

### 3. Results

#### 3.1 Mosquito traps at Lucky bamboo importing companies:

From the 471 trap collections of the 17 Lucky bamboo importing companies during this survey, 67 collections were positive for *Ae. albopictus* (14.2%). In total, 542 adult specimen of *Ae. albopictus* were collected from the traps of these companies, at 15 Lucky bamboo importing companies. Most of these were collected from July until December 2006.

Table 1. Number of *Aedes albopictus* (cumulative for all infested companies) collected at Lucky bamboo-importing companies\* per 4 weeks (2 collections), July 2006-June 2007.

Week nrs.	# <i>Ae. albopictus</i>	# infested companies
27-30 ( $\pm$ July 2006)	185	10
31-34 ( $\pm$ August)	142	9
35-38 ( $\pm$ Sept.)	26	5
39-42 ( $\pm$ end Sept. - half Oct.)	51	4
43-46 ( $\pm$ half Oct. - half Nov.)	11	2
47- 50 ( $\pm$ half Nov. - half Dec.)	78	3
51-2 ( $\pm$ half Dec.- half Jan. 2007)	6	3
3-6 ( $\pm$ half Jan.-half Febr.)	3	1
7-10 ( $\pm$ half Febr. - half March)	21	1
11-14 ( $\pm$ half March-begin April)	11	1
15-18 ( $\pm$ begin April- begin May)	4	2
19-22 ( $\pm$ May)	3	2
23-26 ( $\pm$ June)	2	2

\* There were a total of 16 Lucky bamboo importing companies included in the survey until 41. From week 42 onwards, there were 17.

The peak of the infestation was mid-summer of 2006 (week 28-32), when a total of 13 Lucky bamboo-importing companies (out of the 16 included in the survey at that time) were found positive, relatively high numbers of specimen were collected, and nuisance reports were at its height. The numbers of positive trap collections drastically reduced after December 2006. During the 6-month period from January 2007 until June 2007, a total of 46 specimen were collected from 12 positive collections, against a total of 497 from 55 positive collections in the 6 month period July 2006-December 2006. From a total of 17 Lucky bamboo importing companies that were included in this survey, 15 (88.2%) have been positive for the species at least once during the period of the survey. On most of these *Ae. albopictus*-positive companies, the species was found only once or a few times. Trap nrs. 3, 8, 9, and 14 were found positive only once, and trap numbers 3, 6, 7, 8, 9, 10, 11, 13, 14 and 15 two or three times. A handful of companies were found positive on several different occasions: Trap nr. 4 was found positive on 6 different collections, trap nr. 12 on 8 collections, trap nr. 17 on 9 collections, and trap nr. 16 even on 11 collections. It should be noted however, that infestation levels are not necessarily linked to the number of times that that company has been positive for the presence of *Ae. albopictus*: For instance, trap nr. 6 was positive only on two collection dates (weeks 30 and 32), but at those two collections, a total of 131 mosquitoes were collected, suggesting a heavy infestation at that period in time.

It would be reasonable to suppose that if relatively many Lucky bamboo plants are imported per company per time-scale, the likelihood of (unwillingly) importing *Ae. albopictus* at these companies is also high. This, however, was not the case: The total quantities of imported Lucky bamboo plants per company per year did not coincide with the relative number of *Ae. albopictus* mosquitoes one would expect. Several of the companies that were importing relatively low quantities of Lucky bamboo per year, were among the companies where most mosquitoes were collected, and *vice versa*.

