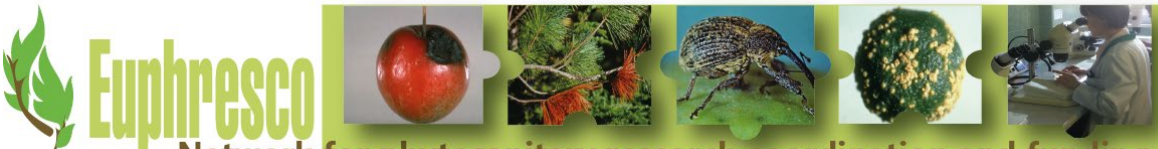

Final Report

Project title (Acronym)
Development and implementation of early detection tools and effective management strategies for invasive non-European and other selected fruit fly species of economic importance (FLY DETECT)

Project duration:

Start date:	2016-04-01
End date:	2019-03-31

Contents

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2. Short project report

2.1. Short executive summary

Insects of the family Tephritidae (Diptera) exert a huge economic impact on fruit and vegetable production worldwide because of direct damage on fruit and vegetable commodities and quarantine regulations. These species are also known as true fruit flies. Within the FLY DETECT project, focus was given on species of tephritid fruit flies that are key pests to a large number of crops in the Mediterranean region, such as *Ceratitis capitata* (Mediterranean fruit fly, Medfly), or are considered as important invasive species, such as *Bactrocera zonata* (Peach Fruit Fly, PFF), *Bactrocera dorsalis* complex (Oriental Fruit Fly, OFF), and *Myiopardalis pardalina* (Melon Fruit Fly, MFF). These species, which are already present in some parts of Europe (e.g. Medfly), or that are a threat as they may become established in the Mediterranean basin (e.g. PFF), pose a risk to the horticultural crops and agriculture of Central and Southern Europe, and are considered quarantine pests for most European countries (cf. EPPO Alert List, A1 or A2 Lists). The pests under study have substantial interceptions in EUROPHYT database each year.

Early detection of the presence of these species in new areas as well as during the season when adults appear is of utmost importance for successful management. For fruit flies, detection is based on the use of specific traps with appropriate lures that attract males or females. There are several traps and attractants combinations that can be used for detection and monitoring of fruit flies. A literature review for traps and attractants revealed a number of trap options and attractants that are being used to trap either male or females or both sexes.

One of the targeted species within FLY DETECT, the Mediterranean fruit fly, has lately expanded its distribution northwards. In northern Italy for example it has become a major pest for apples, jeopardizing the integrated pest management scheme for codling moth. Monitoring activities that took place during the project in Austria and Bulgaria shows that medfly population is present each year in Vienna, while scattered catches occurred in Bulgaria in orchards and warehouses although established overwintering population was not confirmed. Nevertheless, the repeated trap catches in northern areas suggest that there is a constant pressure for northward expansion of the species.

Infestation by tephritid fruit flies can be undetectable during harvest, storage and transport to the point of use, thus, destructive sampling in a large proportion of the commodity is often required. It is known that infested fruits emit specific volatile compounds. The profile of volatiles emitted could be used for the development of technology intense methods of rapid detection of infested fruits. Within FLYDETECT we determined infestation-specific volatile compounds-indicators by headspace- solid phase micro extraction- gas chromatography- mass spectrometry (HS-SPME-GC-MS) in order to develop a rapid, reliable and cost effective method aiming to reduce the time required for a reliable inspection.

2.2. Project aims

Given the huge economic importance of fruit flies on fruit and vegetable production worldwide, there is urgent need for tools and approaches for sustainable management and prevention. A key activity for managing fruit flies' populations is the monitoring with appropriate traps and lures. Solid monitoring data are used for decision making in employment of managing processes. Therefore, within this project it was aimed to explore the current available monitoring tools in order to facilitate the monitoring activities of fruit flies. Moreover, using

appropriate monitoring tools, the expansion of the geographical distribution of the Mediterranean fruit fly was explored. Within FLYDETECT we aimed also to determine infestation-specific volatile compounds-indicators in order to develop a rapid, reliable and cost-effective methods aiming to reduce the time required for a reliable inspection and to avoid the unnecessary destructive sampling.

2.3. Description of the main activities

2.3.1. Literature review on trapping methods for relevant fruit fly species

A literature review on available trapping methods for *Bactrocera zonata* was planned and executed by partner AGES.

The literature study covered scientific publications through searching in databases such as Scopus, CAB abstracts and Google Scholars. Search terms included the scientific names of the species and terms related to trapping and monitoring (traps, monitor, trapping etc.).

2.3.2. Monitoring and surveillance of fruit flies

Ceratitis capitata, which has its origin in Africa, has spread and became an important pest in the Mediterranean countries with widespread distribution. For Central Europe, where climatic conditions do not favour the establishment of tropical fruit flies, few records of earlier occurrences are available. From Austria only records from the city of Vienna were published in the past (Appendix 2). *Bactrocera zonata* and *B. dorsalis*, originally from tropical Asia, are not known to have established populations in Europe. However, frequent interceptions at entry points of the European Union and findings of single individuals in the past (e.g. in Vienna in different years and for *B. dorsalis* in Italy in 2018) indicate the potential risk of entries of those species (EPPO, 2019, Lethmayer, 2011, Nugnes *et al*., 2018).

Monitoring activities of *Ceratitis capitata*, *B. zonata* and *B. dorsalis* took place by partners involved in FLY DETECT project. Specifically, for *C. capitata*, partners AGES in Austria and BFSA in Bulgaria performed monitoring activities by placing different kind of traps in several locations. In Bulgaria traps used during the 2016-2018 surveys were:

- For female attraction - TephriTrap and MacPhail trap, with Biolure and TMA synthetic bait based on Amonium acetate, Putrescine and Trimethylamine. A protein bait with yeast was also used.
- In order to attract the males, paraferomones were used with Delta type traps baited with trimedlure.

In Austria, Tephri trap type traps (Maxitrap®, supplier Sociedad Española de Desarrollos Químicos (SEDQ), S.L., ES) were installed and charged with trimedlure for *Ceratitis capitata* and some traps charged with an attractant for female flies (Femilure-45 (prod. code: PH-180-FM45), supplier Russell IPM Ltd, UK).

For monitoring of *Bactrocera* species, Tephri trap type traps (Austria) or Delta traps (Bulgaria, Greece) baited with methyl eugenol were used.

Monitoring activities took place in Austria in peach trees (*Prunus persica*), which is a major host plant for all fruit fly species covered in the project, or other host plants in allotments and small gardens, but also at fruit and vegetable market areas, near a biogas plant and a composting plant. In other Austrian regions, traps were installed in or close to commercial orchards or small gardens containing host plants. In addition, traps were installed at Vienna International Airport. In Bulgaria, monitoring for Tephritid flies was carried out at the orchards, berry plantations, trade and markets sites, fruit and vegetable storehouses, whereas in Greece

surveillance for *Bactrocera* spp. took place at orchards, points of entry (ports) and central vegetable markets.

2.3.3. Use of volatiles for early detection of fruit flies

As infestation by tephritid fruit flies can be undetectable during harvest, storage and transport to the point of use, it was envisaged to utilize the profile of volatiles emitted by infested for the development of technology intense method of rapid detection of infested fruits. To this end, we offered orange fruits to adults of *C. capitata* for oviposition and subsequently we sampled the headspace volatiles in six different stages of infestation: immediately, 24h, d5, d18, d13 after oviposition and when larvae exit the orange. The collection of headspace volatiles by oranges was achieved using solid phase microextraction (SPME) sampling and absorbent-trap sampling. All samples analysed on a gas chromatography-mass spectrometry (GC-MS) to identify collected volatiles and characterize the profile of infested fruits at different stages of infestation.

2.4. Main results.

2.4.1. Literature review on trapping methods for *B. zonata*

More than 150 references were found and processed in the literature search. Information about available traps and attractants were retrieved and summarised. There are several trapping systems (traps and attractants) available for use for monitoring *B. zonata* males and females. (Appendix 1).

2.4.2. Monitoring activities

Austria

The survey in Austria resulted in catches of more than hundred fruit flies of *Ceratitis capitata* in each season and catches of single individuals of *Bactrocera zonata* and *Bactrocera dorsalis* in different years.

