

## RISK-BASED AND ADAPTIVE INVASIVE MOSQUITO SURVEILLANCE AT LUCKY BAMBOO AND USED TIRE IMPORTERS IN THE NETHERLANDS

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**ABSTRACT.** The detection of *Aedes albopictus* in Lucky bamboo (*Dracaena sanderiana*) greenhouses and *Ae. atropalpus* at used tire importers illustrates that the Netherlands is exposed to the risk of introductions of invasive mosquito species (IMS). In this study we implemented a risk-based and adaptive surveillance (2010–16) in order to detect introductions and prevent potential proliferation of IMS at these locations. Results at Lucky bamboo greenhouses show that interceptions of *Ae. albopictus* occurred every year, with 2010 and 2012 being the years with most locations found positive for this species ( $n = 6$ ), and 2015 the year with the highest percentage of positive samples (4.1%). Furthermore, our results demonstrate that *Ae. japonicus* can also be associated with the import of Lucky bamboo. At used tire companies, IMS were found at 12 locations. Invasive mosquito species identified were *Ae. albopictus*, *Ae. atropalpus*, *Ae. aegypti*, and *Ae. japonicus*, of which *Ae. albopictus* has been found every year since 2010. The proportion of samples containing IMS was significantly higher before application of a covenant between the used tire importers and the Dutch government in 2013 (12.96%) than in the successive 3 years (2014 [6.93%], 2015 [4.24%], 2016 [5.09%], 1-sided binomial test,  $P < 0.01$ ). It is concluded that risk-based and adaptive surveillance is an effective methodology for detection of IMS, and that application of governmental management measures in combination with mosquito control has stabilized the situation.

**KEY WORDS** *Aedes*, import, invasive mosquito species, national policy, vector control

### INTRODUCTION

Increasing international trade, together with international travel, facilitate the expansion of the native range of invasive mosquito species (IMS) (Tatem et al. 2006). The introduction and possible establishment of IMS is a risk for public health due to the ability of these IMS to transmit vector-borne diseases. European countries have witnessed the introduction of IMS, for example, *Aedes albopictus* (Skuse) (Sabatini et al. 1990, Adhami and Reiter 1998), *Aedes aegypti* (L.) (Scholte et al. 2010, Akiner et al. 2016), *Aedes atropalpus* (Coquillett) (Romi et al. 1997, Scholte et al. 2009), *Aedes japonicus* (Theobald) (Schaffner et al. 2009, Versteirt et al. 2009), and *Aedes koreicus* (Edwards) (Capelli et al. 2011, Versteirt et al. 2012). Established populations of IMS in Europe have also been reported to locally transmit pathogens among humans, resulting in cases of dengue and chikungunya in southern France (Gould et al. 2010, La Ruche et al. 2010, Calba et al. 2017), dengue on Madeira island (Portugal) (Sousa et al. 2012), chikungunya

outbreaks in Italy (Rezza et al. 2007, Venturi et al. 2017), and Zika virus in southern France (Giron et al. 2019).

Since 2005, *Ae. albopictus* has been occasionally introduced into the Netherlands at companies that import Lucky bamboo (*Dracaena sanderiana* Mast.) (Scholte et al. 2007). The finding of *Ae. atropalpus* in 2009 at 2 companies that import used tires also illustrates that the Netherlands is exposed to the risk of IMS through international trade. In order to detect introductions and avoid possible proliferation of IMS at these locations, a risk-based and adaptive surveillance has been implemented since 2010. The policy of the government of the Netherlands is to prevent establishment of IMS or to postpone this as long as possible. Several public health responses have been formulated with the aim of reducing the risks of importation of IMS in the country, such as covenants and Commodities Act Decrees for the import of Lucky Bamboo (Staatsblad van het Koninkrijk der Nederlanden 2011, 2014), and covenants with the used tire sector (Staatsblad van het Koninkrijk der Nederlanden 2013, 2016).

Here, we present the results of descriptive studies on a risk-based and adaptive national surveillance of IMS implemented in Lucky bamboo greenhouses and used tire companies in the Netherlands, with focus on the effectiveness of the mosquito monitoring and control operations for the years 2010–16. In addition, we present a revised methodology to assign used tire companies to different risk categories for the import of IMS.

