Final Report Phase 7 Part 1
MEMO results

Final version

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Barcoding of Organisms and Tissues of Policy Concern
Royal Belgian Institute of Natural Sciences

Contents

List of Abbreviations and codes for Points of Entry

Abbreviations

ANB	Agentschap voor Natuur en Bos (Flemisch goverment)
BIP	Border Inspection Post
BG	Biogents® BG-Sentinel trap
BopCo	Barcoding of Organisms and Tissues of Policy Concern
DGO1	Direction Générale Opérationnelle des Routes et des Bâtiments
DNA	DeoxyriboNucleic Acid
2D	Two-Dimensional space
ECDC	European Centre for Disease Prevention and Control
EMS	Exotic Mosquito Species
EXOSURV	Implementation of SURVeillance of EXOtic mosquitoes in Belgium
FASFC	Federal Agency for the Safety of the Food Chain
FLI	Friedrich-Loeffler-Institut
GT	Frommer updraft gravid Trap
ID	IDentification
ITM	Institute of Tropical Medicine, Antwerp
LS	Larval Sampling
MEMO	Monitoring of Exotic MOsquitoes in Belgium
MBD	Mosquito-Borne Diseases
MMT	Mosquito Magnet® Trap
MODIRISK	MOsquito vectors of DIsease: spatial biodiversity, drivers of change, and RISK
NVWA	Nationale Voedsel- en WarenAutoriteit Nederland
OT	Oviposition Trap
PBS	Potential larval Breeding Site
PoE	Point of Entry
QR (code)	Quick Response
QRA	Qualitative Risk Assessment
RBINS	Royal Belgian Institute of Natural Sciences
RFLP	Restriction Fragment Length Polymorphism
RMCA	Royal Museum of Central Africa
SCENA3KOR	Scenario 3 *Aedes koreicus* project
SNPs	Single Nucleotide Polymorphisms
SPW	Service Public de Wallonie (Walloon goverment)
VMM	Vlaamse Milieu Maatschappij
WNV	West-Nile Virus

Codes used for Points of Entry

AB	Tyre company Agrityre & AtoB
AC	Airport Charleroi
AL	Airport Liège
AT	Tyre company ATB Banden
AZ	Airport Zaventem
AZ1	Airport Zaventem (cargo)
AZ2	Airport Zaventem (passenger, luggage handling area)
BA	Tyre company Bridgestone aircraft tire (Europe) s.a.
E0	Parking lot Sprimont/Noidré E25 (A26)
E1	Parking lot Aische-en-Refail E411 (A4)
E2	Parking lot Hondelange E25 (A4)
E3	Parking lot Aubange N52 (A28)
E4	Parking lot Raeren/Lichtenbusch E40 (A3)
E5	Parking lot Wanlin E411 (A4)
E6	Parking lot Shell Marke E17 (A14)
E7	Parking lot Rekkem E17 (A14)
E8	Parking lot Saint-Ghislain E19 (A7)
E9	Parking lot Hensies E19 (A7)
EB	Lucky bamboo company Euro Bonsai
ET	Wholesale market Liège
EU	Allotment garden Eupen
EV	Flower auction Euroveiling
HA	Tyre company Ets Havelange Robert SPRL

MB	Tyre company Maaslandbanden (new name Profile Dilsen-Stokkem Maasland & OTR-EM)
MC	Wholesale market Charleroi
MM	Industrial zone ‘Op de Berg’ Maasmechelen
PA	Port of Antwerp (BIP Kallo)
PG	Port of Ghent (Volvo)
PZ	Port of Zeebrugge
RA	Cemetery Raeren
RO	Cemetery Rocherath - Büllingen
SP	Tyre company Sarri Pneus
VP	Tyre company Visé Pneu

The Partners

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Barcoding of Organisms and Tissues of Policy Concern

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Royal Belgian Institute of Natural Sciences

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- Filip De Block: support start-up (in field and with morphological identifications)
- Adwine Vanslembrouck: reference collection, morphological identification of the collected larvae, support fieldwork

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English summary

The early detection of exotic mosquito species (EMS) along high-risk introduction routes before populations become established is of paramount importance to prevent local transmission of mosquito-borne diseases. Following previous EMS surveillance projects in Belgium, a three-year national active EMS monitoring project (MEMO) started in July 2017 to detect possible foci of introduction and establishment of EMS at an early stage in Belgium.

In 2017, 2018 and 2019, active monitoring was implemented in 20 to 23 different Points of Entry (PoE). The risk of introduction and establishment of the EMS at each PoE was re-evaluated annually to ensure that the monitoring focused on the highest risk sites. Different collection methods were used, including BG-Sentinel and Mosquito Magnet® traps to collect host seeking female mosquitoes, oviposition traps to detect eggs, and larval sampling. The collected specimens were sorted and identified using morphological characteristics. The caught EMS and five percent of all collected mosquitoes were molecularly identified to validate and confirm the morphological identification, and were subsequently deposited in a molecular reference collection. A specific molecular identification pipeline was developed to enable the proper identification of all mosquito species occurring in Belgium. Further, a morphological collection with a fair representation of the species and the most intact specimens sampled during the MEMO project was generated for future reference. Data management was done using the VECMAP® software.

In total of 52478 mosquito (Diptera, Culicidae) specimens, belonging to 31 species (or species complexes) and five genera (*Aedes, Anopheles, Culex, Culiseta, Coquillettidia)* were collected. Three new species for Belgium were discovered, namely *Anopheles daciae, Culex modestus* and *Culiseta longiarealata*. Four EMS were detected: *Aedes albopictus*, *Aedes japonicus*, *Aedes koreicus* and *Anopheles pharoenis.* Two EMS are locally established: *Aedes japonicus* in Natoye (used tyre company Havelange) and *Aedes koreicus* in Maasmechelen (industrial area 'Op de Berg'). *Aedes japonicus* is known to occur in Belgium since 2002. Between 2012 and 2015 a large eradication campaign was implemented in Natoye and *Ae. japonicus* was not collected anymore in 2015 and 2016. However, in 2017 the species was again detected at the same location. The observations of 2018 and 2019 in Natoye indicate that the species is spreading again. Based on microsatellite analysis (DNA markers), this recent population seems to be an admixture of the original population, which survived the eradication action, and a newly introduced genotype from an external source. Also new introductions of *Ae. japonicus* were detected in Maasmechelen and Eupen, the latter of which originated from West Germany. Detected for the first time in 2008, *Ae. koreicus* does not seem to spread fast, but can be considered widely spread in the area around the industrial zone in Maasmechelen now. A first control campaign to reduce the population size started in 2019. *Aedes albopictus* is not yet established in Belgium. During the project, it was collected at seven PoE's in 2018 (five PoE's) and 2019 (four PoE's). Besides known introduction pathways in Belgium such as used tyre and lucky bamboo trade, the species was caught for the first time at multiple parking lots along highways, which is a new introduction pathway for Belgium. A single female of *Anopheles pharoensis* was detected at the airport of Liège by a Mosquito Magnet® trap. This species is a tropical mosquito mostly present in West, East and southern Africa, Egypt, Israel and Syria. In African countries it is a common secondary vector of malaria. The climatic conditions in Belgium are considered as non-suitable for possible establishment of this EMS.

The three-year MEMO project showed that the situation in Belgium is changing. On top of the known introduction pathways, the used tyre and lucky bamboo trade, a new one for Belgium was found, i.e., the passive ground transport. This will be a new challenge in the coming years for Belgium as established populations of *Ae. albopictus* are approaching Belgium and introductions through passive ground transport will become more frequent. Further, during the project we already observed an increase in the percentage of positive PoE's in Belgium, and EMS were introduced into Belgium in all months

between May and October. All these findings stress the need for a permanent and integrated surveillance and control management plan for EMS at national and regional levels setting out clear criteria for surveillance, control actions, methods and strategies with appropriate supervision and evaluation.

Dutch summary

De tijdige detectie van exotische steekmugsoorten (EMS) op introductieplaatsen voordat populaties zich vestigen is van het grootste belang om lokale overdracht van door muggen overgedragen pathogenen te voorkomen. Na eerdere EMS-monitoringsprojecten in België is in juli 2017 een driejarig nationaal actief EMS-monitoringproject (MEMO) van start gegaan om mogelijke introductiehaarden en de vestiging van EMS in een vroeg stadium in België op te sporen.

In 2017, 2018 en 2019 werd de actieve monitoring uitgevoerd in 20 tot 23 verschillende introductieplaatsen (IP). Het risico van introductie en vestiging van EMS voor elke IP werd jaarlijks geëvalueerd om ervoor te zorgen dat de monitoring zich richtte op de sites met het hoogste risico. Er werden verschillende verzamelmethodes gebruikt, waaronder de BG-Sentinel en Mosquito Magnet® vallen voor het vangen van wijfjes-muggen op zoek naar een bloedmaaltijd, ovipositievallen voor het verzamelen van eitjes en larvale bemonstering. De verzamelde muggen werden gesorteerd en geïdentificeerd aan de hand van morfologische kenmerken. De gevangen EMS en vijf procent van alle verzamelde muggen werden moleculair geïdentificeerd om de morfologische identificatie te valideren en te bevestigen. Op basis hiervan werd een moleculaire referentiecollectie aangelegd. Een specifieke moleculaire identificatie-*pipeline* werd ontwikkeld om de juiste identificatie van alle muggensoorten die in België voorkomen mogelijk te maken. Verder werd een morfologische referentiecollectie van de meeste soorten en de meest intacte exemplaren die tijdens het MEMO project werden bemonsterd, opgesteld. Het databeheer werd uitgevoerd met behulp van de VECMAP® software.

In totaal werden 52478 muggen (Diptera, Culicidae) verzameld, behorend tot 31 soorten (of soortcomplexen) en vijf genera (*Aedes, Anopheles, Culex, Culiseta, Coquillettidia*). Er werden drie nieuwe inheemse soorten voor België ontdekt, namelijk *Anopheles daciae*, *Culex modestus* en *Culiseta longiarealata*. Vier EMS werden gevonden: *Aedes albopictus*, *Aedes japonicus*, *Aedes koreicus* en *Anopheles pharoensis*. Twee EMS zijn lokaal gevestigd: *Ae. japonicus* in Natoye (bandenbedrijf Havelange) en *Ae. koreicus* in Maasmechelen (industriezone 'Op de Berg'). *Aedes japonicus* komt sinds 2002 in België voor. Tussen 2012 en 2015 werd een grote bestrijdingscampagne uitgevoerd in Natoye en in 2015 en 2016 werd *Ae. japonicus* niet meer gevangen. In 2017 werd de soort echter opnieuw op dezelfde locatie gevonden. De waarnemingen van 2018 en 2019 in Natoye geven aan dat de soort zich opnieuw verspreidt. Op basis van een microsatellietanalyse (DNA merkers) blijkt deze recente populatie een combinatie te zijn van de oorspronkelijke populatie, die de uitroeiing overleefde, en een nieuw geïntroduceerd genotype van een externe bron. Ook werden nieuwe introducties van *Ae. japonicus* in Maasmechelen en Eupen gedetecteerd, waarvan de laatste afkomstig is uit West-Duitsland. *Aedes koreicus*, voor het eerst gedetecteerd in 2008, lijkt zich niet snel te verspreiden, maar kan nu toch als wijdverspreid worden beschouwd. In 2019 is een eerste campagne gestart om de populatie van deze soort te bestrijden. *Aedes albopictus* is nog niet in België gevestigd. Tijdens het project werd de soort verzameld op zeven IP's: vijf IP's in 2018 en vier IP's in 2019. Naast de bekende introductiewegen voor België, zoals de handel in lucky bamboo plantjes en tweedehandsbanden, werd de soort voor de eerste keer gevangen op verschillende parkeerplaatsen langs snelwegen, wat een nieuwe introductieroute is voor België. *Anopheles pharoensis* werd gedetecteerd op de luchthaven van Luik door een Mosquito Magnet® val. Deze soort is een tropische mug die vooral voorkomt in West-, Oost- en Zuidelijk Afrika, Egypte, Israël en Syrië. In Afrikaanse landen is het een secundaire malariavector. De klimatologische

omstandigheden in België worden als ongeschikt beschouwd voor een eventuele vestiging van deze ESM.

Het driejarige MEMO project toonde aan dat de situatie in België aan het veranderen is. Naast de bekende introductiewegen, de import van lucky bamboo plantjes en tweedehandsbanden, werd een nieuwe route voor België gevonden, namelijk het passieve wegtransport. Dit zal de komende jaren een nieuwe uitdaging zijn voor België, aangezien de gevestigde populaties van *Ae. albopictus* België naderen en introducties via passief wegtransport frequenter zullen plaatsvinden. Verder hebben we tijdens het project al een stijging vastgesteld van het percentage positieve IP's in België en werden de EMS in België geïntroduceerd in alle maanden tussen mei en oktober. Deze bevindingen onderstrepen het belang aan een permanent en geïntegreerd beheersplan voor de monitoring en controle van EMS op nationaal en regionaal niveau, waarin duidelijke criteria voor monitoring, bestrijdingsacties, -methodes en -strategieën met een geschikte supervisie en evaluatie zijn opgenomen.

French summary

Une détection précoce des espèces de moustique exotiques (EMS) au niveau de voies d'introduction à haut risques, avant qu'elles ne puissent s'établir, est d'importance capitale afin de prévenir la diffusion locale de maladies transmises par ceux-ci. A la suite de précédents projets de surveillance de ces EMS en Belgique, un nouveau projet de monitoring actif au niveau national (MEMO) et d'une durée de trois ans a été lancé en juillet 2017, dont le but était de détecter les sites où des introductions peuvent effectivement avoir lieu, et ceux où des EMS se sont déjà antérieurement établis en Belgique.

En 2017, 2018 et 2019, un plan de monitoring actif a été mis en œuvre au niveau de 20 à 23 différents Points d'Entrée (PoE). Le risque d'introduction et d'établissement des EMS connus au niveau de chacun de ces PoEs a été ré-évalué annuellement, afin de s'assurer que le monitoring se concentrait toujours bien sur les sites à plus haut risques. Différentes méthodes d'échantillonnage ont été utilisées, incluant les pièges de type BG-Sentinel et Mosquito Magnet® qui permettent de collecter des moustiques femelles à la recherche d'un hôte pour se nourrir, des pièges pondoirs pour la collecte des œufs, ainsi que des campagnes d'échantillonnage de larves. Les spécimens ainsi collectés ont été triés et identifiés sur base de leurs caractéristiques morphologiques. Tous les spécimens d'EMS, ainsi que cinq pour cent de l'ensemble des moustiques collectés, ont également été identifiés via l'utilisation de techniques ADN afin de valider leurs identifications initiales. Dans le but de réaliser ces identifications sur base de l'investigation de l'ADN des spécimens, et de distinguer les EMS des espèces natives, un pipeline a été développé. Les spécimens contrôlés, et les séquences d'ADN ainsi obtenues, ont été inclus dans une collection de référence pour l'identification moléculaire. Par ailleurs, les spécimens collectés au cours du projet MEMO de la plupart des espèces et qui ont été morphologiquement bien préservés (les plus intacts) ont été sélectionnés et intégrés à une collection morphologique de référence. Tout au long du projet, la gestion des données a été réalisée via l'utilisation du software VECMAP®.

Au total, 52478 moustiques (Diptère, Culicidae), appartenant à 31 espèces (ou complexes d'espèces) et cinq genres (*Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Coquillettidia*), ont été collectés. Trois nouvelles espèces natives ont également été découvert, à savoir *Anopheles daciae*, *Culex modestus* et *Culiseta longiarealata*. Quatre EMS ont détectées: *Aedes albopictus*, *Aedes japonicus*, *Aedes koreicus* et *Anopheles pharoenis*.

Deux EMS sont localement établies: *Aedes japonicus* à Natoye (établissement de pneus Havelange) et *Aedes koreicus* à Maasmechelen (zone industrielle 'op de Berg'). *Aedes japonicus* a été détectée pour la première fois en Belgique en 2002. Entre 2012 et 2015, une grande campagne d'éradication a été mise en place à Natoye et *Ae. japonicus* n'a plus été capturé en 2015 et 2016. Néanmoins, elle a été collectée

à nouveau en 2017 sur ce même site. Les observations effectuées en 2018 et 2019 indiquent que l'espèce se répand à nouveau à Natoye. Des analyses complémentaires sur base de l'utilisation de microsatellites (marqueurs ADN) indiquent que cette population correspond à un mélange entre la population originellement présente, qui aurait survécu à l'éradication, et des individus provenant d'une nouvelle introduction depuis une source externe. Par ailleurs, *Ae. japonicus* a également été introduit à Maasmechelen et à Eupen. Dans ce dernier cas, l'origine de l'introduction serait lié à l'expansion de la population établie en Allemagne de l'ouest. Détectée pour la première fois en 2008, *Ae. koreicus* ne semble pas s'étendre rapidement, mais peut actuellement être considérée comme largement établie dans la région autour de la zone industriel à Maasmechelen. Une première campagne de contrôle a commencé en 2019, et dont le but est de réduire la densité de cette espèce. *Aedes albopictus* n'est actuellement pas établie en Belgique. Au cours du projet, l'espèce a été collectée au niveau de sept PoEs distinct, cinq en 2018 et quatre en 2019. En plus des voies d'introduction bien connues, tel que le commerce de pneus de deuxième main et de "lucky bamboo", l'espèce a été collectée pour la première fois au niveau de plusieurs aires de repos le long des autoroutes, ce qui représente une nouvelle voie d'introduction de l'espèce en Belgique. Enfin, une femelle de l'espèce *Anopheles pharoensis* a été détecté à l'aéroport de Liège via l'utilisation d'un piège de type Mosquito Magnet®. Cette espèce est un moustique tropical qui se retrouve principalement dans les pays de l'ouest, de l'est et du sud de l'Afrique, en Egypte, en Israël et en Syrie. Au sein des pays Africains, il s'agit d'un vecteur secondaire commun de la malaria. Les conditions climatiques de la Belgique sont considérées peu appropriées à l'établissement de cette EMS.

Ces trois années de monitoring (projet MEMO) ont démonstré que la situation en Belgique change. En plus des voies d'introduction principales bien connues (pneus usagés et "lucky bamboo"), *Ae. albopictus* est introduit passivement via le transport routier. Ceci représente un nouveau challenge pour les années à venir puisque des populations établies de cette espèce approche des frontières de la Belgique, et donc que l'introduction via transport routier deviendra certainement plus fréquente. Par ailleurs, le projet a également mis en évidence que le pourcentage de PoEs positif a augmenté au cours du temps, et que des EMS étaient introduites en Belgique au cours de tous les mois entre mai et octobre. Tous ces résultats soulignent la nécessité de développer un programme de surveillance intégré et un plan de contrôle des EMS à un niveau national et régional, avec des critères clairs pour la surveillance et pour les actions, méthodes et stratégies de contrôle sous supervision et évaluation appropriée.

1 Introduction

Mosquitoes and mosquito-borne pathogens constitute an increasing threat to animal and human health in temperate areas. Important drivers for the introduction and spread of exotic mosquito species (EMS) are the significant increase in international trade and tourism, along climatic and ecological changes. Based on the current spread of EMS in Europe [1], on the number of interceptions of these species in Belgium [2] and on the suitability models developed for *Aedes albopictus* in Europe [3, 4], establishment of EMS in Belgium is probably unavoidable. The establishment of EMS is determined by a unique combination of factors related to the species' ecology and genetics, and the environmental and climatic factors. A prerequisite for the control of these species is their early detection before populations get established and a thorough knowledge of their ecology will aid control efforts. Therefore, the Belgian federal authorities and federated entities fund a three-year active monitoring project called *Monitoring of Exotic Mosquitoes in Belgium* (MEMO).

The project is executed by the Institute of Tropical Medicine, Antwerp (ITM) in partnership with the Barcoding of Organisms and Tissues of Policy Concern (BopCo)[1] and the Royal Belgian Institute of Natural Sciences (RBINS). The project started on the 1st of July 2017.

The objectives of the MEMO-project are the following:

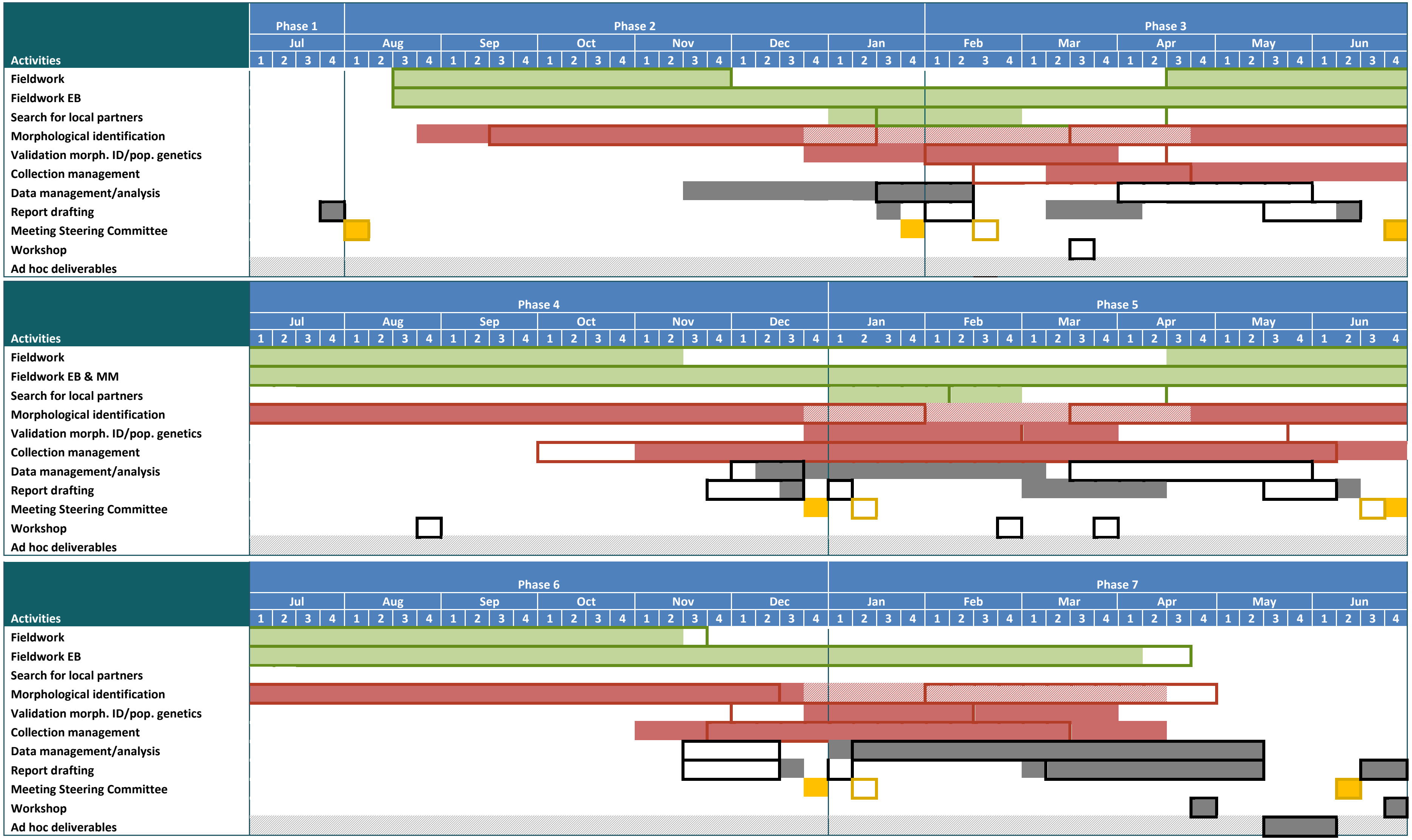
- To actively monitor mosquitoes in order to detect the possible introduction of EMS in Belgium (scenario 1) and quantify the already established local populations of EMS (scenario 2) i.e. the active basic monitoring plan;
- To detect, identify, evaluate and monitor potential import sites or points of entry (PoE's) based on existing passive monitoring data, experience gained and/or other relevant epidemiological or ecological information;
- Possible expansion of the active basic monitoring plan with additional actions: quantifying the possible introduction and spread of newly detected EMS (newly discovered scenario 2 cases through active or passive monitoring (max 2/year));
- To process the collected samples including the morphological and molecular identification of collected samples, appropriate storage, reporting of all collected relevant stages of mosquito species, and the establishment of a morphological and molecular reference collection;
- Analysis of the import risk of EMS in Belgium taking into account the potential PoE's, the ecological profile of the species and climate scenarios;
- To make recommendations for a future, long-term, cost-effective monitoring plan for EMS in Belgium based on the practical experience and the developed risk analysis;
- Responding to ad hoc questions from the contracting authority;
- Transferring the knowledge and skills generated in the project.

Expected outcome comprises the early interception of EMS in Belgium, new DNA barcodes for indigenous and exotic mosquitoes, a morphological and molecular reference collection, and recommendations for a future monitoring plan for EMS in Belgium.

The current report provides a comprehensive account of all activities and results of the MEMO project (part 1). A recommendation for a future cost-efficient monitoring plan for EMS in Belgium is presented (part 2). A complete overview of the overall planning of the three-years monitoring can be found in Figure 1.

[1] The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo - http://bopco.myspecies.info/) is a joint initiative of the Royal Museum for Central Africa and Royal Belgian Institute of Natural Sciences, and is financed by the Belgian Science Policy Office (Belspo) as Belgian federal in kind contribution to the European Research Infrastructure Consortium "LifeWatch".

Figure 1. Overview of the planning (coloured bars) with indication of the actual timing (bold rectangles) of the activities in phases 1 to 7 during the whole project duration (bold vertical line indicates present). Fully coloured: intensive full-time activity. Dashed is less intensive activity. EB=lucky bamboo import company Euro Bonsai.

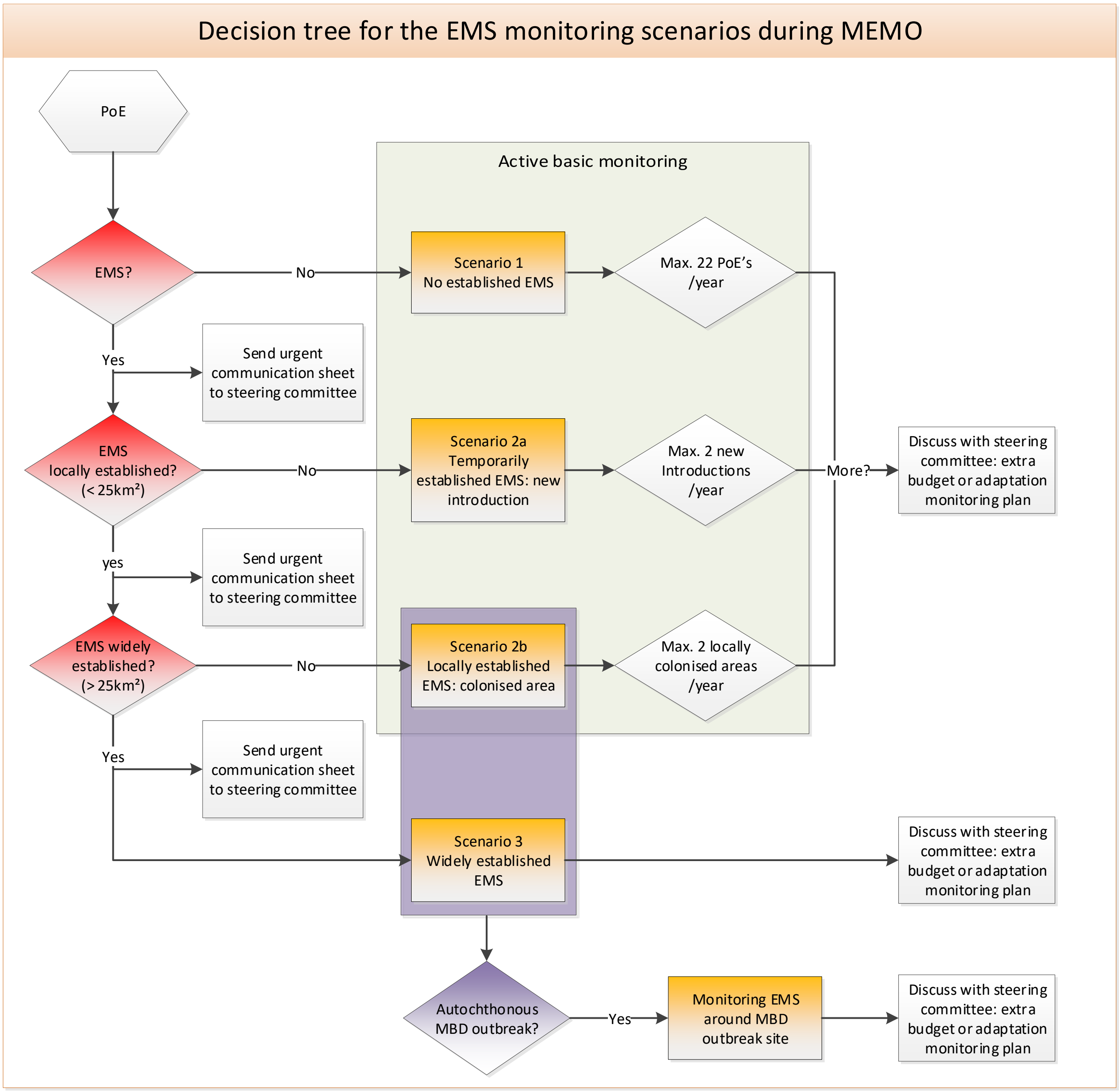


2 Overview of the MEMO monitoring activities

2.1 MEMO decision tree

Depending on the invasion status of the EMS at a PoE, different scenarios were attributed according to the ECDC guidelines [5]: scenario 1, no established EMS but with risk of introduction and establishment; scenario 2, locally established EMS with low risk of spreading into new areas; and scenario 3, widely established EMS with high risk of spreading into new areas. During the MEMO project, we have split up the scenario 2 in two separate scenarios: scenario 2a (new introduction with temporarily established EMS) and scenario 2b (locally established EMS in a colonised area). Figure 2 shows the decision tree for the EMS monitoring scenarios of the MEMO project indicating the scenarios included in the active basic monitoring plan.

Figure 2. Decision tree for the EMS monitoring scenarios during the MEMO-project indicating the active basic monitoring plan (EMS=Exotic Mosquito Species, MBD=Mosquito-Borne Disease, PoE=Point of Entry).



At the start of the MEMO project in 2017, the active basic monitoring plan included 23 fixed PoE's with 21 scenario 1 cases and two scenario 2b cases. The two already defined scenario 2b cases comprised the already colonised areas with *Aedes japonicus* and *Aedes koreicus.* Further, based on new introductions detected by the project or by passive surveillance, two new scenario 2 (a or b) cases could be included per year. When more than four scenario 2 cases occurred in the same year, but also when a scenario 3 case or mosquito-borne disease (MBD) outbreak occurred, the adaptations to the basic monitoring plan or extra budget were discussed with the steering committee.

2.2 Selection and scenario change of the PoE's

2.2.1 Qualitative risk analysis

The active basic monitoring plan included 23 PoE's at the start of the MEMO project in 2017: 11 sites were fixed and listed in Table 2 of the public tender (CES-2016-02) as their import risk was estimated to be very high based on previous monitoring projects. Twelve 12 PoE's needed to be selected from a list of 19 PoE's presented in Table 3 of the public tender (CES-2016-02). To select the 12 PoE's a qualitative risk analysis (QRA) was performed based on the assessment scheme from ECDC [5]. This QRA was updated at least once per year to re-assess the risk of importing and establishing EMS at all monitored PoE's (complete list in Annex 2). Hence, a QRA was performed four times during the MEMO project, in March 2017, February 2018, June 2018 and January 2019 (Figure 3 & Annex 3).

The QRA is based on the ECDC guidelines, more specifically Annex 3, Box A [5], and takes into account a number of factors related to import risk and establishment (Table 1). Per risk factor, categories were defined to which scores were given according to Table 1. First, the necessary information was collected per PoE, based on publicly available data (internet, website) and contact information from the PoE, Federal Agency for the Safety of the Food Chain (FASFC) or customs (by e-mail, telephone). Second, scores were given to each risk factor according to the different categories (Annex 3). Finally, all scores were summed per PoE to give the total QRA score (Figure 3).

In 2017, the QRA was done for the 19 PoE's from which 12 had to be selected (Table 3 of the tender). In February 2018, the QRA was updated for the same 12 previously selected PoE's. Additionally four new potential PoE's were assessed: port of Ghent (Volvo) (PG), parking lot Wanlin (E5), parking lot Marke (E6) and parking lot Saint-Ghislain (E8). Because of extra information or obtained knowledge on the monitored PoE's and the high workload in the first half of 2018, the QRA was updated again in June 2018 for each of the 12 PoE selected in February 2018. At the airport Zaventem (AZ) a new location was chosen and changed from the cargo area (AZ1) to the passenger area (AZ2). To optimise the risk analysis a new risk factor was added in June 2018: 'evidence of import at other European countries'. Finally, the QRA was updated in January 2019 for each of the 12 PoE selected in June 2018 and for two old (monitored before the MEMO project) and two new PoE's. Two old PoE's from the Brussels capital region included the flower auction Euroveiling (EV) and the wholesale market in Brussels (EC). Finally two new parking lots along the highways E411 and E25 following parking lot Hondelange (E2) were also added, more precisely parking lot Aische-en-Refail (E1) along E411 and parking lot Sprimont/Noidré (E0) along E25. The 11 'fixed' PoE's from the tender were revaluated, together with other monitored PoE's, in 2020 as part of the development of the future cost-efficient monitoring plan for EMS in Belgium (see Chapter 1.2.3 in final report Phase 7 part 2 – Recommendation).

Based on the QRA scores, a ranking was made of the PoE's from high risk (highest score) to low risk (lowest score) of importing and establishing EMS. In March 2017 and February 2018, the cut-off score for selection of a PoE was seven, whereas in June 2018 and January 2019 an extra risk factor was added, increasing the cut-off score to nine. In case the number of PoE's exceeded the total number of PoE's that could be monitored, the PoE's with a similar cut-off score were compared and a selection was made based on a finer level of the risk factors (e.g. import volume and frequency of certain goods compared between PoE types), or based on practical arguments (availability of local partners). Repeated evaluation of the PoE's is needed as the risk levels can change over time. The final inclusion of PoE in the monitoring activities should be done in relation to the available financial and human resources.

Table 1. Risk factors for the introduction and possible establishment of EMS with their different categories and scores.

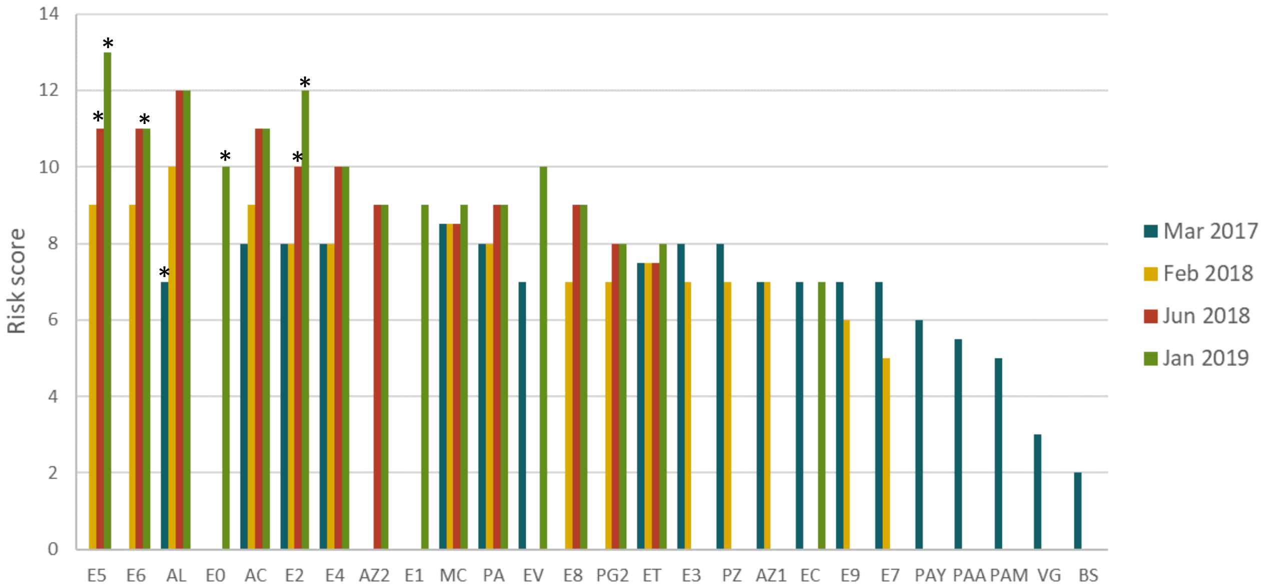
Risk factor	Categories	Score
Import origin	from a EU country not colonised by EMS	0
	from a EU country colonised by EMS	2
	from a non-EU country colonised by/native for EMS	2
	a combination of different origins from above	2
	unknown	1
Import volume/frequency (compared per type of PoE)	small/low	0
	average	1
	big/high	2
	unknown	0,5
Import method	frozen	0
	refrigerated	1
	not frozen or refrigerated	1
	a combination of above	1
	in water and/or gel	2
	unknown	0,5
Import possibility at PoE	not first opening of container/car/passenger cabin	0
	first opening of container/car/passenger cabin	2
	unknown	1
Habitat suitability around PoE	few larval breeding places within 500 m	0
	a lot of larval breeding places within 500 m	1
	plenty of larval breeding places within 500 m	2
	unknown	0,5
Recent import at PoE (2012-2018)	no import of EMS at PoE between 2012 and 2016	0
	recent import at PoE	3
	unknown or no import of EMS at PoE only in 2012 and/or in 2017 and 2018	1
Evidence of import at this PoE type at other countries	no evidence	0
	at non-European countries	1
	at European countries	2

The QRA resulted in the following selection (see also Figure 3):

- **In March 2017**, eight of the 19 PoE's had a score between 8,5 and 7,5 and six PoE had a score of seven points (parking lot Rekkem (E7), parking lot Hensies (E9), airport Liège (AL), AZ1, EC and EV) (Figure 3). Since the import volume at the wholesale market in Brussels (EC) and the flower auction Euroveiling (EV) is lower than the one at the airports Liège (AL) and Zaventem (brucargo) (AZ1), and since the risk of import by car has been documented more frequently (despite the uncertain import risk), EC and EV were not selected for the monitoring. The 12 PoE's that were selected for the monitoring in 2017 were: E2, E3, E4, E7, E9, AL, AZ1, AC, PA, PZ, ET and MC. This selection was part of the ITM offer to the tender.