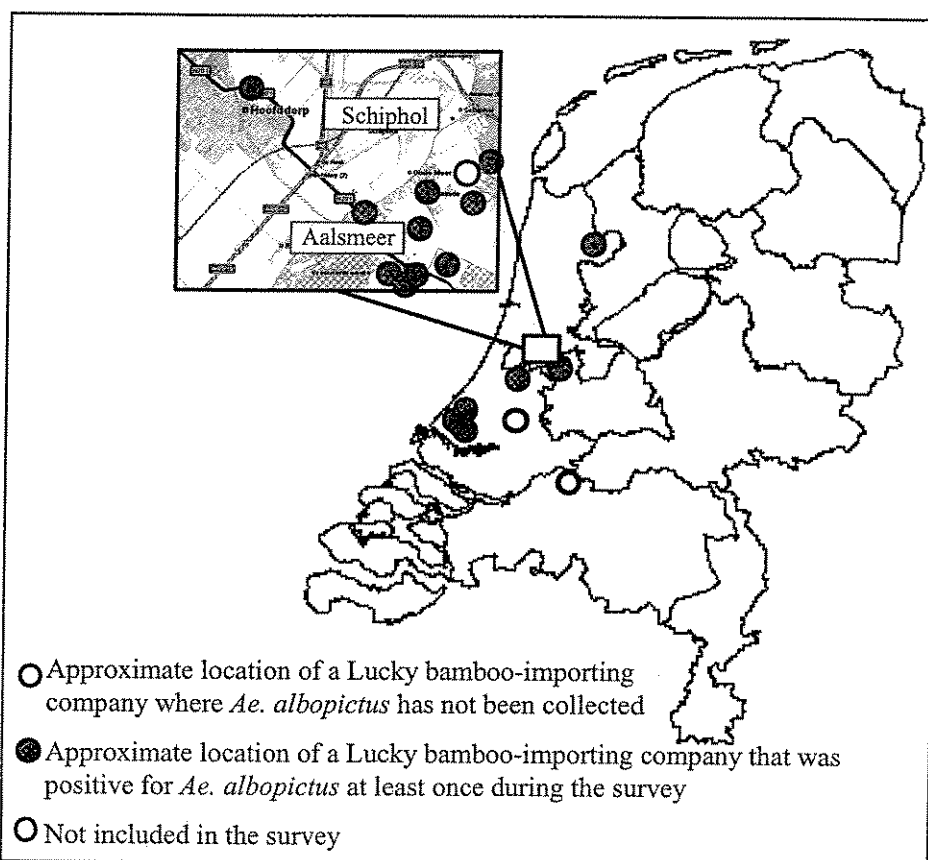


Figure 5. Distribution map of Lucky bamboo-importing companies in the Netherlands where mosquito traps were placed for *Ae. albopictus*.

Table 2. Approximate *Aedes albopictus* infestation levels: cumulative numbers collected at the Lucky bamboo importing companies during the survey 2006-2007.

Cumulative numbers of <i>Ae. albopictus</i>	number of companies
0	2
1 ≤ 5	6
6 ≤ 25	4
26 ≤ 99	2
≥ 100	3



Table 3. Cumulative numbers of *Ae. albopictus* collected per mosquito trap at Lucky bamboo importing companies during the entire duration of the survey.

Trap nr.*	cumulative number of <i>Ae. albopictus</i>
2	0
3	1
4	13
5	32
6	131
7	5
8	1
9	2
10	21
11**	2
12	23
13	0
14	2
15	6
16***	132
17****	111
21	60

\* Trap nrs. 1, 18, & 19 were placed at companies that did not, and never had, imported Lucky bamboo. Trap nr. 20 was placed in the garden of a family. The data of those traps are not depicted in the table, but are presented in the maintext.

\*\* The company where this trap was placed had stopped with importing and storage of Lucky bamboo by early summer 2007.

\*\*\* This company moved from one location to another during the survey; at the initial location where the trap was located, Lucky bamboo had been removed by approximately March.

\*\*\*\* The companies where these traps were placed had stopped with importing and storage of Lucky bamboo by approximately March 2007.

### 3.2 Mosquito traps at other locations:

From the four traps (nrs. 1, 18, 19, and 20) placed in the neighbourhood of Lucky bamboo importing companies, only one trap (trap nr. 19) had been found positive for the presence of *Ae. albopictus*, on a single occasion: In week 30 in 2006, one specimen of *Ae. albopictus* was collected from a trap (trap nr. 19) placed in a glasshouse that did not contain, and never had contained Lucky bamboo. Apparently this mosquito had managed to escape from the glasshouse of the Lucky bamboo-importing company and flew into this neighbouring glasshouse. The trap placed at this neighbouring Lucky bamboo importing company (trap nr. 10) had collected 11 *Ae. albopictus* in that same week, suggesting a relatively heavy infestation level at that moment in time.