While no *C. capitata* individuals were obtained from traps placed in other Austrian regions, despite four individuals, catches occurred at up to eight sampled sites in the city of Vienna. More than one thousand individuals of this species were caught between 2016 and 2018. Molecular analyses confirmed the morphological identification. Molecular sequencing revealed that individuals were in general from a number of genetically different origins. However, results showed that approximately one quarter of the sequences were clonal (including individuals from all three years), therefore indicating the same genetic origin.

Furthermore, survey activities in Austria resulted in catches of single individuals of two *Bactrocera* species in 2016-2018. All individuals were caught in gardens or orchards in urban areas of Vienna while no *Bactrocera* individuals were obtained from traps placed in other provinces. All individuals were caught with traps charged with male attractant. Individuals were identified as *B. zonata* or *B. dorsalis*. However, it is important to note that for identified *B. dorsalis* individuals it was not possible to distinguish between B*. dorsalis* s.l. (following IPPC 2019) and *B. carambolae* with molecular methods (ITS1). Limitations of the current diagnostic protocol to differentiate species of the *B. dorsalis* complex need to be borne in mind when interpreting the results. Molecular sequencing of the *Bactrocera* individuals caught showed that they mostly were genetically different, indicating genetically different origins.

Bulgaria

The monitoring in 2016 took place only in the Plovdiv region. 12 Delta type traps were placed, with an attractant trimedlure at 4 observation points in the area of the villages Kalekovets, Karadjovo, Tsalapitsa and the town Parvenets. Monitoring was carried out from June to October. Visual inspections were made every month. *C. capitata* has not been found. In the Blagoevgrad region, in the framework of monitoring for *Drosophila suzukii, C. capitata* was identified in two apple vinegar traps placed in fruit and vegetable storehouse in the town of Petrich.
In Plovdiv, several individuals of *C. capitata* were found in stored apple fruits from a yard in the town of Sadovo in early November. About the same time, a number of larvae were found in apples, from a private yard in the area of Sofia.
In 2017, no adults of *C. capitata* were captured in all traps employed. In 2018, adults of *C. capitata* were captured in 12 out of 21 traps used for monitoring. A high number of *C. capitata* were captured in the area of Plodviv, where 297 males and 65 females were trapped.

2.4.3. Volatiles from infested fruits

The identification of the volatiles emitted by oranges at different developmental stages from oviposition to final instar larvae exit have shown major differences between healthy and infested fruits. Hexyl hexanoate is the main ester in infested and oviposited fruits. In healthy oranges, Valencene was the predominant compound. The relative high amounts of E-(β)-ocimene in infested and oviposited oranges compared to healthy ones, is associated with damage to the fruit peels of Citrus species.

2.5. Conclusions and recommendations to policy

Survey results for *C. capitata* in Austria indicate repeated entries as most probable origin, however, also raises questions considering a common origin of the individuals that clustered over the years. The potential of *C. capitata* to overwinter in Austria and related hypotheses will be addressed in an international collaboration starting in 2019 (H2020-SFS-2018-2020, “In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies ('OFFSeason' FF-IPM). Considering earlier studies on *Bactrocera* species it is evident that the finding of individuals of *B. zonata* and *B. dorsalis* in Vienna are most probably linked to repeated entries of larvae in infested fruit (cargo or traveller), as Austrian climate is currently unsuitable for their establishment. Survey activities in Austria will proceed in the framework of the Euphresco-Network project 2017-F-236 ‘*Ceratitis capitata*: better knowledge for better risk management (FruitFlyRiskManage)’ .
In Bulgaria the last few years larvae of *C. capitata* have been intercepted in imported fruits and adults trapped in orchards and warehouses, but its establishment in the fields has not been confirmed yet. It is likely that it is hard for *C. capitata* to overwinter in the climatic conditions in Bulgaria. Annual fruit fly inspections will continue in order to show if the Mediterranean fruit fly could overwinter in Bulgaria under currrent climatic conditions.
No individuals of *Bactrocera* species have been caught in traps or found in fruits in Bulgaria and Greece.
The potential of fruit flies to be introduced and further spread in Europe is evidenced by the recent outbreaks of *B. dorsalis* in Italy (Nugnes *et al*., 2018) and the fact that currently, *C. capitata* has become a major pest of apples in the area of Trentino, Italy, jeopardising the applied integrated pest management program against codling moth, *Cydia pomonella* (Zanoni *et al*., 2017). Therefore, monitoring activities for both species being currently not present in

Europe and species that are present and have proven to expanding their distribution northwards, would greatly facilitate the early detection of any new occurrence. Early detection is of utmost importance for successful eradication programs.
Identification of volatiles that discriminate between infested and healthy fruits have been found during this project. There are increasing evidences in the literature that infested fruits are characterised by a different profile of volatile organic compounds in relation to healthy ones (Mas *et al*., 2020). The discrimination between infested and healthy fruits, so far has been attributed to specific volatile compounds. Efforts should be driven towards exploring in detail the effect of other variables such as the cultivar or storage conditions on the emission of these compounds. Odorant-based detection tools (e-noses) are available and could be explored for their ability to detect infested fruits. These tools need to be 'trained' in order to allow a reliable identification of specific volatiles that characterise an infested fruit. This potential is currently under investigation within the EU funded project "In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies (FF-IPM").

2.6. Benefits from trans-national cooperation

Fruit flies of the Tephritidae family are important pest worldwide and there are new evidences supporting the theory that notorious pests such as *C. capitata* are expanding their distribution northwards in Europe. Moreover, outbreaks of *Bactrocera dorsalis* occurred in Europe in 2018 and 2019. Cooperation is an absolutely necessity in order to better understand the drivers for fruit flies' dispersal and develop appropriate management practices. The results of the FLY DETECT project are the motive for further experimentation on early detection, spread and overwintering capacity being currently explored within the EU-funded project "In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies (FF-IPM").

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- Nugnes, F, Russo, E, Viggiani, G, Bernardo, U (2018). First Record of an Invasive Fruit Fly Belonging to *Bactrocera dorsalis* Complex (Diptera: Tephritidae) in Europe. Insects, 9, 182.
- Zanoni S, Baldessari M, de Cristofaro A, Ioriatti C (2017). Susceptibility of selected apple cultivars to the Mediterranean fruit fly. Integrated Protection of Fruit Crops IOBC-WPRS Bulletin Vol. 123, pp. 43-44.

3. Publications

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

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

None.

4. Open Euphresco data

None.

APPENDIX 1. Deliverable: Literature study on trapping methods for *Bactrocera zonata* (established by AGES-AT)

EUPHRESCO project

FLY_DETECT

Development and implementation of early detection tools and effective management strategies for invasive non-European and other selected fruit fly species of economic importance (FLY DETECT)

Deliverable 2.1.

Report on literature review on trapping methods for relevant fruit fly species

Part: *Bactrocera zonata* – peach fruit fly

Author:

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AUSTRIA

Content

Introduction

Information on EUPHRESCO

(See https://www.euphresco.net/ for more details)

Euphresco is a network of organisations funding research projects and coordinating national research in the phytosanitary area. The overall goal of Euphresco is to support coordination and collaboration in the area of phytosanitary research, and to become a strong, long-term network of funders that fully incorporate existing and new members.

Information on the project FLY-DETECT

(See project proposal for more details and original text)

Diptera: Tephritidae have a huge economic impact on fruit and vegetable production worldwide because of their direct damage to fruit and vegetable commodities and indirect effects due to quarantine regulations. The present project focus on those species of fruit flies that are key pests to a large number of crops in the Mediterranean region, such as *Ceratitis capitata* (Mediterranean fruit fly, Medfly), or are considered as important invasive species, such as *Bactrocera zonata* (Peach Fruit Fly, PFF), *Bactrocera dorsalis* (Oriental Fruit Fly, OFF; resp. species of the *B. dorsalis* complex), and *Myiopardalis pardalina* (Melon Fruit Fly, MFF). These species are a threat as they (are/) may become established in the Mediterranean basin, posing a risk to the horticultural crops and agriculture of Central and Southern Europe. The pests under study have substantial interceptions in EUROPHYT database each year.