- **In February 2018**, nine of the 16 PoE's had a score between 10 and 7,5 and five PoE's had a score of seven points (AZ1, PG, E8, PZ & E3) (Figure 3). The import volume and frequency originating from risk countries at the port Zeebrugge (PZ) and parking lot Aubange (E3) was lower than the one at the port of Ghent (Volvo) (PG), airport Zaventem (brucargo) (AZ1) and parking lot Saint-Ghislain (E8). The 12 selected PoE's that were monitored in the first half of 2018 were: E2, E4, E5, E6, E8, AC, AL, AZ1, PA, PG, ET and MC.
- **In June 2018**, six PoE's had a score above 10 and six PoE's had a lower score (PA, AZ2, E8, MC, PG, ET) (Figure 3). To make the monitoring plan more feasible, we suggested to stop the monitoring at the port of Antwerp (Kallo) (PA), the port of Ghent (Volvo) (PG), the wholesale market in Charleroi (MC) and in Liège (ET). At passenger airport Zaventem (AZ2) and parking lot Saint-Ghislain (E8) the assistance of local partners made it possible to keep them in the monitoring plan. The eight selected PoE's that were monitored in the second half of 2018 were: E2, E4, E5, E6, E8, AZ, AL and AC.
- **In January 2019**, eight PoE's had a score above 10 and eight PoE's had a lower score (AZ2, E8, E1, PG, MC, PA, ET, EC) (Figure 3). Six PoE's monitored in 2018 have a high score (> 10), while the parking lot Saint-Ghislain (E8) and the passenger airport of Zaventem (AZ2) are at the top of the lower score list. Two of the new PoE's scored high (flower auction Euroveiling (EV) and parking lot Sprimont (E0)), while parking lot Aische-en-Refail (E1) scores as good as E8 and AZ2. The Wholesale market in Brussels (EC) has the lowest score. During the SC meeting in January 2019 it was decided to add two PoE's with scenario 1 monitoring. Based on the new risk analysis, we suggested to add the flower auction Euroveiling (EV) and parking lot Sprimont (E0) to the monitoring in 2019, making a total of 10 extra PoE's that were monitored in 2019: E0, E2, E4, E5, E6, E8, AC, AL, AZ2 and EV. After the detection of *Ae. albopictus* at parking lot Wanlin (E5), an extra parking lot (Aische-en-Refail (E1)) was added to the monitoring as part of the scenario 2 monitoring of E5.

Figure 3. Qualitative risk analysis of the PoE's during the MEMO project in March 2017, February 2018, June 2018 and January 2019 with indication (*) of the detection of EMS at the PoE's.



PoE: E0=Parking lot Noidré/Sprimont; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; AL=Airport Liège; AZ1=Airport Zaventem (brucargo); AZ2=Airport Zaventem (luggage handling area); AC=Airport Charleroi; PA=Port of Antwerp (Kallo); PG2=Port of Ghent (Volvo); PZ=Port of Zeebrugge; EC=Wholesale market Brussels; ET=Wholesale market Liège; MC=Wholesale market Charleroi; BS=company St-Katelijne-Waver; EV=Flower auction Euroveiling (Neder-Over-Heembeek); VG=garden center Sint-Katelijne-Waver; PAY=Container company Yang Ming; PAM=Container company Maersk; PAA=Container company APL.

To conclude, the QRA gave a good ranking of the highest risk PoE's as exotic *Aedes* species were effectively detected. The QRA, however, needs to be updated after each mosquito season as all risk factors for each PoE can change from year to year. But, it is not always possible to gather the detailed data on import volume and frequency of certain PoE’s, which is also difficult to compare between PoE types. Further, not all PoE details of certain risk factors can be taken into account in the scoring system and should be considered additionally to the QRA.

2.2.2 Scenario change of the PoE’s

Since the start of the MEMO project it was decided that a PoE could only move to a higher level scenario during the season. A downgrade to a lower scenario was only possible between years. As criterion to shift to a new scenario, at least one EMS specimen (adult, pupa, larva or egg) needed to be collected. However, each EMS detection was evaluated based on the monitoring results, the local circumstances (EMS origin, habitat suitability) and the implemented control measures at that moment, before a recommendation for a scenario shift in the season or in the next year was made. There were several possibilities to shift between scenarios:

- If **no EMS was detected at PoE with a scenario 1 monitoring**, the PoE kept its scenario 1 status in the next year.
- If **at least one EMS specimen was detected at a PoE with a scenario 1 monitoring**, a basic scenario 2a monitoring (new introduction, 200 m buffer zone) was implemented as soon as possible in the same season. The PoE kept also its scenario 2 status in the next year after a positive evaluation (i.e. the evaluation of the monitoring results of the previous year, which were found advantageous for EMS spread). If the number of scenario 2 cases exceeded four, an approval for extra budget by the steering committee was needed.
- If **no EMS specimen was detected at a PoE with a scenario 2a monitoring (new introduction, 200 m buffer zone)**, the PoE regained its scenario 1 status in the next year.
- If **at least one EMS specimen was detected at a PoE with scenario 2a monitoring (new introduction, 200m buffer zone)**, an extended scenario 2 a monitoring (new introduction, 500 m buffer zone) was implemented as soon as possible in the same season. The PoE kept also its scenario 2a status in the next year after a positive evaluation. If the number of scenario 2a cases exceeded four, an approval of extra budget by the steering committee was needed.
- If **at least one EMS specimens was detected outside the 25 km² area around the PoE with a scenario 2b monitoring (locally established)**, a scenario 3 monitoring (widely established) was implemented the next year. Again, an approval for extra budget by the steering committee was needed.

After the season in 2018, it was clear that the criterion of the detection of only one EMS specimen to shift between scenarios was too strict and not necessary. This caused the implementation of scenario 2a monitoring at PoE‘s with only one detected EMS specimen during the whole season (such as at the used tyre import company Bridgestone aircraft tire (Europe) s.a. (BA) in 2018). To prevent too many unnecessary scenario 2a cases, more specific criteria were defined to shift between scenarios in January 2019, especially based on a possible evidence of summer reproduction and therefore possible spread:

Criteria to shift **from a basic scenario 1 to a basic scenario 2a monitoring (200 m)** (new introduction):

- Detection of eggs and larvae in two consecutive samplings, or,
- Detection of more than one positive oviposition trap (OT) or potential breeding site (PBS) during one sampling period, or,
- A fair number of adults (> 7) is intercepted.
- For the lucky bamboo import company EB:
    - Detection of all life stages (adults, eggs and larvae/pupae) inside the nursery, or,
    - Detection of eggs or larvae outside the nursery, and thus indicating possible spread in the surroundings.

Criteria to **shift from a basic scenario 2a (200 m) to an extended scenario 2a monitoring (500 m)** on the basis of data collected in the 200m extension zone:

- Detection of eggs and larvae in two consecutive samplings, or,
- Detection of more than one positive OT or PBS during one sampling period, or,
- A fair number of adults (> 7) is intercepted.

Criteria to **backshift from a basic or extended scenario 2a to a basic scenario 1 monitoring**:

- Only applicable from one to the next season: if no eggs, larvae and adults were collected in the 200 m buffer zone and summer reproduction could not be proven at the PoE during the entire season.

Table 2. The PoE's indicating their type, code and scenario status in 2017, 2018 and 2019.

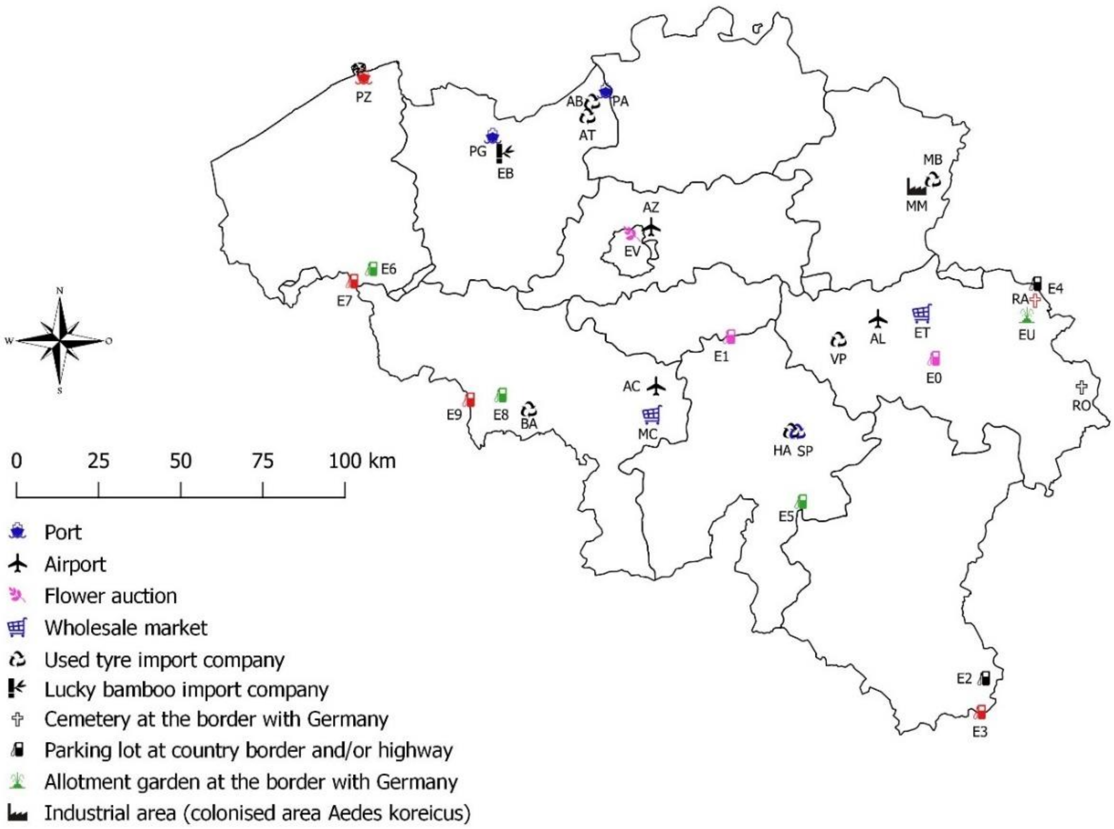
Type of PoE	Code	Scenario in 2017	Scenario in 2018	Scenario in 2019
Airports	AZ1: Zaventem (cargo)	1	-	-
	AZ2: Zaventem (passenger)	-	1	1
	AL: Liège (Avia-partner)	1	1	1
	AC: Charleroi (passenger)	1	1	1
Ports	PA: Antwerp (Border Inspection Post (BIP) Kallo)	1	1[a]	-
	PZ: Zeebrugge (defense marine base)	1	(1)[b]	(1)[b]
	PG: Ghent (Volvo cars)	-	1[a]	-
Platforms for imported used tyres (agriculture, military, industry)	AB: AtoB or Agrityre	1	2a	2a
	AT: ATB banden	1 (2 in 2016)	1	1
	BA: Bridgestone Aircraft tire (Europe) sa	1 (2 in 2016)	2	1
	MB: Maaslandbanden	1	1	1
	VP: Visé Pneu	1	1	1
	SP: Sarri Pneus	1	1	-
	HA: Havelange (also colonised area with *Aedes japonicus*)	2b	2b	2a[c]
Shelters/nursery for imported cutting plants like lucky bamboo	EB: Euro Bonsai	1 (2 in 2016)	1	1
	EV: Euroveiling	-	-	1
Wholesale markets	ET: Marché matinal Liège	1	1[a]	-
	MC: Marché de Gros Marcinelle (Charleroi)	1	1[a]	-
Main parking lots at country borders, highways and road axes originating from colonized areas	E0: E25 (A26) Noidré/Sprimont	-	-	1
	E1: E411 (A4) Aische-en-Refail	-	-	1[d]
	E2: E25 (A4) Hondelange	1	1	1
	E3: E411 (A28) Aubange	1	-	-
	E4: E40 (A3) Raeren	1	1	1
	E5: E411 (A4) Wanlin	-	1	2a
	E6: E17 (A14) Marke	-	1	1
	E7: E17 (A14) Rekkem	1	-	-
	E8: E19 (A7) Saint-Ghislain	-	1	1
	E9: E19 (A7) Hensies	1	-	-
Cemeteries and allotment gardens at the border with Germany	RA: Cemetery Raeren	1	-	-
	EU: Allotment garden Eupen	-	2a[e]	2a
	RO: Cemetery Rocherath - Büllingen	1	1	1
Colonised area with *Aedes koreicus*	MM: industrial area 'Op de Berg' Maasmechelen	2b	3	3 → 1[f]

[a]Monitoring activities were put on hold in June 2018. [b]Monitoring activities completely taken over by Belgian defence. [c]An adapted scenario 2a monitoring was implemented. [d]Monitoring started in September 2019 as part of the scenario 2a monitoring of parking lot Wanlin (E5). [e]At the border with Germany the PoE RA was replaced by the new PoE EU as Ae. japonicus *was detected at that location south of RA. [f]Scenario 3 monitoring activities ended in April 2019 and an adapted scenario 1 was implemented.*

Table 2 and Figure 4 give an overview of all the PoE's monitored during the MEMO project and Table 2 also shows the shift between scenarios.

- In **2017**, 21 PoE's were covered by scenario 1 and two sites (industrial area in Maasmechelen (MM) and used tyre import company Havelange (HA)) fell under scenario 2b.

Figure 4. Map with the PoE's monitored during the MEMO project (red = monitored only in 2017, blue = monitored only in 2017 and 2018, green = monitored only in 2018 and 2019, black = monitored in 2017, 2018 and 2019, pink = monitored only in 2019).



PoE: AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AL=Airport Liège; AT=Tyres ATB; AZ=Airport Zaventem (brucargo (AZ1) and luggage handling area (AZ2)); BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; EB=Lucky bamboo Euro Bonsai; EV=Flower auction Euroveiling; ET=Wholesale market Liège; EU=Allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MC=Wholesale market Charleroi; MM=Industrial area Maasmechelen; PA=Port of Antwerp (Kallo); PG=Port of Ghent (Volvo); PZ=Port of Zeebrugge; RA=Cemetery of Raeren; RO=Cemetery of Rocherath; SP=Tyres Sarri Pneus; VP=Tyres Visé Pneu.

- In the beginning of the **2018** season 20 PoE's were covered by scenario 1, with HA being in a scenario 2b, allotment garden Eupen (EU) in a scenario 2a (new introduction or 'intensive survey') and MM a scenario 3 (separate project SCENA3KOR, financed by ANB). Four new PoE's were monitored, replacing four old PoE's based on a reassessment of the PoE's. These included: port of Ghent (Volvo) (PG), parking lot Wanlin (E5), parking lot Marke (E6), and parking lot Saint-Ghislain (E8). Additionally, at the border with Germany the PoE RA was replaced by the allotment garden Eupen (EU) as *Ae. japonicus* was detected at that location. In June 2018, monitoring at four PoE's (PA, PG, ET and MC) was stopped because of the high workload at other PoE's at that moment and the lower risk for importing EMS. Also in the second half of 2018, two PoE's (used tyre companies Agrityre/AtoB (AB) and BA) shifted from a scenario 1 to a scenario 2a, while at three other PoE's (lucky bamboo import company Euro Bonsai (EB), parking lot E2 and E5) extra monitoring activities were added to the scenario 1 monitoring after the detection of EMS.
- In **2019**, 20 PoE's were monitored: 18 of the 19 PoE's from 2018 were retained for the new monitoring season (one PoE (used tyre import company Sarri Pneus (SP)) did not want to collaborate anymore), together with two newly selected PoE's (parking lot E0 and flower auction EV) based on the updated QRA.

At three PoE's a backshift between scenarios was done as no further detections were made at the PoE or in the buffer zone, with the used tyre company BA shifting back from a scenario 2a to a scenario 1, the used tyre company HA shifting back from a scenario 2b to a scenario 2a, and the industrial area in Maasmechelen (MM) shifting back from a scenario 3 to a scenario 1. This last backshift was based on a decision from the steering committee, indicating that the monitoring at the PoE (MM) based on a scenario 1 would be sufficient to follow the situation of *Ae. koreicus* during the control at MM and to follow-up the new detection of *Ae. japonicus* at MM. During the 2019 season, one PoE (E5) shifted from a scenario 1 to a scenario 2a, while at three PoE's (EB, E0 and E6) extra monitoring activities were added to the scenario 1 after an EMS detection. One PoE (parking lot E1) was added to the monitoring in 2019 as part of the scenario 2a monitoring of E5.

2.3 Fieldwork planning and sampling design

2.3.1 Fieldwork planning

Overall the implementation of the monitoring activities were largely executed as planned (Annex 4). In 2017 and 2018, the fieldwork was differently organised as in 2019. In the first two field seasons, new technical and research staff (two in 2017 and one in 2018) needed to be trained in the field, only one permanent car was available and the new version of VECMAP® (software for targeted surveillance and control of disease vectors, developed by Avia-GIS) was not yet operational. Since 2018, local partners were involved in handling the OT at several parking lots to improve cost-efficiency and sustainability of the monitoring. To ensure efficient sampling in 2017 and 2018, the 23 PoE's were subdivided into eight groups (Annex 4). Each group was visited in one day by one or two teams existing of one or two persons, depending on the amount of work (the number of PoE's to be visited and the area to be monitored (scenario 2 or 3)). In the second half of 2018, flexibility in the number of PoE's and the sampling design was proposed to the steering committee (SC) to decrease the high workload. Also, an extra car was hired by the Institute of Tropical Medicine (ITM) to use primarily for the scenario 2 monitoring. When a scenario 2a (new introduction) was implemented, one extra team of two persons went in the field for the actual scenario 2a monitoring, while the basic monitoring was still done by the original team. Teams included ITM, BopCo and RBINS staff and local partners (Belgian defense, Flanders Environment Agency (VMM) and "Direction Générale Opérationnelle des Routes et des Bâtiments" (DGO1) (Service Public de Wallonie (SPW))). The SC agreed to put on hold four lower risk PoE's, to increase the involvement of the local partners at the parking lots, and to reduce the higher than necessary frequency of larval sampling (LS) in a scenario 2a (new introduction) monitoring. Although this partly decreased the workload, fieldwork days stayed long, especially for scenario 2 and 3 monitoring. In 2019 organisation of the fieldwork was changed to make it more time efficient. Advantages in 2019 were that all technical staff and researchers were fully trained and could independently perform the fieldwork, the new version of VECMAP® was operational and an extra permanent car was made available by ITM to be able to work with two fixed teams. In this way two teams could work independently, which facilitated the work. Further, the PoE's were divided in north and south. The northern PoE's were assigned to a fixed team leaving from Antwerp and the southern PoE's to a fixed team leaving mostly from Brussels. PoE's were divided in 16 groups: each group of PoE's was visited on one day by one team. Team size was adjusted to one or two persons, depending on the amount of work. Loading and unloading the cars was always done on the days without fieldwork. Off course we have to keep in mind that in 2019 we did not have a scenario 3 or a scenario 2b (locally established) compared to in 2018.

2.3.2 Sampling design

The sampling approach followed the requirements set in the public tender CES-2016-02. The planned sampling design for the different scenarios during the MEMO project is presented in Table 3. However, it was stated in the public tender that any improvements or relevant changes to the monitoring protocol could be proposed under certain conditions. During the different phases of the project the approach was evaluated and improvements or changes were suggested based on experiences, new information and the results of the monitoring in that phase.

Table 3. The planned sampling design (collection method, trap density, number of containers or potential breeding sites (PBS) to be sampled, trapping frequency and sampling period) per PoE type or area in each risk scenario.

Risk scenarios	PoE type/area	Collection method	Trap density/# containers or PBS	Trapping frequency	Sampling period
Scenario 1	**Used tyre import companies**	BG	1	every 2 weeks	Apr until Nov
		MMT	1	every 2 weeks	Apr until Nov
		OT	10	every 4 weeks	Apr until Nov
		LS	max 200 containers	every 8 weeks	Apr until Nov
		HLC	15 min	2x/season	Jul & Sep
	Lucky bamboo import company	BG	1	every 2 weeks	Jan until Dec
		MMT	1	every 2 weeks	Jan until Dec
		OT	10	every 4 weeks	Jan until Dec
		LS	max 200 containers	every 8 weeks	Jan until Dec
		HLC	15 min	2x/season	Jul & Sep
	Airports, ports, wholesale markets, shelters for imported cutting plants	MMT	1	every 2 weeks	Apr until Nov
		BG (only at AC)	1	every 2 weeks	Apr until Nov
		OT	10	every 4 weeks	Apr until Nov
		LS	max 10 PBS	every 8 weeks	Apr until Nov
	Main parking lots along highways	OT	10	every 4 weeks	Apr until Nov
		LS	max 10 PBS	every 8 weeks	Apr until Nov
	Cemeteries at the border with Germany	OT	20	every 4 weeks	Apr until Nov
		LS	max 100 containers	2x/season	Aug & Sep
Basic scenario 2a (new introduction)	**PoE**	cfr. scenario 1	cfr. scenario 1, but 3 extra BG	BG & MMT: cfr. scenario 1; OT & LS: every 2 weeks	Apr until Nov
	200 m buffer zone	OT	20	every 2 weeks	Apr until Nov
		LS	max 200 containers	every 2 weeks	Apr until Nov
Extended scenario 2a (new introduction)	**PoE & 200 m buffer zone**	cfr. basic scenario 2a	cfr. basic scenario 2a	cfr. basic scenario 2a	cfr. basic scenario 2a
	500 m buffer zone	BG	3	every 2 weeks	Apr until Nov
		OT	20	every 2 weeks	Apr until Nov
		LS	max 200 containers	every 2 weeks	Apr until Nov
Scenario 2b (locally established, ≤ 25 km²)	**colonised area (≤ 25 km2)**	OT	8	every 4 weeks	May until Oct
		LS	max 200 containers	2x/season	Aug & Sep
	5 km buffer zone	OT	32	every 4 weeks	May until Oct
		LS	max 200 containers	2x/season	Aug & Sep
Scenario 3 (widely established, > 25 km²)	**colonised area (> 25 km²)**	BG	4	every 2 weeks	Jan until Dec
		OT	16	every 2 weeks	Jan until Dec
	5 km buffer zone	OT	32	every 4 weeks	Apr until Nov

BG=BG-Sentinel trap, MMT=Mosquito Magnet® trap, OT=oviposition trap, LS=larval sampling, HLC=human landing collection, AC=airport Charleroi.

Following improvements and changes were made to the sampling design during the MEMO project:

Collection methods

- **Human landing collection (HLC) was not performed**. We suggested to drop the HLC because this collection method would not contribute substantially to the detection of EMS at the PoE's. For this collection method ethics approval is required.

- Since 2018 **oviposition traps (OT)** at all sites were adapted with drainage holes and a chicken wire to prevent the polystyrene piece to be removed by wind, rain or animals. This adaptation was a great success as significantly less polystyrene pieces were lost compared to 2017. Further, to minimise the preparation and trap handling, standing tap water instead of hay infusion was used in the OT for monitoring in 2018 and 2019. Although baiting OT with an oak leaf or hay infusion increases the attraction of *Ae. albopictus* females to the trap [6], in a lot of European countries tap water is used in OT with good results [7-9]. *Aedes japonicus* does not seem to prefer hay or oak infusion above standing tap water [10].
- Since May 2018, two **Frommer updraft gravid traps (GT)** were adapted with a 12/6V converter (3A) to be able to run the traps during two weeks on two 12V 59,7AH batteries. The two GT were added to the sampling scheme in 2018 and 2019, one at the industrial area in Maasmechelen (MM) and one at the allotment garden in Eupen (EU), as this trap is very efficient to collect *Ae. koreicus* and *Ae. japonicus* [11, 12].
- In 2017 and 2018, problems were encountered with six **Mosquito Magnet® traps (MMT)** (thermal failure (3) and manufacturer fault (3)). Traps were sent back to the company FAVEX, who repaired them for free. In the meanwhile old MMT traps could be used from RBINS. Since then, fewer problems occurred and the MMT could still be used.
- In 2019, a **solar panel to provide power to the Biogents® BG-Sentinel trap** (**BG**) was put in place in August-September 2019 at the used tyre import company Agrityre/AtoB (AB) and the industrial area MM to facilitate the fieldwork and increase the sustainability of the monitoring. This set-up proved to be successful.

Scenario 1 (not established) sampling design

- In 2018 and 2019 monitoring at the **cemetery Rocherath (RO)** was done by LS only. We suggested to stop the OT sampling because the LS seemed to be more effective than the OT to detect a new introduction or spread of *Ae. japonicus*. LS was performed only once along the road close to the cemetery of Raeren (RA) in 2017 (September) and 12 larvae of *Ae. japonicus* were detected close to where two OT were located (see Chapter 3.2.2), which were negative during all samplings. Although OT are effective to collect *Ae. japonicus* eggs in areas where established populations occur [10], it seems that this method is less effective in areas with potentially low densities.
- In 2018 and 2019, **local partners assisted in the monitoring at the parking lots**. The VMM assisted in OT handling in Flanders and the DGO1 in Wallonia. A quality control of the OT sampling by the local partners was set-up and consisted of:
    - All local partners received a hands-on training at the parking lot by ITM. All partners received also a written procedure in their language.
    - The preparation and determination of the locations of the OT was conducted by ITM. OT were positioned in the shadow close to or under vegetation or near buildings.
    - ITM was responsible for all preparation (polystyrene pieces, materials needed to perform the collection, labeled and prepaid Bpost packages to send the samples). ITM was owner of all the materials the local partners used to fulfill the sampling.
    - ITM was in permanent contact with the local partners: one day in advance a reminder was sent to the partners, the partners then contacted ITM when the postal package was sent. In this way the work could be followed up and checked by ITM. If the postal packages didn't arrive in time both the local partners could be contacted and the package could be retrieved by the tracking number ITM has. In this manner the execution of the sampling could be controlled. Additionally all local partners needed to write down all actions taken on site on the data forms.
    - Once the samples arrived, one ITM member checked if the package was complete and if the details were filled in on the data forms. The partners needed to deposit the polystyrene pieces,

the wipes used to clean the OT, the filled in data form indicating the status of the trap and the collection details, in the package. ITM provided feedback to the partners.

- Since mid-2018, **LS was dropped from the sampling scheme at the parking lots** and the local partners increased their involvement. OT sampling is the principal method for detecting *Ae. albopictus* along highways in Switzerland [13] and in France [8]. In this way monitoring at the parking lots still continued, but the workload and the ecological footprint of the MEMO project decreased.
- In 2018 and 2019, **Belgian defense was the local partner at the airport Zaventem (luggage handling area) (AZ1)**. ITM provided a training on-site in OT and BG handling during the set-up and LS was trained on the first LS visit. Belgian defense emptied the BG, changed the OT and performed LS during the season. The samples were delivered at ITM soon after collection.
- During the three-year monitoring, sometimes OT sampling at parking lots and LS at other PoE's needed to be shifted to another date or were not performed due to practical reasons (Annex 4).
- In 2018, only OT sampling and LS was performed at the **wholesale market (MC)**. During the monitoring in 2017, very few adults (only 2) were collected with the MMT. Next to the fact that the MMT has a cold temperature indicator (which will shut down the trap when the engine, not the outside temp, reaches 10°C and will restart when the engine reaches 12,7°C), also the general cold temperature of the hangar (refrigerated), where the trap was placed, probably played a role. The containers are opened at a quay with an open space between the containers and the large gates of the hangar. Mosquitoes flying out of the container probably did not enter the hangar because of the cold temperature. At the start of the project it was planned to position the MMT outside the building, but, according to the personnel, the safety could not be guaranteed as there is a lot of vandalism during the night around the wholesale market.
- In June 2019, the scenario at the **industrial area MM** shifted from a scenario 3 (widely established) to a scenario 1 for *Ae. koreicus* and *Ae. japonicus*. The general sampling scheme was adapted in order to take into account the ecology of the targeted species and encountered problems:
    - Because of problems with the MMT during the monitoring in 2018 (scenario 3 monitoring), a BG was placed instead for the scenario 1 monitoring.
    - A GT was added to the sampling design at MM.
- Following the detection of *Ae. albopictus* in 2018, extra LS was conducted at **parking lot Wanlin (E5) and Hondelange (E2)** in March and April 2019 to check overwintering.
- Following the detection of *Ae. albopictus* in August 2019, an extra LS was conducted at the **lucky bamboo company Euro Bonsai (EB)** in September 2019.
- Following the detection of *Ae. albopictus* in September 2019, OT sampling at **parking lot Marke (E6)** was prolonged until the end of October 2019, while OT sampling at **parking lot Sprimont (E0)** was already extended to the beginning of November 2019 for practical reasons. Also at E6, an extra LS was performed in the end of October 2019. The extra OT and LS were performed by ITM, not by DGO1.

Scenario 2a (new introduction) sampling design

- In 2018, **LS frequency was decreased from every two to every four weeks at the PoE and in the 200 m buffer zone.** After the detection of an EMS in a used tyre company in the Netherlands, LS is done in the same week of detection, then after three weeks and then every 6 weeks [14]. After the detection of *Ae. albopictus* in Germany the surveillance included LS once a month [15]. We therefore proposed to lower the frequency of LS in a new scenario 2a case to every four weeks.
- In 2018, the scenario at an **allotment garden EU**, along the monitored road (towards the cemetery Raeren (RA)) close to the border with Germany, shifted from a scenario 1 to a basic scenario 2b (locally established) for *Ae. japonicus*. The general sampling scheme was adapted in order to take into account the ecology of the targeted species:

    - At the allotment garden EU, we used MMT instead of BG. A combination of CO_2 and lure seems to be a better attractant for *Ae. japonicus* than CO_2 or lure alone [10, 16]. Although the BG trap is good at attracting *Ae. albopictus* and *Aedes aegypti*, it is less attractive to *Ae. japonicus* than the MMT [12, 16]. Only two instead of the planned four could be used, due to the limited stock of MMT. In May 2018, a GT was added to the sampling design at EU.
    - OT using polystyrene blocks as oviposition support, as are currently used in the MEMO project, were preferred by *Ae. japonicus* in Switzerland over OT using wooden sticks and seed germination paper [10]. However, in the same study, oak and hay infusions did not increase the attractiveness of OT compared with standing tap water. To minimise the preparation and trap handling, therefore, standing tap water instead of hay infusion was used in the OT for monitoring of *Ae. japonicus* at and around EU.
    - Extra LS was performed once in March 2018 at and around EU to check possible overwintering of eggs and larvae.
    - LS was performed every four weeks instead of every two weeks at and around EU.
- In 2018, the scenario at the **used tyre import company AB** shifted from a scenario 1 to a basic and extended scenario 2a (new introduction) for *Ae. albopictus*. Due to the limited stock of BG, three BG-GAT traps were set-up in the 500 m buffer zone instead. In 2019, a backshift was made to a basic scenario 2a (200 m buffer zone) at and around AB. Extra LS was conducted at and in the 200 m buffer zone around AB in March and April to check overwintering. Further, in May 2019, a BG was moved outside the 200 m buffer zone, close to the offices of Tabaksnatie, after a possible notification of *Ae. albopictus*. Also three extra OT were added to this location in May. However, no *Ae. albopictus* specimens were collected.
- In 2019, the scenario at the **parking lot E5** shifted from a scenario 1 to a basic scenario 2a (new introduction) for *Ae. albopictus*. The general sampling scheme was adapted according to the environment around the parking lot (a lot of forest and fields, few urban areas within 500 m):
    - Scenario 1 monitoring continued as before with assistance of the local partner (10 OT, every four weeks).
    - LS was conducted in September and October 2019 at the E5.
    - To monitor the spread of *Ae. albopictus* along the highway, the next parking lot with service station, Aische-en-Refail (E1), was added to the monitoring. A scenario 1 monitoring was implemented with the set-up of ten OT at E1 between the end of September and the beginning of November 2019. LS was performed in September and October 2019. All the monitoring activities at E1 were performed by ITM, not by DGO1.
- In 2019, the scenario at the **used tyre import company Havelange (HA)** shifted from a scenario 2b (locally established) to a basic and extended scenario 2a (new introduction) for *Ae. japonicus*. The general sampling scheme was adapted in order to take into account the ecology and current situation of the targeted species and the environment (a lot of fields):
    - At HA, no extra BG were set-up, as these are less attractive for *Ae. japonicus* (see above for EU). Due to the limited stock of MMT, no extra MMT could be set-up.
    - In the 200 m and 500 m buffer zones, 10 OT/buffer zone were installed with collections every four weeks.
    - LS was performed every eight weeks instead of every four weeks at and around HA.
    - Extra LS was performed outside the 500 m buffer zone in October, following the detection of the species in September 2019 in the buffer zone. OT sampling was also prolonged until mid-November 2019.
- In February and March 2019, extra LS was conducted at and in the 200 m buffer zone around the **allotment garden EU** to check overwintering of *Ae. japonicus* larvae.

Scenario 3 (widely established) sampling design

- In 2018, the scenario at the industrial area MM shifted from a scenario 2b (locally established) to a scenario 3 (widely established) for *Ae. koreicus*. The general sampling design for a scenario 3 (Table 3) was adapted to the mosquito species *Ae. koreicus*, as trap efficiency differs between species. The OT and the BG used in this and previous projects did not seem to be very efficient for the collection of *Ae. koreicus* [11, 17]. This was confirmed by the results from 2017. Therefore an adapted sampling design was proposed and approved for the scenario 3 case of *Ae. koreicus* at MM.
    - At MM, two MMT were set-up with collections every two weeks between May and October 2018 and in April 2019, and LS was performed every 8 weeks with a maximum of 200 containers. In May 2018, a GT was added to the sampling design and in August 2018 two BG-GAT traps were added to check their efficiency in capturing *Ae. koreicus*.
    - Around MM, in a 6-8 km buffer zone, LS was performed in August and September with a maximum of 200 containers.

2.4 Sample processing and morphological identification

2.4.1 Adult traps

The adult specimens were killed by putting them in a -20°C freezer upon arrival at the lab after collection in the field. Samples were first sorted (i.e. separating mosquitoes from non-mosquitoes) and screened for the presence of EMS. All samples were double checked by two different persons to ensure that no EMS were overlooked. The identification of native adults was performed once all traps from the same sampling period were sorted. Morphological identification was done using dichotomic and digital keys [18, 19]. Samples and specimens were chronologically identified and timely notification and detection of new EMS at PoE's was ensured. The presence of EMS was communicated with the contracting authorities via the urgent notification sheets. The collected rest fraction of each sample was stored in 80% ethanol tubes and was labelled accordingly. Once the MEMO project comes to an end, these rest fractions will be made available for identification and appear in the samples list that is published on the Royal Belgian Society for Entomology website (http://www.srbe-kbve.be/cm/reststaalcollectie-collection-des-restes-0).

2.4.2 Oviposition traps

The collected polystyrene pieces were checked at ITM for the presence of *Aedes* mosquito eggs. If present, a subsample of the eggs (ranging from one to five) from each side of the polystyrene piece was molecularly identified by BopCo. Eggs of other genera can rarely be detected with OT.

2.4.3 Larval sampling

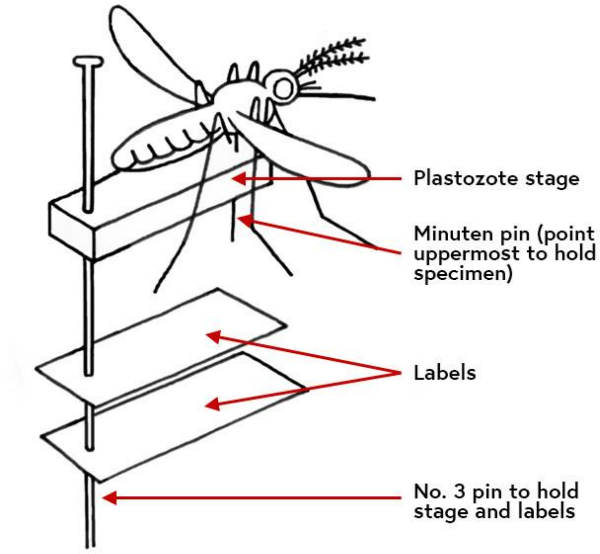
Larvae were transported alive to the ITM laboratory in vials, killed by a thermal shock with hot water (70°C), and finally transferred in 80% (native specimens) or absolute (exotic specimens) ethanol. All larvae were morphologically identified using a digital identification key [19]. Larvae were identified by a staff member of RBINS. Early developmental larval stages and pupal stages, however, cannot reliably be identified to species level. In case of presumed EMS these were molecularly identified to species level.

2.4.4 Morphological reference collection at RBINS.

A collection of the most intact specimens sampled during the MEMO project was generated. It represents the mosquito diversity and different sex and life stages collected at each PoE and each year, i.e. 2017, 2018 and 2019. In total 539 specimens of 23 species or species complexes have been mounted, of which 255 females, 46 males and 231 larvae. Additionally also 4 eggs and three pupae were included as reference material (Annex 5).

Adults were prepared using black enameled pins (No. 6), minute pins type 10, 15 and 20 and white plastozote stage. First a minute pin is pierced through the white plastozote stage. This setup is used to pin the mosquito on the minute pin. Afterwards the black enameled pin is transfixed through the opposite site of the plastozote stage (Figure 5 & Figure 7).

Figure 5. Mounting adult mosquito (https://ugc.futurelearn.com/uploads/assets/8d/e5/8de5b3ef-b54d-43f2-8557-9a21b510d670.jpg).



Larvae, pupae and eggs were slide-mounted using dimethylhydantoin formaldehyde resin, microscope cavity slides and square cover glasses. The abdomen of the larvae is often cut between the 6th and 7th segment in order to mount the anterior part dorsally and the posterior end laterally to maintain key characteristics for the species (Figure 6 & Figure 7).

Figure 6. Mounted larva of *Culiseta annulata* (right)) and *Aedes koreicus* (left) on slide.

Of the most intact specimens, including all EMS and newly detected species for Belgium, high resolution stacking pictures were taken (Figure 8). A digital photo database was build containing pictures of the different species, sex and life stages and will be accessible at http://collections.naturalsciences.be/ssh-entomology/collections/be-rbins-ent-collection-memo-project. Photos of at least one female, male or larvae of 23 encountered species were selected for the database. Photographs were taken with a Canon® EOS 600D camera equipped with a Canon® MP-E 65 mm macro lens, staked with Zerene Stacker software [20] and optimized with Adobe Photoshop® CS3. Observations were done with a Leica® MZ8 stereo microscope. Per specimen, a series of photos was taken of the lateral, dorsal and frontal view in order to make one stacking picture for each side. Of larvae only a dorsal stacking photo was made.