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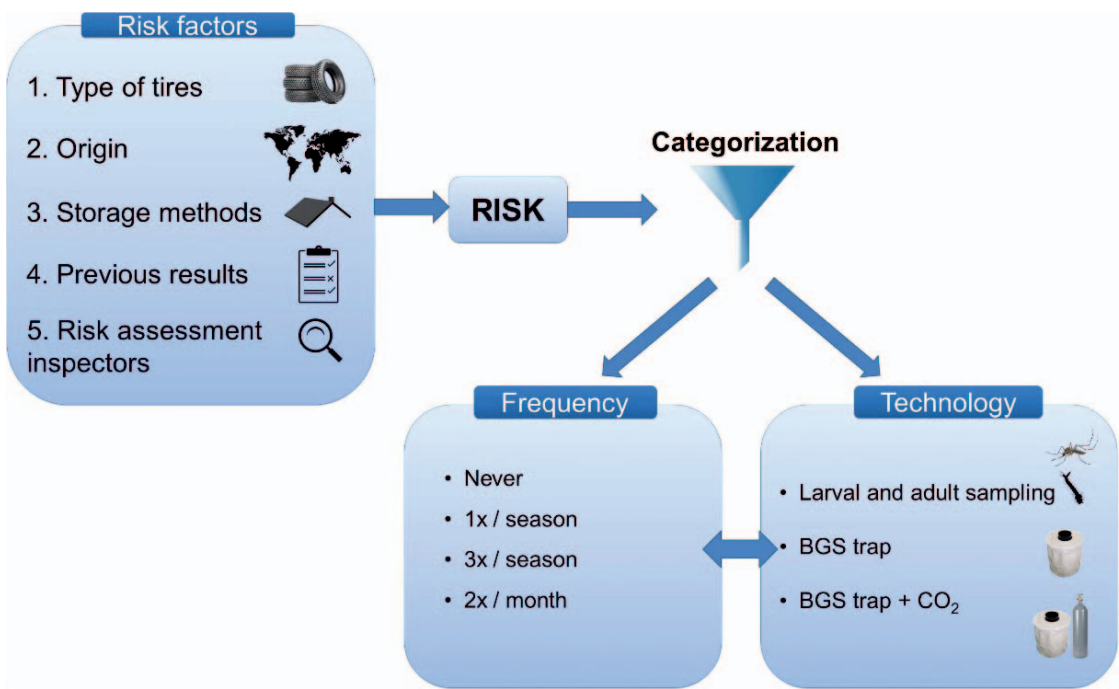


Fig. 1. Flowchart of risk-based and adaptive surveillance for used tire companies.

MATERIALS AND METHODS

Identification of risk locations

The IMS surveillance has been carried out annually in the Netherlands from 2010 onward at Lucky bamboo greenhouses and used tire companies. Regarding Lucky bamboo greenhouses, the Netherlands Food and Consumer Product Safety Authority (NVWA) was asked to provide the locations where Lucky bamboo was imported, processed, and maintained. All traders importing Lucky bamboo of Asian origin were considered potential locations for introduction of IMS and were included in the survey. Regarding used tire companies, we categorized the risk of used tire companies for mosquito introduction. This was done in cooperation with representatives of the tire industry VACO (VACO Bedrijfstakorganisatie voor de banden- en wielencbranche 2016), which provided the locations of companies. Additional information on used tire companies (that were not members of VACO) was searched by systematically identifying tire traders via the internet. The quantitative risk assessment was performed every year for each company. For this assessment, the following risk factors were taken into account (Fig. 1): 1) the type of tires that were imported, 2) the country of origin of the tires, 3) the method of storage, 4) results of findings of IMS during previous surveillance activities, and 5) risk assessment by the inspectors. The 5th risk factor presents the perception of the inspector for risk of IMS introduction after visiting the used tire compa-

ny. The factor scores are relative values used to quantify the risk of importing IMS for the different used tire companies.

Mosquito surveillance

Inside all Lucky bamboo greenhouses, 1 BG-Sentinel trap (BGS-trap; Biogents AG, Regensburg, Germany) and 1 Mosquito Magnet Liberty Plus trap (MM-trap, CO<sub>2</sub> baited; Woodstream Corporation, Lilitz, PA) were run continuously during the entire year (January 1–December 31). Both traps were placed next to each other. The BGS-trap was used with a specific dispenser (BG-Lure cartridge; Biogents AG, Regensburg, Germany), which contains a lure that is a combination of volatile compounds that are also present on human skin (ammonia, lactic acid, and caproic acid). The MM-traps operated by the release of CO<sub>2</sub> and heat as mosquito attractant (Guerenstein and Hildebrand 2008). These traps are advised for routine surveillance of *Ae. albopictus* elsewhere (Hoel et al. 2009). In addition, from May to October, a minimum of 3 oviposition traps (ovitrap) were also deployed immediately outside the greenhouses. This was done to detect *Aedes* IMS that may have left the indoor environment of the greenhouse and forage for suitable oviposition sites outdoors. Ovitrap were small black plastic buckets, filled with hay-infusion and a piece of polystyrene as oviposition support (Velo et al. 2016). Samples from all traps used were collected every 2 wk. If during the course of the study IMS were detected in any of the

deployed traps, larval and adult searches were performed at the greenhouse using fine-mesh aquarium nets for larvae and aspirators for adults. These samples were taken where the inspector suspected to find larvae but did not follow a predefined scheme.