The project aims at (i) developing and implementing novel detection and interception tools and strategies for the above species, (ii) exploring their current geographical distribution in the countries involved in the proposed project as well as their potential range expansion to northern European countries by focusing on basic biological data, (iii) determining the host status of specific fruit of economic importance, and (iv) developing novel pest risk reduction options both at the place of production and at the entry points.

Expected results:

- Overview of the geographical distribution of the fruit-fly species under study in the countries participating in the proposed project
- Development and implementation of tools for early and rapid detection of fruit flies in agricultural commodities
- Management strategies for selected fruit commodities applied at both the place of production and place of entry in order to reduce the risk of introduction and spread within the EU and the Mediterranean region of non-European tephritid fruit flies
- Status of specific fruit (e.g. kiwi fruit) as potential hosts of these pests
- Cold hardiness ability of these pests, which is essential for projecting the potential spread of those species and performing accurate PRAs

Project duration: **01/04/2016 – 31/03/2019**

Background of the deliverable 2.1

WP 2 - Surveillance and management options for fruit flies

Work-package objectives:

Explore their current geographical distribution in the countries involved in the proposed project as well as their potential range expansion to northern European countries by focusing on basic biological data,

determine the host status of specific fruit of economic importance, and
develop novel pest risk reduction options

Task 2.1 Trapping methods for relevant fruit flies

A literature study will be developed on the available trapping methods for certain relevant fruit flies. Participants AGES, BFSA and DAFM will cooperate in performing the literature review on **trapping devices and methodologies**.

The literature study will focus on the species of relevance for the respective partners and with relation to relevant host plant species.

The literature study will cover scientific publications through searching in databases such as Scopus, CAB abstracts and Google Scholars. Key terms will include the scientific names of the species and terms related to trapping and monitoring (traps, monitor, trapping etc.). The terms used for literature search will be defined in cooperation with all participants involved in this task.

During the project meeting on March 8th, 2016 (and subsequent agreements) it was agreed that every

partner works alone on one of the relevant species, which were divided between the partners before. AGES agreed to take over the tasks for *Bactrocera zonata*. The project coordinator will finally combine the files afterwards to end up with one document (pdf). This literature-review basically has the intention to support the project-partners, rather than to support public.

Search terms:

species name (scientific name + synonyms + English common name)

in combination with

Trap – Catch – Monitoring – Detect

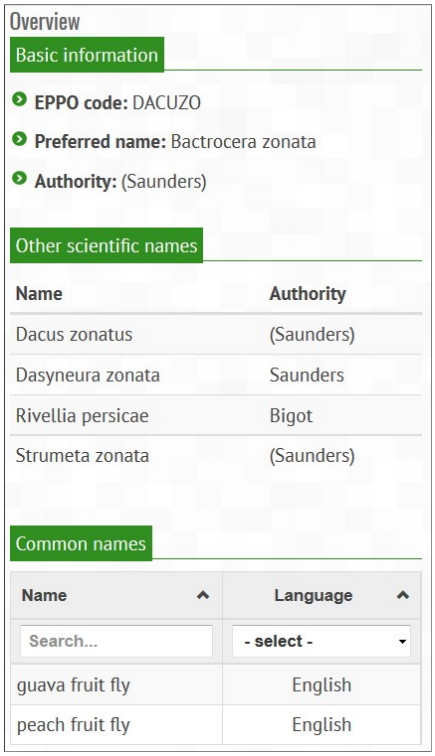
Literature search

Date of search: on 21.09.2016 & 12.12.2017 databases were checked on recent publications and those of relevance were entered in an endnote file (reference manager).

Search terms

Species name (scientific name + synonyms + English common name) (EPPO, 2017)

- *Bactrocera zonata*
- *Dacus persicus*
- *Dacus zonatus*
- *Dasyneura zonata*
- *Rivellia persicae*
- *Strumeta zonata*
- Guava fruit fly
- Peach fruit fly