Figure 7. Overview of the boxes with mounted *Culex* specimens (above, right), mounted *Aedes koreicus* (above, left), mounted larvae (below, right) and an overview of all boxes stored in the conservation room at RBINS (below, left).

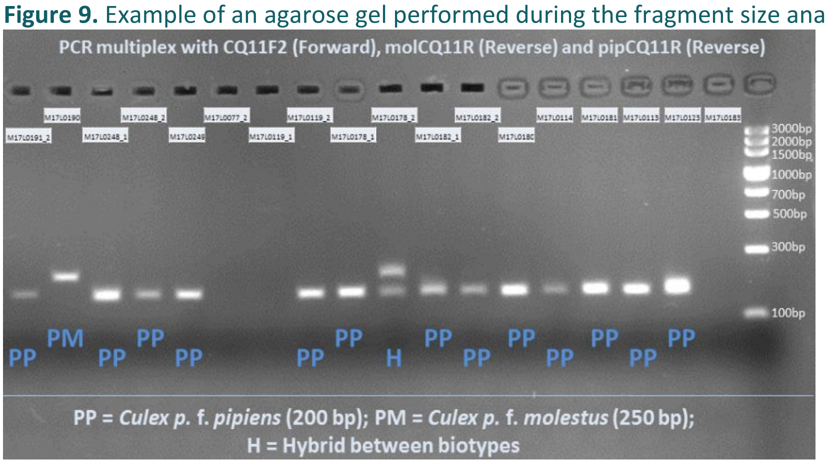
Figure 8. Pictorial overview of the exotic mosquito species and new species in Belgium: *Aedes albopictus* (A-B), *Aedes koreicus* (C-E), *Aedes japonicus* (F-H), *Anopheles pharoensis* (I), *Culex modestus* (J), *Culiseta longiareolata* (K-L).

2.5 Molecular identification of mosquito specimens and collection

Molecular identification of mosquito specimens was conducted by the Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo - the Belgian federal in-kind contribution to LifeWatch). A list of mosquito species occurring in Belgium was established by reviewing the literature [21], to which EMS already present in or of high concern to Europe were added. This list was updated over the course of the project, following the identification of three new species for Belgium in 2017 and 2018 (i.e. *Anopheles daciae*, *Culex modestus* and *Culiseta longiareolata*), and the detection of the new EMS (*i.e. Anopheles pharoensis*). This list currently includes 32 native and 8 exotic species (Figure 10).

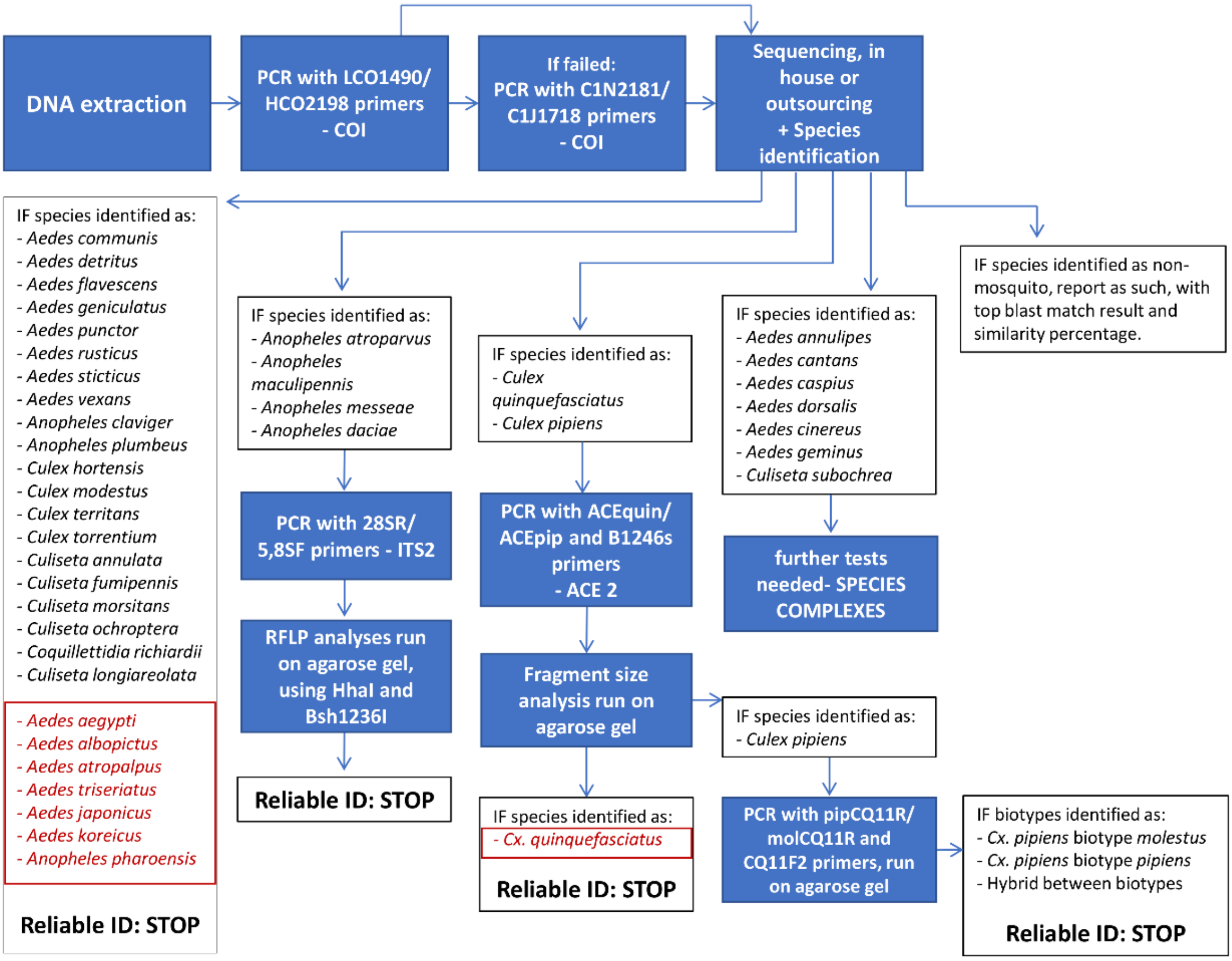
Subsequently, all available DNA sequences for each of these species were downloaded from the online DNA reference databases GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and BOLD (http://www.boldsystems.org/index.php). After filtering the data, each DNA marker was evaluated for its efficiency to provide a reliable species-level identification. DNA barcoding gap analyses and Neighbour-Joining tree constructions were performed for the most promising DNA markers. These markers were selected based on their sequence availability which had to be representative of each species' distribution range to account for the molecular intra-specific variation, and which had to represent most species within the taxa to check if closely related species could be discriminated. Based on these results, BopCo has established an identification pipeline (Figure 10), sometimes including multiple consecutive steps to obtain a molecular identification to species or biotype level. However, most of the time, the amplification and Sanger sequencing of a single DNA marker, the mitochondrial cytochrome *c* oxidase subunit I gene (COI) [22], was found to be sufficient to reach a species-level identification (67,5% of the species). This included all EMS in the elaborated species list (n=8; indicated in red on Figure 10), except for *Culex quinquefasciatus*. For the latter, an extra size fragment analysis of the second intron of the *ace-2* locus was required to discriminate it from its sister species *Culex pipiens*. The investigation of the CQ11 loci additionally allowed to discriminate between *Cx. pipiens* biotypes by performing a size fragment analysis on agarose gel (Figure 9) [23]. Finally, the amplification of the nuclear ribosomal internal transcribed spacer 2 (ITS2) locus [24] was necessary to discriminate between the species of the *Anopheles maculipennis s.l.* complex, of which four representatives are currently recorded in Belgium (i.e. *An. daciae, Anopheles messeae, Anopheles atroparvus* and *Anopheles maculipennis*). Both Sanger sequencing and Restriction Fragment Length Polymorphism (RFLP) of the ITS2 fragment (using a combination of two restriction enzymes [25, 26] allowed for the discrimination between these four species. The RFLP method is, however, less costly and less time-consuming. For seven native species (i.e., *Aedes annulipes, Aedes cantans, Aedes caspius, Aedes dorsalis, Aedes cinereus, Aedes geminus* and *Culiseta subochrea*), no DNA markers were found that allowed an identification to species level, either because they are part of a complex, and/or because of a lack of available DNA reference material in online repositories. The species which are part of a complex were reported as such to ITM when encountered.

Figure 9. Example of an agarose gel performed during the fragment size analysis of the CQ11 locus.



The pipeline displayed in Figure 10 was used to perform: (i) continuous ad hoc molecular species identifications to confirm potential EMS during the monitoring season, and (ii) annual validations of the morphology-based identifications, implemented as quality control measure.

Figure 10. Pipeline established by BopCo for the DNA-based species identifications of mosquitoes collected in Belgium.



2.5.1 Ad hoc molecular species identification

In total 834 mosquito specimens were molecularly identified ad hoc, of which 403 adults, 371 eggs, 56 larvae and four pupae. In addition to confirm the presence of EMS, molecular identification was often necessary to reliably identify native species, mainly due to the loss of diagnostic morphological characteristics.

2.5.2 Quality control of morphological identifications

A subset of the morphologically identified adult and larval specimens were validated annually. In total 2389 specimens were molecularly validated, which represents approximately 5% of the collected adult and larval specimens during each sampling year. Mosquito egg were not included in this validation as these were all molecularly identified when suspected to be an EMS due to the difficult morphological identification. The majority of the specimens were correctly identified: 99.28 % (in 2017), 98.83% (in 2018) and 98.91% (in 2019) (Table 4). The misidentified specimens were rechecked to enable correct morphological identification in the future, and in case the misidentification concerned an EMS, all specimens collected at that particular location and of the same life stage were morphologically re-identified and/or send for additional molecular identification.

2.5.3 Molecular collection and data generation

All samples which were used for molecular identification are stored in 2D smartscan QR boxes. All extracted DNA was dried for long term preservation at room temperature, using the GenTegra® DNA Tube technology. The tissue and DNA material was transferred to the RBINS for integration into the reference collection.

In total, 3209 COI barcodes, 116 ITS2, 284 *nad4* (mitochondrial NADH dehydrogenase subunit 4) and five COII (mitochondrial cytochrome *c* oxidase subunit II) sequences were generated and are in the process of being submitted to the DNA online repository GenBank. Some of these sequences are first molecular contributions to the online repository from specimens collected in Belgium, which belong to the following species: *Culex hortensis, Cx. modestus, An. daciae, An. atroparvus* and *Cs. longiareolata*. Furthermore, sequences of several EMS collected in Belgium will be deposited: *Ae. albopictus*, *Ae. japonicus*, *Ae. koreicus* and *An. pharoensis*.

Table 4. Validation results of the morphological identifications from 2017, 2018 and 2019 (red=differences, green=similarities).

		Morphological identification												
		Aedes annulipes/cantans	*Aedes geniculatus*	*Aedes japonicus*	*Aedes koreicus*	*Aedes vexans*	*Anopheles claviger*	*Anopheles maculipennis s.l.*	*Anopheles plumbeus*	*Coquillettidia richiardii*	*Culex hortensis*	*Culex pipiens/torrentium*	*Culiseta annulata*	*Culiseta longiareolata*
Molecular identification	*Aedes annulipes/cantans*	4												
	Aedes cinereus											2		
	Aedes geniculatus		31	5										
	Aedes japonicus		1	39	7									
	Aedes koreicus				44									
	Aedes vexans					9								
	Anopheles claviger						1							
	Anopheles daciae							2						
	Anopheles maculipennis							1						
	Anopheles plumbeus			4					491					
	Coquillettidia richiardii									9			1	
	Culex hortensis								1		2	1		
	Culex pipiens hybrid between biotypes											30		
	Culex pipiens molestus								2	1		225		
	Culex pipiens pipiens									1		952		
	Culex pipiens s.l.											37		
	Culex pipiens s.s.											37		
	Culex torrentium										1	401	1	
	Culiseta annulata		1									1	39	
	Culiseta longiareolata													1
	No ID											3	1	

2.6 Data management

2.6.1 Field sampling

All the information of the PoE, trap information, sampling information, as well as the morphological identification later in the laboratory, were stored in the data management system VECMAP® with a traceable and unique labelling system. In the field, a unique code was assigned to each sample (a sample is a collection from one trap at one location during one sampling period) that was used during subsequent processing (e.g. M17ATM029AUG30) (Table 5). Sample information was entered by the ITM team in the field via the VECMAP® smartphone application. Local partners wrote down the same information on preformatted paper sheets and these were sent together with the samples to ITM. Upon arrival the data was put in the database by a member of ITM. This same member kept close contact with the local partners to execute the planned schedule. Throughout the monitoring years the format of the different forms was slightly adapted, at the end the data was combined into one uniform sampling database (Figure 11).

Table 5. Code book of the MEMO labelling system.

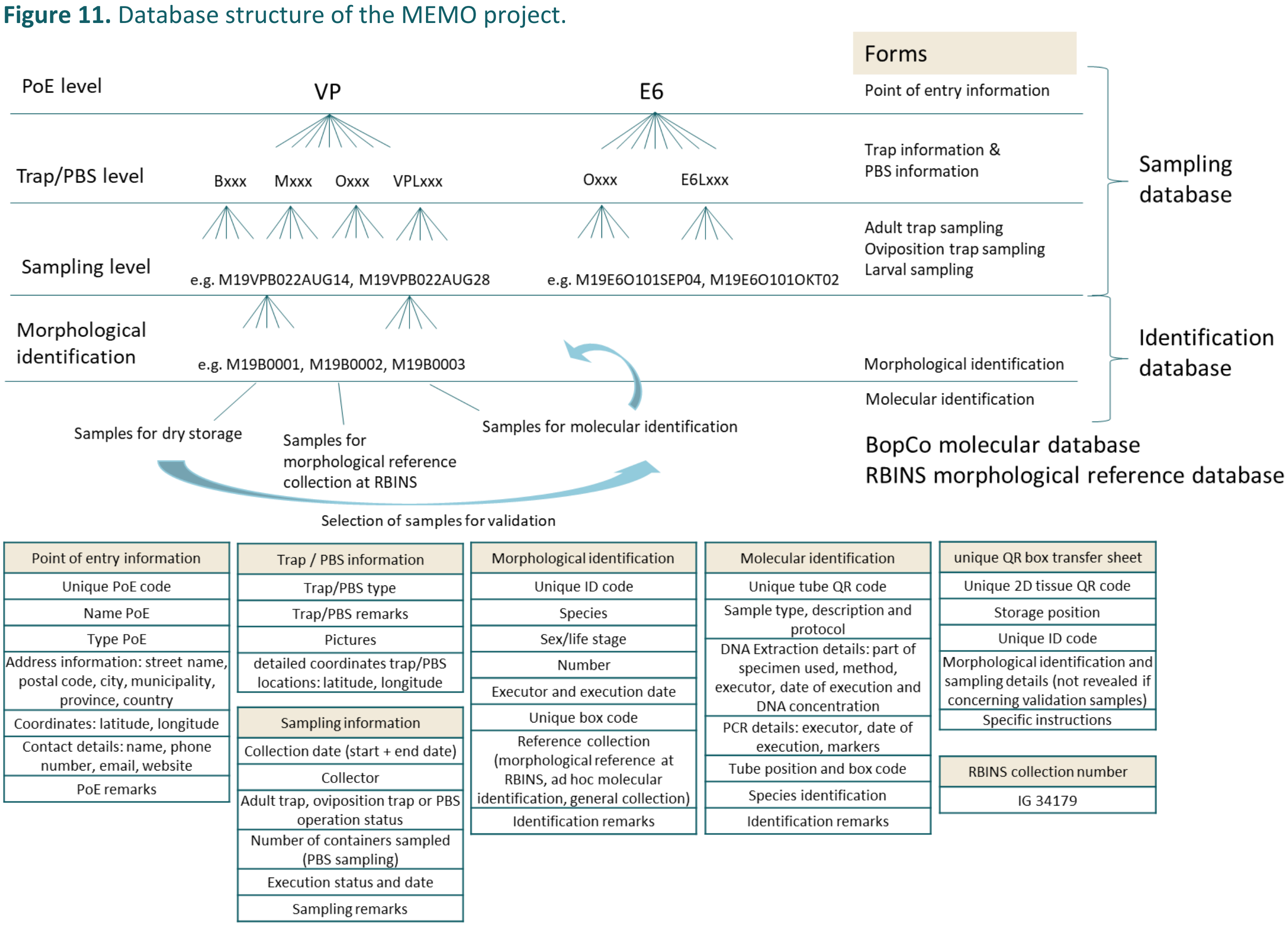
Unique codes	Explanation	Data type	Possible choices
Project code	Abbreviation of the project name MEMO and year	1 capital letter and 2 digits	M17, M18, M19
PoE code[a]	Abbreviation of the PoE name	2 capital letters or 1 capital letter and 1 digit	e.g. AB, EB, AZ
Method code[b]	Abbreviation of the trap or method name	1 capital letter	M, B, O, T, G, L, H
Trap code	Method code + number	1 capital letter and 3 digits	e.g., M001, B005, O203
Date code	Abbreviation of the month in which capture started + day of the month	3 capital letters and 2 digits	e.g. JUN01
PBS code[c]	PoE code + method code of larval sampling (L) + number	3 capital letters and 3 digits	e.g. EBL001
Storage code[d]	Abbreviation of the storage method	2 capital letters	AR, DR, FR, FF, AF
Reference collection code[e]	Abbreviation of the reference collection type	1 capital and 4 small letters	Morph, Molec
Rest fraction code	Underscore + abbreviation of rest fraction (RE)	_ and 2 capital letters	_RE

[a]PoE=Point of Entry; AB=Tyres AtoB or Agrityre; EB=Lucky bamboo Euro Bonsai; AZ=airport Zaventem (the full list of PoE codes is presented in the abbreviation list); [b]M=Mosquito Magnet® trap; B=BG-Sentinel trap; O=Oviposition trap; T=BG-GAT trap; G=Frommer updraft gravid trap; L=Larval sampling; H=Human landing; [c]PBS=Potential breeding site; [d]AR=Alcohol at room temperature; DR=Dry at room temperature; FR=Fresh at room temperature; FF=Fresh frozen (-20°C); AF=Alcohol frozen (-80°C); [e]Morph=Morphological reference collection; Molec=Molecular reference collection.

2.6.2 Species identification

The morphological identifications were entered through the web application of VECMAP®. The mosquito specimens within each field sample were stored in tubes with their unique ID code (e.g. M17M0001) (Table 5), linking it to the unique sample code (Figure 11). These tubes were stored in boxes with a unique box code (e.g. M17EB01DR). Specimens that were transferred for ad hoc molecular identification and morphological reference collection at RBINS were indicated in the identification database (Table 5). This procedure was not followed for the yearly molecular validation as the specimens were randomly selected. These specimens can be traced back in the molecular database (BopCo) via their unique ID code. All specimens for molecular identification (both the molecular validation and the ad hoc specimens) were transferred to 2D smartscan QR boxes of which each tube has its own unique QR code (printed on the bottom of the tubes). The specimens' unique ID code was subsequently printed on a small label and deposited into each respective sample tube to assure full traceability. Information sheets containing the QR box information (unique tube QR code, tube position in the box and box name) and the unique field sample and morphological ID code were shared with BopCo via preformatted Excel® sheets. Morphological identification details of the validation samples (location, species name) were only shared with BopCo upon finalizing the molecular identification for comparison of the respective results. In case of a mismatch the morphological identification was only adapted after the morphological re-identification of the concerned specimen.

Figure 11. Database structure of the MEMO project.



The molecular identifications were performed by **BopCo** in the molecular laboratory of the Royal Museum for Central Africa (RMCA). BopCo uses a standardized preformatted Excel lab-book to follow each step performed in the laboratory, from the DNA extraction all the way through to the sequencing reaction. The information included (Figure 11), but is not limited to, the unique morphological ID code (as provided by ITM), the QR code and position in the 2D barcode box, the storage method, the DNA extraction method, used tissue sample, the PCR and sequencing protocols, reaction details, and the QR code of the dried DNA extract and its position in the respective 2D barcode box. Date and executor are specified for each step.

At **RBINS** the morphological reference collection and the whole MEMO mosquito collection, which will be transferred in the end of the project to RBINS, were given a unique collection code (IG number 34179 for all mosquitoes of the MEMO project), which is linked to the original unique ID code (Figure 11).

2.6.3 Encountered problems and risk mitigations

At the start of the sampling season in 2019 the newest version of VECMAP® (version 2) was not fully operational yet and faced some developing problems. Also the migration of the 2017 and 2018 database to the new version faced some problems. After several meetings with the company Avia-GIS, we worked out a temporary solution and agreed to work with the newest version and give regular feedback for the development. The newest version allows a great flexibility and has some new features. In the newest version of VECMAP® data is internally stored as a hierarchically non-SQL database. In contrast to the old version were no hierarchical structure was possible the new version allowed to build clusters at different levels, such as a PoE level, a trap and PBS level, and a sampling level (Figure 11). Via a personalized R® script the identification database (morphological and molecular database) and sampling database were combined (package dplyr). By combining the databases of BopCo with the stored VECMAP® database a double check of both entered databases was performed frequently, enabling fast exchange and if necessary, apply for the appropriate corrections. As such the original metadata was not altered and in case of peculiarities this can be traced back.

2.6.4 Final material storage

All mosquito material and reference material will be transferred to RBINS and will receive upon arrival their appropriate storage location in repository 3C. Mounted specimens will be stored per species in insect drawers of 30x40 cm (Figure 7). These drawers are placed in oak cabinets, completing the Belgian Culicidae collection naming supplement boxes "MEMO-project 1-2,…". Other samples of mosquitoes that were not included in the morphological collection, will also be stored with the Belgian Culicidae collection. Samples that are preserved in alcohol (such as larvae, pupae and eggs) will be stored in repository 10C completing the Belgian Culicidae collection on alcohol. All extracted DNA was dried for long term preservation at room temperature. The tissue and DNA material, together with the lab-book of BopCo, was transferred to RBINS for integration into the institute collection. The general data storage policy of RBINS is available for consultation on http://collections.naturalsciences.be/collections-rules/.

2.7 Passive monitoring activities

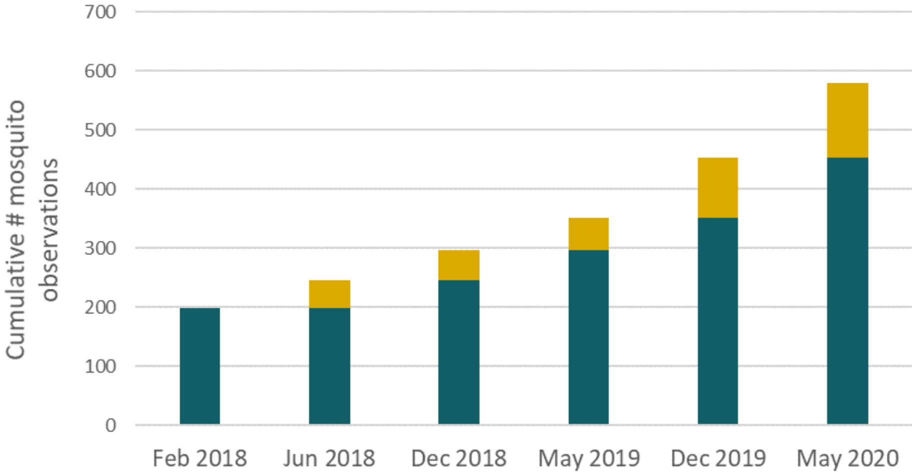
Results for first half of 2020

Since December 2019, 127 extra mosquito observations in www.waarnemingen.be were screened and validated, bringing the total number of observations to 580 over nine years (2010-2020). No EMS were identified. There was one observation of *Ae. albopictus* in Booischot (21/12/2019) without picture (not possible to verify), which probably concerned a *Culiseta annulata* as it was winter. Further, 10 reports of 'tiger mosquitoes' were received at ITM by email since December 2019. Four of the 10 reports were forwarded by NEHAP and RBINS. Four reports did not have a picture or sample, but in three cases it probably concerned the native *Culiseta annulata* or a native *Aedes* sp. Six reports showed photos: three of *Culiseta annulata* and three of other Diptera.

Overall results during MEMO

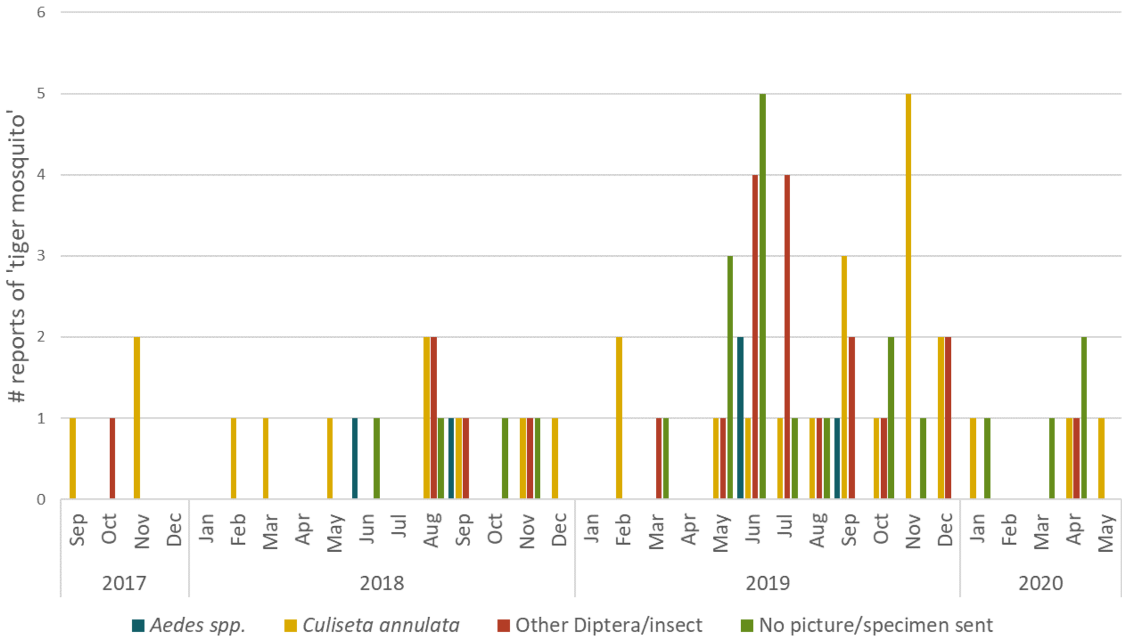
Since the start of the MEMO project the number of mosquito observations almost tripled (Figure 12). Next to *Culex pipiens s.l.*, also a lot of *Culiseta annulata* and native *Aedes* species were observed.

Figure 12. The cumulative number of mosquito observations (green) from www.waarnemingen.be during each reporting phase of the MEMO project with the number of new observations per phase in yellow.



In total 80 '*Ae. albopictus*' or 'tiger mosquito' reports were sent to ITM between September 2017 and May 2020. Most were sent by email (81%), followed by telephone calls (9%), facebook posts (5%), delivered specimens (4%) and twitter (1%). Of the 80 reports, 58 (72,5 %) included a picture. Half of the 'tiger mosquitoes' on the pictures were actually pictures of the native mosquito *Culiseta annulata* (53%), followed by pictures of other Diptera (29%), native *Aedes* spp. (9%), and other insects (9%). The number of received reports increased during the MEMO project (Figure 13). Probably the press releases with the MEMO results sensitised the public and made people more aware of possible exotic *Aedes* species in Belgium. Most reports were sent during summer and autumn when mosquitoes were most active. *Culiseta annulata* was reported often during the winter months, as adults overwinter in houses and are noticed easier, whereas native *Aedes* spp. only during the summer months (Figure 13). These results give already an idea of what to expect when a citizen science project focussing on exotic *Aedes* species will be implemented in Belgium.

Figure 13. The number of reports of 'tiger mosquito' received at ITM per month during the MEMO project with indication if it included a picture or specimen, or not, and the real identification of the picture or specimen.



3 Overview of the MEMO results

3.1 The main results of the MEMO monitoring

3.1.1 General results

A total of 52478 mosquito (Diptera, Culicidae) specimens, belonging to 31 species (or species complexes) and 5 genera (*Aedes, Anopheles, Culex, Culiseta, Coquillettidia)* were collected (Table 7 & Annex 7). Three new species for Belgium, i.e. *An. daciae, Cx. modestus* and *Cs. longiarealata* were found (Table 6). Four exotic mosquito species were detected: *Ae. albopictus* (the Asian tiger mosquito), *Ae. japonicus* (the Asian rock pool mosquito), *Ae. koreicus* and *An. pharoensis* (Table 6). The latter has never been detected during previous EMS monitoring activities in Belgium. Compared to the checklist for Belgium, all mosquito species/complexes were found during the MEMO project except the indigenous species *An. messeae*, *Aedes flavescens*, *Culiseta subochrea* and *Orthopodomyia pulcripalpis* [21].

Most abundant species collected were *Cx. pipiens/torrentium* (65.4%), *Anopheles plumbeus* (17.9%) and *Ae. japonicus* (4.4%). The high number of *Ae. japonicus* is due to the high abundance at the used tyre import company Havelange (HA). It was also collected in an allotment garden in Eupen (EU) and at the industrial area in Maasmechelen (MM). *Aedes albopictus* was found at seven different PoE's: two used tyre import companies (AB and BA), four parking lots (E0, E2, E5, E6) and the lucky bamboo import company (EB). *Aedes koreicus* was found at the industrial area in Maasmechelen where it is established since 2008 and at the used tyre import company (MB) (Table 6). Findings of EMS are described in detail in Chapter 3.2.

Table 6. Overview of the exotic and new species detected at the different PoE's during the MEMO project.

		Points of Entry															
		AB	AL	AT	BA	E0	E2	E5	E6	EB	EU	HA	MB	MM	PA	RO	SP
Exotic species	*Aedes albopictus*	√			√	√	√	√	√	√							
	Aedes japonicus										√	√		√			
	Aedes koreicus												√	√			
	Anopheles pharoensis		√														
new species	*Anopheles daciae*	√		√	√							√	√	√	√		
	Culex modestus	√															
	Culiseta longiareolata				√									√		√	√

AB=Tyres AtoB or Agrityre; AL=Airport Liège; AT=Tyres ATB; BA=tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E2=Parking lot Hondelange; E5=Parking lot Wanlin; E6=Parking lot Marke; EB=Lucky bamboo Euro Bonsai; EU=allotment garden Eupen (=RA); HA=Tyres Havelange; MB=tyres Maaslandbanden; MM=Industrial area Maasmechelen; PA=Port of Antwerp (Kallo); RO=Cemetery of Rocherath; SP=Tyres Sarri Pneus.

The most widely distributed species were *Cx. pipiens/torrentium* (27 out of 31 PoE's), *Aedes geniculatus* and *Culiseta annulata* (15 out of 31 PoE). PoE's with the highest species richness were MM, AB, MB and EU (Annex 6)**.** However, the number and species that can be detected at a PoE highly depends on the sampling period, sampling method and geographical location of the PoE. The main goal of the monitoring was to intercept exotic mosquito species, especially exotic *Aedes* species and, therefore, sampling strategies were adapted to target these species. However, the variety of collection methods used, allowed the collection of 24 indigenous species/species complexes including the rare species *Culiseta fumipennis* (MM) and *Culex territans* (AB & EU). Six native species *Aedes caspius/dorsalis*, *Aedes cinereus/geminus*, *Aedes communis*, *Aedes detritus, An. atroparvus* and *Coquillettidia richiardii* were only collected as adult.

All PoE's were monitored with OT. These traps specifically intent to capture the container breeding exotic *Aedes* mosquitoes. But, also the indigenous *Ae. geniculatus* can be regularly detected. It is a common tree-hole breeding mosquito, often associated with forested areas. It shows a wide host preference, including humans. To be able to discern between the eggs of this and other exotic *Aedes* species, molecular identification was performed.

Table 7. Overview of the collected specimens per species during the MEMO project. In 2020 no mosquito specimens were collected. The newly detected species for Belgium are indicated in bold. EMS are indicated with colour code: blue = *Aedes japonicus*, red = *Aedes albopictus*, green = *Aedes koreicus* and yellow = *Anopheles pharoensis*.

Species	2017	2018	2019	Total
Culex pipiens/torrentium	3918	19280	11178	34376
Anopheles plumbeus	1335	4498	3561	9394
Aedes japonicus	43	511	1732	2286
Aedes geniculatus	8	675	981	1664
Aedes koreicus	62	783	408	1253
Culex spp.	551	285	15	851
Culiseta annulata	104	328	304	736
Aedes albopictus	0	286	200	486
Aedes spp.	5	41	195	241
Coquillettidia richiardii	1	86	117	204
Aedes vexans	3	67	108	178
Anopheles spp.	83	88	0	171
Anopheles claviger	35	46	26	107
*Anopheles maculipennis s.l.**	3	73	3	79
Anopheles maculipennis	0	39	35	74
Culiseta spp.	31	29	0	60
Culex hortensis	14	20	23	57
Aedes annulipes/cantans	0	6	46	52
Anopheles daciae	**1**	**15**	**28**	**44**
Culiseta longiareolata	**4**	**5**	**27**	**36**
Aedes punctor	0	16	17	33
Culiseta morsitans	0	14	13	27
Aedes sticticus	0	5	14	19
Aedes cinereus/geminus	2	13	1	16
Aedes rusticus	0	5	2	7
Aedes communis	0	6	0	6
Anopheles atroparvus	0	3	3	6
Aedes caspius/dorsalis	0	4	0	4
Culex territans	0	0	3	3
*Culex torrentium***	1	2	0	3
Culiseta fumipennis	0	0	2	2
Aedes detritus	1	0	0	1
Anopheles pharoensis	1	0	0	1
Culex modestus	**0**	**1**	**0**	**1**
Total	6206	27230	19042	52478

**The majority of the specimens collected of the* Anopheles maculipennis *complex were molecularly identified up to species level. **Three adults could morphologically be identified as* Culex torrentium, *other specimens are grouped together with* Culex pipiens s.l.

LS was conducted in PBS at the PoE. These included mainly man-made containers but also some natural water bodies or tree holes. In total 17 different PBS types were identified (Table 8). In 12 PBS types we found larvae

(Table 9). *Culex pipiens/torrentium* larvae were found in all types of PBS (Table 9), as previous studies showed the species is very ubiquitously present in a wide variety of breeding sites [27]. The larvae of *Culiseta morsitans* are rarely found in artificial breeding sites [28], however, three larvae were found in tyres at VP. *Aedes albopictus* was only found in drainage holes during the MEMO project. However, during previous monitoring projects in Belgium larvae of this species were also found in used tyres and lucky bamboo vessels [2].

Table 8. The 17 different potential breeding site (PBS) types sampled during the MEMO project.

PBS type	Description
tyre	outer tyre from car, truck, excavator, airplane or other machines
lucky bamboo vessels	containers made of plastic used to store lucky bamboo plants
metal container	container made of metal, zinc, copper, steel or aluminium such as excavator head, drinking trough, can, waste container
puddle	non-permanent water on the street, in car tracks in mud or in forest
drainage	small catch basins on streets, parking spaces, gardens
natural pond	a small permanent water body with reed or other plants along the border looking natural
artificial pond	a small permanent man-made water body with plastic, wooden or stone border not looking natural
plastic sheet	from a large tarp covering materials until plastic foil as waste on the ground
plastic container	container made of hard plastic, such as a bucket, plastic waste (e.g. drinking bottles), plant pot, curver box, drinking trough
cemetery flower vases	vases for flowers in plastic, stone or metal at a cemetery
underground water reserve	large catch basins, waste water basin
ditch	man-made open irrigation/drainage channel in earth, sometimes with concrete walls, often looking natural
stone container	container made of stone, such as flower or plant pot, flower or plant pot dish, construction material, open water reservoir
gutter	man-made open drainage channel made in plastic, stone or metal on ground level or higher
tree hole	hole with standing water at ground level or higher in tree
glass container	container made of glass, such as bottles, drinking glasses
polystyrene container	container made of polystyrene, such as food boxes, packaging material

Table 9. The potential breeding sites (PBS) types that were positive for the different mosquito species that were collected during the MEMO project.

Species	**type of PBS**											
	artificial pond	**cemetery flower vases**	**ditch**	**drainage**	**metal container**	**natural pond**	**plastic container**	**plastic sheet**	**puddle**	**stone container**	**tyre**	**underground water reservoir**
Ae. albopictus				√								
Ae. annulipes/cantans			√			√						
Ae. geniculatus									√		√	
Ae. japonicus				√			√	√	√		√	
Ae. koreicus					√		√	√		√	√	
Ae. rusticus						√						
An. claviger					√						√	
An. daciae					√	√						
An. maculipennis			√		√		√	√			√	
An. maculipennis s.l.	√				√	√	√			√	√	
An. plumbeus								√			√	
Cx. hortensis					√		√				√	
Cx. modestus						√						
Cx. pipiens/torrentium	√	√	√	√	√	√	√	√	√	√	√	√
Cx. territans							√					
Cs. annulata	√		√	√	√	√	√		√		√	
Cs. longiareolata				√			√				√	
Cs. morsitans						√					√	

The observed occurrences of mosquito species collected as adult are summarized in Figure 14. *Culex pipiens/torrentium* was found during the entire monitoring period and abundances peaked in July-August and at the end of the summer (September-October) (

Figure 15). This is similar to what was found during previous monitoring projects in Belgium. However, the number of adult *Cx. pipiens/torrentium* specimens collected varied highly between PoE's and between monitoring years (

Figure 15).

Figure 14. Observed seasonal occurrence of collected adult mosquito species during the MEMO project (data of 2017 until 2019 combined). The blue bar indicates the period during which a species was found.

