RESEARCH AND INNOVATION CENTRE

# REPORT

2023/2024

AGROSYSTEMS AND BIOECONOMY
BIODIVERSITY, ECOLOGY AND ENVIRONMENT
COMPUTATIONAL BIOLOGY
FOOD AND NUTRITION

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FONDAZIONE
EDMUND MACH

EDITORS
Cristina Castellani, Emanuele Eccel, Floriana Marin

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# INDEX

# PRESENTATION

Research into the processes governing our natural environment is expanding rapidly in response to pressing calls for action from primary producers, businesses processing agricultural food products, national and international funding bodies, and the general public, but above all in response to the unescapable knowledge of the metamorphosis affecting ecosystems across our planet.

A careful look at these ongoing studies, often characterized by complex features and methods rarely mentioned in everyday life, reveals how they are a source of new knowledge, but also new questions, encouraging further investigation. The contributions collected in this edition of the Report summarize some of the activities carried out by researchers at the Research and Innovation Centre. With meticulous consideration and analysis of the evidence emerging from natural and agricultural ecosystems, these researchers promote the development of solutions to protect the agrifood and forestry sectors, as well as Alpine flora and fauna, in line with the principles of sustainability promoted by the Province of Trento.

These enquiries have benefits that go far beyond our borders, as witnessed by numerous academic and scientific collaborations and interactions with similar national and international research centres.

For the Fondazione Edmund Mach, research is a primary mission that complements that of education and training in the sectors of agriculture, environment and food, along with our technological consultancy for agricultural operators and winemaking entrusted to the historic cellar and didactic distillery. Together, this expertise has provided and continues to provide continuity that supports primary sector businesses to achieve growing prosperity, at the same time aiming to enhance the territory and environment throughout Trentino.

*San Michele all'Adige, 6 March 2025*

MIRCO MARIA FRANCO CATTANI
President, Fondazione Edmund Mach
(February 2020 – March 2025)

# The Research and Innovation Centre

The Mach Foundation, originally established 150 years ago as the Agricultural Institute of San Michele, was founded with the primary objective of integrating academic education with professional training and experimentation. Subsequently, it expanded its scope to include research, providing crucial support to Trentino agriculture and the protection of the natural environment.

The studies presented in this report, although representing only a selection of the research conducted over the two-year period, offer a significant overview of the wide range of scientific disciplines involved. They highlight the close interconnection between sustainable agriculture, food, biodiversity, ecology, and the environment, themes that define the Trentino territory and its community.

The ambition of this research is not limited to the mere acquisition of knowledge and the impact on agriculture and the environment, but aspires, above all, to generate a positive impact on the people who live in these territories. The search for solutions to local problems increasingly requires a global vision, the adoption of innovative perspectives, and the intensification of collaborations with international research institutions.

In the selection of articles, priority was given to the contributions of young researchers, whose innovative ideas and activities have played a fundamental role in the growth of the Research Center.

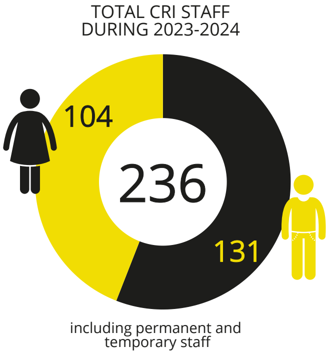
Their contribution is also evident in the ever-increasing multidisciplinary nature of the research conducted, often outside the classic frameworks that define individual disciplines. The use of new technologies, big data, and artificial intelligence represent innovative tools to address research questions, both traditional and emerging, but human capital always remains the cornerstone and indispensable element for any research activity.

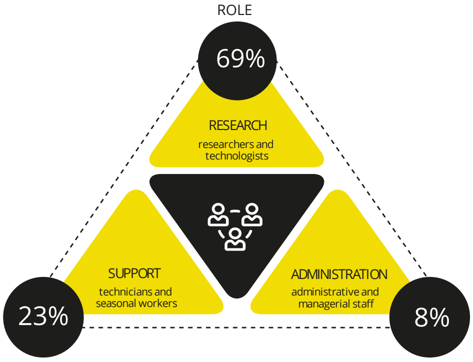
I extend my sincere wishes to all young colleagues, that they may continue to promote the advancement of human knowledge with the same enthusiasm, both within our Center and in other research institutions, through the contribution of new ideas and solutions.

DAMIANO GIANELLE
Director

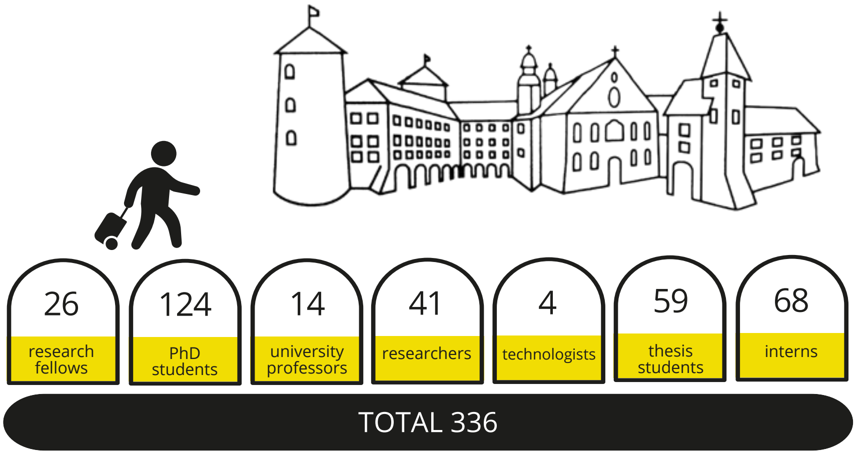
# CRI BY THE NUMBERS

The Sustainable Development Goals (set by the United Nations) where CRI has direct involvement are shown in color

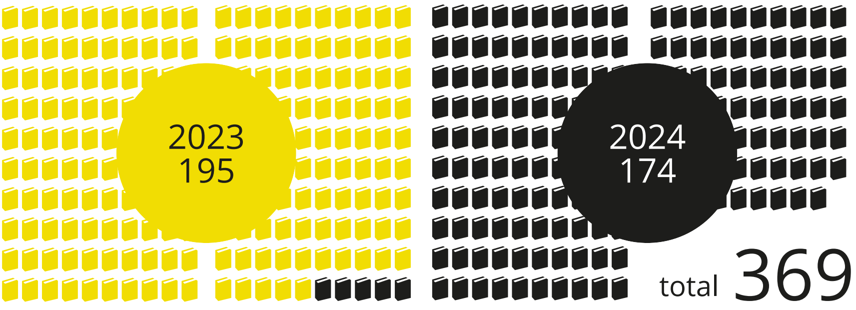




CRI workforce overview: 2023-2024 staff and qualifications

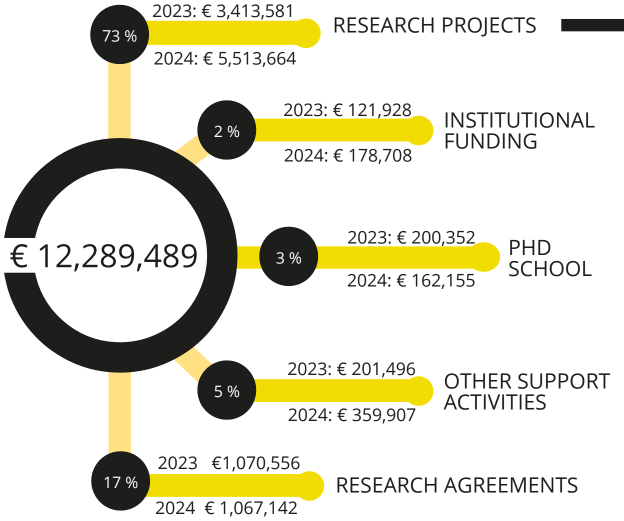


External staff hosted at the centre

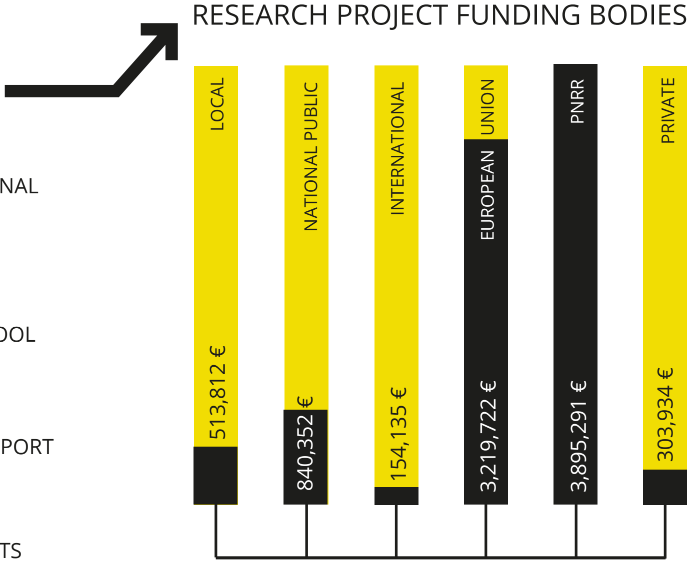


Scientific publications in journals with impact factor

## 2023-2024 BUDGET

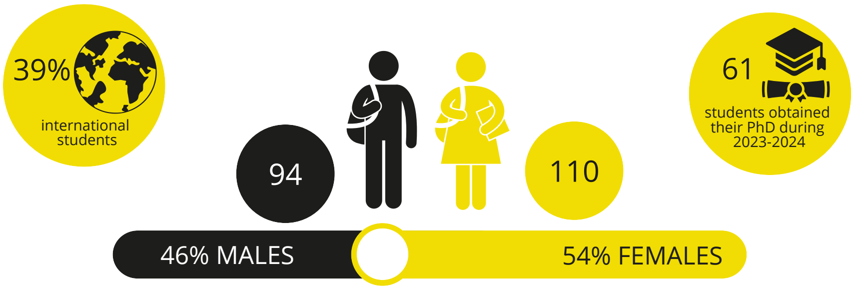


## RESEARCH PROJECT FUNDING BODIES



8,927,246 €

budget related only to 2023-2024 and not to the entire duration of the projects



PHD students supported between 2023-2024

# Research units

In 2023-2024 the Research and Innovation Centre (CRI) was organized into 20 research units divided into thematic areas (Agrosystems and Bioeconomy; Biodiversity, Ecology and Environment; Food and Nutrition; Computational Biology) that collaborate to jointly address the fundamental challenges in the agriculture, environment, and food sectors.

## Agrosystems and Bioeconomy

### Digital Agriculture

This unit focuses on research, development, and innovation to advance the digitization and automation of the agricultural sector. By implementing cutting-edge technologies and automated systems, they aim to enhance efficiency and productivity in agriculture.

### Bioeconomy

Applying multidisciplinary expertise, this unit addresses topics related to the bioeconomy and circular economy. Through experimental projects and specialized consultancy, they promote sustainable practices that deploy biological resources effectively.

### Plant Biology and Physiology

This unit explores the molecular basis and physiology of resistance to biotic and abiotic stresses and fruit quality in grapevine. This knowledge lays the foundations for assisting classical breeding, using novel genomic techniques (gene editing and cis-genesis) and precision agriculture.

### Plant Biotechnology

This unit employs techniques such as cisgenesis and genome editing to enhance plant tolerance to stresses and improve fruit quality. By studying functional genomics in plants, innovative breeding methods are developed without introducing foreign genes.

### Plant Protection

Engaged in both fundamental and applied research, this unit develops innovative and sustainable strategies for protecting agricultural crops. Research aims to reduce reliance on chemical pesticides, thereby positively impacting society and the economy.

**Genetics and Breeding of Fruit Crops**

This unit aims to understand the genetic and molecular bases of fruit quality traits and disease resistance. Applying this knowledge to breeding programs allows the development of new improved fruit varieties.

**Grapevine Genetics and Breeding**

This unit is dedicated to developing new grapevine varieties that can withstand biotic and abiotic stresses. The goal of this research is to promote sustainable viticulture while maintaining high-quality grape production.

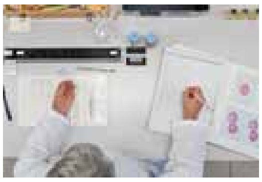
**Genetics and Breeding of Small Fruits**

Focused on crops like blueberries and raspberries, this unit develops new genetic materials with improved characteristics for growers and consumers. The unit tailors varieties to perform well in different environments and conditions.

## Biodiversity, Ecology, and Environment

**Environmental Botany**

This unit studies changes in plant biodiversity and phenology as indicators of responses to environmental stressors and the implications of these changes for human health. Research aims to monitor how plants react to various stress factors to better understand ecosystem dynamics.



**Ecogenomics**

Utilizing comparative and functional genomics, this unit investigates plant biodiversity with a focus on species evolution and adaptation. Research contributes to conservation efforts and understanding plant responses to environmental changes.

**Animal Ecology**

This unit uses bio-sensors and predictive models to study the behavioral adaptations of wildlife to their environment and human-induced changes. Research aims to predict how fauna may respond to future environmental alterations.

**Applied Ecology**

This unit examines the effects of global changes and biodiversity loss on alpine animal species and their ecosystem interactions. Research assess the risk of spreading diseases threatening livestock and human health.

### Forest Ecology

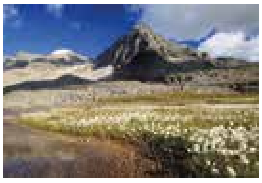
Focusing on interactions between forests and grasslands, and their biotic and abiotic environments, this unit studies the feedback mechanisms of vegetation, biodiversity, and climate against the backdrop of global changes.

### Conservation Genomics

The unit aims to identify the biotic and abiotic factors impacting changes in genetic, genomic and species diversity of wild animal taxa and their microbiota over space and time, providing scientific support for wildlife management and conservation strategies.

### Hydrobiology

This unit conducts research on aquatic biodiversity, studying the ecological characteristics and evolution of water bodies in alpine and peri-alpine regions, and analyzing both temporal and spatial dynamics.



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## Food and Nutrition

### Natural Products Biotechnology

This unit focuses on the biosynthesis of various groups of plant metabolites. Research aims to identify, isolate, and understand the functions and biological roles of these compounds, with applications in health and industry.

### Metabolomics

This unit develops innovative methodologies to characterize the metabolites present in plant and animal tissues. Their work aids the understanding of metabolic processes and how they relate to health and disease.

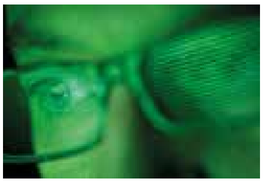
### Sensory Quality

Using a multidisciplinary approach, this unit examines food quality from production to consumption, focusing on consumer perception and sustainability. Research delves into the fundamental processes of how we perceive taste, aroma, and texture.

### Traceability

By applying advanced techniques such as Isotopic Mass Spectrometry and Nuclear Magnetic Resonance, this unit promotes the traceability and authenticity of agri-food products. This research also ensures product integrity and combats food fraud.

# Computational Biology



**Computational Biology**
This unit develops methods for data management, analysis, and modeling to understand the mechanisms governing complex biological phenomena. By utilizing bioinformatics and computational tools, this research supports various research areas in interpreting large datasets.

# Technological Platforms

The Research and Innovation Centre (CRI) hosts **21 state-of-the-art technological platforms** managed by highly qualified personnel. Each platform is supervised by a coordinator who oversees its activities, offering services and research collaborations both within the institution and to external clients. These technological platforms enable cutting-edge research and the development of innovative solutions. The synergy among platforms facilitates an integrated and multidisciplinary approach, essential for addressing complex challenges in the agriculture, environment, and food sectors.

### Aerobiology

Specializing in the detection and study of airborne **pollen and major allergenic and pathogenic spores**, this platform operates in both outdoor and indoor environments. Utilizing advanced technologies, the presence of allergens and airborne pathogens can be monitored, contributing to allergy prevention and a better understanding of atmospheric biological agents. The platform also carries out tests on the **viability and germination** of pollen, providing valuable data for agriculture and public health.

### Volatile Organic Compounds Analysis

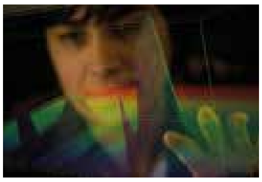
Focusing on the rapid and highly sensitive monitoring of **volatile organic compounds (VOCs)**, this platform employs **mass spectrometry techniques** for direct injection analysis. This allows the identification and quantification of molecules responsible for aromas, flavours, and potential contaminants in food products, beverages, and environments, supporting studies on product quality and authenticity.

### Sensory Analysis

This platform offers comprehensive studies using leading **sensory analysis techniques**, involving trained panels and consumer tests. It evaluates aspects such as taste, aroma, texture and appearance of food products, providing essential feedback for the development of new products and the improvement of existing ones. Understanding consumer preferences is crucial for maintaining high quality standards and market competitiveness.

### Computational Biology

Supporting researchers in experimental design, this platform provides services in **data management, analysis, and interpretation** of biological data. The analysis of genomic, proteomic, and metabolomic data utilizing advanced bioinformatics tools, contributing to the understanding of complex biological mechanisms and accelerating scientific progress across various biological fields.



### Biomass

Equipped with a laboratory dedicated to the **physical, chemical, biological, and energetic characterization** of biomass and derived products, this platform conducts research on bioenergy, biofuels, and biocomposites. It promotes the sustainable use of renewable resources and the development of solutions for the circular economy, aiming to reduce environmental impact and support energy innovation.

### Biotechnology of Fermentation

Active in the creation of **improved fermented food products**, this platform focuses on enhancing organoleptic properties, technological processes, and microbiological safety. New microbial strains and fermentation processes are developed for products such as wine, beer, cheese, and other fermented foods, contributing to innovation and quality in the agri-food sector.

### Biotremology

Specializing in the study of **vibrational communication** in insects, this platform develops methods for **behavioral manipulation** based on applied biotremology techniques. By decoding the vibrational signals used by insects, eco-friendly strategies for controlling agricultural pests can be devised, reducing reliance on chemical pesticides and promoting sustainable pest management practices.

### Plant Tissue Culture

Offering advanced **in vitro culture services** and biotechnological applications primarily for fruit trees, including grapevine, apple, and small fruits, this platform employs techniques such as micropropagation and genetic transformation. It supports genetic improvement programs, germplasm conservation, and the production of healthy, uniform plants for research and agricultural industry needs.

### Animal, Environmental and Ancient DNA

This platform specializes in developing the **extraction and amplification of DNA** from animal, environmental, and ancient biological material, with particular expertise in non-invasive samples. Applications range from conservation genetics and wildlife forensics to phylogeography, contributing to studies on biodiversity estimates, molecular monitoring, and reconstruction of evolutionary dynamics.

### Plant Phenotyping

This platform offers automated systems for **high-throughput plant phenotyping**. Utilizing RGB and hyperspectral imaging techniques, advanced sensors, and controlled growth conditions, parameters such as plant development, stress responses, and resource use efficiency can be monitored, facilitating the selection of improved varieties and accelerating breeding programs.

### Varietal Identification and Grapevine Germplasm Valorization

Ensuring the distribution of **genetic material** for non-commercial purposes, this platform manages the cultivation and characterization of grapevine varieties. Using morphological and molecular analyses, it identifies and enhances grapevine germplasm, supporting biodiversity conservation and genetic improvement programs in viticulture.

### Hydrochemistry

Providing services for **water analysis** and chemical profiling of cyanobacterial toxins, this platform employs advanced analytical methods. It monitors freshwater quality, which is fundamental for safeguarding aquatic ecosystems, managing water resources, and protecting human health from water-related hazards.

### Metabolomics

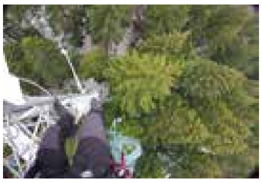
Utilizing innovative methodologies, this platform focuses on the **quantification and characterization of metabolites** in complex matrices such as plant tissues, food products, and biological fluids. Using mass spectrometry techniques, it supports studies on food quality, nutrition, plant physiology, and plant-environment interactions.

### Microfluidics, Microdissection and Microscopy

Equipped with cutting-edge instrumentation, this platform conducts research involving **microfluidics**, **laser microdissection**, and advanced **microscopy**. These techniques enable precise analysis of cells and tissues, manipulation of samples at microscopic scales, and detailed study of fundamental biological processes.

### Micrometeorology

Aiming to measure the exchanges of **energy and matter** (carbon, water, nitrogen) between vegetation and the atmosphere, this platform uses technologies such as **eddy covariance**. It monitors alpine, forest, and agricultural ecosystems, contributing to the understanding of ecological processes and the impact of climate change on the carbon cycle.



### Quarantine

This platform is dedicated to the **rearing of harmful insects and their natural enemies**, both native and exotic, under controlled conditions. It provides a secure environment for studies on regulated organisms, supporting research on biology, biological control, and prevention of the spread of invasive species, essential for biosecurity efforts.

### Bio-logging

Promoting the development of **biosensors and remote sensing technologies** for studying animal ecology, this platform uses devices such as GPS trackers and accelerometers. It monitors behavior, movements, and physiology of wild and domestic animal species, providing valuable data for conservation efforts, wildlife management, and animal welfare studies.

### Sequencing and Genotyping

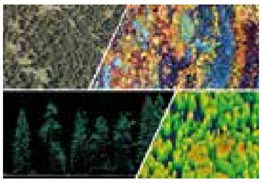
This platform supports genomic, transcriptomic, and metagenomic projects, offering access to **next-generation sequencing (NGS)** technologies and high-density **SNP array analysis**. It contributes to fundamental and applied research in genomics, breeding programs, and molecular biology, facilitating high-throughput genetic analysis.

### Development of Biopesticides and Biofertilizers

This platform identifies, develops, and enhances new **biological fungicides and biofertilizers** for promoting plant growth and protection from pathogens. By leveraging beneficial microorganisms and natural compounds, the platform supports sustainable agriculture practices, reduces chemical inputs, and improves soil health.

### Remote Sensing

Utilizing **remote sensing data** acquired from satellites, aircraft, and drones, this platform estimates biophysical parameters of forest and grassland vegetation. Activities include processing multispectral images and spatial modeling, supporting environmental monitoring, land management, and assessment of climate change impacts.



### Traceability

Specializing in **Isotopic Mass Spectrometry** and **Nuclear Magnetic Resonance (NMR)**, this platform offers services to ensure the **traceability and authenticity** of agri-food products. It helps to fight food fraud, to certify geographical origin, and to verify the quality and purity of foods and beverages, thereby protecting consumers and producers alike.

# BIODIVERSITY, ECOLOGY AND ENVIRONMENT

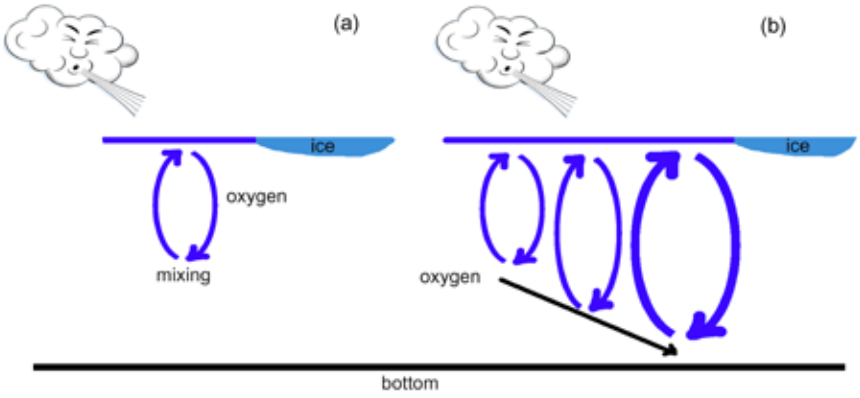
# A long-term perspective on hypolimnetic dissolved oxygen and surface $CO_2$ - Case study of Lake Tovel

ULRIKE OBERTEGGER

Lake Tovel in late January (view from the north shore to the south)

Lake Tovel is a long-term ecological research site (LTER) where physical, chemical, and biological data have been collected since 1995. The monthly sampling during the ice-free period allows the study of climate change effects on this Alpine Lake. Despite its location in a high-altitude, mountain-ringed basin that provides partial shielding from direct solar radiation and associated increases in air temperature, Lake Tovel is demonstrably affected by climate change.

Our studies indicate a delay in ice formation of approximately five days per decade in response to climate change. This influences autumn lake mixing, an important period when superficial and deeper waters are mixed by wind action (Figure 1). Because of the delayed icing, the weak wind at Tovel (mean for autumn < 1 m/s) can mix even the deepest waters (Figure 1B), not possible in previous years 2010-2014 (Figure 1A), when a hypoxic hypolimnion formed (water layer < 30 m). Nowadays the whole lake is well oxygenated, which is good news for fish and other aquatic organisms. Mixing brings oxygen from the surface to deeper layers, and it is probable that nutrients and other dissolved substances from the lake bottom are brought to the surface. A recent study showed that superficial carbon dioxide ($CO_2$) has also increased after 2010-2014, most



Autumn lake mixing driven by wind action. Superficial layers are mixed with lower layers. (a) Before 2010-2016, mixing did not last long enough to reach deeper layers. Oxygen from the surface reaches only intermediate depths (b) With delayed icing, mixing lasts long enough to reach deeper layers. Superficial oxygen reaches deep, bottom layers

probably because of the intense mixing that brings deep-water $CO_2$ to the surface. This deep-water $CO_2$ originates from the decomposition of dead organic material.

More recently, however, mixing has weakened again despite later icing because water temperature on the surface remains high until late autumn. Thus, we have noted again a decreasing oxygen concentration in the hypolimnion and lower $CO_2$ concentrations on the surface with ongoing climate change. Future studies will measure the effect of these changes on lake biota.

**KEYWORDS:** oxygen, $CO_2$, mountain lakes

# A multidisciplinary approach to understanding ecological functions in an Alpine large mammal community

CHARLOTTE VANDERLOCHT
ANDREA CORRADINI
FRANCESCA CAGNACCI

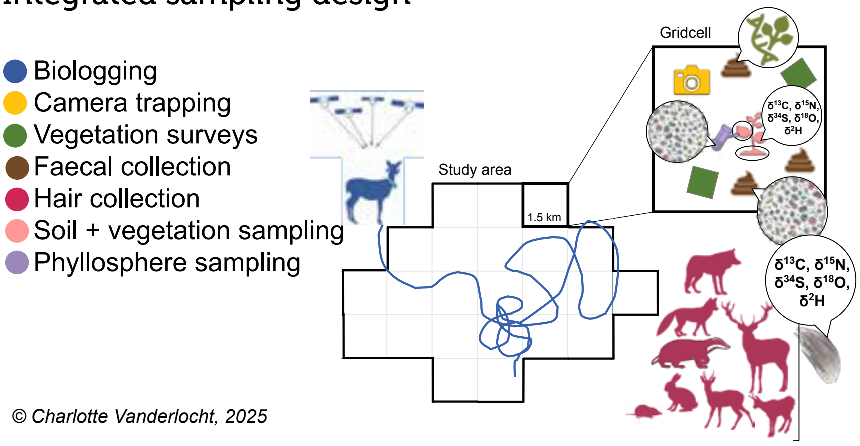
Recent anthropogenic changes have reshaped the Alpine landscape through increased human activity, climatic shifts, and land use modifications. Simultaneously, wolves are gradually recolonising their historical range after a century-long absence. These changes are expected to significantly impact large herbivores (such as red deer, roe deer and chamois), as prey for wolves, game species for hunters, and foragers dependent on vegetation. Indeed, such shifts may affect ungulate behaviour, and thus alter interspecies relationships, triggering cascading ecosystem effects. For example, rising summer temperatures may drive deer into chamois habitats, increasing competition for resources, while nocturnal predation risk from wolves may increase ungulates' diurnal activity, amplifying overlaps with humans, or with each other.

To study these dynamics, we collaborated with Stelvio National Park (co-supervisor Luca Pedrotti) and the Autonomous Province of Bolzano/Bozen to implement a systematic field study in 356 km² along a temporal gradient since wolf recolonisation. Joining expertise from the Animal Ecology (with contributions from Federico Ossi, Simone Dal Farra), Conservation Genomics (co-supervisor Heidi C. Hauffe) and Traceability (Luana Bontempo) Research units, this multidisciplinary effort integrates state-of-the-art methods, including biologging, camera-trapping, non-invasive field sampling, genetic metabarcoding, metataxonomics, and stable isotope analyses.

