# Final Report

| Project title (Acronym) |
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| **Improved knowledge about epidemiology and distribution of priority invasive and (re)emerging arthropod pests in fruit crops and grapevines (e.g. *Aromia bungii*, *Popillia japonica*, *Halyomorpha halys*) (EPIDISARTH)** |

**Project duration:**

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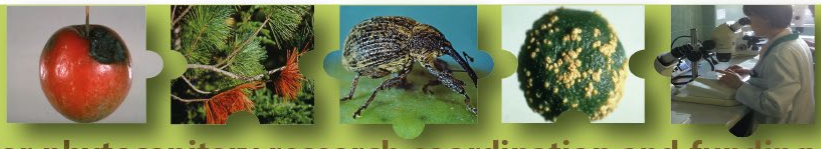
| **Start date:** | 2021-03-01 |
| **End date:** | 2023-02-01 |



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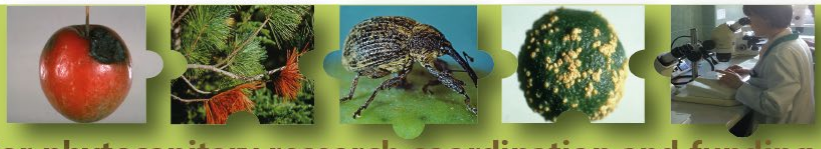

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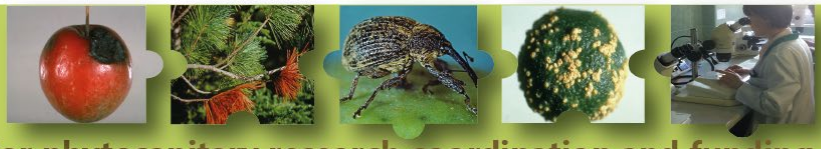

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## 2. Short project report

***Short executive summary***

The EPIDISARTH project focused mainly on the emerging plant pests *Aromia bungii* (Faldermann), *Popillia japonica* (Newman) together with *Halyomorpha halys* (Stål) and to a much lesser extent on *Lycorma delicatula* (White). These species originated from Asia and were introduced into the European Union (EU) except for *L. delicatula*, where they pose a major threat to fruit crops and grapevines. *Aromia bungii* and *P. japonica* are listed as priority quarantine pests within the Commission Implementing Regulation (EU) 2019/2072(2), Annex II, Part B. *Popillia japonica*, in particular, is currently a major concern due to its high populations, polyphagy and significant crop damage. Timely detection is crucial to prevent further pest outbreaks, spread and new infestations.
The objectives of this project were to increase the knowledge about these target pest species with regard to occurrence, phenology, identification and control measures.
The project partners elaborated accurate current data on the target pest species for the regions monitored and up-to-date distribution maps. Such information is useful for targeted and effective control. Surveys were conducted in 2021 and 2022 in fruit crops, vineyards and risk sites such as traffic routes or distribution centres. Different methods for the surveillances in the field were used. Experience was gained on the advantages and disadvantages of different trap types and lures. Additionally, awareness for these emerging plant pests was raised by citizen science initiatives and new mobile phone apps were developed to report possible findings. Through citizen science engagement additional records for *H. halys* were collected and a larger geographical area was covered. Monitoring activities by experts provided better insight into the presence or confirmed absence of the target pest species in the participating partner countries. Information on pest occurrence is important to support the early detection and management of these new plant pests in the European Union.

A major outcome of the cooperation in this project was the exchange of biological material for the generation of barcoding DNA sequences from *A. bungii*, *P. japonica* and *L. delicatula* for the molecular identification and the preparation of a diagnostic protocol (in cooperation with R. Mouttet, P. Rousse, Anses and J.C. Streito, CBGP-INRAE) for the reliable identification of *A. bungii* larvae and adults. This diagnostic protocol will be very valuable for plant protection organisations and National Reference Laboratories (NRLs) for diagnosing the pest.
Extensive research on behaviour and integrated control options of *P. japonica* has been

conducted by the Plant Protection Service of Lombardy in cooperation with universities of Brescia, Padua, Turin and Verona in the GESPO project (https://sites.google.com/unibs.it/gespo-project-en) and in the PRECONFITOLOMB project, funded by Regione Lombardia.

***Project aims***

The overall aim of this project was to collect actual data on the occurrence (WP2: Surveillance of target pests), molecular diagnostic (WP3: Molecular and morphological diagnostic of target pests) and control measures (WP4: Potential containment and control measures of target pests) for *A. bungii*, *P. japonica* and *H. halys* in the partner countries of this project. The various tasks and activities of the project were intended to increase knowledge, to support the official survey activities and contribute to the control of these pests in the EU. One goal was to raise awareness for the importance of these pest species and to involve the general public in the monitoring through citizen science activities. For this purpose, special mobile phone apps are already available in e.g. Italy (FitoDetective), France (Agiir, available at http://ephytia.inra.fr/fr/P/128/Agiir) and Serbia (HHBug-Monitor, in collaboration with Italian colleagues, Fondazione Edmund Mach). Other important aims were to improve molecular diagnosis and to characterise the genetic variation of *H. halys* populations. For *A. bungii* a diagnostic protocol was established to improve identification.