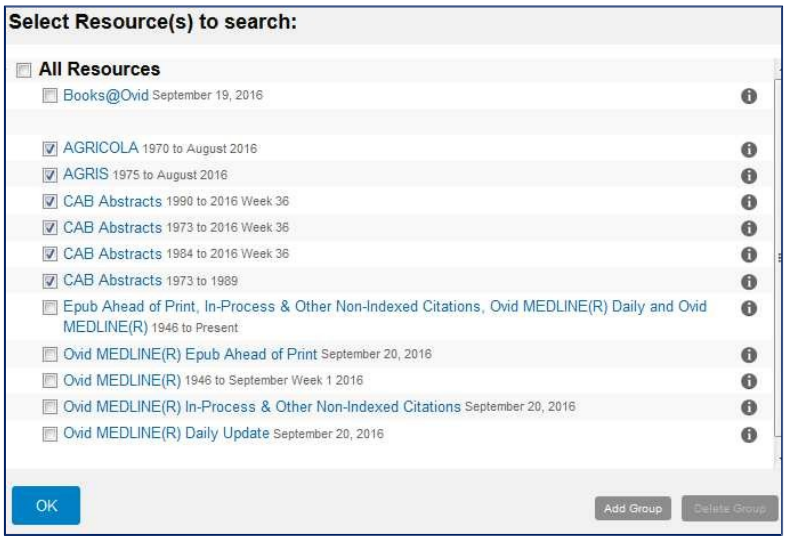


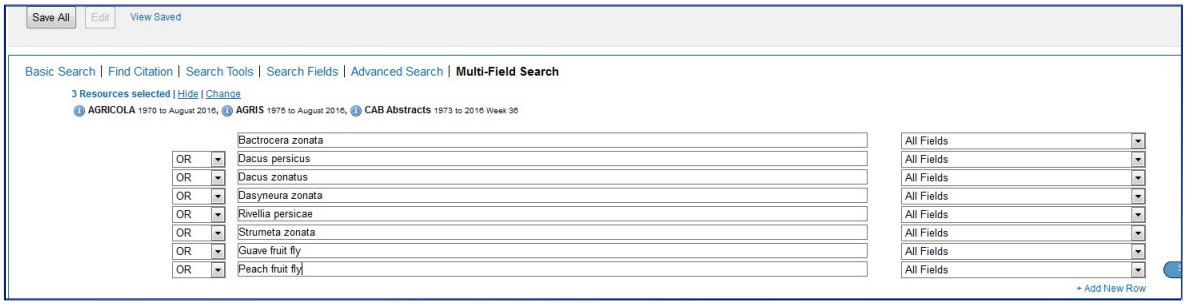
in combination with

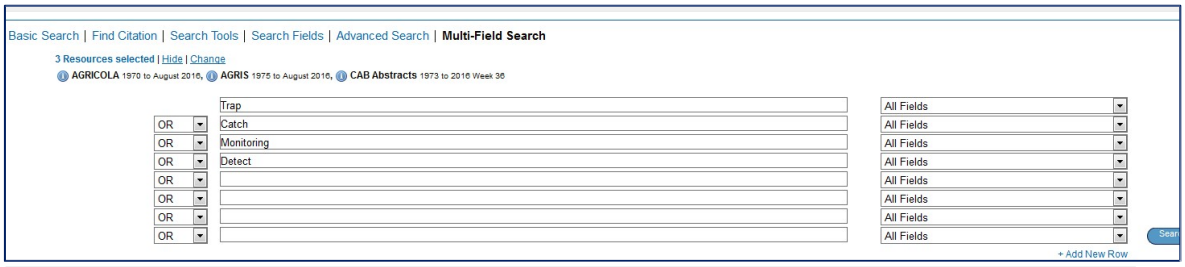
- trap
- catch
- monitoring
- detect

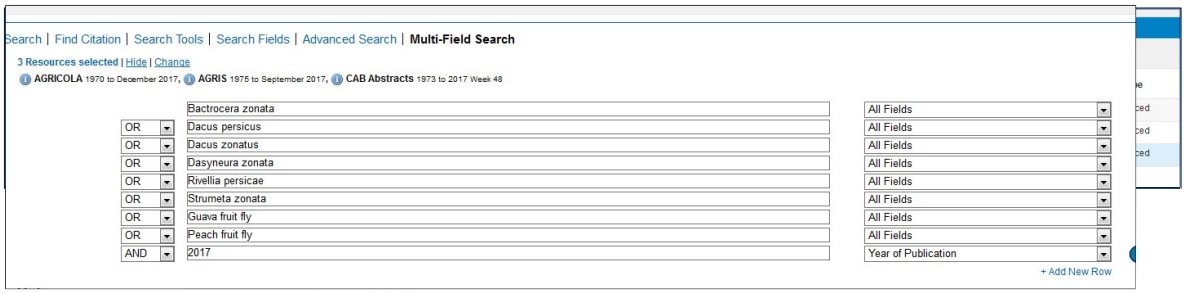
Search execution

CAB Abstracts, Agricola, Agris





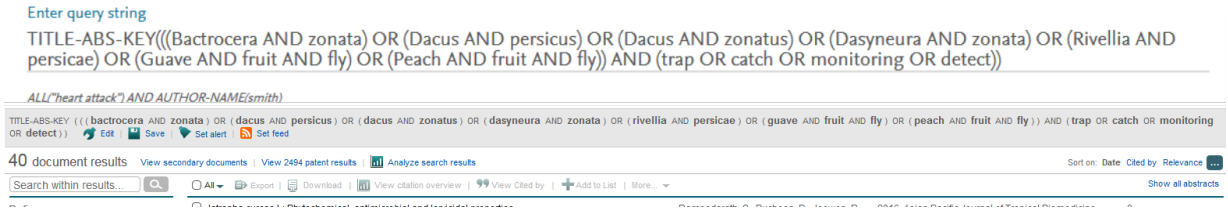




Scopus

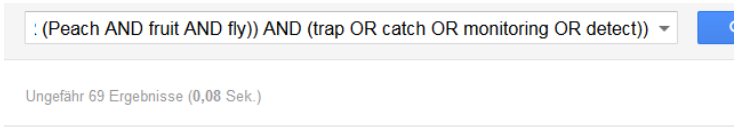
Search term combination:

TITLE-ABS-KEY(((Bactrocera AND zonata) OR (Dacus AND persicus) OR (Dacus AND zonatus) OR (Dasyneura AND zonata) OR (Rivellia AND persicae) OR (Guava AND fruit AND fly) OR (Peach AND fruit AND fly)) AND (trap OR catch OR monitoring OR detect))



Google Scholar

(((Bactrocera AND zonata) OR (Dacus AND persicus) OR (Dacus AND zonatus) OR (Dasineura AND zonata) OR (Rivellia AND persicae) OR (Guava AND fruit AND fly) OR (Peach AND fruit AND fly)) AND (trap OR catch OR monitoring OR detect))



Relevant references were included in the reference manager, in case they were not already included (no duplication).

Other Sources

Relevant references from other sources, which were not found with the above search history, were considered when available, for example:

- documents or recommendations of international organizations
- references used in other sources
- books and relevant online documents

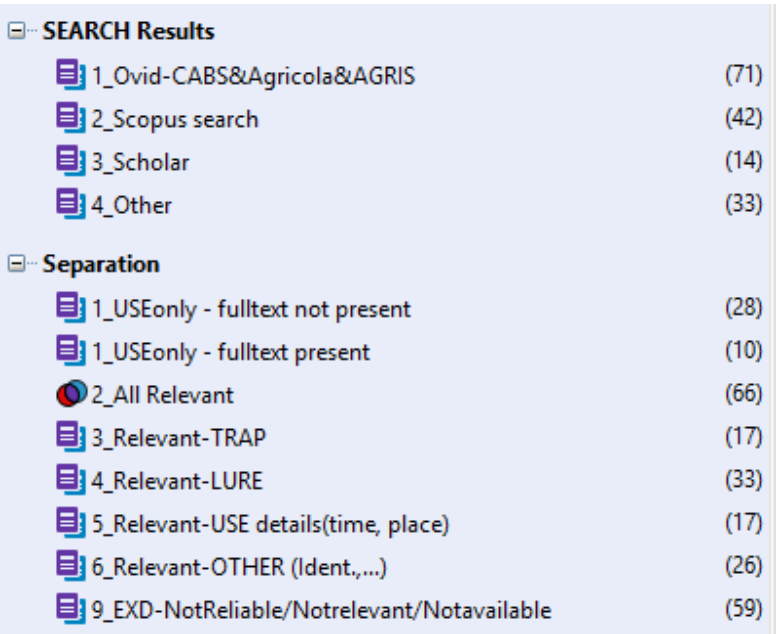
Processing of search output

In order to screen and manage the output of the literature search, which was saved in a reference manager file, references were separated in different groups.

After screening of the references (title, keywords, abstract) on their relevance for the search purpose, they were either

- sorted out (e.g. when trapping devices were only used for other purposes but not for testing the device or trapping system, or in case the reference seemed not to be reliable)
- or they were placed in one of the separation-groups containing references with relevant content on trap/lure/use details/others.

For all references classified as *relevant* full texts were organised when possible. If no full text documents could be organized (ordering via library services was not possible), the reference was sorted out as *not available*.



Resulting references

More than 150 references were found and processed in the literature search.
Those references can be found below.

(Abdel-Galil et al., 2010, Abdellah, 2007, Abraham and Jayanthi, 2001, Abraham et al., 2002, Agarwal and Kumar, 1999a, Agarwal and Kumar, 1999b, Agarwal et al., 1999, Agrawal and Deepa, 2013, Ahmad and Afzal, 1977, Ahmad et al., 2005a, Ahmad et al., 2005b, Ahmad and Begum, 2017, Alberti et al., 2009, Al- Eryan, 2008, Aljazzar, 2013, Alzubaidy, 2000, Annonymus, 1984, Babu and Shashidhar, 2003, Bagheri et al., 2017, Bateman and Morton, 1981, Bisane, 2017, Bonne et al., 2001, Botton et al., 2002, CABI, 2017, Carroll et al., 2002onwards, Cayol et al., 2002, Chambers, 1977, Chaudhry and Jamal, 2000, Choudhary et al., 2012, Cunningham, 1989, Darwish et al., 2014, Darwish et al., 2015, Deepa and Neeraja, 2010, Deepa et al., 2009, Divender and Ranjeet, 2000, Dowell and Odomelam, 1984, Draz et al., 2016, Drew, 1974, Drew and Hooper, 1981, EFSA, 2007, El-Gendy, 2012a, El-Gendy, 2012b, El-Gendy, 2013, Elnagar et al., 2010, EPPO, 2005, EPPO, 2010, EPPO, 2013, EPPO, 2017, Escudero-Colomar et al., 2008, FAO/IAEA, 2000, FAO/IAEA, 2003, FAO/IAEA, 2013, FAO/IAEA, 2018, Fletcher, 1989, Fui et al., 2013, Galande and Ukey, 2011, Galande et al., 2010, Grewal and Kapoor, 1987, Gupta et al., 1990, Howlett, 1912, Howlett, 1915, Hurtrel et al., 2002, IPPC, 2016, Ishtiaq et al., 1999, Iwahashi, 2001, Jamwal et al., 2015, Jitendra et al., 2014, Kakar et al., 2016, Katsoyannos, 1989, Kawashita et al., 2004, Khan et al., 2003, Khan et al., 2002, Khan et al., 2005, Khan and Muhammed, 2017, Khan et al., 2015, Khosravi et al., 2012, Khuhro et al., 1999, Mahmood and Mishkatullah, 2007, Malumphy, 2007, Manzar and Srivastava, 2004, Marwat et al., 1992, Marwat and Khalid, 1993, McPhail, 1939, Mo`tamedi Niya et al., 2008, Mogahed and Abdelmaksoud, 2016, Monteiro et al., 2007, Montes et al., 2011, Monzó et al., 2009, Moustafa, 2009, Muhammad et al., 2000, Muhammad et al., 2004, Nagaraj et al., 2014a, Nagaraj et al., 2014b, Nagaraj et al., 2014c, Nagaraj et al., 2014d, Ni et al., 2012, Othman and Aulaqi, 2011, Pal et al., 2015, Pal et al., 2012, Papadopoulos et al., 2001a, Papadopoulos et al., 2001b, Papadopoulos et al., 2003, Pasini et al., 2014, Patel and Patel, 1998, Qureshi et al., 1981, Qureshi et al., 1976, Qureshi et al., 1991, Qureshi et al., 1992, Rafi et al., 2016, Rajitha and Shashidhar, 2006, Rajitha and Viraktamath, 2005, Rampadarath et al., 2016, Rao and Sonali, 2016, Ravikumar and Viraktamath, 2006, Ravikumar and Viraktamath, 2007, Riaz et al., 2015, Rizk et al., 2014, Roomi et al., 1993, Rousse et al., 2007, Saafan, 2005b, Saafan, 2005a, Saafan et al., 2005b, Saafan et al., 2005a, Saafan et al., 2000, Sanjeev et al., 2008, Sarada et al., 2001, Satarkar et al., 2009, Satarkar et al., 2006, Sciarretta and Trematerra, 2011, Sewoosunkur et al., 2000, Shaked et al., 2017, Shankar et al., 2010, Sharma et al., 2015, Shashidar and Babu, 2004, Siddiqui et al., 2003, Singh et al., 2013, Singh and Sharma, 2013, Singh et al., 2007, Solangi et al., 2014, Solangi et al., 2015, Sookar et al., 2006, Souza-Filho et al., 2009, Steiner, 1957, Steyskal, 1977, Stonehouse et al., 2002, Talib et al., 1995, Tan, 1998, Thakur et al., 2013, Tokushima et al., 2010, Venkatachalam et al., 2014, Verghese et al., 2013, Verma and Nath, 2006, Viraktamath and Ravikumar, 2006, White and Elson-Harris, 1992, Wong et al., 1991, Ye and Liu, 2005, Ye and Liu, 2007, Zahran et al., 2017, Zhang et al., 2013)