At used tire locations, manual larval and adult searches, and mosquito trap sampling were carried out every 2 wk during the period in which mosquitoes in the Netherlands are most active, i.e., from mid-April to mid-October. For larval sampling, between 50 and 100 used tires, accessible for the inspector and distributed over the property of the company, were inspected for the presence of mosquito larvae during inspection. When mosquito adults were detected during larval sampling, these were collected using sweep nets and aspirators. The BGS-traps, baited with BG-Lure and CO<sub>2</sub> (<http://www.bg-sentinel.com>), were also used during surveillance. The surveillance strategy was adapted over the course of the study, mostly informed by findings of IMS from the previous years and by the advances in IMS detecting methods. For example, less labor-intensive and more sensitive collection methods, such as BGS-traps with CO<sub>2</sub>, replaced the larval sampling as a main method for detecting IMS at the high-risk companies from 2013 onward.

Following our adaptive approach, if an IMS was detected during the surveillance within the used tire company limits, more intensive inspections were initiated here, as well as within a predefined perimeter of 500 m from the limits of the company. This consisted of increased frequency of deployment of traps (from every 2 wk to every week), increased number of traps (minimum of 3 traps outside the limits of the company), as well as sampling and subsequent removal of potential larval habitats for container-breeding *Aedes*. If an IMS was detected during the surveillance within the 500-m perimeter, but outside the company limits, the sampling area was increased by including a new circular search area of 500-m radius from the new finding site. Two BGS-traps were deployed, and sampling and subsequent removal of potential larval habitats for container-breeding *Aedes* was performed in the increased area. The increase of the sampling area at a location stopped when no more IMS specimens were detected outside the company.

### Sample identification

Samples were labeled in the field with a unique code, sealed, and sent to the laboratory of the Centre for Monitoring of Vectors of the National Reference Centre for species identification. Mosquitoes were counted and morphologically identified using appropriate keys (Schaffner et al. 2001, Becker et al. 2010). Samples containing only indigenous mosquito species were considered as “negative samples” and samples containing IMS were considered as “positive samples.” The abundance of indigenous mosquitoes was recorded from 2013 onward. Each mosquito that

was morphologically identified as an IMS was validated by a different mosquito specialist. From 2014 onward, IMS identifications were also validated by using a real-time polymerase chain reaction specifically developed for the IMS surveillance (van de Vossen et al. 2015).

All data from each sampling location were included in VecBase (a tailor-made Oracle database application; Oracle Corporation, Redwood City, CA). Data on the annual numbers of imported used tires from 2010 until 2016 were provided by the CBS (CBS-Statistics-Netherlands 2017). Data on the annual numbers of imported Lucky bamboo plants from 2010 up to 2016 were provided by the phytosanitary authorities of the Netherlands (NVWA).

### IMS control

As stipulated in the Lucky Bamboo commodity act (Staatsblad van het Koninkrijk der Nederlanden 2011), importers need to apply mosquito larvicide to the water in which they place the Lucky bamboo stems after shipment. Additionally, if an IMS was found during surveillance, the importer was notified by the NVWA and was advised to apply adulticide in the greenhouse.

In case an IMS was detected at a used tire company, the site was treated by spraying *Bacillus thuringiensis israelensis* de Barjac (*Bti*) (VectoBac WG; Valent BioSciences LLC, Libertyville, IL) against larvae inside the tire stacks and deltamethrin (aqua K-Othrine; Bayer Environmental Sciences, Leverkusen, Germany) against adult mosquitoes. Larval control of the surrounding area (predefined perimeter of 500 m) consisted of removal of potential larval breeding sites for container-breeding *Aedes* spp., or treatment with either *Bti* space spray (VectoBac WG; Valent BioSciences) or with *Bti*-*Bacillus sphaericus* Neide granules (Vectomax FG; Valent BioSciences). One week later, adulticiding was repeated once. Larviciding was repeated every 2–3 wk until the end of October, when ambient conditions for IMS became generally unfavorable.

## RESULTS

### Surveillance in Lucky bamboo greenhouses

Between 2010 and 2016, 3,022 samples were taken at 13 companies that were importing Lucky bamboo into the Netherlands (Table 1). Since 2010, *Ae. albopictus* was found every year, with 2010 and 2012 being the years with most locations found positive for this species ( $n = 6$ ), and 2015 the year with the highest percentage of positive samples (4.1%) (Table 1 and Fig. 2). Annually, the number of *Ae. albopictus* captured varied strongly (e.g., 82 captured in 2010 and 2 specimens in 2014). At the same time, the total number of imported plants as reported to the phytosanitary authorities strongly decreased from 2011 onward (Fig. 2). However, this

Table 1. Results of the Lucky bamboo monitoring in the Netherlands from 2010 to 2016: number of locations inspected, number of locations with invasive mosquito species (IMS), number of samples taken per year, and number of samples per year with IMS.