***Description of the main activities***

The project was divided in three technical work packages, dealing with surveillance, molecular and morphological diagnostic, containment and control measures.

**Work-package 2. Surveillance of target pests**

Information about sampling and detection methodologies (e.g. trap types, lures, visual detection, etc.) for *A. bungii*, *P. japonica* and *H. halys* was collected by the project partners in a common excel sheet, including available information on advantages, disadvantages and costs (task 2.1). Expert survey was done in orchards, vineyards and risk areas using different methods and materials. For this, either new mobile phone applications were developed or existing ones were improved to report the detection of the target pests. Using the data from expert monitoring and citizen science reports, up-to-date distribution maps were produced. In the following section, the materials and methods are described in more detail for each pest species (task 2.2).

***Aromia bungii***

Expert survey: black cross-vane traps and 4 component multi-funnel traps with a specific lure for *A. bungii* (*A. bungii* P661 from ChemTica Internacional) were tested. In North-Italy, 12 traps were installed in an infested area from June 13$^{th}$ - August 13$^{th}$ in 2022 with a control once per month. In addition, visual inspections of trees for sawdust and exit holes were performed. In Belgium, 4 component multi-funnel traps (Andermatt; 15 traps) and black cross-vane panel traps (WitaPrall; 10 traps) were set up in 9 provinces from Flanders and Wallonia in *Prunus* orchards from May to September/October in 2021 and 2022. In Austria,

black cross-vane panel traps (WitaPrall; 3 traps) were installed from July to October 2021 in a risk area in Tyrol along a transit route from/to the infested area in Bavaria, Germany.

Citizen science: a new mobile application was developed by INRAE for *A. bungii* for citizen scientists to send photos and report their findings: http://ephytia.inra.fr/fr/C/27328/Agiir-Le-longicorne-a-col-rouge. The new app contains the most important information about the biology, introduction history and identification characteristics of *A. bungii* adults and larvae with photos as well as for non-target longhorn beetles.

***Popillia japonica***

Expert survey: *P. japonica* has been occurring on the Azores for a long time (since the 1970s) and only recently in northern Italy (2014). On the Azores, *P. japonica* surveillance has been conducted on nine islands. A net of traps was implemented and maintained in the field during the period of appearance of the adults (from May to October) on all the islands. The data obtained permitted to know on each island the amount of *P. japonica* and its fluctuations during their period of occurrence, and even to identify the places with bigger captures in the field, as a way to help the decision-makers to implement better IPM strategies dealing with this priority pest. It was also possible to compare adult population levels for 2021 and 2022 on all islands.

In Lombardy (Italy), a special focus of the trapping was to detect the beginning of adults' emergence, to track the adult population during the flight season and to support survey activities. 12 home-made traps baited with lures (pheromone + kairomones) were tested and installed homogeneously within the infested zone with 1 control once a week from the end of May until the end of adults' flight. First adults were detected on May 25$^{th}$ 2022.
The surveillance of *P. japonica* in Austria and Belgium was conducted with funnel traps (Pherobank BV; 1 or 2 traps/site) with a specific lure for *P. japonica* (N°50313, Pherobank BV), deployed from May/July to September, along high-traffic routes (road, rail and port), distribution centres, in orchards and suitable habitats for egg laying.

Citizen science: a new mobile application was developed by INRAE for *P. japonica* for the reporting of citizen scientists: http://ephytia.inra.fr/fr/C/27002/Agiir-Le-scarabee-japonais

***Halyomorpha halys***

Expert survey: different trap types and methods to monitor *H. halys* in urban areas and in the fields were tested by the partners in combination with the specific lure from Trécé (Trécé Pherocon BMSB dual lure). The trap types tested were e.g.: Dead-Inn black pyramid trap (AgBio Inc., 4 ft. height), sticky traps (Trécé Inc.), Rescue stink bug traps and home-made traps as well as the beating tray method and visual detection. The seasonal dynamics and the host plants of *H. halys* (adults and larvae) were studied in two locations in the urban area of Ljubljana (Slovenia) and all plant species in the area (40 m x 40 m) of 6 pheromone traps in two locations (3 traps/location) were recorded. Weather data were collected for predicting the phenology of *H. halys*. Monitoring of *H. halys* egg parasitoids was conducted by PPS Regione Lombardia (Italy) and the University of Novi Sad (Serbia).

Citizen science: the mobile application developed by INRAE for *H. halys* for the reporting of citizen scientists was updated and completed: https://ephytia.inrae.fr/fr/C/20532/Agiir-Punaise-diabolique.

