ESTIMATES OF PUBLIC HEALTH RISKS THROUGH IMPORT OF *AEDES ALBOPICTUS* VIA *DRACAENA'S* IN THE NETHERLANDS

In Dutch: Inschatting risico's voor de volksgezondheid door import van *Aedes albopictus* via *Dracaena's* in Nederland.

Part II – Investigation in the establishment of *Aedes albopictus*

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Final report, April 2008

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Summary
Between September 2005 and October 2007 studies were done in six horticultural companies in the Netherlands to assess the presence of the exotic *Aedes albopictus* and to determine whether the species had become established. Adult mosquitoes were sampled with liberty plus carbon dioxide traps. The presence of eggs and larvae was studied by ovitraps and inspection of water collections. All traps ran continuously, and were inspected for presence of mosquitoes at 14d intervals. A total of 198 adult *Ae. albopictus* were collected, as well as thousands of specimens of other mosquito species and *Culicoides* species. 95% of *Ae. albopictus* were caught indoors. *Aedes albopictus* was not found in the ovitraps, while on a few occasions *Culex pipiens* larvae were found in these traps. On one occasion, larvae of *Ae. albopictus* were found indoors in a flower pot containing Lucky bamboo shoots. All ditches and other water collections sampled indoors and outdoors did not reveal the presence of *Ae. albopictus*. It is concluded that during the study no evidence for the establishment of *Ae. albopictus* was found, and it appears that the adult *Ae. albopictus* found originated from imports from abroad. *Culex pipiens* was the most abundant mosquito species found in each study site with evidence of local reproduction.

Introduction

The Asian tiger mosquito, *Aedes albopictus*, was sighted in the Netherlands for the first time in the summer of 2005. The mosquito was found in greenhouses where plants imported from abroad were kept and prepared for wholesale to commercial flower- and plant shops. In one company the workers complained about biting mosquitoes (Scholte et al. 2007). Staff from the Plant Protection Service and the Laboratory of Entomology of Wageningen University identified the mosquitoes as *Aedes albopictus*. This was the first time that this mosquito species had been reported this far North in Europe. It was soon suspected that the mosquitoes had accidentally arrived as hitchhikers with shipments of Lucky bamboo (*Dracaena senderiana*) from China. Such shipments were arriving in the Netherlands in huge quantifies. The plants were unloaded in greenhouses in the Netherlands, and prepared for sale to the public. In California, such shipments had been responsible for the accidental introduction of *Aedes albopictus* in that state (Linthicum et al. 2003). Elsewhere, *Aedes albopictus* has invaded huge geographic areas, becoming definitely established in the United States, the Caribbean, South and Central America, southern Europe and West Africa. Occasionally, the species is seen in North Australia.

As *Aedes albopictus* is an excellent vector of several arboviral diseases (Gratz 2004), the potential of esablishment of this mosquito must not be underestimated. For example, in 2007 Italy witnessed its first epidemic of Chikungunya virus, transmitted by *Aedes albopictus* (Angelini et al. 2007). The Dutch Ministry of Health, Welfare and Sports (VWS) commissioned a study to investigate the introduction of *Aedes albopictus* in the Netherlands, and to assess the health risks associated with these introductions. As part of this greater study, the current study concerns an investigation in the establishment of *Aedes albopictus* in and around sites of introduction. The study was conducted from June 2006 through November 2007. Data collected between September 2005 and June 2006 are also included.

Material and Methods

Selection of study sites

Study sites consisted of horticultural companies specialized in the importation of plants and flowers from abroad for wholesale to flowershops and other markets. These companies are usually housed in huge greenhouses, sometimes the size of several soccer fields. The study was initiated in September 2005 in three companies near Amsterdam. When it became clear that the first company (company A) soon stopped trading in plants, another company was added (company D). Later in the study companies E and F were included, and sampling at company C was terminated.

