutting plants (e.g. lucky bamboo), fruits or vegetables; one main parking lot near a highway at the country border; two ports; and three airports. One tyre company stopped its activities in 2014 and was replaced by another. At each of the 12 potential PoEs, adult mosquitoes were collected continuously using one CO_2-baited trap (Mosquito Magnet™ Liberty Plus or Executive (MMLP), Woodstream Corporation, Lititz, PA, USA), which was emptied every 2 weeks. Between five and nine oviposition traps were baited with an oak leaf infusion, placed at each site and sampled every month. Additionally, a larval search was carried out every 2 months. At each potential PoE, all potential larval breeding sites (PBS) were inspected. A potential breeding site is defined as a single vessel or a group of the same vessels (e.g. a stock of tyres, lucky bamboo containers in the same shelter) in which mosquito larvae can develop. Per sampling period, an average of 145 tyres was inspected for larvae at each tyre company and an average of 90 vessels at the lucky bamboo nursery.

Intensified surveillance

Routine surveillance was intensified upon detection of an IMS. MMLP traps were emptied weekly during a period of intensified surveillance. At each PoE, two to five BG-Sentinel traps (Biogents, Germany) baited with BG-lure (without CO_2) were set up and one of the traps was situated near the MMLP trap. BG-Sentinel traps were also run continuously and emptied weekly. Between four and ten additional oviposition traps were set up (indoor and outdoor when breeding sites were located inside a building) and inspected every 2 or 4 weeks depending on the PoE. Further, the frequency of larval sampling was increased to every 2 weeks. If the IMS was detected in open air, a 200-m buffer zone was also surveyed. In 2014, between one and four data loggers (Hygropuce, Waranet Solutions, France) were used at each infested PoE to collect temperature data. Missing temperature and rainfall data from 2013 and 2014 were provided by the Royal Meteorological Institute (RMI).

Lucky bamboo shipments

Between September 2013 and August 2014, incoming shipments of lucky bamboo were inspected at a border inspection post in the port of Antwerp for the presence of IMS larvae and eggs (38 out of 43 shipments were inspected). Since the expiration of the exemption on the biocide legislation in the Netherlands in June 2013, all lucky bamboo shipments destined for a lucky bamboo import company in Belgium arrived at the port of Antwerp instead of at the port of Rotterdam in the Netherlands. These diversions stopped in the end of August 2014. For each shipment, up to 20 boxes or trays containing water and/or up to 60 plastic bags containing hygroscopic gel were sampled and checked for larvae and eggs.

Identification

Morphological identification of adult mosquitoes and larvae was carried out using taxonomic keys (Schaffner et al. 2001; Becker et al. 2010; ECDC 2012). Morphological