Our results show that, although typically crepuscular, red deer increase their daytime summer activity by almost 18% in response to wolf presence, meaning that their activity overlaps more with humans. In autumn, red deer and roe deer modify their diet and spatial behaviour to avoid human hunters, possibly overriding previous natural effects of the wolf. However, to avoid human distur-

Cameratrap images of red deer (top left), Alpine chamois (top right), roe deer (bottom right) and wolf (bottom left) in the Stelvio National Park

## Integrated sampling design



*© Charlotte Vanderlocht, 2025*

bance, both red deer and chamois are active in areas far from humans: this avoidance pattern, together with rising summer temperatures, may explain why red deer and chamois are now seen together more often in high-elevation summer ranges. But their ecological niches remain distinct: chamois hair samples contain distinctly less heavy nitrogen isotopes ($\delta^{15}N$) than red deer, suggesting differences in diet quality or protein uptake. Ongoing genomic investigations on precise taxonomic composition of diet and gut microbiota profiles will help to disentangle the roles of food selection and digestion capacities of these ruminants. Through this work, we provide a detailed view of the interactions between large mammals and humans in Alpine anthropogenic landscapes.

**KEYWORDS:** interspecies relationships, anthropogenic effects

**SPECIES LIST:** *Cervus elaphus, Capreolus capreolus, Rupicapra rupicapra, Canis lupus*

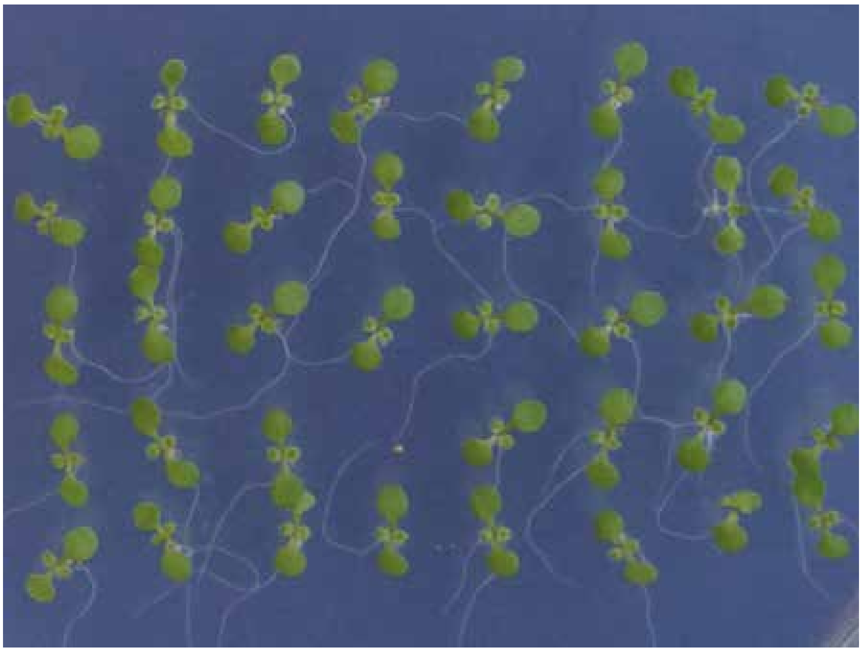
# Functional characterization and possible use of a novel isoprene synthase

JIAMEI YU
MINGAI LI
CLAUDIO VAROTTO

Single flower of *Copernicia prunifera*

Isoprene (2-methyl-1,3-butadiene) is the single most abundant biological volatile organic compound (BVOC) in the atmosphere. Every year about 500 million tonnes of carbon in the form of isoprene are released in the air, most of which is produced by plants. The reasons for such an expenditure of energy and carbon from plants have been mainly associated with the fact that isoprene protects photosynthesis from excessive temperatures. More recent studies suggested that plant isoprene emission can also offer some protection from drought, although the molecular mechanisms underlying this capacity are until now only partly understood. The Arecaceae family constitute a very interesting plant model system to study the role of isoprene in drought tolerance, as the high isoprene emissions of these tree species could be among the reasons for their success in neotropical dry forests, characterized by recurrent and extended periods of drought stress.

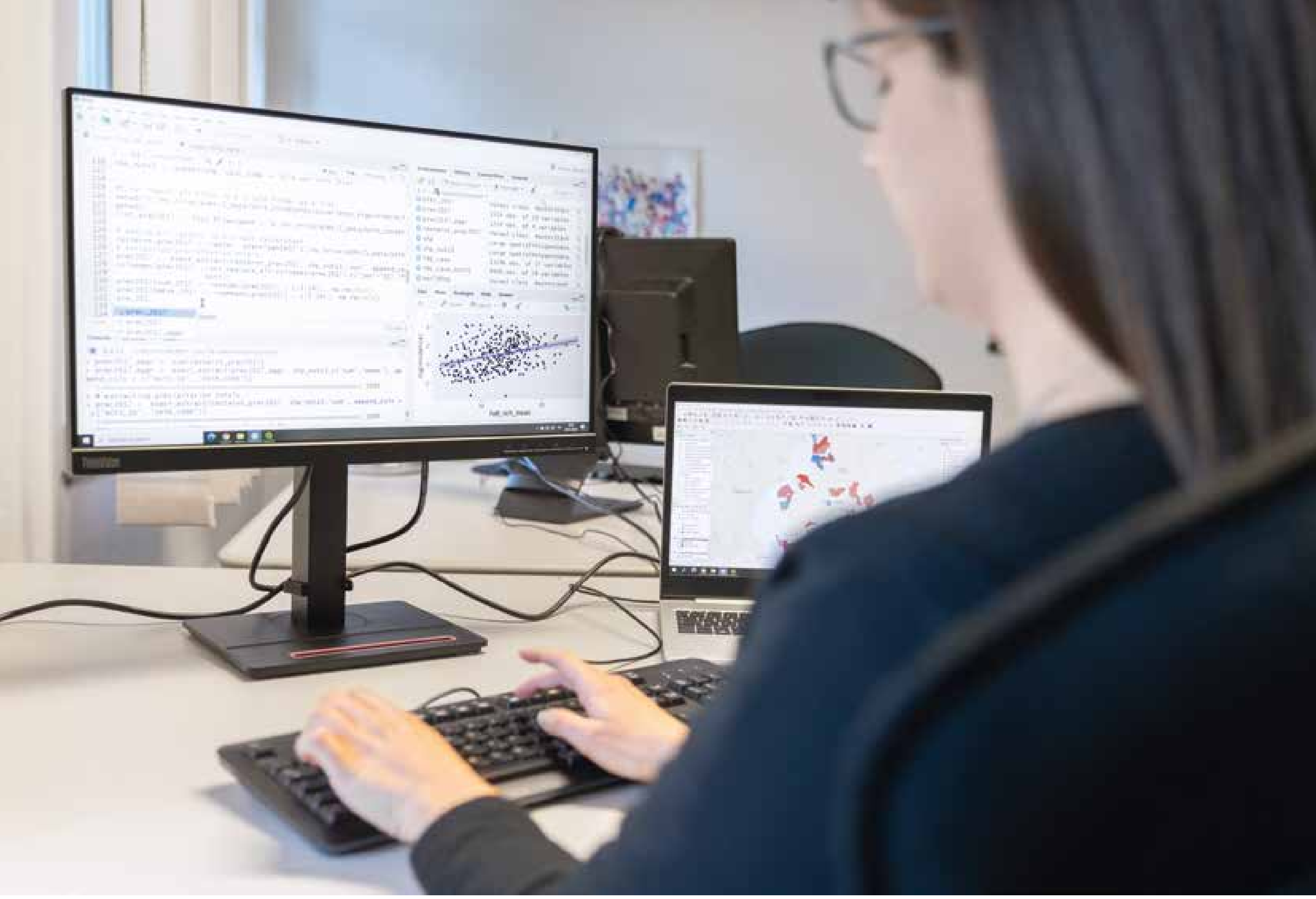
To test whether isoprene synthases (IspS), the enzymes necessary for isoprene biosynthesis from Aracaeceae, are involved in drought tolerance and could be used in the genetic improvement of crops, we isolated and characterized the *CprISPS* gene from *Copernicia prunifera* by overexpressing it in the model plant *Arabidopsis thaliana*. The high levels of isoprene emission attained in *Arabidopsis* conferred enhanced tolerance to water limitation throughout plant growth and development, from germination to maturity. The plants germinated better



Germination test of *Arabidopsis thaliana* transformed with *Copernicia prunifera* isoprene synthase

and had improved rates of cotyledon/leaf greening, higher water usage efficiency and survival than the control *Arabidopsis* plants under drought stress. This increased drought tolerance was accompanied by a different regulation of many genes characterized in previous studies and known to be key players in drought responses in plants. The results obtained are relevant to demonstrating that *CprISPS* can significantly enhance drought tolerance in *Arabidopsis* and possibly other non-isoprene-emitting species, and indicate that isoprene emission could be an important defence mechanism in tree species for coping with water limitation.

**KEYWORDS:** isoprene, VOCs, water stress
**SPECIES LIST:** *Copernicia prunifera*



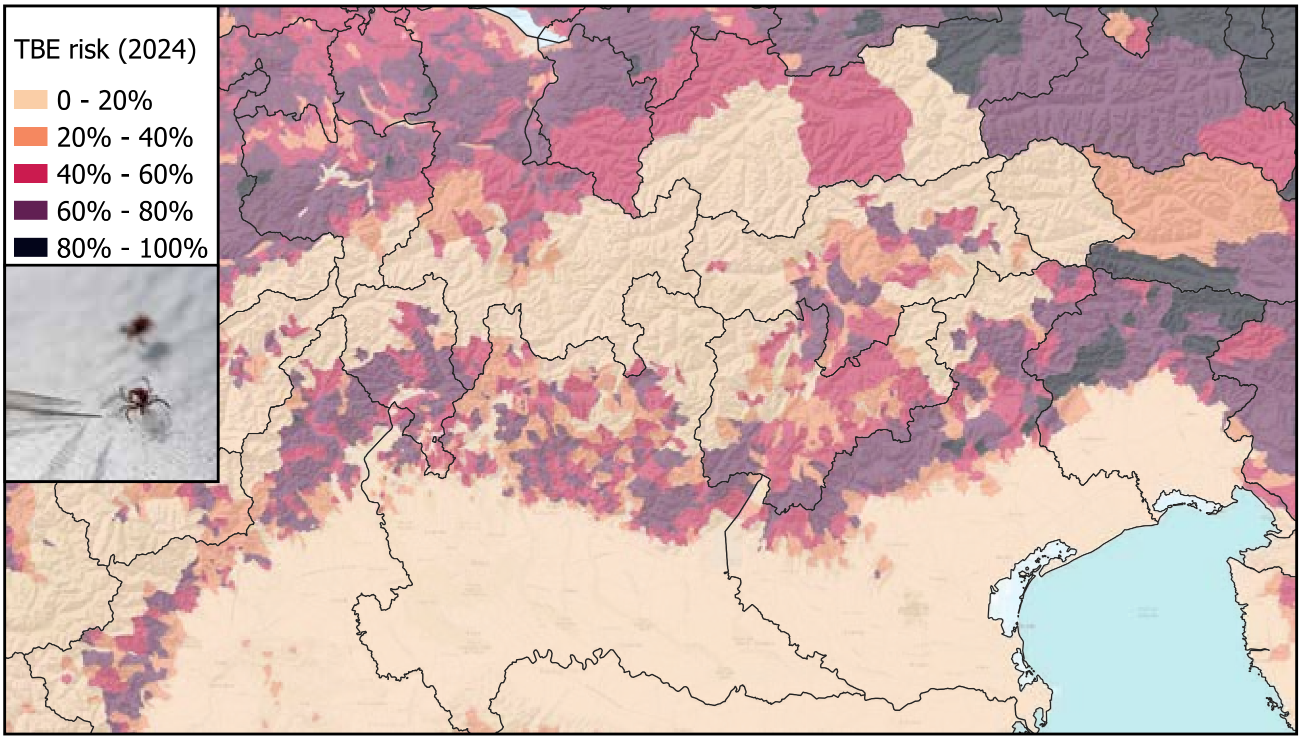
# From data to action: a modeling framework to support tick-borne encephalitis surveillance and prevention in Europe

FRANCESCA DAGOSTIN
DANIELE DA RE
ANNAPAOLA RIZZOLI

Tick-borne encephalitis (TBE) is one of the most serious neurological diseases transmitted by tick bites in Europe. In addition to the bite of infected ticks, the virus can also be contracted through the consumption of unpasteurised milk products. Patients may experience flu-like symptoms in mild cases, while severe cases can lead to life-threatening complications. Despite the availability of a vaccine, the number of human TBE infections in Europe is increasing, with new areas at risk. In Italy, human infections currently appear to be focused in the pre-Alpine and Alpine areas of Friuli-Venezia Giulia, Veneto and Trentino-Alto Adige/Südtirol.

Surveillance efforts for TBE aim to track the distribution of infections in the human population. Within this context, mapping the probability of presence of TBE, ideally at the municipal level, is essential for targeted public health interventions.

To this end, within the context of the European H2020 project MOOD, which aims to develop innovative tools for the early detection of infectious disease threats, an artificial intelligence model to predict the presence of human TBE cases was developed. By integrating epidemiological data with environmental and climatic factors, the model generates annual maps that highlight the regions and municipalities at risk.



Predicted TBE risk of human infection in the alpine area (2024)

According to this study, such areas are characterised by a rapid drop in autumnal temperatures and high levels of rainfall during summer, which promote tick survival and activity. Forested areas, by providing a suitable habitat for ticks and their hosts, are also associated with TBE presence. Finally, human behaviour can act in synergy with these ecological factors by increasing the likelihood of encountering infected ticks, as people who engage in recreational or professional outdoor activities in green areas are at increased risk of tick bites.

The risk maps developed within this study can be explored in detail at https://app.mood-h2020.eu/ and will be regularly updated and made accessible to stakeholders and public health authorities. This tool will be useful to inform targeted public health interventions, including awareness campaigns, vaccination programmes and complementary risk assessments, which are essential to inform and educate local populations in endemic regions, but also visitors,on how to take appropriate prophylactic measures for avoiding tick bites and tick-borne infections in areas where TBE is emerging.

This study is being conducted in collaboration with Dr. Giovanni Marini and Dr. Valentina Tagliapietra (FEM), Dr. Maria Grazia Zuccali (Azienda Provinciale Servizi Sanitari di Trento) and Dr. Nahuel Fiorito (Unità Locale Socio Sanitaria Dolomiti di Belluno).

**KEYWORDS:** tick-borne encephalitis, modeling, modelli statistici, *One Health*
**SPECIES LIST:** *Ixodes ricinus*

Female of *Conocephalum conicum*, Serbia

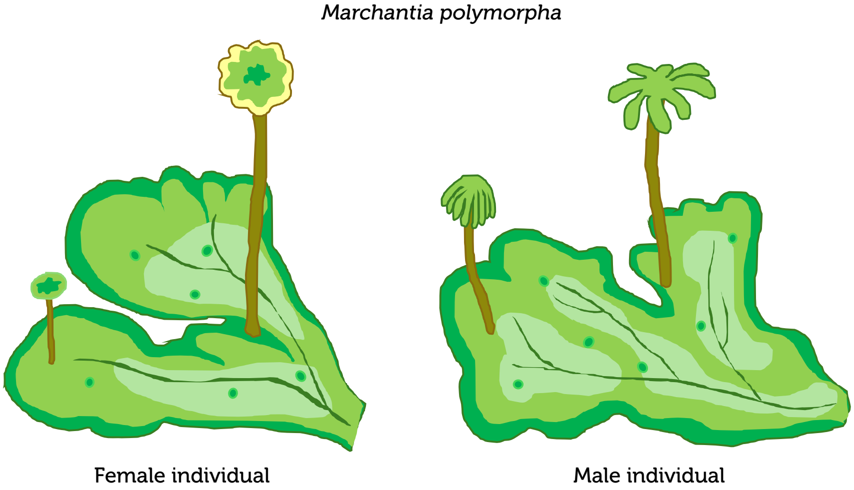
# Sex determination in bryophytes

DJORDJE BOZOVIC
MINGAI LI
CLAUDIO VAROTTO

Unlike most flowering plants, which are often hermaphroditic, possessing both male and female reproductive organs within the same flower, many bryophytes, an ancient group of non-vascular plants including mosses and liverworts, are dioecious, exhibiting separate sexes. This sexual dimorphism, also common in animals, is believed to confer an evolutionary advantage by maximizing genetic diversity and avoiding the deleterious effects of inbreeding.

The recent sequencing of the genome of several bryophyte species has just started to unravel the genes responsible for sexual separation in bryophytes, but it is already providing very interesting insights the genetic control of this feature, which has probably evolved independently several times during the about 450 million years (i.e. since the appearance of the bryophytes).

Unfortunately, many bryophyte taxa worldwide are threatened with extinction: about 20% of species are endangered in Europe, and more than 50% at the global level. The reproductive system and the ratio of male versus female individuals in populations can also significantly impact the genetic variability of bryophyte species and thus, their capacity to cope with environmental stresses like climate and land use changes. Currently, however, there is still a significant gap in our knowledge about the relationships between sex and threat status in bryophytes. We are currently working on the development of genetic markers for sex determination, which will help provide fundamental information on the reproductive systems and distribution of sexes in European bryophyte



Schematic representation of female and male plants of *Marchantia polymorpha*

populations. In turn, this information will be of paramount importance for the conservation of bryophytes, an often-overlooked priority in plant biodiversity conservation strategies at both European and global levels.

**KEYWORDS:** mosses, liverworts, extinction risk, molecular markers
**SPECIES LIST:** *Conocephalum conicum, Marchantia polymorpha*

High altitude aerobiological sampling at the Rifugio Val di Fumo (Province of Trento, Italy)

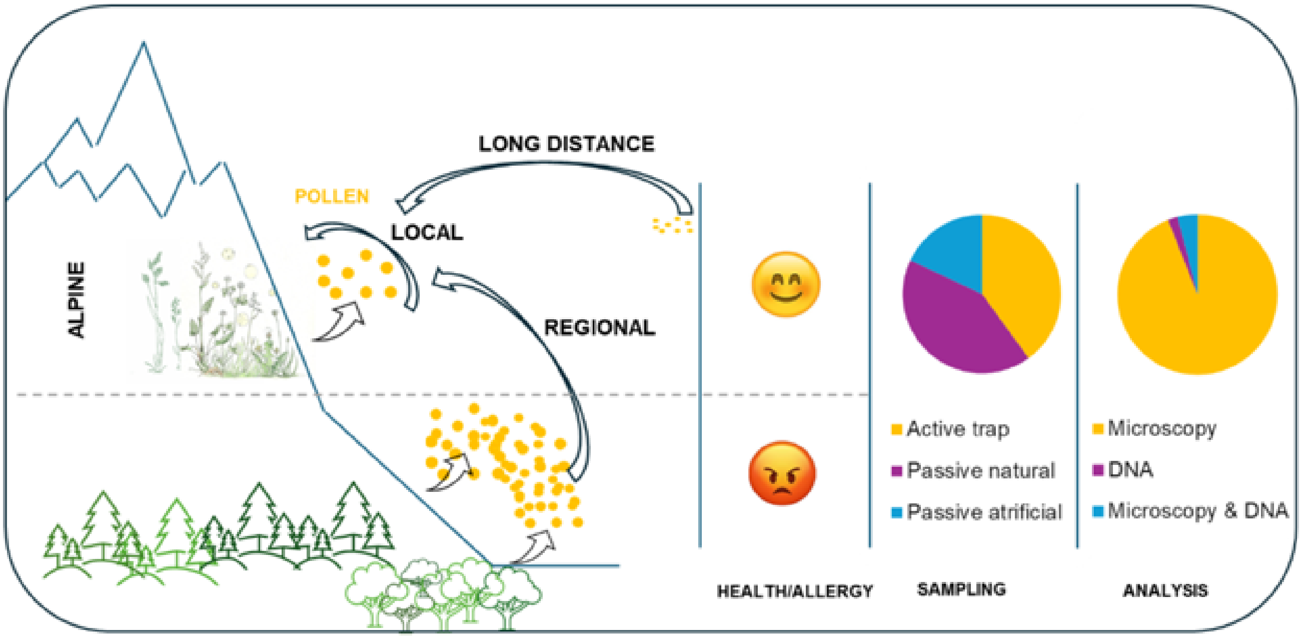
# Exploring the airborne secrets of alpine ecosystems: pollen, biodiversity, and climate change

FRANZISKA ZEMMER
ANTONELLA CRISTOFORI
ELENA GOTTARDINI

Alpine ecosystems, with their unique cold-adapted plant communities, are biodiversity hotspots and natural laboratories for studying interactions between vegetation, climate, and airborne particles. These high-altitude regions have become focal points for aerobiology. A review article published by the FEM Environmental Botany unit has examined methods, findings, and implications of aerobiological research in alpine biomes, focusing on the role of pollen in biodiversity, climate modeling, and human health.

Aerobiological studies in alpine areas use two key sampling methods: deposition and volumetric sampling. Deposition sampling is simple and effective for remote regions, while volumetric sampling requires an accessible infrastructure. Pollen identification relies largely on microscopic techniques, which are detailed but time-intensive. Molecular methods, like DNA analysis, offer added insights but remain underutilized in alpine research. Bridging traditional and modern techniques could enhance understanding of airborne pollen biodiversity.

Pollen bioaerosol reflects surrounding vegetation but also includes contributions from distant sources carried by wind. This mixture complicates data interpretation, as pollen from lower elevations or far-off valleys often appears in high-altitude samples. To address this, researchers are defining pollen sources as local, regional, or long-distance, enabling clearer insights into biodiversity and climate interactions.



Sources of pollen in the alpine environment, health impacts, and methodologies for sampling and analysis

Alpine habitats are vulnerable to rising temperatures, with plant communities shifting to higher elevations or vanishing entirely. Pollen, a proxy for vegetation, offers valuable data for monitoring these changes. Aerobiological studies, though, remain underexplored in predicting biodiversity impacts.

Airborne pollen affects human health, linking to allergies and asthma. In alpine regions, understanding pollen patterns is vital as outdoor tourism thrives. Aerobiological research can improve allergy forecasts and strategies to protect sensitive populations. Aerobiology offers a lens into alpine ecosystems, revealing interactions between vegetation, climate, human health, and airborne particles. By integrating traditional and modern methods, researchers can decode pollen bioaerosols, addressing biodiversity, climate, and health challenges. As global warming threatens alpine environments, these studies are important for protecting fragile ecosystems and uncovering insights into the planet's future.

**KEYWORDS:** alpine environment, pollen, biodiversity
**SPECIES LIST:** Poaceae, Cyperaceae, Pinaceae

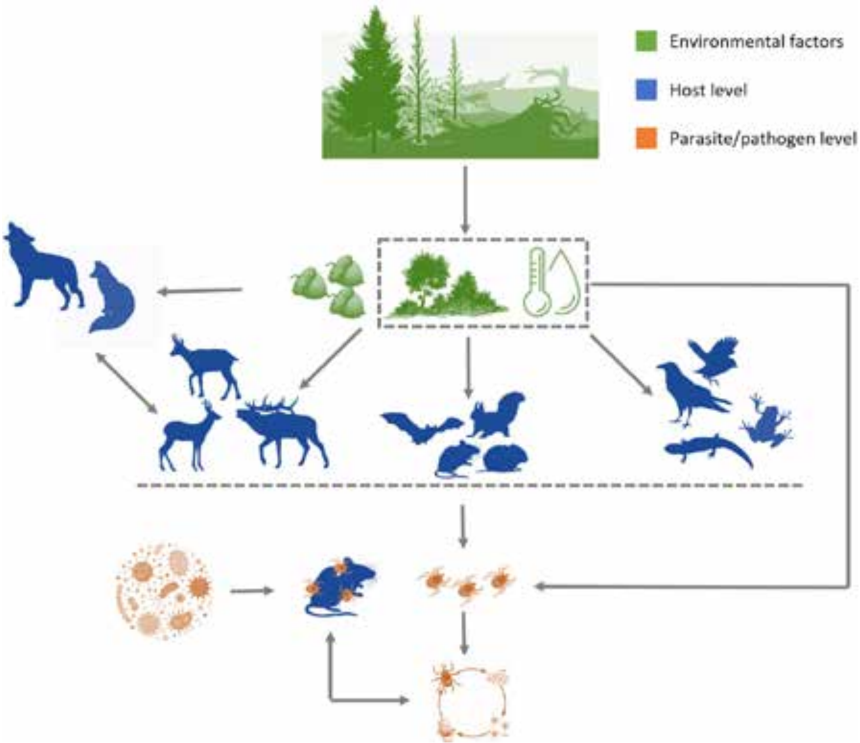
# Climate-induced habitat change alters patterns of host diversity and risk of tick-borne diseases: a *One Health* perspective

GIULIA FERRARI
FAUSTA ROSSO
VALENTINA TAGLIAPIETRA

Forest damage, with windthrows caused by the Vaia windstorm on October 29, 2018 in Eggental, Karer Pass, South Tyrol, Italy (© lookphotos / Seehauser, Othmar)

Extreme weather events can influence disease transmission risk by altering demographic parameters and geographic distribution of pathogens, vectors and hosts together with environmental variations that might affect communities and contact rates at a local scale. We aimed to assess the impacts of a catastrophic meteorological event, specifically the Vaia windstorm that occurred in the North-Eastern Italian Alps in 2018, on the infection pathways underlying vector-borne disease dynamics by investigating the health threats posed by tick-borne transmitted zoonotic pathogens.

An integrated multi-disciplinary approach based on *One Health* concepts (an approach that recognizes the interconnectedness of human, animal, and environmental health) was implemented in the Natural Park of Paneveggio-Pale di San Martino (Autonomous Province of Trento, Italy), combining camera trapping, Capture-Mark-Recapture live-trapping of small mammals, tick collection and high-throughput molecular methods. This assessment was performed during 2023 and 2024 to investigate changes in host community and activity; tick density and pathogen prevalence within a control-treatment experimental framework (undisturbed forests vs Vaia windthrow sites) at two different altitudes (1100 m and 1600 m a.s.l.).



Ecological and epidemiological interactions observed in the context of Vaia windstorm. In green the environmental factors altered by this extreme event (temperature and humidity, vegetation structure and available food resources). In blue the hosts affected by the environmental changes. In orange vectors and pathogens (© Ferrari G.)

Our results highlighted that sites affected by the Vaia windstorm exhibited a reduced diversity in meso- and large mammal communities that changed their activity patterns to the novel heterogeneous habitats. Moreover, these sites exhibited a higher presence of ticks and small mammal species promoting their interactions. We observed heterogeneity in the prevalence of the tick-borne pathogens found in host-seeking ticks, identifying agents for various zoonoses. The *Borrelia burgdorferi s.l.* complex (the group of bacteria that causes Lyme disease) was more associated with forested areas, while *Anaplasma phagocytophilum* (the agent of human granulocytic anaplasmosis), *Babesia spp.* (parasites that cause babesiosis), and *Rickettsia spp.* (bacteria that cause various rickettsial diseases) were related to Vaia sites. These shifts in population and community composition of host species with different epidemiological roles may have consequences on pathogen spread.

In conclusion, in this study, funded by PNRR-NBFC (Piano Nazionale di Ripresa e Resilienza - National Biodiversity Future Centre) and the EU project BE PREP, we observed the impacts of climate-driven extreme events on wildlife animal hosts communities, presence and behaviour, on the distribution of vector populations and on zoonoses transmission risk, which might have consequences on humans and ecosystems health. We acknowledge contributions of D. Arnoldi, G. Cervantes and the unit leader A. Rizzoli.