Mosquito collections

Adult mosquitoes were collected with liberty plus traps (American Biophysics Co., Rhode Island, USA)(Fig 1). These are counterflow traps, releasing CO_2 as mosquito bait by the catalytic burning of propane gas (Kline 2002). These traps can run continuously, and propane gas tanks need to be replaced approx .once every 3 weeks. In each company one trap was placed indoors, in the vicinity of Lucky bamboo plants. In company B, due to its large size, two traps were placed indoors. The number of traps placed outdoors varied from one to four (Table 1). In company A the outdoor trap was placed at 10 m from the back entrance to the company, where trucks were being unloaded. In company B, one trap was next to the main entrance, two traps on the parking lot (50 m from the buildings) and one trap at a corner (10 m from the building) facing an open agricultural field. At company E one trap was placed next to the entrance to the building, and two traps at the edge of a parking lot, approx. 25 m from the building. Both traps were adjacent to a drainage ditch and faced an agricultural field.
At company F two traps were placed on the parking lot, at 10 m from the building. A third trap was placed in the garden of a farm neighbouring the company. At companies C and D mosquitoes were collected only indoors.
Monitoring of aquatic stages of mosquitoes was done in companies B, F and G, each with 10 ovitraps (Bellini et al. 1996) of which 5 were placed indoors, and 5 outdoors. The traps were emptied once every 2 weeks and the oviposition strips inspected for presence of eggs. In addition, during each site visit a thorough inspection for larvae was conducted in standing water indoors as well as in ditches and water containers outdoors. Larvae were collected with a standard dipper (in ditches) and with suction tubes.

Taxonomic identification
All larval and adult mosquito samples were taken to the laboratory in Wageningen and identified to species. Larvae, when appropriate, were allowed to develop into adult mosquitoes in the laboratory for better identification.

Results

Adult collections
The number of weeks of continuous sampling for mosquitoes at each of the six companies varied from 20 tot 118 (Table 1). Company B was sampled from September 2005 until the end of October 2007. *Aedes albopictus* was found in five of the six companies. A schedule of sampling and the resulting overall catch of *Aedes albopictus* per company is presented in Figure 1. In companies A and F *Aedes albopictus* was found on one occasion only. In company B *Ae. albopictus* was found on 18 occasions, and in company E on 22 occasions. 42% of all *Ae. albopictus* collected was found at company B, and 51% at company E. The number of *Ae. albopictus* per trap varied from zero (on most occasions) to 21 per trap per 14 days (Table 2). In company B *Ae. albopictus* was found for the first time 40 weeks after surveillance began. Subsequently, for 22 weeks there were weekly collections, then 14 weeks no *Ae. albopictus* was found, whereafter *Ae. albopictus* catches became more irregular (Table 2). In company E *Ae. albopictus* was found from the start of the surveillance until 76 weeks into the study, after which, upto week 100, no *Ae. albopictus* was found any longer.
Of the 198 adult *Ae. albopictus* found during this study, 95% were collected indoors. Two *Ae. albopictus* were found outside of company B on 9 February 2006, several months after the company had closed for business. The other outdoor collections were made at times when the companies were importing Lucky bamboo.

Table 1 – Summary of the number of weeks mosquitoes were collected in liberty plus traps per company, distributed over indoor (In) and outdoor (Out) collections. The pooled number of *Aedes albopictus* is indicated behind each company and trap site.

	Trap weeks	no. traps indoors	In	In	no. traps outdoors	Out	Out	Out	Out
Company A	94	1	0	na	1	2	na	na	na
Company B	118	2	69	5	4	6	0	3	0
Company C	64	1	1	na	na	na	na	na	na
Company D	20	1	0	na	na	na	na	na	na
Company E	100	1	95	na	3	5	0	1	na
Company F	24	1	na	0	3	0	1	0	na

Oviposition searches

All bi-weekly inspections of the oviposition traps did not reveal the presence of *Ae. albopictus* during the period of June 2006 – October 2007. During the entire study period mosquito eggs were not found. *Ae. albopictus* larvae were found once on 19 September 2006 at company E indoors in water in a pot containing Lucky bamboo shoots. This was at a time when the highest number of adult *Ae. albopictus* were found here. All other searches for larval stages of *Ae. albopictus* were negative. By contrast, larvae of *Culex pipiens* were frequently found in the oviposition traps placed outdoors. Large numbers of *Cx pipiens* were also found in drainage ditches and used tyres and other receptacles of rainwater.

Other insect species

A large number of insect species was collected in the liberty plus traps (Table 3). These included 11 mosquito species of 5 genera and 15 species of the genus *Culicoides* and the stable fly *Stomoxys calcitrans* (data not shown). The latter includes several vectors of bluetongue, an arbovirus that affects sheep and cattle. *Culex pipiens* was the most abundant species found. *Culicoides obsoletus* s.l. was the most frequent bluetongue vector found. Trap F4 at company F collected very large numbers of *Cx pipiens* and *Culicoides festivipennis* (Table 3). These mosquito and midge collections were not restricted to the outdoor traps, but a large number of species was also collected indoors