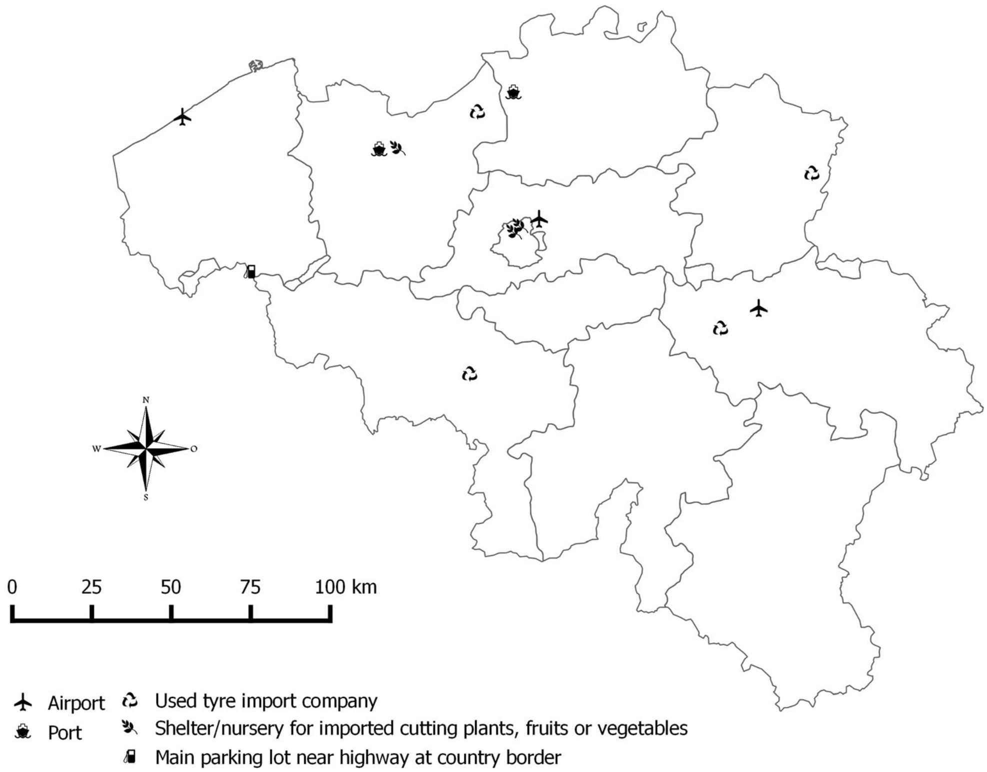


Fig. 1 Map of Belgium with the location of the 13 potential points of entry (PoEs) surveyed for invasive mosquito species in 2013 and/or 2014

identification of adults, eggs and larvae of *Ae. albopictus* was confirmed by PCR and sequencing. The ITS2 gene of the rDNA was amplified using 5.8S (5′-ATCACTCGGCTCGTGGATCG-3′) and 28S (5′-ATGCTTAAATTTAGGGGGTAGTCAC-3′) universal primers which flank the ITS2 gene (Djadid et al. 2007). A reverse primer specific for the *Ae. albopictus* 28S sequence was used to perform a semi-nested PCR (R28Salbo: 5′-TTGCGGGTGTTTTGTGTGTCGTC-3′) (De Jong et al. 2009). DNA was run on 2 % agarose gels stained with ethidium bromide, and bands were visualised by UV trans-illumination. PCR products were cloned, and DNA sequencing of selected clones was carried out by Vlaams Instituut voor Biotechnologie (VIB, Department of Molecular Genetics, University of Antwerp) on an automated sequencer. The obtained sequences were compared with sequences published in GenBank.

Results

Routine surveillance

During routine surveillance at the 12 potential PoEs, a total of 6775 adult mosquitoes (2013: 3332; 2014: 3443) and 6537 larvae (2013: 2134; 2014: 4403) were collected (Table 1). Four larvae of the IMS *Aedes* (*Finlaya*) *koreicus* (Edwards 1917) (*Hulecoeteomyia koreica* sensu Reinert et al. 2006) were collected in May 2014 in plastic containers next to a used tyre import company, located 5.3 km from an area known to be colonised by this mosquito. *Ae. albopictus* was detected at two of the 12 potential PoEs.

Intensified surveillance

A male *Ae. albopictus* was collected in a MMLP trap between 2 and 7 July 2013 by the University of Liège at a used tyre import company surveyed by ITM (Boukraa et al. 2013). The male was morphologically identified on 23 August 2013, and ITM was notified the same day. After notification, ITM intensified surveillance at this PoE. On 4 September 2013, the morphological identification was confirmed with PCR and the authorities were notified. On 10 September 2013, the Flemish Ministry of Environment notified the mayor of Vrasene, but no actions were taken. As it was almost winter and obtaining an exemption on the biocide legislation would take too much time, the Flemish Ministry of Environment decided not to take immediate chemical control actions. This wait-and-see strategy permitted an investigation of possible overwintering of the population. Intensified surveillance took place between August and November 2013 (Table 2) and between February and October 2014 (Table 3). On two occasions in 2013, a 200-m buffer zone extending from the point of first detection was inspected for the presence of *Ae. albopictus*, but none was found outside the tyre company

Table 1 Number of samplings, total specimens and presence of invasive mosquito species (IMS) per collection method during the routine surveillance at each type of potential point of entry (PoE) in 2013 and 2014