### 3.3 Presence of larvae:

On three occasions, at two different Lucky bamboo importing companies, *Aedes albopictus* larvae were collected from the transport/storage boxes containing the plants plus a layer of water at the bottom: in week 38 (2006), 32 larvae were collected, and in week 41 two larvae at the same company. In that same week (41), 24 larvae were collected from another Lucky bamboo importing company.



### 3.4 Manually collected adult mosquitoes:

On five occasions, flying *Ae. albopictus* mosquitoes were collected manually (either with a suction tube or with a net) in 4 different infested Lucky bamboo importing companies. In all cases, these manual collections coincided with the presence of relatively large numbers of *Ae. albopictus* in the mosquito traps for that period. On many other occasions, flying *Ae. albopictus* were observed but were not collected manually, either due to time-pressure or because the mosquitoes were difficult to collect.

### 3.5 Sightings by the public:

There have been numerous reports coming in from the public about supposed tigermosquitoes, in houses, gardens, and, in one case, out in a forest. Except for one case that is described below, all reported mosquitoes were endemic species. Most sightings were on mosquitoes that had a yellow-brown striped abdomen which, after asking the caller why he/she had supposed it was an Asian tigermosquito, had brought up an association with the coloring pattern of a tiger. So rather than knowing the true patchy black and white coloration pattern of *Ae. albopictus*, almost all callers assumed the species would have yellow brown stripes, somewhat like tigers.

Only in one case collected mosquitoes by the public were actually identified as *Ae. albopictus*. This case was a family living next door to a heavily infested Lucky bamboo importing company (where trap nr. 21 was placed), who had experienced severe nuisance of what they described as 'aggressive day-time biting mosquitoes', during the summer months of 2006 in and around the house. They had manually collected several specimen which, upon identification, proved to be *Ae. albopictus*. Ironically, although these people have been the only ones to actually have collected *Ae. albopictus*, they did not know about the existence of 'tigermosquitoes', and they did not report their findings on their own initiative. It was only by chance that inspectors came to 'chat' with them, as they were investigating mosquito biting nuisance on and around a particularly heavy infested Lucky bamboo importing company. After this finding, the immediate surroundings of the company have been investigated several times for the presence of *Ae. albopictus*, but the species was not found again (no larvae, no adults), and the affected family did not experience mosquito biting nuisance after august 2006.

This case, and the one described above in the paragraph '*mosquito traps at other locations*' showed that *Ae. albopictus* does not limit itself to the glasshouses, but may exit these glasshouses, sometimes apparently in relative large numbers and cause nuisance in neighboring areas.

### 3.6 Biting nuisance

Nuisance was reported repeatedly by PD inspectors themselves while visiting infested Lucky bamboo importing companies. Also, when PD inspectors asked employees if they had experienced mosquito biting nuisance, were answered with a 'yes' on 36 occasions. Strangely enough, only in 13 cases of these reports coincided actually with the presence of *Ae. albopictus* collected from the trap at that company that particular period. On the other hand it also occurred that the trap had collected *Ae. albopictus* mosquitoes, but that the employees answered 'no' to the question if they had experienced mosquito biting nuisance. In particular, two cases are worthwhile mentioning; in the first case a total of 26 specimen and in the second even 67 specimen were collected from the traps at that period, which is considered as heavy infestation, but yet the answer to the question about experiencing nuisance remained a clear 'no'. The same response was given on a few occasions when the PD inspector himself reported to have seen many *Ae. albopictus* flying around and had experienced severe nuisance while being at the company. Nuisance is apparently too subjective a term, because it was witnessed by both PD inspectors as myself that in several cases the arms, neck, and underarms of employees were covered in red bumps from mosquito bites, and that several *Ae. albopictus*

were continuously encircling the employees in search for a bloodmeal. In one case a family living at 50 meters from a Lucky bamboo importing company (where trap nr. 10 was placed) had repeatedly reported severe mosquito biting. From the trap (trap nr. 20) that was placed for four months in the garden of this family at very close proximity to the window of the sleeping room, many endemic Culicidae were collected (which explains the mosquito nuisance), but *Ae. albopictus* was never collected.