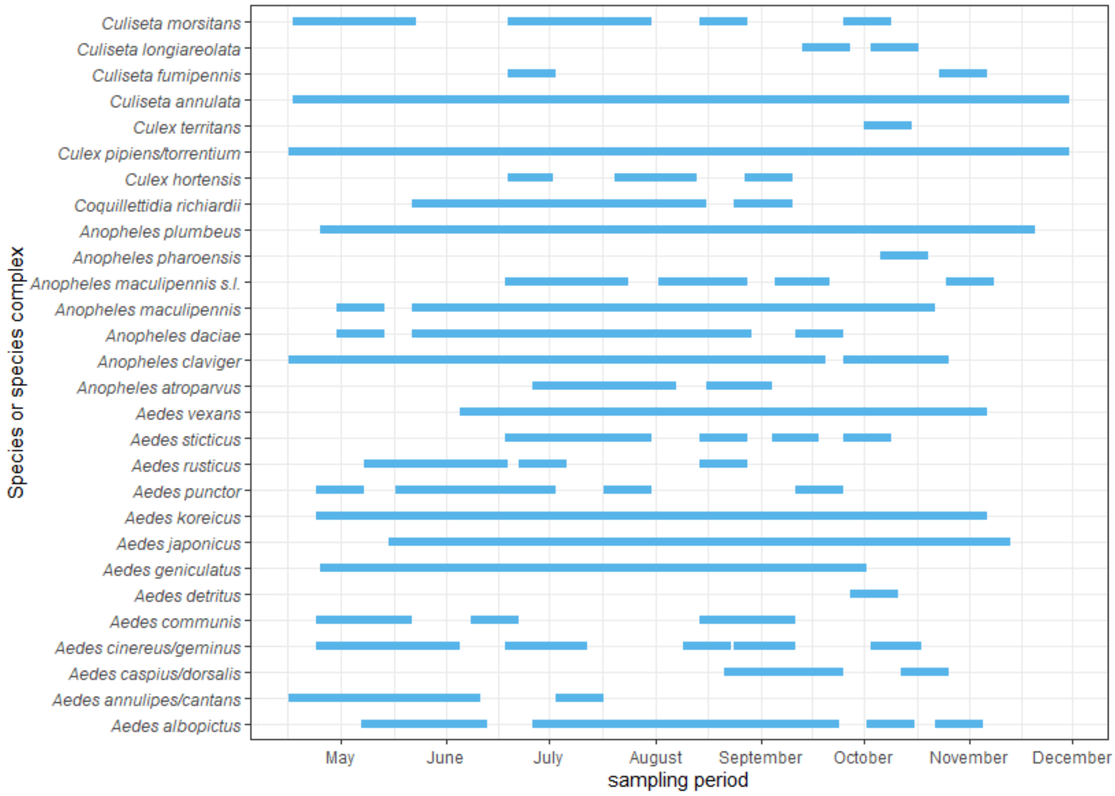
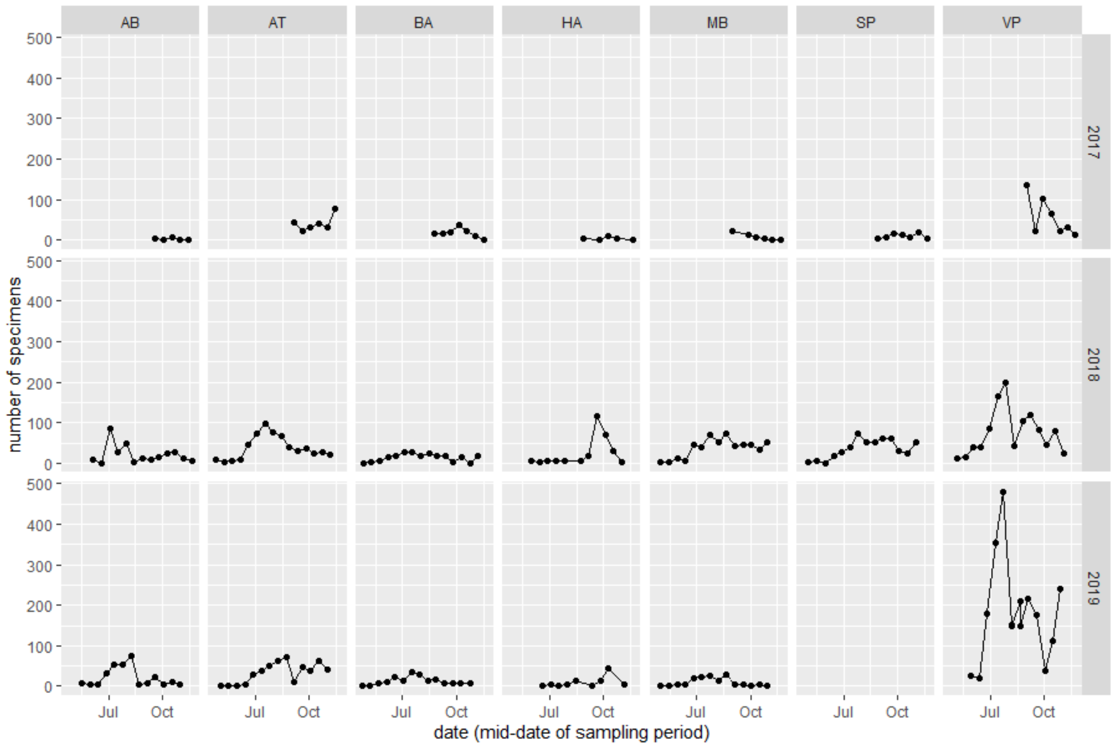


Figure 15. The monthly number of adult *Culex pipiens/torrentium* specimens collected with the BG-Sentinel and Mosquito Magnet® trap at the seven used tyre import companies in 2017, 2018 and 2019.



AB=Tyres AtoB or Agrityre; AT=Tyres ATB; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; HA=Tyres Havelange; MB=Tyres Maaslandbanden; SP=Tyres Sarri Pneus; VP=Tyres Visé Pneu.

3.1.2 New species for Belgium and species complexes

Culex modestus

One larva of *Cx. modestus*, which has been morphologically and genetically confirmed, was detected mid-September 2018 in a natural pond near the used tyre import company AB. The pond contained reed vegetation and was almost completely dried out at the time of the collection. In addition, larvae of *Cs. annulata* (n=1), *An. maculipennis s.l*. (n=1) and *Cx. pipiens/torrentium* (n=4) were found in the same pond. The pond is part of a corridor network of multiple breeding sites for endangered species (e.g. the Natterjack toad, *Epidalea calamita*) that occur in the port area. The port area surrounding AB is rich in wetlands and brackish marshes, which is the preferred habitat of *Cx. modestus*. The species is widely distributed in the Paleartic region and is considered as a common species in Southern and Central Europe. The larvae inhabit fresh to slightly saline water and are mainly found in ground pools, ponds, swamps, rice fields and marshes [18]. The species is often associated with wetland reed beds and in Southern Europe they are abundant in wetlands with increased salinity (e.g. Camargue wetlands) [29, 30]. *Culex modestus* is one of the main vectors for West Nile virus (WNV) in Europe [31, 32]. It is an ornithophilic species but regularly feeds upon humans and other mammals.

The port area surrounding AB is rich in wetlands and is developed to host both migratory and resident bird populations. The increasing restoration and protection of wetlands near urbanized areas (such as ports) could create a suitable habitat for this vector species [27]. *Culex modestus* could be present at other wetland areas in Belgium, but these breeding sites are not routinely sampled for the presence of mosquito larvae. However, the species was not collected in Flanders during the water quality monitoring between 1997 and 2009 [27], nor during the MODIRISK project [33]. In Southern England the species was detected for the first time after an extensive search of marsh areas in 2010 [34].

Culiseta longiareolata

Culiseta longiareolata was detected at four different PoE's (Table 10) and has been morphologically and genetically confirmed. The species was detected for the first time in Belgium in 2017 during LS activities to evaluate the possible spread of *Ae. koreicus* at the industrial area at Maasmechelen (MM). It was also detected at two used tyre companies (BA and SP) and near a cemetery in Rocherath (RO). Larvae were collected in small plastic containers, used tyres and drainage holes. Adult specimens were collected with the BG, which was positioned next to the MMT.

Culiseta longiareolata is widely established in the Mediterranean region and is considered as a thermophilic species [18, 35]. Recently, the species was also detected in the Netherlands (A. Ibáñez-Justicia, personal communication), Germany, Austria, Slovenia and Luxembourg [35-38]. It is a container breeding species, which rarely occurs in natural water bodies and can tolerate a high degree of pollution [18]. Larvae of this species have been reported at used tyre companies in other European countries [28, 39]. In Luxembourg, larvae were found in similar breeding sites as in Belgium [36]. However, as this species is ornithophagous and rarely bites humans [18], it probably remained undetected with the most commonly used monitoring methods. It is considered as a vector of parasites among birds [18]. The species is not known to transmit pathogens to humans nor is considered as a nuisance. Although the species is widely distributed across Europe, it is hardly present in online DNA reference databases. Therefore, we produced multiple barcodes (COI, COII and ND4) from the collected specimens. In Belgium, we could not reconfirm the species at the same locations. But, the presence at geographically distinct locations and during multiple years suggest that the species is probably more common than detected so far.

Table 10. The collection period, number of specimens, life stages and trapping method (number of containers and potential breeding site type or attractants between brackets) for *Culiseta longiareolata* per PoE where it was collected.

PoE	Collection period	Number of specimens and life stages	Trapping method
MM	5/09/2017	4 larvae	LS (2 plastic containers)
BA	3/10/2018-17/10/2018	1 male	BG (BG-lure + CO_2 from MMT*)
SP	13/09/2018-29/09/2018	1 female	BG (BG-lure + CO_2 from MMT*)
	30/08/2018	3 larvae	LS (87 tyres)
RO	5/09/2019	27 larvae	LS (3 drainage holes)

LS=Larval sampling; BG=BG-Sentinel trap; MMT=Mosquito Magnet® trap. MM=Industrial area Maasmechelen; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; SP=Tyres Sarri Pneus; RO=Cemetery Rocherath.

Anopheles maculipennis complex

Three species of the *An. maculipennis* complex have been reported from Belgium in the past, i.e. *An. messeae*, *An. maculipennis* s.s. and *An. atroparvus* [21]. Of these, *An. atroparvus* has not been observed in Belgium for years. This is possibly linked to surface water pollution [40] and/or to the loss of suitable resting sites for hibernation [41]. Traditionally, species of this complex were diagnosed by egg morphology, which is considered not reliable [18]. Since 1999, molecular analysis of the ITS2 locus is commonly used for species identification [42]. During the molecular validation in 2018, two specimens from the *An. maculipennis* species complex were processed by DNA-based identification. One ITS2 BLAST result returned as *An. daciae*, a species within the complex which was described in 2004 from Romania [25], and which was not known to occur in Belgium. Apart from the morphology, the species description was based on five discriminative single nucleotide polymorphisms (SNPs) in the ITS2 locus [25]. Since its formal description, *An. daciae* was found to occur in Germany [43-45], Greece [46], Poland [47], Serbia [48], Iran [49], Italy [50], Turkey [51] and England [26, 52], indicating that *An. daciae* is distributed over wide parts of Europe.

Following this first detection of *An. daciae* in Belgium, the *An. maculipennis* complex was further investigated. Specimens collected during the MEMO project and morphologically assigned to the species of the complex were shipped to BopCo for molecular analysis. This concerned one specimen collected in 2017, 47 specimens in 2018 and 67 specimens in 2019 (n_{tot}=115). Subsequent examination of these specimens, which were previously identified as *An. maculipennis* s.s. and *An. messeae*, revealed the presence of *An. atroparvus* (n = 6, three collected in 2018, and three in 2019, all at location AB). This species occurs in coastal areas or at inland breeding sites with an elevated salinity [44] and is known to be a major malaria vector in Europe [53]. The distinction between the different species in the complex is therefore of high relevance [44].

For the identification of *An. daciae* the suitability of the Restriction Fragment Length Polymorphism (RFLP) method [25, 26] using the enzymes HhaI (GCG↓C) and Bsh1236I (CG↓CG) was tested, as it is less expensive than Sanger sequencing. RFLP allows to identify species based on unique cutting patterns of restriction enzymes in specific regions of the DNA (Figure 16). Since HhaI is not able to discriminate between *An. daciae* and *An. messeae*, while Bsh1236I cannot distinguish *An. messeae* and *An. maculipennis*, both RFLP reactions should be used in combination in order to discriminate between the four species of the complex. Since the detection of *An. daciae* in 2018, the molecular pipeline for the identification of *An. maculipennis* s.l. members (Figure 10) has been adapted and all *An. maculipennis* s.l. re-identified. The results indicated that all specimens previously thought to belong to *An. messeae* turned out to be *An. daciae* (Table 11).

Figure 16. Picture of the 3% agarose gel RFLP patterns obtained after HhaI and Bsh1236I enzyme digestions of ITS2 amplicons.

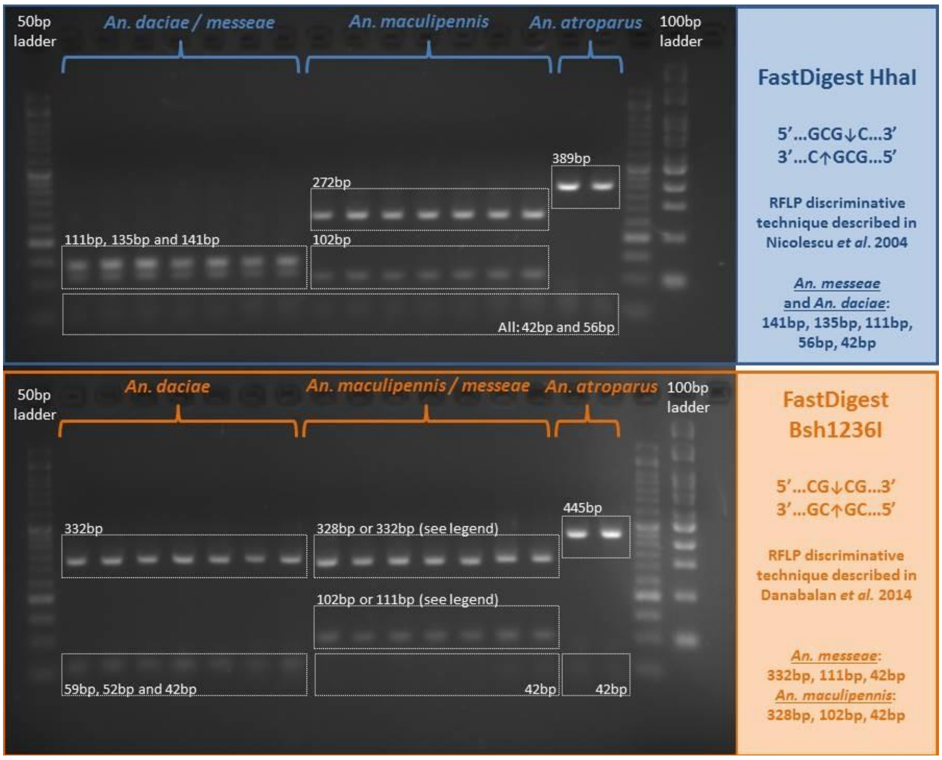


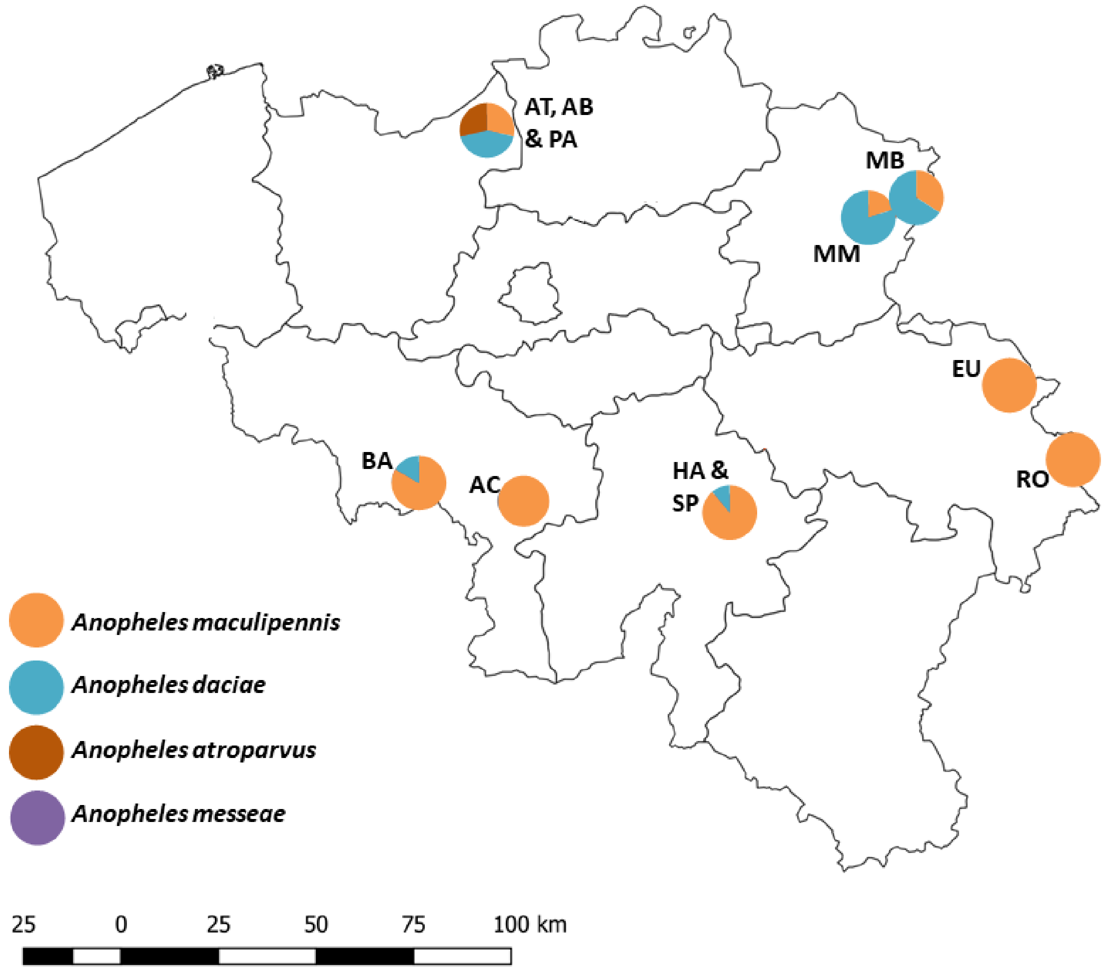
Table 11. The molecular identification results for the specimens of the *Anopheles maculipennis* species complex caught in Belgium for each of the PoE's where the complex was found (as indicated in Figure 17).

Geographical location	*An. maculipennis*	*An. daciae*	*An. messeae*	*An. atroparvus*	Total
Natoye (HA & SP)	8	1	0	0	9
Frameries (BA)	5	1	0	0	6
Rocherath (RO)	4	0	0	0	4
Eupen (EU)	25	0	0	0	25
Maasmechelen (MM)	1	4	0	0	5
Dilsen-Stokkem (MB)	15	29	0	0	44
Vrasene and Kallo (AT, AB & PA)	6	9	0	6	21
Charleroi (AC)	1	0	0	0	1
Total	**65**	**44**	**0**	**6**	**115**

AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AT=Tyres ATB; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; EU=Allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MM=Industrial area Maasmechelen; PA=Port Antwerp (Kallo); RO=Cemetery Rocherath; SP=Tyres Sarri Pneus.

Anopheles maculipennis s.s. and *An. daciae* were found at several PoEs, whereas *An. atroparvus* was only found at one PoE (Figure 17). A study in Serbia found that *An. maculipennis* s.s. larvae were recorded predominantly in manmade breeding sites [48]. In fact, the species was mainly found in water with higher quantity of ammonia and mud, which is in accordance with its findings in predominantly manmade containers. *An. messeae/daciae* might, however, have a preference for clean water [45, 48]. Therefore, a bias in the sampling might have resulted in the collection of more *An. maculipennis* s.s. specimens during MEMO, since manmade breeding sites were particularly targeted for the detection of EMS.

Figure 17. Map of Belgium showing PoE locations where specimens of the *Anopheles maculipennis s.l.* species complex were captured. The pie charts represent the proportion of the specimens of each species collected at the PoE.



AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AT=Tyres ATB; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; EU=Allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MM=Industrial area Maasmechelen; PA=Port Antwerp (Kallo); RO=Cemetery Rocherath; SP=Tyres Sarri Pneus.

Culex pipiens/torrentium

Reliable identification of *Cx. pipiens s.s.* (the Northern house mosquito), *Cx. quinquefasciatus* (the Southern house mosquito) and sibling species *Cx. torrentium* based on morphological characteristics is difficult [18]. According to previous monitoring surveys, *Cx. pipiens* is the most abundant mosquito species in Belgium [54], followed by *Cx. torrentium* and *An. plumbeus* [55]. In Europe, *Cx. pipiens* and *Cx. torrentium* are considered main vectors of the WNV [56, 57]. Within *Cx. pipiens*, two biotypes can be distinguished without morphological distinction: biotype *pipiens* and biotype *molestus* [58]. The biotypes of *Cx. pipiens* were described based on differences in behavioural traits. Autogeny (first oviposition without a prior blood meal), stenogamy (ability to mate and breed in confined spaces), not overwintering in diapause and anthropophily (preference of biting human hosts) differentiates the biotype *molestus* from *pipiens*. The biotype *pipiens* is mainly ornithophilic (preference of biting bird hosts), anautogenous and eurygamous [18, 58]. The distinct behaviour and physiology may influence their vector status. Additionally, due to incomplete reproductive isolation, hybrids between biotypes have been observed, displaying intermediate behavioural traits. However, this 'new' opportunistic biting behaviour could pose a threat as a bridge vector between birds and mammals [59]. Therefore, the occurrence *of Cx. torrentium*, *Cx. pipiens* (and its biotypes and hybrids) in Belgium using DNA-based techniques is of importance. The microsatellite locus CQ11 is currently used for molecular discrimination of the two biotypes [23]. Hybrids of *Cx. pipiens pipiens* and *Cx. pipiens molestus* are characterized by amplification of molecular fragments of both biotypes. Following the established molecular identification pipeline (Figure 10), *Cx. pipiens* and *Cx. torrentium* can be distinguished based on the investigation of the genetic variations in the COI locus.

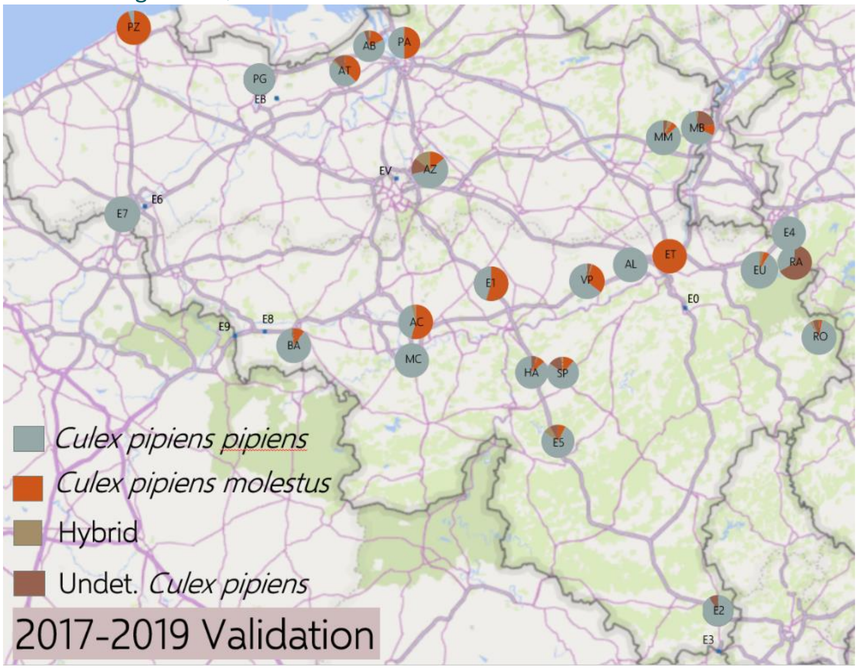
During the three years of the MEMO project, 1694 specimens were processed of which 403 (23.9%) were assigned to *Cx. torrentium* (Table 12). Within *Cx. pipiens*, the biotype *pipiens* was clearly predominant (74.2%), while 30 specimens (0.02%) were identified as hybrids. The geographic distributions of the different species (Figure 18 A) and biotypes (Figure 18 B) are displayed on the map of Belgium.

Table 12. The molecular identification results for the specimens morphologically identified as *Culex pipiens/torrentium*, and their absolute number over the different years (validation steps).

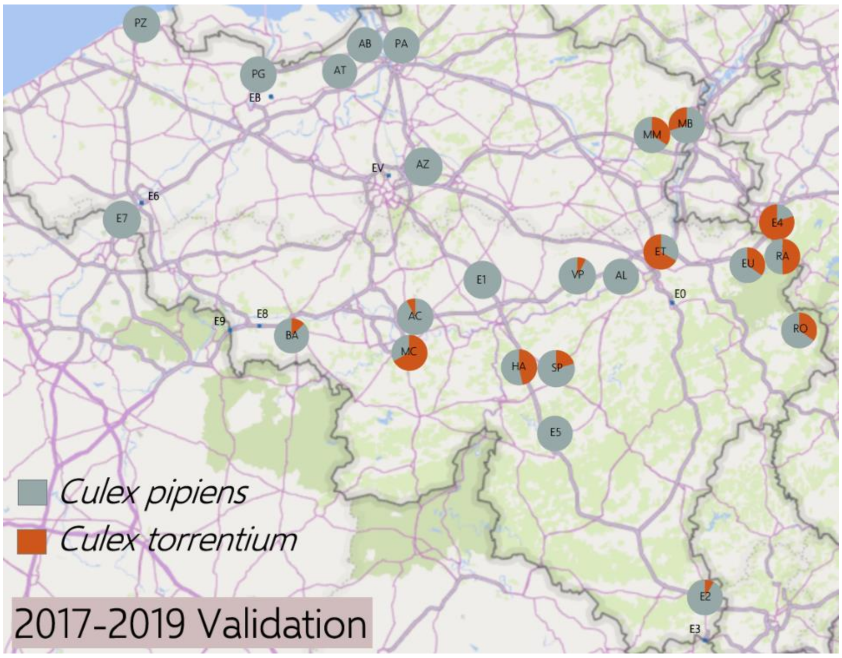
Species/biotype	2017 validation		2018 validation		2019 validation		Total	
	Absolute number	%	Absolute number	%	Absolute number	%	Absolute number	%
Culex pipiens* f. *molestus	47	**24,4**	102	**10,6**	79	**14,6**	228	**13,5**
Culex pipiens* f. *pipiens	74	**38,3**	530	**55,3**	352	**64,9**	956	**56,4**
***Culex pipiens* hybrid between biotypes**	4	**2,1**	16	**1,7**	10	**1,8**	30	**1,8**
***Culex pipiens* undetermined biotype**	21	**10,9**	38	**4**	8	**1,5**	67	**4**
***Culex pipiens* s.l.**	8	**4,1**	0	**0**	0	**0**	8	**0,5**
Culex torrentium	39	**20,2**	273	**28,5**	93	**17,2**	405	**23,9**
Total	193	**100**	959	**100**	542	**100**	1694	**100**

Until now, for nine *Cx. pipiens s.s.* individuals originating from five different locations in Belgium (Frameries (BA), Maasmechelen (MM), Kallo (AB), Vrasene (AT), and Zeebrugge (PZ)), full genome sequencing data has been generated within the ongoing *Cx. pipiens* population genomic project (PipPop, http://mcbridelab.princeton.edu/wp-content/uploads/2018/08/PipPopSampleRequest-V2.pdf). Researchers of the McBride lab at the University of Princeton are analysing populations across the global distribution range to study the evolutionary history of this important disease vector and its biotypes. In the future extra genomic data and results on the Belgian populations are expected, which will be subject for publication.

Figure 18. Map of Belgium showing PoE locations where specimens of A. *Culex pipiens* and *Culex torrentium*, and B. the different biotypes of *Cx. pipiens*, were captured. The pie charts represent the proportion of the specimens identified by A. COI DNA barcoding and B. screening the CQ11 locus.



A.



B.

3.2 Focus on EMS detections

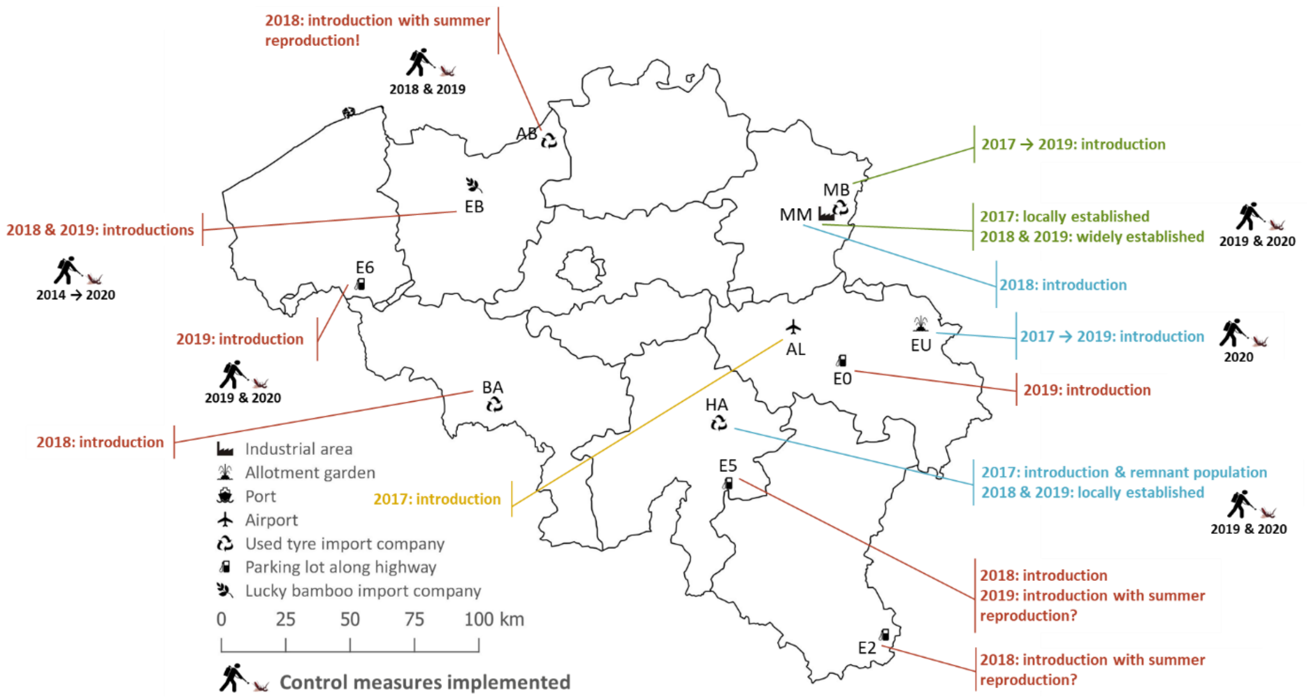
We found 4025 specimens belonging to four EMS: *Ae. albopictus* (n =486), *Ae. japonicus* (n =2285), *Ae. koreicus* (n =1253) and *An. pharoensis* (n=1). *Aedes albopictus* was collected at the tyre companies AB and BA, the lucky bamboo company EB and the parking lots Hondelange (E2), Wanlin (E5), Sprimont (E0) and Marke (E6); *Ae. japonicus* at the tyre company Havelange (HA), the allotment garden near Eupen (EU) and the industrial area 'Op de Berg' in Maasmechelen (MM); *Ae. koreicus* at the industrial area 'Op de Berg' of Maasmechelen (MM) and the tyre company Maaslandbanden (MB); and *An. pharoensis* at the cargo airport in Liège (AL) (Table 13 & Figure 19).

Table 13. Overview of the number of exotic mosquito species collected during the monitoring activities between 2017 and 2020. Results are presented per life stage and species (scenario 1, 2 and 3 monitoring included; red=*Aedes albopictus*, blue=*Aedes japonicus*, green=*Aedes koreicus*, yellow=*Anopheles pharoensis*).

	Ae. albopictus			*Ae. japonicus*			*Ae. koreicus*			*An. pharoensis*	Total
PoE	adult	larva	egg	adult	larva	egg	adult	larva	egg	female	EMS
AB	28	10	35	0	0	0	0	0	0	0	73
BA	1	0	0	0	0	0	0	0	0	0	1
E0	0	0	69	0	0	0	0	0	0	0	69
E2	0	0	151	0	0	0	0	0	0	0	151
E5	0	3	116	0	0	0	0	0	0	0	119
E6	0	0	64	0	0	0	0	0	0	0	64
EB	9	0	0	0	0	0	0	0	0	0	9
EU	0	0	0	5	24	199	0	0	0	0	228
HA	0	0	0	455	353	1199	0	0	0	0	2007
MB	0	0	0	0	0	0	7	0	0	0	7
MM	0	0	0	50	0	0	481	436	329	0	1296
AL	0	0	0	0	0	0	0	0	0	1	1
Total	**38**	**13**	**435**	**510**	**377**	**1398**	**488**	**436**	**329**	**1**	**4025**

PoE: AB=Tyres AtoB or Agrityre; AL=Airport Liège; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E2=Parking lot Hondelange; E5=Parking lot Wanlin; E6=Parking lot Marke; EB=Lucky bamboo Euro Bonsai; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MM=Industrial area Maasmechelen; EU=Allotment garden Eupen.

Figure 19. Overview of the exotic mosquito species detections between 2017 and 2020 in Belgium with locations of the positive points of entry (PoE), indication of the invasion status per year and of implemented control measures. (color code: red = *Aedes albopictus*, blue = *Aedes japonicus*, green = *Aedes koreicus*, yellow = *Anopheles pharoensis*)



PoE: AB=Tyres AtoB or Agrityre; AL=Airport Liège; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E2=Parking lot Hondelange; E5=Parking lot Wanlin; E6=Parking lot Marke; EB=Lucky bamboo Euro Bonsai; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MM=Industrial area Maasmechelen; EU=Allotment garden Eupen.

3.2.1 *Aedes albopictus*

During the MEMO project, *Ae albopictus* was introduced in Belgium between 2017 and 2019 through three import pathways: used tyre trade, lucky bamboo trade and the passive ground traffic. In 2018, *Ae. albopictus* was detected at two used tyre import companies (AB and BA), the lucky bamboo import company (EB) and two parking lots (E2 and E5). In 2019, the species was detected at EB and at three parking lots (E0, E5 and E6).

Used tyre import company Agrityre and AtoB (AB)

At the used tyre import company AB, two adult traps (one MMT and one BG) were set-up outdoors in the vicinity where containers with imported used tyres were unloaded. The collection of eggs (10 OT) was performed both inside and outside the hangar with used tyres, while LS was performed only outside. At this PoE monitoring was performed from the beginning of September until mid-November 2017, from mid-April until the end of November 2018 and from mid-April until the beginning of November 2019.

In total 73 *Ae. albopictus* specimens, including 26 females, two males, 10 larvae and 35 eggs**,** were collected at AB during the MEMO project, all in 2018 (Table 14 & Figure 20). At the premises of the tyre company AB, 24 females, two males and 16 eggs were collected. The first detection and most other detections at AB were done with the BG. It is remarkable that no larvae were found in the used tyres stored outside. The fact that a lot of tyres were dry during the summer and that the turnover of tyres was high at AB, which probably explains this absence. Upon the first findings, a basic scenario 2a (new introduction) monitoring was implemented in a 200 m buffer zone around the location of first detection in the beginning of August 2018. This resulted in the detection of two females, 10 larvae and 19 eggs in this buffer zone. Of the 10 larvae, two were collected in drainage holes during LS and eight in OT. Mid-September monitoring activities were also conducted in a 500 m buffer zone (extended scenario 2a monitoring). But, no additional specimens were found. The repeated findings of all life stages (eggs, larvae, adults) and overlapping sampling periods indicated local reproduction at the site in 2018 (Figure 20). Repeated control measurements (including both larviciding and adulticiding) have been implemented in 2018 and 2019 to prevent establishment and future spread of the species. After mid-September 2018 the species was no longer detected. Also during the scenario 2a (new introduction) monitoring activities performed in 2019 at both the used tyre company and the 200 m buffer zone no evidence of local establishment was found.

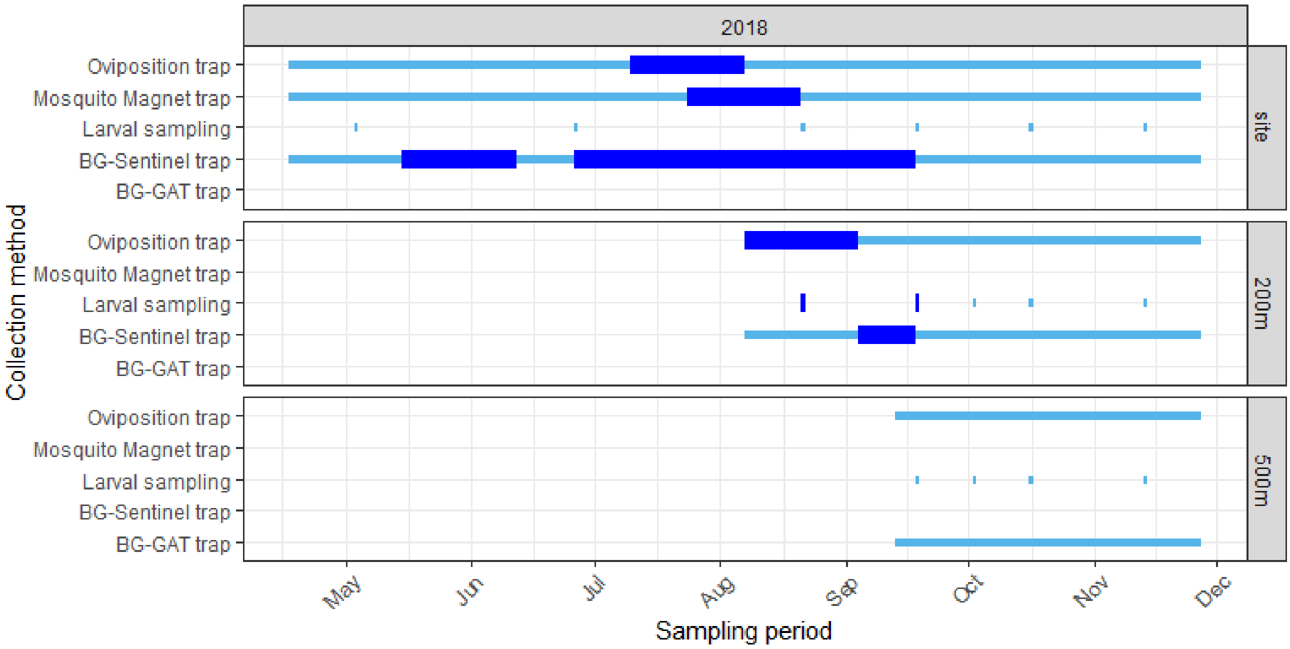
Table 14. Overview of the collected *Aedes albopictus* specimens per location, date and collection method at the used tyre import company Agrityre/AtoB (AB) in 2018 during the MEMO project.