PoE		Platforms of imported used tyres		Shelters/nurseries for imported plants/fruits/vegetables		Parking lot at the country border		Ports		Airports	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Number of sites		3[d]	3[d]	3	3	1	1	2	2	3	3
MMLP	No. samplings[a]	43/45	30/36	29/44	28/36	15/15	12/12	27/29	24/24	40/42	27/36
	Total specimens	2398	2635	46	63	147	168	487	462	254	115
	Presence IMS adults	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
OT	No. samplings[b]	106/120	106/114	106/120	81/90	37/40	23/30	77/89	48/60	109/115	84/90
	Presence IMS eggs	Negative	Negative	Negative	2/90 (*Ae. albopictus*)	Negative	Negative	Negative	Negative	Negative	Negative
LS	No. samplings[c]	20 (7 PBS)	25 (10 PBS)	23 (7 PBS)	29 (10 PBS)	7 (2 PBS)	9 (3 PBS)	23 (7 PBS)	22 (9 PBS)	37 (14 PBS)	42 (16 PBS)
	Total specimens	1775	3508	62	536	22	54	225	75	50	230
	Presence IMS larvae	4 (*Ae. albopictus*)	4 (*Ae. koreicus*)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

MMLP Mosquito Magnet Liberty Plus (CO_2) trap, *OT* oviposition trap, *LS* larval sampling, *PBS* potential breeding site (= a single vessel or a group of the same vessels (e.g. a stock of tyres, lucky bamboo containers in the same shelter) in which mosquito larvae can develop)

[a] No. effective samplings (period of 2 weeks)/no. samplings planned (an effective sampling is a sampling without any problems such as malfunctioning of the trap)

[b] No. samplings with polystyrene piece recovered/no. samplings planned

[c] No. samplings with number of PBS sampled in parentheses

[d] One used tyre import company surveyed in 2013 was replaced by another company in 2014 due to a stop of its activities

(Table 2). In 2013, a total of one female and 17 larvae were collected at the tyre company in three tyres stored for more than 1 year (Table 2). In 2014, no adults, larvae, pupae or eggs of *Ae. albopictus* were collected at the same company

Table 2 Dates, methods and results of the intensified surveillance at the used tyre import company in Vrasene (Province of East Flanders, Belgium) in 2013

BG-Sentinel trapping					
Start date	End date	Number of traps	Location	# *Aedes albopictus*	
				♀	♂
29 Aug 2013	30 Aug 2013	4	Tyre company	1	0
16 Sept 2013	20 Sept 2013	5	200-m buffer zone	0	0
7 Oct 2013	10 Oct 2013	5	200-m buffer zone	0	0
14 Nov 2013	18 Nov 2013	5	Tyre company	0	0
Larval sampling					
Date	# PBS	# Tyres/vessels	Location	# *Aedes albopictus*	
				Larvae	Pupae
29 Aug 2013	1	200	Tyre company	1	0
10 Sept 2013	1	115	Tyre company	2	0
16 Sept 2013	9	103	200-m buffer zone	9[a]	0
7 Oct 2013	1	4	Tyre company	0	0
10 Oct 2013	8	85	200-m buffer zone	1[a]	0
16 Oct 2013	1	150	Tyre company	4	0
14 Nov 2013	1	2	Tyre company	0	0
18 Nov 2013	1	312	Tyre company	0	0

PBS potential breeding site (= a single vessel or a group of the same vessels (e.g. a stock of tyres, lucky bamboo containers in the same shelter) in which mosquito larvae can develop)

[a] Larvae only found in tyres at the premises of the tyre company

Table 3 Start date, end date, number of traps and samplings, total specimens and presence of *Ae. albopictus* per collection method during the intensified surveillance at the used tyre import and lucky bamboo company in the Province of East Flanders (Belgium) in 2014

Intensified survey 2014		Used tyre company	Lucky bamboo company
Aim		Investigate overwintering	Investigate introduction
MMLP	Start date	10 March 2014	6 October 2014
	End Date	8 October 2014	8 December 2014
	No. traps	1	1
	No. samplings[a,d]	25/30[e]	7/8
	Total specimens	1715	12
	Presence *Ae. albopictus* adults	Negative	Negative
BG	Start date	3 March 2014	21 October 2014
	End date	8 October 2014	8 December 2014
	No. traps	4	2
	No. samplings[a]	111/124	14/14
	Total specimens	7416	41
	Presence *Ae. albopictus* adults	Negative	2 (1♀ and 1♂)
OT	Start date	15 April 2014	6 October 2014
	End date	8 October 2014	12 January 2015
	No. traps	9	15
	No. samplings[b]	45/54[e]	74/75[d]
	Presence *Ae. albopictus* eggs/larvae	Negative	2 larvae in infusion
LS	Start date	10 February 2014	6 October 2014
	End date	30 October 2014	8 December 2014
	No. samplings[c,d]	19[e] (1 PBS[a])	33 (6 PBS)
	Total specimens	3355	72
	Presence *Ae. albopictus* larvae/pupae	Negative	15 (11 pupae and 4 larvae)