While many of the references were classified as *not relevant / not reliable* or full texts were not available, variable numbers of references can be considered when searching for information on *B. zonata* trapping, with respect to: traps / lures / use details / other relevant information. Those references can be found below.

Relevant references for *B. zonata* trapping – TRAPS

(Aljazzar, 2013, Draz et al., 2016, El-Gendy, 2012a, FAO/IAEA, 2013, Grewal and Kapoor, 1987, IPPC, 2016, Katsoyannos, 1989, Rajitha and Viraktamath, 2005, Ravikumar and Viraktamath, 2006, Ravikumar and Viraktamath, 2007, Rizk et al., 2014, Satarkar et al., 2006, Shaked et al., 2017, Shankar et al., 2010, Sookar et al., 2006, Steiner, 1957, Steyskal, 1977)

Relevant references for *B. zonata* trapping – LURE

(Agarwal and Kumar, 1999b, Agrawal and Deepa, 2013, Ahmad and Afzal, 1977, Ahmad et al., 2005a, Ahmad et al., 2005b, Aljazzar, 2013, Bagheri et al., 2017, Bateman and Morton, 1981, Chambers, 1977, Cunningham, 1989, Drew, 1974, Drew and Hooper, 1981, El-Gendy, 2012b, El-Gendy, 2013, FAO/IAEA, 2003, FAO/IAEA, 2013, Howlett, 1912, Howlett, 1915, IPPC, 2016, Kakar et al., 2016, Khan et al., 2015, McPhail, 1939, Moustafa, 2009, Nagaraj et al., 2014d, Pal et al., 2012, Qureshi et al., 1976, Riaz et al., 2015, Rizk et al., 2014, Roomi et al., 1993, Sarada et al., 2001, Satarkar et al., 2006, Sookar et al., 2006, Steiner et al., 1965, Verghese et al., 2013)

Relevant references for *B. zonata* trapping – USE (time, place …)

(Agarwal and Kumar, 1999a, Agrawal and Deepa, 2013, Darwish et al., 2014, Darwish et al., 2015, Draz et al., 2016, El-Gendy, 2012a, EPPO, 2010, FAO/IAEA, 2003, FAO/IAEA, 2013, IPPC, 2016, Ishtiaq et al., 1999, Manzar and Srivastava, 2004, Pal et al., 2012, Rizk et al., 2014, Sarada et al., 2001, Siddiqui et al., 2003, Singh and Sharma, 2013)

Relevant references for *B. zonata* trapping – Other potentially relevant information (Identification, internat. recommendations…)

This section includes references, which could support in identification, include international recommendations, or could be helpful in any other means.

(Ahmad and Afzal, 1977, Bateman and Morton, 1981, CABI, 2017, Drew, 1974, Drew and Hooper, 1981, EFSA, 2007, EPPO, 2005, EPPO, 2010, EPPO, 2017, FAO/IAEA, 2000, FAO/IAEA, 2003, FAO/IAEA, 2013, Fletcher, 1989, Howlett, 1915, IPPC, 2016, McPhail, 1939, Ni et al., 2012, Steiner, 1957, Tan, 1998, Verma and Nath, 2006, White and Elson-Harris, 1992)

In addition added, due to recent publication: (FAO/IAEA, 2018)

Literature review on *Bactrocera zonata* trapping

At this point, it is important to note that, due to the limited resources for this in-kind EUPHRESCO project, it was only possible to establish the literature search and screening of the references on their relevance (see above). The collected references can now support further activities, like detailed reviews on *Bactorcera zonata* trapping, which however was not possible to achieve in the framework of this project.

Nevertheless, some information is provided below.

Fruit flies, including those of the genus *Bactrocera*, utilize visual cues to locate vegetation and plant structure. Therefore, shape, size, color of foliage and fruit, darkness of silhouette and other visual cues are of importance for them to localize their host plants and suitable fruit. Objects mimicking these visual characters will attract fruit flies and have proved to be suitable for trapping purposes. In general, spherical models were more attractive for both sexes than other shapes, when placed in a hostplant. At least in laboratory trials, as oviposition devices those with convex surfaces (like spheres or hemispheres) and a size comparable of that of the host fruit were preferred for egg laying. While in the laboratory the shape and size of an oviposition device are of primary importance, in the field the color of a device to attract fruit flies would be equal important. Yellow, orange and green were the most attractive colors tested, and also fluorescent colors are highly attractive for several fruit flies (Katsoyannos, 1989).

However, the attractiveness of colors might be diverse and of different importance between fruit fly species. A number of investigations tried to reveal the preferences concerning the color of trapping devices for *B. zonata* in field studies. While some of them revealed clear preferences for one or more colors compared to others, no preferences were found in other studies. E.g. red cylinder traps caught significantly more *B. zonata* compared to black, transparent, orange, green, yellow, blue or white traps of the same design in one study in Indian orchards (Ravikumar and Viraktamath, 2007). However, no preference for a color between transparent, red, green and orange was found in another study carried out with different shapes of traps in another Indian orchard (Rajitha and Viraktamath, 2005). Comparing white to yellow Jackson traps in Egypt, still another study found significant preferences for white over yellow as trap color (El-Gendy, 2012a).

According to international recommendations (FAO/IAEA, 2018) (which also refer to some of those publications that resulted from the literature search like (Katsoyannos, 1989, White and Elson-Harris,

1992, FAO/IAEA, 2003)) trapping systems for fruit fly surveys require attractants (e.g. pheromones, parapheromones or food attractants), killing agents (and a preservative) and devices for trapping.

Commonly used attractants for *Bactrocera zonata* are

Methyl eugenol (ME)

Three-component (3C) synthetic food attractant, mainly for female captures (ammonium acetate, putrescine, trimethylamine) and

Ammonium acetate (AA)

To catch male *B. zonata* flies, the attractant ME can be used with a great variety of different traps (for details on the traps please see Annex 1 at page 31 of (FAO/IAEA, 2018)), namely:

CH - ChamP Trap

ET – Easy Trap

JT – Jackson Trap (or Delta Trap)
LT – Lynfield Trap

MM – Maghreb-Med or Morocco trap

ST – Steiner Trap

TP – Tephri Trap

YP – Yellow Panel Trap

To catch female *B. zonata* flies, either the attractant 3C should be used in MLT (Multilure trap) or protein attractants can be used in the following trap types

McP – McPhail Trap type

MLT – Multilure Trap

When using one of the above-mentioned attractants, it is important to consider the field longevity and the required service intervals in relation to the survey type. The details of the following graph are part of table 4 on page 10 of (FAO/IAEA, 2018), where more details can be found.

