

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

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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 (Stegomia) 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.

Key words

Tiger mosquito – overwintering – surveillance – control – tyres – lucky bamboo

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).

Aedes 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 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 (PoE). 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 PoE (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 PoE 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 PoE adult mosquitoes were collected continuously using one CO₂-baited trap (Mosquito Magnet™ Liberty Plus or Executive (MMLP), Woodstream Corporation, Lititz, PA, USA), which was emptied every two 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 two 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 were inspected for larvae at each tyre company, and an average of 90 vessels at the lucky bamboo nursery.

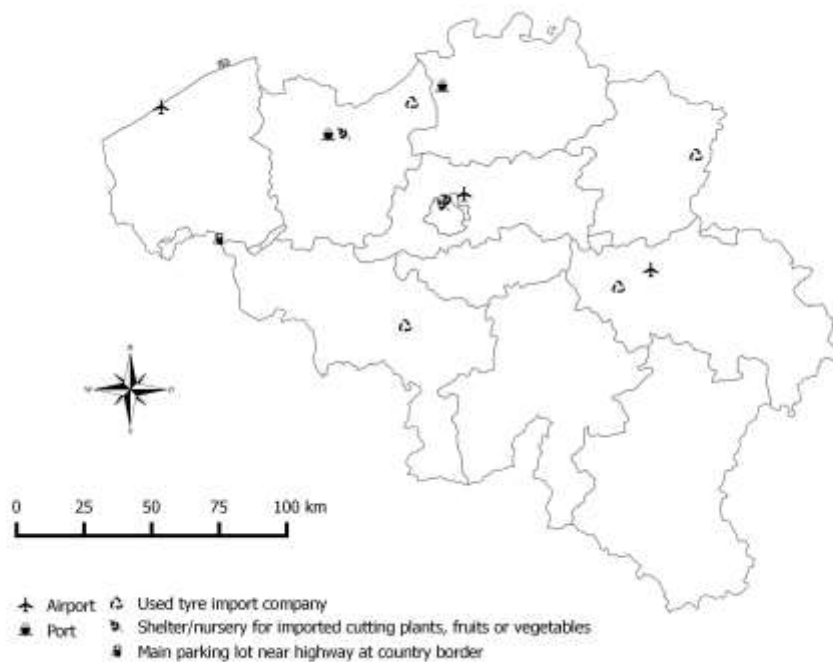


Fig. 1 Map of Belgium with the location of the 13 potential points of entry (PoE) surveyed for invasive mosquito species in 2013 and/or 2014

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, 2-5 BG-Sentinel traps (Biogents, Germany) baited with BG-lure (without CO₂) 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 4-10 additional oviposition traps were set up (indoor and outdoor when breeding sites were located inside a building) and inspected every two or four weeks depending on the PoE. Further, the frequency of larval sampling was increased to every two weeks. If the IMS was detected in open air, a 200m buffer zone was also surveyed. In 2014, between 1-4 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 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'-TTGCGGGTGT TTTGTGTGTCGTC-3') (De Jong et al. 2009). DNA was run on 2% agarose gels stained with ethidium bromide, and bands were visualized by UV trans-illumination. PCR products were cloned and DNA sequencing of selected clones was carried out by VIB (Vlaams Instituut voor Biotechnologie, 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 PoE, 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 5.3km from an area known to be colonised by this mosquito. *Aedes albopictus* was detected at two of the 12 potential PoE.

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 200m buffer zone extending from the point of first detection was inspected for the presence of *Ae. albopictus*, but none were found outside the tyre company (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 one year (Table 2). In 2014, no adults,

Table 1 Number of samplings, total specimens, 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/greenhouses 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 ^c | 3 ^c | 3 | 3 | 1 | 1 | 2 | 2 | 3 | 3 |
| MMLP^a | N° samplings^b | 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^a | N° samplings^c | 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 (<i>Ae. albopictus</i>) | negative | negative | negative | negative | negative | negative |
| LS^a | N° samplings^d | 20 (7 PBS ^a) | 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 (<i>Ae. albopictus</i>) | 4 (<i>Ae. koreicus</i>) | negative | negative | negative | negative | negative | negative | negative | negative |

^aMMLP = Mosquito Magnet Liberty Plus (CO₂) 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)