MMLP Mosquito Magnet Liberty Plus (CO_2) trap, *BG* BG-Sentinel trap, *OT* oviposition trap, *LS* larval sampling, *PBS* potential breeding site (= a single vessel or a group of the same vessels (e.g. a stock of tyres, lucky bamboo containers in the same shelter) in which mosquito larvae can develop)

[a] No. effective samplings (period of 1 week)/no. samplings planned (an effective sampling is a sampling without any problems such as malfunctioning of the trap)

[b] No. samplings with polystyrene piece recovered/ no. samplings planned

[c] No. samplings with number of PBS sampled in parentheses

[d] Sampling frequency of the MMLP traps was 1 week instead of the standard 2 weeks and of larval sampling 2 weeks instead of the standard 2 months, while ovitraps at the lucky bamboo company were collected every 2 to 5 weeks instead of every 4 weeks

[e] MMLP and OT samplings and LS from routine surveillance included

(Tables 1 and 3). The most abundant mosquito species collected during the routine and intensified surveillance at this site in 2013 and 2014 (total specimens: larvae: 4355, adults: 10,775) were *Anopheles* (*Anopheles*) *plumbeus* (Stephens 1828) (larvae 33 %, adults (MMLP and BG-Sentinel) 72 %), *Culex pipiens* (Linnaeus 1758)/*torrentium* (Martini 1925) (larvae 59 %, adults (MMLP and BG-Sentinel) 19 %), *Aedes* (*Finlaya*) *geniculatus* (Olivier 1791) (larvae 6 %, adults (MMLP and BG-Sentinel) 8 %) and *Culiseta* (*Culiseta*) *annulata* (Schrank 1776) (larvae 1.6 %, adults (MMLP and BG-Sentinel) 0.5 %). At this PoE, the mean January temperature in 2014 was 6.7 °C, the mean spring temperature in 2014 was 14.4 °C, the annual temperature in 2013 (July)–2014 (August) was 12.4 °C, and the annual rainfall in 2013 (July)–2014 (August) was 1025 mm.

Another introduction of *Ae. albopictus* was detected in a lucky bamboo company in Lochristi (Province of East-Flanders). One indoor ovitrap was found to be positive (23 eggs) during the sampling period from 11 August until 8 September 2014 (Table 1). The same indoor ovitrap was also positive (one egg) during the next sampling period from 8 September until 6 October 2014 (Table 1). After confirming the identity by PCR of the first eggs on 1 October 2014, the authorities were notified and surveillance was intensified (Tables 3 and 4). Two larvae were collected in two ovitrap containers during the sampling period from 6 until 21

Table 4 Egg, larval, pupal and adult samplings; mosquito control; and findings of *Ae. albopictus* at the lucky bamboo company in Lochristi (Province of East Flanders, Belgium), October 2014–January 2015

	2014												2015	
Week	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**1**	**2**
Eggs		2[a]												
Larvae					4									
Pupae					11									
Females					1									
Males							1							

Egg, larval, pupal and adult sampling (light gray cells); mechanical and chemical control (Pyretrex®) (dark gray cell); chemical control (VectoMax®) (gray cell)