Common name	Acronym	Formulation	Field longevity[1] (weeks)	Survey programme			
				Monitoring/Detection		Delimiting/Verification	
				Inspection[2] (days)	Service[3] (rebait) (weeks)	Inspection[2] (days)	Service[3] (rebait) (weeks)
Para-pheromones							
Methyl eugenol	ME	Polymeric plug	4–10	7–14	8–10	3–7	4
		Liquid	4–8	7–14	6–8	3–7	4
Food-based attractants							
Torula yeast/borax	PA	Pellet	1–2	7–14	2	2–3	1
Protein derivatives	PA	Liquid	1–2	7–14	2	2–3	1
Ammonium acetate	AA	Patches	4–6	7–14	5–6	2–3	4
		Liquid	1	7–14	1	2–3	1
		Polymer	2–4	7–14	3–4	2–3	2
Ammonium acetate Putrescine Trimethylamine	3C	Long-lasting patches	18–26	7–14	24–26	2–3	18

[1] Based on half-life; attractant longevity is indicative only; actual timing should be supported by field testing and validation
[2] Inspection refers to interval between checking traps for target fruit fly captures
[3] Service refers to rebaiting period of the trap based on half-life of the attractant. Other factors such as weathering of traps, density of flies trapped and longevity of killing agents are not considered.

Information on killing agents are also available in the suggested publication (see page 11), as well as details on the trapping procedure (chapter 5, page 12 of (FAO/IAEA, 2018)).

Table 6b. Trap densities for *Bactrocera* spp. responding to methyl eugenol (ME), cuelure (CUE) and food attractants[1] (PA — protein attractants)

Scenario	Trap type[2]	Attractant	Trap density[2] / square km			
			Production area	Marginal	Urban	Points of entry[4]
A. Monitoring surveys, no control	JT/ST/TP/LT/MLT/McP/TP	ME/CUE/PA	0.5–1.0	0.2–0.5	0.2–0.5	0.2–0.5
B. Monitoring surveys for suppression	JT/ST/TP/LT/MLT/McP/TP	ME/CUE/PA	2–4	1–2	0.25–0.5	0.25–0.5
C. Monitoring surveys for eradication	JT/ST/TP/MLT/LT/McP/TP	ME/CUE/PA	3–5	3–5	3–5	3–5
D. Detection surveys for exclusion exclusion (includes intensive trapping)	CH/ST/LT/MLT/McP/TP/ YP	ME/CUE/PA	1	1	1–5	3–12
E. Delimitation surveys after incursion in addition to detection survey[5]	JT/ST/TP/MLT/LT/McP/YP	ME/CUE/PA	2–20	2–20	2–20	2–20
F. Verification surveys after eradication of pest outbreak[6]	JT/ST/TP/MLT/LT/McP/YP	ME/CUE/PA	5–10	5–10	5–10	5–10

1 *Bactrocera zonata*, *Z. cucurbitae* (3- and 2-component attractants and other ammonium-based synthetic food attractants).
2 Different traps can be combined to reach the total number.
3 Refers to the total number of traps.
4 Including other high-risk sites.
5 This range includes high-density trapping in the immediate area of the detection (core area) and decreasing towards the surrounding trapping zones (**Section 6, Figure 3**).
6 Applies only to core area and first surrounding zone (**Figure 3**).

Trap type

CH	ChamP trap	MLT	Multilure trap
JT	Jackson trap	ST	Steiner trap
LT	Lynfield trap	TP	Tephri trap
McP	McPhail trap	YP	Yellow panel trap

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APPENDIX 2. Deliverable: Report on the fruit fly in Austria (established by AGES-AT)

EUPHRESCO project

FLY_DETECT

Development and implementation of early detection tools and effective management strategies for invasive non-European and other selected fruit fly species of economic importance (FLY DETECT)

Deliverable 2.2.

Reports on distribution and presence of relevant fruit fly species in participating countries based on national monitoring results.

Survey on *Ceratitis capitata* and *Bactrocera* spp. in Austria, 2016 - 2018

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AUSTRIA

Content

Introduction

Information on EUPHRESCO

(See https://www.euphresco.net/ for more details)

Euphresco is a network of organisations funding research projects and coordinating national research in the phytosanitary area. The overall goal of Euphresco is to support coordination and collaboration in the area of phytosanitary research, and to become a strong, long-term network of funders that fully incorporate existing and new members.

Information on the project FLY-DETECT

(See project proposal for more details and original text)

Diptera: Tephritidae have a huge economic impact on fruit and vegetable production worldwide because of their direct damage to fruit and vegetable commodities and indirect effects due to quarantine regulations. The present project focus on those species of fruit flies that are key pests to a large number of crops in the Mediterranean region, such as *Ceratitis capitata* (Mediterranean fruit fly, Medfly), or are considered as important invasive species, such as *Bactrocera zonata* (Peach Fruit Fly, PFF), *Bactrocera dorsalis* (Oriental Fruit Fly, OFF; resp. species of the *B. dorsalis* complex), and *Myiopardalis pardalina* (Melon Fruit Fly, MFF). These species are a threat as they (are/) may become established in the Mediterranean basin, posing a risk to the horticultural crops and agriculture of Central and Southern Europe. The pests under study have substantial interceptions in EUROPHYT database each year.

The project aims at (i) developing and implementing novel detection and interception tools and strategies for the above species, (ii) exploring their current geographical distribution in the countries involved in the proposed project as well as their potential range expansion to northern European countries by focusing on basic biological data, (iii) determining the host status of specific fruit of economic importance, and (iv) developing novel pest risk reduction options both at the place of production and at the entry points.

Expected results:

- Overview of the geographical distribution of the fruit-fly species under study in the countries participating in the proposed project
- Development and implementation of tools for early and rapid detection of fruit flies in agricultural commodities
- Management strategies for selected fruit commodities applied at both the place of production and place of entry in order to reduce the risk of introduction and spread within the EU and the Mediterranean region of non-European tephritid fruit flies
- Status of specific fruit (e.g. kiwi fruit) as potential hosts of these pests
- Cold hardiness ability of these pests, which is essential for projecting the potential spread of those species and performing accurate PRAs

Project duration: **01/04/2016 – 31/03/2019**

Background of the deliverable 2.2

WP 2 - Surveillance and management options for fruit flies

(See project proposal for more details and original text)

Work-package objectives:

> Explore their current geographical distribution in the countries involved in the proposed project as well as their potential range expansion to northern European countries by focusing on basic biological data,
>
> determine the host status of specific fruit of economic importance, and
> develop novel pest risk reduction options

Task 2.2 National monitoring activities on relevant fruit flies

(See project proposal for more details and original text)

National activities were scheduled for Bulgaria and Austria for the following species:

- *Ceratitis capitata*
- *Bactrocera zonata*
- *B. dorsalis*

Furthermore, provision of the data from the national monitoring in Greece was agreed by partner BPI.

Report on the fruit fly survey in Austria, 2016-2018

Introduction

Ceratitis capitata, the Mediterranean fruit fly (medfly) which has its origin in Africa, has spread and became an important pest in the Mediterranean countries with widespread distribution. For Central Europe, where climatic conditions do not favour the establishment of tropical fruit flies, few records of earlier occurrences are available. From Austria only records from the city of Vienna were published in the past (Böhm, 1958, Glaeser, 1979, Watzl, 1932). *Bactrocera zonata*, the Peach fruit fly, and *B. dorsalis*, the Oriental fruit fly, originally from tropical Asia, are not known to have established populations in Europe. However, frequent interceptions at entry points of the European Union and findings of single individuals in the past (e.g. in Vienna in different years and for *B. dorsalis* in Italy in 2018) indicate the potential risk of entries of those species (EPPO, 2019, Lethmayer, 2011, Nugnes et al., 2018).

Material and methods

Sampling sites and survey period

In Vienna, traps were installed in peach trees (*Prunus persica*), which is a major host plant for all focussed fruit fly species, or other host plants in allotments and small gardens, but also at fruit and vegetable market areas, near a biogas plant and a composting plant. In the other Austrian regions, traps were installed in or close to commercial orchards or small gardens containing host plants. In addition, traps were installed at Vienna International Airport (point of entry; Lower Austria). See Table 1 for number of sampling sites per region and year. In order to cover the season's fruiting periods the survey period was approximately from June to October at most of the sites in 2016, 2017 and 2018.

Table 1. Number of sampling sites in Austrian regions (2016–2018).