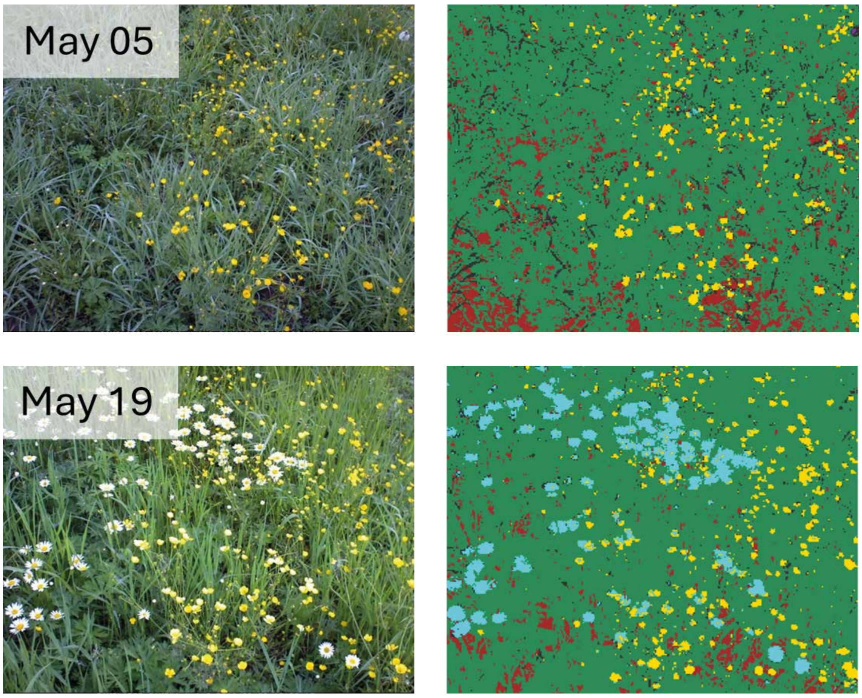
**KEYWORDS:** climate change, host-vector-pathogen dynamics, *One Health*
**LISTA SPECIE CITATE:** *Apodemus* spp., *Ixodes ricinus, Cervus elaphus*

# Climate changes (also) phenology: the impact of climate change on plant development cycles

DAVIDE ANDREATTA
LUCA BELELLI MARCHESINI
MICHELE DALPONTE

Variability in autumn phenology of larch plants at San Giuliano Lakes (Photo: Michele Dalponte)

Climate change affects the functioning of plant communities in several ways. Over the past two years, the FEM Forest Ecology unit has analyzed changes in plant phenology, i.e., the stages of their annual cycles such as the onset of spring leafing and autumn senescence. The ability to regulate the timing of these events is a result of the evolution of each species and population within a given soil and climate context. Therefore, temporal shifts in the occurrence of the different phenological phases is a clear indicator of climate change. Research led to the development of new methods for phenological monitoring applied, for instance, to the identifying of grassland flowering phenology at the single species level using a time series of images, and the potential of below-canopy transmitted light measurements for monitoring forest phenology. One initial study opens new possibilities for understanding phenology regulation in experimental setups and for evaluating ecosystem services. A second study confirmed a good correlation between satellite data with that observed under the canopy. In other studies, satellite data were used to monitor phenology at regional-continental scales. The analysis of changes in phenology over the past twenty years in European mountains showed an earlier onset of the growing season and a marked delay in the end of the growing season, with specific patterns depending on altitude and vegetation type (forest/primary



Automatic image classification for the analysis of grassland species flowering phenology

grassland). In addition, it was found that the lengthening of the growing season does not seem to result in an increase in ecosystem productivity. This divergence may be partly explained by numerous climate anomalies in recent decades, as evidenced by the analysis of the phenological response of Trentino forests to extended late frost (2019) and drought (2022) events. As climate anomalies are projected to continue in the coming decades, a better understanding of phenological response is crucial for predicting the response of different species to the new climate.

**KEYWORDS:** phenology, forest, climate change, grassland

# Pollen as a risk indicator for Tick-Borne Encephalitis

GIOVANNI MARINI
ANTONELLA CRISTOFORI
ELENA GOTTARDINI

Nymph of *Ixodes ricinus*

This study investigated the relationship between the amount of airborne pollen of some forest plants and the incidence of tick-borne encephalitis (TBE) in the Province of Trento. TBE is caused by a Flavivirus that affects the human central nervous system and is primarily transmitted through the bite of infected ticks, particularly the castor bean tick (*Ixodes ricinus*) in western Europe.

The tick's life cycle includes four developmental stages (egg, larva, nymph, and adult) and typically spans three years. Larvae and nymphs feed on small to medium-sized mammals, while adult females feed on larger mammals, such as ungulates.

TBE is present in many countries, with an average of about 3,000 cases reported annually in Europe. The spread of the virus depends on the density of infected ticks, which is influenced by the availability of rodent hosts. Rodent populations are, in turn, affected by the availability of food resources, such as the seeds produced by some tree species, whose production varies across years.

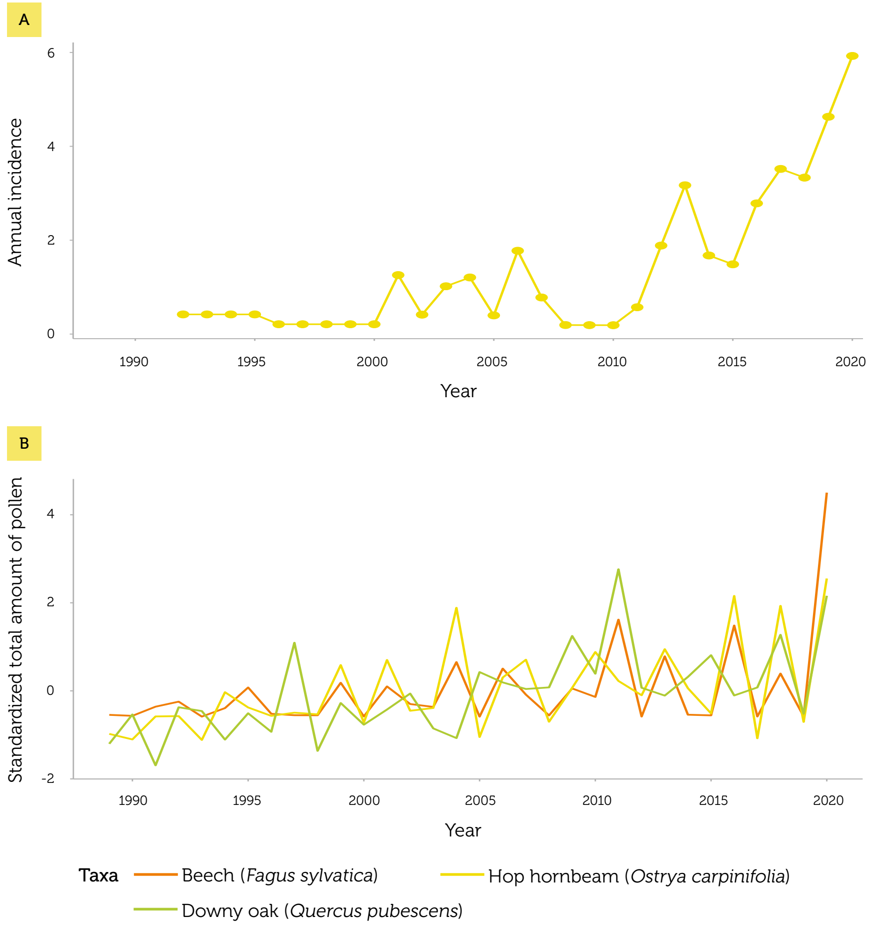
Using a dataset of pollen concentrations derived from 30 years of aerobiological monitoring (1989–2020), we examined the correlation between the amount of airborne pollen from seven tree species widely present in the Province and the annual TBE cases recorded in Trentino from 1992 to 2020. Pollen was collected using a Hirst-type sampler located on the campus of the Fondazione Edmund Mach and analyzed using conventional optical microscopy methods.

Our results show a strong positive correlation, with a two-year delay, between the amount of pollen from hop hornbeam (*Ostrya carpinifolia*) and downy oak (*Quercus pubescens*) and TBE incidence. Beech (*Fagus sylvatica*) pollen also showed a correlation, though weaker, likely due to the limited atmospheric dispersal of this species' pollen.

A high pollen load in a given year correlates with increased seed production (year 0) and consequently greater food availability for rodents, which become more numerous the following year (year 1). This positively affects tick population growth and virus circulation, leading to a higher incidence of TBE cases two years after the pollen event.

Our analysis could be replicated on a larger scale, and if the results are confirmed, it could enable the implementation of an early warning system for TBE transmission risk.

The study was carried out in collaboration with Fabiana Cristofolini, Francesca Dagostin, Annapaola Rizzoli and Valentina Tagliapietra (FEM), and Silvia Molinaro and Maria Grazia Zuccali (Azienda Provinciale Servizi Sanitari della Provincia di Trento).



A: number of recorded TBE human cases per 100,000 population per year in the province of Trento (1992-2020).
B: Standardized total amount of pollen for beech, hop hornbeam and downy oak

**KEYWORDS:** tick-borne encephalitis, pollen, public health
**SPECIES LIST:** *Ixodes ricinus, Ostrya carpinifolia, Fagus sylvatica*

Egg clutches, tadpoles, and young frogs release DNA traces in the aquatic environment, which can then be detected and analyzed with specific protocols, providing important information on the presence and genetic diversity of amphibians

# The memory of water: genetic diversity of an alpine amphibian measured from eDNA

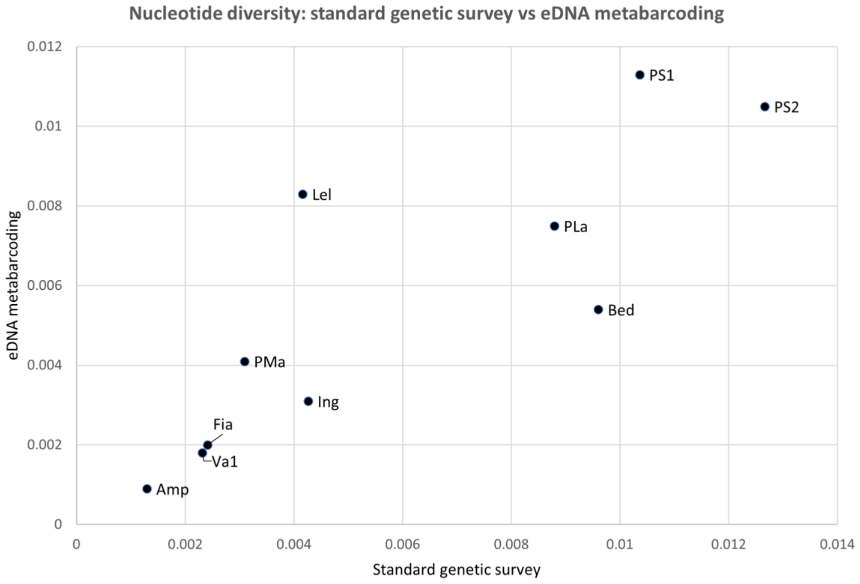
LUCIA ZANOVELLO
MATTEO GIRARDI
HEIDI C. HAUFFE

Since the dawn of time, human civilizations have been irresistibly attracted by aquatic environments, resulting in the urbanization of riverbanks and lakeshores as well as the draining of smaller wetlands. As well as being a source of drinking water and food, and a vital support for the defense and trade of local human populations, these water bodies represent also an invaluable source of wild biodiversity. Thanks to recent technological advancements in the field of genetics for the conservation of species at risk of extinction, we have the confirmation that these wetlands constitute real "*pools*" of knowledge, capable of providing essential information for the management of these environments and the animal species that live in them. Starting from a simple sample of water, in fact, it is possible to extract DNA and learn which species live in the wetland and the surrounding area, without harming or even disturbing them.

We used environmental DNA (eDNA) samples from water and highly innovative laboratory methods at the CRI Animal, Environmental and Antique DNA Platform to monitor the biodiversity of Alpine amphibians, a group of shy animals defined as critically endangered on a global level. This research, developed thanks to funding from the UNESCO Biosphere Reserve Alpi Ledrensi and Giudicaria (ACQUAVIVA project), and in collaboration with MUSE – Museo delle Scienze, Trento, allowed us to test the life stages and conditions in which these genetic techniques are more effective, as well as the possibility of measuring the genetic diversity of these animals.

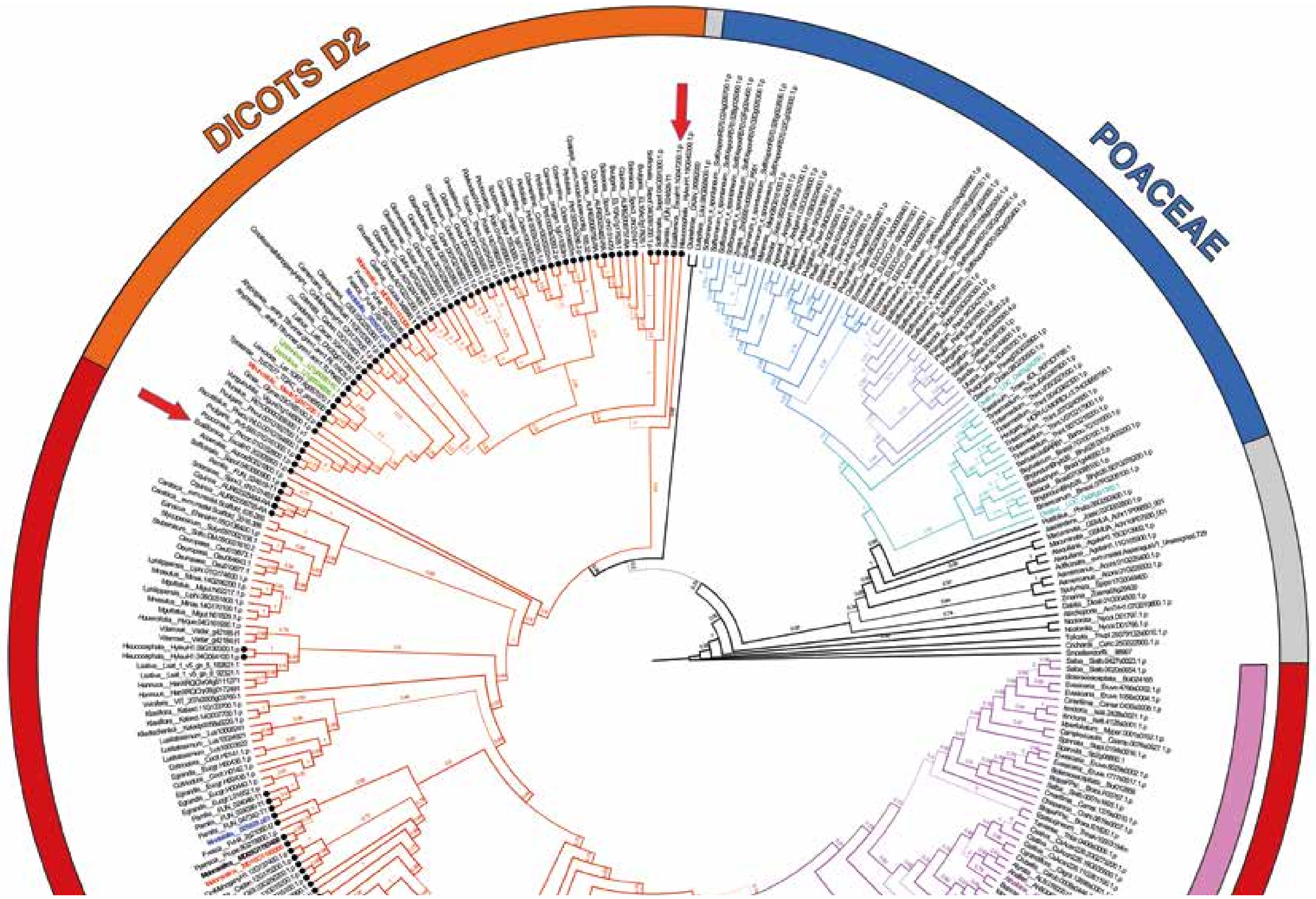
The mountain frog *Rana temporaria* was chosen for this research because it is widespread in Trentino and an important model species for "classical" studies on genetic diversity. This species also shows local declines due to the climate crisis and habitat degradation. Using a fragment of the Cytochrome Oxidase I (COI) gene as a genetic marker, we demonstrated that our eDNA-based protocol is able to provide estimates of traditional genetic diversity indices (i.e. number of haplotypes, haplotype and nucleotide diversity) with high reliability compared to classical genetic methods (which require tissue).

This study has therefore demonstrated for the first time, at a global level, that even in the case of amphibians, it is possible to obtain information on genetic variability from eDNA samples, paving the way for new monitoring programs and bringing genetic diversity to the forefront of conservation.



Scatterplot of the nucleotide diversity index values of *Rana temporaria* populations in the Province of Trento Italy, for two datasets: x-axis: previous standard tissue-based genetic survey; y-axis: this study (based on eDNA)

**KEYWORDS:** environmental DNA, amphibians, genetic diversity
**SPECIES LIST:** *Rana temporaria*



Cladogram of PCS proteins from fully-sequenced plant genomes

# Ancient duplication and functional differentiation of phytochelatin synthases in plants

MINGAI LI
CLAUDIO VAROTTO

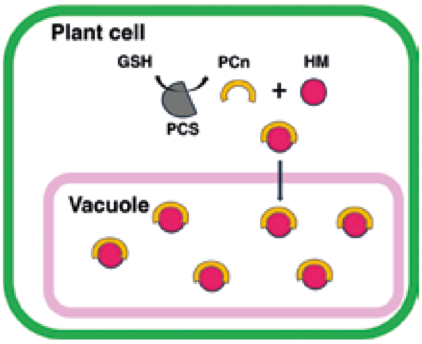
Some metals are essential for plant growth and development, while other heavy metals, such as cadmium and lead, or metalloids like arsenic are toxic even at very low concentrations, because they compete with endogenous metals necessary for proper biological processes. Therefore, tight regulation of heavy metal accumulation in plants is an important mechanism to maintain plant fitness.

Phytochelatins (PCn) are small peptides rich in cysteine, widely present not only in plants, but also in other organisms. PCn biosynthesis is catalyzed by the phytochelatin synthase (PCS) enzyme. PCn bind the heavy metals and the heavy metal-PC complex is then transported into the vacuoles, a large compartment of plant cells, where the heavy metals can be detoxified. Despite the paramount importance in metal(loid) detoxification of phytochelatin synthase (PCS) genes, no comprehensive analysis of their evolutionary patterns has been carried out in land plants in general and in crops in particular.

Through a large-scale analysis of flowering plant genomes, we uncovered a so far undetected ancient gene duplication present in many plant species resulting in two types of *PCS* genes called *D1* and *D2*. By complementing the lack of PCn production in a mutant of *Arabidopsis thaliana* with either the *D1* or *D2* gene copy from two model crop species, apple (*Malus domestica*) and barrel medic (*Medicago truncatula*), we observed that the *D2* copies promoted better plant growth, phytochelatin and glu-

Seeds of *Medicago truncatula*

tathione production, as well as higher element contents under heavy metal(loid) stress. *In vitro* analysis of the D1 or D2 purified PCS proteins confirmed the whole-plant observations, clearly showing a higher activity of D2 type proteins compared to D1 type proteins. As the phytochelatin detoxification pathway is the most important heavy metal(loid) detoxification identified to date in vascular plants, the discovery and first characterization of D2 type genes in additional species will provide deeper insights into the functional differences between the two *PCS* copies. Furthermore, this discovery may provide a new tool for the improvement of the capacity to detoxify heavy metals and metalloids making crop species more tolerant to these harmful elements.



Schematic representation of heavy metal (HM) detoxification by phytochelatins (PCn) inside plant cells. The biosynthesis of PCn is catalyzed by phytochelatin synthase (PCS). The formed PCn::metal complexes are transported to vacuoles, where the metal ions are detoxified

**KEYWORDS:** heavy metals, metalloids, detoxification, duplicated genes
**SPECIES LIST:** *Malus x domestica, Medicago truncatula*

# The fingerprint of tropospheric ozone on forests in Europe

ELENA GOTTARDINI

Tropospheric ozone is a powerful oxidant that can cause physiological and biochemical damage to forest vegetation, leading to reduced productivity and, ultimately, negative effects on the entire ecosystem.

The only specific and visible evidence of ozone's impact on vegetation consists of peculiar visible foliar symptoms (VFS). These symptoms have been reproduced under (semi-)controlled conditions in various species, and their field assessment has been widely standardized across Europe within the framework of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). Data on ozone concentrations and VFS collected between 2005 and 2018 at approximately 100 intensive forest monitoring sites under ICP Forests, were subjected to in-depth analysis by a team of experts. The study encompassed 20 European countries spanning five biogeographical regions, analyzing spatiotemporal variations in ozone concentrations, VFS frequency, and their interrelationships.

Ozone concentrations, which were significantly higher in Alpine and Mediterranean regions, remained essentially stable during the study period, with a trend toward decrease in Alpine-Boreal sites and increase in Atlantic sites.

Foliar symptoms were observed in more than one-third of the broadleaved species monitored in Europe, with no significant differences between biogeographical regions. Overall, and with the exception of an increase recorded in Atlantic sites,

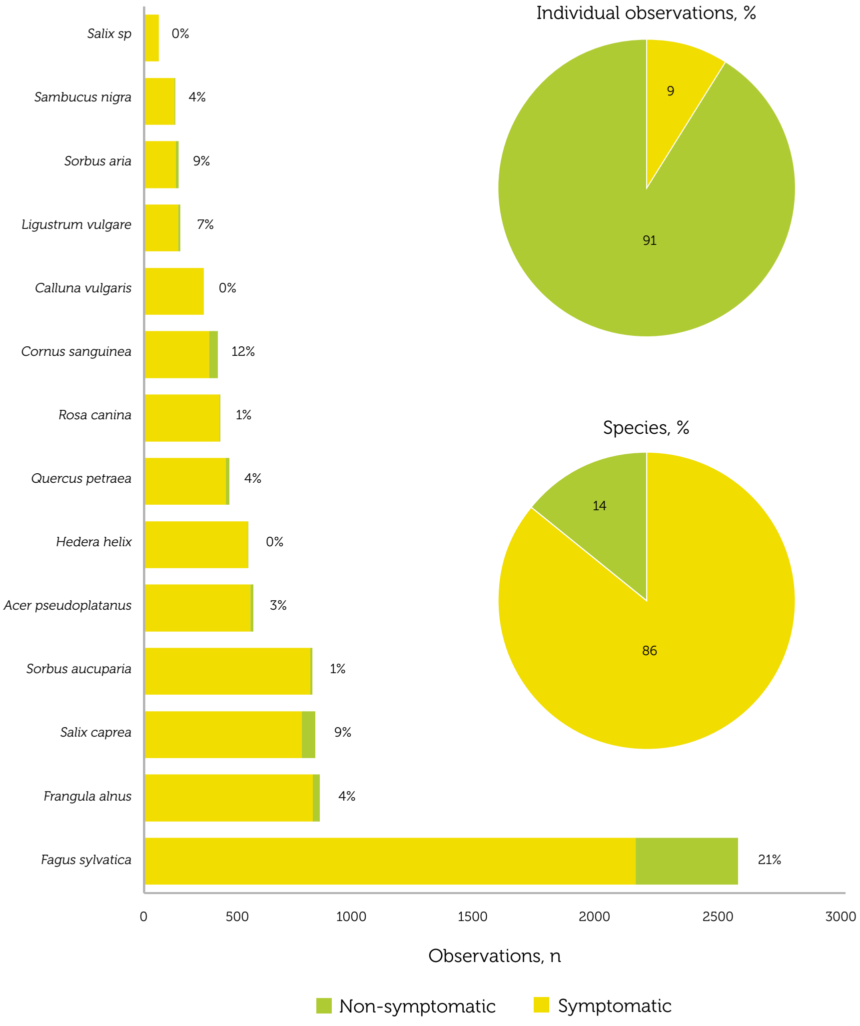
Foliar symptoms caused by ozone

the frequency of VFS remained unchanged or slightly decreased over the study period. A positive relationship between ozone concentrations and VFS was also observed at the European level.

Species exhibiting a higher frequency of VFS corresponded to those previously identified as ozone-sensitive in controlled exposure experiments. Furthermore, VFS frequency appeared to be modulated by species-specific traits, such as specific leaf area and leaf thickness.

Despite ozone concentrations indicating a gradient of increasing risk from North to South, with critical areas in the Alpine and Mediterranean regions, VFS highlight a higher risk to vegetation in the Alpine and Continental areas compared to the Mediterranean regions.

This study underscores the importance of considering local vegetation composition and species-specific ozone sensitivity for a more comprehensive understanding of ozone's ecological impacts and the development of effective mitigation strategies.



List of common species in the four biogeographic regions and relative number of observations, distinguished between non-symptomatic (yellow) and symptomatic (green); for each species, the percentage value of symptomatic observations is also reported

**KEYWORDS:** tropospheric ozone, visible foliar symptoms, forest, monitoring
**SPECIES LIST:** *Fagus sylvatica, Viburnum lantana*

# The study of biodiversity in Alpine waters: a multidisciplinary approach

SARA VETTORAZZO

Lake Ampola (TN)

Alpine waters are unique ecosystems rich in biodiversity, and today they are highly threatened by climate change and human pressures. The melting of the cryosphere, shifts in precipitation patterns, and warming waters are profoundly impacting the quantity and the chemical, physical, and biological quality of water resources. Additionally, anthropogenic activities are contributing to eutrophication and the introduction of invasive species, causing significant ecological damage. In this context, understanding and monitoring the ecological consequences of these transformations is essential.

Within the BioAlpEC (Biodiversity of Alpine Ecosystems in a Changing World) project, developed within the framework of the National Biodiversity Future Centre (NBFC) and involving eight research units of the Research and Innovation Centre, the Hydrobiology unit aims to expand knowledge of Alpine water biodiversity through a multidisciplinary, multi-scale approach. In particular, the research concentrates on a wide physiographic and altitudinal gradient of environments, with particular focus on understudied ecosystems such as small Alpine water bodies and proglacial lakes. The project involves the analysis of various organisms and communities, from microscopic (bacteria, microalgae, fungi) to macroscopic (copepods, invasive bivalve molluscs) scales.

Specific techniques are employed to address different research questions: barcoding is used to genetically characterise individual organisms (copepods and molluscs), enriching reference databases with new sequences of species of interest; metabarcoding is applied to analyse community diversity (bacteria, diatoms, fungi) from environmental DNA collected in different environments and/or time periods; and metagenomics is used to characterise the functionality of certain organisms, including cyanobacteria and their toxin-coding genes.

The first results, published in 2024, include studies on bacterial communities in small water bodies and peat bogs, and a metagenomic analysis of cyanobacteria. Further data will be made available in 2025. The knowledge generated through this project will enhance our understanding of Alpine freshwater biodiversity in the context of climate change, contributing to an important step towards their conservation. A special thanks to the Hydrobiology unitstaff, all involved in the BioAlpEC project: Adriano Boscaini, Maria Cristina Bruno, Leonardo Cerasino, Ulrike Obertegger, Monica Tolotti and Nico Salmaso. This project is funded by the National Recovery and Resilience Plan (PNRR; MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 - D.D. 1034 17/06/2022, CN00000033, Project title "National Biodiversity Future Center - NBFC").

Sampling of *Dreissena polymorpha* (an invasive bivalve mollusk) using a Ponar grab - Lake Ledro (TN), November 2024

**KEYWORDS:** biodiversity, alpine waters, multidisciplinarity

# Monitoring of invasive alien mosquito species in the Autonomous Province of Trento

DANIELE ARNOLDI
ENRICO INAMA
GONZALO CERVANTES

In recent decades, three invasive mosquito species have been spreading throughout Europe: the tiger mosquito (*Aedes albopictus*), the east Asian mosquito (*Ae. koreicus*) and the rock pool mosquito (*Ae. japonicus*). Among these, *Ae. albopictus* is the most invasive and has colonized several Mediterranean countries. Concurrently, Europe has seen an increase in human infections transmitted by these mosquito vectors, notably chikungunya and dengue, which are primarily vectored by *Ae. albopictus*. Climate change and globalization increase the risk of introducing both new invasive mosquito species and the exotic pathogens they may carry. The increased health risks and nuisance caused by these mosquitoes have required intensified research and monitoring of these species, in the Province of Trento as well.

In 2023 and 2024, FEM carried out adult mosquito sampling in 16 sites located in 9 municipalities in Trentino between April and October. The primary surveillance tool was the BG-Sentinel trap, specifically designed to capture *Aedes* mosquitoes, particularly the tiger mosquito. Traps were positioned in urban and peri-urban environments and activated for 24 hours every two weeks.

The most represented species is the tiger mosquito with 82% of the total captured specimens followed by the common house mosquito (*Culex pipiens*) with 17% and the east Asian mosquito with 1%. Furthermore, specimens of rock pool mosquitoes were identified at the larval stage. The population density of this species is still very low, so the capture of adult individuals is not effective.

When comparing 2023 and 2024, a similar trend of the tiger mosquito population is highlighted but the onset of a significant presence of adult mosquitoes occurred a couple of weeks later in 2024 (week 26, Fig. 1). This is probably due to the unstable weather conditions from March to June 2024 (2.4 times accumulated rainfall). Subsequently, 2024 presents a more abundant population, with a population peak (first half of September) double that of 2023.