[a] Larvae found in infusion of ovitraps

October 2014 (Table 4). To avoid further proliferation in the ovitraps, they were subsequently treated with the larvicide VectoMax® (Sumitomo Chemicals). On 4 November 2014, four larvae and 11 pupae were collected in lucky bamboo vessels in the nursery and one female was trapped in a BG-Sentinel trap close to those vessels (Table 4). The owner was advised to eliminate the breeding sites by emptying the vessels on a permeable surface and cleaning them, which he immediately did. He also treated the nursery with the registered and permitted adulticide, Pyretrex® fogger (Edialux), in the same week (Table 4). This time, the Flemish Ministry of Environment decided to take chemical control actions, and an exemption on the biocide legislation for VectoMax® was obtained in that same week. Despite no larvae being collected on 13 November 2014, all possible breeding places inside the nursery were treated with the larvicide VectoMax® (Table 4). In the following weeks, only a single male was caught with the BG-Sentinel trap close to the vessels, but no more larvae were collected. The ovitraps were also negative in the second week of January 2015. Other mosquito species collected at this site in 2013 and 2014 (indoors and/or outdoors), during the routine and intensified surveillance, were *Cx. pipiens/torrentium*, *Cs. annulata*, *Anopheles* (*Anopheles*) *maculipennis* s. l. (Theobald 1911), *Anopheles* (*Anopheles*) *claviger* (Meigen 1804) and *Ae. geniculatus*. The mean, maximum and minimum temperatures in the nursery were 16.6, 32.7 and 12.2 °C, respectively, and the mean relative humidity was 83 % between 6 October 2014 and 12 January 2015.

Lucky bamboo shipments

During the inspection of 38 lucky bamboo shipments at the port of Antwerp, a total of five *Ae. albopictus* larvae were collected on two occasions (Online Resource 1). The first collection of one larva in gel-transported lucky bamboo on 21 November 2013 was described in detail by Demeulemeester et al. (2014). The second collection of four fourth-instar larvae from polystyrene boxes containing water and lucky bamboo plants was made on 8 August 2014. Two larvae were successfully reared to the adult stage, while two other larvae were used for molecular identification. The destination of this shipment was the same lucky bamboo company in East Flanders at which *Ae. albopictus* was collected in 2014. As there is no regulation in Belgium concerning *Ae. albopictus*, the shipments continued to their destination without any control measures.

Discussion

The increased detection of *Ae. albopictus* demonstrates that Belgium, like its neighbouring countries, is at risk of invasion. A general trend arising from a comparison of several models is that climatic conditions in western and central Europe, including Belgium, will provide a suitable habitat for this IMS within the next decades (Fischer et al. 2014). In the Netherlands, climatic conditions between 1997 and 2007 were already favourable for the overwintering of diapausing eggs of temperate strains of *Ae. albopictus* (Takumi et al. 2009). In the present field study, the 2013–2014 temperatures and rainfall at the tyre company were, according to several models, suitable for overwintering of *Ae. albopictus* (Fischer et al. 2014). The location of the tyre company in a peri-urban area should also favour its proliferation (Medlock et al. 2006). Although this IMS was reproducing at the site during summer 2013, it either failed to overwinter at the tyre company or was not detected in 2014.

The most probable reason for the failure of overwintering in Belgium is a low frequency of introductions resulting in a small population lacking genetic variation. The success of populations of *Ae. albopictus* colonising the USA and Italy is based on several successive introductions, each with large numbers of individuals (Urbanelli et al. 2000). Bad timing of the production of diapausing eggs and their subsequent hatching in spring is also one of the possible reasons for the failure of overwintering. Although diapause of *Ae. albopictus* is under fine and rapid selective control, the critical photoperiod is geographically determined and initial populations will be incompletely adapted to local conditions (Focks et al. 1994). The origin of import at the used tyre company in Belgium could not be identified with certainty, but it was probably a temperate strain of *Ae. albopictus* from the USA (New York) or the Netherlands. The critical photoperiod for autumn diapause of *Ae. albopictus* (13.5 h, Fischer et al. 2014) occurred in Belgium on 1 September 2013 (mean temperature September 15.2 °C), while the spring photoperiod for hatching (11.5 h, Fischer et al. 2014) occurred on 11 March 2014 (mean temperature March 9.4 °C). It is possible that diapausing eggs were laid in early September 2013 and hatching occurred in March 2014, when it was still too cold to support development (mean temperature threshold for activity in spring 10.5–11 °C (Medlock et al. 2006)). Alternatively, hatching may have occurred much later increasing egg mortality by desiccation. When oviposition of diapausing eggs occurs in early autumn and initial hatching in late spring, the length of diapause might lead to egg desiccation resulting from low temperatures and relative humidity (Medlock et al. 2006). Mean relative humidity and rainfall at the tyre company were 68 % and 24.5 mm in March 2014 (normal values 79 % and 70 mm, RMI 2015) and 68 % and 11.1 mm in April 2014 (normal values 73 % and 51.3 mm, RMI 2015), which might have led to desiccation of the diapausing eggs.