^bN° effective samplings (period of 2 weeks) / n° samplings planned (an effective sampling is a sampling without any problems such as malfunctioning of the trap)

^cN° samplings with polystyrene piece recovered / n° samplings planned

^dN° samplings with number of PBS sampled in parentheses

^eOne used tyres import company surveyed in 2013 was replaced by another company in 2014 due to a stop of its activities

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 | # <i>Aedes albopictus</i> | |
| | | | | ♀ | ♂ |
| 29-08-2013 | 30-08-2013 | 4 | tyre company | 1 | 0 |
| 16-09-2013 | 20-09-2013 | 5 | 200m buffer zone | 0 | 0 |
| 7-10-2013 | 10-10-2013 | 5 | 200m buffer zone | 0 | 0 |
| 14-11-2013 | 18-11-2013 | 5 | tyre company | 0 | 0 |
| Larval sampling | | | | | |
| Date | # PBS^a | # Tyres/vessels | Location | # <i>Aedes albopictus</i> | |
| | | | | larvae | pupae |
| 29-08-2013 | 1 | 200 | tyre company | 1 | 0 |
| 10-09-2013 | 1 | 115 | tyre company | 2 | 0 |
| 16-09-2013 | 9 | 103 | 200m buffer zone | 9 ^b | 0 |
| 7-10-2013 | 1 | 4 | tyre company | 0 | 0 |
| 10-10-2013 | 8 | 85 | 200m buffer zone | 1 ^b | 0 |
| 16-10-2013 | 1 | 150 | tyre company | 4 | 0 |
| 14-11-2013 | 1 | 2 | tyre company | 0 | 0 |
| 18-11-2013 | 1 | 312 | tyre company | 0 | 0 |

^aPBS = 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)

^bLarvae only found in tyres at the premises of the tyre company

larvae, pupae or eggs of *Ae. albopictus* were collected at the same company (Tables 1 & 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: 10775) were *Anopheles (Anopheles) plumbeus* (Stephens 1828) (larvae: 33%, adults (MMLP & BG-Sentinel): 72%), *Culex pipiens (Linnaeus 1758)/torrentium (Martini 1925)* (larvae: 59%, adults (MMLP & BG-Sentinel): 19%), *Aedes (Finlaya) geniculatus* (Olivier 1791) (larvae: 6%, adults (MMLP & BG-Sentinel): 8%) and *Culiseta (Culiseta) annulata* (Schrank 1776) (larvae: 1.6%, adults (MMLP & 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 1025mm.

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 (1 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 & 4). Two larvae were collected in two ovitrap containers during the sampling period from 6 until 21 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

Table 3 Start date, end date, number of traps and samplings, total specimens, 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^a | Start date | 10 March 2014 | 6 October 2014 |
| | End Date | 8 October 2014 | 8 December 2014 |
| | N° traps | 1 | 1 |
| | N° samplings^{b,e} | 25/30 ^f | 7/8 |
| | Total specimens | 1715 | 12 |
| | Presence <i>Ae. albopictus</i> adults | negative | negative |
| BG^a | Start date | 3 March 2014 | 21 October 2014 |
| | End Date | 8 October 2014 | 8 December 2014 |
| | N° traps | 4 | 2 |
| | N° samplings^b | 111/124 | 14/14 |
| | Total specimens | 7416 | 41 |
| | Presence <i>Ae. albopictus</i> adults | negative | 2 (1♀ & 1♂) |
| OT^a | Start date | 15 April 2014 | 6 October 2014 |
| | End Date | 8 October 2014 | 12 January 2015 |
| | N° traps | 9 | 15 |
| | N° samplings^c | 45/54 ^f | 74/75 ^e |
| | Presence <i>Ae. albopictus</i> eggs/larvae | negative | 2 larvae in infusion |
| LS^a | Start date | 10 February 2014 | 6 October 2014 |
| | End Date | 30 October 2014 | 8 December 2014 |
| | N° samplings^{d,e} | 19 ^f (1 PBS ^a) | 33 (6 PBS) |
| | Total specimens | 3355 | 72 |
| | Presence <i>Ae. albopictus</i> larvae/pupae | negative | 15 (11 pupae & 4 larvae) |

^a MMLP = Mosquito Magnet Liberty Plus (CO₂) 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)

^b N° effective samplings (period of 1 week) / n° samplings planned (an effective sampling is a sampling without any problems such as malfunctioning of the trap)