	2016	2017	2018
Vienna	4	8	14
Lower Austria	3	5	7
Upper Austria	1	1	1
Burgenland	2	3	3
Styria	3	3	5
Carinthia	4	4	4
Salzburg	0	1	0
Tirol	0	2	2
Total	**17**	**27**	**36**

Traps and lures

Tephri trap type traps (Maxitrap®, supplier Sociedad Española de Desarrollos Químicos (SEDQ), S.L., ES) were installed and charged with trimedlure for *Ceratitis capitata* or methyl eugenol for *Bactrocera zonata* and *B. dorsalis* (supplier Pherobank B.V., NL) to lure male fruit flies. In addition, traps charged with an attractant for female flies (Femilure-45 (prod. code: PH-180-FM45), supplier Russell IPM Ltd, UK) were installed at some sites in 2017 and 2018.

Identification and molecular analysis

Morphological identification was carried out using identification keys and diagnostic protocols. Identification of the caught individuals was confirmed with molecular diagnostic methods for all *Bactrocera* individuals and for some *Ceratitis* individuals. Sequencing data of transcribed spacer 1 (ITS 1) PCR amplicons (for *C. capitata*) or of the cytochrome oxidase I (COI) PCR amplicons (for *Bactrocera* spp.) were compared using the software Geneious 10.1.3 (Biomatters Ltd.), and alignment trees were built to identify genetic differences to determine matching origins of the individuals (Boykin et al., 2014, Carroll et al., 2002onwards, David and Ramani, 2011, Drew and Hancock, 1994, EPPO, 2011, EPPO, 2013, IPPC, 2019, Lethmayer, 2011, Malumphy, 2007, Nugnes et al., 2018, Van Houdt et al., 2010, Virgilio et al., 2014, White and Elson-Harris, 1992).

Results

The survey in Austria resulted in catches of more than hundred fruit flies of *Ceratitis capitata* in each season (see Table 2) and catches of single individuals of *Bactrocera zonata* and *Bactrocera dorsalis* in different years.

While no *C. capitata* individuals were obtained from traps placed in other Austrian regions, despite four individuals (see Table 2), catches occurred at up to eight sampled sites in the city of Vienna. More than one thousand individuals of this species were caught between 2016 and 2018. Molecular analyses confirmed the morphological identification. Molecular sequencing revealed that analysed individuals were in general from a number of genetically different origins. However, results showed that approximately one quarter of the sequences were clonal (including individuals from all three years), therefore indicating the same genetic origin.

Table 2. Number of catches of adult *Ceratitis capitata* in Austrian regions (2016–2018).
(n.a. = not applicable)

	2016	**2017**	**2018**
Vienna	764	115	182
Lower Austria	1	0	0
Upper Austria	0	2	0
Burgenland	1	0	0
Styria	0	0	0
Carinthia	0	0	0
Salzburg	n.a.	0	n.a.
Tirol	n.a.	0	0
Total	**766**	**117**	**182**

Furthermore, survey activities in Austria resulted in catches of single individuals of two *Bactrocera* species in 2016-2018. All individuals were caught in gardens or orchards in urban areas of Vienna while no *Bactrocera* individuals were obtained from traps placed in other provinces. All individuals were caught

with traps charged with male attractant. Individuals were identified as *B. zonata* or *B. dorsalis*. However, it is important to note that for identified *B. dorsalis* individuals it was not possible to distinguish between *B. dorsalis s.l.* (following IPPC 2019) and *B. carambolae* with molecular methods (ITS1). Limitations of the current diagnostic protocol to differentiate species of the *B. dorsalis* complex need to be borne in mind when interpreting the results. Molecular sequencing of the *Bactrocera* individuals caught showed that they mostly were genetically different, indicating genetically different origins.

Notes and Outlook

Detailed results of our survey activities from the present project, together with the results from our previous findings will be published soon (for *Ceratitis capitata*: Egartner et al. 2019, IOBC Bulletin*, in publication*; for *Bactrocera* spp.: Egartner et al. 2019, *in preparation*).

Considering earlier studies on *Bactrocera* species it is evident that the finding of individuals of *B. zonata* and *B. dorsalis* in Vienna are most probably linked to repeated entries of larvae in infested fruit (cargo or traveller), as Austrian climate is unsuitable for their establishment (De Villiers et al., 2016, Hong et al., 2015, Ni et al., 2012, Stephens et al., 2007, White and Elson-Harris, 1992).

Survey results for *C. capitata* also indicate repeated entries as most probable origin, however, also raises questions considering a common origin of the individuals that clustered over the years. The potential of *C. capitata* to overwinter in Austria and related hypotheses (Papadopoulos et al., 1996, Papadopoulos et al., 1998) will be addressed in an international collaboration starting in 2019 (H2020-SFS-2018-2020, “In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies ('OFF-Season' FF-IPM).

Survey activities in Austria will proceed in the framework of the Euphresco-Network project “FruitFlyRiskManage” (more details: https://www.euphresco.net/) in 2019.

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APPENDIX 3. Survey of fruit flies in Bulgaria

REPUBLIC OF BULGARIA

MINISTRY OF AGRICULTURE, FOOD AND FORESTRY
BULGARIAN FOOD SAFETY AGENCY

CENTRAL LABORATORY FOR PLANT QUARANTINE

EUPHRESCO project FLY DETECT

Development and implementation of early detection tools and effective management strategies for invasive non-European and other selected fruit fly species of economic importance (FLY DETECT)

WP2 Surveillance and management options for fruit flies

Task 2.2 National monitoring activities on relevant fruit flies

Survey on fruit flies in Bulgaria, 2016 - 2018

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Introduction

The monitoring for the Mediterranean fruit fly *Ceratitis capitata* is an essential part of the programs for the survey and control of quarantine and invasive pests in the country. It is carried out within the framework of a European project for technical cooperation with the participation of phytosanitary inspectors from the Regional Directorates and the Central Laboratory of Plant Quarantine (CLPQ) of the Bulgarian Food Safety Agency (BFSA).

Material and methods

The monitoring for *C. capitata* and other Tephritid flies was carried out at the following observation points:

- Orchards - plums, cherries, sour cherries, peaches, nectarines, apricots, apples, quinces, pears, figs, walnuts, almonds.
- Berry crops - currants, gooseberries, bilberries, cranberries, blackcurrant, chokeberries.
- Exchanges and markets, fruit and vegetable storehouses - exotic and other crops - citrus, papaya, guava, avocado, pomegranate, carambola, mango, jujube, sugar-apple, watermelon, melon, cucumber, zucchini, tomato, grape, coffee etc.

The following types of traps were used during the 2016-2018 surveys:

- For female attraction - TephriTrap and MacPhail trap, with Biolure and TMA synthetic bait based on Amonium acetate, Putrescine and Trimethylamine. A protein bait with yeast was also used.
- In order to attract the males paraferomones were used with Delta type traps – methyl eugenol for species of the genus *Bactrocera* and trimedlure, for species of the genus *Ceratitis.*
- In some observation points Yellow Sticky Traps were also placed.

The use of traps in orchards and berry crops allows us to follow the pest's biology, moment of first occurrence and peak of infestation compared to the local conditions. Pheromone traps and

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those with a three-component attractant were placed from May to November, more often in mixed-type gardens. Traps were placed on different trees at a distance of 25-30 meters apart, at a suitable height (1-1.5 m), on the south or east side of the tree, in more shady places - away from leaves and fruits. Every two to four weeks, the traps were inspected and the individuals captured in them were sent to the CLPQ for analysis. All identifications were made based on morphological characteristics.

Inspections in markets and fruit and vegetable warehouses were carried out throughout the year whenever hosts of the pests were present.

Results

The monitoring in 2016 took place only in the Plovdiv region. 12 Delta type traps were placed, with an attractant trimedlure at 4 observation points in the area of the villages Kalekovets, Karadjovo, Tsalapitsa and the town Parvenets. Monitoring was carried out from June to October. Visual inspections were made every month. *C. capitata* has not been found.

In the Blagoevgrad region, in the frame of monitoring for *Drosophila suzukii*, *C. capitata* was identified in two apple vinegar traps placed in fruit and vegetable storehouse in the town of Petrich.

