The REACT Newsletter

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REACT

Editorial

Dear Readers,

Welcome to the first issue of our REACT newsletter. As the coordinator of the REACT program, part of the European Union's Horizon Research and Innovation Programme, I'm pleased to guide you through our venture into innovative ecological pest control, with a particular focus on invasive fruit fly species in Europe.

The purpose of this newsletter is to provide a brief explanation of the REACT program. This initiative applies the advanced Sterile Insect Technique (SIT) in a manner that is customized to suit the specific agricultural requirements of Europe. Our approach offers a more sustainable alternative to conventional pest control methods for dealing with invasive species.

We cover several key areas in this issue. The work program and milestones section provides the roadmap of our work packages, ranging from ecological studies to the development of genetic sexing strains combined with socio-economic analysis and tailored communication and dissemination activities. It's a blend of complex science and practical applications – like a good coffee, strong and effective.

Our research on *Bactrocera* species in space is a unique highlight. Yes, you read that right - we're taking pest control to astronomical levels. This study examines gene detection in microgravity, adding a bit of cosmic intrigue to our earth-bound challenges. In simpler terms, we are studying how genes behave in fruit flies in space, which could help us better understand how to control pests on Earth.

Pesticides and their impacts are also under our microscope. We're not just discussing reducing pesticide use but actively pursuing alternatives. This issue brings insights from a range of experts, including our team members, from seasoned researchers to enthusiastic PhD students.

Additionally, we have included snapshots from recent events and field trips to showcase the collaborative nature of REACT. The program is not just about lab coats and data; it also involves real fieldwork and coffee breaks.

In summary, since November 2022, the REACT program has gone beyond being just a scientific endeavour. It is a commitment to a greener future, where fruit flies might need to consider finding a new occupation.

Thank you for joining us on this journey.
Enjoy reading!

Kind regards,

Marc F. Schetelig
Coordinator of REACT | Giessen, November 2023

Introduction

For the past year, the REACT consortium has been working on **the development of SIT applications** aimed at controlling two invasive fruit fly species: the oriental fruit fly (*Bactrocera dorsalis*) and the peach fly (*Bactrocera zonata*). Among the novelties and innovations of the project is that Sterile Insect Technique (SIT) applications are to be developed that are suitable for European agriculture with small-scale structured cultivation areas. An important first step in the development of efficient pest management methods is to **understand the ecological drivers of pest invasions** and the factors that contribute to their spread and containment.

The first year of the **four-year research project** is now over and work is in full swing in each of the **eight work packages**. This newsletter is intended to provide an **insight into the exciting work of the project** and inform interested readers about the ambitious research project.

In this Newsletter:

Imprint: The REACT Newsletter is produced by Oikoplus GmbH, Vienna, as part of the communication and dissemination activities of the project.

Utilizing a pest to fight itself

REACT aims to break innovative ground in the ecological control of invasive insect pests in Europe and worldwide.

Invasive species of fruit flies pose a significant risk to agriculture. They feed on a wide range of fruits and vegetables, causing **significant damage to crops**. The feeding damage reduces the quality and market value of the produce.

The spread of invasive pests in Europe can lead to trade restrictions and quarantine measures, impacting European fruit and vegetables export. **In its approach to develop ecological pest mitigation tools, the REACT project focuses on two fruit fly species: *Bactrocera dorsalis* and *Bactrocera zonata,*** and draws on experience from other parts of the world.