Year	No. locations inspected	No. locations with IMS	No. samples taken per year <sup>1</sup>	No. samples per year with IMS <sup>1</sup>
2010	11	6	649	15
2011	7	3	353	11
2012	7	6	325	12
2013	6	2	321	3
2014	5	1	266	2
2015	5	4	293	12
2016	6	3	312	7

<sup>1</sup> Using BG-Sentinel and Mosquito Magnet Liberty Plus traps.

did not seem to coincide with a decline in the number of positive samples containing *Ae. albopictus* intercepted every year. A binomial test indicated that the proportion of positive samples obtained using only BGS-traps and MM-traps in 2013 (0.93%) and 2014 (0.75%) was significantly lower than in 2010 (2.31%), 2011 (3.11%), 2012 (3.69%), and 2015 (4.09%),  $P < 0.01$  (1-sided).

A total of 200 specimens of *Ae. albopictus* were captured, mostly by BGS-traps ( $n = 148$ ) and MM-traps ( $n = 48$ ). In 2013, an adult female of *Ae. japonicus* was collected with a MM-trap. Two adult specimens of *Ae. albopictus* were captured using aspirators (1 in 2010 and 1 in 2016) and 2 larvae of *Ae. albopictus* were captured in a container with water and Lucky bamboo at arrival at the company in 2010. No IMS were found in ovitraps placed in the immediate surroundings of the greenhouses.

Surveillance at used tire companies

Between 2010 and 2016, 43 companies that trade in used tires were identified and the quantitative risk assessment assigned these locations as “high,” “medium,” or “low risk” for introduction of IMS. From 2010 to 2016, a total of 7,104 samples were taken and >260,000 mosquito specimens were collected (Table 2). Invasive mosquito species were found at 12 locations (28%). At 9 locations (21%), IMS were also found within the 500-m buffer zone immediately outside the premises of the company. Except for 2012, positive samples were found outside the premises of the tire companies every year (Table 3). Invasive mosquito species found were *Ae. albopictus*, *Ae. atropalpus*, *Ae. aegypti*, and *Ae. japonicus*, of which *Ae. albopictus* has been routinely found every year since 2010. *Aedes aegypti* was found only in 2010 and *Ae. atropalpus* in 2010 and 2011. Both species were no longer detected after mosquito

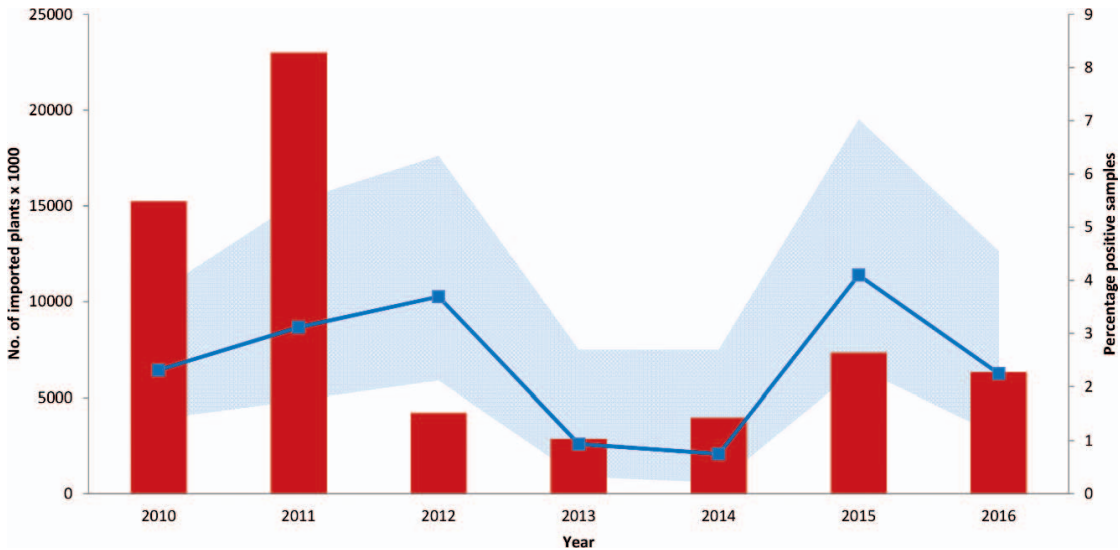


Fig. 2. Number of imported Lucky bamboo plants in the Netherlands, 2010–16 (red bars; source: Netherlands Food and Consumer Product Safety Authority [NVWA]), and percentage of positive samples using BG-Sentinel (BGS) and Mosquito Magnet Liberty Plus (MM) traps (blue line). Blue area represents 95% confidence interval of the percentage of positive samples.