***Lycorma delicatula***

Expert survey: monitoring of *L. delicatula* was started in Lombardy (Italy) in 2021. A visual inspection of *Ailanthus altissima* was done and brown sticky traps (hand-made) were placed on trunks of susceptible trees in risk areas, e.g. at Malpensa airport and companies that import stones from third countries.

Citizen science: a new mobile application was developed by INRAE for *L. delicatula* for the reporting of citizen scientists: https://ephytia.inrae.fr/fr/C/27321/Agiir-Le-fulgore-tachete.

**Work-package 3: Molecular and morphological diagnostic of target pests**

Task 3.1: Molecular diagnostic of target pests (INRAE)

The specimens of the target species were collected by partners and sent to INRAE-CBGP for sequencing. DNA was extracted using a non-destructive protocol preserving the integrity of the studied specimens. All voucher specimens are preserved within the CBGP Continental Arthropod Collection (Montpellier, France: https://doi.org/10.15454/D6XAKL). This approach allows the morphological re-examination and identification in case of discrepancy between morphological and molecular identifications. To ensure that the methods developed could be used by any European laboratory including non-entomological ones, the COI standard barcode (Hebert et al., 2003) was chosen, a technique that can easily be applied and currently mastered by most laboratories worldwide. DNA standard barcodes were produced and validated accounting for available published sequences (by BLAST: Basic Local Alignment Search Tool) and phylogenetic reconstruction including species of related taxonomic groups. All sequences are freely available through the Arthemis database (https://arthemisdb.supagro.inrae.fr/) and deposited in the EPPO-Q-bank database (https://qbank.eppo.int/) making possible reliable identification of these four pests and lookalike species at all stages.

Task 3.2: Diagnostic protocols (INRAE)

A diagnostic protocol for the identification of larvae and adults of *Aromia bungii* was developed based on specimens and sequences available from the INRAE collections (Montpellier) and specimens from the Plant Protection Service laboratory of the Lombardy Region. Morphological keys for both adults and larvae were developed. Molecular identification by barcoding is also possible thanks to the sequences produced and validated in task 3.1.

Task 3.3. Assessing the genetic diversity of *H. halys* population in Belgium (CRAW & pcfruit)

DNA extraction was performed on legs using a chelex extraction method. We amplified the cytochrome oxydase subunit I (COI) and II (COII) regions of the mtDNA (COI: LCO 1490/HCO 2198 primers, COII: HhalysCO2F/R primers). Resulting sequences were compared to public databases using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Genetic diversity was investigated among localities, regions and at the Belgian scale through haplotype networks and genetic diversity indexes (haplotype, h, and nucleotide, π, diversity). We tested for the presence of a genetic structure in Belgium by performing a Mantel test on genetic and geographic distance matrices between collected specimens. Furthermore, demographic history using Tajima's D and Ramos-Onsins and Rozas's R2 statistics was also investigated. Analyses were performed on each locus (COI and COII) separately, and on the concatenated sequences of COI + COII. We also compared the haplotypes found in Belgium to published sequences from the studies of Cesari et al. (2018) and Yan et al. (2021).

## Work-package 4: Potential containment and control measures of target pests

### *Popillia japonica*

The focus of this work package was to investigate different control options for *P. japonica*. Several extensive trials for the integrated control of *P. japonica* larvae and adults were conducted by PPS Lombardy in collaboration with several universities (Brescia, Padua, Verona and Turin). To suppress adult populations, different control measures were performed on high-value crops with nets as physical barriers or Long-Lasting Insecticide-treated Nets (LLINs) covered with insecticides (pyrethroids) in an attract-and-kill setup. Different weed mulching products (coconut fiber, jute fabric and wood chips) for potted plants were tested to prevent beetle oviposition in container-grown nursery stocks. The entomopathogenic nematode *Heterorhabditis bacteriophora* and the entomopathogenic fungus *Metarhizium anisopliae* were tested as biocontrol agents against *P. japonica* larvae in the soil.

### *Halyomorpha halys*

In Serbia, parasitism of *H. halys* eggs by native egg parasitoids was studied, whereas in northern Italy the spread and parasitism rate of *H. halys* eggs by the released parasitoid *Trissolcus japonicus* (Scelionidae) was recorded. *Trissolcus japonicus* was released in 34 sites in Lombardy. Two releases of the parasitoid were carried out at each site (100 females and 10 males) per year.

### *Main results - Surveillance activities*

### *Halyomorpha halys*

France, INRAE: Monitoring is performed since 2012 by citizen sciences via AGIIR mobile phone application (http://ephytia.inra.fr/fr/P/128/agiir). A new updated and more complete version was supplied for the project. A large number of occurrences were collected with the application throughout France, making it possible to track the expansion of *H. halys* and show that *H. halys* is present over the vast majority of the country. Since 2021, damage has been reported in France in apple, pear, kiwi and hazelnut orchards in particular.