In cases where many *Ae. albopictus* were collected from a trap, or when mosquito biting nuisance was experienced by the PD inspector or by the employees, the PD inspector investigated the near surroundings of the company and asked neighbours about mosquito biting nuisance. In one case (near the company with trap nr. 21) a neighbouring family had experienced mosquito biting nuisance for several weeks. They even collected and kept several specimens, which were later confirmed by specialists to be indeed *Ae. albopictus* (see also the above paragraph 'sightings by the public').

Despite repeated suggestions of PD inspectors to the employees of *Ae. albopictus*-infested companies to protect themselves from mosquito bites using DEET-based products, this advice was largely ignored. Only one company mentioned to have used such products.

### 3.7 Insecticides

Most of the affected companies were helpful in providing information on the use of insecticides. Unfortunately, however, employees on several companies either evaded the questions, or were unwilling reveal if they used insecticides, and if so, what product, or how often they used it. From the companies that were helpful in answering these questions, a total of 12 had used insecticides at least once. The majority seemed to spray insecticides for the duration of a few weeks. In one case the company said it had sprayed only once, and one company said it had sprayed for a relatively long period (at least two months). Only in three cases *Ae. albopictus* mosquitoes were found from companies that had mentioned to have sprayed that period. In the majority of the cases when the companies had used insecticides, no mosquitoes were collected, suggesting that on the whole, the control methods were quite effective. Although the information acquired on insecticide use contains many gaps over the entire period of the survey, the available data show that when insecticides were said to have been used the species diversity and total number of insects in these trap collections were drastically reduced: Normally, when no insecticides are used, the trap collections contain a variety of small flying insects such as mosquitoes (Culicidae), moth flies (Psychodidae), and fungus gnats (Sciaridae). The finding of only dust and no (or hardly any) insects in trap collections, suggests that insecticides were used. Only in two cases did the company say what product was used: in one case Decies, and in the other case Malathion. To our knowledge, no company had made use of the mosquito larvicide *Bacillus thuringiensis israelensis*, the product that had been granted exemption by the ministry of VROM to be used for larval control of *Ae. albopictus* in Lucky bamboo importing companies. Apparently, companies have not made use of the distributed PD/WUR advises on mosquito control options\*.

In most cases, insecticides were used when mosquito nuisance was experienced. However, in the cases when the question 'did you experience mosquito biting nuisance?' was responded by 'yes', only approximately half of the cases the employee said to have used insecticides during that period. One company had said to spray after a new load of Lucky bamboo arrives, as a preventive method. The other companies apparently only sprayed when the biting nuisance was becoming too much.

\* 'Advies ter bestrijding van *Aedes albopictus* in kassen in Nederland' (auteurs Susanne Sutterlin<sup>1</sup>, Ernst-Jan Scholte<sup>2</sup>, en Willem Takken<sup>3</sup>), dated 09-01-2007.

## 4. Discussion

The finding that *Ae. albopictus* has been present on the majority of the Lucky bamboo importing companies in the Netherlands is reason for concern. The fact that the species has been found on 67 out of the 471 collections, dispersed over time and location shows that the initial finding of several specimen in the summer of 2005 was not an exception, and that introductions occur regularly. Although the number of *Ae. albopictus*-positive trap collections and the numbers of specimen collected have diminished drastically since December 2006, the species is still found at different Lucky bamboo importing companies. The finding that several Lucky bamboo-importing companies were found positive for *Ae. albopictus* at different periods, with intervals in between where the species was not found, strongly suggests that the species was introduced repeatedly. This is further supported by repeated observations from Lucky bamboo-importing companies that either stop trading and storing Lucky bamboo, or move from one location to another: after the removal of the Lucky bamboo from a location, we continued monitoring up to 6 months afterwards, but no *Ae. albopictus* mosquitoes were ever found in those 'abandoned' glasshouses.