^c N° samplings with polystyrene piece recovered / n° samplings planned

^d N° samplings with number of PBS sampled in parentheses

^e Sampling frequency of the MMLP traps was one week instead of the standard two weeks, and of larval sampling two weeks instead of the standard two months, while ovitraps at the lucky bamboo company were collected every two to five weeks instead of every four weeks

^f MMLP and OT samplings, and LS from routine surveillance included

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 | | | | | | | |

^aLarvae found in infusion of ovitraps

| | |
|--|---|
| | Egg, larval, pupal and adult sampling |
| | Mechanical and chemical control (Pyretrex®) |
| | Chemical control (VectoMax®) |

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 temperature in the nursery were 16.6°C, 32.7°C 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 US 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 US (New York) or the Netherlands. The critical photoperiod for autumn diapause of *Ae. albopictus* (13.5h, Fischer et al. 2014) occurred in Belgium on 1 September 2013 (mean temperature September:

15.2°C), while the spring photoperiod for hatching (11.5h, 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 24.5mm in March 2014 (normal values: 79% and 70mm, RMI 2015) and 68% and 11.1mm in April 2014 (normal values: 73% and 51.3mm, 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. *Culex 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, 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 2km periphery, creating favourable microhabitats, with at least three *Azalea* nurseries within a perimeter of 200m 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 Zhangjiang (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 treat 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 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.

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Electronic Supplementary Material

Online Resource 1 Inspected lucky bamboo shipments, between September 2013 and August 2014, with inspection date, mode of transport, number of samples inspected and number of positive samples for *Ae. albopictus* (NA = info not available).

| Date | Water/gel | # inspected samples | # positive samples |
|------------|-----------|---------------------|--------------------|
| 19-09-2013 | Gel | 30 | 0 |
| 25-09-2013 | Gel | 30 | 0 |
| 25-09-2013 | Water | 12 | 0 |
| 3-10-2013 | Water | 38 | 0 |
| 9-10-2013 | Water | 38 | 0 |
| 31-10-2013 | Gel | 30 | 0 |
| 21-11-2013 | Gel | 60 | 1(1 larva) |
| 27-11-2013 | Gel | 30 | 0 |
| 27-11-2013 | Gel | 30 | 0 |
| 27-11-2013 | Water | 30 | 0 |
| 11-12-2013 | Water | 25 | 0 |
| 18-12-2013 | Gel | 40 | 0 |
| 18-12-2013 | Gel | 40 | 0 |
| 2-01-2014 | NA | 0 | 0 |
| 16-01-2014 | Gel | 40 | 0 |
| 24-01-2014 | Water | 20 | 0 |
| 3-02-2014 | Water | 20 | 0 |
| 3-02-2014 | Gel | 40 | 0 |
| 6-02-2014 | Water | 12 | 0 |
| 14-02-2014 | Gel | 40 | 0 |
| 17-02-2014 | Water | 15 | 0 |
| 19-02-2014 | Gel | 30 | 0 |
| 19-02-2014 | Gel | 30 | 0 |
| 25-02-2014 | Water | 18 | 0 |
| 18-03-2014 | Gel | 40 | 0 |
| 18-03-2014 | Gel | 40 | 0 |
| 25-03-2014 | Gel | 40 | 0 |
| 25-03-2014 | Gel | 40 | 0 |
| 1-04-2014 | Gel | 30 | 0 |
| 1-04-2014 | Water | 10 | 0 |
| 9-04-2014 | Water | 10 | 0 |
| 9-04-2014 | Gel | 40 | 0 |
| 9-04-2014 | Gel | 40 | 0 |
| 23-04-2014 | NA | 0 | 0 |
| 23-04-2014 | NA | 0 | 0 |

| Date | Water/gel | # inspected samples | # positive samples |
|-------------|------------------|----------------------------|---------------------------|
| 28-05-2014 | Water | 8 | 0 |
| 28-05-2014 | Water | 12 | 0 |
| 11-06-2014 | NA | 0 | 0 |
| 25-06-2014 | Water | 15 | 0 |
| 30-07-2014 | Water | 6 | 0 |
| 30-07-2014 | Gel | 40 | 0 |
| 8-08-2014 | Water | 12 | 2 (4 larvae) |
| 14-08-2014 | Water | 0 | 0 |