Slovenia, Univ. of Ljubljana: Seasonal dynamics of the brown marmorated stink bug was investigated at two different suburban areas in Ljubljana – Laboratory Field of Biotechnical Faculty and Rakovnik. Regarding both experimental years (2021 and 2022), first individuals (adults) occurred in the first week of April. The highest number of adults was recorded in September, in both experimental years at both areas. The stink bugs were monitored in three different developmental stages: young larvae, old larvae and adults. Young larvae have occurred from June till beginning of September in 2022, and from the beginning of July till mid-September in 2021. Egg clusters of the pest were recorded on industrial hemp (*Cannabis sativa* L.) and soybean (*Glycine max* L.).

Serbia, University of Novi Sad: *H. halys* was recorded for the first time in Serbia in October 2015. Since 2018, Dead-Inn black pyramid traps from AgBio and Trécé lures were used for its monitoring all over the country. In 2021, 42 traps were set in different environments, urban areas and in the fields. Parallel with those activities, the monitoring of egg parasitoids was also conducted. The results showed establishments and spreading of *H. halys* and few parasitoid species which are native to Europe.

Italy, PPS Lombardy: *H. halys* is widespread throughout the Lombardy region. Therefore, the surveillance has been focused on the sites where the samurai wasps, *Trissolcus japonicus*, were launched. Different types of traps were tested.

Austria, AGES: In 2021 and 2022 the occurrence of the *H. halys* was surveyed near apple (organic or conventional), cherry, pear and apricot (conventional) orchards and hazelnut plantations in seven provinces. *H. halys* appeared in higher numbers only in the urban area of Vienna with its near surroundings in Lower Austria. In 2022, comparatively high numbers of *H. halys* were found in Leibnitz, a settlement in Styria. In contrast, the numbers were very low or zero in the rural areas monitored in 2021 and 2022. As for economic impact, no damages were reported by the farmers or by the Chambers of Agriculture who participated in the monitoring. AgBio Dead-Inn black pyramid traps and Wita-Prall traps with the BMST Trécé lure were used for monitoring. Significantly more adults (1023) than nymphs (363) were caught in 2022 with both trap types making it difficult to track phenology. Based on the trap catches, it was not possible to determine the number of generations with certainty. Therefore, only an estimate can be made on the basis of the data and it seems possible that at least a partial second generation is formed in Austria. Both types of traps are very well suitable for monitoring, however, handling of the AgBio pyramid trap is easier than the WitaPrall trap because it has only one collection container. Only a few non-target species were found in the traps such as spiders, snails, amber wood cockroach and beetles. In spring and autumn, nymphs and adults of *Nezara viridula* were also observed.

Belgium, pc-fruit and CRA-W: *H. halys* was observed in multiple locations in Belgium, with the majority of detections in the northern part of the country (Flanders region). A total of 174

*H. halys* specimens were detected during the 2021-2022 monitoring activities of pcfruit and CRA-W.

Azores, Univ. of Azores: *A. bungii*, *H. halys* and *L.delicatula* are not present in the Azores archipelago but *H. halys* has been recorded on the mainland of Portugal (Grosso-Silva et al., 2020).

***Popillia japonica***

Azores, Univ. of Azores: There is a contingency plan regarding *P. japonica* elaborated by the phytosanitary authority in Portugal (DGAV) that deals with the introduction of this pest in mainland Portugal because this pest is only recorded on eight of the nine islands of Azores. For *P. japonica* the trap used was the Ellisco japanese beetle trap (funnel + can; producer: Trécé; origin: USA) and the attractant was PHEROCON® JB Lure (Japanese Beetle Floral Lure) also from Trécé. The last two years of *P. japonica* monitoring on the Azores islands showed an increase in the population from 2021 to 2022 on the eight islands monitored.

France, INRAE: A monitoring program is implemented by the NPPO, supplemented since 2021 by citizen monitoring via the AGIIR application. The application dedicated to *P. japonica* was developed as part of the project and launched in July 2021. To date no reports of *P. japonica* have been confirmed in France. The app contains the most important information about the pests' biology, introduction history and identification characteristics of *P. japonica* adults with photos as well as information on common lookalike non-target European beetles.

Austria, AGES and Belgium, pc-fruit & CRA-W: In 2021 and 2022 no specimens of *P. japonica* were detected during the surveillance in Austria and Belgium.

***Aromia bungii***

Italy, PPS Lombardy: In 2022, 12 *A. bungii* individuals were captured in a survey in an infested area in North-Italy using cross-vane traps with a specific lure (P661-Lure E2,cis-6,7-epoxynonenal).

France, INRAE: A monitoring program is implemented by the NPPO, supplemented since 2021 by citizen monitoring via the AGIIR application. The application dedicated to *A. bungii* was developed as part of the project and launched in October 2022. To date no reports of *A. bungii* have been confirmed in France. The new app contains the most important information about the pest's biology, introduction history and identification characteristics of *A. bungii* adults and larva with photos as well as for other non-target longhorn beetles.

Austria, AGES and Belgium, pc-fruit & CRA-W: In 2021 and 2022 no specimens of *A. bungii* were detected during the surveillance in Austria and Belgium.