Currently, invasive mosquitoes are widespread over the Province of Trento. The tiger mosquito has colo-

Detailed view of *Aedes koreicus*

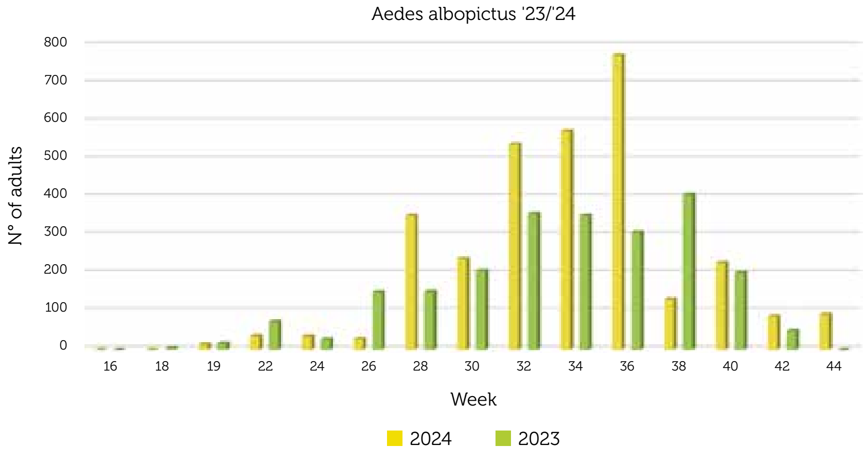
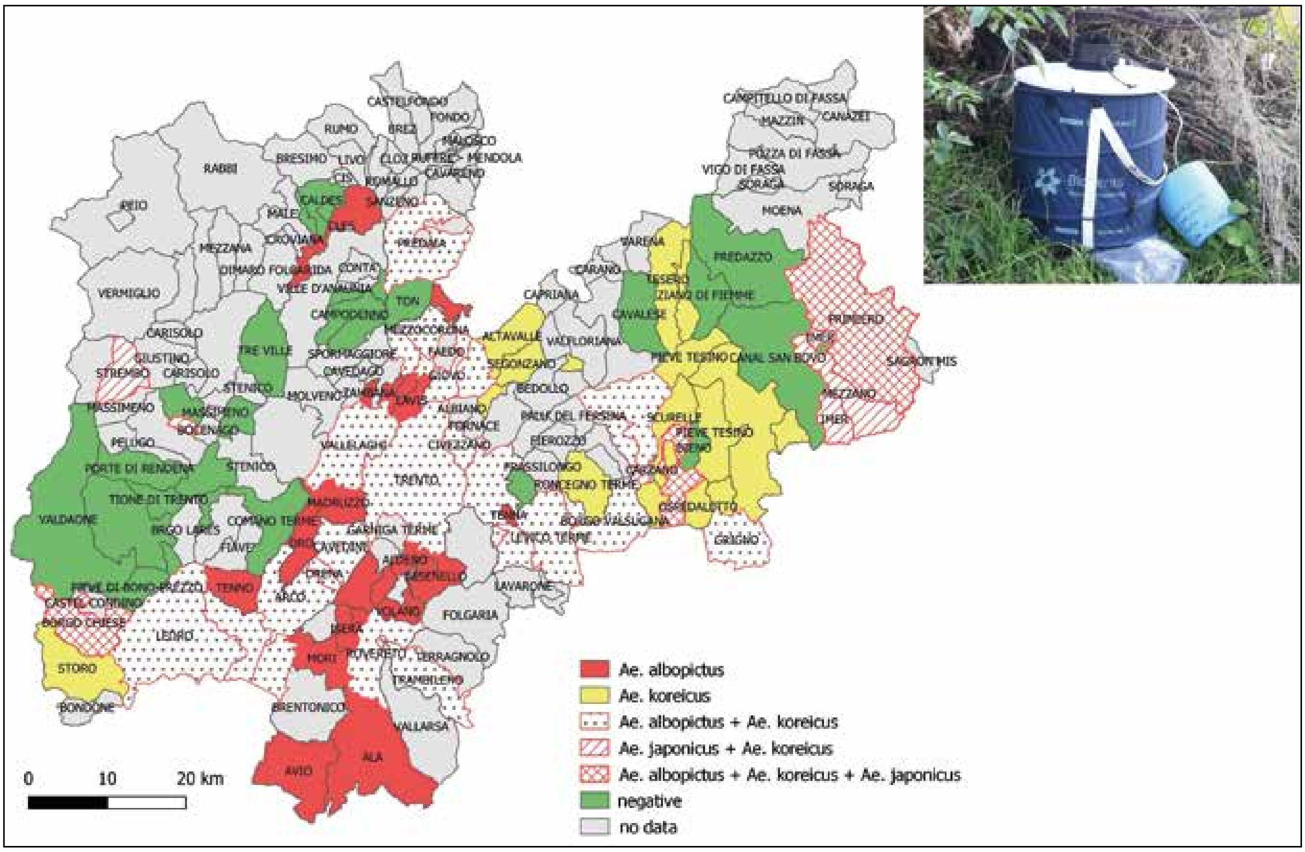


Fig. 1: Trend of the tiger mosquito (*Aedes albopictus*) population in 2023 and 2024

nized the entire province at altitudes below 600 m a.s.l, while the East Asian mosquito has a greater altitudinal distribution and can be found up to 1500 m a.s.l. Finally, the rock pool mosquito has a scattered distribution due to its recent introduction but, given its ecological similarities with *Ae. koreicus*, further spread in the coming years is expected (Fig. 2).

Fig. 2: Distribution of invasive mosquito species in the province of Trento. In the box BG-sentinel trap



🟥 Ae. albopictus
🟨 Ae. koreicus
⋯ Ae. albopictus + Ae. koreicus
▨ Ae. japonicus + Ae. koreicus
▩ Ae. albopictus + Ae. koreicus + Ae. japonicus
🟩 negative
⬜ no data

**KEYWORDS:** invasive alien mosquitoes, monitoring, Trentino

**SPECIES LIST:** *Aedes albopictus, Aedes koreicus, Aedes japonicus*

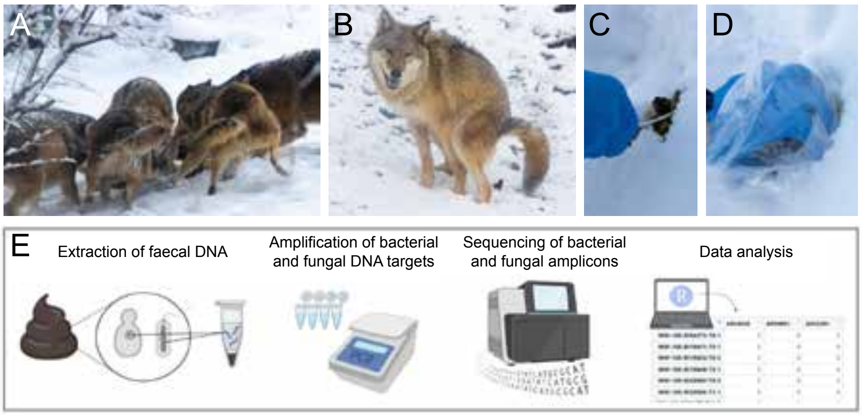
# They are what they eat: how diet affects the gut flora of wild animal species

GIULIO GALLA
ELENA SGARABOTTO
HEIDI C. HAUFFE

Wolves hosted at the Spormaggiore Wildlife Park – BELPARK (photo of E. Sgarabotto)

Microorganisms that live in the mammalian gut ('microbiota' - bacteria, fungi, viruses) are known to contribute to host health. However, although well-studied in humans, the effect of dietary changes on the gut microbiota composition and function of wild animals living in human-impacted environments is still largely unknown. The Conservation Genomics Research unit staff (G. Galla, N. Flux, E. Sgarabotto, H.C. Hauffe, S. Casari, C. Rossi, and M.U. Scholz) studied nine healthy adult wolves at the Spormaggiore Wildlife Park – BELPARK in collaboration with park personnel (A. Marcolla and M. Hirber) and veterinarian (R. Guadagnini) to investigate the effect of changes in diet on gut microbiota function in wild mammals (Project METALUPO).

The wolves (which normally eat a mixed diet) were offered a diet of livestock meat (whole sheep purchased from a local butcher) for one month followed by deer meat (roadkill carcasses donated by a local park) for one month. Fresh fecal samples were collected daily over a three-month period and kept in -80°C freezers until analyses using metataxonomy (i.e a sequencing-based method allowing the taxonomic profiling of all microorganisms present in a sample). In this model system, we found that the pack gut microbiota was very diverse, consisting of a small core of bacteria and fungi shared by all animals (109 types of bacteria out of a total 6,248 and 30 fungi out of 2,365), while a large number of microorganisms were detected at



Methodology adopted in this study.
A: wolf pack feeding on the provided diet; B:D faecal sampling using non-invasive approaches. E: main analytical steps of the metataxonomic approach used to study the wolf intestinal bacterial and fungal communities. (photos by E. Sgarabotto)

much lower abundance, or even only identified in single wolves (604 types of bacteria and 1131 types of fungi). These results confirm that variation in gut microbiota between individuals is quite high, even in a small family of closely related individuals sharing the same environment.

The change in diet was associated with substantial changes in the bacterial and fungal taxa hosted by the wolf gut, with bacteria showing a quick but transient increase in richness following a new diet, while the number of fungi grew more steadily as the diet changed from their commercial diet, to sheep to deer. Moreover, while the composition of the gut fungal community was closely associated with diet and show significant differences between different diets, changes in the composition of the gut bacterial community appear to be only marginally associated with diet, an emerging pattern in our wildlife microbiota research suggesting that bacteria has more closely evolved with mammalian hosts and are more resilient to change. Further studies will investigate if and how the changes in gut microbiota taxa lead to changes in gut function.

This study will be used to inform veterinary and wildlife management strategies for the grey wolf,
and will add to the growing body of knowledge investigating the importance of the diversity and functional potential of the intestinal microbiota for mammal conservation.

**KEYWORDS:** gut microbiota, wildlife animals, diet
**SPECIES LIST:** *Canis lupus*

# Tradition and innovation in aerobiology at FEM

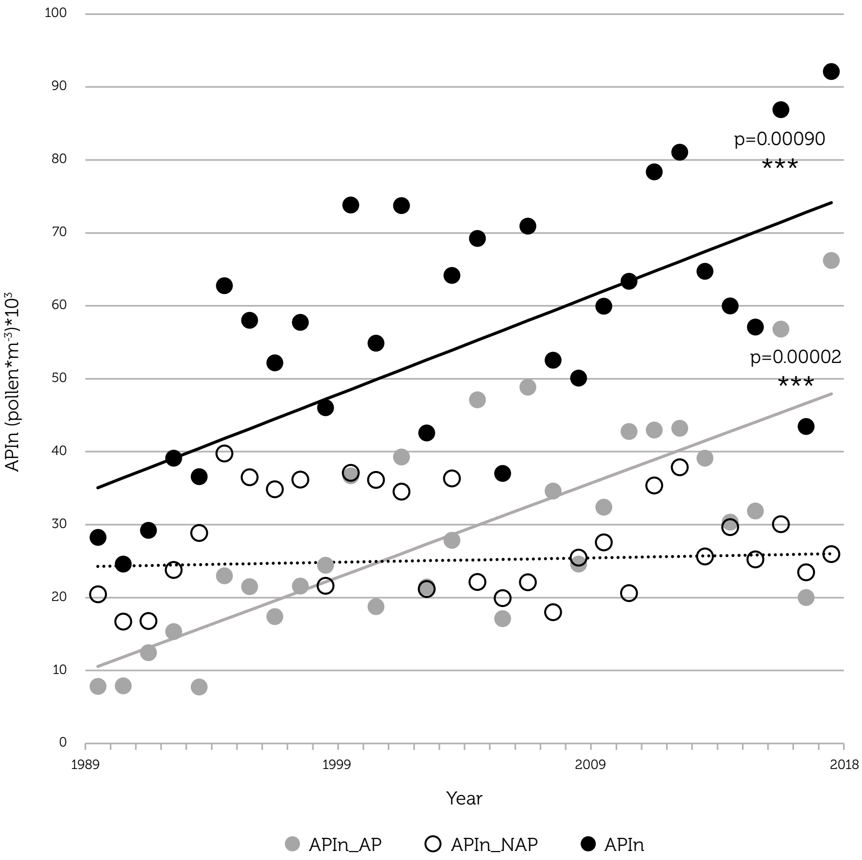
FABIANA CRISTOFOLINI
ANTONELLA CRISTOFORI
ELENA GOTTARDINI

The automatic and real-time instrument for sampling and analysis of the bioaerosol Poleno Mars (Swisens), with which the measurement of pure pollen is achieved through a special particle atomizer

Aerobiological monitoring has been carried out at FEM for the last 35 years, and has allowed us to identify variations in the pollination spectrum and the pollination curves, which reflect variations in vegetation and in flowering phenology. These parameters are also closely linked to global change. The analysis of airborne pollen produced by anemophilous (wind-pollinated) plants provides information on the biological component of ambient air, the plant biodiversity of the study area and the phenological phases of flowering. The standard method (UNI EN 16868:2019), now applied on a global scale, involves the use of a volumetric Hirst-type sampler and a microscopic analysis of collected samples. The long time series of data on daily pollen concentrations for over 50 different types of pollen (taxa) allows us to estimate the response of vegetation to global changes.

Our results, consistent with those found in other study areas, show an increase in the quantities of pollen, primarily from trees, and an earlier beginning of the phenological phase of flowering, on average five days every 10 years, for some of the taxa investigated. The study highlights the impact of global changes, in particular, the rise in minimum temperatures which have resulted in a decrease in the number of frost days, is an important cofactor in determining the increase in pollen quantities. Pre-flowering temperatures are also positively correlated with an earlier pollen release.

In addition to these important results obtained by analysing histor-



Trends in real time pollen quantities (Annual Pollen Integral) from 1989 to 2018, showing a significant increase for total pollen (black) and tree plant pollen (grey). The pollen produced by herbaceous plants (white) shows no significant trend

ical data series collected through conventional sampling and analysis methods, the Research unit of Environmental Botany has been committed to research and apply the most innovative techniques and instruments in the field of aerobiology.
Thanks to the collaboration with the Animal, Environmental and Antique DNA, Sequencing and Genotyping, and Computational Biology platforms, our research on plant and fungal biodiversity has developed through methods of DNA metabarcoding from environmental DNA, which has led both to an increase of knowledge and to the development of a network of international collaborations. In parallel, the CATS project has been developed, thanks to the collaboration with the Bolzano Environment and Climate Protection Agency and the Swiss company Swisens. This project will exploit the potential of the new bioaerosol automatic and real-time sampling and analysis system Poleno Mars, installed at the FEM campus.

**KEYWORDS:** pollen, temperature, time trends
**SPECIES LIST:** *Ostrya carpinifolia*, Urticaceae, Cupressaceae

# Development of species-specific DNA barcodes for plantains

FURRUKH MEHMOOD
MINGAI LI
CLAUDIO VAROTTO

*Plantago* (plantains, Plantaginaceae) is a cosmopolitan genus including over 250 species used as functional foods, forage, and ingredients in traditional medicines. Some *Plantago* species are relevant in the nutraceutical and pharmaceutical sectors because of the mucilaginous substance, called psyllium, obtained from the seed husk, that can be used as a functional food and dietary supplement improving intestinal health. Different *Plantago* species are utilized in human nutrition as ingredients in baked products, salads or soups or as animal feed helping improve their overall health. Among the different plantains, *Plantago lanceolata* L. is a common forb in Europe and Western Asia. Currently, one of the most common uses of *P. lanceolata* is in the treatment of skin diseases and oral and upper respiratory tract affections and its use is recognized as safe for use in the pharmacopeias of several countries. According to a report prepared by the World Health Organization, the herbal products market is vigorously growing and will reach a value of USD 5 trillion worldwide by 2050. Given their relatively simple morphology, *Plantago* species are relatively difficult to distinguish from each other, leading to possible misidentification and qualitative variability through the herbal medicinal value chain of plantain products.

Inflorescence of *Plantago lanceolata*

To solve this problem, we carried out whole plastome sequencing of four *Plantago* species for the development of highly robust and reliable molecular markers able to distinguish the valuable *P. lanceolata* from the closely related species *P. argentea*, *P. atrata* and *P. maritima*, which are not used as herbal ingredients.

By comparing the newly sequenced plastome accessions with those present in public databases, we were able to identify chloroplast genes likely involved in the adaptation of plantains to their environment and deepen our general understanding of the timing and patterns of evolution of different European plantain species. Additionally, we identified hotspots of sequence divergence in the chloroplast genomes and developed a novel PCR-based marker that selectively discriminates *P. lanceolata* from any other *Plantago* species. Given the high sensitivity, ease of use and robustness of this new molecular marker, it is now possible to reliably identify *P. lanceolata* from similar *Plantago* species, thus setting a solid foundation in the future for routine identification of contaminations or adulterations of plantain herbal products.

**KEYWORDS:** herbal products, plantains, molecular markers
**SPECIES LIST:** *Plantago lanceolata*

# A new arsenic bioindicator from a model species of liverwort

MINGAI LI
CLAUDIO VAROTTO

Inorganic arsenic (As) is a recognized carcinogen, considered the most hazardous element at the global level due to its ubiquitous presence as an environmental pollutant of either natural or anthropic origin. Human exposure through intake of contaminated water or food (especially rice) is a major health concern worldwide. It has been estimated that between 94 and 220 million people worldwide are exposed to potentially toxic concentrations of As just from naturally contaminated ground water, without counting anthropic sources like mining, metallurgy, energy production, agriculture and various types of industries.

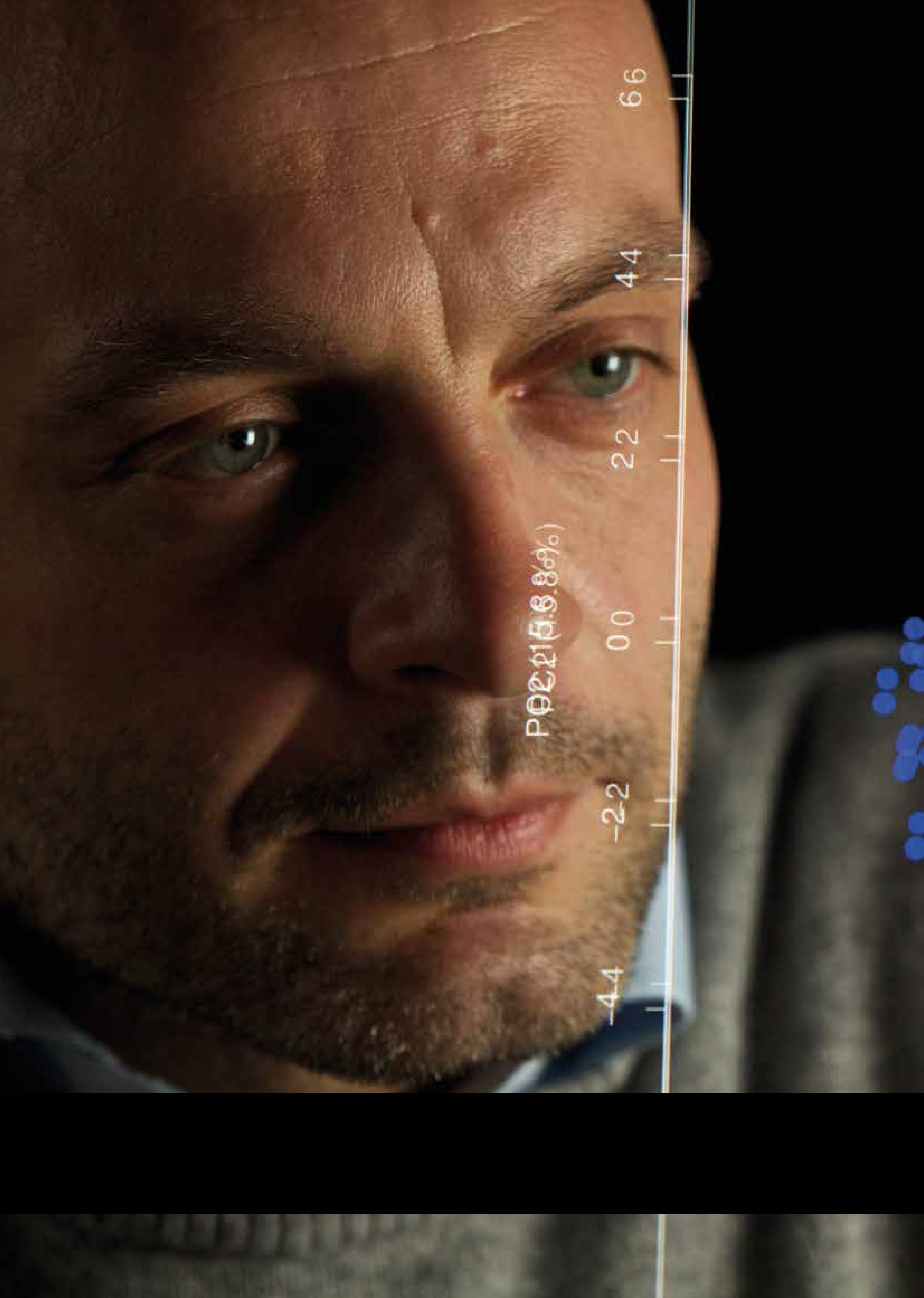
Plants can also suffer from As toxicity and, therefore, have evolved various systems of As detoxification. In gymnosperms and all earlier evolved lineages of embryophytes (ferns and horsetails, lycopods and bryophytes), as well as in fungi and some bacteria, one such detoxification system (interestingly not found in angiosperms) relies on the active extrusion of AsIII from the cell mediated by the arsenic compounds resistance 3 (ACR3) protein.

Among bryophytes, liverworts are a species-rich monophyletic clade with the majority of species having small genomes with low levels of genetic redundancy due to a low frequency of whole genome duplications during their evolutionary history. The most important model species among liverworts is by far *Marchantia polymorpha* L., owing to its short life cycle, easy propagation with both sexual and asexual systems, high quality genome assembly, and a complete set of functional genomics tools.

The arsenic-specific ACR3 transporter plays pivotal roles in As detoxification in yeast and a group of ancient vascular plants, the ferns. Despite putative *ACR3* genes being present in the genomes of bryophytes, it is currently unknown whether these genes have the same relevance in bryophytes as in yeast and ferns. We characterized the *MpACR3* gene from *M. polymorpha* through functional approaches. These analyses demonstrated the pivotal function of ACR3 in As detoxification in bryophytes. Moreover, we found that *Mpacr3* mutants generated by genome editing are extremely susceptible to As at levels that can be found in drinking water in some countries. Based on this observation, we propose that *Mpacr3* mutants could be used in developing countries as low-cost and low-technology visual bioindicators to detect As pollution in water.

*Marchantia polymorpha* plant with a gemma cup containing gemmae for asexual reproduction

**KEYWORDS:** arsenic, bryophytes, detoxification
**SPECIES LIST:** *Marchantia polymorpha*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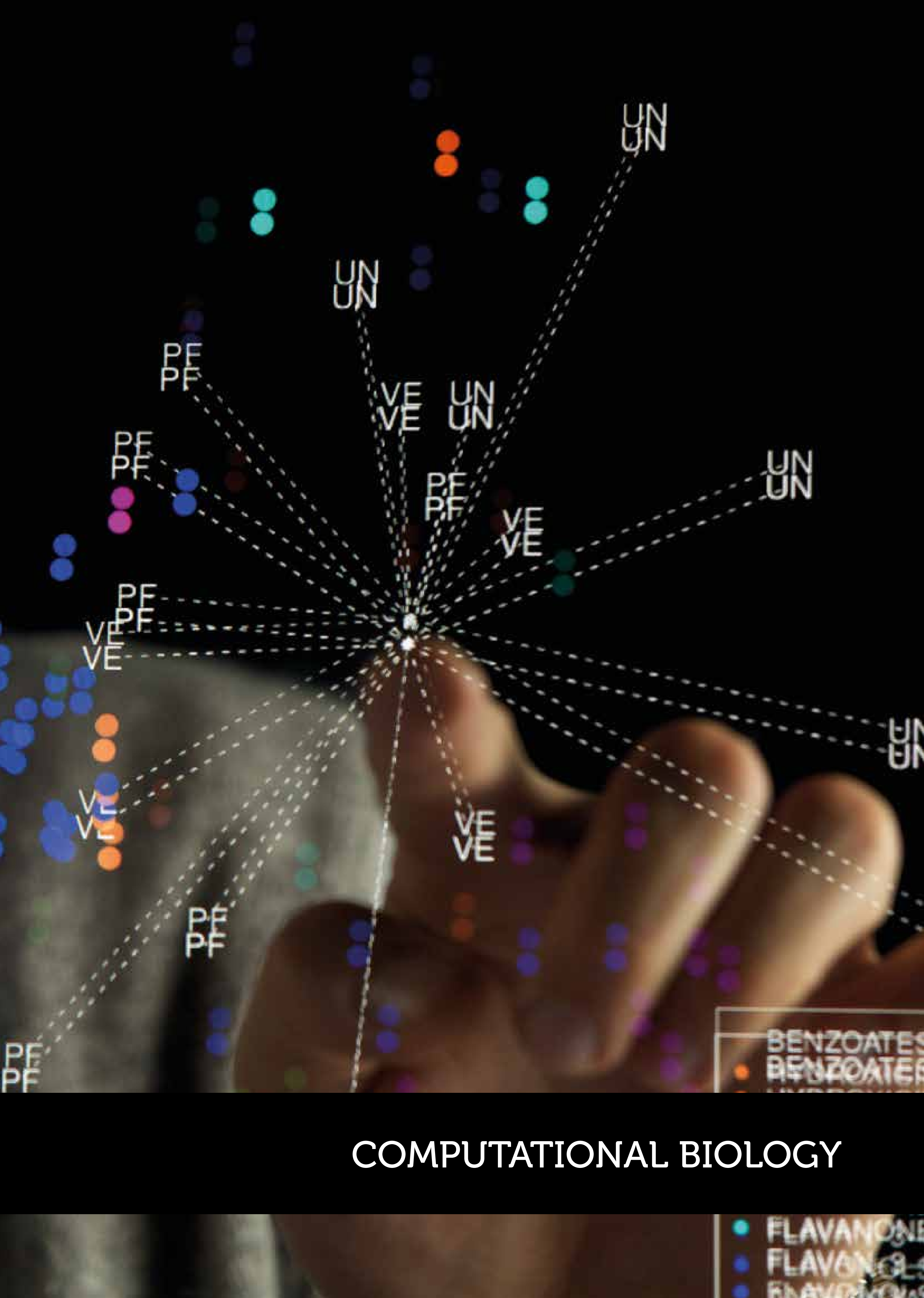


# COMPUTATIONAL BIOLOGY

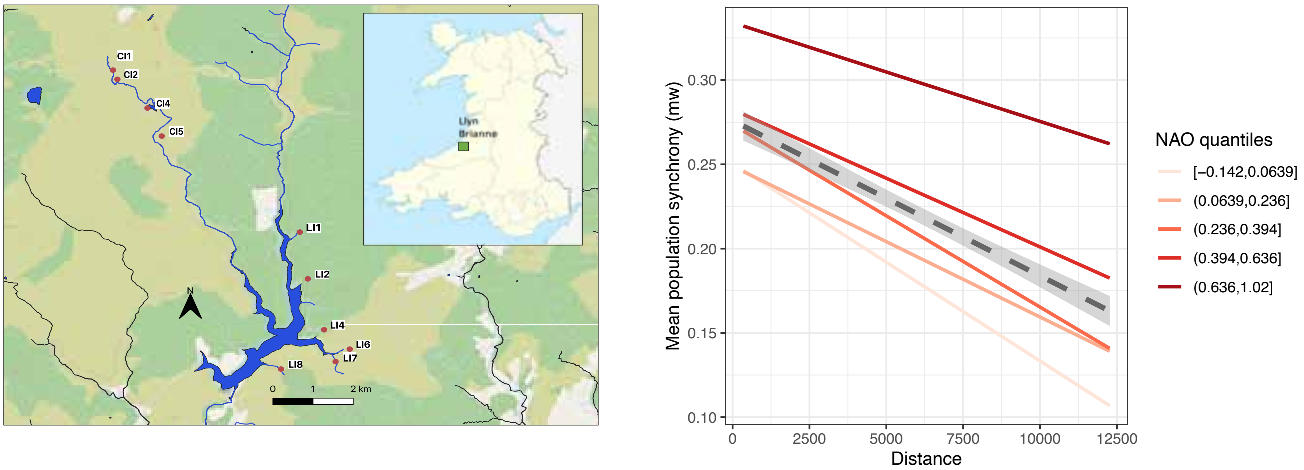
# Climatic effects on the stability of stream ecosystems

STEFANO LARSEN

Mayfly of the genus *Ecdyonurus*, typical dwellers of stony mountain streams

Predicting the effects of climate change on the dynamics of natural ecosystems is an imperative that presents significant challenges, mainly due to the scarcity of long-term data series for decadal-scale analysis. Historical data on the abundance of populations and communities in natural environments are still scarce. In this study, we leveraged a unique dataset where the abundance of aquatic insects has been continuously monitored for over 40 years in a dozen streams in central Wales (UK). We hypothesized that important clues about the effects of climate change on this biodiversity could emerge by understanding the influence of other large-scale atmospheric processes, such as the North Atlantic Oscillation (NAO). Similar to "El Niño", although operating on different timescales and through different mechanisms, the NAO has broad effects in the North Atlantic area, reflecting cyclical variations in atmospheric pressure between the Azores and Iceland, with a periodicity of about 6-8 years. In Western Europe, positive phases of the NAO lead to rainy winters with mild temperatures, while negative phases are associated with colder and drier winters. In addition to being crucial in ecosystem processes, the NAO serves as a useful indicator of general climatic conditions, and above all represents a valid analog of the ongoing climate change that favors mild and rainy winters and hot summers.