Year	Location	Start date	End date	Trap	Number of collected *Ae. albopictus*
2018	tyre company	15 May	29 May	BG	1M
		29 May	12 June	BG	2F
		26 June	10 July	BG	2F
		10 July	24 July	BG	4F
		10 July	7 August	OT	16E
		24 July	7 August	BG & MMT	9F (6 in BG, 3 in MMT) & 1M
		7 August	21 August	BG & MMT	4F (2 in BG, 2 in MMT)
		21 August	4 September	BG	2F
		4 September	18 September	BG	1F
	200 m buffer zone	7 August	21 August	OT	19E & 7L
		21 August	21 August	LS	1L
		21 August	4 September	OT	1L
		4 September	18 September	BG (2 separate BG)	2F
		18 September	18 September	LS	1L

BG=BG-Sentinel trap, MMT=Mosquito Magnet® trap, OT=Oviposition trap, LS=Larval sampling, M=male, F=female, L=larva, E=egg.

Agrityre and AtoB (AB) are tyre wholesalers in aircraft tyres (ground use), used tyres, retreated tyres and various new custom-made agricultural tyres. It is located in the port of Antwerp (Kallo) and trades tyres from all over the world, mainly from Europe, the Middle East (Israel, Turkey), Russia and Africa. From AB, tyres are transported to the PoE ATB Banden (AT), the used tyre import company in Vrasene, where *Ae. albopictus* was found in 2000, 2013 and 2016 [2, 60, 61]. There is also a lot of traffic between AB and the PoE Bridgestone Aircraft Tire (Europe) s.a. (BA), where *Ae. albopictus* was found in 2016 [60] and 2018. These three companies have very similar activities that facilitate import of mosquitoes. Mosquitoes can even potentially be transported among them. In addition, AB is linked with a used tyre import company in the French department of Aisne in the Hauts-de-France region in northern France, where *Ae. albopictus* is established (notification of France to Belgium on 29 September 2017). The high number of trucks and freight trains arriving and departing at or close to AB increase the risk for spread of this exotic mosquito species. Therefore, although control measures seemed successful and no *Ae. albopictus* specimens were collected in 2019, it will be important to continue the monitoring and subsequent control at AB in the future.

Figure 20. The time frame during which *Aedes albopictus* was collected per collection method and per location at and around the used tyre import company AB in 2018 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. albopictus*).



Used tyre import company Bridgestone Aircraft Tire (Europe) s.a. (BA)

At the used tyre import company BA, two adult traps (one MMT and one BG) were set-up outside close to the area where containers with imported used tyres were unloaded. The collection of eggs (10 OT) and LS was performed outside the hangar with used tyres. At this PoE, monitoring was performed from the beginning of September until the beginning of November 2017, from mid-April until the end of October 2018 and from mid-April until the beginning of November 2019.

A single female *Ae. albopictus* was collected at the used tyre import company BA during the MEMO project. It was collected with the MMT between the 30th of May and the 13th of June. In the beginning of August 2018 a basic scenario 2a (new introduction) monitoring was implemented in a 200 m buffer zone around the location of the detection. No control measures were implemented by the Walloon authorities (SPW). However, neither on the tyre company nor in the surroundings evidence of additional specimens were found. Therefore, the PoE regained its scenario 1 status in 2019.

Bridgestone Aircraft Tire (Europe) s.a. (BA) is an aircraft tyre retreading plant in Frameries in the province of Hainaut. BA trades tyres from all over the world, including Africa, Western Asia (Middle East (Turkey, Israel)), Russia and Europe. *Aedes albopictus* was found on the BA site for the first time in 2016, the year in which exotic mosquito monitoring at this PoE started (during the FASFC project) [60]. In June and August 2016, a total of three females and eight eggs were collected at the company site and in the surrounding area. Neither mechanical nor chemical control measures were implemented either by the company nor by the Walloon authorities (SPW) in 2016. In 2017, however, the BA site was cleaned by the owner. Used tyres are not stored outside anymore, which considerably decreases the number of breeding containers and, consequently, also the risk of establishment of *Ae. albopictus*. Introductions of *Ae. albopictus* at BA are still possible as the company frequently trades with the used tyre import company AB, where this mosquito species was detected in 2018. It is well possible that the single female collected at BA arrived with a container coming from AB. Although no *Ae. albopictus* specimens were collected in 2019, it will be important to continue the monitoring at BA in the future.

Lucky bamboo import company Euro Bonsai (EB)

At the lucky bamboo company EB the adult traps (one BG and one MMT) were located inside the nursery next to the arrival of lucky bamboo shipments. The collection of eggs (10 OT) and larvae was performed both inside and outside the nursery. At this PoE monitoring was performed continuously from the beginning of September 2017 until the beginning of March 2020.

In total nine *Ae. albopictus* adults, including five females and four males, were collected at the lucky bamboo import company EB during the MEMO project (Table 15 & Figure 21). In 2018, three males and two females were collected during four distinct sampling events. On 8 October 2018, LS was also performed in the broad vicinity of the plant nursery (approximately in a 500-700 m radius), but only larvae of *Cx. pipiens/torrentium* were collected. In 2019, two consecutive adult samplings were positive in August, respectively one male and two females were collected. In addition, one female was collected in the beginning of October 2019. The BG, located next to the MMT, collected most *Ae. albopictus* specimens and both males and females.

Table 15. Overview of the collected *Aedes albopictus* specimens per year, date and collection method at the lucky bamboo import company Euro Bonsai (EB) during the MEMO project.

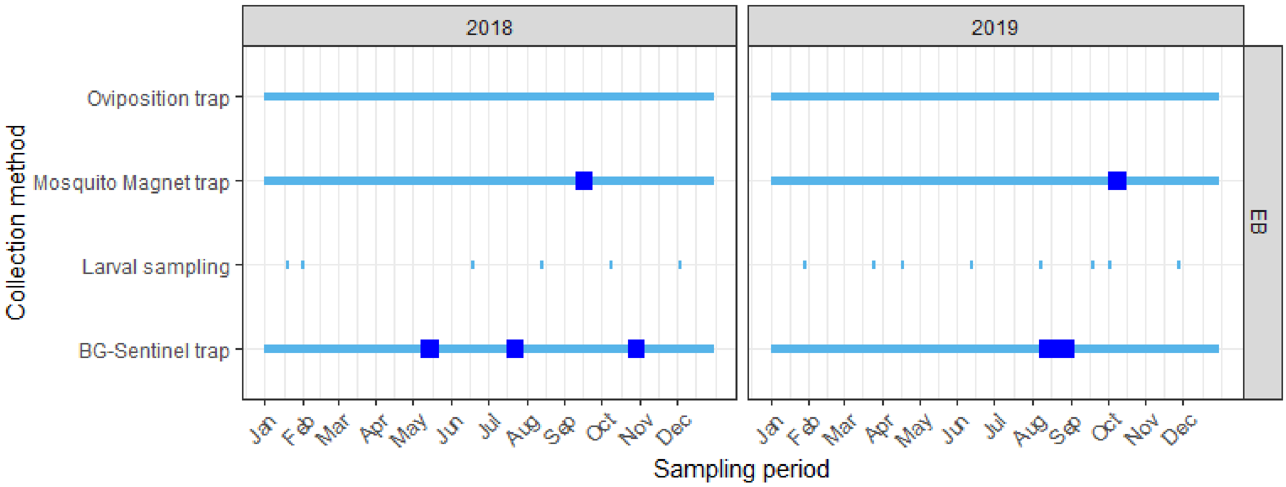
Year	Start date	End date	Trap	Number of collected *Ae. albopictus* adults
2018	7 May	23 May	BG	2M
	16 July	30 July	BG	1M
	10 September	24 September	MMT	1F
	22 October	5 November	BG	1F
2019	7 August	21 August	BG	1M
	21 August	4 September	BG	2F
	2 October	16 October	MMT	1F

BG=BG-Sentinel trap, MMT=Mosquito Magnet® trap, M=Male, F=Female.

Control measurements were frequently applied in the nursery between 2017 and 2020 to prevent reproduction and possible establishment of *Ae. albopictus* in the nursery. It is possible that the adult mosquitoes hatched during transport and were intercepted upon opening of the containers in the nursery. Another option is that the imported eggs or larvae developed in the nursery after placing the lucky bamboo plants in fresh water. During each LS visit, the lucky bamboo vessels were inspected for the presence of mosquito larvae, but none were collected. The fact that no developmental stages (larvae or eggs) and only a limited number of adults in distinct sampling periods were collected, indicates that the observations of *Ae. albopictus* were independent introductions at the nursery. During previous monitoring projects in Belgium *Ae. albopictus* larvae and eggs were detected at this company [2]. However, at that time control measures were not yet preventively implemented and often after a first detection only, probably allowing summer reproduction.

The company Euro Bonsai (EB) is a wholesaler in bonsai and lucky bamboo located in Lochristi, East Flanders. Since 2012, EMS have been monitored at this company [17, 60, 62-64]. In 2013 and 2014, *Ae. albopictus* was intercepted in the port of Antwerp where the shipments with lucky bamboo, destined for the lucky bamboo import company EB, were inspected at the border inspection post of the FASFC [2, 62, 63, 65]. *Aedes albopictus* was found at EB in 2014, 2015 and 2016 [60, 63, 64] and again in 2018 and 2019.

Figure 21. The time frame during which *Aedes albopictus* was collected per collection method and per year at the lucky bamboo import company EB in 2018 and 2019 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. albopictus*).



Since 2014, annual control measures have been implemented at EB, both mechanical and chemical. At the beginning of 2017, the Flemish authorities (ANB) prepared a control plan and an exemption for one year was obtained for the use of VectoBac®. It was decided to treat preventively at the lucky bamboo company EB and the used tyre import company AT. In 2017, PBS at EB were treated with VectoBac® on 29 March and 20 April. On 15 February 2018 another exemption on the biocide regulation of up to 550 days was obtained for the use of the biocides VectoMax G® and Aqua-K-Othrine® in Belgium. In September 2018 an authorisation for eight year was given to use an alternative larvicide AquaBac® 200 g, as this product is already registered on the Belgian market in contrast to VectoBac® and VectoMax G®. Since January 2019 only AquaBac® was used as mosquito larvicide at EB. The preventive control measures in 2017, 2018 and 2019 seemed to prevent summer reproduction at EB. It will be important to continue this preventive control at EB during the mosquito season (April – October) to avoid the establishment of *Ae. albopictus* in the surrounding area.

Main parking lots along highway (E0, E2, E5, E6)

At all parking lots, monitoring was executed with 10 OT. In 2017 and in the beginning of the season in 2018 LS was performed every eight weeks. Since July 2018 it was only implemented upon the finding of an EMS or to check potential overwintering or early occurrence during the next monitoring season at the same location. Trap locations remained constant over the monitoring years. The scenario 2a (new introduction) monitoring was adapted and included extra LS and the inclusion of the next main parking lot along the highway.

At the **parking lot Hondelange (E2)** monitoring was performed from the end of August until mid-November 2017, from the beginning of May until the beginning of December 2018 and from mid-April until mid-November 2019. In total 151 *Ae. albopictus* eggs were detected at the parking lot E2 in 2018 during the MEMO project

(Figure 22 & Table 16). These eggs were collected during four consecutive sampling periods (June, July, August and September 2018) within four different OT. The fact that the same OT was positive for *Ae. albopictus* for three consecutive times and that at the end of August, three additional OT were positive indicates that summer reproduction could have occurred at E2. However, no larvae were found. LS was performed in May, August and October 2018. Drainage holes, small containers (plastic, metal and stone), an artificial pond and an underground water reservoir were inspected. Only 74 *Cx. pipiens/torrentium* larvae were collected. After mid-September, no eggs were collected anymore. During the monitoring activities performed in 2019 no evidence of establishment nor of new introductions was found.

Table 16. Overview of the collected *Aedes albopictus* specimens per year, parking lot, date and collection method during the MEMO project.

Year	PoE	Start date	End date	Trap	Number of collected *Ae. albopictus* specimens
2018	**E2**	28 May	25 June	OT	62E
		25 June	23 July	OT	62E
		23 July	20 August	OT	2E
		20 August	17 September	OT	25E
	E5	31 July	20 August	OT	56E
2019	**E0**	5 September	3 October	OT	69E
	E5	4 July	1 August	OT	2E
		1 August	29 August	OT	40E
		29 August	3 September	OT	18E
		3 September	3 September	LS	3L
	E6	4 September	2 October	OT	64E

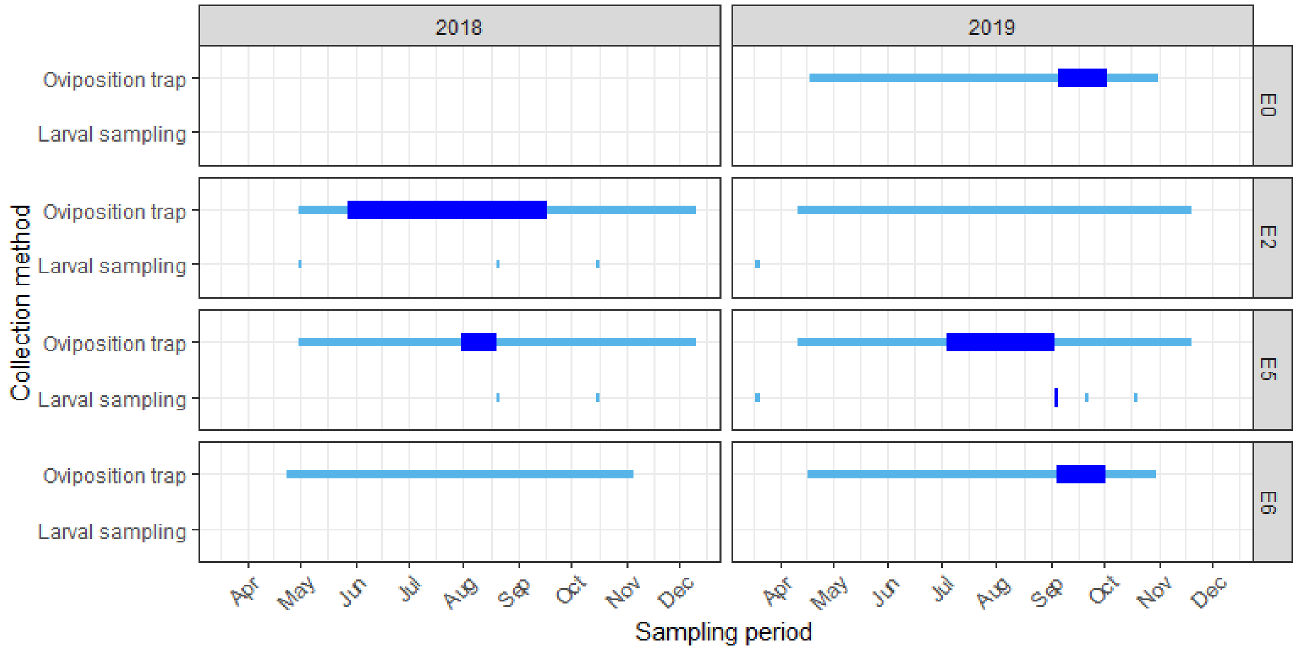
OT=Oviposition trap, LS=Larval sampling, L=Larva, E=Egg.

At the **parking lot Wanlin (E5)** monitoring was performed from the beginning of May until the beginning of December 2018 and from mid-April until mid-November 2019. In total 119 *Ae. albopictus* specimens, including 116 eggs and three larvae, were detected at the parking lot E5 in 2018 and 2019 during the MEMO project (Figure 22 & Table 16). In August 2018, 56 eggs of *Ae. albopictus* were collected. In August and October 2018, multiple drainage holes and one large stone container were checked for the presence of mosquito larvae. In August 2018, 33 larvae of *Cx. pipiens/torrentium* were collected from a drainage hole. No *Ae. albopictus* larvae were found. In 2019, 42 *Ae. albopictus* eggs were found in two consecutive samplings conducted in July (n=2) and August (n=40). In addition, 18 eggs of *Ae. albopictus* were also collected between 29 August and 3 September 2019. Larvae were searched four times (in March, September (2x) and October). On 3 September 2019, three larvae of *Ae. albopictus* were found together with *Cx. pipiens/torrentium* larvae in one drainage hole located close-by the positive OT.

At the **parking lot Sprimont/Noidré (E0)** monitoring was performed from mid-April until the beginning of November 2019. In total 69 *Ae. albopictus* eggs were detected at this parking lot in 2019 (Figure 22 & Table 16). The eggs were collected in September 2019 in one OT, close to the entrance of the restaurant.

At the **parking lot Marke (E6)** monitoring was performed from mid-April until mid-November 2018 and from the beginning of April until the end of October 2019. In total 64 *Ae. albopictus* eggs were detected at this parking in 2019 (Figure 22 & Table 16). The eggs were collected in September 2019 in one OT, located close to the entrance of the restaurant.

Figure 22. The time frame during which *Aedes albopictus* was collected per collection method, per year at the parking lots E0, E2, E5 and E6 in 2018 and 2019 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. albopictus*) (E0 was only monitored in 2019).



Control measures (larviciding) at all positive parking lots was advised by ITM in the urgent notification sheets. Only at parking lot E6 control measures were implemented by the Flemish authorities (ANB) in 2019. At E2, E5 and E0 control measures were planned by the Walloon government (SPW) in 2020, but were cancelled because of the Covid-19 pandemic. As the borders were closed cross-border traffic decreased a lot, consequently decreasing the risk of *Ae. albopictus* import through passive ground transport in 2020.

The parking lot Wanlin (E5) is located on the E411 and the parking lots Hondelange (E2) and Sprimont (E0) are located on the E25. Both highways are coming from the E25 through Luxembourg and from the Alsace region in France and Baden-Württemberg in Germany, where a population of *Ae. albopictus* is established [1, 66]. The Belgian-Luxembourg border was crossed by 47300 vehicles per day via this motorway in 2016 and the traffic intensity around Sprimont averaged 32900 vehicles per day in 2017 [67]. The parking lot Marke (E6) is located on the E17, coming from an area in France (departments of Val-de-Marne, Seine-Saint-Denis, Hauts-de-Seine, Seine-et-Marne, Essonne, Aisne) that is colonized by *Ae. albopictus*. This Belgian-French border was crossed by approximately 35500 vehicles per day in 2017 via this motorway and the traffic intensity around Marke was 72400 vehicles per day in 2017 [67]. Because of their good infrastructure (restaurant, shower, petrol station), E0, E2, E5 and E6 are important resting places for tourists and truckers.

Overall, for vehicles coming from the areas in France and Germany which are colonized by *Ae. albopictus*, the monitored parking lots are often the first stop after crossing the border. The introduction of *Ae. albopictus* at these parking lots in 2018 and 2019 is probably due to the human-facilitated ground traffic [7, 68]. These findings confirm the presence of a new *Ae. albopictus* import pathway for Belgium and need to be taken into account when deciding on a future monitoring and control plan. It will be important to monitor these and other parking lots along the highways coming from areas colonised by *Ae. albopictus* to follow the introduction of *Ae. albopictus* through this import pathway.

3.2.2 Aedes japonicus

Aedes japonicus was found at the used tyre import company Havelange (HA) in Natoye, in an allotment garden in Eupen (EU) and at the industrial area 'Op de Berg' in Maasmechelen (MM).

Used tyre import company Havelange (HA)

Aedes japonicus was first detected in 2002 at a used tyre import company Havelange (HA), located in the village Natoye, and was reconfirmed in the 2003 and 2004 [69]. In 2008 and 2009, it was considered well established at HA and was also intercepted once at the used tyre import company Sarri Pneus (SP), 2 km southeast from the initial detection site [12, 69]. No further dissemination was detected at that moment. This was in strong contrast to the fast spread observed in other established European populations [70]. In 2012, the first control measures were implemented (mechanical source reduction and larviciding), which drastically reduced the population size, but the species was still present and detected up to 2 km in south-western direction [17, 71]. Subsequently a large eradication program was executed between 2013 and 2015 after which the population was considered eliminated [72, 73]. Monitoring in 2017 focused on confirming the elimination at and around the used tyre import company HA. Therefore, a scenario 2b (locally established, at HA site and in a 2 and 5 km buffer zone) monitoring (Table 17) was implemented between mid-August and the end of November 2017, and between the end of April and the beginning of November 2018. No *Ae. japonicus* specimens were collected in the buffer zone around HA whereas a limited number of specimens were found at HA. An adapted basic scenario 2a (new introduction, at HA and in a 200 m buffer zone) monitoring was implemented between mid-April and mid-November 2019 to check for a possible new spread (Table 17). The collection of *Ae. japonicus* eggs in the 200 m buffer zone in 2019, led to the implementation of an extended scenario 2a monitoring (new introduction, at HA site and in a 200 and 500 m buffer zone) around the new detection location.

In total 2007 *Ae. japonicus* specimens, including 455 adults, 353 larvae and 1199 eggs, were collected at and around the used tyre import company Havelange (HA) during the MEMO project (Table 17). In 2017 and 2018, respectively 31 and 251 *Ae. japonicus* specimens were collected during the scenario 2b monitoring (locally established, at HA site and in a 2 and 5 km buffer zone) (Table 17 & Figure 23). The new detection at HA indicated that there was a remnant population or that a new introduction occurred. To investigate the possible origin a microsatellite analysis was performed (see below). No evidence of spread in the surrounding 2 and 5 km buffer zones was detected. At the used tyre import company Sarri Pneu (SP), where scenario 1 monitoring activities were executed in 2017 and 2018, *Ae . japonicus* was not collected.

In 2019, 1725 *Ae. japonicus* specimens were collected during the adapted basic and extended scenario 2a monitoring (new introduction, at HA site and in a 200 m and 500 m buffer zone) (Table 17 & Figure 24). In the forest 'Bois Henrard', southwest of HA, 58 larvae were collected from standing water in plastic sheets covering woodpiles. No specimens were found in the private gardens in northeast direction. As in 2012 *Ae. japonicus* was spreading southwest [17], the monitoring was extended to the gardens of the private houses bordering the forest in the village Vincon at about 750 m southwest from HA (other side of forest). In the garden of one of these private houses, 12 *Ae. japonicus* larvae were collected from a plastic container. The inspection of PBS at a farm at 1,3 km southwest from HA, where *Ae japonicus* was found in 2012, was still negative for this species. It seems the forest 'Bois Henrard' forms a good 'shrub-corridor' for the mosquito to spread through compared with the meadows at the other side of HA. Several studies indicate that *Ae. japonicus* uses forest edges to spread [70, 74] and that their breeding sites are mainly distributed in urban and suburban area, whereas adults are more distributed in the forest [10].

Table 17. Overview of the collected *Aedes japonicus* specimens per year, location and collection method during the MEMO project.

Year	Location	Monitoring design	Trap	Number of collected *Aedes japonicus* specimens
2017 (Scenario 2b)	**tyre company**	1BG, 1MMT, 10OT, LS	BG	4F
			MMT	2F
			OT	21E
			LS	4L
	surrounding 2 and 5 km buffer zone	40 OT, LS	OT	0E
			LS	0L
2018 (Scenario 2b)	**tyre company**	1BG, 1MMT, 10OT, LS	BG	8F, 2M
			MMT	16F
			OT	160E, 15L
			LS	50L
	surrounding 2 and 5 km buffer zone	40 OT, LS	OT	0E
			LS	0L
2019 (Scenario 2a)	**tyre company**	1BG, 1MMT, 10OT, LS	BG	139F, 12M
			MMT	268F, 4M
			OT	850E, 32L
			LS	151L
	surrounding 200m and 500m buffer zone + additional LS in private gardens in close by hamlet	200 m: 10OT, LS	OT	168E
			LS	31L
		500 m: 10OT, LS	OT	0E
			LS	58L
		800 m: LS	LS	12L

BG=BG-Sentinel trap, MMT=Mosquito Magnet® trap, OT=Oviposition trap, LS=Larval sampling, M=Male, F=Female, L=Larva, E=Egg.

Figure 23. The time frame during which *Aedes japonicus* was collected per collection method, per year and per location at the used tyre import company HA during the scenario 2b (locally established, at HA site and in a 2 and 5 km buffer zone) monitoring in 2017 and 2018 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. japonicus*).

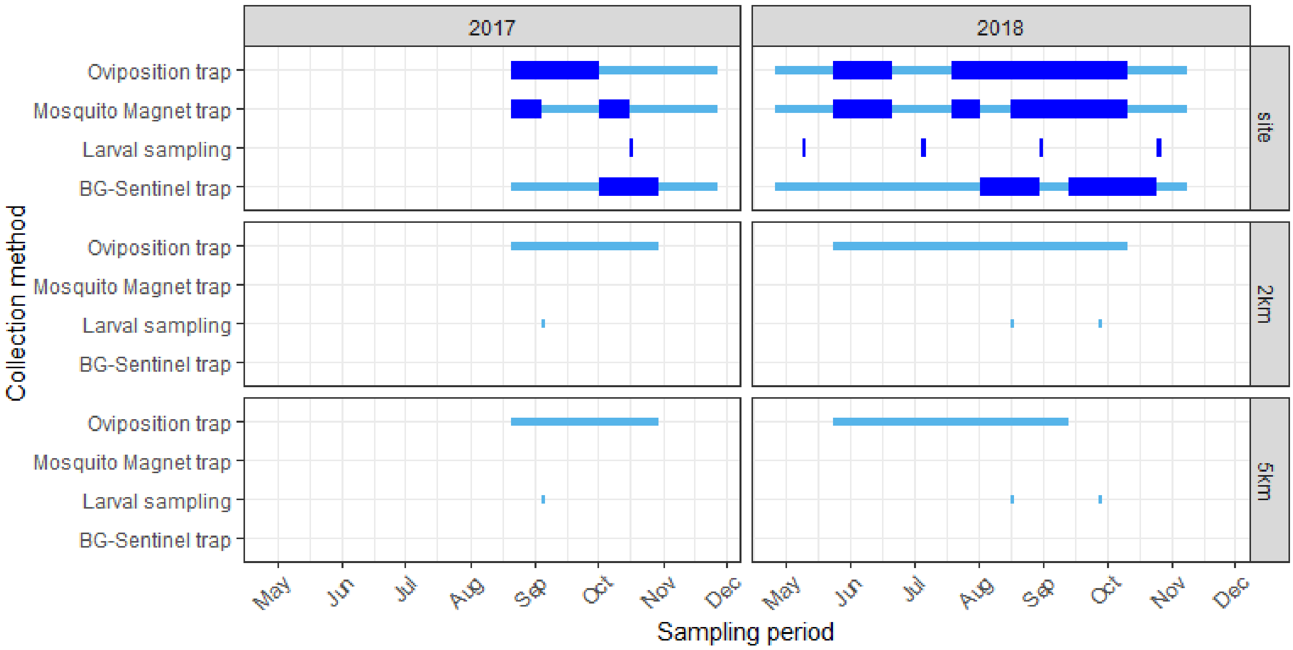
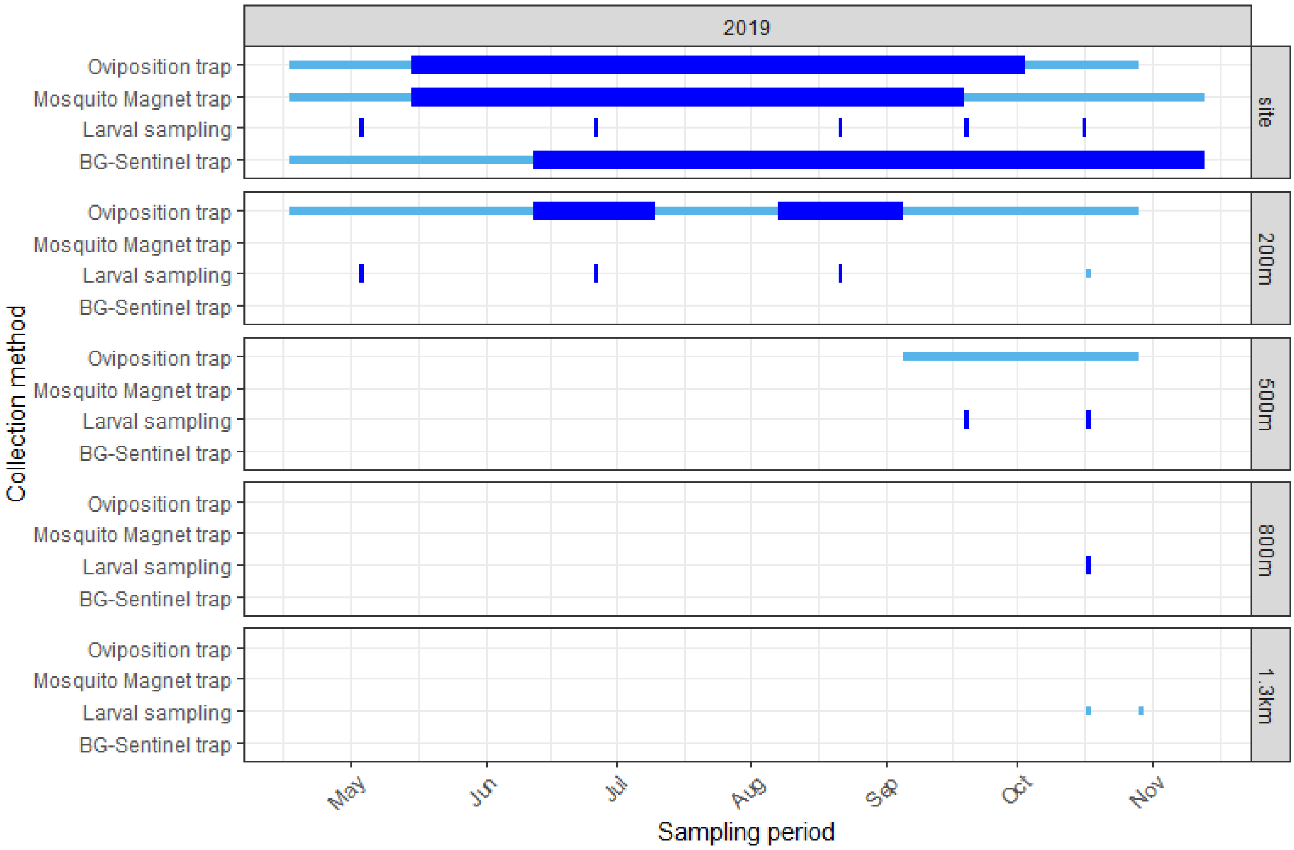
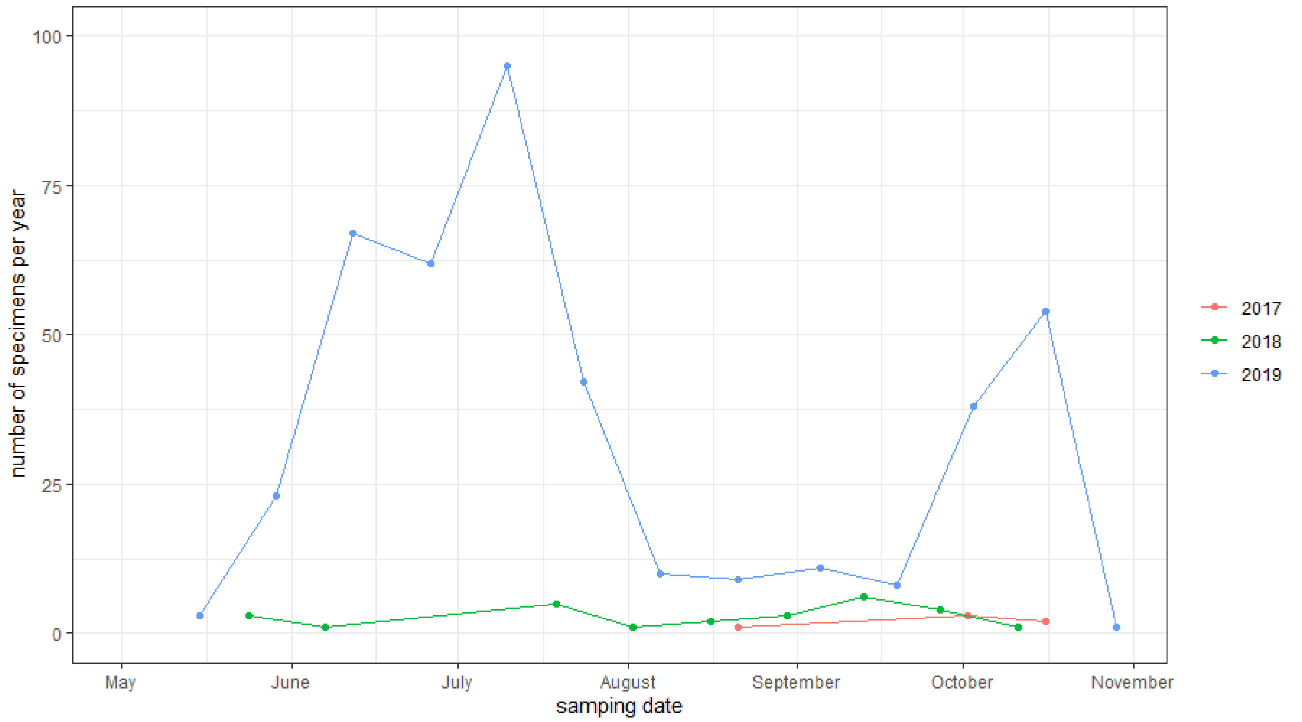


Figure 24. The time frame during which *Aedes japonicus* was collected per collection method and per location at the used tyre import company HA during the adapted basic and extended scenario 2a (new introduction, at HA site and in a 200 m and 500 m buffer zone) monitoring in 2019 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. japonicus*).



Adults, eggs and larvae were found during the entire monitoring period (in general from mid-April to end-October every year, except in 2017 monitoring started in August). The abundance of the species at the HA site increased during the three monitoring years. Adults peaked between early June and mid-July, and in October (Figure 25). Furthermore, the number of positive oviposition samplings during comparable sampling periods increased from 12% in 2017 to 13% in 2018 to 32% in 2019. In 2019, *Ae. japonicus* was identified in all adult samplings collected.

Figure 25. Seasonal abundance of adult *Aedes japonicus* collected with the BG-Sentinel and Mosquito Magnet® trap at the used tyre import company HA per year during the MEMO project.



The detection of *Aedes japonicus* at the tyre company HA from 2017 onwards, could indicate a revival of remnant individuals or a new introduction event that took place. By interviewing the company owner we suspected possible introductions from the established population in Germany (Hesse), where a used tyre company is located from which used tyres are regularly imported. To retrieve more insights in this matter, we have performed a microsatellite analysis (see below for details). Results indicate a possible new introduction from an external source at HA after the eradication process. However, the link with the West German *Ae. japonicus* population was not supported. However, the original population has survived the eradication, as an admixture between the original and a new genotype cluster occurred after the eradication in 2017.

To conclude, in 2019 the population of *Ae. japonicus* at the tyre company HA has increased strongly compared to 2017 and 2018. The detection of eggs and larvae in the forest 'Bois Henrard', together with the detection of larvae in a plastic container in a garden at Vincon (Ciney) at a distance of 750 m from HA, confirm that *Ae. japonicus* spreads again through the forest in southwest direction as it was the case in 2012. This spread seems faster than in the past. The combination of new and original genetic material could develop a stronger *Ae. japonicus* population able to spread faster. Control measures were advised at the HA site, as well as in the 500 m buffer zone around HA, and in particular the forest 'Bois Henrard' and the hamlet Vincon. An information meeting with the villagers of Natoye and the mayor of the municipality Hamois, to which Natoye belongs, has been organised by the Walloon gouvernment (SPW) on 26 September 2019 and a first clean-up campaign was organised in the 500 m buffer zone around HA on 2 October 2019. However, the number of interested citizens was low. The control campaign has started again in May 2020. Especially the used tyre company HA will need to take mechanical measures (e.g. stock tyres inside, cover tyres outside, remove old tyres) to prevent imported *Ae. japonicus* to be able to reproduce, as new introductions will probably keep occurring at HA.

Allotment garden in Eupen (EU)

Since 2008 *Ae. japonicus* is considered established in Germany. The four original populations of *Ae. japonicus* in Germany [75] started to merge in 2017 [76]. Meanwhile, its population density is largest in southwest Germany and its distribution area reaches up to the border with Belgium [76]. We can expect further colonisation along the German-Belgian border in the future. Therefore, in 2017 an adapted scenario 1 monitoring, which covered the roads and highway that pass through 'De Hoge Venen' nature reserve in Belgium (network of 40 OT between the cemetery in Raeren (RA) and the cemetery in Rocherath (RO) and LS along this network in September) was implemented. Also at the parking lot Raeren/Lichtenbusch (E4), at the German border, a scenario 1 monitoring was implemented. In 2017, RA, RO and E4 were monitoring between mid-August and the end of November 2017. Based on the detections of *Ae. japonicus* in German cemeteries, inspection of local cemeteries RA (2017 only) and RO were executed twice a year, but no presence of *Ae. japonicus* was detected. However, *Ae. japonicus* larvae were found in an allotment garden in Eupen, south of the cemetery in Raeren (RA), (new PoE code = EU), which showed that the species is present in the canton of Eupen. An adapted basic scenario 2a monitoring (new introduction, 200 m buffer zone) was implemented between mid-May and mid-October 2018 around the point of first detection at the allotment garden in Eupen (EU) to assess its spread. As only a low number and limited spread was detected at EU in 2018, the same basic scenario 2a (new introduction, 200 m buffer zone) monitoring was implemented between the end of April and the end of October 2019.