In Plovdiv - several individuals of *C. capitata* were found in stored apple fruits from a yard in the town of Sadovo in early November. About the same time a number of larvae were found in apples, from a private yard in the area of Sofia.

Details about the observation points and the trapping period are presented in Table 1.

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Table 1. Observation points, period of setting and checking the traps in 2016

District	Number of points	Observation point	Trap type and attractant	Period of setting the traps	Result Trapped flies	
					♀	♂
Plovdiv						
Kalekovets	1	*Malus domestica*	Delta trap/trimedlure	June-October	Negative	
Karadjovo	1	*Prunus avium*	Delta trap/trimedlure	June-October	Negative	
	1	*Prunus domestica*	Delta trap/trimedlure	June-October	Negative	
Tsalapitsa	1	*Rubus ideaus*	Delta trap/trimedlure	June-October	Negative	
Parvenets	1	Trade for fruits and vegetables	Delta trap/trimedlure	June-October	Negative	
Sadovo	1	*Malus domestica*	fruits	November	7	3
Blagoevgrad						
Petrich	1	Trade for fruits and vegetables	Apple vinegar and soap	July-August	2	-
Sofia city						
Orlandovci	1	*Malus domestica*	fruits	November	1 larva of *C. capitata*	

In 2017, monitoring was carried out in the following areas of the country: Blagoevgrad, Burgas, Varna, Kyustendil, Pazardzhik, Plovdiv, Pleven, Sliven and Sofia. Traps were set at 19 observation points: in 18 orchards and 1 warehouse for fresh fruits and vegetables.

The traps used during this period were Delta trap with trimedlure (T), TefriTrap with bait Biolure (3C) and MacPhail trap with food bait (PB)

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Visual inspections were carried out in 20 premises trading with fresh fruits and vegetables from import, other Member States and local production. Fruits and vegetables from 12 different countries have been inspected, most of the consignments being from Greece and Turkey. There were no symptoms of fruit fly infestation. 95 samples were sent to CLPQ for identification of fruit flies. No *C. capitata* was found. Details about the observation points and the trapping period are presented in Table 2.

Table 2. Observation points, period of setting and checking the traps in 2017

District	Number of points	Observation points	Attractant type			Period of setting the traps	Result
			3C	PB	T		Trapped flies
Blagoevgrad	3	*Prunus persica*	3	2	5	July-August	Negative
Burgas	2	*Malus domestica; Prunus persica*		1	6	August-September	Negative
Varna	1	*Prunus domestica*	1	1	5	July-November	Negative
Kyustendil	4	*Prunus domestica, Vitis vinifera Prunus persica; Prunus armeniaca Prunus avium Malus domestica*	3	2	8	June-September	Negative
Pazardzhik	2	*Malus domestica*	2	1	4	August-November	Negative
Pleven	1	*Prunus domestica, Prunus persica Prunus avium*	2	2	6	June-October	Negative
Plovdiv	3	*Prunus avium*	3	2	8	June-August	Negative
Sliven	2	*Prunus persica*			7	July-October	Negative
Sofia	1	*Prunus persica*		2	1	June-October	Negative

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In 2018 monitoring was carried out in the following areas of the country: Blagoevgrad, Burgas, Varna, Vidin, Kardzhali, Kyustendil, Pazardzhik, Plovdiv, Pleven, Sliven, Silistra and Stara Zagora.

As a result of the monitoring fruit flies were identified in 21 traps as follows:

- *Ceratitis capitata* in 12 traps (5 Delta traps with trimedlure, 2 Delta traps with methyl eugenol and 5 Mc Phail with Biolure);
- *Drosophila suzukii* in 2 McPhail traps with Biolure;
- *Rhagoletis cerasi* in 5 Yellow Sticky Traps - Rebell (R);
- *Carpomya vesuviana* in 2 traps (1 MacPhail with Biolure and 1 Yellow Sticky Trap);
- *Carpomya sp*. in 2 traps (1 Delta trap with trimedlure and 1 Delta trap with methyl eugenol).

The presence of fruit flies in orchards was detected in the period from August to October 2018 on the territory of seven districts. *C. capitata* was identified in four of them.

In the region of Kyustendil, 6 specimens of the pest were found in a Delta type trap with trimedlure attractant set in apple garden.

In one MacPhail trap with Biolure from Pazardzhik, set in apple garden in the village of Glavinitsa, 11 female and 3 male specimens of *C. capitata* were identified.

In the region of Plovdiv, *C. capitata* was detected in 6 traps of different types: 2 MacPhail traps with Biolure, 2 Delta traps with trimedlure and 2 Delta traps with methyl eugenol.

No damage of the fruit has been observed during the visual inspections. The pest was only caught in traps. A particularly strong infestation was reported in the Delta trap with trimedlure on apple trees in Plovdiv, where 281 female and 28 male specimens of Mediterranean fruit fly were identified.

On the territory of Sliven Mediterranean fruit fly was caught in 2 orchards - peaches in the village of Glufishevo and mixed garden in the village of Samuilovo. In the peach garden a male specimen of *C. capitata* was identified in a Delta TML trap. In the mixed garden there were MacPhail traps with Biolure, two of which had a total of 21 male and 18 female specimens. Inspections in markets and exchanges were conducted on the territory of 9 districts - Blagoevgrad, Burgas, Varna, Vidin, Kardzhali, Plovdiv, Pazardzhik, Sofia and Stara Zagora. 190 visual inspections were carried out in commercial premises and 86 fruit flies were caught.

43 Delta traps with trimedlure (T), 23 Delta traps with methyl eugenol (ME), 12 MacPhail type traps with Biolure (3C) and 8 Yellow Sticky Traps were used.

Live larvae of the Mediterranean fruit fly were found in two consignments of oranges, imported from Egypt in a warehouse under customs control on the territory of Burgas. Details about the observation points and the trapping period are presented in Table 3.

Table 3. Observation points, period of setting and checking the traps in 2018

District	Number of points	Observation points	Attractant type			Period of setting the traps	Result Trapped fly	
			3C	ME	T		♀	♂
Blagoevgrad	3	*Prunus persica;* *Rubus ideaus;*	2	6	9	June - September	-	-
Burgas	5	*Prunus domestica;* *Prunus persica;*	2	2	13	July - October	-	-
Varna	3	*Malus domestica;* *Pyrus communis;* *Prunus avium;* *Prunus domestica;* *Prunus persica;*	9	2	9	June - November	-	-
Vidin	9	*Malus domestica;* *Pyrus communis;* *Prunus avium;* *Prunus domestica;* *Prunus persica;* *Prunus cerasus;* *Prunus armeniaca;*	8	4	8	June - November	-	-
Kardzhali	3	*Malus domestica;* *Pyrus communis;* *Prunus domestica;* *Prunus persica;*	6		7	June - October	--	-
Kyustendil	11	*Malus domestica;* *Pyrus communis;* *Prunus avium;* *Juglans regia*	8	4	8	May - October	-	6
Pazardzhik	2	*Malus domestica;* *Prunus avium;* *Prunus domestica;* *Prunus persica;*	7	6	6	June - December	11	3

Pleven	6	*Malus sp.;* *Prunus domestica;* *Prunus persica;* *Prunus avium;*	2	4	6	June - October	-	-
Plovdiv	7	*Malus domestica;* *Prunus persica;* *Prunus armeniaca;*	16	10	10	June - October	297	65
Sliven	3	*Prunus avium;* *Prunus domestica;* *Prunus persica;*	6	4	4	June - September	18	22
Silistra	6	*Prunus avium;* *Prunus domestica;* *Prunus persica;*	12	10	12	June - October	-	-
Stara Zagora	3	*Prunus persica;* *Prunus avium*	5	8	6	June - September	-	-

Conclusions:

- In the last few years individuals of *C. capitata* have been intercepted in imported fruits and trapped in orchards and warehouses, but its establishment in the fields has not been confirmed yet. *C. capitata* is not able to overwinter in the climatic conditions in Bulgaria. The main limiting factor is the long period of low temperatures from December to the end of February.
- No individuals of *Bactrocera* species have been caught in traps or found in fruits.
- Due to contradictory results, it is not possible to determine with certainty which type of traps and attractants are most effective for catching the flies.
- Annual fruit fly inspections will continue in order to show if the Mediterranean fruit fly could overwinter in the country under our climatic conditions.