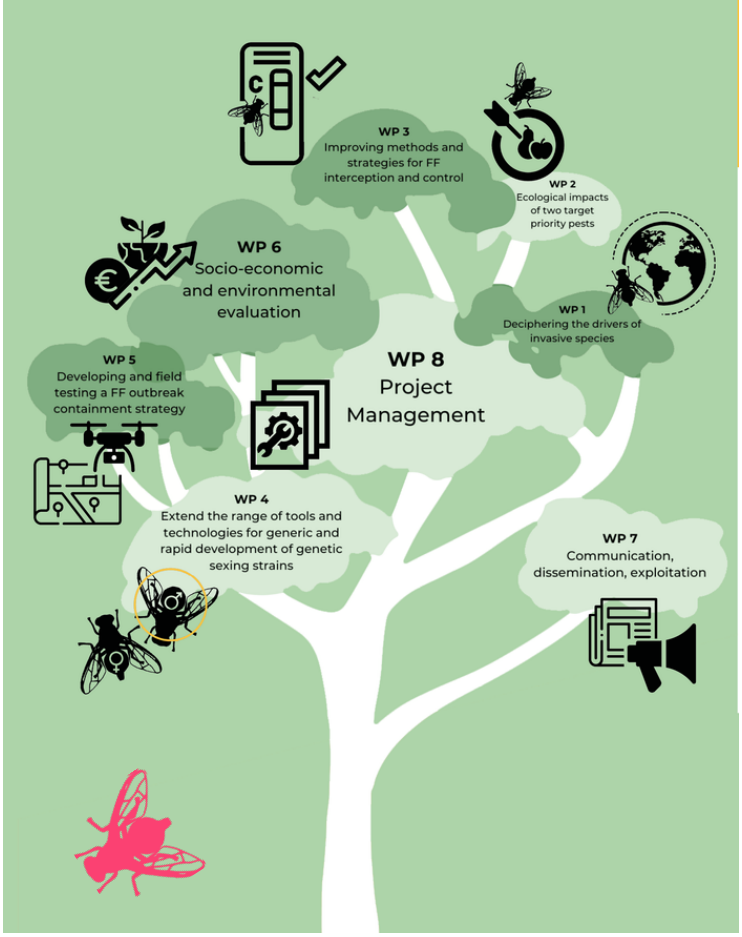
Aruna Manrakhan is a Research Entomologist at Citrus Research International in South Africa. "We had *Bactrocera dorsalis* reaching the South African northern borders in 2010 and we eradicated it following an approved action plan," she explains. "Eventually, there were more incursions and the pest became established in the northern parts of the country. Some southern parts of the country are still free of *Bactrocera dorsalis*. So **we have interesting scenarios with regard to pest presence and pest absence within the country that could benefit REACT.**" The status of *Bactrocera dorsalis* in South Africa allows important information to be drawn about the control of the pest species.

To mitigate the risks posed by fruit flies, European fruit growers rely on a **combination of cultural practices, such as crop rotation and the use of insect-proof netting, as well as chemical controls, such as pesticides**. However, developing **resistance to pesticides by fruit fly** populations is a growing concern, **highlighting the need for alternative control methods, such as the Sterile Insect Technique, which REACT utilizes in its approach.**

Photo: Antoine Franck,CIRAD

Work Program

The **work program of REACT** compiled of eight work packages takes this into account. Alongside the **deciphering of the drivers** of invasive species (WP1), the **ecological impacts** of the two target priority pests will be analyzed (WP2) in order to inform the **improvement of methods** and strategies for their interception and control (WP3). The project aims at **extending the range of tools and technologies** for generic and rapid development of genetic sexing strains (WP4) and **developing and field testing** a fruit fly outbreak containment strategy (WP5). A **socio-economic and environmental evaluation** of SIT-based tools and strategies for eradication of invasive fruitflies (WP6) as well as ongoing project **communication, dissemination and exploitation** of the project results (WP7) and a project management work package (WP8) complete REACT's work program.



Project Milestones

MARCH 2023
Milestone 0.1: Consortium Agreement
Milestone 0.2: Kick-off meeting (KOM)

JUNE 2023
Milestone 6.1: Relevance of selected list of parameters for socio-economic analysis for the project development tools and strategies validated.
Milestone 7.1: Plan for dissemination and explotation including communication developed.

October 2023
Milestone 4.1: Initial bioinformatic pipeline for Work Package 4 generation of Genetic Sexing Strains in *Ceratitis capitata* developed.