***Lycorma delicatula***

Italy, PPS Lombardy: no specimens of *L. delicatula* were detected during the surveillance in 2021 and 2022 in North-Italy.

France, INRAE: An AGIIR application dedicated to *L. delicatula* was developed as part of the project and launched in October 2022. The app contains information about the pests' biology, and identification characteristics. To date no reports of *L. delicatula* have been confirmed in France.

**Main results - Molecular identification**

***Aromia bungii***

A diagnostic protocol for the identification of *A. bungii* was prepared.

France, INRAE: Specimens of *A. bungii* are rare. We sequenced 2 specimens and recovered 3 sequences given by EURL (France). These sequences have been compared to 13 sequences of *A. bungii* from BOLD/GenBank and validated. They are available from the EPPO-Q-bank database, completed by 6 sequences of *A. moschata* as lookalike species.

A morphological and molecular diagnostic protocol was developed and drafted as part of the project by Jean-Claude Streito (INRAE, France), Raphaëlle Mouttet and Pascal Rousse (Anses, France) and Matteo Zugno (Regione Lombardia, Italy). It was first submitted to the EPPO Panel on Diagnostics in Entomology and revised by the authors. The proofreading process is in progress for a publication as an EPPO diagnostic protocol in the EPPO Bulletin.

***Halyomorpha halys***

France, INRAE: We sequenced 17 specimens of *H. halys* completed by 1 sequence from EURL (France). These sequences have been compared to 67 sequences of *H. halys* and *H. pictus* from BOLD/GenBank and validated. They are available from the EPPO-Q-bank database, completed by 8 sequences of *Rhaphigaster nebulosa* (Pentatomidae) as lookalike species.

Belgium: On the 99 samples analysed (captures from CRA-W and pcfruit, and additional Belgian samples from the University of Gent, UGent), all but one sequences perfectly matched publicly available *H. halys* sequences, confirming pest identity. A single sequence perfectly matched *Rhaphigaster nebulosa* (Pentatomidae) sequences, a native lookalike stinkbug.

Genetic diversity of *H. halys* in Belgium: In total, 99 specimens from 19 populations were analyzed. Here, only the results from the concatenated sequences (COI + COII) are reported. Ninety-three sequences were retrieved from the concatenation of COI and COII fragments and 11 distinct haplotypes were isolated from them. All haplotypes found in this

study (for COI and for COII) were already described by previous authors (although some combinations of these haplotypes in concatenated sequences were unique to Belgium).

The haplotype diversity (i.e. the probability that two randomly chosen haplotypes are different; Nei & Tajima, 1981) over all sequences is h = 0.78. This is quite high compared to other invaded countries. Furthermore, a relatively large number of mutations separate some of the haplotypes. The nucleotide diversity (i.e. the average number of nucleotide differences per site between two DNA sequences from the population/sample of interest; Nei & Li, 1979) is π = 0.003. Neutrality tests based on Tajima's D and Ramos-Onsins and Rozas's R2 statistics are not significant. This indicates that no genetic signal of demographic expansion is present in Belgian population of *H. halys* (very likely because introduction is too recent). Regarding the distribution of haplotypes over Belgium, we found a significant isolation-by-distance signal, hence genetic structuring, between pairs of individuals. This pattern could have arisen due to multiple introductions of *H. halys* from different origins followed by short-distance dispersal events. Finally, we compared the sequences of Belgian samples to previously published sequences from native (China and Japan) and invaded countries. Many Belgian sequences shared haplotypes with other invaded countries and with China. Interestingly, other rarer sequences were only isolated from specimens from Japan and/or Italy. In conclusion, the high level of genetic diversity and the haplotypes found in Belgium suggest repeated introductions of *H. halys* (with Italy as a very likely source population).

***Popillia japonica***

France, INRAE: We sequenced 26 specimens of *P. japonica* from USA, Canada, Switzerland and Azores completed by 1 sequence of *P. mutans* from EURL (France). These sequences have been compared to 57 sequences of *P. japonica* from BOLD/GenBank and validated. They are available from the EPPO-Q-bank database, completed by 1 sequence of *Phyllopertha horticola* (Scarabaeidae) as lookalike species.

***Lycorma delicatula***

France, INRAE: We have produced for the project 17 sequences from China completed by 2 USA sequences from EURL (France). These sequences have been compared to 26 sequences of *L. delicatula* from BOLD/GenBank and validated. They are available from the EPPO-Q-bank database, completed by 5 sequences of *Metcalfa pruinosa* (Flatidae) as lookalike species.

**Main results - Potential containment and control measures of target pests**

Extensive research on integrated control options of *P. japonica* has been conducted in cooperation by PPS Lombardia and several universities in North-Italy. The results of these studies have been published in various journals (https://sites.google.com/unibs.it/gespo-project-en).

