First interception of *Aedes* (*Stegomyia*) *albopictus* in Lucky bamboo shipments in Belgium

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Abstract: Six gel-and five water-transported *Dracaena braunii* shipments originating from the South coast of China were screened for exotic mosquito species during a surveillance project on exotic vectors in Belgium. In November 2013, a live *Aedes* (*Stegomyia) albopictus* larva was detected in a gel substrate. This is the first direct evidence of the importation of *Ae. albopictus* on gel-transported Lucky bamboo. It also confirms that the importation risk of *Ae. albopictus* by transport of ornamental bamboo plants remains. In addition to the registration of appropriate biocides, a structured and permanent surveillance programme is needed in Belgium to allow for the early detection of invasive mosquito species and the timely implementation of control measures. ***Journal of the European Mosquito Control Association* 32: 14-16, 2014**

Keywords: Tiger mosquito, *Aedes albopictus*, *Dracaena braunii*, biocide legislation, surveillance, Belgium

Introduction

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse, 1894), is considered to be the most invasive mosquito species in the world (Gratz, 2004). It is a known vector of *Dirofilaria*, and also arboviruses including dengue, chikungunya, Eastern equine encephalitis, La Crosse, Venezuelan equine encephalitis, West Nile and Japanese encephalitis (Gratz, 2004). It was originally indigenous to South-East Asia, but has since spread to Africa, the Middle East, the Americas and Europe (Knudsen, 1995).

Aedes albopictus first appeared in Belgium on 31st October 2000 at a used tyre company in Vrasene, near Antwerp, when a single pupa and larva were collected (Schaffner *et al.*, 2004). Following further inspections and short term surveillance projects between 2001 and 2012, *Ae. albopictus* was not considered to be established in Belgium, and was assumed to have died-out naturally (Schaffner, unpublished data; Deblauwe *et al.*, 2012; Versteirt *et al.*, 2012). However, in July 2013 a male *Ae. albopictus* was collected at the same tyre company as the previous record (Boukraa *et al.*, 2013). Since then, 18 specimens have been found at the same company in Vrasene by the Institute of Tropical Medicine, suggesting a new and persistent introduction of this species.

Individuals of this species are primarily introduced into new geographic areas via the international trade in used tyres (Knudsen, 1995), and through the import of Lucky bamboo (*Dracaena braunii)* plants. The latter route has been responsible for introducing *Ae. albopictus* into California, U.S. (Madon *et al.*, 2003) and the Netherlands (Scholte *et al.*, 2007). In Belgium, the introduction of this mosquito species has only so far been detected through the import of used tyres.

Following the collection of the first *Ae. albopictus* specimens from a Lucky bamboo nursery in the Netherlands (Scholte *et al.*, 2007), it was proposed that transporting the plants on gel rather than in water would provide a more effective way to reduce the presence of *Ae. albopictus* in Lucky bamboo imports. In California, shipments of *Dracaena* spp. in standing water were prohibited following the identification of *Ae. albopictus*, and the import of plants has since only been permitted on gel (Madon *et al.*, 2003). This appears to have prevented further importations of *Ae. albopictus* in Lucky bamboo (Scholte *et al.*, 2008). However, in the Netherlands, 70% of nurseries where adult *Ae. albopictus* have been found, claimed to have imported plants on gel only (Scholte *et al.*, 2008).

Other means to prevent further importations of *Ae. albopictus* were proposed by the mandatory treatment of shipping containers with larval biocides shortly before shipment.

The Lucky bamboo commodities act (DWJZ/SWW-3039313) was created after the collection of more than 500 *Ae. albopictus* specimens at nurseries in the Netherlands between 2005 and 2009. The act states that "*Lucky Bamboo plants shall only enter the territory of the Netherlands free of Asian tiger mosquitoes. The plants have to be transported in sealed containers. These containers must be opened in a secluded area. The plants shall not leave the room unless they have been adequately treated with both an appropriate biocide to make the plants free of Asian tiger mosquito larvae, and an appropriate biocide to make the plants free of adult Asian tiger mosquitoes.*" (WTO, 2011).

In Belgium and the Netherlands however, the biocide legislation prohibits the use of biocides against invasive mosquitoes. An exemption on this legislation is therefore necessary before certain larvicidal products can be used. In June 2013, the exemption expired for the Netherlands. Since this expiration, some Lucky bamboo shipments destined for Dutch import companies, along with all shipments destined for a plant import company in Belgium, have been deviated via the port of Antwerp.