Table 2. Number of samples processed, number of mosquito specimens processed, species, and number of specimens of invasive mosquito species (IMS) captured at used tire companies in the Netherlands from 2010 to 2016.<sup>1</sup>

Year	No. samples (no. samples with IMS)	No. mosquito specimens, total (A, L, P, E)	No. locations with IMS (no. with IMS outdoors)	No. samples with IMS inside company using standardized BGS-trap + CO <sub>2</sub>	IMS name	No., total (A, P, L)
2010	1,116 (43)	3,393 (915A, 2,442L, 226P, 10E)	6 (3)	Not applicable	<i>Aedes atropalpus</i> <i>Ae. aegypti</i> <i>Ae. albopictus</i>	325 (121A, 203L, 1P) 13 (13A) 23 (15A, 8L) 361
Total 2011	1,466 (14)	864 (52A, 809L, 3P)	4 (2)	Not applicable	<i>Ae. atropalpus</i> <i>Ae. albopictus</i>	22 (21A, 1L) 30 (22A, 5L, 3P) 52
Total 2012	752 (10)	7,738 (5,103A, 2,638L, 3E)	5 (0)	Not applicable	<i>Ae. albopictus</i>	27 (27A)
Total 2013	1,137 (31)	24,657 (12,415A, 12,238L, 4P)	9 (7)	21	<i>Ae. albopictus</i> <i>Ae. japonicus</i>	74 (46A, 28L) 1 (1A) 75
Total 2014	1,150 (18)	64,163 (53,008A, 11,047L, 108P)	6 (1)	12	<i>Ae. albopictus</i> <i>Ae. japonicus</i>	31 (31A, 16L) 1 (1L) 48
Total 2015	863 (9)	45,292 (18,509A, 26,472L, 311P)	6 (1)	7	<i>Ae. albopictus</i> <i>Ae. japonicus</i>	11 (11A) 1 (1A) Total 12
Total 2016	620 (12)	118,364 (109,403A, 527E, 8,254L, 180P)	4 (1)	8	<i>Ae. albopictus</i>	14 (14A) Total 14
Totals	7,104	264,471	12 (9)		<i>Ae. aegypti</i> <i>Ae. atropalpus</i> <i>Ae. albopictus</i> <i>Ae. japonicus</i>	13 (1 year) 347 (2 years) 210 (7 years) 3 (3 years) Total 573

<sup>1</sup> A, adults; P, pupae; L, larvae; E, eggs; BGS, BG-Sentinel; CO<sub>2</sub>, carbon dioxide.



Table 3. Results of monitoring and control of invasive mosquito species (IMS) at used tire companies in the Netherlands from 2010 to 2016.