*Halyomorpha halys* has become a globally invasive pest, as a serious threat to agricultural production and a notorious nuisance pest in urban areas. Considerable efforts have been made so far to develop effective pest control measures to prevent crop damage. Due to the damage caused by *H. halys* and subsequent economic losses, the use of insecticides has substantially increased in commercial orchards, disrupting established integrated pest management programs for different crops in the USA and Europe. More frequent pesticide application, including broad-spectrum insecticides, has affected beneficial arthropods and caused secondary pest outbreaks. Many research groups and technicians around the world have been working feverishly over the last 10 years to find effective, feasible and environmentally acceptable ways of managing this pest. Exclusion nets have proven to be a promising tool to prevent *H. halys* from damaging nectarines and apples. The attract-and-kill strategy was effective at managing low to moderate *H. halys* populations in apple orchards. Long-lasting insecticide-treated nets (LLINs) were tested in pear orchards. Although the method was not proven for *H. halys* control itself, it is proposed as part of an IPM program, which contributes to a significant reduction of pest population in the crop. In past years, much attention has been given to alternative methods for long-term management. Biological control of *H. halys* with egg parasitoids is considered one of the most viable long-term solutions. Numerous studies on natural enemies have been carried out in the USA and Europe. Overall findings show that three principal groups of hymenopteran parasitoids are able to attack *H. halys* eggs in invaded areas: Scelionidae (*Telenomus* spp., *Trissolcus* spp. and *Gryon* spp.), Eupelmidae (*Anastatus* spp.) and Encyrtidae (*Ooencyrtus* spp.). However, so far, the level of parasitism by native species is too low to reduce pest population below economic thresholds. *Anastatus bifasciatus* (Geoffroy) is the most widespread native egg parasitoid in Europe capable of developing on viable *H. halys* eggs and is considered as the most suitable candidate for augmentative biological control in Europe.
In the Lombardy region, *T. japonicus* has begun to settle, but slowly and unevenly. The northern provinces of the region seem to have more favorable agro-meteorological conditions for the parasitoid. The parasitization levels of *H. halys* eggs is highly variable, ranging from 0% to 32.5%. At regional level, the total average parasitization is estimated at around 9%, which is decidedly insufficient for a good control and reduction of *H. halys* populations.

***Conclusions and recommendations to policy makers***

It is necessary to continue monitoring of *P. japonica* adult populations in the countries and regions where this pest already occurs, but also in countries where it is not yet present. A global IPM strategy is needed to test and apply different means of control methods and mass trapping against this pest in order to reduce adult and larvae populations in the affected areas and to prevent *P. japonica* spread to new areas. Countries close to already affected areas with *P. japonica* should create and implement contingency plans to deal with the introduction of this priority pest. In North-Italy, 12 *A. bungii* individuals were captured in a survey in an infested area using cross-vane traps with a specific lure (P661-Lure E2,cis-6,7- epoxynonenal). Accordingly, this method proved to be effective. As regards the progress of the programme for biological control of *H. halys* through the use of its natural antagonist *Trissolcus japonicus*, it is noted that after three years of releases, *T. japonicus* is becoming established in Lombardy, but slowly and preferentially in the northern areas of the region. It would therefore be necessary to continue the releases for a few more years.

*Benefits from trans-national cooperation*

The cooperation was very fruitful, although the partners did not know each other personally and the exchange only took place in the virtual project meetings and via email. Valuable experiences were exchanged between the partners and synergy effects were created. Expert knowledge about a target pest species already occurring in one country was passed on to colleagues who had no direct experience with it so far. Expertise with different types of traps and attractants were openly shared within the project group (e.g. best site for placing traps, etc.). In addition, partners provided valuable biological material for molecular genetic analyses. The cooperation in this project made it possible that *A. bungii* samples from the Lombardy region were made available to colleagues from INRAE for development of the diagnostic protocol. The diagnostic protocol was produced and written by French and Italian specialists. *P. japonica* larval samples from the Azores were provided for molecular diagnosis by INRAE. Collaboration has been very close and constructive all the way. *H. halys* sequences were also exchanged between Belgium and French patners.

# 3. Publications

*The scientific publications linked to the project should be included* in extenso *in the report, and clear reference to them should be given. It is recommended to contact the Euphresco Secretariat to explore the opportunities for dissemination via publication. The research consortium is responsible for the publication strategy for a given project.*

## 3.1. Article(s) for publication in the EPPO Bulletin

*Publication in the EPPO Bulletin is not automatically granted and is dependent on the quality of the article. If an article is accepted, charges could apply to cover publication costs.*

## 3.2. Article for publication in the EPPO Reporting Service

*Publication in the EPPO Reporting Service is not automatically granted and is dependent on a number of criteria, among which the novelty and the quality of the information proposed. Only references to the Euphresco project (and no reference to the individual scientists) will be given in the published article.*

## 3.3. Article(s) for publication in other journals

*The research consortium is solely responsible for publishing project outputs in peer-reviewed scientific journals.*

**Scientific journals**

Borgogno Mondino E., Lessio F., Bianchi A., Ciampitti M., Cavagna B. and Alberto Alma (2022). Modelling the spread of *Popillia japonica* Newman (Coleoptera: Scarabaeidae) from a recently infested area. Entomologia Generalis DOI: 10.1127/entomologia/2022/1370.