The reason for the observed decline in number of infested companies and number of collected specimen is unclear. It may have to do with natural fluctuation patterns of high and low population densities of the mosquito species in the area of origin. Southern China (Guangdong and Guangxi Provinces) have mostly subtropical climates with dry and wet seasons. Normally, mosquito population densities drop in dry periods, to grow to large population sizes during the wet season. Eggs of *Aedes* mosquitoes have the ability to survive long periods of draught, and will hatch only when emerged into water. In fact, in many areas of the world, *Aedes* mosquitoes are associated with rising water levels in river delta's; some of which are called 'floodwater' mosquitoes: eggs that are deposited at grass or plant-stems in e.g. river delta will only hatch when the river floods the surrounding plains. It is possible that in relatively dry periods few *Ae. albopictus* females are present, resulting in low numbers of eggs being deposited. The relatively low number of *Ae. albopictus* that we found since December 2006 may be associated to the dry season in the Lucky bamboo production area. The dry period in those areas is from approximately November-December to March-April. If the dry season would be the main reason for the low numbers of mosquitoes found since December, this would also mean that the *Ae. albopictus* population density are expected to increase from May till December due to frequent rains. Another possibility is that from summer 2006 onwards, producers of Lucky bamboo in the production area make increasingly more use of insecticides, or use more efficient insecticidal products or methodologies. Another possibility is that the plants are rubbed clean using a towel to remove adhered mosquito eggs before they are shipped to Europe. It is difficult to obtain clear information about the mosquito control practices in the production area of Lucky bamboo in China. Furthermore, the trade in Lucky bamboo is also quite flexible: groups of plants from different growers are sometimes mixed to meet the required quantities of Lucky bamboo orders from a Chinese exporting company or a Dutch importing company. These different growers may have different approaches and efficiencies in mosquito control methods. However, from the accompanying documents it is not always possible to trace back the precise origin of all plants.

The findings that 1) all companies that had been found positive for *Ae. albopictus*, remained so for only limited periods of time, 2) most companies had been positive only once or for a few times, and 3) the continued absence of the species at locations where lucky bamboo was not present at that company any more, suggests that imported *Ae. albopictus* does not survive for a long time at the companies. This can be either due to unfavourable conditions for the mosquito to survive for long periods and/or to reproduce itself, or otherwise because the company used insecticides.