FEBRUARY 2024
Milestone 2.1: Amplicon sequencing pipeline developed and microbial profiles determined.

APRIL 2024
Milestone 1.1: Sequencing for population diversity and microbiome studies completed.
Milestone 2.2: Acquisition of data for meta analysis on ecological impacts of *Bactrocera dorsalis* and *Bactrocera zonata* conducted.
Milestone 3.1: Metabolomics pipeline and male quality markers in *Ceratitis capitata* developed.

APRIL 2024
Milestone 4.2: Y chromosome targeting developed and evaluated as proof-of-principle in *Ceratitis capitata.*
Milestone 5.1: Emergency-response rearing facility established and productivity assured.

SEPTEMBER 2024
Milestone 1.2: Parameters for population dispersal studies collected.

OCTOBER 2024
Milestone 1.3: Parameters for developing simulation models for *Bactrocera dorsalis* and *Bactrocera zonata* collected.
Milestone 3.2: Fruitfly interception protocol developed and evaluated.

OCTOBER 2024
Milestone 5.2: Area tailored *Ceratitis capitata* containment strategy developed and validated.
Milestone 7.2: Reaching sufficient response and interest for the project (indicator values in Dissemination, Exploitation and Communication Plan).

How does REACT contribute to pesticide use reduction?

The use of SIT could help reaching Europe's declared goal to reduce the use of pesticides in agriculture.

Ana Larcher on the use of pesticides in agriculture

Pesticides are there to fight invasive species of insects and weeds. However, they have the potential to contaminate our soils, the crops they should protect, and the water we drink. In addition, they often target not only the the pests they are intended to, but also other vegetation and beneficial species. **Ana Larcher, a researcher at the Lisbon University Institute**, addresses the topic of pesticide use in agriculture, explaining why the approach of the REACT project is likely to innovatively exploit the potential of another technique for pest control.

Antonios Avgoustinos explaining REACT's SIT methodology

But why is REACT so innovative, even though many other initiatives have taken on the challenge of eradicating pesticide use? **Antonios Avgoustinos, a researcher at the Hellenic Agricultural Organisation**, explains from his professional perspective what makes REACT stand out.

Of course, novel technologies in pest control potentially carry many risks. **Cristina Borghesi, a PhD student at the Justus-Liebig University Giessen**, presents the agricultural benefits of the REACT project.

Cristina Borghesi on the advantages of SIT from a farmer's perspective

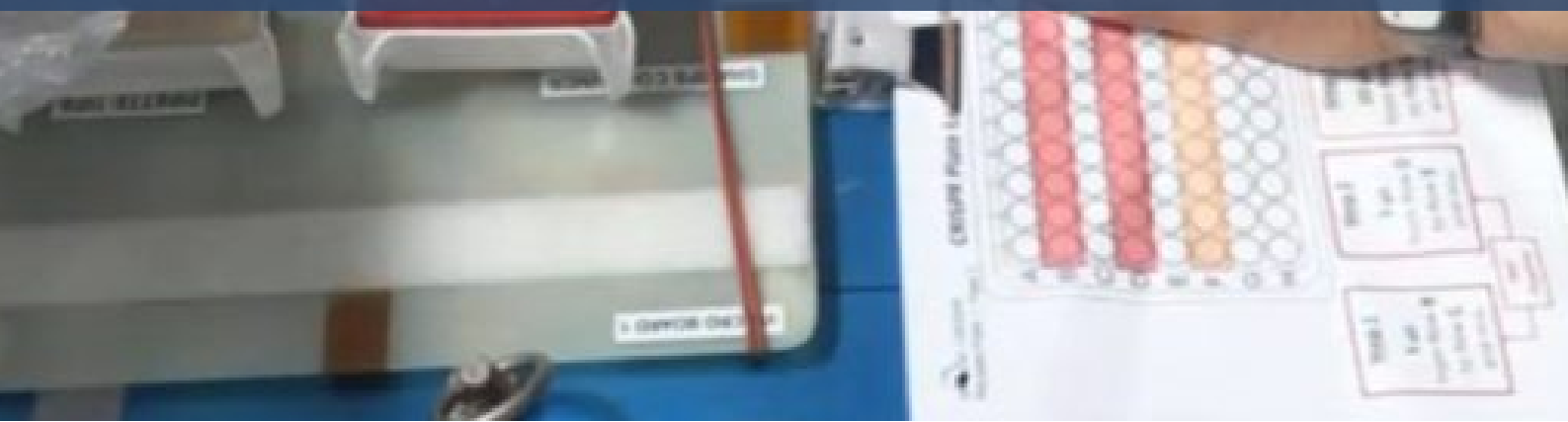
Bactrocera zonata as space invader: Gene detecion in microgravity