Year	No. of companies where IMS were found (indoors) (outdoors) by year	Municipality	Risk category (Cat.)	Date of 1st detection	Detection method <sup>2</sup> (1st)	Date of last finding	Company positive next year? Y/N
2010	6 (3)	Oss <sup>1</sup>	High	Jul. 26, 2010	(As), L, BGS	Aug. 23, 2010	Y
		Weert 1 <sup>1</sup>	High	Aug. 24, 2010	(L), As, MM	Sep. 27, 2010	Y
		Weert 2	High	Sep. 7, 2010	(L), As, MM	Sep. 21, 2010	Y
		Montfoort	High	Sep. 28, 2010	(L), As	Oct. 5, 2010	N
		Heijningen <sup>1</sup>	High	Jul. 21, 2010	(L), As, BGS	Sep. 3, 2010	N
		Oosterhout	High	Jul. 26, 2010	(As)	Aug. 6, 2010	Y
2011	4 (2)	Oss <sup>1</sup>	High (Cat. 1)	Jul. 19, 2011	(As), BGS	Sep. 29, 2011	Y
		Weert 1	High (Cat. 1)	Jul. 13, 2011	(BGS)	Jul. 25, 2011	Y
		Weert 2	High (Cat. 1)	Jul. 28, 2011	(L), BGS	Aug. 3, 2011	Y
		Oosterhout <sup>1</sup>	High (Cat. 1)	Aug. 15, 2011	(L, As), BGS	Oct. 5, 2011	N
		Oss	High (Cat. 1)	Aug. 2, 2012	(BGS), As	Aug. 16, 2012	Y
2012	5 (0)	Weert 1	High (Cat. 1)	Jul. 10, 2012	(BGS), As	Jul. 20, 2012	Y
		Weert 2	High (Cat. 1)	Jul. 20, 2012	(BGS + CO <sub>2</sub> )	Jul. 20, 2012	Y
		Montfoort	High (Cat. 1)	Aug. 6, 2012	(BGS + CO <sub>2</sub> )	Aug. 29, 2012	Y
		Heijningen	High (Cat. 1)	Jul. 5, 2012	(As), BGS	Jul. 25, 2012	N
		Oss <sup>1</sup>	High	Jul. 8, 2013	(BGS + CO <sub>2</sub> ), BGS	Jul. 18, 2013	Y
2013	9 (7)	Weert 1 <sup>1</sup>	High	Jul. 23, 2013	(BGS + CO <sub>2</sub> ), BGS	Jul. 23, 2013	Y
		Weert 2	High	Jul. 23, 2013	(BGS + CO <sub>2</sub> )	Jul. 23, 2013	N
		Montfoort <sup>1</sup>	High	Jul. 23, 2013	(BGS + CO <sub>2</sub> ), BGS	Aug. 27, 2013	N
		Assen <sup>1</sup>	High	Aug. 9, 2013	(BGS + CO <sub>2</sub> ), BGS	Sep. 3, 2013	Y
		Almere	High	Jul. 22, 2013	(BGS + CO <sub>2</sub> )	Aug. 5, 2013	N
		Hardenberg <sup>1</sup>	High	Aug. 2, 2013	(BGS + CO <sub>2</sub> ), L	Aug. 14, 2013	Y
		Emmeloord <sup>1</sup>	High	Aug. 1, 2013	(BGS + CO <sub>2</sub> ), L, BGS	Sep. 24, 2013	Y
		Lelystad <sup>1</sup>	High	Aug. 1, 2013	(BGS + CO <sub>2</sub> ), BGS	Aug. 21, 2013	Y
		Oss	High	Jun. 17, 2014	(BGS + CO <sub>2</sub> )	Aug. 13, 2014	Y
2014	6 (1)	Weert 1	High	Jun. 17, 2014	(BGS + CO <sub>2</sub> )	Jun. 17, 2014	N
		Assen	High	Jun. 16, 2014	(BGS + CO <sub>2</sub> ), L	Sep. 17, 2014	Y
		Hardenberg	High	Sep. 22, 2014	(HL), L	Sep. 22, 2014	N
		Emmeloord <sup>1</sup>	High	Jun. 11, 2014	(BGS + CO <sub>2</sub> , HL), BGS	Jul. 14, 2014	Y
		Lelystad	High	Sep. 23, 2014	(BGS + CO <sub>2</sub> )	Sep. 23, 2014	Y
		Oss	High	Jul. 1, 2015	(BGS + CO <sub>2</sub> )	Jul. 1, 2015	N
2015	6 (1)	Montfoort	High	Jul. 13, 2015	(BGS + CO <sub>2</sub> )	Jul. 13, 2015	N
		Assen <sup>1</sup>	High	Aug. 10, 2015	(BGS + CO <sub>2</sub> ), As, HL	Aug. 20, 2015	Y
		Emmeloord	High	Jul. 13, 2015	(BGS + CO <sub>2</sub> )	Aug. 11, 2015	Y
		Lelystad	High	Jul. 15, 2015	(BGS + CO <sub>2</sub> )	Jul. 15, 2015	Y
		Etten-Leur	High	Jul. 13, 2015	(BGS + CO <sub>2</sub> )	Jul. 13, 2016	Y
		Assen	High	Jun. 24, 2016	(BGS + CO <sub>2</sub> )	Jul. 7, 2016	Not applicable
2016	4 (1)	Etten-Leur	High	Aug. 2, 2016	(BGS + CO <sub>2</sub> )	Sep. 13, 2016	Not applicable
		Emmeloord <sup>1</sup>	High	Jun. 22, 2016	(BGS + CO <sub>2</sub> ), BGS	Sep. 12, 2016	Not applicable
		Lelystad	High	Jun. 23, 2016	(BGS + CO <sub>2</sub> )	Sep. 6, 2016	Not applicable
Total	40						

<sup>1</sup> Found outdoors.

<sup>2</sup> L, larval sampling; As, aspirator; BGS, BG-Sentinel trap; CO<sub>2</sub>, carbon dioxide; MM, Mosquito Magnet Liberty Plus trap; HL, human landing collection.

control was applied at the locations. *Aedes japonicus* was found during surveillance outside a company that was located in the municipality of Lelystad, where an established population of this species was detected in 2013 (Ibañez-Justicia et al. 2014), and also at 2 used tire companies in 2014 and 2015.