Our analyses show how prolonged warm and humid conditions during the positive phases of the NAO lead



Left panel: map of the study area (Wales, UK). Right panel: decay of mean population synchrony with geographic distance and as a function of the NAO values. Periods with large and positive NAO phases are associated with increased population synchrony, thus reducing overall community stability

to a synchronization of population dynamics among different rivers. In other words, the temporal variation in the abundance of different species becomes similar among separate streams, thus reducing regional ecological stability. Linear models using wavelet analysis (a statistical technique for analyzing time series data at different frequencies) have shown how climate patterns linked to the North Atlantic Oscillation explain up to 50% of the variation in species synchrony on time scales of 4-6 years. This synchronization occurred independently of the local characteristics of the different streams but was less marked where insect communities were more functionally diverse.

The study indicates that protecting and restoring functional diversity in riverine communities could improve ecological stability and resilience to warmer and wetter conditions, analogous to the ongoing climate changes.

Streams in central Wales (UK), photo by Steve Ormerod

**KEYWORDS:** climate change, ecological stability, aquatic insects

# ‘Golden Delicious’ haplotypes offer new perspectives for breeding

LUCA BIANCO
DIEGO MICHELETTI
PAOLO FONTANA

The ‘Golden Delicious’ apple is a true symbol of Trentino’s agriculture. Primarily cultivated in the Non and Sole valleys, this apple variety, with its characteristic golden yellow colour, has become a benchmark for the Region and the entire Italian fruit-growing landscape.

The importance of ‘Golden Delicious’ goes beyond its simple production and consumption. In recent years, scientific research has focused on this variety, sequencing its genome. This achievement was a fundamental step in understanding the genetic mechanisms that determine the qualitative characteristics of the apple, such as taste, colour, texture, and disease resistance. In 2010, Fondazione Edmund Mach led a consortium that resulted in the first sequencing of the Golden Delicious genome. In 2017, it participated in the efforts that produced an improved version of the genome of a haploid accession (GDDH13). Now, researchers at FEM have used the most innovative sequencing techniques to reach a new level: two copies of each chromosome (haplotypes) have been decoded, and the genetic variants present in each of them have been cata-

A ‘Golden Delicious’ apple

logued. This level of detail will allow for a more precise identification of the genes responsible for the characteristics that make the 'Golden Delicious' unique and desirable, to trace the origin of these characteristics more precisely in the variety's pedigree, and to analyze in greater depth the dynamic behaviour, in terms of gene expression, of the two haplotypes in response to ripening and biotic/abiotic stresses.

Knowledge of the 'Golden Delicious' genome offers numerous opportunities to improve the quality and productivity of apples throughout Trentino. For example, through marker-assisted selection techniques, it is possible to identify and select plants with desirable characteristics, such as greater disease resistance or a longer storage capacity. In addition, the genome sequences can be used to develop new apple varieties with innovative characteristics, such as a more intense flavour or greater resistance to climate change.

**KEYWORDS:** genome, haplotypes, 'Golden Delicious'
**SPECIES LIST:** *Malus x domestica*

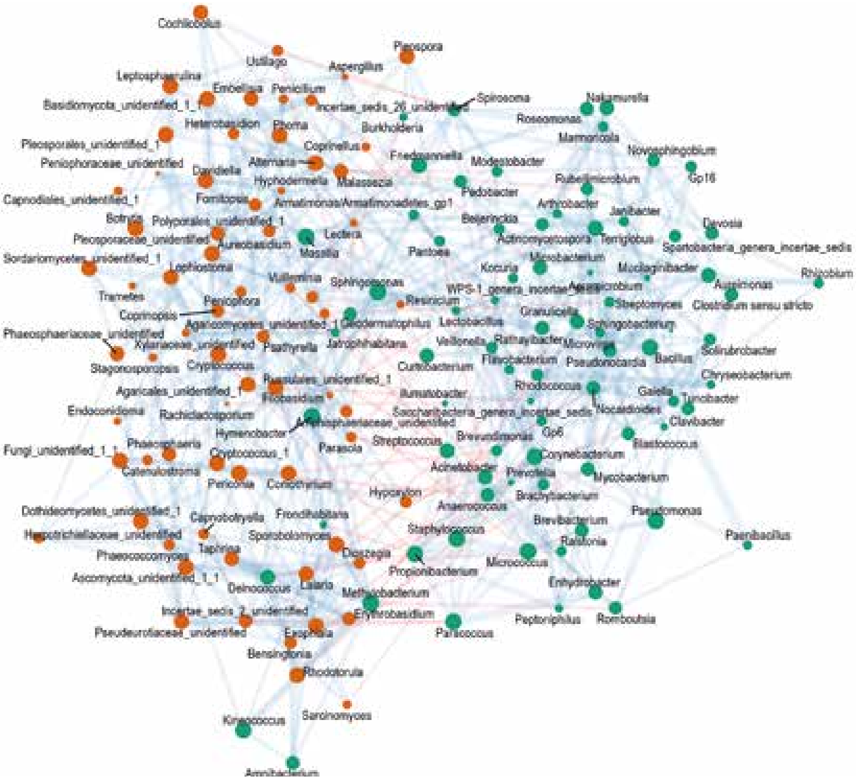
The FEM blueberry collection

# The microbiome of the FEM blueberry collection

CLAUDIO DONATI
MATTEO GIESE
BRIAN FARNETI

Plants are colonized by a host of microorganisms, such as bacteria and fungi, which form complex communities. These communities, known as microbiotas (with their associated genomes called microbiomes), play a crucial role in the health and development of plants. Each plant tissue creates a set of specific environmental conditions that drive the formation of a specific microbial community. The structure and composition of plant microbiotas are influenced by a variety of factors, including interaction with the host and environmental and climatic factors. Understanding how plants regulate the composition of the colonizing microbiota in the different compartments is the first step towards manipulating the microbiome to increase productivity and resilience to abiotic stresses such as drought and to pathogens.

We have begun a comprehensive characterization of the microbiomes that colonize the different tissues of blueberry plants in the FEM collection. In an initial study, we focused on the above-ground portion of the plants, including leaves, fruit surfaces, and fruit pulp. We found that the specific types of microorganisms living on a blueberry plant vary depending on several factors, including the plant's genetic makeup and the specific tissue being studied. Despite plant-to-plant variability, for each tissue, we were able to identify a subset of taxa that were widespread across different plants and formed a tissue-specific core microbiome. These core taxa accounted for the dominant fraction of each



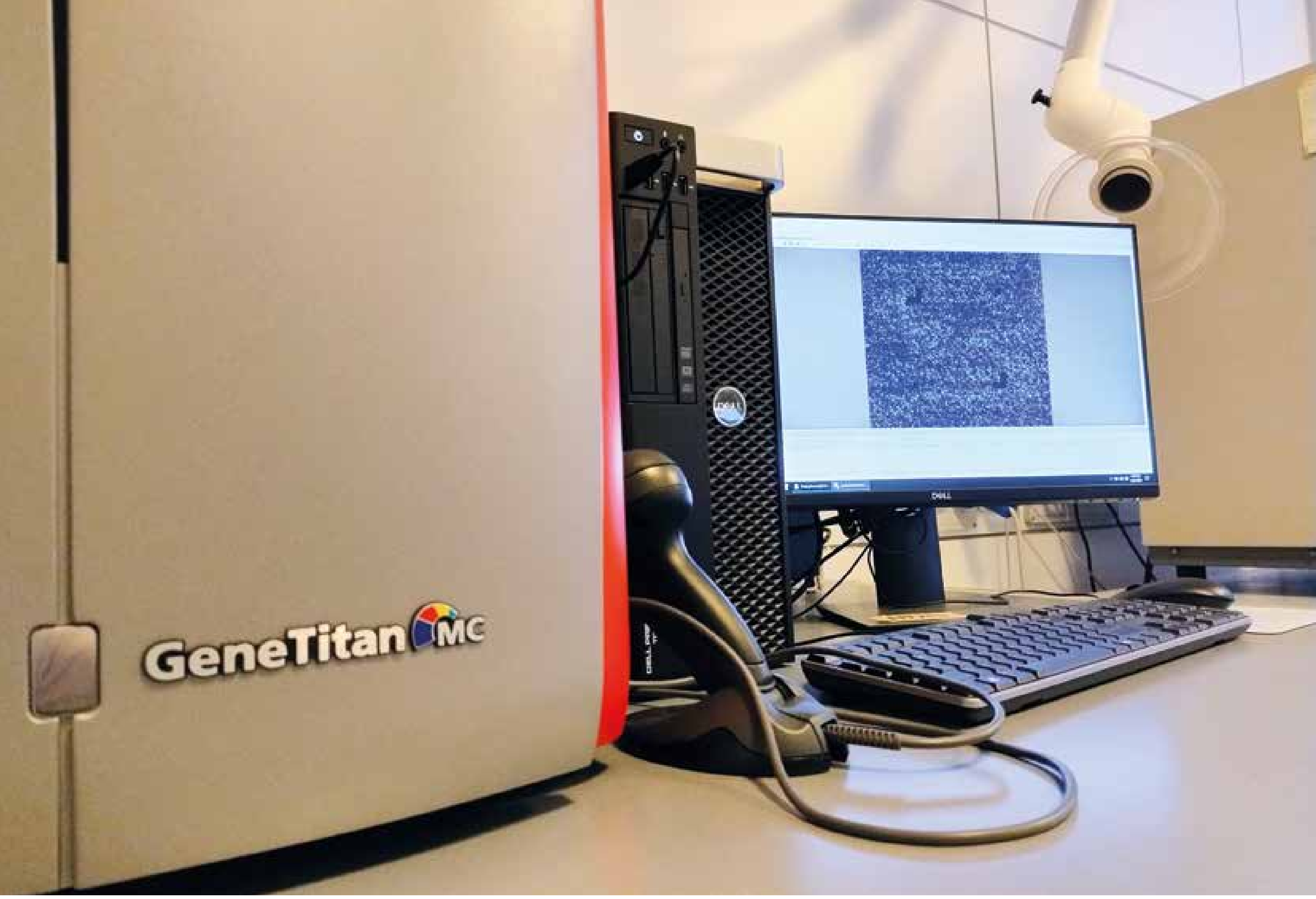
The microbiomes that colonize the leaves of the blueberry plants in the FEM collection

microbiome. Importantly, this core microbiome was conserved across two consecutive years of sampling, demonstrating stability over time. We also observed a significant overlap of core taxa between fruit and leaf surfaces, and, to a lesser extent, with the fruit pulp.

Several key findings emerged from this study. First, we observed a considerable diversity of microbes, with blueberries hosting a diverse array of microorganisms, and different parts of the plant supporting distinct microbial communities. Second, the genetic makeup of a blueberry plant significantly influences the composition of its microbiome, demonstrating that plant genetics matter in shaping these microbial communities. Third, in each analyzed tissue, we found a set of core taxa that accounted for the dominant fraction of the microbiota, highlighting the importance of these core taxa in the overall microbial community structure. Finally, while the microbiota can change over time, these core groups of microorganisms remain relatively stable, suggesting a consistent and important role for them within the plant.

Understanding the blueberry microbiota and microbiome can help us develop new strategies to improve crop yields and protect plants from diseases. By manipulating the microbial communities associated with blueberries, we may be able to enhance their resistance to pests and improve the quality of the fruit.

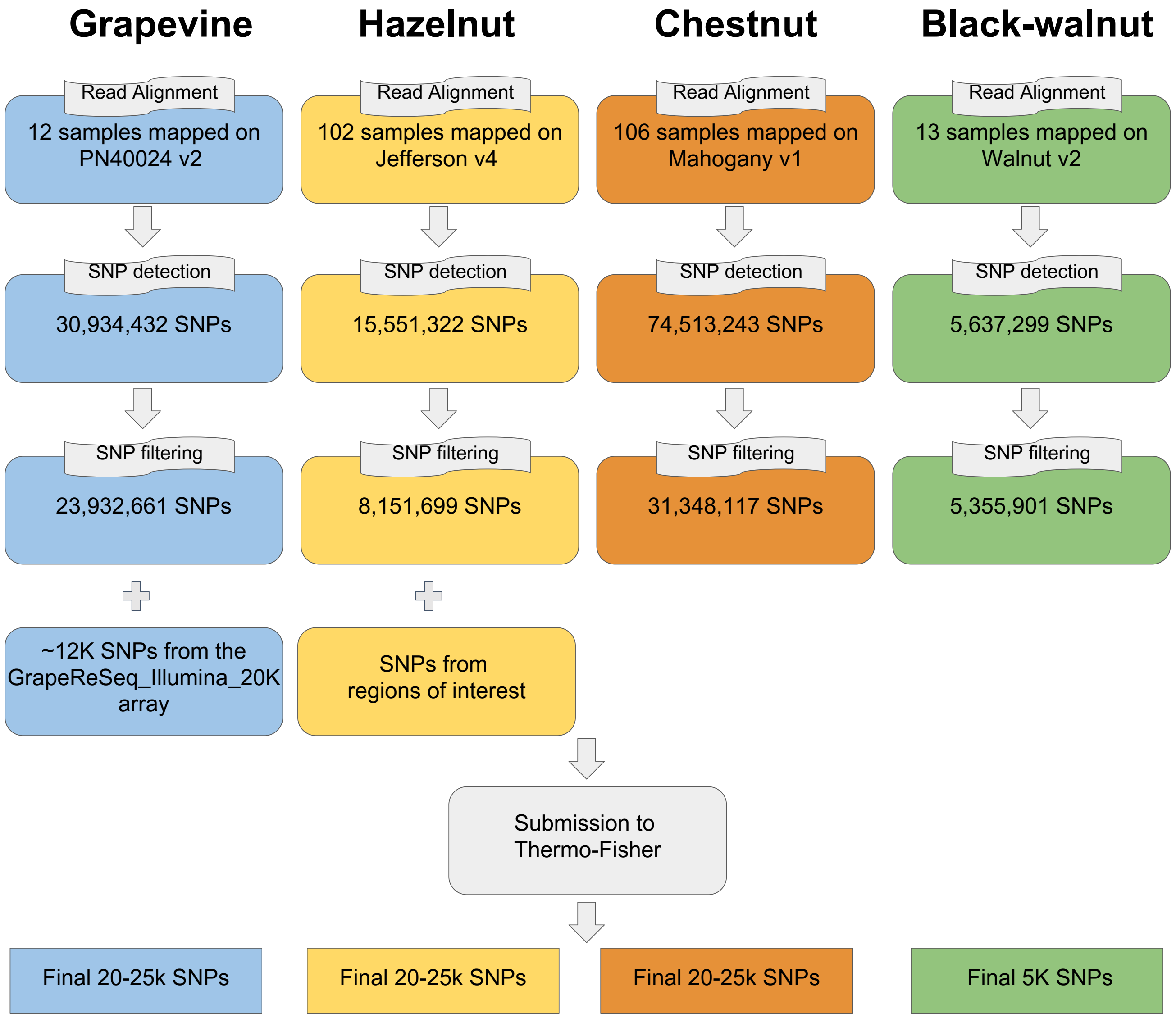
**KEYWORDS:** plant microbiome
**SPECIES LIST:** *Vaccinium* spp.



# High-throughput genotyping array for grape, black walnut, chestnut, and hazelnut

DIEGO MICHELETTI
MICHELA TROGGIO
LUCA BIANCO

Genotyping arrays are powerful tools that enable the simultaneous analysis of thousands of genetic variants across the genome. They are routinely used in many crop and fruit tree species for several applications, including genetic diversity studies and association mapping. More recently, they have gained popularity as tools for accelerating breeding. Here, we present a novel ThermoFisher Axiom high-throughput genotyping array designed for four economically important plant species: grapevine, black walnut, chestnut, and hazelnut. This multispecies array incorporates over 70,000 single nucleotide polymorphisms (SNPs) strategically selected to capture the genetic diversity within each species and facilitate high-resolution genotyping across all four genera. The development of this array involved whole-genome sequencing of 12 to 106 accessions per species, enabling the identification and selection of high-quality SNPs representative of the genetic variation within each species. A standardized bioinformatics pipeline was employed for SNP discovery and filtering. The array comprises between 20,000 and 25,000 SNPs for grape, hazelnut, and chestnut, and approximately 5,000 SNPs for black walnut. Manufactured on the ThermoFisher Axiom platform, this tool offers a cost-effective solution for high-throughput genotyping. This platform provides a powerful tool for advancing genetic research and breeding programs in these important fruit tree species. It enables genome-wide association



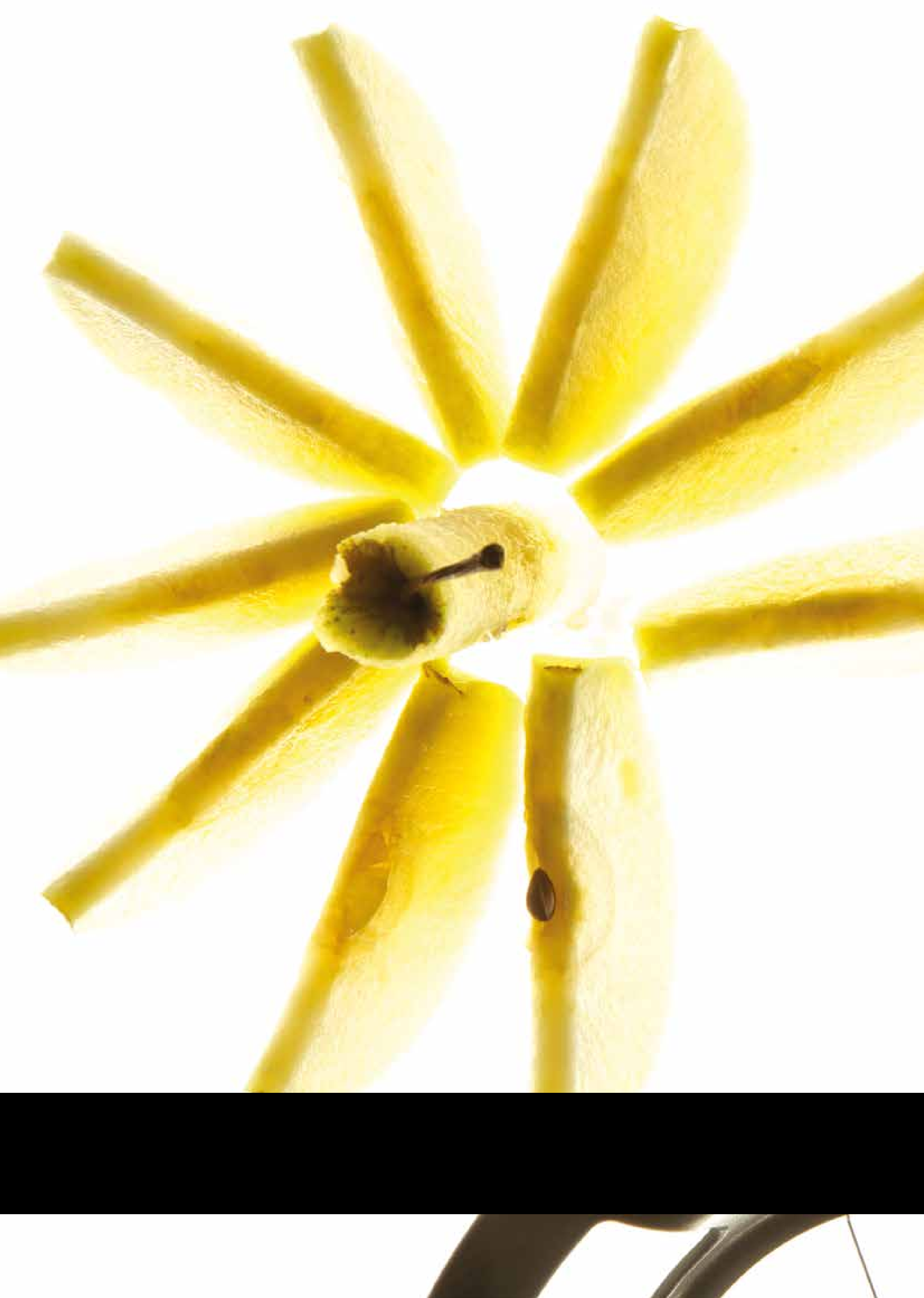
Flowchart demonstrating how SNPs were selected for the Thermo-Fisher array

studies (GWAS) to identify genetic loci associated with key agronomic traits and supports the construction of high-density genetic maps essential for marker-assisted selection (MAS) programs, which accelerate the development of improved cultivars with desirable traits.

The array was developed through a collaborative effort led by FEM, involving contributions from other institutions including the University of Bologna, Marche Polytechnic University, University of Turin, CNR, University of Missouri, The American Chestnut Foundation, Oregon State University, USDA, Rutgers University, and the University of California Davis. The array is exclusively available through the FEM genotyping platform.

**KEYWORDS:** SNPs genotyping, breeding

**SPECIES LIST:** *Vitis vinifera, Corylus avellana, Castanea sativa, Juglans nigra*



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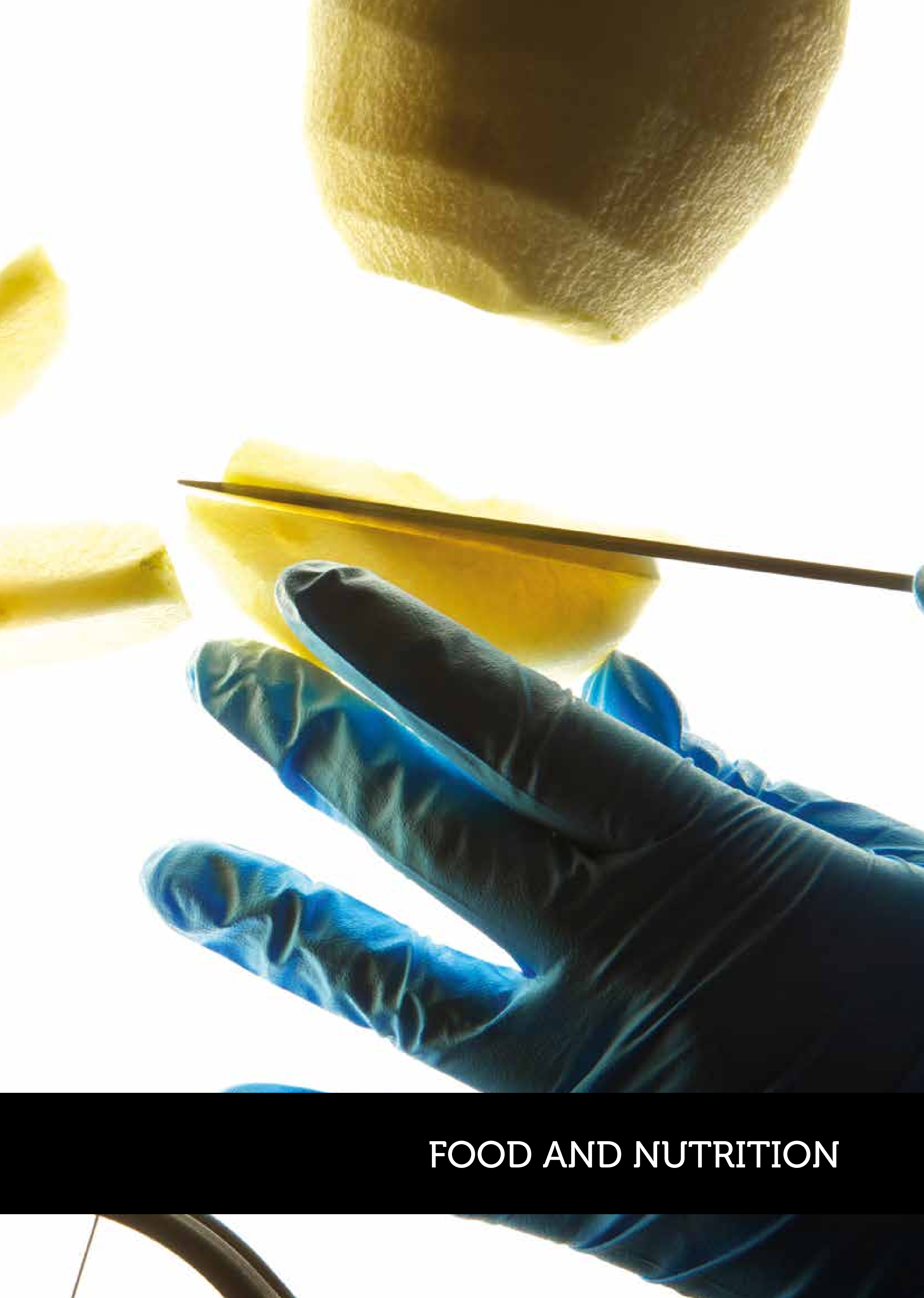


# FOOD AND NUTRITION

# Discovering Trentino's maize: how local varieties shape flour and polenta quality

MICHELE PEDROTTI

Different flint maize varieties

Maize is one of the most widely cultivated cereals in the world, with a vast range of varieties suited to specific culinary and industrial uses. Introduced from the Americas, vitreous maize (*Zea mays indurata*) was brought to Veneto shortly before the mid-1500s, becoming a fundamental staple of rural diets in northern Italy due to its usage in preparing 'polenta' (maize porridge). In Trentino, polenta symbolizes tradition, culture, and local identity.

In the Trentino region, several landraces of vitreous maize are cultivated, each with unique characteristics. Understanding these differences is crucial for preserving and enhancing cultural heritage and agrifood biodiversity. A study carried out at FEM explored how local landraces and post-harvest characteristics influence the quality and nutritional composition of maize flour and polenta. The investigation analyzed four indigenous varieties (three from Trentino and one from Veneto): 'Nostrano di Storo', 'Spin di Caldonazzo', 'Dorotea di Primiero', and 'Marano Vicentino', for a total of 28 samples obtained from local producers. The analyses were conducted using advanced methods at the Volatile Compounds Platform of FEM, focusing on physical properties, macronutritional composition, and aromatic profiles.

The analyses revealed significant differences in fatty acid profiles, which are also reflected in the aromatic profile, with variations depending on the variety and cooking processes. Cooking polenta induced significant changes, including the formation of new aromatic compounds and the reduction of others, mainly due to evaporation. Certain classes of aromatic compounds, crucial for the final quality, were more concentrated in the cooked product. It is noteworthy that both flour and pre-cooked polenta samples were found to have fewer aromatic compounds after industrial cooking, confirming the superior organoleptic quality of polenta prepared with raw flour.

The study highlights significant qualitative differences among vitreous maize varieties, emphasizing the importance of traditional Trentino varieties. Moreover, it demonstrated how cooking modifies both nutritional and qualitative characteristics, at least partially preserving the peculiarities of local varieties. These findings contribute to the enhancement and preservation of landrace maize varieties, promoting the diversity and uniqueness of traditional food products. This research represents an important step in deepening knowledge of a symbolic food of Trentino tradition while supporting the safeguarding of local agri-food heritage.