A final possible explanation for the failure of overwintering is competition between *Ae. albopictus* and native mosquito species. *Cx. pipiens*/*torrentium*, *An. plumbeus* and *Ae. geniculatus* were well represented at the used tyre company. However, there is a strong and highly asymmetrical interspecific competition between larvae of *Ae. albopictus* and *Cx. pipiens*, with *Ae. albopictus* clearly superior to *Cx. pipiens* at 25 °C (Carrieri et al. 2003; Costanzo et al. 2005). No data are available on the competition of *Ae. albopictus* and *An. plumbeus* or *Ae. geniculatus*. Like *Ae. albopictus*, *An. plumbeus* is an aggressive human biter, is active during daytime and breeds in used tyres, and its hatching is dependent on flooding (Becker et al. 2010; Dekoninck et al. 2011). The high abundance of *An. plumbeus* at the used tyre company might make it a possible competitor of *Ae. albopictus*. More studies on the diapause/longevity of *Ae. albopictus* eggs in northern temperate climatic conditions and on the competition with endemic species in western and central Europe are warranted to assess the potential for this IMS to overwinter.

Although the introduction of the presumably tropical strain of *Ae. albopictus* in the lucky bamboo nursery presents a smaller risk than the introduction of temperate strains at the used tyre import company, its establishment is still possible. The constant and suitable climate, the many available breeding sites and the human blood hosts at the nursery provide excellent environmental conditions for this species, while during summer, it can disperse outdoors to other nurseries (Scholte et al. 2007). Additionally, Qualls et al. (2013) demonstrated that *Ae. albopictus* can readily survive long enough to complete a gonotrophic cycle by lucky bamboo plant tissue feeding only. The use of ovitraps in the Belgian nursery, which was thought to be outcompeted by the high abundance of oviposition sites in a nursery (Scholte et al. 2008), was effective in first detecting *Ae. albopictus*. Its introduction probably occurred in August 2014 when the first eggs were found, and the population survived and reproduced until November 2014 when the only male was found. Climatic conditions in the nursery were favourable for survival of the tropical strain of *Ae. albopictus*, even in winter (Waldock et al. 2013). More than 25 plant nurseries are present within a 2-km periphery, creating favourable microhabitats, with at least three *Azalea* nurseries within a perimeter of 200 m from the lucky bamboo company. In the Netherlands, preventative and curative indoor control measures are effective in preventing both indoor and outdoor establishment of this IMS (Scholte et al. 2010). In Belgium, the mechanical and chemical control measures also seemed successful.

The introduction of *Ae. albopictus* in August 2014 coincided with the detection of larvae in a shipment of lucky bamboo, originating from Zhanjiang (Guangdong, China) and destined for the lucky bamboo company in East-Flanders. It is most likely that the tiger mosquitoes captured at the nursery in summer and autumn 2014 originated from this shipment. In August 2014, the diversions of lucky bamboo shipments via the port of Antwerp ceased and shipments have started again to arrive at Rotterdam from where they are transported to the Dutch importer who treats them with biocides before arriving in Belgium. This is likely to reduce the risk of introducing *Ae. albopictus* to Belgium. However, surveillance at the nursery is still necessary to intercept invasive mosquitoes from occasionally untreated, but infested shipments following port deviations or from consignments transported on a gel substrate (Demeulemeester et al. 2014), which escape control measures.

At the moment there is no permanent and national surveillance and control plan for IMS in Belgium. Following the four detections of *Ae. albopictus* in 2013 and 2014, immediate control measures were implemented only once, in contrast to the standard procedure in the Netherlands, where one single *Ae. albopictus* specimen is enough to implement control measures (Scholte et al. 2010, 2012; WTO 2011). Immediately following the water/gel sampling and before the results of the analysis, the two positive lucky bamboo shipments continued to their destination. The lack of any regulation to avoid establishment of IMS at lucky bamboo and used tyre import companies in Belgium increases the risk for their introduction. Consequently, without any IMS control policy in Belgium, the future establishment of this mosquito will not be slowed down. At the moment, Belgium is not ready to intervene effectively. The registration of effective biocides not only within Belgium, but also within other European countries, remains a problem. Although the most recent exemption on the biocide legislation was quickly obtained, no national/regional protocols for IMS management are available. These protocols should state who is responsible for the implementation and costs of control measures and the manner in which such measures should be implemented. However, a national Belgian vector surveillance policy is in the pipeline. We urge the Belgian authorities not to wait too long with the implementation of this permanent vector surveillance and control plan.