In total 228 *Ae. japonicus* specimens, including five adults, 24 larvae and 199 eggs, were collected at and around the allotment garden in Eupen (EU) during the MEMO project (Table 18). In 2017, 12 *Ae. japonicus* larvae were collected during the adapted scenario 1 monitoring between RA and RO (Table 18). On 20 September 2017, these larvae were collected from rainwater barrels at an allotment garden in Eupen (EU). In 2018, 210 *Ae. japonicus* specimens, including all life stages, were collected during the adapted basic scenario 2a monitoring (new introduction, 200 m buffer zone) at and around EU (Table 18 & Figure 26). Five adults and six larvae were detected at EU with the first specimens (larvae) collected at the end of June. In the 200 m buffer zone, one OT collected 199 eggs, but no other life stages were found. In 2019, only six *Ae. japonicus* larvae were collected during the adapted basic scenario 2a monitoring (new introduction, 200 m buffer zone) at and around EU (Table 18 & Figure 26). Four larvae were detected at EU in plastic sheet and a tyre, while two larvae were found in a puddle in the 200 m buffer zone. The first larvae were collected early in May. The presence of *Ae. japonicus* in the 200 m buffer zone later in the year in 2018 and 2019 could indicate summer reproduction of the species. Both in 2018 and 2019 additional LS was also executed in early spring to investigate possible overwintering of the species at EU (Figure 26). However, as most PBS were still covered under a tick ice layer, egg hatching and larval development were unlikely to occur or detected if present [77]. The limited number of specimens, the absence of adults, and no evidence of egg laying females (i.e. no eggs were collected with OT) in 2019 suggest the absence of an established population. The monitoring results rather point to the phenomenon of multiple introductions at the site.

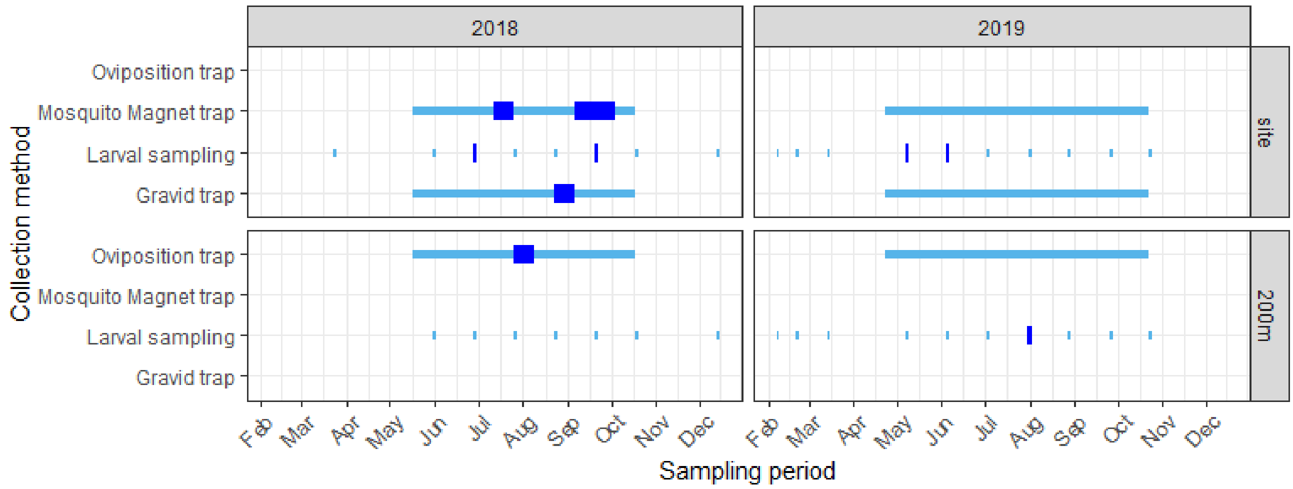
The species is probably introduced from the nearby established West German populations via human-mediated ground transport. Alternatively, the established West German population could also have crossed the border by natural spread. To investigate the possible West German origin of the *Ae. japonicus* individuals found near the Belgian-German border, we have conducted a preliminary microsatellite analysis (see below for details). The results suggest indeed a relation between the collected individuals at EU and the population in West-Germany.

Table 18. Overview of the collected *Aedes japonicus* specimens at the allotment garden in Eupen (RA & EU) per year, per PoE, per location (monitoring design between brackets), per date and per collection method during the MEMO project.

Year	PoE	Location	Start date	End date	Trap	Number of collected *Ae. japonicus* specimens
2017	**RA**	**allotment garden (LS)**	20 September	20 September	LS	12L
2018	**EU**	**allotment garden (2MMT, 1GT, LS)**	28 June	28 June	LS	5L
			12 July	26 July	MMT	2F
			23 August	6 September	GT	1F
			6 September	20 September	MMT	1F
			20 September	20 September	LS	1L
			20 September	4 October	MMT	1F
		200m buffer zone (22OT, LS)	26 July	9 August	OT	199E
2019	**EU**	**allotment garden (2MMT, 1GT, LS)**	7 May	7 May	LS	3L
			4 June	4 June	LS	1L
		200m buffer zone (22OT, LS)	31 July	31 July	LS	2L

MMT=Mosquito Magnet® trap, GT=Frommer updraft gravid trap, OT=Oviposition trap, LS=Larval sampling, F=Female, L=Larva, E=Egg.

Figure 26. The time frame during which *Aedes japonicus* was collected per collection method, per year and per location at the allotment garden EU during the adapted basic scenario 2a (new introduction, 200 m buffer zone) monitoring in 2018 and 2019 during the MEMO project (light blue lines = sampling period; dark blue = detection of *Ae. japonicus*).



To conclude, at this moment no established *Ae. japonicus* population is yet present at the allotment garden EU. But, the population in Germany is well established and has the tendency to spread fast [75, 76]. It can be soon expected that the species spreads across the border, not only by the repeated introduction by passive ground transport, but also by natural spread. As the German authorities don't have the intention to implement control for *Ae. japonicus*, it might be ineffective to implement control in Belgium with the aim of eradication. Control measures with the aim of diminishing the population in case of nuisance is possible but should be discussed with the neighbouring countries Luxembourg and Germany to come up with a common strategy. For 2020 a sensitisation campaign will be set-up by the Walloon government (SPW) at EU to inform the gardeners and inhabitants about *Ae. japonicus* and to recommend preventive measures (e.g. covering the rainwater barrels).

Industrial area 'Op de Berg' in Maasmechelen (MM)

During the scenario 3 monitoring (widely established, 6-8 km buffer zone) for *Ae. korecius* at the industrial area 'Op de Berg' in Maasmechelen (MM) between mid-April 2018 and mid-April 2019 (see Chapter 3.2.3), *Ae.*

japonicus was detected for the first time at this PoE. Between mid-June and mid-July 2018, 50 *Ae. japonicus* adults were intercepted at MM (more precisely the APK company) with the GT (Table 19). No other life stages nor detections were made. To investigate the possible origin of the *Ae. japonicus* individuals found at MM, we have conducted a preliminary microsatellite analysis (see below for details). The collected individuals are genetically different from the specimens collected at EU and HA, and from the German populations. Therefore, its introduction route is still unknown. It was the first time both *Ae. japonicus* and *Ae. koreicus* were present at the same location in Belgium. Co-occurrence of *Ae. japonicus* and *Ae. koreicus* was found in Germany, Switzerland and Slovenia [78]. Both species can occupy the same breeding sites. The new discovery of *Ae. japonicus* at MM happened during the regular molecular validation of the morphologically identified samples in March 2019. All 2018 samples from MM were reidentified morphologically afterwards and 48 extra specimens were tested molecularly. Both species are morphologically very similar and adults missing specific characteristics should be identified by DNA barcoding.

Table 19. Overview of the collected *Aedes japonicus* specimens at the industrial area in Maasmechelen (MM, APK company) per date and collection method in 2018 during the MEMO project.

Year	PoE	Start date	End date	Trap	Number of collected *Ae. japonicus* specimens
2018	**MM**	19 June	3 July	GT	1F & 8M
		3 July	17 July	GT	24F & 17M

GT=Frommer updraft gravid trap, F=Female, M=Male.

Aedes japonicus *population genetic structure and diversity investigation*

Elucidating the relationships between the Belgian *Ae. japonicus* specimens and the West German population is of great interest as it might help customizing control efforts in Belgium. Using highly polymorphic DNA regions (microsatellites and the mitochondrial NADH dehydrogenase subunit 4 (*nad4*) locus), the objectives were to determine 1) if the mosquito specimens collected along the Belgian border at the allotment garden in Eupen (EU) and at the industrial area in Maasmechelen (MM) were introduced from the nearby existing West German population, and 2) if the population at the used tyre import company Havelange (HA) after the eradication program represents a new introduction and/or results from undetected specimens that escaped eradication. Therefore, the *nad4* fragment and seven microsatellite loci were scored in 278 and 292 specimens of *Ae. japonicus,* respectively, including specimens collected in well-identified German population clusters [79], and during the EXOSURV project and the eradication campaign undertaken at HA (Table 20).

1) Geographic analysis: introduction source along the Belgian border

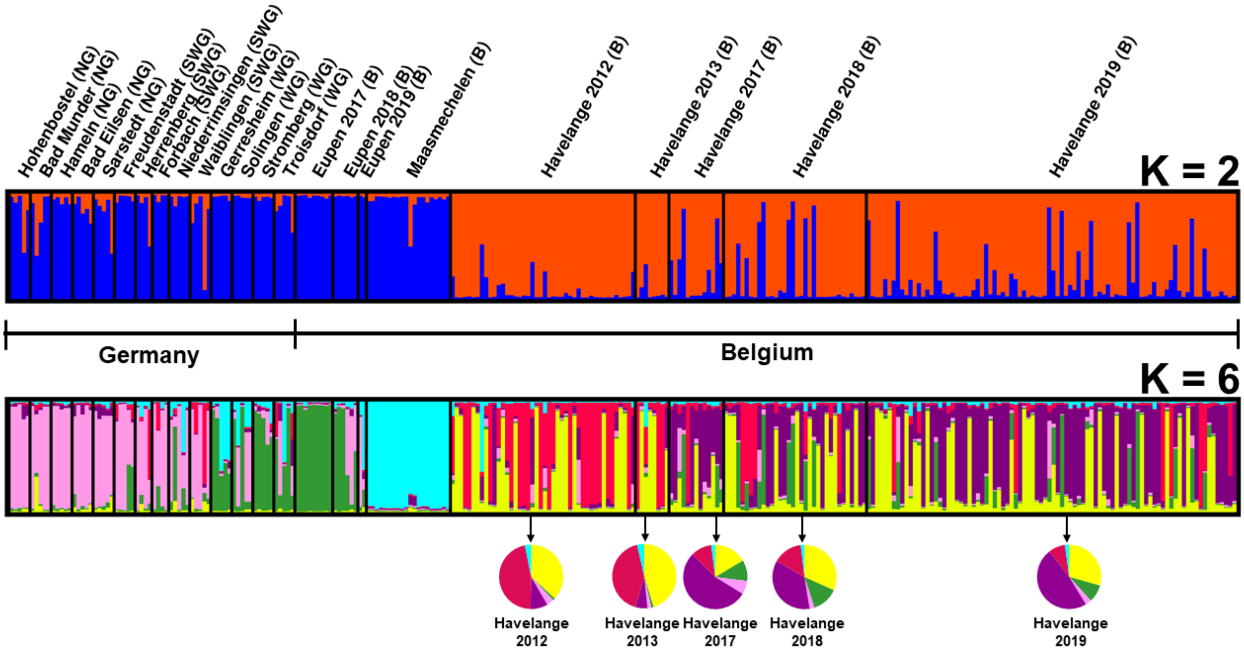
Based on the *nad4* locus, a lack of structure in both Neighbour-Joining tree and minimum spanning network constructions is likely linked to the randomness of international introduction events, with specimens possibly originating from diverse populations. Bayesian cluster analysis of the microsatellite data, however, identified two (highest posterior probability for K = 2) and six (second highest posterior probability for K = 6) genotypic clusters (Figure 27). At K = 2, the specimens from HA are separated from all others. At K = 6, four genotype groups corresponded with geographical populations, with different degrees of admixture: (1) Maasmechelen (MM), (2) North and South West Germany, (3) West Germany and Eupen (EU), and (4) Havelange (HA) (Figure 27 & Figure 28).These results first suggest a relation between Eupen (EU) and the population of West Germany, which is in agreement with the prediction that the species might cross the border with Belgium in a near future [75]. Despite the extensive monitoring efforts at the PoE's targeted over MEMO, only few *Ae. japonicus* specimens were collected at EU (see above). Therefore, the species is not believed to be established and to overwinter yet, and specimens collected between years could result from multiple introductions. Second, at K = 6, Maasmechelen (MM) forms a distinct genotypic cluster, with only one recorded *nad4* haplotype at that PoE (H1 – Figure 29). However, despite intensive monitoring efforts, only few adult *Ae. japonicus* were trapped. Since these two trapping dates are close to each other, it is possible that the specimens derive from the same single introduction and reproduced on site. A bias in the analyses is therefore assumed by over-sampling across siblings. Further monitoring and a higher number of genome wide DNA markers would help gain insight in the introduction pathways and origin of *Ae. japonicus* into Belgium.

Table 20. Sample information of the *Aedes japonicus* specimens analysed, including their geographical origin, the year of collection and the number of specimens analysed.

Country	State/ Province	Location	Year	N_{nad4}	N_M	Life stage at collection	Surveillance project
North Germany	Lower Saxony	Hohenbostel	2017	5	5	L: 5	FLI [76]
North Germany	Lower Saxony	Bad Münder	2017	5	5	L: 5	FLI [76]
North Germany	Lower Saxony	Hameln	2017	5	5	L: 5	FLI [76]
North Germany	Lower Saxony	Bad Eilsen	2017	5	5	L: 5	FLI [76]
North Germany	Lower Saxony	Sarstedt	2017	5	5	L: 5	FLI [76]
South West Germany	Baden-Württemberg	Freudenstadt	2016	5	5	L: 5	FLI [76]
South West Germany	Baden-Württemberg	Herrenberg	2016	4	4	L: 4	FLI [76]
South West Germany	Baden-Württemberg	Forbach	2017	4	4	L: 4	FLI [76]
South West Germany	Baden-Württemberg	Niederrimsingen	2017	5	5	L: 5	FLI [76]
South West Germany	Baden-Württemberg	Waiblingen	2017	5	5	L: 5	FLI [76]
West Germany	Rhine-Westphalia	Gerresheim	2016	5	5	L: 5	FLI [76]
West Germany	Rhine-Westphalia	Solingen	2016	5	5	L: 5	FLI [76]
West Germany	Rhineland-Palatinate	Stromberg	2017	5	5	L: 5	FLI [76]
West Germany	Rhine-Westphalia	Troisdorf	2017	4	5	L: 5	FLI [76]
Belgium (Wallonia)	Liège	Eupen (EU)	2017	9	9	L: 9	MEMO
Belgium (Wallonia)	Liège	Eupen (EU)	2018	5	6	L: 2; A: 4	MEMO
Belgium (Wallonia)	Liège	Eupen (EU)	2019	2	2	L: 2	MEMO
Belgium (Flanders)	Limburg	Maasmechelen (MM)	2018	19	20	A: 20	MEMO
Belgium (Wallonia)	Namur	Havelange (HA)	2012	44	44	L: 35, E: 9	ExoSurv [4]
Belgium (Wallonia)	Namur	Havelange (HA)	2013	8	8	L: 8	Avia-GIS [72]
Belgium (Wallonia)	Namur	Havelange (HA)	2017	13	13	A: 4, L: 3, E: 6	MEMO
Belgium (Wallonia)	Namur	Havelange (HA)	2018	31	34	A: 20, L: 14	MEMO
Belgium (Wallonia)	Namur	Havelange (HA)	2019	80	88	A: 45, L: 43	MEMO
Total				**278**	**292**		

N_{nad4}=Number of specimens for which nad4 sequences were obtained, N_M=Number of individuals genotyped for the seven microsatellites, A=Adult, L=Larvae, E=Egg.

Figure 27. Clusters for both K = 2 and K = 6, inferred with Structure® v2.3.4 software, after Evanno et al. [80] correction. The cluster membership of each individual is shown by the color composition of the vertical lines, with the length of each color being proportional to the estimated membership coefficient.



WG=West Germany; SWG=South West Germany; NG=North Germany; B=Belgium.

Figure 28. Map of Belgium and Germany displaying the Bayesian cluster analysis results for K = 6 per sampling locality, based on seven microsatellite loci. Each pie chart represents one sampling location. Colors of the pie chart represent the mean assignment probabilities for all individuals collected at that location to each clusters, following the same color code as in Figure 27.

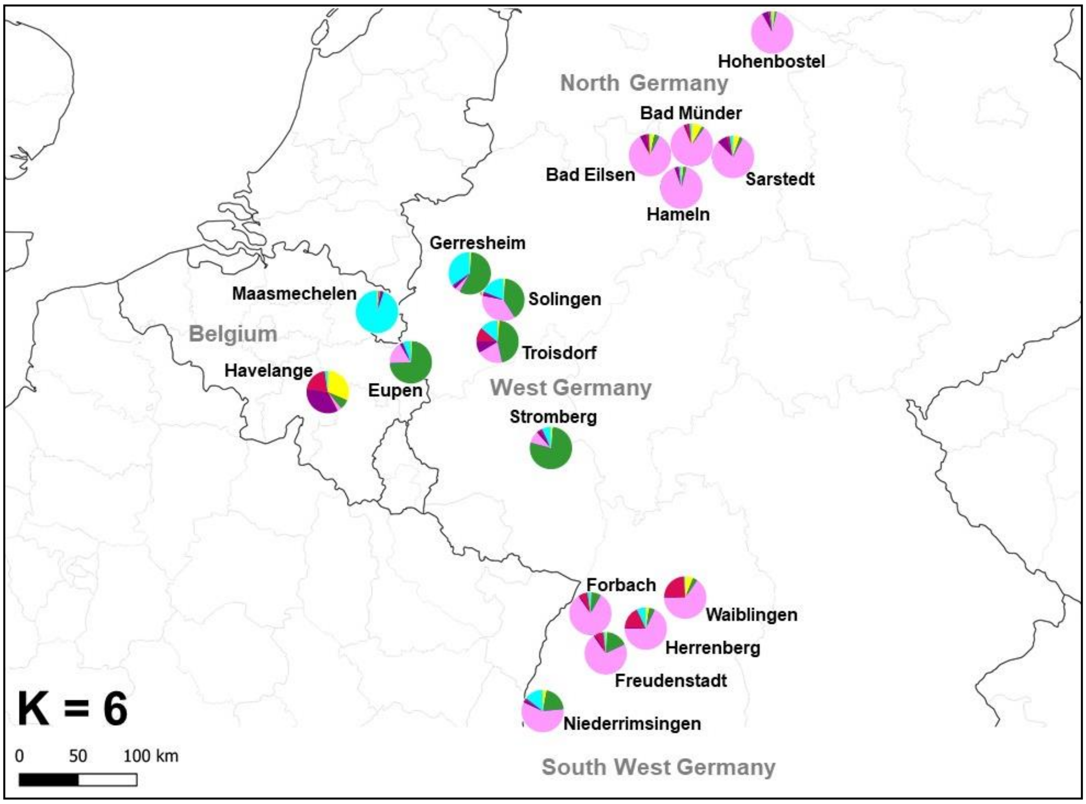
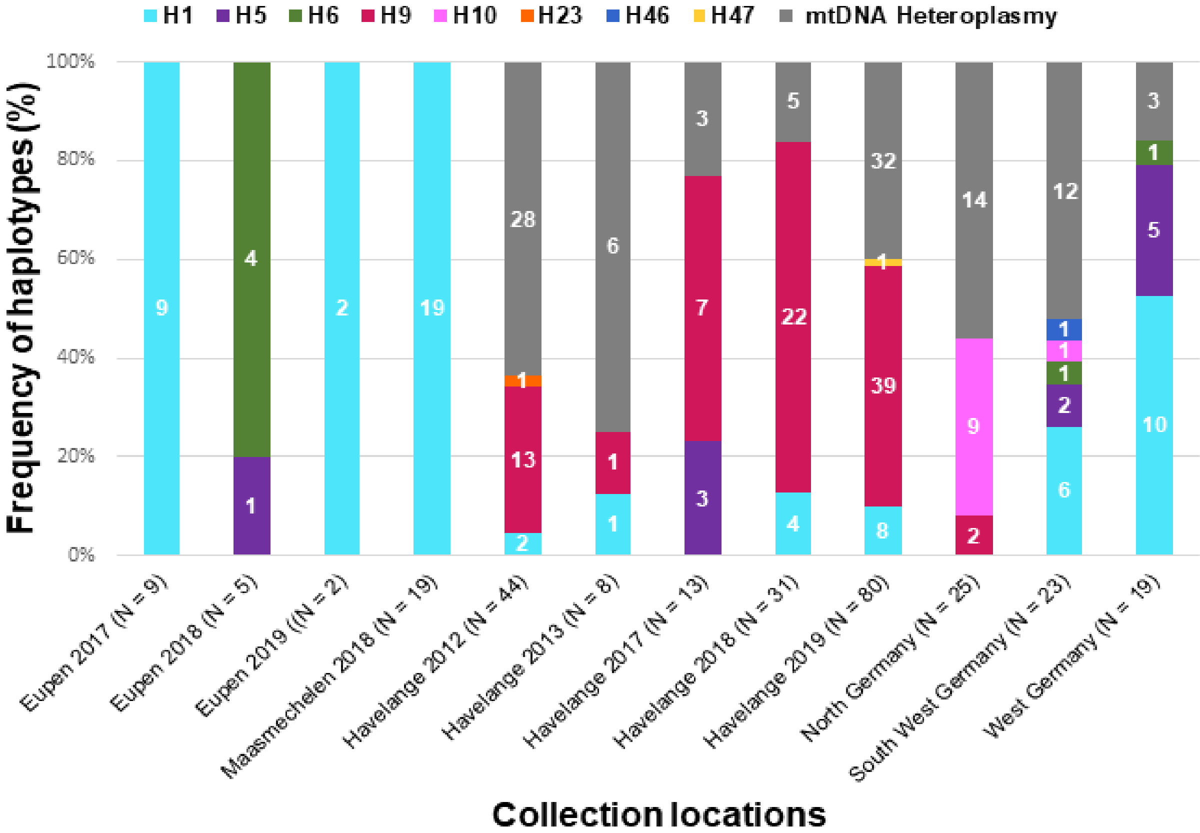


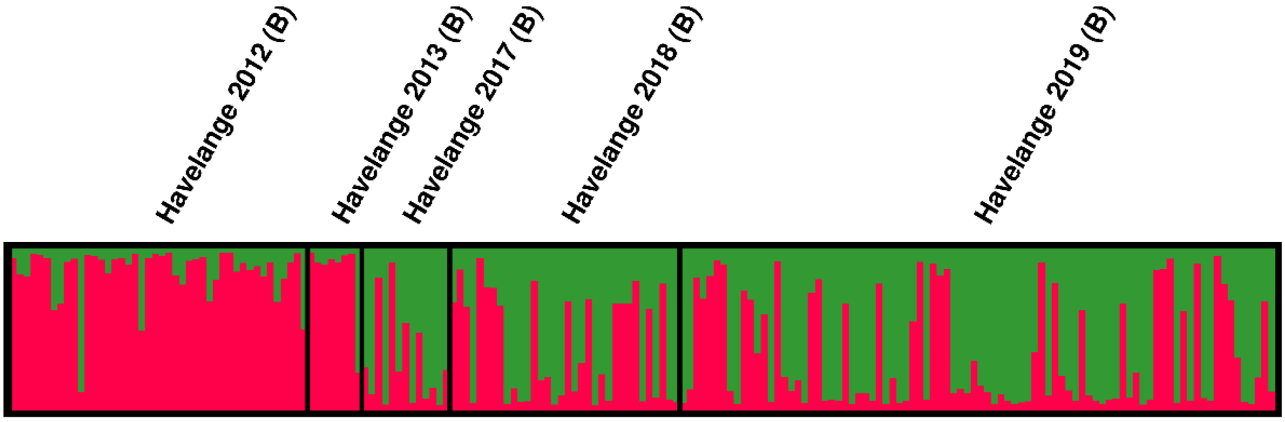
Figure 29. Frequency of nad4 haplotypes at the different collection locations in Belgium and Germany. Naming of haplotypes according to [79, 81]. The white bold numbers on the bars represent the number of specimens assigned to each haplotype.



2) Temporal analysis at Havelange

The results of the genetic investigation indicate that the Havelange (HA) population is significantly differentiated from all other populations included (Figure 27), both for *nad4* (high prevalence of haplotype H9) and the microsatellite data. The HA population also showed a clear difference between its genotypic microsatellite make-up in 2012-2013 and 2017-2019, i.e. before and after the eradication in 2014-2015, as suggested by the significant F_{ST} values and the Bayesian clustering in Figure 30. This was, however, not accompanied by a difference in the *nad4* data, with haplotype H9 being still predominant (Figure 29). In 2017-2019 the population had an increased allelic richness and number of private alleles compared to 2012-2013 (10 *versus* one, respectively). Between 2017 and 2019, 59 specimens displayed one or more private alleles, while only three specimens were recorded with a private allele in the time period 2012-2013. These results would indicate that there may be a new introduction from an external source at HA after the eradication process. The population present in 2012-2013 is, however, believed to have survived the eradication in 2014-2015 since admixture between the two genotype clusters identified based on microsatellite data was identified (Figure 30). The forest next to the tyre company, where the species has also been collected over different monitoring projects, might have played a role of refuge for *Ae. japonicus* [54]. Indeed, in its natural distribution range in East Asia, the species is usually found in forested areas [82].

Figure 30. Clusters for *K* = 2 at the used tyre company Havelange (HA), inferred with Structure® v2.3.4 software, after Evanno *et al.* [80] correction. The cluster membership of each individual is shown by the color composition of the vertical lines, with the length of each color being proportional to the estimated membership coefficient.



Considering the international movement of goods and people, and the colonising behaviour of *Ae. japonicus* in Germany, it is to be expected that further introductions will occur in Belgium. To further investigate the population genetic relationships and changes in the allelic frequencies over time in the frame of surveillance programmes, thorough sampling of all *Ae. japonicus* populations, including representatives of its native and invasive ranges, additionally to the use of genome wide genetic data, would be required.

3.2.3 Aedes koreicus

Since 2008, *Ae. koreicus* is established at the **Industrial area 'Op de Berg' in Maasmechelen (MM)**. Monitoring focused on assessing the spread of this EMS and monitor the local abundance in the industrial zone. Therefore, a scenario 2b (locally established, 1,5 and 5 km buffer zone) monitoring was implemented between the end of August and the end of October 2017. Because of the detections of both larvae and adults of *Ae. koreicus* outside the perimeter of 2,8 km (> 25 km²) in 2017, a scenario 3 monitoring (widely established, 6-8 km buffer zone) was implemented between mid-April 2018 and mid-April 2019, which was financed separately by the Flemish authorities (ANB) (SCENAKOR3 project). Based on decisions of the steering committee and on the fact that no further spread was detected, a scenario 1 monitoring (at PoE) was implemented between mid-June and the beginning of November 2019, mainly to check the efficiency of the implemented control measures at the PoE in 2019 and the introduction of *Ae. japonicus* at MM.

In total 1246 *Ae. koreicus* specimens, including 481 adults, 436 larvae and 329 eggs, were collected at the industrial area in Maasmechelen (MM) during the MEMO and SCENA3KOR project. In 2017, 60 *Ae. koreicus* specimens were collected during the scenario 2b monitoring (locally established, 1,5 and 5 km buffer zone) (Table 21 & Figure 32). Eight *Ae. koreicus* larvae were found in a tyre located in a forest 3,8 km from MM and 52 larvae were also detected at MM (company Walkro) in the colonised area (1,5 km buffer zone). Furthermore, two females were found during the monitoring activities at the used tyre import company MB, located 5,4 km northwest of MM (see further).

During the scenario 3 monitoring (the SCENA3KOR project) in 2018 and 2019, 793 *Ae. koreicus* specimens, including 427 adults and 366 larvae, were collected at the MM site (Table 21, Figure 31 & Figure 32). The species was not collected in the 6-8 km buffer zone around MM, only at the industrial area MM (company APK). It was found in early spring (26 March 2019) and reached its peak during summer (end of June and July 2018) (Figure 31), which is two months earlier than in 2009 [11]. The species was active until the end of October. The GT captured more adults than the MMT during the peak season, but the MMT collected more specimens before and after the peak. The same was seen in 2009 and can be explained by the fact that the GT reflects the oviposition activity, while the MMT reflects more the female host-seeking activity [11].

Table 21. Overview of the collected *Aedes koreicus* specimens per scenario, location and collection method at and around the industrial area in Maasmechelen (MM) during the MEMO project.

Project	Location	Monitoring design	Trap	Collected *Aedes koreicus* specimens
Scenario 2b (2017)	**1.5 km buffer zone**	8 OT, LS	OT	0
			LS	52L
	5 km buffer zone	32 OT, LS	OT	0
			LS	8L
Scenario 3 (2018-2019)	**industrial site**	2MMT, 2BG-GAT, 1GT, LS	MMT	119F, 17M
			GT	143F, 144M, 86L
			BG-GAT	2F, 2M
			LS	280L
	6-8 km buffer zone	LS	LS	0L
Scenario 1 at APK (2019)	**industrial site (restricted to APK site)**	1 BG, 1GT, 10 OT, LS	BG	10F, 10M
			GT	26F, 8M
			OT	329E
			LS	10L

BG=BG-Sentinel trap, MMT=Mosquito Magnet® trap, GT=Frommer updraft gravid trap, OT=Oviposition trap, LS=Larval sampling, F=Female, L=Larva, E=Egg.

Figure 31. Seasonal abundance of adult *Aedes koreicus* at the industrial area in Maasmechelen (MM) per trap type during the MEMO project. All traps were located at the APK company, except one Mosquito Magnet® trap, located at company Walkro in 2018.

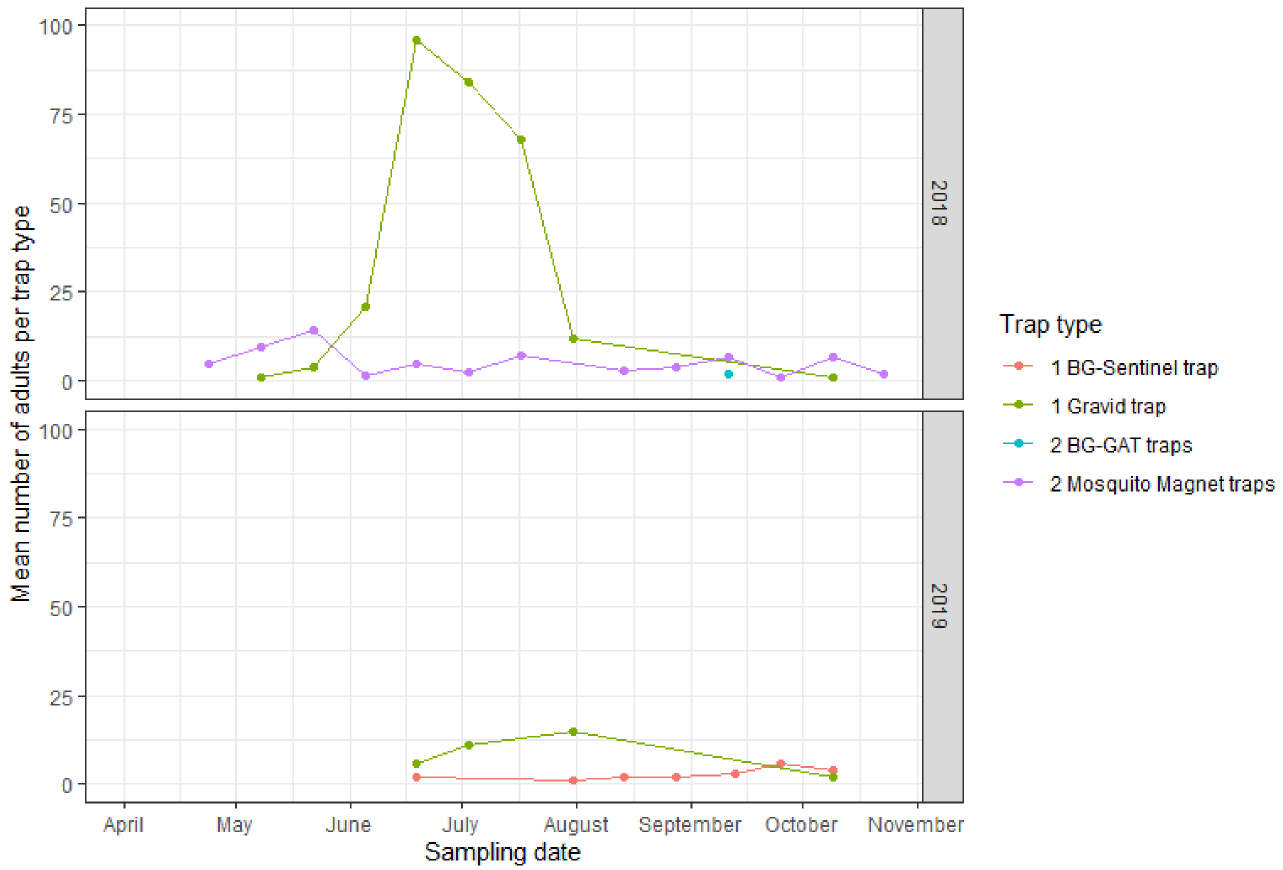
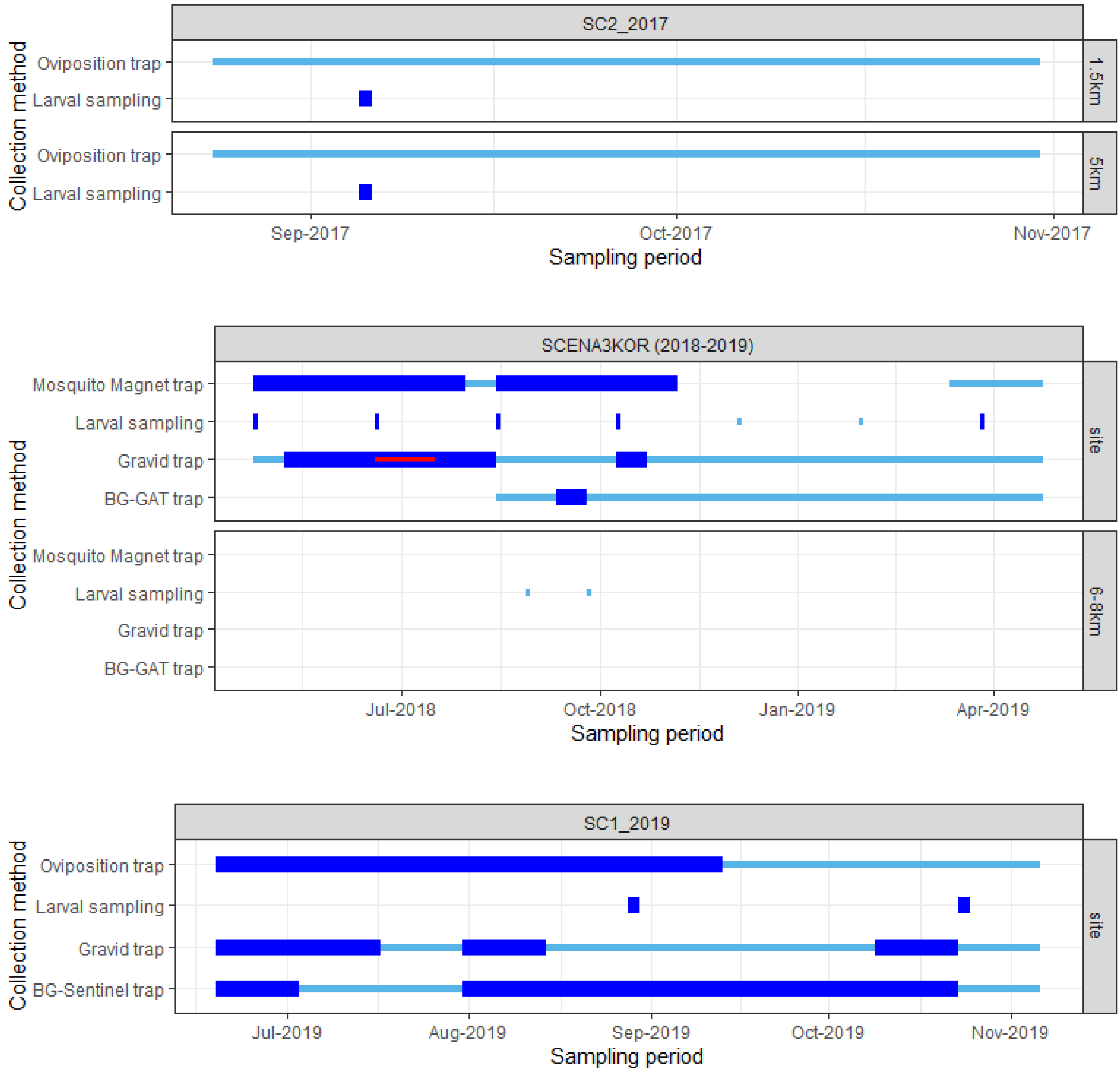


Figure 32. The time frame during which *Aedes koreicus* was collected per collection method, per scenario and per location at the industrial area in Maasmechelen (MM) during the MEMO and SCENA3KOR project (light blue lines = sampling period; dark blue = detection of *Ae. koreicus*; red line = detection of *Aedes japonicus*).



SC2_2017=Scenario 2b (locally established) between August and October 2017, SCENA3KOR (2018-2019)=Scenario 3 between April 2018 and April 2019; SC1_2019=Scenario 1 between June and October 2019.

During the scenario 1 monitoring in 2019, 393 *Ae. koreicus* specimens, including 54 adults, 10 larvae and 329 eggs, were collected at the MM site (Table 21, Figure 31 & Figure 32). *Aedes koreicus* adults were active between the start of the sampling in June and October 2019 (Figure 31 & Figure 32). The last individual of *Ae. koreicus* (larva) was collected in a plastic container on 23 October 2019 during the last LS event. The GT collected a lot less adults in 2019 than in 2018 (Figure 31). This reduced number of adults may be correlated with the sensitization and clean-up by the APK company and the control measures taken by ANB. In contrast to 2017, the OT collected a lot of *Ae. koreicus* eggs. This might be because in 2019 the OT were placed at the hotspot of the population, the APK company, whereas in 2017 they were placed more at the border and around the APK company. Further, the number of PBS decreased after a clean-up at the APK site, which might have made the OT more attractive.