A team of Israeli researchers has tested gene detecting in space. Among other samples, they used *Bactrocera zonata*.

While the **REACT project is primarily concerned with protecting agriculture from invasive, harmful fruit flies,** there are already thoughts about how to protect the cultivation of plant organisms in space in the future, namely during long-term manned space missions. **One of the fruit fly species targeted by REACT has therefore already been part of a series of tests on the International Space Station (ISS).**

In April 2022, the first private orbital space flight took off from Cape Canaveral to the ISS. **Israeli astronaut Eytan Stibbe embarked on this flight to complete the "Rakia" mission**, which included dozens of scientific experiments. As part of this mission, **Gur Pines and a team of scientists tested the collateral activity of Cas12a and RPA amplification in microgravity**. The series of tests **targeted three organisms** from different branches of the tree of life: *Escherichia coli, Saccharomyces cerevisiae*, and *Bactrocera zonata*.

The aim of the tests in microgravity was to find out, **if Cas12a genetic detection could contribute to medical and hygienic safety during manned space missions in the future. Gur Pines**, who in REACT works on developing rapid tests to identify fruit flies at the larval stage, **explains why the fruit fly *Bactrocera zonata* was one of the organisms tested on board** the international space station and **what scientific insights were gained from the space mission.**



How did *Bactrocera zonata* qualify as a valuable organism to be tested during the mission?z qualify as a valuable organism to be tested during the mission?

To test the technology's specificity under microgravity conditions, my collaborator from Tel Aviv University, Dr. David Burstein, and I selected to detect three organisms representing different branches of the tree of life, with potential relevance to future space missions. The first two were microorganisms, namely *E. coli* (bacteria) and *S. cerevisiae* (yeast), representing potential pathogens threatening the astronauts' health. We selected the third organism to represent an agricultural pest since longer missions will probably require space farming at some capacity. While we hope that earth-derived pests will never leave Earth, with space travel becoming more and more accessible, we cannot rule this option from ever happening, unfortunately. We have been working with *B. zonata* for a while, so it was a natural choice.

Eytan Stibbe on the International Space Station (Photo: Rakia Mission)

What was the greatest challenge to overcome in preparing the testing on ISS (maybe the storage of experiment components?)

Preparing for such an experiment had many challenges. This was our first (and hopefully not the last) experiment sent to the International Space Station. The time we had from the announcement that our experiment was selected to be performed onboard the ISS until the expected launch was very short, and it was unclear whether we would be prepared in time. To be honest, we did not know what we got ourselves into. We had to complete the experiment's protocol and simplify it as much as possible while considering restrictions imposed by microgravity and NASA. For example, our initial design was to have all the reagents in powder form to simplify shipping, and the first step would be to rehydrate the different reagents. But it soon occurred to us that would not be possible since the powder would float away when the containers were opened, so eventually, the experiment was shipped as frozen liquid. This is also the place to acknowledge BioServe Space Technologies from Boulder, Colorado, who helped us with so many things, including the experimental design, protocol simplification, and Eytans training. Also, the experiment was part of the first-ever commercial flight to the ISS, which required a lot of coordination with many different private, commercial, and governmental entities since everything was done for the first time.