**KEYWORDS:** flint maize, polenta, volatilome
**SPECIES LIST:** *Zea mays indurata*

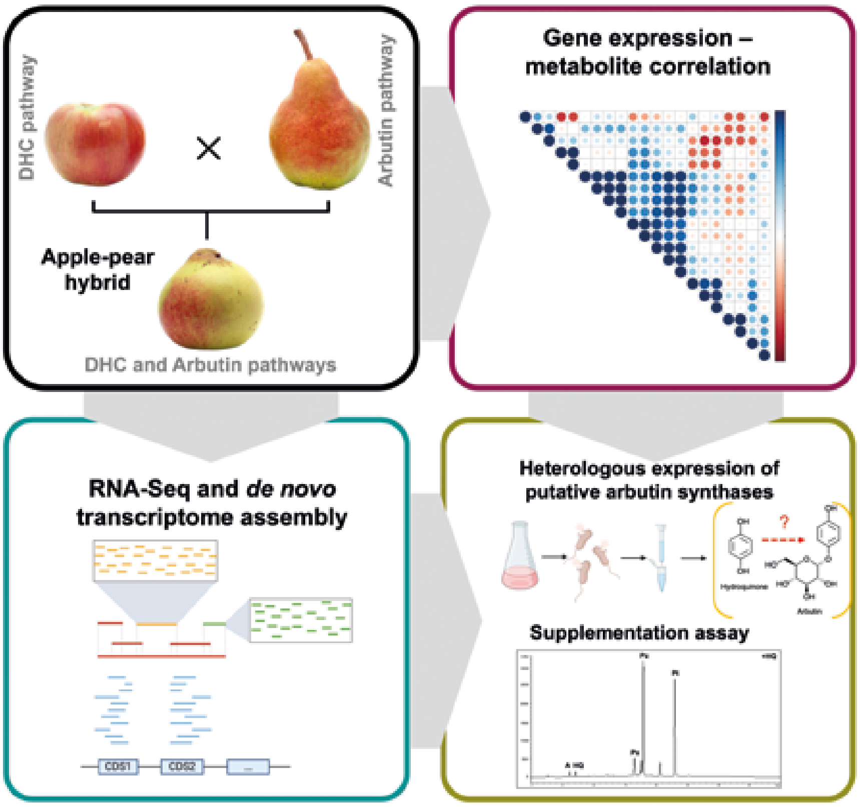
From left to right: apple, pear and apple-pear hybrid (photo of M. Malnoy)

# Dihydrochalcone and arbutin biosynthesis in an apple-pear hybrid

SIMON MIRANDA
MICKAEL MALNOY
STEFAN MARTENS

Apples (*Malus* × *domestica* Borkh.) and pears (*Pyrus communis* L.) are valuable crops with many beneficial properties for nutrition and human health. Despite their evident differences, both are closely related species within the Rosaceae family. Apples and pears accumulate distinctive natural compounds that are rarely found in nature, which have many beneficial properties and uses. Apple is characterized by accumulating phloridzin, a type of molecule from a class named dihydrochalcones. Meanwhile, arbutin is a natural compound characterizing pears. Dihydrochalcones exhibit anticancer, antioxidant and neuroprotective properties, and they were used in Asian traditional medicine as an antipyretic remedy. More recently, researchers have demonstrated that phloridzin could protect against type 2 diabetes by blocking glucose transport. Arbutin, in turn, has been used in traditional medicine as a natural antiseptic treatment for urinary infection. In addition, arbutin is used in the cosmetic industry as a skin lightener.

In plants, it is still unclear what benefits arbutin and dihydrochalcones confer to pears and apples, respectively. It has been proposed that they could be involved in plant defence against fire blight and apple scab infection caused by *Erwinia amylovora* and *Venturia inaequalis*, respectively. Phloridzin and arbutin, and their precursors phloretin and hydroquinone, could act as antioxidant molecules promoting a better response during infection with diseases. However, the genes responsible for producing



Genes were identified from a de novo transcriptome obtained for apple-pear hybrid, and characterized by expression and biochemical analyses

these specialized metabolites are only partially known.

Fondazione Edmund Mach has a unique germplasm collection, in which a hybrid obtained from crossing an apple female parent and a pear is conserved. We have previously demonstrated that this hybrid exhibits intermediate properties of both parents, from genetic background to metabolite profile. In our latest publication, we used this unique genetic resource at FEM to assemble a de novo transcriptome of the hybrid. This tool, combined with molecular analyses, allowed us to discover genes that could be involved in the biosynthesis of metabolites, which could serve as the basis for future confirmation of potential physiological roles. To this end, we are currently using genome editing at FEM to further study candidate genes.

The authors thank Marion Koop, Andrea Angeli, and Jorge Lagrèze for their contribution to this work.

**KEYWORDS:** apple, pear, hybrid, natural compounds
**SPECIES LIST:** *Malus x domestica, Pyrus communis*

# How to preserve the aroma of Gewürztraminer: the role of pH and temperature in aromatic wines

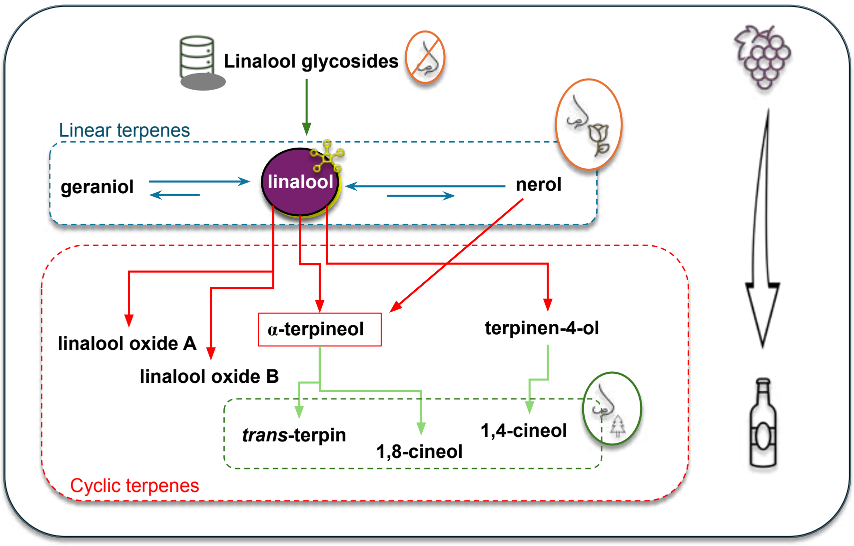
SILVIA CARLIN
PANAGIOTIS ARAPITSAS
FULVIO MATTIVI

Gewürztraminer grapes

This study focused on Gewürztraminer, also known as Traminer Aromatico, a wine of great significance for the Trentino-Alto Adige/Südtirol Region. An emblematic product of South Tyrol, Gewürztraminer is also widely produced in Trentino. This wine is renowned for its distinctive aroma, featuring notes of rose, lychee, and spices. Our research provides a solid scientific foundation showing the decisive influence of pH and storage temperature in shaping the wine's aromas.

More specifically, our study investigated the dynamics of volatile compounds in wine, analyzing how terpenoids - essential for Gewürztraminer's aromatic profile - evolve over time under varying temperature and pH conditions. Through a rigorous theoretical approach, the study details all interconversion processes, i.e., the simultaneous transformation of terpenes that significantly impacts sensory characteristics. From a theoretical perspective, the constructed model represents the most refined work conducted to date on terpene transformation.

In practice, the results of this research, conducted on a sample of wines provided by Fondazione Edmund Mach, highlight that higher pH levels and lower storage temperatures are essential for preserving terpenoids and thus maintaining Gewürztraminer's distinctive aroma for a longer time. These findings not only enhance the scientific understanding of wine aromas, but also provide practical guidelines for the



Terpenoids interconversion reactions

optimal preservation of one of the Region's iconic wines.

Currently, some types of Gewürztraminer on the market already exhibit pH levels above the standard range of 3–3.5. According to the experimental results, wines with pH values around 3.8 could achieve an extended shelf life of 8–10 years.

This work is the result of a collaboration among an interdisciplinary team of researchers from FEM, the University of Trento, and the University of West Attica, Greece. It was recently published in the Food Research International journal, marking a significant breakthrough in oenological research.

**KEYWORDS:** terpenoids, kinetics, wine
**SPECIES LIST:** *Vitis vinifera*

# Decision-making by European consumers about food expiry date labels and implications for household food waste

MICHELE PEDROTTI
ISABELLA ENDRIZZI

Recent estimates from Eurostat show that over 58 million tons of food waste are generated annually in the EU, equal to 131 kg per inhabitant and a market value loss of 132 billion euros. Notably, the consumption stage has been identified as the largest contributor to food waste, with 54% of waste occurring at the household level, translating to 70-79 kg per person per year. Consumers discard food for various reasons, including fresh produce no longer deemed optimal, leftover or unused food in the food service sector or household, plate waste, over-purchasing, improper storage, and misinterpretation or misuse of food expiry date labels. Among these factors, labels with expiration dates plays a significant role in influencing consumer behavior and decisions to discard food. This underscores the need to raise consumer awareness as a critical strategy to reduce food waste, as well as a deeper understanding of how consumer knowledge, attention, and behavior regarding expiry date labels vary across different segments of the population.

To explore this, we conducted a study to identify varying involvement patterns with expiry date labels and their impact on food waste decisions among EU consumers. The study surveyed 1507 consumers (54.6% female, mean age = 44.9 years) across six EU countries (Belgium, France, Germany, Italy, Spain, Sweden), ensuring representation across different age groups. Participants completed an online questionnaire assessing their knowledge, attention, and behavioral use of the different expiry date labels, as well as their reported domestic food waste behaviors, sociodemographic factors, and food management habits.

Results indicate that 75% of European consumers understand the difference between 'use by' and 'best before' dates, with over 80% accurately identifying their meanings. Increased knowledge is associated with reduced household food waste, although this factor alone is not enough to significantly lower waste levels. More importantly, improved behavioral use of expiry date labels emerged as a significant predictor of reduced food waste.

The study identifies two distinct consumer clusters: Information-driven who rely heavily on expiry date labels for food management, and Perception-driven who use sensory cues for decision-making. This partitioning offers valuable insights for designing targeted interventions to improve expiry date label usage and reduce household food waste.

Finally, this investigation highlights the importance of our everyday actions and reminds us that, as citizens, we can truly make a difference. Reducing household food waste is a crucial first step toward a more sustainable future, as discarding food also means wasting all the resources used to produce, transport, transform, store, and cook it!

The authors wish to thank Danny Cliceri (University of Trento) for his valuable contribution.

More than 50% of food waste occurs in the household

**KEYWORDS:** food waste, consumer, expiry date

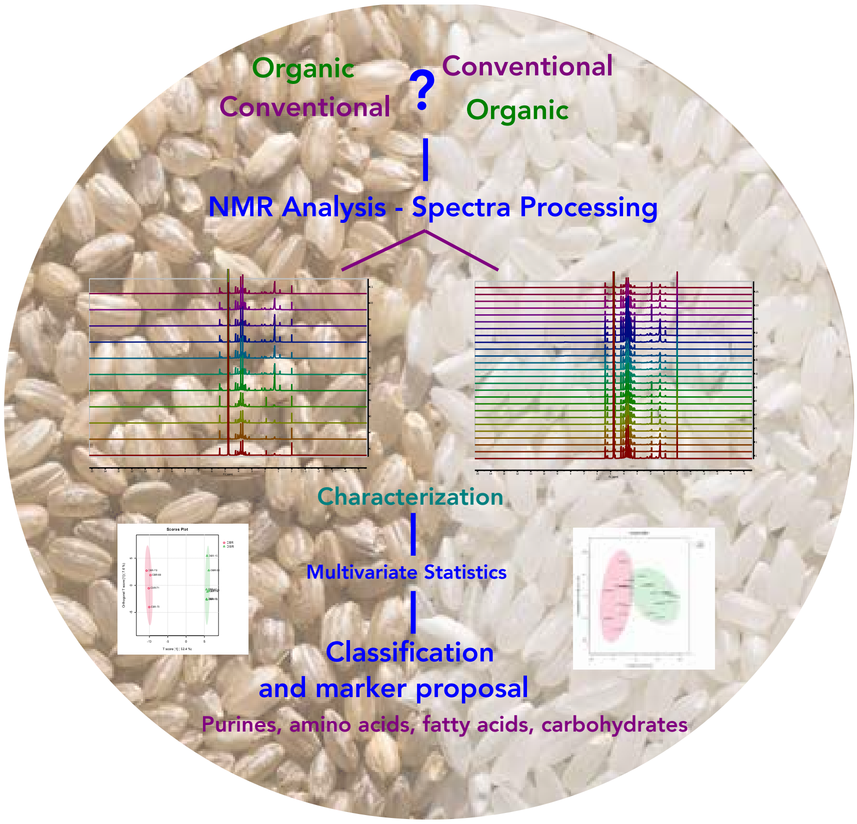
# NMR metabolomic fingerprinting for the differentiation of organic and conventional Italian brown and white rice

FEDERICO BRIGANTE
PAVEL SOLOVYEV
LUANA BONTEMPO

Rice is an important source of dietary nutrients, including carbohydrates, vitamins, and minerals, and shows differences in both nutritional and sensory properties depending on the milling process applied. In brown rice production, only the outermost layer of the grain is removed, making brown rice significantly richer in nutrients found in the bran (including essential fatty acids, fiber, thiamine, B vitamins, and important minerals) compared to white rice, which loses the bran and germ during polishing.

This study aimed to classify Italian brown and white rice based on cultivation systems (organic and conventional) through untargeted metabolomics via hydrogen nuclear magnetic resonance, and proposes a set of metabolites for validation and future authenticity testing.

A group of metabolites was selected as discriminant for both types of Italian rice using multivariate statistical methodologies. This group of metabolites included β-Glucose, α-Glucose, asparagine, and glutamate/glutamine. Asparagine and glutamate/glutamine showed higher relative intensity in organic rice samples, while β-Glucose was more intense in conventional rice samples. Interestingly, α-Glucose was the only metabolite that showed higher relative intensity in both organic brown rice and conventional white rice samples. This could be linked to factors such as the degree of milling,



Workflow of the research

the cultivation system, and intrinsic factors like the distribution of glucose anomers in each rice variety. Sucrose and α-Maltose were selected as discriminating molecules for brown rice, with higher intensity in conventional brown rice, while scyllo-inositol (a type of sugar) was selected for white rice, showing higher intensity in conventional white rice samples.

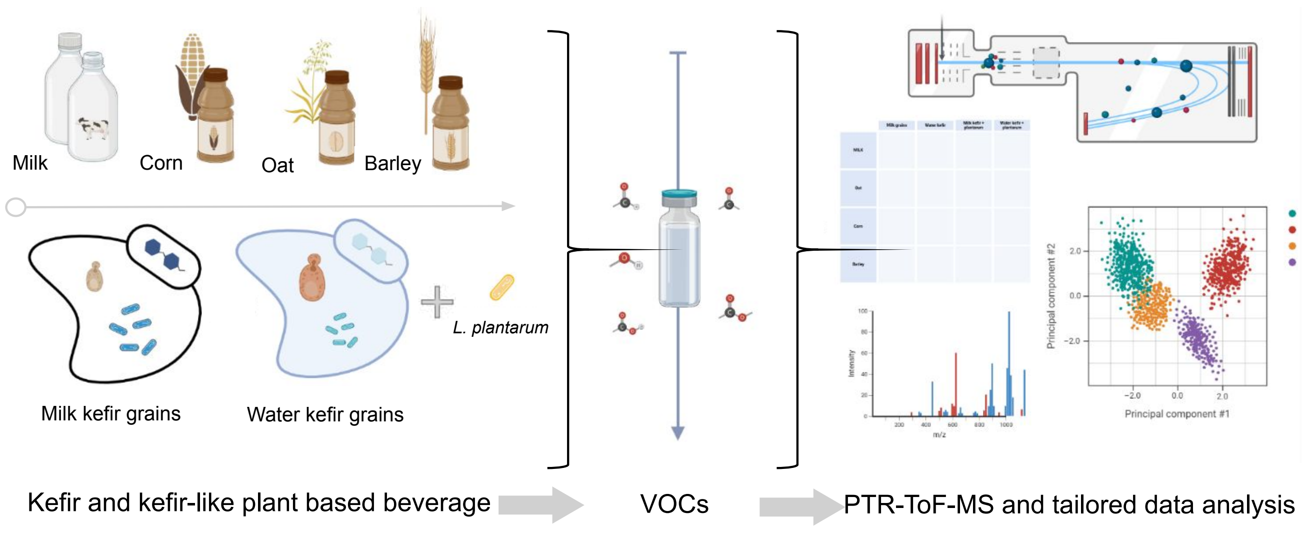
**KEYWORDS**: organic rice, metabolomics, cultivation systems
**SPECIES LIST**: *Oryza sativa*

PTR-ToF-MS in use: automatic analysis of volatile organic compounds in milk kefir and fermented cereal-based beverages

# Innovative cereal-based kefir: balancing nutritional quality and aroma profile

MARTINA MORETTON
IULIIA KHOMENKO
ANTONIA CORVINO

Functional beverages represent a rapidly expanding market among emerging food categories. Milk kefir, a traditional Caucasian fermented functional drink, is recognized for its health benefits. Traditionally, it is produced through fermentation with kefir grains, which are symbiotic communities of lactic acid bacteria, yeasts, and other microorganisms. Similar to milk kefir, water kefir, with a different microbial composition, is another traditional fermented beverage. Recent innovations aim to create kefir-like products with improved sensory, nutritional, and functional properties, promoting the use of plant-based ingredients and higher fiber content compared to traditional kefir. In this context, volatile organic compounds (VOCs) are valuable for monitoring bioprocesses, assessing matrix quality, and serve as sensory biomarkers. Direct injection mass spectrometry, particularly proton-transfer-reaction coupled with time-of-flight mass spectrometry (PTR-ToF-MS), enables direct, eco-friendly, real-time VOC analysis, making it ideal for fermentation monitoring. This study examined the volatile profile of novel fermented beverages using cereals, barley, corn, and oats, as fermentable substrates, comparing the results with traditional milk-based kefir. Each fermentation was conducted with or without the addition of *Lactoplantibacillus plantarum*, a microorganism selected for its ability to produce riboflavin. PTR-ToF-MS enabled continuous monitoring of volatilome variations throughout the fermentation process. The study allowed the determination



of the volatile profile associated with microbiotas, identifying compounds of technological and sensory interest, and the analysis of the interaction between the starter and the fermented matrix in the development of VOCs.

The focus of this work is of multifaceted relevance, spanning the formulation of novel functional products with high nutritional quality, sensory evaluation, fermentation monitoring, and the use of VOCs to analyze combinations of microorganisms and matrices. The integrated technological approach proposed in this study proves valuable for developing innovative fermented products designed to meet emerging consumer demands. Research supported by OnFoods and funded by the European Union Next-GenerationEU (PNRR-MISSION 4 COMPONENT 2, INVESTMENT 1.3–D.D. 1550 11/10/2022, PE00000003).

**KEYWORDS:** VOCs, fermented beverage, functional products

# Methyl salicylate (MeSA) in Italian white wines: a hidden aromatic potential

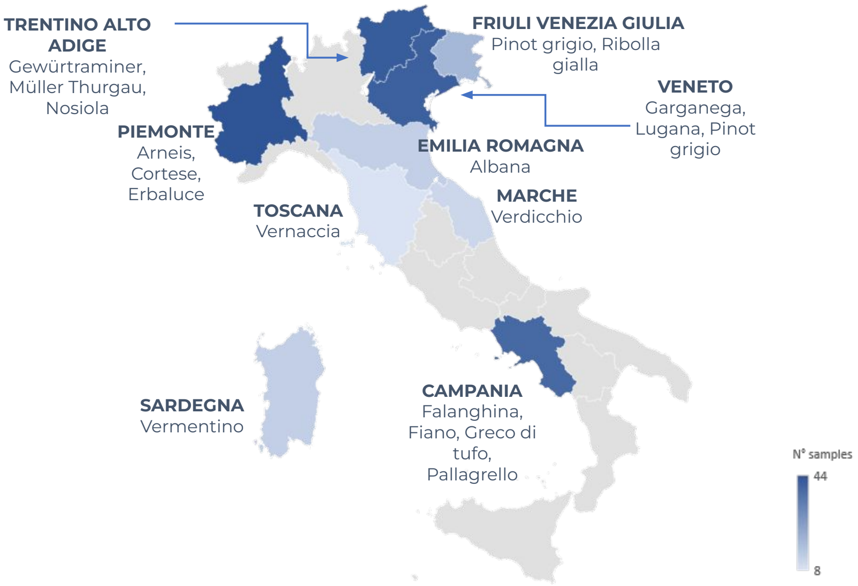
DOMENICO MASUERO
SILVIA CARLIN
FULVIO MATTIVI

Methyl salicylate (MeSA) in its free form is an aromatic compound that contributes to the distinctiveness of certain wines, often adding balsamic notes that are also associated with sweet fruity nuances. While this molecule is found in high concentrations in plants like wintergreen (genus *Gaultheria*), as well as in the Genera *Camellia*, *Polygala*, *Filipendula*, and *Passiflora*, where it imparts a sweet, balsamic aroma. In wine MeSA is primarily present bound to one or more carbohydrates, forming various glycosylated forms.

This study aimed to comprehensively evaluate the MeSA content in some of the most representative Italian white wine varieties. Both free MeSA, analyzed using GC-MS/MS, and glycosylated MeSA, measured via UHPLC-MS/MS, including monoglycosides, diglycosides, and triglycosides, were studied in 246 single-varietal wine samples produced from 18 grape varieties across nine Italian regions.

The results confirmed that MeSA glycosides are present at significant concentrations in Verdicchio and Lugana wines and were quantified for the first time in all other varieties. Notably, Erbaluce was also found to have relatively high levels of glycosylated MeSA. The most abundant forms were the monoglucoside (MeSA–glucoside) and the diglycosides (MeSA–gentiobioside and MeSA–violutoside), while the triglycosylated form was absent in all samples.

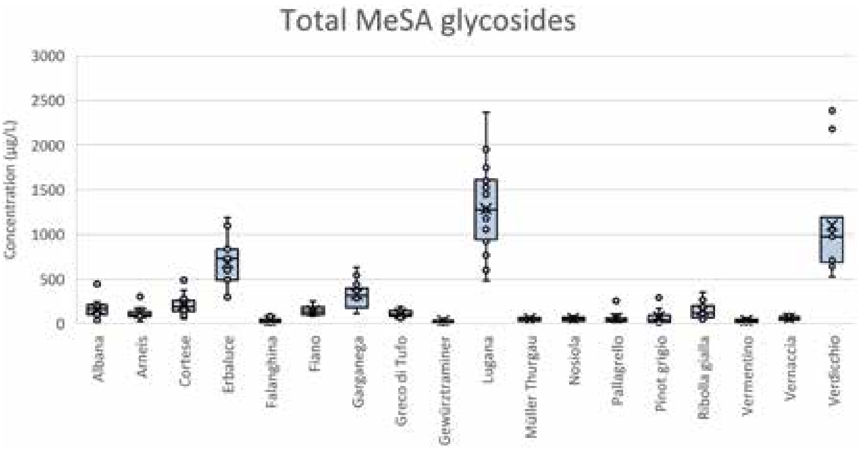
In addition to varietal screening, the study also assessed the evolution of MeSA during wine aging, subjecting a subset of samples from each variety to an accelerated aging protocol. The glycosylated forms were positively associated with an increase in free MeSA content during aging. According to the results, 'Lugana', 'Verdic-



Map of Italy showing the regional distribution of sampled varieties

chio', and 'Erbaluce' are the varieties that release the highest amounts of free MeSA during aging, with the effect being enhanced by lower pH levels. This release of hidden potential could further enrich the aromatic bouquet of the wine, offering greater consumer appeal and added value to the bottle.

This research was conducted by Fondazione Edmund Mach in collaboration with six Italian universities (Bologna, Naples, Padua, Turin, Trento, and Verona).



Boxplot of total MeSA glycoside concentrations in the cultivars tested

**KEYWORDS:** methyl salicylate, aroma, glycosides
**SPECIES LIST:** *Vitis vinifera*

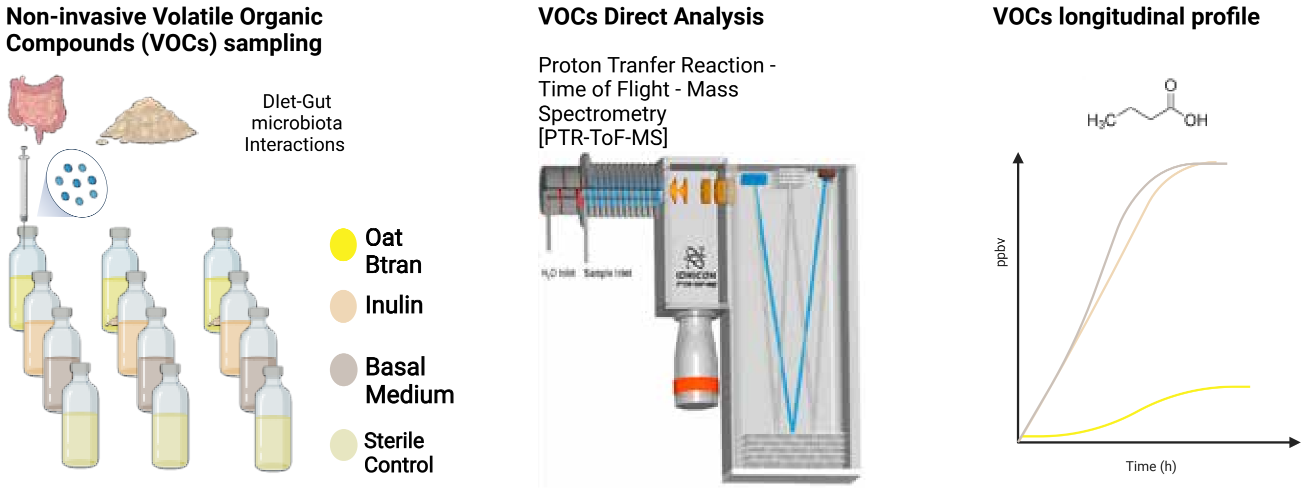
SPME-GC-MS analysis of an intestinal sample enriched with oat bran. The SPME needle (red head) extracts volatile compounds from the headspace of the glass vial, where the volatile metabolites produced by the sample accumulate

# Non-invasive VOC detection to monitor gut microbiota metabolism *in-vitro*

ANDREA DELL'OLIO
IULIIA KHOMENKO
EMANUELA BETTA

This study implemented an innovative and non-invasive approach to monitor volatile organic compounds (VOC) during the *in vitro* fermentation of dietary fibers with human gut microbiota. Two substrates were analyzed: inulin, a well-known soluble prebiotic, and oat bran, a matrix rich in insoluble fibers with prebiotic potential. The substrates were incubated in a minimal gut medium (a culture medium that simulates the human intestinal environment) with a stabilized fecal inoculum (a preparation of feces used to introduce the microbiota). The techniques used, HS-SPME-GC-MS (Headspace-Solid Phase Microextraction coupled with Gas Chromatography-Mass Spectrometry) and PTR-TOF-MS (Proton Transfer Reaction-Time of Flight Mass Spectrometry), allowed detailed and continuous monitoring of the temporal evolution of VOCs without interfering with the biological processes involved. By applying the RM-ASCA (Repeated Measures-ANOVA Simultaneous Component Analysis) statistical method, it was possible to clearly discriminate the metabolic profiles generated by the studied substrates.