At the **used tyre import company Maaslandbanden (MB, 5,4 km from MM)** the two adult traps (one MMT and one BG) were located close to the used tyres stocked outside. The collection of eggs (10 OT) and larvae was also performed outside. At this PoE monitoring was performed from the end of August until the end of November 2017, from mid-April until the beginning of November 2018 and from mid-April until the beginning of November 2019. In total seven *Ae. koreicus* females were collected at the used tyre import company MB during the MEMO project (Table 22). In 2017, two female *Ae. koreicus* were collected in October. In 2018, three females were collected in September and October. In 2019, two females were collected in May, which was the first time that *Ae. koreicus* was detected so early in the season at MB. The species was also collected in September-October 2019. In all three years of the MEMO project, the numbers of *Ae. koreicus* adults captured at MB were low and no larvae were found. Although a new introduction at MB cannot be excluded, a spread of the population from MM seems more plausible as the detection of the species was always in the same northeast direction, in which also larvae were found in 2017 (in tyre in the forest).

Table 22. Overview of the collected *Aedes koreicus* specimens per year, date and collection method at the used tyre import company Maaslandbanden (MB).

Year	Start date	End date	Trap	Number of collected *Ae. koreicus* adults
2017	3 October	28 June	MMT	2F
2018	11 September	25 September	MMT	1F
	25 September	9 October	MMT	1F
	9 October	23 October	MMT	1F
2019	22 May	5 June	MMT	1F
	25 September	9 October	MMT	1F

MMT=Mosquito Magnet® trap, F=Female

In conclusion, *Aedes koreicus* was first detected in Belgium in 2008 during the MODIRISK project in a forest next to the industrial area 'Op de Berg' in Maasmechelen (MM) [11]. Although the import route of this EMS into Belgium is unknown, it could have happened via international trade because there is a large industrial zone in the area [11]. During the MODIRISK project, a longitudinal study was carried out between April and October 2009 to assess the degree of establishment and the population dynamics of this new EMS. Larvae and eggs were collected up to 4 km to the east of the point of first detection. Adults were caught from May to the beginning of October with a peak in August and September 2009. Larvae were also found in early October 2009. *Aedes koreicus* breeds in a variety of PBS: metal containers (such as old construction equipment (mainly excavator heads)), small and large tyres, plastic containers (such as buckets, trays) and plastic sheets. The establishment of *Ae. koreicus* in Maasmechelen was reaffirmed in 2010 (personal communication F. Schaffner). In 2011, several larvae were found again 4 km east of the point of first detection (personal communication F. Schaffner). In 2012, during the EXOSURV project, and in 2013, during the FASFC project, *Ae. koreicus* was found within a distance of 1 km from MM, where the species was first detected [2, 17, 62, 71]. Although in 2014, during the FASFC project, four *Ae. koreicus* larvae were caught 5,3 km from the industrial area [2, 63, 83], no larvae were found in 2015 outside a radius of 1.5 km around the point of first detection [64]. In 2017, however, during the MEMO project, larvae were found at 3,8 km from the point of first detection and for the first time also adults at 5,4 km (MB). In 2018 and 2019 adults were again collected at 5,4 km (MB). The current monitoring data confirm the observations of 2009, 2011 and 2014 that this species occurs more than 2,8 km from the point of first detection. These observations show that the population of *Ae. koreicus* is already widespread (i.e., in an area of more than 25 km^2). However, the numbers of *Ae. koreicus* adults captured at MB are low and no larvae were found anymore. Furthermore, it was also not detected during LS in the 6-8 km buffer zone around MM. The late detections at MB most likely indicate that *Ae. koreicus* is not established at the site itself but that individuals are most probably spreading from the industrial area MM during the season. The species is thus widespread, but the hot spot remains in the industrial area. It is still not clear why the species did not disperse faster. The limited spread found in the northeast of the industrial area might be explained by the presence of the forest 'Mechelse Bos', which forms a good 'shrub-corridor' for the mosquito to spread compared with the open terrain of the sand quarry and the heath at the other sides of the industrial area. In general, mosquitoes prefer to fly through bushes and shrubs and avoid open terrain [5].

Although the vector status of *Ae. koreicus* in unclear, it is not known how it will evolve in the future [84], therefore the precautionary principle could be used to justify control measures. Because of the slow, but widespread, dispersion, eradication is becoming difficult. Control measures should be aimed at reducing the population to density levels that prevent possible disease transmission. An important factor in the control plan is the environmental management of the 'Op de Berg' industrial area. Plans exist to transform the industrial area into a nature reserve so that it can be integrated into 'De Hoge Kempen' National Park [85]. It is important to reduce the *Ae. koreicus* population as much as possible in the years before the movement of all companies and private owners to other locations. Eggs and larvae could be moved also to these locations, which would spread this EMS over a larger area. A first step is the mechanical control of PBS, such as tyres, plastic containers, plastic sheet. Larger (metal containers) or non-removable PBS (drainage) could be treated with a biological larvicide. As the first larvae can be found in the end of March, larviciding should start soon enough. Communities, companies and private owners should be involved in this first step to have a long-term effective control. Before and after control measures monitoring of the efficiency of control actions by experts is needed.

Following these recommendations, the Flemish authorities (ANB) implemented a sensitisation campaign with the communities on 6 July 2018. In 2019 a control campaign was implemented for the first time at the industrial area MM, the hotspot area of *Ae. koreicus*. The fact that less adult *Ae. koreicus* were captured at the APK company in 2019 is probably due to both the clean-up by the company and the control measures taken by ANB. It will be important to continue the control measures in the next years to minimize the spreading of the species after the movement of the companies by 2035.

3.2.4 Anopheles pharoensis

At Liège airport (AL) monitoring was executed with one MMT, 10 OT and LS (Table 23). The adult trap (MMT) was placed indoors in a hangar next to the transport band where cargo is unloaded. The OT were placed both indoors, close to the transport band, and outdoors, along the platform where airplanes land and are opened. Trap locations remained constant over the monitoring years. In total six different types of PBS were sampled: drainage holes, metal and plastic containers, plastic sheet, tyres and an underground water reservoir. Between 6 and 20 October 2017, one female *An. pharoensis* was collected with the MMT at AL (Table 23). Only larvae of *Cx. pipiens/torrentium* were found in the drainage holes in 2018 (n=3) and 2019 (n=1).

It is the first time an exotic species was detected at this PoE. Monitoring activities have been implemented since 2012, except for 2016 [2, 60, 62-64, 71]. The airport is located about 9 km West of the city of Liège in Belgium and mainly focusses on cargo transport. In Belgium, the airport of Liège is the largest cargo airport and ranked 8th in Europe in 2018. Goods are mainly imported from Ethiopia, Uganda, Kenya, USA, Qatar and Israël. In 2017, the airport of Liège had a record year with 717000 tonnes of goods handled and this growth further continued in 2018 and 2019, with respectively 870000 tonnes and 900000 tonnes of transported goods [86, 87]. This fact indicates the increasing risk of importing EMS through large cargo airports.

Table 23. The collection methods, number of samplings and number of traps or potential breeding sites (PBS) used to monitor the airport of Liège (AL) and the mosquito species collected.

Airport Liège (AL)				
		2017	**2018**	**2019**
MMT	N° traps	1	1	1
	N° samplings	7	14	9
LS	N° PBS	4	6	4
	N° containers	14	35	25
	N° samplings	4	13	7
OT	N° traps	10	10	10
	N° samplings	40	70	68
Species	*Anopheles pharoensis*	1F		
	Anopheles plumbeus	2F		
	Culex pipiens/torrentium	10F, 1M	19F, 3L	13F, 1L
	Culiseta annulata	1F	1F	
	Aedes caspius/dorsalis		1F	
	Aedes rusticus		1F	

MMT=Mosquito Magnet® trap, LS=larval sampling, OT=oviposition trap, M=male, F=female, L=larva.

Anopheles pharoensis is a tropical mosquito species mostly present in West-, East- and southern Africa, Egypt, Israel, and Syria [18]. It prefers marshes, swamps, rice fields and ponds, especially those with abundant grassy or floating vegetation [88]. The larvae are primarily found in large vegetated fresh water swamps, but also breed along lake shores and among floating plants. The females feed predominantly on domestic animals, especially bovids [89, 90], but enter houses readily at night and bite humans. It can disperse long-distances from nine to 70 km [88, 91]. The species is adapted to dry environmental conditions [92]. *Anopheles pharoensis* is a well-known vector of malaria in Egypt [18]. In African countries it is a common secondary vector of malaria [88, 93]. This species is also a vector of Rift Valley fever virus (RVFV). In a recent assessment of the probability of entry of RVFV into the European Union through active or passive movement of vectors, the species together with *Ae. aegypti, Mansonia uniformis, Aedes mcintoshi, and Cx. quinquefasciatus* were highest ranked among 39 potential RVFV vectors based on their distribution in the African at-risk countries, their potential role as vector, and their behavioural and ecological traits influencing the risk of transportation [94]. The finding of this species at AL confirms that EMS can enter via cargo transport [95], however the ecological preference of this EMS are dry environments, consequently the climatic conditions in Belgium are considered as non-suitable for establishment.

4 Key messages and conclusion

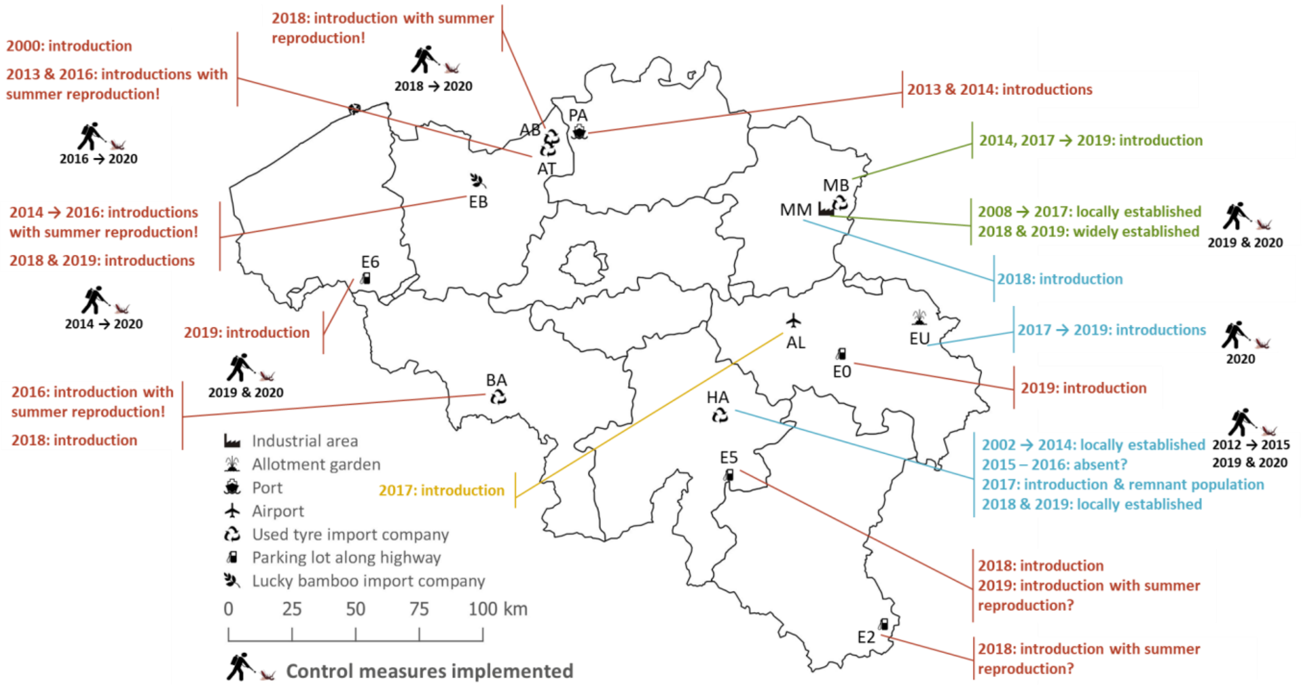
The **most important key messages** from the MEMO project results are summarised below.

Where do EMS enter Belgium?

Since the first EMS detection in Belgium in 2000 at the used tyre import company AT [61], four EMS were detected at 14 PoE's (Figure 33). The percentage of positive PoE's in Belgium is increasing (Figure 34), especially of *Ae. albopictus* and *Ae. japonicus*. Both species are gaining more and more territory in neighbouring countries (Germany [15, 76, 96, 97], the Netherlands [98], France [66, 99], United Kingdom [9, 68, 100]). The increasing population of *Ae. albopictus* in France and Germany led to the new introductions at the parking lots in Belgium in 2018 and 2019. Results from mosquito surveillance programs conducted in southern England and Germany indicate ongoing introductions of *Ae. albopictus* via ground vehicular traffic from nearby established populations [9, 96]. Adult mosquitoes 'hitchhike' along major road networks via passenger cars, trucks or campers and it is the suggested way of spread along the Mediterranean coast to Spain, inland France and Germany [97, 99, 101]. The detection of this species at parking lots in Belgium is probably also due to human-facilitated ground traffic coming from colonised areas in France and Germany. During the MEMO project, it was the first time that this import route was detected in Belgium for *Ae. albopictus.* In the meantime introductions through the used tyre and lucky bamboo trade continue and stay important. Similarly, the increasing population of *Ae. japonicus* in Germany has led to the new introductions at the allotment garden in Eupen (EU) in 2017, 2018 and 2019. At EU, *Ae. japonicus* most probably entered through human-facilitated ground traffic originating from colonised areas in West Germany. The import route of the new genetic strain of *Ae. japonicus* at the used tyre import company Havelange (HA) in 2017 was probably long distance import from an unknown external source through the used tyre trade. The origin of *Ae. japonicus* at MM remains unknown.

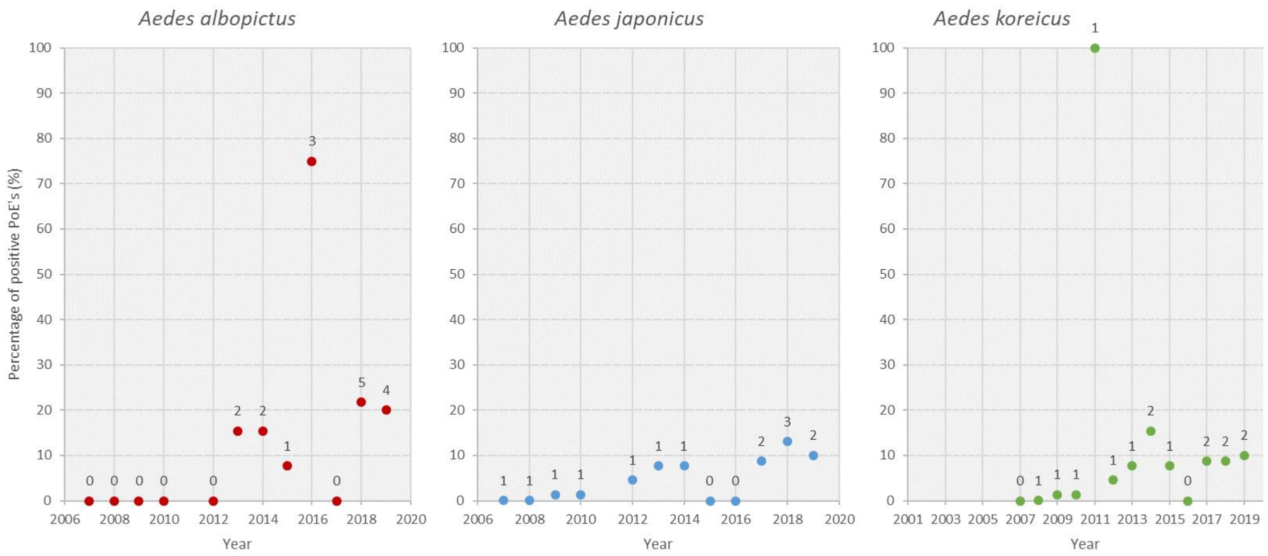
Hence, during the MEMO project a new introduction pathway for Belgium was found, the passive ground transport, which is short-distance transport from *Aedes* populations that are well adapted to our north-western European climate. Therefore, the chance for establishment of *Ae. albopictus* in Belgium highly increases. Further, using passive ground transport exotic *Aedes* species can enter Belgium at a lot of different locations (parking lots, camping places, cities, …). **On top of the still important long-distance EMS import with well-defined PoE's (especially the used tyre and lucky bamboo import companies) now also short-distance import with less defined PoE's occurs**, which has implications for the future monitoring programme.

Figure 33. Overview map of the exotic mosquito species detections between 2000 and 2020 in Belgium with locations of the positive points of entry (PoE), indication of the invasion status per year and of implemented control measures. (color code: red = *Aedes albopictus*, blue = *Aedes japonicus*, green = *Aedes koreicus*, yellow = *Anopheles pharoensis*)



PoE: AB=Tyres AtoB or Agrityre; AL=Airport Liège; AT=Tyres ATB; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E2=Parking lot Hondelange; E5=Parking lot Wanlin; E6=Parking lot Marke; EB=Lucky bamboo Euro Bonsai; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MM=Industrial area Maasmechelen; EU=Allotment garden Eupen.

Figure 34. Percentage of positive points of entry (PoE's) for *Aedes albopictus*, *Aedes japonicus* and *Aedes koreicus* per year between 2007 and 2020 (the number of positive PoE's is indicated above the dot).

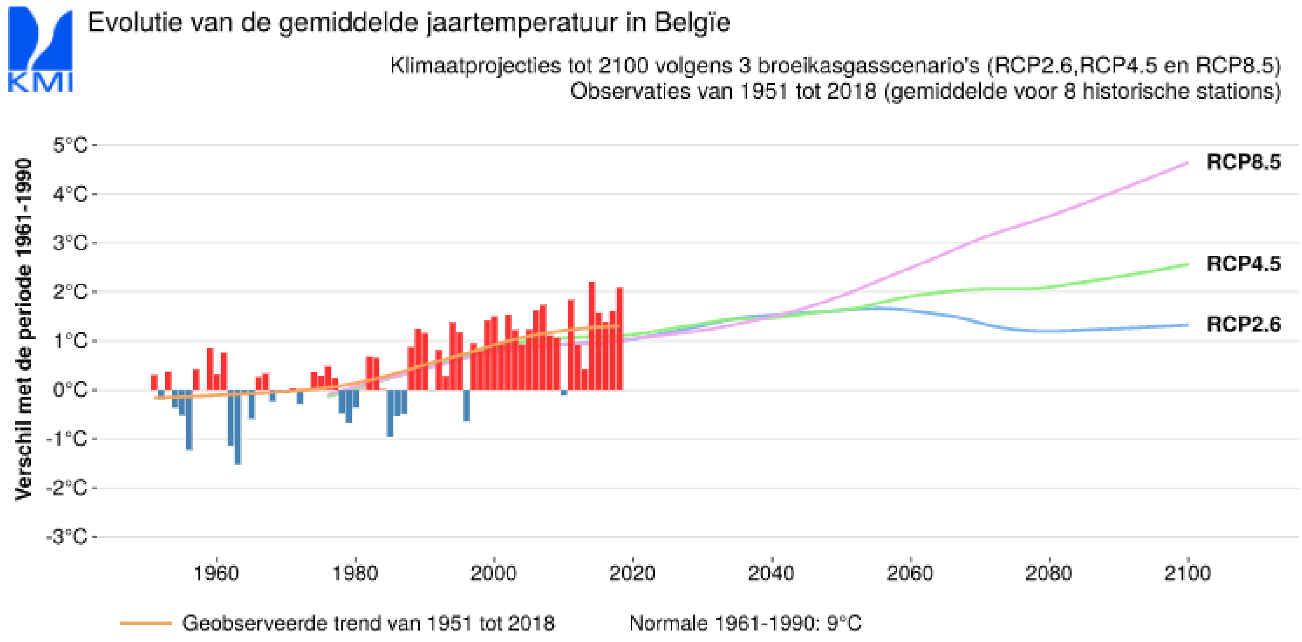


*Number of PoE's surveyed per year: 2007: 975; 2008: 975; 2009: 75; 2010: 74 (MODIRISK project (2007 – 2010)); 2011: 1 (*Ae. koreicus *detected by F. Schaffner in 2010 and 2011 at industrial area); 2012: 22 (EXOSURV project); 2013 – 2015: 13; 2016: 4 (FASFC project (2013 – 2016),* Ae. japonicus *monitored and controlled by Avia-GIS between 2013 and 2016); 2017 – 2018: 23 (MEMO (2017 – 2019)).*

When do EMS enter Belgium?

During the MEMO project, EMS were introduced into Belgium **in all months between May and October**. For *Ae. albopictus* summer reproduction occurred at a used tyre import company (AB) and probably also at two parking lots (E2 and E5). In case local reproduction took place, specimens were found until September. Although multiple *Ae. albopictus* introductions occur and summer reproduction events are observed in Belgium, no overwintering has yet been detected in the past [2] nor during the MEMO project. However, as the average temperature in Belgium is increasing since 1990 and will probably increase even more, especially during the winter [102] (Figure 35), the Belgian weather will become only more suitable for *Ae. albopictus*. **It is only a matter of time before a population becomes established in Belgium.**

Figure 35. Evolution of the average year temperature in Belgium until 2100 according to three global warming scenarios [102].



What collection method is most efficient for EMS?

All collection methods used during the MEMO project collected EMS. Larval sampling (LS) and oviposition trap (OT) sampling collected all exotic *Aedes* species. **Oviposition traps** were not efficient to detect the spread of *Ae. koreicus*, but they did work well to detect the presence of this species at the colonised PoE and to detect the spread of *Ae. japonicus* and *Ae. albopictus*. At parking lots OT also detected *Ae. albopictus* introduction. **Larval sampling** was a very efficient method to detect the spread of the three exotic *Aedes* species. Depending on the EMS species, specific **adult trapping methods** are preferred. The most efficient adult trap for the early detection of *Ae. albopictus* was the BG-Sentinel trap (BG, in combination with CO_2), which is also the gold standard trap for this species [103-105]. Although it was often positioned next to the Mosquito Magnet® trap (MMT, for the CO_2), it captured most individuals. It was also the best collection method for the first detection of *Ae. albopictus* at the used tyre and lucky bamboo import companies. This is logic as it captures the imported host-seeking females or emerged females from imported eggs or larvae. The most efficient adult trap for *Ae. koreicus* was the Frommer updraft gravid trap (GT), confirming previous findings during other monitoring projects [11]. Both the MMT and the GT performed best when collecting *Ae. japonicus*. **As the early detection of *Ae. albopictus* is the main aim of future monitoring, it will be important to continue to use the appropriate collection methods at the PoE's (BG with CO_2, OT and LS).**

The following **operational conclusions** can be drawn from the MEMO project.

The increased introduction of EMS through passive ground transport creates a situation where EMS can enter everywhere in the country with more less-defined and even unknown PoE's in the future. The whole geographical area of Belgium will need to be covered and this is not possible through active monitoring alone. **To make future EMS monitoring more sustainable, passive monitoring will be needed to supplement the active monitoring and make it more focused.** However, the active monitoring at well-known PoE's (e.g. used tyre and lucky bamboo import companies) is still important and needs to continue too.

Active monitoring showed to have a high cost and workload. Especially during the field work workload is high. A large part of the time during the fieldwork goes to driving back and forth to the PoE. In a lot of countries, local partners are trained to handle traps or collect larvae to make active monitoring more sustainable and cost-efficient. In the Netherlands, inspectors from the Netherlands Food and Consumer Product Safety Authority (NVWA) are assisting in active monitoring. In England, local Public Health units handle the traps. During the MEMO project, the feasibility of the assistance of local partners was tested at some PoE's (Belgian Defence at airport Zaventem, directorate of roads (DGO1) at parking lots in Wallonia and Flanders Environment Agency (VMM) at a parking lot in Flanders). Good preparation of the material, training of the local partners, and follow-up is pivotal. Overall, few problems were encountered and samples were mostly delivered in time. The detections of *Ae. albopictus* at the parking lots was done in collaboration with our local partners. **The assistance of local partners in active EMS monitoring showed to be feasible during the MEMO project and should be further developed and officialised in the future monitoring project to make it sustainable and cost-efficient.** Especially when the number of introductions and thus scenario 2 monitoring (both new introduction as locally established) cases will increase.

To conclude, exotic *Aedes* species currently enter Belgium through three pathways: used tyre trade, lucky bamboo trade and passive ground transport. The last one is new for Belgium and creates a new situation with both well-known PoE's and less-defined PoE's. The control management actions at well-known PoE's with long-distance introductions are more straightforward than at less-defined PoE's with short-distance introductions. This will be a new challenge in the coming years for Belgium as established populations of *Ae. albopictus* and *Ae. japonicus* are approaching Belgium and introductions through passive ground transport will become more frequent. This stresses the importance of EMS monitoring and especially of the subsequent control actions in Belgium. It is important to prepare a long-term EMS monitoring programme, following the MEMO project, which takes into account the increase of the number of positive PoE's, the still unknown PoE's and the link with arbovirus surveillance. But what is even as important, is a clear and complete control management plan. Currently, the decision and implementation of control of invasive *Aedes* mosquitoes in Belgium is often ad hoc and not based on different epidemiological and entomological risk scenarios such as described in [106]. Also involvement of local authorities (municipalities, provinces and local Public Health units), social mobilisation and cross-border collaboration is still lacking. Although political awareness is present now, there is an urgent need for an integrated surveillance and control management plan at national and regional levels setting out clear criteria for action, control methods and strategies with appropriate supervision and evaluation.

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6 Annexes

6.1 Annex 1: Proposition of a communication in Layman's terms

Waarom vangen we exotische muggen?

Exotische steekmuggen zoals de tijgermug verspreiden zich door globalisering, en klimaatsopwarming. De tijgermug komt oorspronkelijk uit Zuidoost-Azië maar kon zich intussen in Europa en Amerika vestigen dankzij haar uitstekende aanpassingsvermogen. Ze wordt ook gespot in ons land. De tijgermug is een klein en vinnig beestje dat naast pijnlijke beten ook virusziektes kan overdragen. Als we de exotische muggen op tijd opsporen, kunnen we ze beter bestrijden. Ook als ze erin slagen zich hier te vestigen, is het belangrijk dat we de populaties nauwgezet opvolgen.

Daarom werd het project “Monitoring van Exotische Steekmuggen in België” (MEMO) opgestart vanuit de Belgische overheden. Het project liep drie jaar (juli 2017- juni 2020) en werd gefinancierd door de Vlaamse, Waalse en Brusselse overheden en de FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu in het kader van het nationale samenwerkingsakkoord in de beleidsdomeinen milieu en gezondheid (NEHAP). Het ITG voerde het project uit in samenwerking met het Koninklijk Belgisch Instituut voor Natuurwetenschappen (KBIN) en Barcoding of Organisms and Tissues of Policy Concern (BopCo).

Hoe zijn we te werk gegaan?

Het MEMO project heeft op 20 importplaatsen, verspreid over heel België, muggenvallen gezet. Importplaatsen zijn plaatsen waar exotische muggen mogelijk ons land kunnen binnenkomen, zoals bandencentrales, tuincentra, parkings langs de snelweg vanuit onze zuidelijke buurlanden, havens en luchthavens. We gebruikten verschillende vallen en vangstmethoden om onze kans op de vondst van exotische muggen te vergroten. We identificeerden de muggen op basis van uitwendige kenmerken en gebruikten een genetische analyse om de identificatie te bevestigen. De verzamelde muggen werden ook in een collectie op het KBIN ondergebracht als referentie voor verder onderzoek. Voor het databeheer gebruiken we de VECMAP®-applicatie.

Wat zijn onze resultaten na drie jaar intensieve monitoring?

In totaal hebben we tijdens het MEMO project meer den 52000 steekmuggen gevangen. We spotten drie nieuwe inheemse soorten voor België en vier soorten exotische steekmuggen.

We hebben de tijgermug, *Aedes albopictus*, op zeven verschillende plaatsen in ons land gespot. Deze soort reist graag mee met lucky bamboo-plantjes en tweedehandsbanden, dat blijkt ook uit onze resultaten. Tijdens het onderzoek ontdekten we eveneens dat tijgermuggen ons land bereikten via toegangswegen vanuit onze buurlanden Frankrijk en Duitsland. In de provincies Luxemburg, Namen, Luik en West-Vlaanderen vonden we immers eitjes op snelwegparkings. Belgen die tijdens een autovakantie door landen reizen waar deze mug gevestigd is, kunnen de ongewenste passagier dus onbewust meebrengen. Het was de eerste keer dat deze soort België op die manier bereikte. We hebben de Aziatische bosmug, *Aedes japonicus,* aangetroffen in Namen, aan de Duitse grens in Eupen en een keer in de omgeving Maasmechelen. De waarneming in Eupen houdt verband met gevestigde populaties van *Aedes japonicus* net over de grens in Duitsland. *Aedes koreicus* werd al in 2007 gespot in België en is nu gevestigd in onze contreien (omgeving Maasmechelen). De populatie is echter klein en leidt niet tot overlast. Eén exemplaar van de soort *Anopheles pharoensis*, een Afrikaanse malariamug, werd gevonden op de luchthaven van Luik. De overlevingskans in België is echter klein aangezien het Belgische gematigde klimaat niet geschikt is voor deze soort.

Conclusie

Onze bevindingen tonen aan dat monitoring van exotische steekmuggen in België aan maatschappelijke relevantie blijft winnen. Tijdens het MEMO project hebben we op verschillende plaatsen exotische steekmuggen gespot, waaronder de tijgermug. Deze tijgermug zal zich verder verspreiden naar onze contreien. Enkele tijgermuggen in België betekent niet dat we ons meteen aan een virusuitbraak moeten verwachten, maar als de mug zich kan vestigen en de populatie muggen groeit dan is de kans ook groter dat er lokale overdracht van virussen op gang komt. Dat toont nogmaals het belang aan van de monitoring van exotische steekmuggen en de daaropvolgende preventie en bestrijding in België.

6.2 Annex 2: List with the monitored Points of Entry (PoE)

Type of PoE	Code	Municipality	Province	Latitude	Longitude
Airports	AZ: Zaventem (Brucargo)	Zaventem	Flemish Brabant	50,899057	4,4837193
	AL: Liège (Avia-partner)	Grâce-Hollogne	Liège	50,637785	5,431387
	AC: Charleroi (bagage handling area)	Charleroi	Hainaut	50,469761	4,471325
Ports	PA: Antwerp (Border Inspection Post (BIP) Kallo)	Beveren	East Flanders	51,278834	4,276499
	PZ: Zeebrugge (defense marine base)	Bruges	West Flanders	51,337907	3,203598
	PG: Ghent (Volvo cars)	Ghent	East Flanders	51,159808	3,780577
Platforms for imported used tyres (agriculture, military, industry)	AB: AtoB or Agrityre	Beveren	East Flanders	51,251559	4,217719
	AT: ATB banden	Beveren	East Flanders	51,213511	4,193505
	BA: Bridgestone Aircraft tire (Europe) sa	Frameries	Hainaut	50,412026	3,924913
	MB: Maaslandbanden	Dilsen-Stokkem	Limburg	51,016278	5,695222
	VP: Visé Pneu	Villers-Le-Bouillet	Liège	50,585328	5,259748
	SP: Sarri Pneus	Hamois	Namur	50,335863	5,071636
	HA: Havelange (also colonised area with *Ae. japonicus*)	Hamois	Namur	50,339201	5,04574
Shelters/nursery for imported cutting plants like lucky bamboo	EB: Euro Bonsai	Lochristi	East Flanders	51,111951	3,8360827
	EV: Euroveiling	Brussel	Brussel	50,888243	4,382161
Wholesale markets	ET: Marché matinal Liège	Liège	Liège	50,648771	5,621613
	MC: Marché de Gros Marcinelle (Charleroi)	Charleroi	Hainaut	50,390757	4,451212
Main parking lots at country borders, highways and road axes originating from colonised areas	E0: E25 (A26) Sprimont/Noidré	Sprimont	Liège	50,524368	5,667709
	E1: E411 (A4) Aische-en-Refail	Éghezée	Namur	50,600066	4,7924409
	E2: E25 (A4) Hondelange	Messancy	Luxembourg	49,643818	5,830286
	E3: E411 (A28) Aubange	Aubange	Luxembourg	49,550703	5,808331
	E4: E40 (A3) Raeren	Raeren	Liège	50,717536	6,117821
	E5: E411 (A4) Wanlin	Houyet	Namur	50,144183	5,079253
	E6: E17 (A14) Marke	Kortrijk	West Flanders	50,799111	3,252428
	E7: E17 (A14) Rekkem	Menen	West Flanders	50,764356	3,172908
	E8: E19 (A7) Saint-Ghislain	Saint-Ghislain	Hainaut	50,451261	3,8047735
	E9: E19 (A7) Hensies	Hensies	Hainaut	50,438789	3,670263
Cemeteries and allotment garden at the border with Germany	RA: Cemetery Raeren	Raeren	Liège	50,675553	6,110327
	EU: Allotment garden Eupen	Eupen	Liège	50,632351	6,0732128
	RO: Cemetery Rocherath - Büllingen	Büllingen	Liège	50,432578	6,29456
Colonised area with *Ae. koreicus*	MM: industrial area 'Op de Berg' Maasmechelen	Maasmechelen	Limburg	50,995255	5,621248

6.3 Annex 3: Qualitative risk analysis of the PoE's during the MEMO project

PoE	Import origin				Import volume/frequency				Import method				Import possibility at PoE				Habitat suitability around PoE				Recent import at PoE (2012-2018)				Evidence of import at this PoE type in other countries				Total risk score			
	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019	Mar 2017	Feb 2018	Jun 2018	Jan 2019
MC	2	2	2	2	0,5	0,5	0,5	1[b]	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1			0	0	8,5	8,5	8,5	9
AC	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	0	1[a]	1	1	1	1	1	1			2	2	8	9	11	11
E2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3[b]			2	2	8	8	10	12
E3	2	2			1	0[a]			1	1			1	1			2	2			1	1							8	7		
E4	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1			2	2	8	8	10	10
PA	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	0	0	0	0			1	1	8	8	9	9
PZ	2	2			1	0[a]			1	1			2	2			1	1			1	1							8	7		
ET	2	2	2	2	0,5	0,5	0,5	1[b]	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1			0	0	7,5	7,5	7,5	8
AL	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	0	0	0	0	0	3[a]	3	3			2	2	7	10	12	12
AZ1	2	2			2	2			1	1			2	2			0	0			0	0							7	7		
E7	2	2			2	0[a]			1	1			1	1			1	1			0	0							7	5		
E9	2	2			1	0[a]			1	1			1	1			1	1			1	1							7	6		
EC	2			2	1			1	1			1	2			2	1			1	0			0				0	7			7
EV	2			2	1			1	1			2[b]	2			2	1			1	0			0				2	7			10
PAY	2				0,5				2				0				0,5				1								6			
PAA	2				1				1				0				0,5				1								5,5			
PAM	2				0,5				1				0				0,5				1								5			
VG	0				0				1				0				1				1								3			
BS	0				0				0				0				1				1								2			
AZ2			2	2			2	2			1	1			2	2			0	0			0	0			2	2			9	9
E0				2				2				1				1				1				1				2				10
E1				2				2				1				1				0				1				2				9
E5		2	2	2		2	2	2		1	1	1		1	1	1		2	2	2		1	1	3[b]			2	2		9	11	13
E6		2	2	2		2	2	2		1	1	1		1	1	1		2	2	2		1	1	1			2	2		9	11	11
E8		2	2	2		1	1	1		1	1	1		1	1	1		1	1	1		1	1	1			2	2		7	9	9
PG		2	2	2		2	2	2		1	1	1		2	2	2		0	0	0		0	0	0			1	1		7	8	8

PoE: E0=Parking lot Sprimont/Noidré; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; AL=Airport Liège; AZ1=Airport Zaventem (brucargo); AZ2=Airport Zaventem (luggage handling area); AC=Airport Charleroi; PA=Port of Antwerp (Kallo); PG2=Port of Ghent (Volvo); PZ=Port of Zeebrugge; EC=Wholesale market Brussels; ET=Wholesale market Liège; MC=Wholesale market Charleroi; BS=company St-Katelijne-Waver; EV=Flower auction Euroveiling (Neder-Over-Heembeek); VG=garden center Sint-Katelijne-Waver; PAY=Container company Yang Ming; PAM=Container company Maersk; PAA=Container company APL. [a]*Updated scores in February 2018: AL: recent import of* Anopheles pharoensis*; AC: presence of a cemetery within 500m of the luggage handling area at the airport; PZ: no cruise ships from southern Europe and low frequency of marine ships entering the port; E3, E9 & E7: low frequency of passenger cars stopping at these parking lots.* [b]*Updated scores in January 2019: MC & ET: daily import of products from southern Europe confirmed by some of the importers at the wholesale market; EV: import of stems from Italy in water increases annually; E2 & E5: detection of* Aedes albopictus *in 2018. Selected PoE's indicated in red.*

6.4 Annex 4: Schematic overview of the fieldwork conducted in 2017, 2018 and 2019.

Colour code: Yellow=the start of the field sampling in 2017; Red=assistance of local partners; Bleu=scenario 3 monitoring at and around MM (SCENA3KOR project for ANB); Green=extra monitoring activities (often added following new EMS detections); Black arrows=shifts of sampling events; Cross=non-executed sampling events.

Collection methods: A=adult traps (Mosquito Magnet® trap (MMT), BG-Sentinel trap (BG), Frommer updraft gravid trap (GT) and BG-GAT traps); E=egg sampling (oviposition traps (OT)); L=larval sampling. ^{I}Installation of the adult and egg traps. ^{R}Removal of the adult and egg traps.

Scenarios: SC2a=scenario 2a monitoring; SC2b=scenario 2b monitoring; SC3=scenario 3 monitoring.

Field teams: ITM=Institute of Tropical Medicine; DGO1=Directorate of roads (Wallonia); VMM= Flanders Environment Agency; Defense=Belgian Defense. PoE: PoE's: AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AL=Airport Liège; AT=Tyres ATB; AZ=Airport Zaventem; BA=tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; EB=Lucky bamboo Euro Bonsai; ET=Wholesale Liège; EV=Flower auction Euroveiling; HA=Tyres Havelange; MB=tyres Maaslandbanden; MC=Wholesale Charleroi; MM=Industrial area Maasmechelen; PA=Port of Antwerp; PG=Port of Ghent; PZ=Port of Zeebrugge; RA=Cemetry of Raeren; EU=allotment garden Eupen; RO=Cemetery of Rocherath; SP=Tyres Sarri Pnues; VP=tyres Visé Pneu.