Chartois M., Streito J.-C., Pierre É., Armand J.-M., Gaudin J. & Rossi J.-P., (2021). A crowdsourcing approach to track the expansion of the brown marmorated stinkbug *Halyomorpha halys* (Stål, 1855) in France. Biodiversity Data Journal 9: e66335. doi: 10.3897/BDJ.9.e66335.

Gilioli G., Sperandio G., Simonetto A., Colturato M., Battisti A., Mori N., Ciampitti M, Beniamino B., Bianchi A., Gervasio P. (2022). Modelling diapause termination and phenologyof the Japanese beetle, Popillia japonica. Journal of Pest Science (2022) 95:869–880 https://doi.org/10.1007/s10340-021-01434-8.

Glazer I., Santoiemma G., Battisti A., De Luca F., Fanelli E., Troccoli A., Tarasco E., Sacchi S., Bianchi A., Gilioli G., Gherardo P., Mori N. (2022). Invasion of *Popillia japonica* in Lombardy, Italy: Interactions with soil entomopathogenic nematodes and native grubs. Agricultural and Forest Entomology, 24: 600–608. DOI: 10.1111/afe.12524.

Laznik, Ž., Trdan, S. (2021). Management methods for marmorated stink bug (*Halyomorpha halys* [Stål, 1855], Hemiptera, Pentatomidae). Acta agriculturae Slovenica, 117, 1: 1-11 [in Slovenian].http://ojs.aas.bf.uni-lj.si/index.php/AAS/article/view/2106/473.

Lessio F., Pisa C. G., Picciau L., Ciampitti M., Cavagna B. and Alberto Alma (2021). An immunomarking method to investigate the flight distance of the Japanese beetle. Entomologia Generalis, Vol. 42 (2022), Issue 1, 45–56. DOI: 10.1127/entomologia/2021/1117.

Mori N., Santoiemma G., Glazer I., Gilioli G., Ciampitti M., Cavagna B., Battisti A. (2021) Management of *Popillia japonica* in container-grown nursery stock in Italy Phytoparasitica. DOI https://doi.org/10.1007/s12600-021-00948-2.

Santoiemma G., Battisti A., Gusella G., Cortese G., Tosi L., Gilioli G., Sperandio G., Ciampitti M., Cavagna B., Mori N. (2021). Chemical control of *Popillia japonica* adults on high-value crops and landscape plants of northern Italy. Crop Protection, DOI 10.1016/j.cropro.2021.105808.

Simonetto A., Sperandio G., Battisti A., Mori N., Ciampitti M., Cavagna B., Bianchi A., Gilioli G. (2022). Exploring the main factors influencing habitat preference of *Popillia japonica* in an area of recent introduction. Ecological Informatics https://doi.org/10.1016/j.ecoinf.2022.101749.

Sperandio G., Simonetto A., Gervasio P., Ciampitti M., Cavagna B., Bianchi A., Battisti A., Santoiemma G., Mori N., Gilioli G. Population growth and adult phenology of the Japanese beetle during an ongoing invasion process. Submitted to Biological Invasions BINV-S-23-0006.

Streito J.-C., Chartois M., Pierre É., Dusoulier F., Armand J.-M., Gaudin J. & Rossi J.-P., (2021). Citizen science and niche modeling to track and forecast the expansion of the brown marmorated stinkbug *Halyomorpha halys* (Stål, 1855). Scientific Reports 11: 11421. https://doi.org/10.1038/s41598-021-90378-1.

Berteloot O.H., Kuhn A., Peusens G., Beliën T., Hautier L. & De Clercq P. Distribution and genetic diversity of *Halyomorpha halys* (Hemiptera: Pentatomidae) in Belgium. *In preparation*

**Scientific conference**

Bohinc, T., Jelnikar, J., Strauss, G., Sinkovič, T., Trdan, S. 2022. Occurrence of brown marmorated stink bug (*Halyomorpha halys*, Hemiptera, Pentatomidae) in urban area (Ljubljana, Slovenia). In: GEROWITT, Bärbel (ed.). Working Group "Landscape management for functional biodiversity": Proceedings of the 9th meeting at Milan (Italy), 7-10 June, 2022. Darmstadt: IOBC/WPRS, 2022. IOBC-WPRS Bulletin, Vol. 156, 2022: 109-112.

Batistič, L., Bohinc, T., Trdan, S. 2022. Efficacy of trap crops and biopesticides to control brown marmorated stink bug [*Halymorpha halys* (Stål), Hemiptera, Pentatomidae] in apple orchards. In: GEROWITT, Bärbel (ur.). Working Group "Landscape management for functional biodiversity" : Proceedings of the 9th meeting at Milan (Italy), 7-10 June, 2022. Darmstadt: IOBC/WPRS, 2022. IOBC-WPRS Bulletin, Vol. 156, 2022: 104-108.