The results obtained showed a significant increase in short-chain (SCFA, Short-Chain Fatty Acids) and medium-chain fatty acids (MCFA, Medium-Chain Fatty Acids) during oat bran fermentation, confirming its prebiotic potential. For inulin, a different kinetic pattern was observed, characterized by a slower but more continuous production of specific VOCs such as

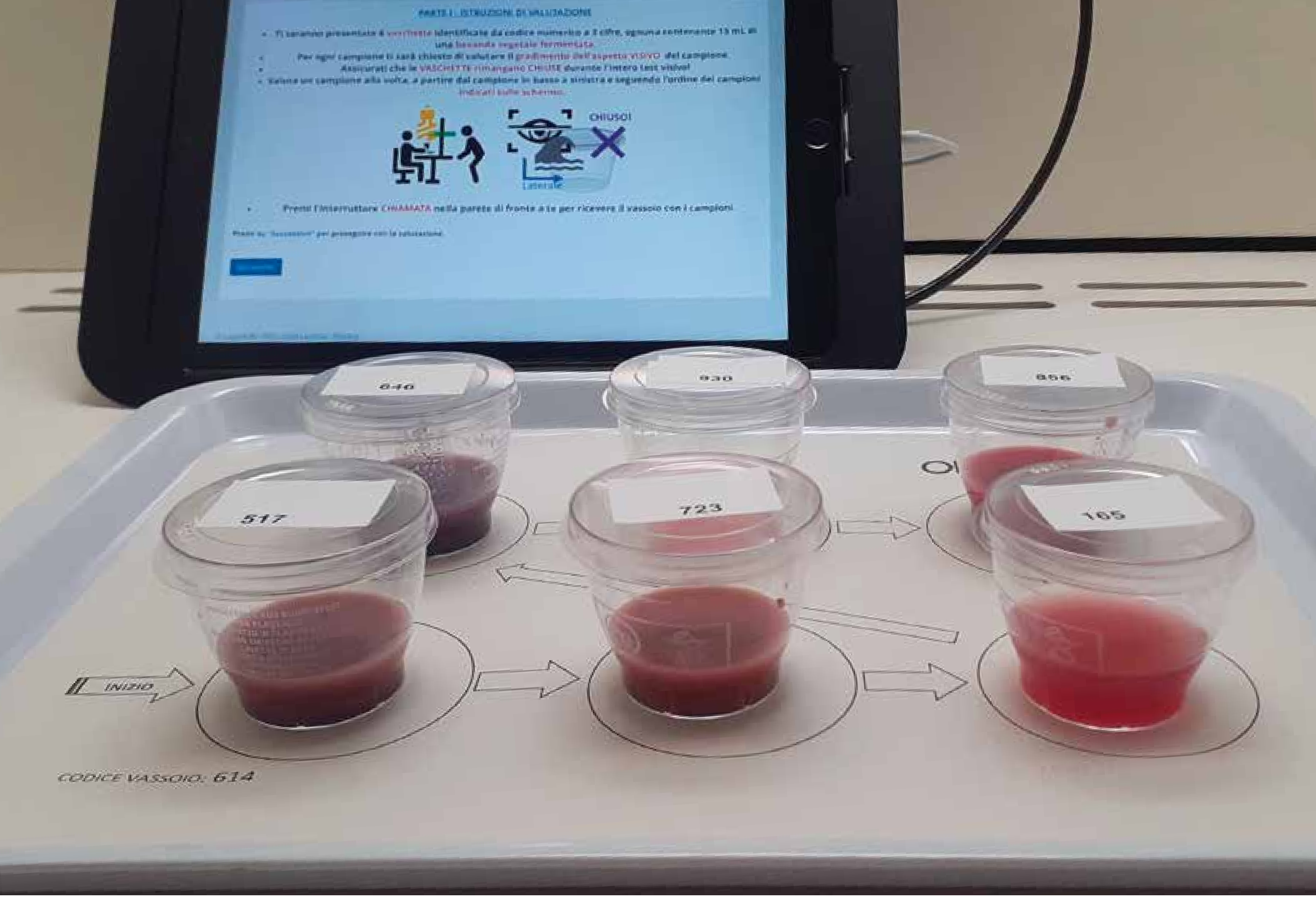


Left: Non-invasive sampling of volatile organic compounds (VOCs) in an in vitro colonic model. Center: Schematic of thePTR-TOF-MS system, used for continuous and highly sensitive monitoring of VOCs. Right: Longitudinal tracking of VOCs with an example of the temporal production profile of propionic acid, expressed in parts per billion by volume (ppbv) as a function of incubation time

alcohols and aldehydes. The most distinctive volatilomic profile was recorded after 12 hours of fermentation, suggesting a critical time point for evaluating interactions between microbiota and substrates.

This study demonstrates how the temporal analysis of VOCs can offer new perspectives in studying interactions between gut microbiota and dietary fibers. The approach used can be applied not only to better understand metabolic dynamics but also to effectively evaluate new prebiotics and investigate, more generally, the interaction between food matrices and gut microbiota. These results represent a significant step forward toward a more detailed and applicable understanding of microbiota metabolism, opening new possibilities for research and development in the field of functional foods and personalized nutrition.

**KEYWORDS:** gut microbiota, prebiotics, VOCs
**SPECIES LIST:** *Homo sapiens*

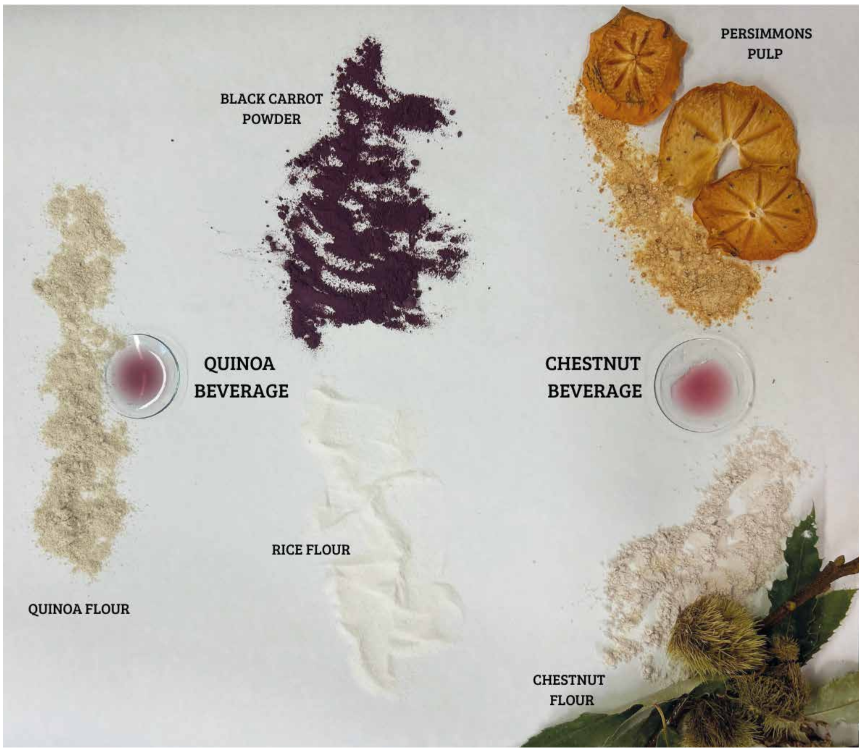


Sensory analysis of fermented beverages by flash-profile method

# Development of a novel fermented beverage from chestnut and quinoa, as a source of fibers and polyphenols

GIORGIA SECCHI
ANDREA MANCINI
ELENA FRANCIOSI

The increasing focus on healthy and sustainable nutrition has stimulated the development of products that combine taste, health, and innovation. This study focused on fermented beverages, known for their probiotic benefits and ability to improve nutrient bioavailability, using chestnuts and quinoa as key ingredients. Chestnut flour, derived from the fruit of the chestnut tree, a plant widely found in Trentino, is a valuable source of complex carbohydrates, fibers, minerals, and antioxidant polyphenols. Quinoa flour, obtained from quinoa seeds, is highly regarded for its exceptional nutritional profile, rich in high-quality plant proteins, fibers, and bioactive flavonoids. The beverage formulation includes rice flour (2%) as a base, dehydrated persimmon pulp (4%) for fiber and natural sugars, and black carrot powder (1%) to increase polyphenol content and enhance the color. The beverages were fermented using a commercial *Lactobacillus casei* strain (SACCO) through a controlled process to enhance their organoleptic and nutritional qualities. Fermentation enriched the beverages with probiotics, improving the digestibility of complex nutrients, and reducing potential anti-nutrients. To assess the quality of the product at different times of fermentation (0 h, 24 h, 48 h, and 7 days) different analyses were conducted. Total polyphenols were quantified using the Folin-Ciocalteu spectrometric method; the microbial community was evaluated through plate counts, and chemical composition was studied using nuclear magnetic resonance (NMR) spectroscopy (analysis carried out by F. I. Brigante). Volatile compounds were also investigated (analysis carried out by M. Moretton). Finally, pilot acceptability and sensory profile were assessed using the flash-profile method (evaluation carried out by B. D. Galli). The results showed that the fermentation process enhances sensory appreciation and promotes the development of descriptors of interest. Thanks to their nutritional profile and health benefits, fermented beverages based on chestnut and quinoa flour represent an interesting novelty in the field of functional foods. This study serves as a promising starting point for future developments, with the potential to improve formulations and further study ingredients to optimize the product's organoleptic, nutritional, and consumer acceptability properties. Research supported by ONFoods and received funding from the European Union Next-GenerationEU (PNRR-MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3–D.D. 1550 11/10/2022, PE00000003).



**KEYWORDS:** fermented beverage, chestnut, quinoa
**SPECIES LIST:** *Lactobacillus casei, Castanea sativa, Chenopodium quinoa*

# Semi-automated lipidome profiling of grapes by high-resolution mass spectrometry

MAR GARCIA-ALOY
DOMENICO MASUERO
PIETRO FRANCESCHI

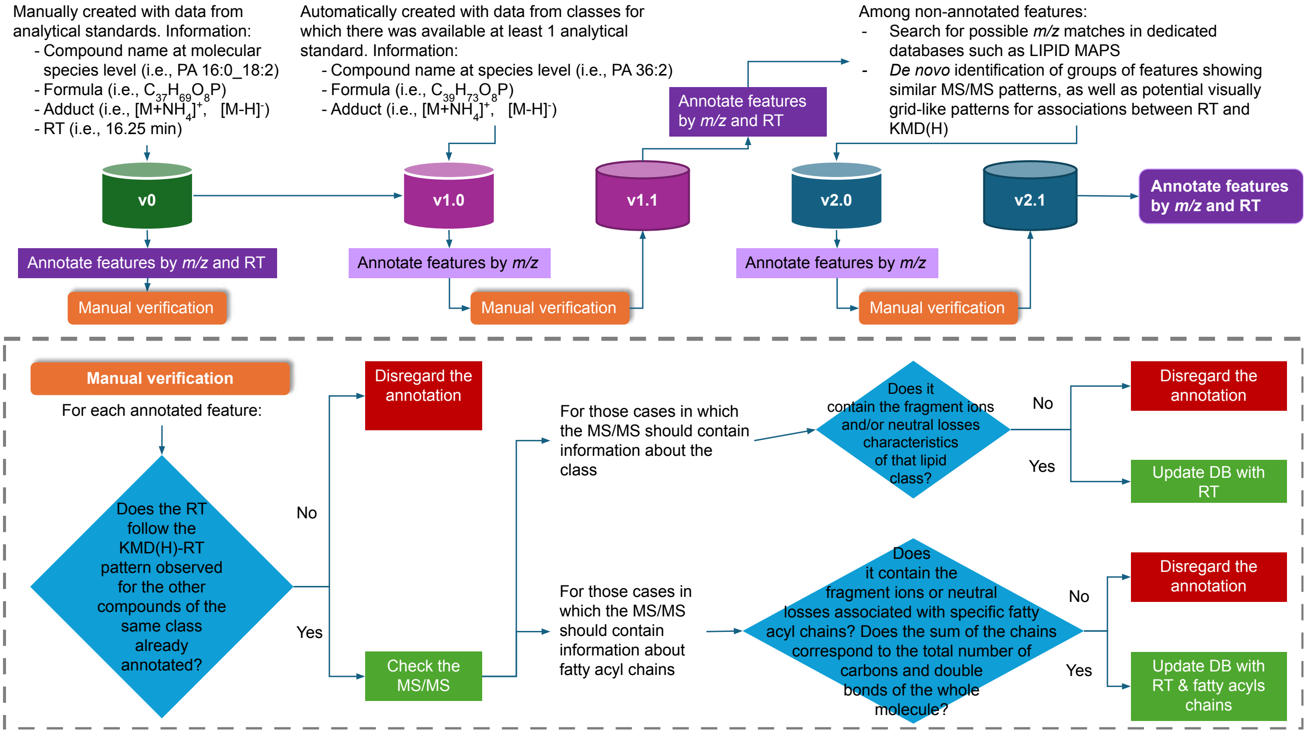
HPLC-ORBITRAP XL (High-Resolution Mass Spectrometer): High-performance liquid chromatography (HPLC) system coupled with a high-resolution Orbitrap mass spectrometer, designed for untargeted analysis of primary and secondary metabolites in biological samples (human and animal) and plant matrices

Lipids are important structural components of plant cell membranes and play a fundamental role in a wide range of essential biological functions, such as growth, development, and stress responses. In grapes, they also serve as a crucial nutritional substrate for yeast metabolism during winemaking, significantly impacting the sensory properties of wine.

The aim of this study was to develop a methodology based on high-resolution mass spectrometry for the fingerprinting of the lipid component. Specifically, a pipeline was designed to enable a semi-quantitative analysis of the lipidome through a semi-automatic and accurate identification process of the detected lipid species, integrating diverse and complementary chemical properties. This approach involves creating an interactive database populated with both theoretical information and experimentally observed data, following a workflow that allows a more comprehensive characterization of the lipidome, applicable to different biological matrices.

The developed methodology was applied for the characterization of the grape lipidome, providing a more complete insight into the lipid composition of grapes. The grape lipidome proved to be complex, containing a wide range of lipid species belonging to several different lipid classes. Most of the lipids annotated in grape samples were (lyso)glycerophospholipids and glycerolipids, although free fatty acids, hydroxyceramides, and sitosterol esters were also identified.

One of the main contributions pro-



Workflow for the generation of the database that is later used to perform compound annotation (adapted from the original publication)

vided by our research was the ability to bring novel knowledge on the previously undiscovered diversity of lipids since, unlike previous lipidomics profiling studies, going far beyond the description of compounds belonging to the "classical" lipid classes. In particular, the proposed pipeline enabled the identification of a series of methylated glycerophosphates not previously observed in grapes. Another non-typical lipid class found in the analyzed samples consisted of acylated monogalactosyldiacylglycerols. Therefore, this approach is expected to enhance the coverage of lipid classes, facilitating a better understanding of grape biology.

In summary, this study presents a strategy for in-depth lipidomic profiling of grapes, which can be transferred and adapted to generate the reference lipidome for grapes as well as for other species.

**KEYWORDS:** lipidomics, grape, mass spectrometry
**SPECIES LIST:** *Vitis vinifera*

Lavender essential oil

# Sniffing out the truth: unveiling lavender essential oil authenticity with GC-MS/MS and stable isotopes analysis

ALBERTO RONCONE
MAURO PAOLINI
LUANA BONTEMPO

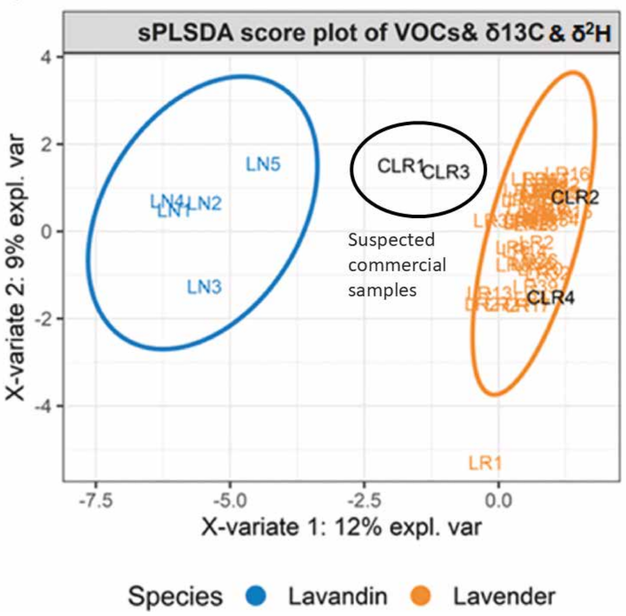
Two recent studies coordinated by the Traceability unit have developed a combined analytical approach to characterize and differentiate lavender essential oil from lavandin oil (a non-edible hybrid), and to detect adulteration with synthetic compounds.

Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) and gas chromatography coupled with isotope ratio mass spectrometry (GC-IRMS) were used to analyze volatile organic compounds (VOCs) and their isotopic ratios ($\delta^{13}C$ and $\delta^{2}H$). The developed GC-MS/MS method allowed for the quantification of 71 VOCs in both essential oils, achieving a 60% reduction in analysis time compared to conventional methods. The GC-IRMS analysis provided isotopic ratios for 32 VOCs for carbon and 30 for hydrogen.

Lavender essential oil is primarily composed of monoterpenes and their derivatives, such as linalool and linalyl acetate, which contribute to its characteristic sweet, floral aroma. In contrast, lavandula oil contains higher concentrations of eucalyptol and camphor, resulting in a more pungent scent.

Fraudulent practices may involve not only substituting the high-value lavender oil with a lower-value oil (such as lavandula oil) but also adulterating a low-value oil by adding synthetic compounds. This process typically involves using a neutral base oil to which synthetic compounds mimicking the characteristic components of lavender oil are added. In such instances, relying solely on the qualitative and quantitative analysis of VOCs may not be sufficient to distinguish the adulterated product from authentic lavender oil. Isotopic analysis provides a crucial solution: the isotopic ratios of synthetic VOCs differ significantly from those of naturally derived VOCs, enabling the identification of fraudulent additions through isotopic measurements. In this study, artificially adulterated samples were prepared, and the method successfully identified all samples containing more than 15% synthetic VOCs. Furthermore, the analyses revealed that some commercial oils labeled as "lavender essential oil" were, in fact, blends containing lavandin oil and synthetic compounds.

These studies demonstrate that the combined use of conventional mass spectrometry and isotope ratio mass spectrometry represents a significant advancement in the authentication of essential oils. The developed techniques enable differentiation between lavender and lavandin oils, as well as the detection of adulteration with synthetic compounds. This analytical approach can also be applied to other essential oils and natural products to ensure their authenticity.



sPLSDA score plot of VOCs and stable isotope data

**KEYWORDS:** lavender essential oil, IRMS, VOCs

**SPECIES LIST:** *Lavandula angustifolia, Lavandula intermedia*

# Towards healthier eating habits in adolescents

LARA FONTANA
LEONARDO MENGHI
ISABELLA ENDRIZZI

During the 2023-24 school year, students at the FEM high school participated in a comprehensive study investigating food perception, preferences, and dietary behaviors among adolescents, as well as the biological (e.g., genetics, oral microbiota), non-biological (e.g., socio-cultural environment), and psychological (e.g., emotions, beliefs) factors influencing these choices. Understanding these influences is crucial for developing effective strategies to promote healthier eating habits in this age group. This research aims to identify key factors influencing adolescent food choices and evaluate the effectiveness of interventions designed to promote healthier dietary behaviors.

This activity is part of two research projects funded by the European Union and promoted by Fondazione Edmund Mach and the University of Trento: the PRIMA PROmedLIFE project, focused on developing innovative Mediterranean foods to promote health, and Spoke 2 of the iNEST ecosystem (PNRR), dedicated to nutrition and health.

The experimental activities involved students in sensory evaluations of innovative snacks made with Mediterranean ingredients (developed by PROmedLIFE partners) and two model foods (grapefruit juice and chocolate pudding) designed to elicit four increasing levels of sweetness.

Validated questionnaires assessed psychological traits related to food choices, and biological samples (saliva and tongue swabs) were collected for metagenomic (microbial community analysis) and genetic analyses (genetic variations associated with taste perception) to investigate the influence of the oral microbiome on individual taste and flavor perception and its subsequent impact on adolescent food choices.

A four-week intervention was implemented, involving the distribution of healthy snack bars as an alternative to traditional break-time snacks and the concurrent implementation of food education programs. The educational modules focused on promoting healthy eating patterns, addressing diet quality, food sustainability, and strategies to overcome food neophobia (reluctance to try new foods) through engaging sensory activities. These initiatives aimed to encourage adolescents to explore new foods and adopt more varied and healthier dietary patterns.

The intervention study will also be replicated in Greece and Tunisia, enabling an international evaluation of the results.

The FEM high school proved to be an ideal setting for testing strategies to promote more conscious and sustainable food choices and to support basic research, thanks to daily interactions, educational opportunities, and the active participation of students and their teachers.

Students during sensory education lessons

**KEYWORDS**: eating habits, adolescents, food education

# Feed your microbes: the psychobiotic diet to deal with stress

ANDREA ANESI
FULVIO MATTIVI
URSKA VRHOVSEK

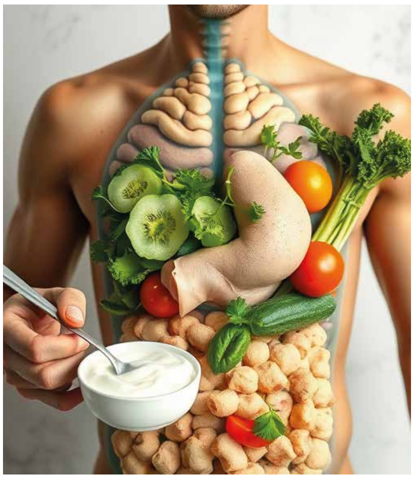
The human gut microbiota is composed of over 500 species of bacteria, yeasts, and viruses coexisting in a state of dynamic equilibrium. The microbiota performs numerous functions for human health, such as protecting the intestinal mucosa, regulating immune defenses against pathogens, aiding digestion, and influencing cognitive and mental functions through the gut-brain axis. Disruption of this balance can induce a state of low-grade chronic inflammation, which may initially manifest as various intestinal disorders and, in the long term, predispose individuals to the development of several diseases, including severe conditions.

This study assessed the effect of a psychobiotic diet, based on high consumption of fermented and prebiotic foods, to promote gut balance and the psycho-physical well-being of individuals. The innovative approach of the study lies in evaluating the impact of an entire dietary approach, based on the combination of different food components, rather than focusing on a single food as in most previous studies performed elsewhere.

Here, 24 healthy adult participants, recruited in the Cork area (Ireland) between 2018 and 2021, were instructed to consume a specific diet including fermented foods for a 4-week period. A control group of 21 participants received instructions regarding a standard diet.

Participants who adhered most closely to the psychobiotic diet showed a greater reduction in perceived stress, although no significant differences were found between the two groups at the end of the intervention. The psychobiotic diet led to slight changes in the microbial profile, indicating that a four-week period is sufficient to induce modifications, but also suggesting that a longer period is needed to determine more evident changes. Some changes in the fecal lipid profile, potentially indicative of improved gut barrier integrity, were observed. Concurrently, the reduction in certain toxic urinary catabolites derived from tryptophan metabolism, known for their potential neurotoxic effects, suggests a possible long-term benefit for mental health.

Despite the limitations of the small sample size and short study duration, which preclude establishing a definitive causal relationship between diet, microbial changes, and perceived stress, the findings suggest the potential of nutritional approaches based on the consumption of fermented and prebiotic foods in the prevention and treatment of human mental health conditions.



**KEYWORDS:** gut microbiota, psychobiotic diet, mental health
**SPECIES LIST:** *Homo sapiens*

# AGROSYSTEMS AND BIOECONOMY

Apple micropropagation in *in vitro* culture

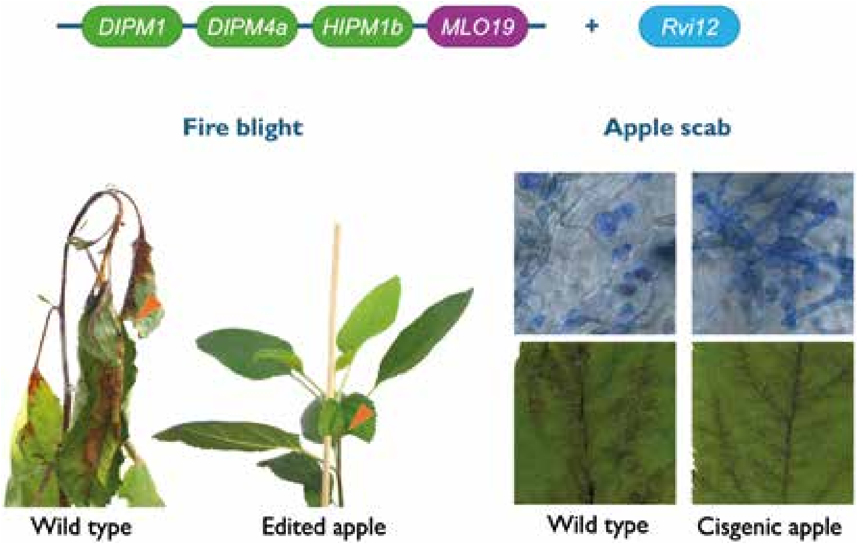
# Dual NBT approach: cisgenesis and gene editing to improve resistance to multiple pathogens in apple

SIMON MIRANDA
VALERIA GUALANDRI
MICKAEL MALNOY

Fire blight, a devastating disease caused by the gram-negative bacterium *Erwinia amylovora*, poses a serious threat to many plants in the Rosaceae family, particularly apples (*Malus x domestica* Borkh). Similarly, apple production is negatively impacted by fungal diseases such as apple scab, caused by *Venturia inaequalis*, and powdery mildew. Current management of these diseases relies heavily on intensive applications of antibiotics and fungicides during the growing season, as sustainable control options remain unavailable.

To address this challenge, breeding approaches targeting genes related to susceptibility and resistance offer a promising path to developing more resilient apple cultivars. New Breeding Techniques (NBTs), such as CRISPR/Cas9 gene-editing and cisgenesis, have emerged as tools with significant potential to contribute to these improvements. Encouragingly, regulatory frameworks in some regions are now leaning towards exempting gene-editing and cisgenesis from traditional GMO regulations, potentially accelerating their adoption in agricultural practices.

In our study, we used an excisable genetic cassette to simultaneously edit three fire blight susceptibility genes and one powdery mildew susceptibility gene in two commercial apple cultivars. Additionally, we employed cisgenesis to introduce an apple scab resistance gene (namely, *Rvi12*) from a wild apple species. We obtained regenerant apple lines and assessed the editing efficiency



Fire blight (left) and apple scab (right) symptoms can be reduced by gene-editing susceptibility genes and introducing a resistance gene via cisgenesis, respectively

across the target genes. The resulting lines displayed varying editing profiles, with modifications ranging from a single to all target susceptibility genes. In addition, we evaluated *Rvi12* gene expression levels in cisgenic lines, as well as the copy number of inserted cassettes.

Selected lines were subjected to infection tests for fire blight, apple scab, and powdery mildew. Our findings showed that significant reductions in fire blight progression was found in lines with edits in all susceptibility genes. Preliminary results from *V. inaequalis* inoculation indicate that symptoms of apple scab can be reduced in a number of cisgenic lines. These results highlight the potential of gene editing and cisgenesis to develop apple cultivars more resilient to multiple pathogen challenges, paving the way for more sustainable apple production.