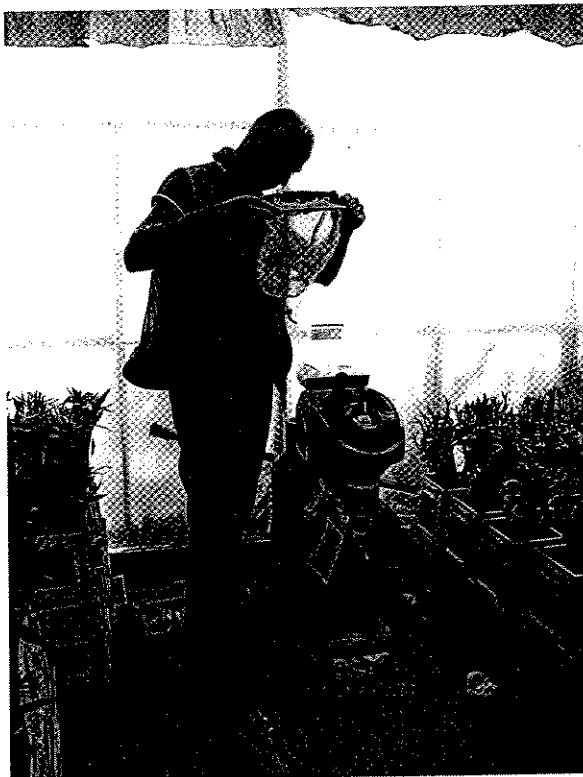
What does the presence or absence of the mosquitoes in the traps actually mean? When *Ae. albopictus* was present in the trap collections, one can say that the species had been present there in the period since the last trap collection. However, when no specimen of *Ae. albopictus* were present in the trap collections, one can not say 'the species was absent in the period since the last trap collection. There is always the possibility that the species was present, but the trap did not collect any specimen. Maybe the trap was not working in such cases? Possible, but not likely in the majority of the cases. The findings that during the periods in which no *Ae. albopictus* was present in the trap collections, other entomofauna such as other (endemic) mosquito species and moth flies was present in the collections indicates that the traps were working. However, it has occurred on a number of occasions that the traps were not working properly. In those cases, the trap collections were not included into the database. The trap that was used is a powerful trap to collect Culicidae mosquitoes, used all over the world in entomological studies on mosquitoes. However, it is unlikely that the trap will collect all *Ae. albopictus* mosquitoes that are present in the area. The more mosquitoes present, the more likely the trap is to become positive for *Ae. albopictus*. A high number of *Ae. albopictus* in a trap collection is therefore a strong indication for a relatively high infestation level, and a low number of collected specimen for a low infestation level. This is supported by the experience of the PD inspectors: when they experienced mosquito biting nuisance while visiting a Lucky bamboo importing company for trap collection, and saw mosquitoes flying that looked very much like *Ae. albopictus*, trap collections of that company from that period contained relatively large numbers of *Ae. albopictus*. On the other hand are the absence of biting nuisance and/or sightings of active flying *Ae. albopictus* no measure for the absence of the species. When there is a low infestation level, it is unlikely to witness the presence of a specimen. In those cases, the trap is more likely (but no guarantee) to 'betray' the presence of the species.

It is difficult to determine the status of the larvae that were collected. There are several possible scenario's. 1) they could be larvae that were transported as larvae directly from China. 2) they could be the results of eggs that were deposited on the plants or the boxes in China above the waterlevel in the boxes. Since eggs of *Aedes* species can survive long periods without water and will hatch only when emerged in water, it may well be possible that during transport to the Netherlands they survived as dormant eggs, only to hatch when in the Netherlands extra water was added to the boxes. 3) gravid female *Ae. albopictus* were 'hiding' in the plants or boxes in China. They were transported with the plants to the Netherlands and laid their eggs during transport or in the Dutch greenhouses upon arrival. 4) the eggs are the result of local breeding and oviposition. In my opinion, scenario 2 is the most likely scenario, followed by scenario 4. Scenario 1 is less likely, because the transport has a duration of approximately a month, and although larval development is temperature dependent, is unlikely to takes more than 3 weeks from 1<sup>st</sup> to 4<sup>th</sup> larval stage, especially since the larger part of the travel is in (semi) tropical climates where temperatures are not low. When in 2001 *Ae. albopictus* was introduced into California by import of Lucky bamboo from China the Californian authorities restricted import of these plants to import 'on gel' only. No import 'on water' was allowed anymore. This seemed to have contributed in preventing further import (S. Klüh, *personal communication*). When in October 2006 the Lucky bamboo import method (on water or on gel) per company was linked to the numbers of *Ae. albopictus* collected at the companies, it became apparent that import 'on water' was not linked to less *Ae. albopictus* import. In fact, of all the companies, only two claimed to be importing 'on water', of which one both 'on water' as 'on gel', and one 'on water' only. Both these companies have been found positive for *Ae. albopictus* on different collection dates, but the relatively few specimen that were collected (9 in total) suggest low infestation levels. The vast majority of the companies claimed to import on gel only. These companies made up for more than 98% of the collected *Ae. albopictus*.

The top 8 of companies that were infested most often and where most *Ae. albopictus* specimen were collected, imported only on gel. Apparently, the import of Lucky bamboo on gel does not prevent import of *Ae. albopictus*. Possibly, the eggs on the stems of the plants are still able to hatch when, upon arrival in the Netherlands, the plants are transferred to boxes filled with water. Another possibility, although less likely, is that larvae survive on the small layer of water that occasionally is present on top of the gel.

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PD inspector changing the net of a mosquito trap