Bohinc, T., Sinkovič, T., Strauss, G., Trdan, S. 2022. Seasonal dynamics and host plants of brown marmorated stink bug (*Halyomorpha halys* [Stål], Hemiptera, Pentatomidae) in Ljubljana. In: Trdan, S. (ed.). Lectures and papers presented at the 15th Slovenian Conference on Plant Protection with International Participation, Portorož, March 1-2, 2022. Ljubljana, Plant Protection Society of Slovenia: 380-390 [in Slovenian]
https://dvrs.bf.uni-lj.si/wp-content/uploads/45Bohinc-et-al.-Halyomorpha-P1.pdf

Lethmayer, C. & Strauß, G. 2022: Monitoring activities on non-native pests in fruit crops in Austria. - IOBC (WPRS)-joint meeting PheroFruits 2022, 25.09.-29.09.2022, Girona (ESP)

Peusens, G., Belien, T., De Vis, F., Bangels, E., Alhmedi, A., Thys, T., Berteloot, O., De Clercq, P., Bylemans, D. (2022). Evaluation of crop protection agents with a chemical or physical mode of action against stink bugs and side effects on *Trissolcus basalis*. Poster at the PheroFruits 2022 symposium - Joint Meeting of the IOBC/WPRS Working Groups “Pheromones and other semiochemicals in integrated production” & “Integrated Protection of Fruit Crops”. 25-29/09/2022. Girona, Spain.

Streito Jean-Claude, Pierre Éric, Genson Guénaëlle, Rossi Jean-Pierre, Cruaud Astrid, Rasplus Jean-Yves 2022. Arthemis: a web-interface database for the identification of pest and beneficial Arthropods for agriculture in Europe. International Conference on DNA Barcoding and Biodiversity, Sofia (Bulgaria) 25-27 May 2022. Oral communication 27V2022.

**Oral presentations**

Strauß, G. & Lethmayer, C.: Vorstellung des EUPHRESCO-EPIDISARTH-Projekts zur Marmorierten Baumwanze, Japankäfer und dem Asiatischen Moschusbockkäfer. Österreichische Pflanzenschutztagung (Presentation of the EUPHRESCO-EPIDISARTH project on *Halyomorpha halys*, *Popillia japonica* and *Aromia bungii*. Austrian Plant Protection Conference), 30.11.2021, online

Strauß, G. 2021: Euphresco EPIDISARTH project: deep into epidemiology and distribution of priority pests in fruit crops and grapevines (*A. bungii, P. japonica, H. halys, L. delicatula*). EURL for Insects & Mites annual workshop, 24.11.2021, online

Strauß, G. 2022: EPIDISARTH. EPPO Panel on Diagnostics in Entomology, 15.09.2022, online

**MSc Thesis at the Univ. of Ljubljana, Biotechnical Faculty (S. Trdan as supervisor)**
Bastistič, L. 2022. Research on efficacy of different trap crops and environmentally acceptable methods of controlling the brown marmorated stink bug (*Halyomorpha halys* [Stal], Hemiptera, Pentatomidae) in an orchard: M. Sc. thesis. Ljubljana: 51 p. https://repozitorij.uni-lj.si/Dokument.php?id=153428&lang=slv

**Specialist journal/Professional paper**
Laznik Ž., Trdan, S. 2021. Marmorated stink bug is spreading across Slovenia - how to live with it?. Kmečki glas. 22. Sept. 2021, 78, 38: 27. [in Slovenian – orig. title: Marmorirana smrdljivka se širi po Sloveniji - kako živeti z njo?]

**Press release**
Streito & Rossi, 2021. Expansion de la punaise diabolique en France: le rôle capital des sciences participatives pour le suivi des espèces envahissantes. Communiqué de presse 210601 INRAE.

**Interview (S. Trdan)**
Levičnik, Vesna. Over stink bugs successfully with pheromone lures: marmorated stink bug. Dnevnik online. 17. Nov. 2022, https://www.dnevnik.si/1043001084/lokalno/ljubljana/nad-smrdljivce-uspesno-s-feromonskimi-vabami [in Slovenian - orig. title: Nad smrdljivce uspešno s feromonskimi vabami: marmorirana smrdljivka.

# 4. Open Euphresco data

All sequences produced and validated during the project are freely accessible on the following bases:

The EPPO-Qbank database: https://qbank.eppo.int/project/EuphrescoEPIDISARTH

Arthemis DB@se: https://arthemisdb.supagro.inrae.fr/

AGIIR occurrences of *Halyomorpha halys* in France were published and are freely available (Chartois et al., 2021: e66335. doi: 10.3897/BDJ.9.e66335.)

AGIIR phone app are available freely on any platform such as PlayStore and App Store.