Table 2 - Collections of adult *Aedes albopictus*

Company	A		B						C	D	E				F			
	Binnen	Buiten	Binnen	Binnen	Afvalbak	Ingang	Park.plaats #1	parkplaats #2			Binnen	ingang	Hoek N	Hoek Z	Kas	Treurberk	Treurwilg	Achtertuin
26/08/2005											1							
02/09/2005												1						
23/09/2005												2						
03/10/2005											2							
07/10/2005																		
13/10/2005																		
03/11/2005											1							
11/11/2005																		
02/12/2005											11							
09/12/2005											1							
22/12/2005											9							
06/01/2006											3							
16/01/2006											6							
27/01/2006											2							
09/02/2006		2									1							
23/02/2006											1							
03/03/2006																		
15/03/2006																		
30/03/2006											1							
18/04/2006																		
02/05/2006			1															
16/05/2006			6								2							
01/06/2006			8															
15/06/2006			8															
29/06/2006		X			1													
12/07/2006			1															
27/07/2006			7								1	1						
09/08/2006			5															
24/08/2006			8				1											
07/09/2006			9		2						12							
19/09/2006			5				2				21							
05/10/2006											16			1				
19/10/2006											1	X		X				
02/11/2006												1						
16/11/2006		X										X						
30/11/2006			X						X		2							
14/12/2006		X											X					
28/12/2006									X		1			X				
11/01/2007		X	1						X				X					
25/01/2007													X	X				
08/02/2007		X	X										X					
22/02/2007		X					X											
08/03/2007									X									
22/03/2007			1									X						
05/04/2007			3						X			X						
18/04/2007			4	5	X							X						
03/05/2007			1		X				X			X						
16/05/2007					3				X								1	
31/05/2007									X									
14/06/2007									1								X	
28/06/2007					X	X												
12/07/2007																		
26/07/2007																		
09/08/2007			1													X		
23/08/2007					X													
06/09/2007																		
20/09/2007									X									
04/10/2007									X									
18/10/2007									X							X		

Legend: X = trap failure occurred during the collection period

Figure 2 - Time schedule of mosquito collections and division of traps over the six Lucky-bamboo importing companies