Acknowledgments We thank the Federal Agency for the Safety of the Food Chain (FASFC) for financing the surveillance in 2013 and 2014, the University of Zurich (Francis Schaffner) for MALDITOF mass spectrometry of the first *Ae. albopictus* larva and the used tyre import and lucky bamboo company, together with the other potential points of entry, for their collaboration.

References

Becker N (2008) Influence of climate change on mosquito development and mosquito-borne diseases in Europe. Parasitol Res 103(1):S19–S28

Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010) Mosquitoes and their control. Springer, Heidelberg

Becker N, Geier M, Balczun C, Bradersen U, Huber K, Kiel E, Krüger A, Lühken R, Orendt C, Plenge-Bönig A, Rose A, Schaub GA, Tannich E (2013) Repeated introduction of *Aedes albopictus* into Germany, July to October 2012. Parasitol Res 112(4):1787–1790

Boukraa S, Raharimalala FN, Zimmer J-Y, Schaffner F, Bawin T, Haubruge E, Francis F (2013) Reintroduction of the invasive mosquito species *Aedes albopictus* in Belgium in July 2013. Parasite 20: 54

Carrieri M, Bacchi M, Bellini R, Maini S (2003) On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. Environ Entomol 32(6):1313–1321

Costanzo KS, Mormann K, Juliano SA (2005) Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). J Med Entomol 42(4):559–570

De Jong L, Moreau X, Dalia J, Coustau C, Thiery A (2009) Molecular characterization of the invasive Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Corsica. Acta Trop 112(3):266–269

Deblauwe I, Sohier C, Schaffner F, Rakotoarivony LM, Coosemans M (2014) Implementation of surveillance of invasive mosquitoes in Belgium according to the ECDC guidelines. Parasit Vectors 7:201

Dekoninck W, Hendrickx F, Van Bortel W, Versteirt V, Coosemans M, Damiens D, Hance T, De Clercq EM, Hendrickx G, Schaffner F, Grootaert P (2011) Expanded distribution associated with the use of man-made larval habitats of the day active mosquito *Anopheles plumbeus*, experimental vector of West Nile virus and a potential vector of human malaria in Belgium. J Med Entomol 48(4):924–928

Demeulemeester J, Deblauwe I, De Witte J, Jansen F, Hendy A, Madder M (2014) First interception of *Aedes* (*Stegomyia*) *albopictus* in Lucky bamboo shipments in Belgium. J Eur Mosq Control Assoc 32:14–16

Djadid ND, Gholizadeh S, Tafsiri E, Romi R, Gordeev M, Zakeri S (2007) Molecular identification of palearctic members of *Anopheles maculipennis* in northern Iran. Malar J 6:6

ECDC (European Centre for Disease Prevention and Control) (2012) Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm, Sweden. http://ecdc.europa.eu/en/publications/publications/ter-mosquito-surveillance-guidelines.pdf. Accessed 23 Mar 2015

ECDC (European Centre for Disease Prevention and Control) (2015) Mosquito maps. Stockholm, Sweden. http://www.ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx. Accessed 23 Mar 2015

Fischer D, Thomas SM, Neteler M, Tjaden NB, Beierkuhnlein C (2014) Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections: comparison of mechanistic and correlative niche modelling approaches. Euro Surveill 19(6).

Focks DA, Linda SB, Craig GB Jr, Hawley WA, Pumpuni CB (1994) *Aedes albopictus* (Diptera: Culicidae): a statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. J Med Entomol 31(2):278–286

Gould EA, Gallian P, De Lamballerie X, Charrel RN (2010) First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! Clin Microbiol Infect 16(12):1702–1704

Harrus S, Baneth G (2005) Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. Int J Parasitol 35(11-12):1309–1318

Kampen H, Kronefeld M, Zielke D, Werner D (2013) Further specimens of the Asian tiger mosquito *Aedes albopictus* (Diptera, Culicidae) trapped in southwest Germany. Parasitol Res 112(2):905–907

Medlock JM, Avenell D, Barrass I, Leach S (2006) Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. J Vector Ecol 31(2):292–304

Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, Van Bortel W (2012) A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector Borne Zoonotic Dis 12(6):435–447

Qualls WA, Xue RD, Beier JC, Müller GC (2013) Survivorship of adult *Aedes albopictus* (Diptera: Culicidae) feeding on indoor ornamental plants with no inflorescence. Parasitol Res 112(6):2313–2318

Reinert JF, Harbach RE, Kitching IJ (2004) Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zool J Linnean Soc 142(3):289–368

Reinert JF, Harbach RE, Kitching IJ (2006) Phylogeny and classification of Finlaya and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. Zool J Linnean Soc 148(1):1–101

Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S, Magurano F, Silvi G, Angelini P, Dottori M, Ciufolini MG, Majori GC, Cassone A (2007) Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370(9602):1840–1846

RMI (Royal Meteorological Institute) (2015) http://www.meteo.be/meteo/view/nl/1124386-Voorbije+maanden.html. Accessed 23 Mar 2015

Schaffner F, Mathis A (2014) Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis 14(12):1271–1280

Schaffner F, Angel G, Geoffroy B, Hervy J-P, Rhaiem A, Brunhes J (2001) The mosquitoes of Europe/Les moustiques d'Europe. An identification and training programme/Logiciel d'identification et d'enseignement. Didactiques. Montpellier: IRD Editions and EID Méditerranée. CD-ROM.

Schaffner F, Van Bortel W, Coosemans M (2004) First record of *Aedes* (*Stegomyia*) *albopictus* in Belgium. J Am Mosq Control Assoc 20(2):201–203

Schaffner F, Fontenille D, Mathis A (2014) Autochthonous dengue emphasises the threat of arbovirosis in Europe. Lancet Infect Dis 14(11):1044

Scholte EJ, Dijkstra E, Blok H, De Vries A, Takken W, Hofhuis A, Koopmans M, De Boer A, Reusken C (2008) Accidental importation of the mosquito *Aedes albopictus* into the Netherlands: a survey of mosquito distribution and the presence of dengue virus. Med Vet Entomol 22(4):352–358

Scholte EJ, Den Hartog W, Dik M, Schoelitsz B, Brooks M, Schaffner F, Foussadier R, Braks M, Beeuwkes J (2010) Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010. Euro Surveill 15(45)

Scholte EJ, Dik M, Ibañez Justicia A, Den Hartog W, Schoelitsz B, Brooks M, Braks M, Steeghs M (2012) Findings and control of two invasive exotic mosquito species, *Aedes albopictus* and *Ae. atropalpus* (Diptera: Culicidae) in the Netherlands, 2011. Eur Mosq Bull 30:1–14

Scholte EJ, Jacobs F, Linton Y-M, Dijkstra E, Fransen J, Takken W (2007) First record of *Aedes (Stegomyia) albopictus* in the Netherlands. Eur Mosq Bull 22:5–9

Takumi K, Scholte EJ, Braks M, Reusken C, Avenell D, Medlock JM (2009) Introduction, scenarios for establishment and seasonal activity of *Aedes albopictus* in the Netherlands. Vector Borne Zoonotic Dis 9(2):191–196

Urbanelli S, Bellini R, Carrieri M, Sallicandro P, Celli G (2000) Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity (Edinb) 84(3):331–337

Versteirt V, Boyer S, Damiens D, De Clercq EM, Dekoninck W, Ducheyne E, Grootaert P, Garros C, Hance T, Hendrickx G, Coosemans M, Van Bortel W (2012) Nationwide inventory of mosquito biodiversity (Diptera: Culicidae) in Belgium, Europe. Bull Entomol Res 103(2):193–203

Waldock J, Chandra NL, Lelieveld J, Proestos Y, Michael E, Christophides G, Parham PE (2013) The role of environmental variables on *Aedes albopictus* biology and chikungunya epidemiology. Pathog Glob Health 107(5):224–241

Werner D, Kampen H (2015) *Aedes albopictus* breeding in southern Germany, 2014. Parasitol Res 114(3):831–834

WTO (World Trade Organisation) (2011) Committee on Sanitary and Phytosanitary Measures. Notification. G/SPS/N/NLD/70. http://policy.mofcom.gov.cn/spstbt/en/NLD70.doc. Accessed 23 Mar 2015