Here we present the first direct evidence of *Ae. albopictus* being imported into Belgium on gel-transported Lucky bamboo.

Materials and Methods

Eleven shipments of *Dracaena braunii* were inspected for the presence of mosquito larvae and eggs, on eight days, between August 2013 and January 2014. In five of these shipments, plants were transported in trays containing water, and in the remaining six, plants were transported in plastic bags containing hygroscopic gel. These bags were fixed around the base of plants to prevent the roots from drying out.

One of the shipments was destined for a Lucky bamboo import company in the Netherlands, and the other ten shipments for a Belgian company in the province of Oost-Vlaanderen.

Upon arrival at the border inspection post of the Federal Agency for the Safety of the Food Chain (FASFC), trays of Lucky bamboo were randomly selected for sampling of water or gel. Between 12 and 60 samples were collected from each shipment (Table 1), and the samples were taken to the Institute of Tropical Medicine (ITM), Antwerp, for analysis.

Morphological identification of mosquito larvae was carried out using taxonomic keys (Schaffner *et al.*, 2001; ECDC 2012) and confirmed by PCR. The ITS2 region of the rDNA gene was amplified using 5.8S (5'-ATCACTCGGCTCGTGGATCG-3') and 28S (5'-ATGCTTAAATTTAGGGGGTAGTCAC-3'), universal primers which flank the ITS2 gene (Collins *et al.*, 1987; 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 gel. Gels were stained with ethidium bromide and bands were visualized by UV trans-illumination

Results

On the 21st November 2013, a live third instar *Ae. albopictus* larva was found in a shipment of Lucky bamboo transported on gel (Table 1). The shipment originated from Guangzhou, a town in the south-eastern province of Guandong in China, and was intended for delivery to the import company in Belgium. No other mosquito larvae or eggs were detected in any of the remaining samples.

Table 1: Inspected Lucky bamboo shipments with inspection date, destination, mode of transport, number of samples inspected and number of positive samples for *Ae. albopictus.*

Date	Destination	Water/gel	# inspected samples	# positive samples
19 Sep-13	The Netherlands	Gel	30	0
25 Sep-13	Belgium	Gel	30	0
25 Sep-13	Belgium	Water	12	0
03 Oct-13	Belgium	Water	38	0
09 Oct-13	Belgium	Water	38	0
31 Oct-13	Belgium	Gel	30	0
21 Nov-13	Belgium	Gel	60	1(larva)
27 Nov-13	Belgium	Gel	30	0
27 Nov-13	Belgium	Gel	30	0
27 Nov-13	Belgium	Water	30	0
11 Dec-13	Belgium	Water	25	0

Discussion and conclusion

At most, 60 gel-filled bags were screened from each shipment of Lucky bamboo. This represents only 1/200th of the total cargo of each container. The likelihood that more gel-filled bags contain mosquito larvae is therefore high.

The limited data presented in this study indicates that there is still a risk of importing *Ae. albopictus* eggs or larvae into Europe via consignments of Lucky bamboo, even on a gel substrate. Although no water is found in the gel-bags, the gel is well hydrated and appears to offer a suitable medium for the short-term survival of mosquito larvae, and possibly for the eclosion of mosquito eggs during transport. Hygroscopic gel is likely a better substrate for transporting Lucky bamboo than water, but it does not alleviate the risk of importing a dangerous disease vector.

The ability of *Ae. albopictus* to colonise new geographic areas has led to its predicted spread in Europe (Medlock *et al.*, 2012). Using a Geographic Information Systems (GIS)-based model, a study in the U.K. revealed that abiotic risk factors would permit establishment of *Ae. albopictus* throughout large parts of lowland U.K. (Medlock *et al.*, 2006). A parallel study concluded that climatic conditions do not appear to preclude establishment of *Ae. albopictus* in the Netherlands (Takumi *et al.*, 2009). Indeed some temperate strains of *Ae. albopictus* have been proven to survive the cold winters in the northern hemisphere through the production of cold-resistant eggs (Severini *et al.*, 2008).