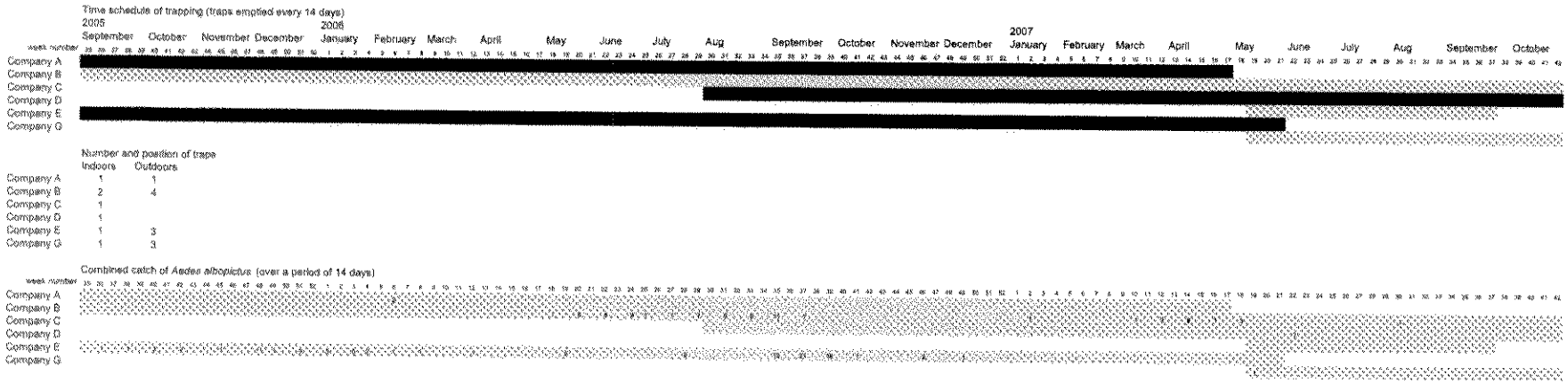

Table 3 - Total insects (species) collected per trap

		Ae albopictus	Aedes cinereus	Aedes caspius	Aedes punctor	Aedes vexans	Culex pipiens	Culex modestus	Culiseta annulata	An. maculipennis	An. claviger	Coquillettidia richardii	Culicoides obsoletus	Culicoides dewulfi	Culicoides chiopterus	Culicoides pulicaris	Culicoides punctatis	Culicoides circumscriptus	Culicoides festivipennis	Culicoides minitissimum	Culicoides stigma	Culicoides salinarius	Culicoides albicans	Culicoides nubeculosis	Culicoides impunctatus	Culicoides newsteady
Indoor collections																										
Company A	Trap A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Company B	Trap B1	69	0	0	0	0	63	0	0	0	0	0	2	0	2	3	2	0	0	0	0	0	0	0	0	0
	Trap B2	5	0	0	0	0	7	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0
Company C	Trap C1	1	0	0	0	0	5,442	0	20	21	6	1	6	0	4	1	1	1	16	15	0	0	0	0	0	0
Company D	Trap D1	0	0	1	1	0	59	0	54	10	3	1	36	0	10	13	0	2	1	1	0	0	0	0	0	0
Company E	Trap E1	95	0	0	0	0	262	0	4	1	0	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0
Company F	Trap F1	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Outdoor collections																										
Company A	Trap A2	2	2	0	0	0	1,576	0	20	4	0	0	21	0	2	2	0	0	0	1	0	0	0	0	0	0
Company B	Trap B3	6	0	0	0	0	334	0	15	1	1	0	13	1	0	5	3	0	10	3	0	0	0	0	1	0
	Trap B4	0	0	0	0	0	280	0	9	0	2	1	2	0	7	4	3	2	7	2	0	0	0	0	0	0
	Trap B5	3	0	0	1	1	1,339	0	40	46	15	12	75	20	8	0	0	0	6		0	0	46	0	4	1
	Trap B6	0	0	0	0	0	60	0	3	0	0	0	6	1	1	0	0	0	0	0	0	0	0	0	0	0
Company E	Trap E2	5	2	0	0	0	537	0	4	12	26	6	23	0	0	0	0	0	0	1	3	0	0	0	0	0
	Trap E3	0	0	0	0	0	767	0	35	84	1	7	11	0	2	1	0	0	1	0	0	0	0	0	0	0
	Trap E4	1	0	0	0	0	373	8	0	1	0	0	3	0	3	1	0	0	0	0	0	0	0	0	0	0
Company F	Trap F2	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Trap F3	0	0	0	0	0	350	0	4	0	0	0	5	2	0	3	3	0	5	0	0	0	0	0	0	0
	Trap F4	0	0	0	0	0	62,649	0	1	0	0	0	9	1	0	0	0	5	3,345	0	0	0	1	2	0	0
	Trap F5	0	0	0	0	0	1,007	1	0	3	2	0	16	1	1	0	0	0	4	0	0	0	4	1	0	0

Discussion

This study was undertaken to investigate the possibility that *Ae. albopictus* might become established in the Netherlands. The frequent sightings and recorded collections of *Ae. albopictus* in this study, and in the longitudinal surveillance study, suggest that under favourable climatic conditions *Ae. albopictus* can survive in the Netherlands. In warm summers, the species can in theory deposit viable eggs. Indeed, in southern Europe *Ae. albopictus* has become firmly established in Albania, Italy, France and Spain, with frequent incursions into southern Switzerland. At least in Italy the invasion and establishment of *Ae. albopictus* has been attributed to the import of used tyres. It is known that such tyres can contain very large number of *Ae. albopictus* eggs which survive periods of desiccation (Knudsen et al. 1996). In northern Italy *Ae. albopictus* overwinters in the egg stage, and can tolerate periods of frost. Population growth, however, occurs mostly in the summer when mean temperatures are favourable for egg and larval development.

Our study has shown that the hypothesis of current establishment of *Ae. albopictus* in or near the companies studied must be rejected. Establishment of the species in the vicinity of our traps should have demonstrated an increase in numbers of *Ae. albopictus* in the collections. This has not been the case. In company B, where on 5 separate occasions *Ae. albopictus* was recorded in outdoor traps, 3 of these collections were made next to the main entrance of the company building. At times when mosquito densities were "high", it is likely that several of them escaped to the outdoors. Carbon dioxide is an attractive compound for *Ae. albopictus*, and hence several escapees could be trapped. Similarly, at company E 4 of the 5 outdoor collections were made in the trap placed next to the entrance/exit door. We conclude that based on adult collections, no indications of establishment have been noted, neither indoors nor outdoors.