How did you prepare astronaut Eytan Stibbe for doing the experiments?

Within the short time to launch, we had to schedule training sessions for our astronaut, Mr. Eytan Stibbe, for whom this was one of 44 scientific experiments he performed in various disciplines. Eytan is not a trained biologist, and we had to start with the basics. Eytan took the training very seriously and trained until he felt confident that he could conduct the experiment successfully. After all, we had only one shot at this. The initial training took place in Israel and was led by Dr. Dan Alon, then a Ph.D. student, who, together with Dr. Karin Mittleman, did all the required experimental preparations. The second training phase was done in the US by BioServe personnel who also helped us adapt our protocol and who accompanied him during the experiment remotely.

When did you learn about the results of the experiments in space? Was it only after the completion of the mission, or were results sent back to Earth even during the mission?

We had the privilege to witness the experiment being conducted in real-time from the Rakia Mission Control room in Tel Aviv. Due to the experiment's complexity, it was done in two different sessions, the first verifying the specificity and the second testing the sensitivity. Witnessing our experiment unfold was very exciting, especially since I brought my kids, and I got to see their enthusiasm during the process. A few hours after the experiment was concluded, the results were transmitted to Bioserve, who transferred them to us. We were super excited! Then, Dan and Karin peered into the data, and within a few minutes, we knew that the experiment worked.

The paper recently published based on the tests outlines how future space missions could benefit from CRISPR-based diagnostics. However, did the experiments also show results that have implications for the implementation of testing methods on Earth?

CRISPR-based detection is a fantastic technology. It is simple to perform, very specific, and also very sensitive. With discoveries and applications being made regularly, I believe this technology will be expanded in ways we can't imagine. Our microgravity experiment signifies how universal and robust this system is and its potential to be performed under extreme conditions.

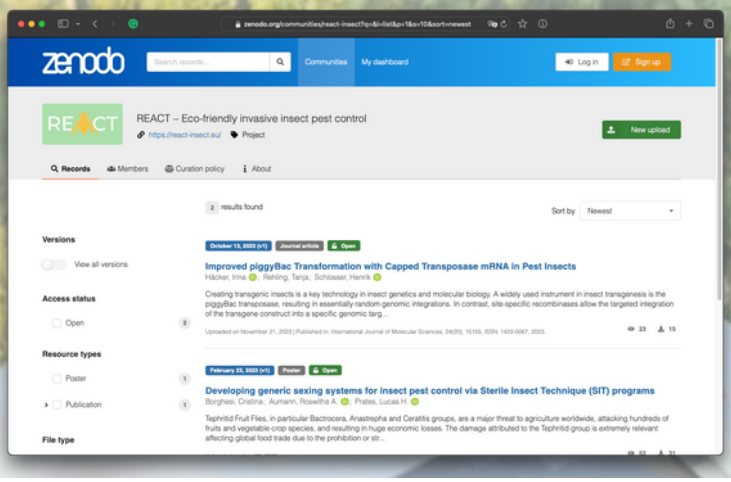
Were there any learnings from the experiment, that REACT could draw upon, maybe for example, in the design of simple testing tools or testing kits?

Our microgravity experiment helped to establish these genetic-based diagnostics as a universal platform. While other methods exist, we hope to further develop and simplify this CRISPR-based approach to allow the detection of everything that has DNA or RNA, let it be a virus, pathogenic bacterium, or an agricultural pest.

Gur Pines, researcher at the Agricultural Research Organization, Israel

This interview was conducted by Thomas Stollenwerk of Oikoplus GmbH, Vienna in August 2023 for the REACT project.

News and Events



Find all scientific publications of REACT on **Zenodo**: https://zenodo.org/communities/react-insect

You can find REACT on **iNaturalist**, the world's largest community for environmental observation.