Although the strains of *Ae. albopictus* imported with *Dracaena braunii* are tropical, outdoor establishment of the mosquito might be possible if no preventative and curative control measures are taken in nurseries housing Lucky bamboo in the Netherlands (Scholte *et al.*, 2010; Takumi *et al.*, 2009) and other temperate countries like Belgium. No control measures are currently in place in Belgium owing to the difficulty in obtaining an exemption of the biocide legislation, which increases the risk of *Ae. albopictus* becoming established. We therefore urge the registration of appropriate insecticides for use against invasive mosquitoes. We also recommend the implementation of a structured and permanent surveillance programme in Belgium, similar to those already in use in neighbouring countries (e.g. CNEV 2012; VWA 2013).

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References

Boukraa, S., Raharimalala, F.N., 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

Centre national d'Expertise sur les Vecteurs. Optimisation de la surveillance et du contrôle d'*Aedes albopictus* en France. Montpellier: CNEV (2012) http://www.cnev.fr/index.php/publications-et-outils/avis-du-cnev/981-rapport-relatif-a-la-surveillance-et-au-controle-daedes-albopictus Accessed on November 2013

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 Tropica* **112**, 266–269

Deblauwe, I., Sohier, C. and Coosemans, M. (2012) ExoSurv: implementation of surveillance of exotic mosquitoes in Belgium. Report for the Federal and Regional governments of Belgium, 130pp. Available online: http://www.health.belgium.be/internet2Prd/groups/public/@public/@nehap/documents/ie2divers/19094720.pdf

Djadid, N.D., Gholizadeh, S., Tafsiri, E., Romi, R., Gordeev, M. & Zakeri, S. (2007) Molecular identification of palearctic members of *Anopheles maculipennis* in northern Iran. *Malaria Journal* **2007**, 6. doi:10.1186/1475-2875-6-6

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

Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus. Medical and Veterinary Entomology* **18**, 215– 227

Knudsen, A.B. (1995) Global distribution and continuing spread of *Aedes albopictus. Parassitologia* **37**, 91–97

Madon, M.B., Hazelrigg, J.E., Shaw, M.W., Kluh, S. & Mulla, M.S. (2003) Has *Aedes albopictus* established in California? *Journal of the American Mosquito Control Association* **19**(4), 297-300

Medlock, J.M., 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. *Journal of Vector Ecology* **31**, 292-304

Medlock, J.M., Hansford, K.M., 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 and Zoonotic Diseases* **12**(6), 435–447

Schaffner, F., Angel, G., Geoffrey, B., Hervy, J.P., Rhaiem, A. & Brunhes, J. (2001) The Mosquitoes of Europe. An Identification and Training Programme (CD Rom). IRD Éditions & EID Méditerrannée, Montpellier

Schaffner, F., Van Bortel, W. & Coosemans, M. (2004) First record of *Aedes (Stegomiya) albopictus* in Belgium. *Journal of the American Mosquito Control Association* **20**, 201-203

Scholte, E.J., Jacobs, F., Linton, Y.M., Dijkstra, E., Fransen, J. & Takken W. (2007) First record of *Aedes* (*Stegomyia*) *albopictus* in the Netherlands. *European Mosquito Bulletin* **22**, 5–9

Scholte, E.J., 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. *Medical and Veterinary Entomology* **22**(4), 352-358

Scholte, E.J., 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 Surveillance*, **15**(45):pii=19710

Severini, F., Di Luca, M., Toma, L. & Roma, R. (2008) *Aedes albopictus* in Rome: results and perspectives after 10 years of monitoring. *Parassitologica* **50**(1-2), 121–123

Skuse, F. (1894) The banded mosquito of Bengal, Indian Museum. Notes **3**, 20

Takumi, K., Scholte, E.J., Braks, M., Reusken, C., Avenell, D. & Medlock, J.M. (2009) Introduction, Scenarios for establishment and seasonal activity of *Aedes albopictus* in The Netherlands. *Vector- Borne and Zoonotic Diseases* **9**, 191-196

Versteirt, V., Boyer, S., Damiens, D., De Clercq, E.M., 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. *Bulletin of Entomological Research* 103, 193–203

VWA: Nederlandse Voedsel- en Warenautoriteit: Vectoren 2013 Available online: http://www.vwa.nl/onderwerpen/bacterien-ziekteverwekkers/dossier/vectoren/centrum-monitoring-vectoren Accessed on November 2013