All ovitrap collections were negative throughout the study. It is possible that occasionally larvae of *Ae. albopictus* might have developed into mature mosquitoes, as demonstrated by our one-time collection of larvae in a pot containing Lucky bamboo and some water (Fig 1). This was indoors in a greenhouse. These larvae may have arisen from eggs imported with the plants from China. They could also have arisen from eggs deposited by female mosquitoes imported from China. However, in a total of 538 trap weeks (this is the pooled number of weeks that the indoor traps operated), and bi-weekly inspections of water collections and oviposition traps, only once were larvae of *Ae. albopictus* found. Should an establishment have occurred, several and serial larval findings should have been made in or around the same location. This was not the case.

In a recent simulation study (K. Takumi et al., unpublished) on the potential for survival of *Ae. albopictus* in the Netherlands, it was found that the climatic conditions in the Netherlands are suitable for survival of the species in parts of the country. The current study area lies within the boundaries of the suitable area. The summer of 2006 was the warmest summer on record, with an average temperature of 22.5°C. This coincided with several high catch periods of *Ae. albopictus* at the companies under study. Therefore, the environmental conditions for establishment of the species in 2006 appeared ideal and

were better than in 2007, when the frequency of introductions was much less than in 2006.

The most likely reason why establishment has not occurred during our study is the relatively low density of *Ae. albopictus* populations in the locations studied. The largest catch of *Ae. albopictus* over a 2-week period was 21 mosquitoes (Table 2), whereas in most cases only one mosquito was found. However, as the efficiency of the traps is not known, and the greenhouses in which the lucky bamboo plants were being kept were large, the relative number of *Ae. albopictus* in the greenhouses may have been high at several times, the more so as staff working in the greenhouses complained about mosquito bites. It is unlikely that these bites derived from *Cx pipiens*, which is an ornithophilic species. The probability that newly emerged adult *Ae. albopictus* encountered mating partners is considered very small. For this to occur, high densities of the species are needed within a relatively confined area (Ricklefs and Miller 1999).

It was noted on several occasions that mosquito control was undertaken by spraying with an insecticide. It is not known which insecticides were used, but for several weeks after such activities the number of mosquitoes in the greenhouse was very low, and this may have also kept any development of *Ae. albopictus* under control.

On several occasions the traps experienced a mechanical failure (Table 2). Such failures occurred mostly because of low outdoor temperatures (< 5°C) shutting down the catalytic converter. This may not have resulted in missed collections, as at these ambient conditions mosquitoes are no longer active outdoors, and indeed one would not have expected a mosquito to be caught by the trap under such circumstances. At other times, traps failed occasionally because of poor batteries. The mechanical failure of the traps has also been found in other (winter) studies on arthropod vectors in the Netherlands (Takken et al., unpublished data) and in the UK (Hutchinson et al. 2007).

Once an establishment of an exotic mosquito species has occurred, it may take some time before this is observed. For example, it is not known for how many months or years *Ae. albopictus* had been present in Genoa, Italy, before the first adult mosquito was reported in 1990. Similarly, the species may have been present in Albania long before it was reported for the first time (Adhami and Reiter 1998). Therefore, in order to be sure that no establishment of *Ae. albopictus* can occur, a continuous surveillance for the presence of *Ae. albopictus* will be necessary as long as the importation of Lucky bamboo continues uncontrolled. Plans for placing the plants into quarantaine before release are being developed, but it remains to be seen how effective such measures will be, and vigilance for the presence of *Ae. albopictus* is strongly recommended. The measures taken by the Ministry of Health, Welfare and Sports in November 2006 to reduce the import of *Ae. albopictus* appear to be effective as the number of new findings of *Ae. albopictus* has dropped sharply since early 2007 (E.-J. Scholte, personal communication).

It is concluded that the current study did not demonstrate the establishment of *Ae. albopictus* in or around the companies that were studied. At the same time, over a period of 118 collection weeks (Sept 2005 – Oct 2007), *Ae. albopictus* was found on 76 of the 118 weeks, suggesting a very regular introduction of the species from abroad. The frequent introductions are a cause for concern, as theoretically *Ae. albopictus* can survive in the Netherlands under current climate conditions. However, it is assumed that large

numbers of mosquitoes need to be present at the same time for establishment to occur. Studies are needed to estimate the population density of adult *Ae. albopictus* required within a geographically-defined region in order for an establishment of this mosquito species to be successful.

Acknowledgments

We thank the anonymous owners and managers of the companies discussed in this report for their cooperation and interest in the work undertaken on their premises. We are grateful to Niels Verhulst for having assisted with the mosquito collections. Annette de Boer (RIVM), Chantal Reuskens (RIVM), Marion Koopmans (RIVM) and Ernst-Jan Scholte (Plant Protection Service) are thanked for a pleasant collaboration and asisstance in the course of the study.

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