In order to evaluate the impact of the adaptive surveillance strategy over time, we compared the annual proportions of samples containing IMS collected by BGS-trap with CO<sub>2</sub> at used tire locations

considered as “high risk.” This proportion was significantly higher (1-sided binomial test,  $P < 0.01$ ) before the application of the covenant in 2013 (12.96%) than in the successive 3 years (2014 [6.93%], 2015 [4.24%], and 2016 [5.09%]) (Fig. 3).

DISCUSSION

The study demonstrates that eggs of *Aedes* IMS are repeatedly introduced at specific locations that

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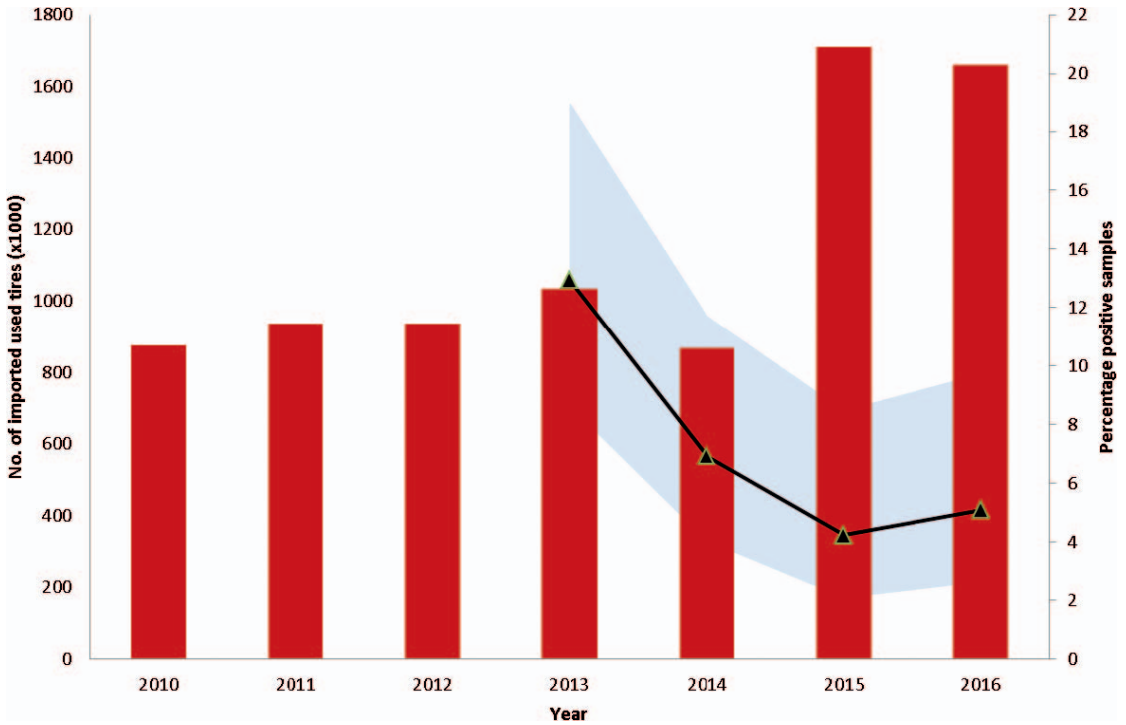


Fig. 3. Number of imported used tires at the used tire companies in the Netherlands (2010–16) (red bars; source: CBS-Statistics-Netherlands), and percentage of positive samples using BG-Sentinel (BGS) traps with CO<sub>2</sub> (black line). Blue area represents 95% confidence interval of the percentage of positive samples.

are under surveillance in the Netherlands. Under favorable conditions, these eggs hatch and develop into adults. Furthermore, occasional introductions at used tire companies were effectively kept under control after application of interventions against the larval and adult stages of IMS. Finally, since the measures specified in a covenant came into effect, our results suggest that the introduction of IMS at used tire and Lucky bamboo companies has stabilized and this process is not expanding.

At the Lucky bamboo greenhouses surveyed, results show that, even with the preventive measures mentioned in the commodities act (Staatsblad van het Koninkrijk der Nederlanden 2011, 2014), *Ae. albopictus* eggs still hatch and develop to the adult stage in spite of the biocide treatment. However, the numbers of *Ae. albopictus* specimens intercepted from 2010 onward were considerably lower than our earlier results starting in 2006, in which hundreds of *Ae. albopictus* were captured and caused nuisance to the nursery workers (Scholte et al. 2007, 2008). One factor that may explain the observed reduction of *Ae. albopictus* is the use of hygroscopic gel instead of water for transporting Lucky bamboo. However, this hygroscopic gel does not entirely eliminate the risk of importing *Ae. albopictus*. In a study in Belgium, a live 3rd instar of *Ae. albopictus* was found in a shipment of Lucky bamboo transported on gel (Demeulemeester et al. 2014). After the commodities

act came into effect in 2012, we initially observed few positive findings at the greenhouses but results from 2015 demonstrate that the measures need to remain accurately implemented by the Lucky bamboo importers.