2017

		2017																																																			
Group	PoE	Jan				Feb				Mar					Apr				May					Jun				Jul				Aug					Sep				Oct				Nov					Dec			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	AT,AB,PA,AZ															A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^I,E^I		A, L(AT)		A,E		A,L		A,E		A		A^R,E^R					
2A	MC,AC,BA															A,E				A,E				A,E				A,E				A,E				A,E				A,E				A,E				A^R,E^R					
2B	MC,AC																	A,L				A				A,L				A				A^I,X,E^I				A				A,L				A							
2C	BA																	A,L				A				A,L				A				A^I,→,E^I				A,L				A,L				A							
	E9																	E,L				E				E,L				E				E^I,X				E				E,L				E^R							
3	EB	A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^I,E^I		A,L		A,E		A,L		A,E		A		A,E		A,L		A,E	
	PZ															A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^I,E^I		A		A,E		A,L		A,E		A		A^R,E^R					
	E7																	E,L				E				E,L				E				E,L				E				E,L				E^R							
4	E2,E3															E				E,L				E				E,L				E				E^I,X				E				E,L				E^R					
5	RA,RO																		E				E				E				E				E^I,X				E,L				E				E^R						
	E4																		E,L				E				E,L				E				E^I,X				E				E,L				E^R						
6	MB																A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A^I,X,E^I		A,E		A		A,E		A,L		A,E		A		A^R,E^R				
	MM (SC2b)																				E				E				E				E,L				E,L				E												
7	VP,ET,AL																A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A^I,X,E^I		A,E		A		A,E		A,L		A,E		A		A^R,E^R				
8	HA,SP																A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A^I,X,E^I		A,E		A		A,E		A,L		A,E		A		A^R,E^R				
	HA (SC2b)																				E				E				E				E,L				E,L				E												

2018

Group	PoE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
		Jan				Feb				Mar					Apr				May					Jun				Jul				Aug					Sep				Oct				Nov					Dec			
1A	AB																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A^R,E^R				
	AB (SC2a)																																A^I,E^I		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A^R,E^R				
	AT																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E^R		A^R						
	PA																A^I,E^I		A,L		A,E		A		A^R,E^R,L																												
1B	AZ																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E^R		A^R						
2A	AC																A^I,E^I				A,E				A^R,E^R				A^I,E^I				A,E				A,E				A,E				A,E^R								
	MC																E^I,L				E				E^R,L																												
2B	AC																		A,L				A				X				A				A,L				A				A,L				A^R						
	BA																		A,L				A				A,L				A				A,E,L				A,E,L				A,E,L				A^R						
2C	BA																A^I,E^I				A,E				A,E				A,E				A,E				A,E				A,E				A,E^R								
	BA (SC2a)																																A^I,E^I		A,E,L		A,E		A,E,L		A,E		A^R,E^R,L										
	E8																E^I,L				E				E,L				X				→	E			E				E				→	→	E^R						
3A	EB	A		A,E,L		A,L		A,E		A		A,E,L		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E	
	EB (omg)																																	X				→	→	→	→	L											
	PG																	A^I,E^I		A,L		A,E		A		A^R,E^R,L																											
3B	E6																	E^I,L				E				E				E				E				E				E				E^R							
4	E2																		E^I,L				E				E				E				E,L				E				X,L				E				E^R		
	E5																		E^I,L				E				E				→	E			E,L				→	E			X,L				E				E^R		
5A	EU (SC2a)												L								A^I,E^I		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A^R,E^R,L										
	RO																																L				L																
5B	E4																E^I,L				→	E			E,L				E				→	E			E				E		E^R										
6	MB																	A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
	MM (SC3)																	A^I,L		A		A		A		A,L		A		A		A		A,L		A		A		A		A,L		A		A		A		A,L		A	
	MM (SC3)																																			L				L													
7	VP,AL																	A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
	ET																	A^I,E^I		A,L		A,E		A		A^R,E^R																											
8	HA,SP																	A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
	HA (SC2b)																					E^I				E				E				E →	→	L		X →	→	L		E^R											

2019

Group	Field team	PoE	Jan				Feb				Mar					Apr				May					Jun				Jul				Aug					Sep				Oct				Nov					Dec			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	ITM1	AB (SC2a)												L			L	A^I,E^I		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A^R,E^R								
2	ITM1	EB	A		A,E		A,L		A,E		A		A,E		A,L		A,E	A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E,L		A,L		A,E		A		A,E		A,L		A,E		A
3	ITM1/VMM	E6																E^I				E				E				E				E				E				E				E,L		E^R						
4	ITM1/DGO1	E8																E^I				→	E			E				→	E			→	→	E		→	→	→	E	X				E^R								
5	ITM1/Defense	AZ																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E^R		A^R						
6	ITM2	BA																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,X		→	A^R,E^R							
		AC																A^I,E^I		A →	→	A,E	→	A,L		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,X		→	A^R,E^R							
7	ITM2	HA																A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A^R,E^R						
		HA (SC2a)																E^I		L		E				E		L		E				E		L		E				E		L		E		E^R						
		HA (SC2a)																																				E		E,L		E		E,L		E,L		E^R						
8	ITM2	RO																																L				L																
9	ITM2/DGO1	E0																E^I				E				E				E				E				E				E				→	E^R							
		E4																E^I				E				E				E				E				X				→	E			→	→	E^R						
10	ITM1	EV																	A^I,E^I,L	←	← A		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
11	ITM1	AT																	A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
		AB												L			L		A^I,E^I		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A^R,E^R							
12	ITM1	MB																	A^I,E^I		A,L		A,E		A,L	←	A,E	←	← A		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
		MM (SC3)	A		A		A,L		A		A		A		A,L		A		A^R																																			
		MM (SC1)																									A^I,E^I		A,X		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
13	ITM2	EU (SC2a)						→	→	L			L						A^I,E^I		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A,E,L		A,E		A^R,E^R,L									
14	ITM2	VP,AL																	A^I,E^I		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A,E		A		A,E		A,L		A^R,E^R							
15	ITM2/DGO1	E2												L		E^I,L	←				E				E				E				E				E				E				E				E^R					
		E5												L		E^I,L	←				E				E				E				E				E				→	E			E				E^R					
16	ITM1	E5 (SC2a)																																				L		L				L										
		E1																																						E,L				E,L			E^R	←						

6.5 Annex 5: Overview of the mosquito specimens included in the morphological reference collection at RBINS

		Point of entry																										
	Species	AB	AC	AL	AT	AZ	BA	E2	E3	E4	E5	E7	E8	EB	ET	EU	HA	MB	MC	MM	PA	PG	PZ	RA	RO	SP	VP	Total number of specimens
	Ae. albopictus	2																										2
	Ae. annulipes/cantans	1																										1
	Ae. caspius/dorsalis	1		1																1								3
	Ae. cinereus/geminus																	2		1								3
	Ae. communis																	1									1	2
	Ae. geniculatus				8		2										2	2								10	3	27
	Ae. japonicus																7			6								13
	Ae. koreicus																	2		10								12
	Ae. punctor															4		6		2								12
	Ae. rusticus																	2										2
Females (n=255)	*Ae. sticticus*																	1										1
	Ae. vexans																	10		2							4	16
	An. claviger				3		2									1		1			1	1	1				2	12
	An. maculipennis s.l.	1																2		2								5
	An. pharoensis			1																								1
	An. plumbeus	1			8		1										6	2		1						10		29
	Cq. richiardii															6		2		3								11
	Cx. hortensis																										1	1
	Cx. pipiens/torrentium	7	1	2	5	2	7							1	2	8	3	4		5	4	2	4			6	7	70
	Cx. torrentium						1																					1
	Cs. annulata	2		1	2		2									2		2		2	6		2			1	2	24
	Cs. morsitans				1															6								7
	Ae. geniculatus				1												2											3
	Ae. japonicus																7			1								8
	Ae. koreicus																			4								4
Males (n=46)	*An. maculipennis s.l.*	1																								2		3
	An. plumbeus	1			1												2									1		5
	Cq. richiardii															2												2
	Cx. pipiens/torrentium	2	1		1		1							2		1	1	6		1						2	2	20
	Cs. longiareolata						1																					1
	Ae. albopictus	4									1																	5

	Species	Point of entry																										Total number of specimens
		AB	AC	AL	AT	AZ	BA	E2	E3	E4	E5	E7	E8	EB	ET	EU	HA	MB	MC	MM	PA	PG	PZ	RA	RO	SP	VP	
Larvae (n=231)	*Ae. annulipes/cantans*												1															1
	Ae. geniculatus				5												7			1						8	1	22
	Ae. japonicus															2	5											7
	Ae. koreicus																			19								19
	An. claviger				4																					1		5
	An. maculipennis s.l.	2					4										2			3	1				2		1	15
	An. plumbeus						8									3	5			2						2		20
	Cx. hortensis														5		4										1	10
	Cx. modestus	1																										1
	Cx. pipiens/torrentium	3	2	2	3	3	3	2	1	8	2	2			5	2	8	4	4	11	1	2	1	3	4	17	3	96
	Cs. annulata	1			3		4		1				2		2	2	2	2		2	1					2	2	26
	Cs. longiareolata																			1						1		2
	Cs. morsitans									1																	1	2
Eggs (n=4)	*Ae. japonicus*																2											2
	An. spp.													2														2
Pupae (n=3)	*Ae. spp.*																			1								1
	Cx. pipiens/torrentium															1												1
	Cx. spp.													1														1
		30	**4**	**7**	**45**	**5**	**36**	**2**	**2**	**9**	**3**	**2**	**3**	**6**	**14**	**34**	**65**	**51**	**4**	**87**	**14**	**5**	**8**	**3**	**6**	**63**	**31**	**539**

PoE code: AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AL=Airport Liège; AT=Tyres ATB; AZ=Airport Zaventem; BA=tyres Bridgestone Aircraft Tire (Europe) sa; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; EB=Lucky bamboo Euro Bonsai; ET=Wholesale market Liège; EU=allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MC=Wholesale market Charleroi; MM=Industrial area Maasmechelen; PA=Port of Antwerp (Kallo); PG=Port of Ghent (Volvo); PZ=Port of Zeebrugge; RA=Cemetery of Raeren; RO=Cemetery of Rocherath; SP=Tyres Sarri Pneus; VP=Tyres Visé Pneu.

6.6 Annex 6: Mosquito biodiversity at the PoE's

	Point of Entry (PoE)																														
	AB	AC	AL	AT	AZ	BA	E0	E1	E2	E3	E4	E5	E6	E7	E8	E9	EB	ET	EU	EV	HA	MB	MC	MM	PA	PG	PZ	RA	RO	SP	VP	31 PoE
Ae. albopictus	√					√	√		√			√	√				√															7
Ae. annulipes/cantans	√			√							√				√				√													5
Ae. caspius/dorsalis	√		√																					√								3
Ae. cinereus/geminus																			√			√		√								3
Ae. communis				√																		√		√							√	4
Ae. detritus																											√					1
Ae. geniculatus	√			√		√	√		√			√			√		√	√	√		√	√		√						√	√	15
Ae. japonicus																			√		√			√				√				4
Ae. koreicus																						√		√								2
Ae. punctor																			√			√		√								3
Ae. rusticus			√								√										√	√										4
Ae. sticticus				√		√													√		√	√		√							√	7
Ae. vexans	√																		√			√		√							√	5
An. atroparvus	√			√																												2
An. claviger	√			√		√											√		√		√	√			√	√	√			√	√	12
An. daciae	√			√		√															√	√		√	√							7
An. maculipennis	√	√				√													√		√	√		√					√	√		9
An. maculipennis s.l.	√					√													√		√	√		√	√				√	√	√	10
An. pharoensis			√																													1
An. plumbeus	√		√	√		√											√	√	√		√	√		√						√	√	12
Cq. richiardii	√																		√			√		√							√	5
Cx. hortensis																		√	√		√										√	4
Cx. modestus	√																															1
Cx. pipiens/torrentium	√	√	√	√	√	√		√	√	√	√	√		√			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	27
Cx. territans	√																		√													2
Cx. torrentium						√													√			√		√							√	5
Cs. annulata	√		√	√		√				√					√			√	√		√	√		√	√		√			√	√	15
Cs. fumipennis																								√								1
Cs. longiareolata						√																		√					√	√		4
Cs. morsitans	√			√							√													√							√	5
Total	**17**	**2**	**6**	**11**	**1**	**12**	**2**	**1**	**3**	**2**	**4**	**3**	**1**	**1**	**3**	**0**	**5**	**5**	**17**	**1**	**12**	**17**	**1**	**20**	**5**	**2**	**4**	**2**	**4**	**8**	**13**	

PoE code: AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AL=Airport Liège; AT=Tyres ATB; AZ=Airport Zaventem; BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; EB=Lucky bamboo Euro Bonsai; EV=Flower auction Euroveiling; ET=Wholesale market Liège; EU=Allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MC=Wholesale market Charleroi; MM=Industrial area Maasmechelen; PA=Port of Antwerp (Kallo); PG=Port of Ghent (Volvo); PZ=Port of Zeebrugge; RA=Cemetery of Raeren; RO=Cemetery of Rocherath; SP=Tyres Sarri Pneus; VP=Tyres Visé Pneu.

6.7 Annex 7: The number of individuals (N° indiv) of all mosquito species captured per PoE and per sampling method, with indication of the number of traps and samplings and the EMS species captured

Collection methods: MMT=Mosquito Magnet® trap; BG=BG-Sentinel trap; GT=Frommer updraft gravid trap; BG-GAT=gravid Aedes trap; LS=larval sampling; PBS=potential breeding site; OT=oviposition traps.

For larval sampling, the number of potential breeding sites (N° PBS) with the total number of inspected containers (N° containers) and samplings (N° samplings) (i.e. total number of times the PBS were sampled) is given.

For adult sampling, the number of samplings (N° samplings) is the number of samplings (period of 2 weeks) correctly executed/number of samplings planned.

For egg sampling, the number of samplings (N° samplings) is the number of samplings (periods of 2 or 4 weeks) with polystyrene piece found back in trap/number of samplings planned.

Scenario 1 and 2 (or 3 (MM)) monitoring included.

Presence of EMS is indicated in colour: red=*Aedes albopictus*; blue=*Aedes japonicus*; green=*Aedes koreicus*; yellow=*Anopheles pharoensis*

PoE code: AB=Tyres AtoB or Agrityre; AC=Airport Charleroi; AL=Airport Liège; AT=Tyres ATB; AZ=Airport Zaventem (brucargo (AZ1, 2017) and luggage handling area (AZ2, 2018 & 2019)); BA=Tyres Bridgestone Aircraft Tire (Europe) sa; E0=Parking lot Sprimont/Noidré; E1=Parking lot Aische-en-Refail; E2=Parking lot Hondelange; E3=Parking lot Aubange; E4=Parking lot Raeren; E5=Parking lot Wanlin; E6=Parking lot Marke; E7=Parking lot Rekkem; E8=Parking lot Saint-Ghislain; E9=Parking lot Hensies; EB=Lucky bamboo Euro Bonsai; EV=Flower auction Euroveiling; ET=Wholesale market Liège; EU=Allotment garden Eupen; HA=Tyres Havelange; MB=Tyres Maaslandbanden; MC=Wholesale market Charleroi; MM=Industrial area Maasmechelen; PA=Port of Antwerp (Kallo); PG=Port of Ghent (Volvo); PZ=Port of Zeebrugge; RA=Cemetery of Raeren; RO=Cemetery of Rocherath; SP=Tyres Sarri Pneus; VP=Tyres Visé Pneu.

2017		Used tyre import company							Lucky bamboo company	Airport			Port & Whole sale market				Parking lot at country border and highway					Border with Germany		Colonised area *Ae. koreicus*	Total
		AB	AT	BA	HA	MB	SP	VP	EB	AC	AL	AZ	PA	PZ	ET	MC	E2	E3	E4	E7	E9	RA	RO	MM	
Adults	**N° indiv**	**19**	**696**	**131**	**98**	**62**	**681**	**399**	**7**	**34**	**15**	**5**	**62**	**402**	**13**	**2**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**2626**
BG	N° indiv	11	163	40	10	33	30	121	1	34															443
	N° traps	1	1	1	1	1	1	1	1	1															9
	N° samplings	5\6	6\6	7\7	7\7	7\7	7\7	6\7	10\10	7\7															62\64
MMT	N° indiv	8	533	91	88	29	651	278	6		15	5	62	402	13	2									2183
	N° traps	1	1	1	1	1	1	1	1		1	1	1	1	1	1									14
	N° samplings	4\6	5\6	7\7	4\7	7\7	7\7	6\7	9\9		7\7	5\6	6\6	6\6	5\7	5\7									83\95
Larvae	**N° indiv**	**36**	**122**	**82**	**619**	**173**	**153**	**176**	**0**	**2**	**0**	**22**	**56**	**27**	**73**	**39**	**0**	**4**	**115**	**22**	**0**	**140**	**169**	**1006**	**3036**
LS	N° PBS	3	2	4	37	1	3	3	5	6	4	4	3	5	3	7	4	4	9	4	2	6	4	24	*17
	N° containers	74	427	45	254	152	75	93	1202	62	14	10	8	29	9	10	6	21	13	6	7	128	219	128	2992
	N° samplings	3	4	8	37	1	3	3	10	6	4	4	3	5	3	7	4	4	9	4	2	7	4	24	159
Eggs	**N° indiv**	**0**	**38**	**1**	**136**	**0**	**84**	**50**	**15**	**0**	**0**	**12**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**60**	**0**	**0**	**148**	**544**
OT[1]	N° traps	10	10	10	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	20	20	40	290
	N° samplings	23\30	25\30	37\47	116\161	28\40	32\40	36\40	55\60	33\40	40\40	25\30	22\30	12\31	32\41	36\42	13\30	17\30	19\30	13\30	19\30	35\60	23\60	63\116	954\1088
Total		55	856	214	853	235	918	625	22	36	15	39	118	429	86	41	0	4	115	22	60	140	169	1154	6206

2018		Used tyre import company							Lucky bamboo company	Airport			Port & Whole sale market				Parking lot at country border and highway					Border with Germany		Colonised area *Ae. koreicus*	Total
		AB	AT	BA	HA	MB	SP	VP	EB	AC	AL	AZ	PA	PG	ET	MC	E2	E4	E5	E6	E8	EU	RO	MM	
Adults	**N° indiv**	**556**	**2033**	**333**	**1454**	**704**	**2418**	**1072**	**30**	**67**	**22**	**8**	**81**	**11**	**3**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**276**	**n.a.**	**1098**	**10166**
BG	N° indiv	505	460	229	87	391	673	471	27	67		8													2918
	N° traps	4	1	4	1	1	11	1	1	1		1													26
	N° samplings	38\40	15\15	30\30	14\14	14\14	14\14	14\14	24\24	13\13		11\15													187\193
MMT	N° indiv	46	1573	104	1367	313	1745	601	3		22		81	11	3							128		398	6395
	N° traps	1	1	1	1	1	1	1	1		1		1	1	1							2		2	16
	N° samplings	10\16	14\15	12\15	11\14	14\14	13\14	14\14	25\25		14\14		4\4	4\4	2\4							20\22		28\28	
GT	N° indiv																					148		688	836
	N° traps																					1		1	2
	N° samplings																					10\11		14\19	24\30
BG-GAT	N° indiv	5																						12	17
	N° traps	3																						2	5
	N° samplings	14\15																						20\20	34\35
Larvae	**N° indiv**	**521**	**397**	**1259**	**2287**	**1233**	**1173**	**868**	**11**	**96**	**3**	**36**	**0**	**45**	**110**	**123**	**74**	**10**	**33**	**0**	**6**	**3620**	**857**	**2926**	**15688**
LS	N° PBS[1]	19	4	17	47	3	4	5	12	14	6	6	3	1	4	7	5	12	2	3	7	14	5	45	*17
	N° containers	620	774	170	1065	216	316	506	1961	275	35	77	8	62	17	20	27	22	39	7	49	607	501	898	8272
	N° samplings	60	10	40	66	8	13	13	36	17	13	24	3	2	7	9	14	12	5	3	7	65	10	75	512
Eggs	**N° indiv**	**43**	**132**	**3**	**546**	**15**	**205**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**160**	**1**	**72**	**0**	**0**	**199**	**n.a.**	**n.a**	**1376**
OT	N° traps	50	10	30	50	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	22			322
	N° samplings	330\341	58\70	195\200	219\238	69\70	68\70	52\70	123\130	52\60	70\70	70\70	20\20	17\20	20\20	19\20	58\70	64\70	66\70	62\70	49\60	230\242			1911/2051
Total		1120	2562	1595	4287	1952	3796	1940	41	163	25	44	81	56	113	123	234	11	105	0	6	4095	857	4024	27230

2019-2020		Used tyre import company						Lucky bamboo company	Airport			Flower auction	Parking lot at country border and highway							Border with Germany		Colonised area *Ae. koreicus*	Total
		AB	AT	BA	HA	MB	VP	EB	AC	AL	AZ	EV	E0	E1	E2	E4	E5	E6	E8	EU	RO	MM	
Adults	**N° indiv**	**1328**	**1323**	**221**	**3407**	**289**	**2615**	**20**	**39**	**13**	**6**	**23**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**610**	**n.a.**	**905**	**10799**
BG	N° indiv	1244	374	108	828	112	1427	12	39		6	23										765	4938
	N° traps	4	1	1	1	1	1	1	1		1	1										1	14
	N° samplings	55\56	14\14	14\14	15\15	13\14	12\14	29\30	14\14		14\15	13\14										9\10	202\210
MMT	N° indiv	84	949	113	2579	177	1188	8		13										220		5	5336
	N° traps	1	1	1	1	1	1	1		1										2		1	11
	N° samplings	11\14	11\14	13\14	15\15	11\14	12\14	29/30		9\14										22\26		2\3	135\158
GT	N° indiv																			390		135	525
	N° traps																			1		1	2
	N° samplings																			12\13		15\18	27\31
BG-GAT	N° indiv																					0	0
	N° traps																					2	2
	N° samplings																					16\16	16\16
Larvae	**N° indiv**	**374**	**335**	**81**	**1327**	**236**	**374**	**0**	**1**	**1**	**21**	**0**	**n.a.**	**158**	**0**	**n.a.**	**187**	**0**	**n.a.**	**2346**	**361**	**43**	**5845**
LS	N° PBS	6	5	2	65	4	5	4	6	4	2	5		3	2		5	1		12	7	9	*17
	N° containers	826	406	49	596	267	218	2334	111	25	11	155		21	2		90	2		798	341	152	6404
	N° samplings	41	13	6	101	14	14	28	14	7	2	11		5	2		11	0		84	14	18	385
Eggs	**N° indiv**	**0**	**0**	**0**	**1661**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**138**	**0**	**52**	**0**	**60**	**64**	**11**	**19**	**n.a.**	**392**	**2398**
OT	N° traps	33	10	10	30	10	10	10	10	10	10	10	10	10	10	10	10	10	10	22		10	255
	N° samplings	444\453	69\70	69\70	176\180	70\70	70\70	145/150	68\70	68\70	70\70	68\70	64\70	18\20	65\80	53\60	77\81	66\70	45\60	283\286		49\50	2037/2120
Total		1702	1658	302	6395	525	2989	20	40	14	28	23	138	158	52	0	247	64	11	2975	361	1340	19042

6.8 Annex 8: List of outputs and ad hoc deliverables

6.8.1 Scientific and press output

- **Peer reviewed papers**
  - Ibáñez-Justicia A, Smitz N, den Hartog W, van de Vossenberg B, De Wolf K, Deblauwe I, Van Bortel W, Jacobs F, Vaux AGC, Medlock JM, Stroo A. **Detection of Exotic Mosquito Species (Diptera: Culicidae) at International Airports in Europe**. *Int. J. Environ. Res. Public Health* **2020**, *17*, 3450.
  - De Wolf K *et al.* **First detections of *Culiseta longiareolata* in Belgium and the Netherlands** (working title). Collaboration with Centre for Monitoring of Vectors in the Netherlands. In preparation.
  - De Wolf K *et al.* **First detection of the West Nile vector *Culex modestus* in Belgium** (working title). In preparation.
  - De Wolf K *et al.* **Updated checklist of the mosquito fauna in Belgium including a molecular identification workflow** (working title). In preparation.
  - Deblauwe I *et al.* **Overview of invasive *Aedes* mosquito surveillance in Belgium between 2007 and 2020** (working title). In preparation.
  - Deblauwe I *et al.* **Monitoring approaches of exotic *Aedes* mosquitoes in Belgium.** In preparation.
  - Smitz N *et al.* **Molecular identification of species of the *Anopheles maculipennis* group (Diptera: Culicidae) in Belgium** (working title). In preparation.
  - Smitz N *et al.* **Population genetic structure of the Asian bush mosquito, *Aedes japonicus* (Diptera, Culicidae), in Belgium suggests multiple introductions** (working title). In preparation.
  - Vanderheyden A *et al.* **Distribution of WNV vector *Culex pipiens* (Diptera, Culicidae) in Belgium with reference to biotypes *Cx. p. pipiens* and *Cx. p. molestus*** (working title). In preparation.
- **Presentations at meetings**
  - Smitz N, Deblauwe I, Dekoninck W, De Wolf K, Verlé I, De Witte J, Vanslembrouck A, Meganck K, Gombeer S, Van Bourgonie YR, De Meyer M, Backeljau T, Coosemans M, Van Bortel W: **Monitoring of Exotic Mosquitoes in Belgium (MEMO). Molecular validation of morphological species identifications** In: *24th Congress of Zoology: Genotype-phenotype map: from model systems to ecosystems.* Wageningen University, the Netherlands; 2017.
  - Van Bortel W: **Detection and identification of mosquito species (Culicidae) in Belgium**. In: *LifeWatchbe users and stakeholders meeting.* Flanders Marine Institute, Belgium; 2018.
  - De Wolf K, Smitz N, Deblauwe I, Vanslembrouck A, Meganck K, Gombeer S, Van Bourgonie YR, Verlé I, Schneider A, De Witte J, Dekoninck W, Backeljau T, De Meyer M, Van Bortel W: **The state of the art of the exotic mosquito species (Diptera: Culicidae) in Belgium: new invasion pathways detected for Belgium.** In: *21st European Society for Vector Ecology (E-SOVE)*: '*Arthropod Vector Science for the benefit of society: Educate, Empathize, Engage*'. Palermo, Italy; 2018.
  - Deblauwe I, De Wolf K, Verlé I, Schneider A, De Witte J, Vanslembrouck A, Dekoninck W, Smitz N, De Meyer M, Backeljau T, Van Bortel W: **Exotic mosquito species surveillance in Belgium: an overview from 2007 – 2018**. In: *21st European Society for Vector Ecology (E-SOVE)*: *Arthropod Vector Science for the benefit of society: Educate, Empathize, Engage*. Palermo, Italy; 2018.

- Vanslembrouck A, De Wolf K, Deblauwe I, Verlé I, Schneider A, De Witte J, Smitz N, De Meyer M, Van Bortel W, Backeljau T, Dekoninck W: **Constructing a morphological reference collection of native and exotic mosquito species in Belgium**. In: *21st European Society for Vector Ecology (E-SOVE)*: *Arthropod Vector Science for the benefit of society: Educate, Empathize, Engage*. Palermo, Italy; 2018.
- Deblauwe I, De Wolf K, Smitz N, Verlé I, Schneider A, De Witte J, Vanslembrouck A, Dekoninck W, Meganck K, Gombeer S, Van Bourgonie YR, Backeljau T, De Meyer M, Van Bortel W, Pollet M: **Belgium on the lookout for exotic mosquito species (Diptera: Culicidae).** In: *9th International Congress of Dipterology*. Windhoek, Namibia; 2018.
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- **Press and other communication**
   - Press release on 3 October 2017. Announcement of the MEMO project and demonstration of traps in the field. (https://www.itg.be/E/Article/institute-of-tropical-medicine-on-the-lookout-for-tiger-mosquitoes). In response to the press release different interviews were given and press articles are published.
   - Press release on 7 June 2018. Results 2017 (https://www.itg.be/E/Article/no-tiger-mosquitoes-spotted). In response to the press release different interviews were given and press articles are published.
   - Interview with Isra Deblauwe in magazine 'Libelle': « Hallo wereld: muggenplaag ». In June 2018.
   - Interview with Isra Deblauwe in Radio 1 programme 'Weetikveel' on 31 July 2018 about mosquitoes in general.
   - Interview with Isra Deblauwe in P3-magazine from ITM: « The tour of Belgium: in search of exotic mosquitoes ». In October 2018.
   - Press release on 22 November 2018. Results 2018 (https://www.itg.be/E/Article/new-sightings-of-tiger-mosquito-in-belgium). In response to the press release different interviews were given and press articles are published.
   - Announcement of MEMO web page on the ITM website on 22 November 2018: https://www.itg.be/E/memo-mosquito-monitoring.
   - Science day at ITM on 25 November 2018: « Who is the mosquito? » (https://www.itg.be/E/Event/dag-van-de-wetenschap)
   - Interview with Isra Deblauwe in magazine 'Knack': « Exotische muggen, overdragers van tropische ziekten komen naar ons land. Hoed u voor de tijgermug ». In January 2019.
   - Press release on 5 March 2019. Scientists predict spread of tropical mosquitoes. https://www.itg.be/E/Article/scientists-predict-spread-of-tropical-mosquitoes. In response to the press release different interviews were given and press articles are published.
   - Interview with Katrien De Wolf in magazine 'Goed Gevoel': « Laat ze een toontje lager zoemen ». In May 2019.
   - Press release on 11 June 2019. Start of field season and demonstration of traps in the field (https://www.itg.be/E/Article/tiger-mosquito-travels-down-the-motorway). In response to the press release different interviews were given and press articles are published.
   - Interview with Wim Van Bortel in magazine 'Humo': « De tropen in Europa: de verraderlijke streken van virussen, vleermuizen, tijgermuggen en reuzenteken ». In July 2019.

- Article by Nathalie Smitz for BFSO Magazine : « BopCo – MEMO: Monitoring of Exotic Mosquitoes; L'ADN, un outil pour l'identification d'espèces de moustiques exotiques recensés en Belgique ». In August 2019.
- Press release on 28 November 2019. Results 2019 (https://www.itg.be/E/Article/tiger-mosquitos-spotted-in-belgium-in-2019). In response to the press release different interviews were given and press articles are published.
- Interview with Isra Deblauwe in magazine 'Goed Gevoel' (will appear mid-July 2020). In June 2020.
- Podcast for EOS, interview with Isra Deblauwe: « Waarom moeten muggen mij altijd hebben? » (https://www.eoswetenschap.eu/natuur-milieu/waarom-moeten-muggen-mij-altijd-hebben?fbclid=IwAR1Xqc7HJOtzE2JztjKqSsLJlLj0JN1f4TpnL_--rlDALaHp_XC3bduiOt8). In June 2020.
- Interview with Katrien De Wolf in Radio 2 (Vlaams Brabant & Brussel) programme 'Start je dag' on 18 June 2020 about mosquitoes in general.

6.8.2 Ad hoc deliverables

- **VectorNet**
  - On 22 May 2018 the detection of *Aedes japonicus* at HA and RA and of *Ae. koreicus* at MM were submitted to VectorNet.
  - On 29 November 2018 the detections of *Ae. albopictus* at AB, BA, EB, E2 and E5, of *Ae. japonicus* at HA and EU and of *Ae. koreicus* at MM were submitted to VectorNet.
  - Attendance of the VectorNet Kick-off meeting 10-12 March 2020, Amersfoort, the Netherlands
  - The EMS detections of 2019 will be submitted to VectorNet in June 2020.
- **Workshops**
  - **"Introduction to the ecology & morphological identification of mosquitoes" for the Belgian Defence** was organised on 22 and 23 March 2018 at ITM.
  - **"Introduction to DNA barcoding" for the members of the SC** was organised on 30 August 2018 at RMCA.
  - **"Introduction to the identification of mosquito species using DNA-based technologies" for the ITM staff** was organised on Monday 25 and Tuesday 26 February 2019 at RMCA.
  - **"Challenges in vector surveillance & control"** was organised **for the staff of ITM**, German and Nepalese colleagues on 27, 28 and 29 March 2019 at ITM.
  - **MEMO Webinar was organised on 28 April 2020:** the results and lessons learned from the MEMO project were presented, experiences from neighbouring countries (Germany and the Netherlands) was presented and recommendations were given for future EMS surveillance in Belgium.
- **Input and advice for the regional/local governments on control management**
  - Meeting with **ANB** on the management and approach of *Ae. koreicus* control in Maasmechelen on 8 March 2018.
  - Revision of the **SPW** control plan of *Ae. japonicus* at Havelange (PoE HA) in December 2018.
  - Meeting with **SPW and Mayor of Hamois** on the management and approach of *Ae. japonicus* control at Havelange (PoE HA) on 26 September 2019.
  - Synchronisation of monitoring and control at PoE AT, AB, EB and MM: information exchange between Rentokil (ANB) and ITM at the PoE premises in 2019.
- **Field visits by external people**

- **Marc Pollet** joined the field team at the used tyre import companies HA and SP on 16 August 2018.
- **Wietse Den Hartog** from the Centre for Monitoring of Vectors in the Netherlands joined the field team at the industrial area MM and the used tyre import company MB on 14 August 2018.
- **Olivier Beck** from Environment Brussels joined the field team at the flower auction Euroveiling on 16 July 2019.

- **Communication of the results to the PoE**
  - Between 7 and 18 May 2018 an e-mail was sent to each PoE (including communities in case of a scenario 2b monitoring) with an overview of the results of 2017.
  - Between 7 February and 16 May 2019 an e-mail was sent to eight PoE (E2, E4, E5, E6, E8, AZ, AC, AB) with an overview of the results of 2018. The other PoE will get a small report on the results of 2018 together with the results of 2019 in June 2020.
- **Ad hoc questions by email**:
  - Request by Dominique Wagner and Daniel Reynders to produce an overview of the control measures of exotic mosquitoes in Belgium on 22 February 2018.
  - Review of the exotic mosquito species for the TrIAS (Tracking Invasive Alien Species) project on 17 April 2018 and 8 January 2020.
  - Parliamentary question regarding *Ae. albopictus* on 22 May 2018.
  - Request by Dominique Wagner to produce a map of Belgium with a summary of all exotic mosquito species detections in 2018 (and past) and the current scenario's and implemented control measures.
- **Training Schools**
  - Isra Deblauwe participated and shared experiences and knowledge of mosquito surveillance in the 1st AIM-COST training school 'Harmonizing *Aedes* Invasive Mosquito (AIM) surveillance across Europe'; Akrotiri, Cyprus, 13-17 Jan 2020.
  - Adwine Vanslembrouck participated in the 2nd training school 'Digitisation and data management challenges in small collections'. Organized within the MOBILISE COST Action; Warsaw, Poland, 13-14 Feb 2020.
- **Exhibition**
  - Information, photo material and two specimens of *Ae. albopictus* are displayed at the exhibition "CO_2-Het klimaatverhaal" from the Flemish government. The exhibition will run at three educative centres ('De Vroente', 'De Helix' and 'De Duinpanne') between February 2020 and December 2022 (https://omgeving.vlaanderen.be/de-vroente-opent-klimaattentoonstelling-1).

6.9 Annex 9: MEMO material inventory list

Collection method	Description part	Supplier	Number	Trap code
Mosquito Magnet® trap (MMT)	Independence Power Head MMT (2017)	FAVEX	10	M033-M042
	Executive Power Head MMT (2019)	FAVEX	5	M044-M048
	Bloc Battery MMT	FAVEX	24	
	Long Battery MMT	FAVEX	5	
	Base Support Pole MMT	FAVEX	15	
	Base Support MMT	FAVEX	15	
	Executive Bug Bag MMT (plastic box)	FAVEX	5	
	Bug Bag MMT	FAVEX	28	
	Remote Battery Charger MMT	FAVEX	15	
Frommer updraft gravid trap (GT)	Frommer Updraft Gravid Trap (black bucket)	John W. Hock Company	2	G006-G007
	DC-DC converter 12V-6V (Velleman CARS2000)	Cada Batterijen bv	2	
	Aspiration fan GT	John W. Hock Company	2	
	Collection chamber GT	John W. Hock Company	2	
	Base stand G	John W. Hock Company	2	
BG-GAT trap	BG-GAT Trap Black bucket with drainage holes (2018)	BIOGENTS AG	10	T001-T010
	BG-GAT catch bag	BIOGENTS AG	36	
	BG-GAT Sticky cards	BIOGENTS AG	120	
	BG-GAT ring	BIOGENTS AG	10	
	BG-GAT Funnel	BIOGENTS AG	10	
	BG-GAT Transparant chamber	BIOGENTS AG	10	
	BG-GAT Sticky card holder	BIOGENTS AG	10	
BG-Sentinel trap (BG)	BG-Sentinel BG-2 (2017) trap body + trap cover	BIOGENTS AG	16	B009-B024
	BG-Sentinel BG-2 (2018) trap body + trap cover	BIOGENTS AG	2	B025-B026
	BG-Sentinel BG-2 intake funnel	BIOGENTS AG	20	
	BG-Sentinel BG-2 catch bag	BIOGENTS AG	62	
	BG-Sentinel BG-2 funnel net	BIOGENTS AG	28	
	BG-Sentinel BG-2 rain cover + 3 sticks	BIOGENTS AG	18	
	BG-Sentinel BG-2 connection plug socket	BIOGENTS AG	6	
	BG-Sentinel BG-2 adapter connection battery cable	Cada Batterijen bv	22	
Oviposition trap (OT)	OT ELHO® without drainage holes	N/A	16	
	OT ELHO®	N/A	191	
Larval sampling (LS)	Dipper telescopic stick (model 320)	John W. Hock Company	2	
Battery charging material	Gel Battery (GF 12 50V accu)	Cada Batterijen bv	88	
	Battery Boxes (new 2019)	Cada Batterijen bv	18	
	Battery Boxes	Cada Batterijen bv	13	
	Battery Boxes stretchers	Action Belgium BVBA	60	
	Gel Battery (DAB 12-55EV,grey)	Cada Batterijen bv	12	
	Long Battery Boxes (per 3 batteries)	Cada Batterijen bv	2	
	Battery Charger VICTRON	Cada Batterijen bv	6	
	Battery Charger MASCOT	Cada Batterijen bv	3	
	Gel Battery Cable	Cada Batterijen bv	17	