19-20 January 2023:
Kick-off Meeting, Gießen, Germany

Just a few weeks after the official launch of the project, the international project team followed the invitation of the project coordinator Prof. Dr. Marc Schetelig and met in Giessen to explore the possibilities of long-term and pesticide-free methods of pest control in the context of crops. The kick-off meeting included a first semi-public session where members of the Advisory Board were invited to comment on the project strategies, methods, results, and outcome invited to comment on the project strategies, methods, results, and outcomes.s.

Photos: REACT

21 April 2023:
Work Visit to IAEA Labs, Seibersdorf, Austria

Project coordinator Marc Schetelig visited the IAEA's insect laboratory in Seibersdorf to discuss the REACT approach with Kostas Bourtzis and other scientists at the lab. The visit highlights the importance of the IAEA's expertise for the project. "We're hopping on technologies that have been successfully developed by the IAEA labs for decades and applied around the world on large scales highly successfully. Therefore, the discussions and exchange of ideas with our colleagues at the IAEA have been important for the field of SIT and the REACT consortium is happy to contribute with the novel strategies developed for insecticide-free pest control."

Photos: REACT

30 April 2023:
Field trip: Sampling insects in South Africa

George Tsiamis of the Department of Environmental Engineering at the University of Patras, Greece, visited the colleagues from Citrus Research International (CRI) in South Africa. The purpose of the field trip was to collect samples of *Bactrocera dorsalis* in order to study the microbiome of the species.

"With the assistance of Aruna Manrakhan we were able to initiate a culture-dependent approach using natural and laboratory populations and also collect samples that will be analyzed using the MinION platform. In addition to the sampling, I also visited the CRI center to learn more about their research on citrus pests and diseases. I had the opportunity to meet with several researchers and scientists, and we discussed the potential for collaboration between our organizations. Overall, the trip to South Africa was a success. I was able to collect valuable samples of Bactrocera dorsalis and to make important connections with researchers at CRI."

Photos: REACT 2023

Photo: REACT

13 May 2023:
Work Package 1 Workshop, Vienna, Austria

During a work meeting in Vienna, researchers discussed findings on the behavior of the fly species *Bactrocera dorsalis* and *Bactrocera zonata.* The focus was on the current state of research on the insect species concerned and the question of how the insects overwinter.

Workshop organizer David Nestel of the Volcani Institute: *"The workshop intention was to discuss current knowledge, plan, and coordinate, activities within the work on Deciphering the drivers of invasive species."* The workshop was also attended by colleagues from the International Atomic Energy Agency (IAEA), one of them being involved as part of the advisory board.

23-25 August 2023:
Stakeholder Meeting, Santarém, Portugal

The demand for innovative methods in insect control became very clear to the REACT team in the summer when Portuguese farmers proactively contacted REACT coordinator Marc Schetelig to invite him to Portugal to see the consequences of the spread of the olive fly there.

During a field trip in the Portuguese district of Santarém, REACT coordinator Marc Schetelig, Evelyn Vollmeister and Ana Carvalho of the REACT project team were able to get an idea of the damage caused by olive flies (*Bactrocera olea*) in agricultural production. The scientists were able to discuss possible applications of the Sterile Insect Technique with representatives of regional producers as well as the Portuguese Ministry of Agriculture and regional authorities.

Photos: REACT

6 September 2023: Stakeholder Meeting, Naousa, Greece

At a local stakeholder meeting in Naousa, Greece, members of the REACT consortium presented their novel approach in fighting invasive insect pests to local farmers. In the region in the Greek province of Macedonia, the first field trials are due to start in 2024 to test the SIT application using Medfly (*Ceratitis capitata*).

Photos: REACT

9-12 October 2023: Annual Meeting, Nafplio, Greece

During the first REACT Annual Meeting in Nafplio, Greece (Oct 9-12, 2023), the members of the consortium had the opportunity to inform each other about the status of their work in the individual work packages, exchange experiences, and discuss many technical and scientific questions.

Photos: REACT 2023

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