Our finding of *Ae. japonicus* is the first to demonstrate that this species can be associated with the import of Lucky bamboo. *Aedes japonicus* has never been associated with trade products other than used tires before, the only other described mode of long-distance dispersal being passive transport inside horse trailers in the USA (Gaspar et al. 2012). *Aedes japonicus*, similar to *Ae. albopictus*, is native to southern China (Tanaka et al. 1979) and has a similar oviposition behavior by laying eggs in bamboo stumps (Sota et al. 1994). This may explain the presence of *Ae. japonicus* eggs in the Lucky bamboo imported from southern China. Furthermore, our results confirm for the 1st time, the introduction of *Ae. japonicus* with imported used tires in the Netherlands (Tables 2 and 3). The finding of a larva of *Ae. japonicus* in tires suggests that established populations in the surroundings of the municipality of Lelystad (Ibañez-Justicia et al. 2018) may have built up from successive introductions of infested tires at the used tire location located at this municipality, and before the surveillance activities were introduced in used tire companies in 2010.

The sampling strategy for detecting IMS at the used tire companies is presented here as an “adaptive sampling strategy.” This strategy is characterized by application of adjustments in the sampling methods, frequency of inspections, and sampling areas over the course of the study, mostly informed by findings of IMS at these locations, the change in the risk category of the locations for introduction of IMS, and by the advances in IMS detection methods. Although the use of larval sampling and aspirators can be effective for detecting IMS, the current use of mosquito traps for detecting adults (e.g., BGS-traps) and especially the addition of CO<sub>2</sub> is considered advantageous because 1) traps containing lures attract and capture IMS throughout the day in comparison with fortnightly larval sampling inspections; 2) the effectivity of the sampling of adult IMS using aspirators strongly depends on the choice of days with good weather for sampling (e.g., no rain, adequate temperature, no wind); 3) traps can detect IMS adults emerging from tire stacks that are not accessible for adequate larval sampling; and 4) the addition of CO<sub>2</sub> increases the chance of detecting IMS that are less attracted to the BG-Lure (such as *Ae. japonicus*).

From 2010 to 2016, the earliest detection of an adult IMS was June 11, 2014, in Emmeloord (Table 3). At other locations (e.g., Assen, Weert, and Oss), IMS were also found later that same month. These early findings may be related to the exceptionally warm weather in the spring of 2014, as 2014 was recorded as the hottest year in 3 centuries (<https://www.knmi.nl/kennis-en-datacentrum/achtergrond/2014-record-warm>).

From 2011 to 2016, 25 out of 34 introductions occurred at companies that were found positive the year before, suggesting that at these locations, new sets of used tires containing eggs of IMS were introduced, leading to new findings the following year. It is not known whether the IMS successfully overwintered at these companies during the sampling years, or if it was reintroduced each year through transport of infested tires. It is, therefore, recommended to study the lower temperature thresholds that 1) could limit the ability of *Ae. albopictus* to establish permanent populations over multiple years and 2) prevent population buildup during the warmer periods of the year.

Two mosquito species found during the surveillance were introduced with used tires from North America (Scholte et al. 2009, 2010): *Ae. aegypti* and *Ae. atropalpus*. In the study period, *Ae. aegypti* was no longer detected after the 1st finding and after subsequent mosquito control applied in 2010. Similarly, *Ae. atropalpus* was found in 2009 (Scholte et al. 2009), 2010, and 2011, and following mosquito control it was no longer detected at any of the sites. In comparison with the number of used tires imported from areas in southern Europe where *Ae. albopictus* has established, the number of used tire shipments from North America is considered small.

Data on used tire volumes imported into the Netherlands show that, except in 2014, the annual numbers of used tires imported are higher than in our 1st year of study (2010; Fig. 3), eventually leading to more possibility for introduction of infested tires. On the other hand, and as shown in the results, after the application of the preventive measures laid down in the covenant, from 2014 we expected a reduction in the risk for introduction of IMS at the used tire companies. Whether this is an effect that can be ascribed to the application of the covenant remains unknown. Although the application of the preventive measures has not completely eliminated the risk of introduction, it seems to have stabilized the situation and thus kept the introductions under control.

Overall, it can be considered that the yearly results of categorizing locations for used tires has resulted in detection of IMS in the country. Results show that all findings have been recorded at locations that were categorized as “high” risk in our risk analysis. In conclusion, data on introductions of IMS provided by surveillance are needed to evaluate risks, as well as to assess the effectiveness of surveillance and control measures.

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