**KEYWORDS:** fire blight, apple scab, gene editing
**SPECIES LIST:** *Malus x domestica, Erwinia amylovora, Venturia inaequalis*

# Cuticle and cell wall: a fundamental barrier in grapevine defence against biotic and abiotic stressors

CARLOTTA PIRRELLO
STEFANIA PILATI
GIULIA MALACARNE

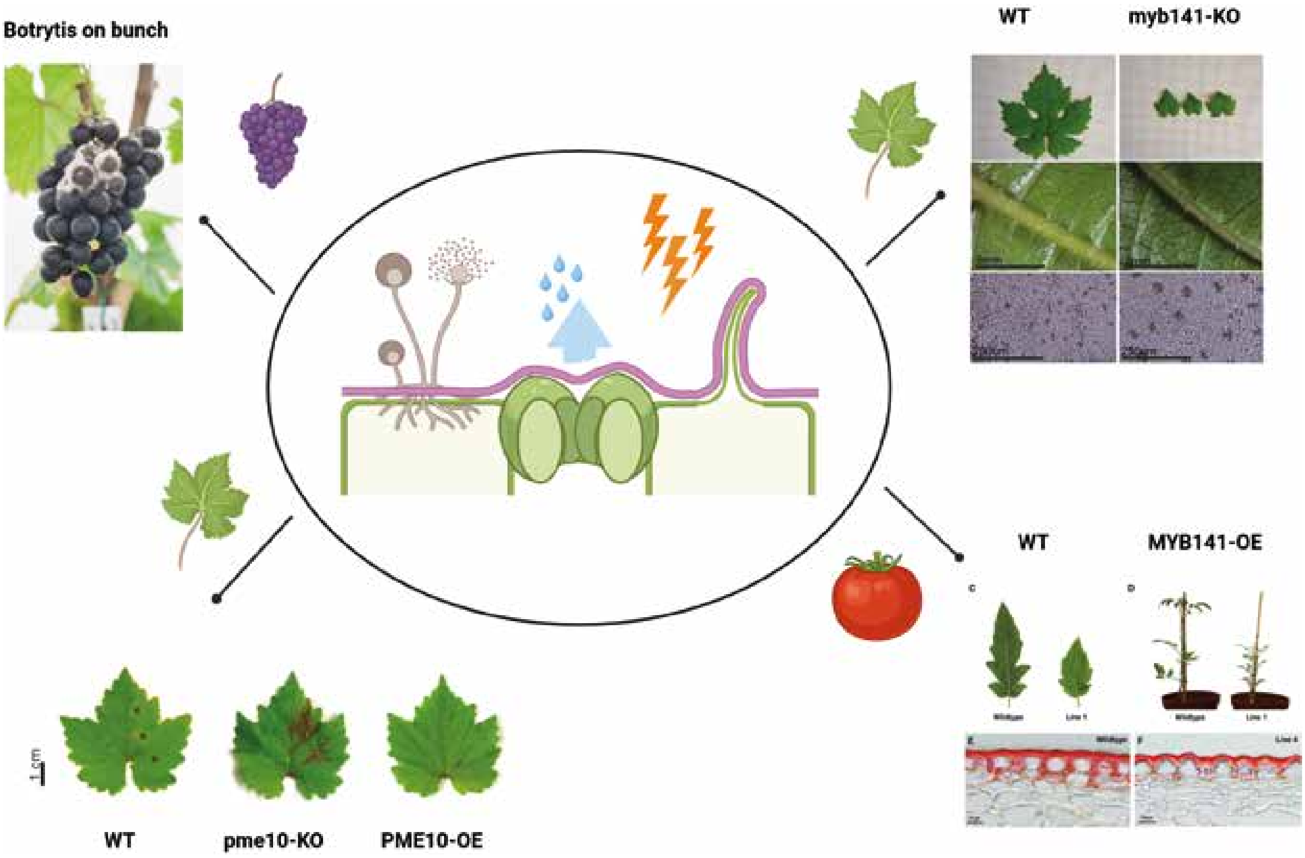
In grapevine leaves and fruit, the cuticle and the cell wall of epidermal cells represent the first physical barrier against pathogen penetration and regulate gas exchange between the plant and the external environment. Therefore, their molecular characterisation is crucial for developing innovative strategies to protect grapevines from biotic and abiotic stressors. This aligns with the goals of sustainable viticulture, which aims to reduce dependence on chemical fungicides and promote environmentally friendly agricultural practices.

*Botrytis*-infected bunch

Jorge Lagreze's PhD research investigated grapevine genes involved in cell wall remodelling during the interaction of grapevine with *Botrytis cinerea*, focusing on pectin methyl esterases (PME). Silencing and overexpression of the *VviPME10* gene, whose expression is significantly induced by fungal presence, demonstrated its key role in *Botrytis* resistance. *VviPME10*-mediated modulation of pectin integrity not only strengthens the cell wall as a physical barrier but may also function as a signaling mechanism, enhancing the plant's capacity to recognize and respond to pathogen attack.

The effect of VviERF45, a transcriptional regulator, on cuticle structure was also examined. Leaves from grapevines overexpressing VviERF45 exhibited reduced deposition of epicuticular wax. Subsequently, the downstream regulator VviMYB141 was identified, influencing wax composition (a phenotype observed in tomato fruit used as a model system), trichome development, and epidermal cell size, including stomatal guard cells (phenotypes observed in grapevine leaves). While the fruit cuticle affects grape quality by limiting wilting and decay during storage and transport, these morphological traits influence leaf transpiration and thus plant water use efficiency. These factors are increasingly relevant in the context of more frequent stress events driven by climate change.

In conclusion, *VviPME10* represents a novel and promising molecular marker for grapevine *Botrytis* resistance, with significant implications for both fundamental plant biology and applied agricultural research. Ongoing studies of cuticle biosynthesis are expected to identify promising candidates for developing or selecting plants with enhanced tolerance to water stress or for producing grapes with improved shelf life.



Representation of the main results obtained from the characterization of grapevine genes involved in cell wall remodeling *PME 10* and in the regulation of the wax composition of the cuticle and the formation of trichomes and epidermal cells *MYB141*

**KEYWORDS:** cell wall, cuticle, biotic and abiotic stresses

**SPECIES LIST:** *Vitis vinifera, Botrytis cinerea*

# Unveiling key pathways in chilling injury: a comparative study of superficial scald in ‘Granny Smith’ and ‘Ladina’ apple varieties

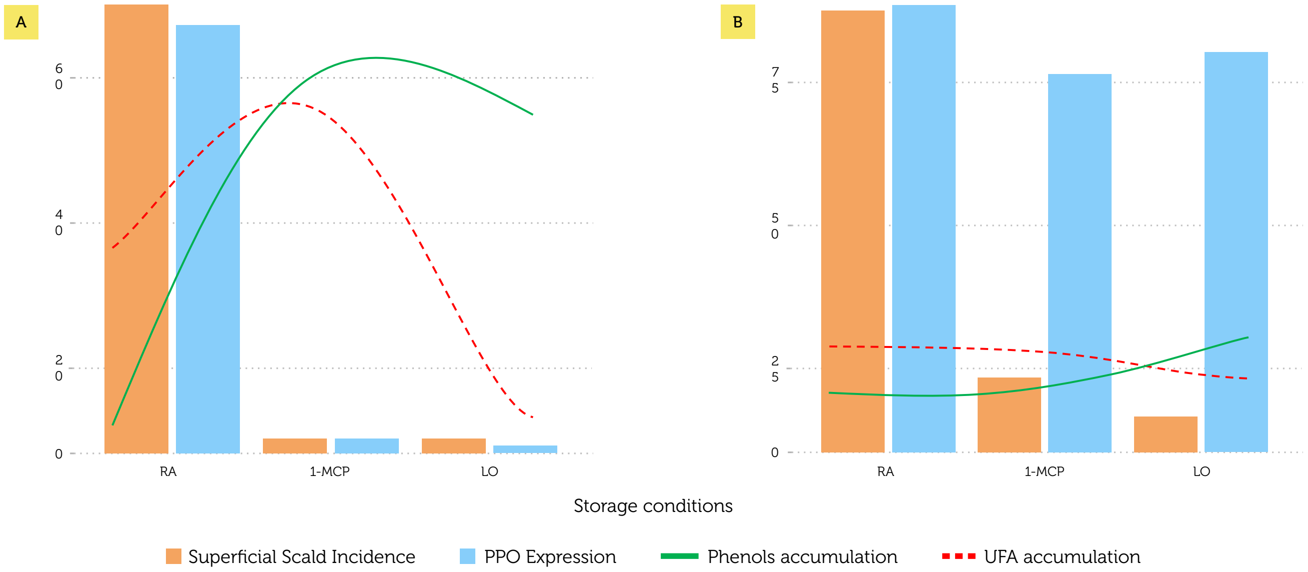
LORENZO VITTANI
FRANCESCA POPULIN
NICOLA BUSATTO

Superficial scald, a chilling injury that affects apples during storage, presents a significant challenge to preserving fruit quality. Characterized by dark blemishes on fruit skin, this disorder is driven by oxidative stress and enzymatic browning, leading to fruit loss and economic impacts.

This study, conducted in collaboration with Fabrizio Costa (University of Trento), investigates the molecular and metabolic mechanisms underlying scald in two apple cultivars, ‘Granny Smith’ and ‘Ladina’, while assessing two preventive treatments: the application of the gaseous hormone 1-Methylcyclopropene (1-MCP) and Low-Oxygen (LO) storage.

Striking differences emerged between the cultivars. In ‘Granny Smith,’ both treatments were highly effective, reducing scald incidence to less than

Effect of postharvest cold storage on apple quality. The left panel shows a fresh green apple prior to storage, while the right panel shows an apple after extended cold storage, exhibiting superficial scald symptoms, with a detailed inset highlighting the affected peel tissue structure



Incidence of superficial scald (orange bars), polyphenol oxidase (PPO) expression (blue bars), phenols accumulation (green continuous line), and unsaturated fatty acids (UFA) levels (red dotted line) in two apple varieties, 'Granny Smith' (A) and 'Ladina' (B), under three storage conditions: regular atmosphere (RA), 1-MCP treatment, and low oxygen (LO). The graph illustrates the interplay between physiological and biochemical factors in the development of superficial scald and its mitigation through different storage strategies

1%. By contrast, 'Ladina' retained symptoms in 9–20% of fruit. These differences were traced to metabolic and genetic factors, particularly the composition of phenolic compounds. 'Granny Smith' accumulated antioxidants such as catechin and epicatechin in response to treatments, which combat oxidative stress and scald development. Conversely, 'Ladina' showed elevated levels of chlorogenic acid, a compound that reacts with the enzyme polyphenol oxidase (PPO) to produce browning. While 1-MCP and low oxygen suppressed PPO expression in 'Granny Smith', their effects were less pronounced in 'Ladina', leaving it more susceptible.

Treatments in 'Granny Smith' promoted the accumulation of unsaturated fatty acids, such as linolenic and oleic acids, which stabilize cell membranes and enhance cold tolerance. By contrast, 'Ladina' exhibited higher levels of saturated long-chain fatty acids, which can destabilize membranes, contributing to scald symptoms.

The treatments suppressed ethylene production in both cultivars, suggesting that ethylene is not directly involved in controlling the disorder; instead, postharvest strategies like 1-MCP or LO may affect superficial scald by modulating pathways such as phenolic or lipid metabolism.

This study emphasizes the importance of cultivar-specific approaches to scald prevention. While 1-MCP and low oxygen were highly effective for 'Granny Smith', they offered only partial protection for 'Ladina'.

By tailoring postharvest treatments to the unique genetic and metabolic traits of each cultivar, producers can significantly extend storage life and improve apple quality, thereby reducing food waste and meeting consumer demands for high-quality fruit.

**KEYWORDS:** superficial scald, postharvest, chilling injuries
**SPECIES LIST:** *Malus x domestica*

# FruitDiv: exploiting the untapped potential of fruit tree wild diversity for sustainable agriculture

DAVIDE BUSETTI
LUCA BIANCO
MICHELA TROGGIO

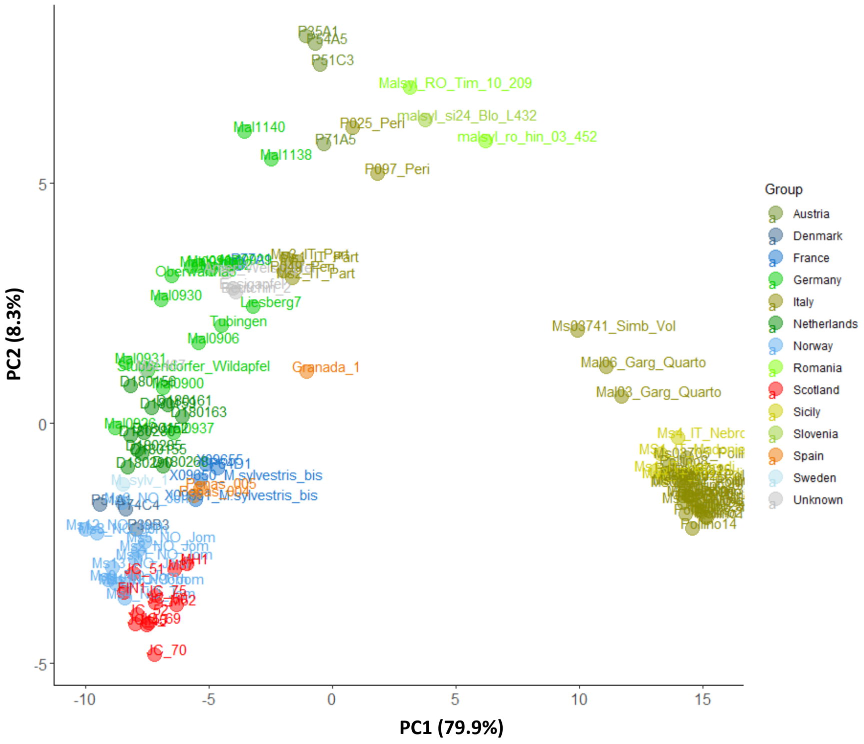
Launched on January 1, 2024, FruitDiv is a Horizon Europe project focused on the potential of fruit tree Crop Wild Relatives (CWR) to enhance sustainable agriculture. Targeting pome (*Malus* spp., *Pyrus* spp.) and stone (*Prunus* spp.) fruits, the project aims to monitor, characterize, conserve, and utilize the genetic diversity of these wild relatives.

CWR are wild species closely related to cultivated crops, which have often maintained traits like pest- and disease resistance, drought tolerance, and adaptability to climate change. Exploiting this genetic diversity is crucial for crop improvement, sustainable practices, and food security, aligning with the European Green Deal, Biodiversity, and Farm to Fork strategies, which aim to reduce pesticide use and risks. With the long lifespan of fruit trees and a production landscape dominated by a few cultivars, research and conservation into CWR is vital for adapting the existing crops to global changes.

FruitDiv aims to deliver several key outcomes, starting with a comprehensive inventory of pome and stone fruit CWR across Europe to improve their representation in genetic resource programs and enhance conservation and use through *in-situ*, *ex-situ*, and on-farm strategies establishing European-wide CWR collections.

The FEM Research units of Fruit Crop Genetics and Breeding, and Computational Biology, in collaboration with the Metabolomics platform, are actively involved in the consortium's activities, which include 26 institutions from 14 European countries. By using cutting-edge genomic and phenomic techniques, including pangenomic-assisted breeding and CWR-adapted prediction models, FEM's efforts are

FEM researchers, in collaboration with the University of Basilicata, during the sampling activities in 2024 in the Simbruini Mountain Regional Park (left) and Pollino National Park (right)



2D-PCA plot showing the overall genetic variance among *Malus sylvestris* populations sampled to date, measured using 50K Axiom® SNP array technology. Italian samples are clearly separated from non-Italian samples along the second principal component (PC2)

primarily focused on the genetic and phenotypic characterization of apple CWR, coordinating the sampling of various *Malus sylvestris* (the European wild apple) populations. Interestingly, the genetic data analyzed so far have identified the Pollino National Park (Basilicata) as an extraordinary site that hosts natural populations of wild *Malus sylvestris*, which are distinctly different from those found in the rest of the continent. In addition, FEM is involved in managing and organizing the extensive data generated over the five-year duration of the project. The consortium also collaborates on developing new breeding strategies that employ CWR as breeding parents, combining marker-assisted introgression for traits of interest, such as resilience, resistance to pests and pathogens, and nutritional and fruit quality traits, with genomic prediction. By integrating innovative methodologies and fostering collaboration, FruitDiv aims to unlock the full potential of fruit tree CWR, ensuring a resilient and sustainable agricultural future.

**KEYWORDS:** Crop Wild Relatives (CWR), biodiversity, breeding
**SPECIES LIST:** *Malus sylvestris, Malus x domestica*

# Cross-breeding: making viticulture more sustainable

MARCO STEFANINI
LUCA ZULINI
SILVIA VEZZULLI

F1P54 resistant variety obtained by crossing with 'Chardonnay'

In recent years, the main challenges that viticulture is facing revolves around the ability of plants to adapt to climate change and exhibit resistance to major diseases, as well as making grapevine cultivation increasingly sustainable.

During grapevine evolution, crossbreeding naturally results in new individuals with a recombination of traits, providing the basis for selection and propagation.

Grapevine genetic improvement through crossbreeding is the most suitable approach to maintaining a vast genetic biodiversity, defining the parents to be used in crosses and evaluating all the progeny, according to the selection objective.

At Fondazione Edmund Mach, genetic improvement programs for grapevine have been active for many years, and aim to obtain new grape varieties resistant to the most important fungal diseases, such as downy mildew, powdery mildew, and black rot.

More recently, crossbreeding programs have involved crosses among the main *Vitis vinifera* varieties and parents with different genetic sources of resistance (pyramiding). This approach results in plants with resistance barriers much more stable against the fungus.

The parents that provide resistance are selected to obtain progeny with five sources of resistance against downy mildew and powdery mildew, in addition to resistances against other infections, with the highest possible level of homozygosity, to increase the breeding efficiency.

The progeny are phenotypically selected in a controlled environment

Crosses artificially infected with downy mildew under greenhouse conditions

that facilitates fungal development. The non-symptomatic individuals are preserved, evaluated for the quality and verified for the presence of the molecular markers associated with resistances.

Annually, the crossbreeding programs involve around 100 different cross combinations, the phenotypic selection of 7000-8000 seedlings, 300 genotypes under selection, and 50 microvinifications to be evaluated. The criteria for the selection of the parents are also defined according to the objectives of Trentino viticulture. For example, a population of 'Chardonnay' progeny has been obtained with different phenologies, aromatic profiles or acidity levels. In addition, in collaboration with Consorzio Innovazione Vite (CIVIT), four resistant varieties have been registered ('Valnosia', 'Charvir', 'Nermantis' and 'Termantis') and four genotypes have been deposited to the CPVO (Community Plant Variety Office), awaiting evaluation on their trait stability. Moreover, another four genotypes are likely to be sent to follow the same procedure required for enrollment in the National Register of Wine Grape Varieties of Italy.

This activity was possible thanks to the contribution of all the staff of the Grapevine Physiology and Breeding unit.

**KEYWORDS:** cross-breeding of vines, climate change, resistances
**SPECIES LIST:** *Vitis vinifera*

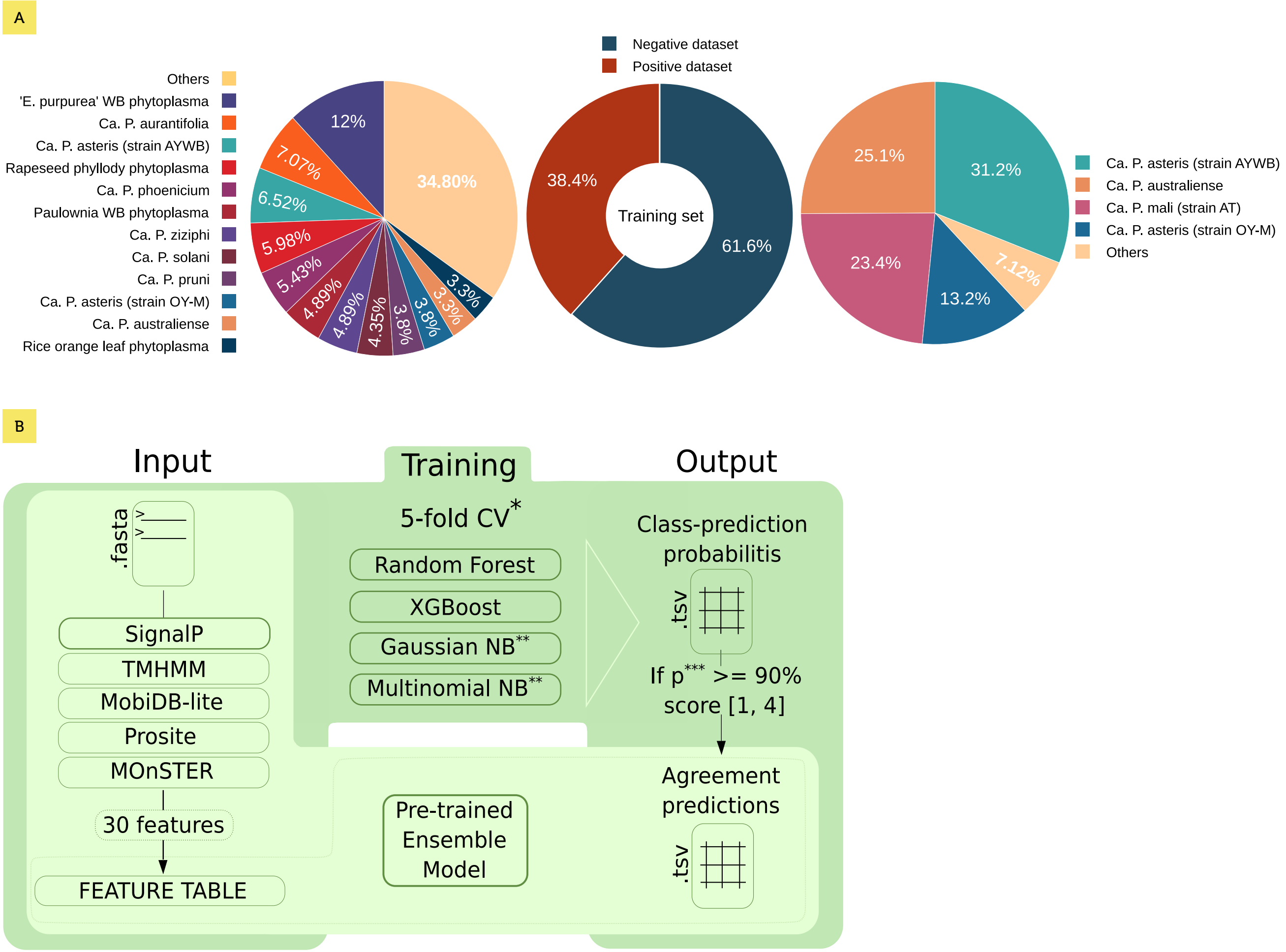
Apple tree affected by apple proliferation (photo by Erica Candioli)

# Apple proliferation phytoplasma: variants, effectors, and pathogenicity

MIRKO MOSER
GIULIA CALIA
ALESSANDRO CESTARO

Plants are constantly subject to attack by pathogenic organisms, and many plant diseases have been noted for centuries. However, in the case of phytoplasmoses, although they had been described symptomatically, the identification of the causal agent only occurred in the 1960s with the discovery of phytoplasmas. These microorganisms, lacking a cell wall, colonize the phloem - the network of vessels that transports elaborated sap to all parts of the plant - causing a variety of symptoms, from leaf yellowing to the deformation of leaves and flowers, leading to a progressive decline of the plant. To colonize and dominate host plants, phytoplasmas produce specialized proteins called ‘effectors’. These proteins enter plant cells and disrupt essential cellular processes, favoring the pathogen. They influence the plant’s metabolism, development, and defenses. In their insect vectors, phytoplasmas appear to alter the capacity for acquisition and transmission, and affect the insect’s reproductive ability, although the mechanisms involved remain poorly understood. The genetic variability of phytoplasmas has been associated with differences in virulence and symptomatology, with various strains characterizing each type of phytoplasma. In recent years, we have focused on sequencing the genomes of several strains of *Candidatus Phytoplasma mali*, the phytoplasma responsible for apple proliferation, to study their functions, with particular attention to effectors. While some effectors have been characterized in the past, it is hypothesized that the total number is significantly higher. Identifying new effectors is crucial to understanding the molecular mechanisms underlying phytoplasma-induced diseases and analyzing their interactions with insect vectors. To this end, we recently developed an advanced bioinformatic tool, LEAPH (ensembLe model for Effector clAssification in PHytoplasmas), based on a machine learning model. This tool analyzes phytoplasma protein sequences and predicts with high accuracy which proteins are most likely to be effectors. LEAPH opens new avenues for research, identifying candidate effectors on which to focus experiments to study their functions and modes of action in host plants. Despite significant progress, research on phytoplasmas and their effectors still faces many challenges. The complexity of plant-pathogen interactions, the genetic variability of phytoplasmas, and the lack of efficient experimental models remain major obstacles, but new technologies are paving the way for important discoveries. The FEM colleagues Claudio Donati and Diego Micheletti also contributed to this work.

Training dataset composition and LEAPH workflow



**KEYWORDS:** apple proliferation, effectors, LEAPH

**SPECIES LIST:** *Malus x domestica, Candidatus Phytoplasma mali*

Guyot apple training systems

# Environmental impact of Spindle and Guyot apple training systems in the mountain region of Trentino

MOE THAE OO
LUCA TOMASI

The environmental impacts of agri-food production are being increasingly studied due to sustainability concerns and resource depletion. Life Cycle Assessment (LCA) has become a key methodology for evaluating the environmental footprint of agri-food systems throughout their life cycles. In mountainous regions tailored LCA approaches are crucial for integrating existing databases with site-specific data for accurate evaluation.

Apple cultivation is a significant agricultural activity in Trentino, where innovative systems, such as Guyot, have been introduced to optimise resource use, yield, and fruit quality. However, understanding their environmental impact remains limited. Within the PNRR i-NEST project, a PhD scholarship at the University of Padua in Circular Economy was co-financed. Research will the environmental performance of the Spindle and Guyot training systems. This project identifies environmental hotspots within the apple production chain to promote sustainable practices in the Trentino apple industry.

This LCA follows the ISO 14040/14044 standards, using a cradle-to-farm-gate approach, covering processes from nurseries to apple delivery at the warehouse (Fig. 1). Primary data were gathered from local apple growers, the FEM experimental farm, and a nursery company, supplemented with secondary data from sources, such as Ecoinvent and WFLCB. The functional unit was defined as one kilogram of fresh apple. Emissions from agricultural inputs were calculated using mod-

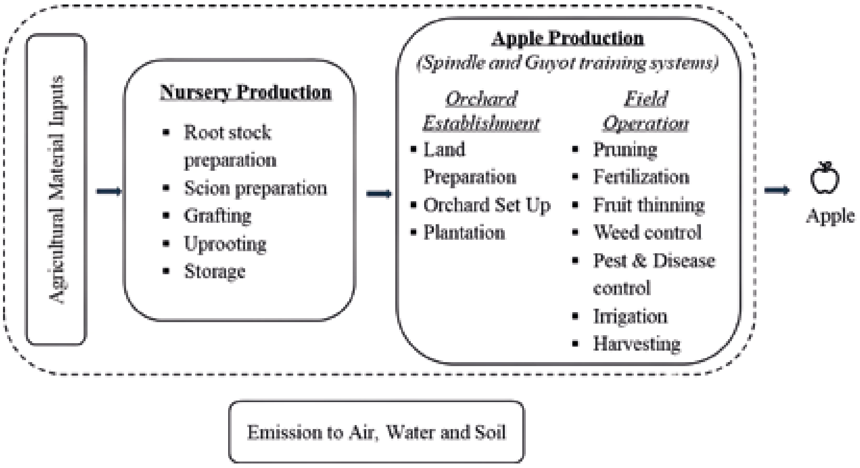


Fig. 1: System boundaries of the research

els, and system modelling and impact analysis were conducted with SimaPro (v9.6) using the EF 3.1 method.

Preliminary findings highlight significant differences in the environmental profiles of the Spindle and Guyot apple training systems (Fig. 2). The Spindle system shows higher resource use, including energy, land, and water, while the Guyot system has slightly greater impacts on terrestrial eutrophication and climate change due to differences in fertilizer application and energy use. These trade-offs underscore the need for optimization strategies: reducing resource intensity in the Spindle system and refining fertilizer and energy management in the Guyot system.

This study provides environmental hotspots, offering actionable insights for improving sustainable apple cultivation in mountainous regions. While focusing on apple orchards, the findings have broader implications for perennial crops and can inform regional agricultural policies. By adopting a life-cycle perspective, this study supports data-driven decisions that enhance mountain agriculture's environmental performance and align with global sustainability goals.

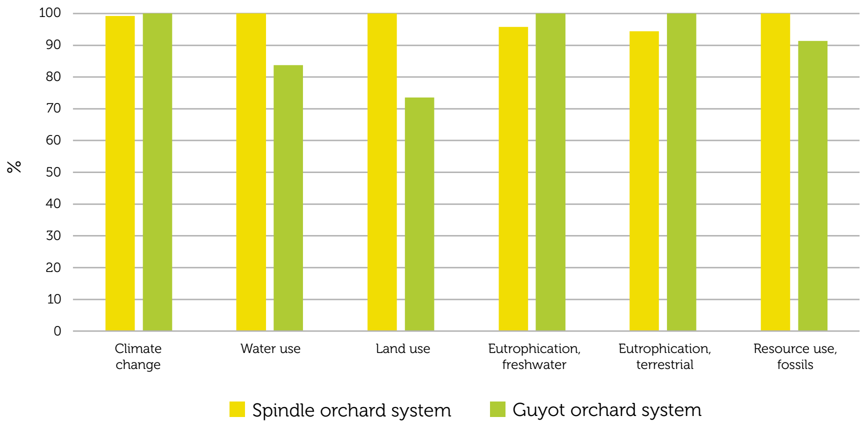
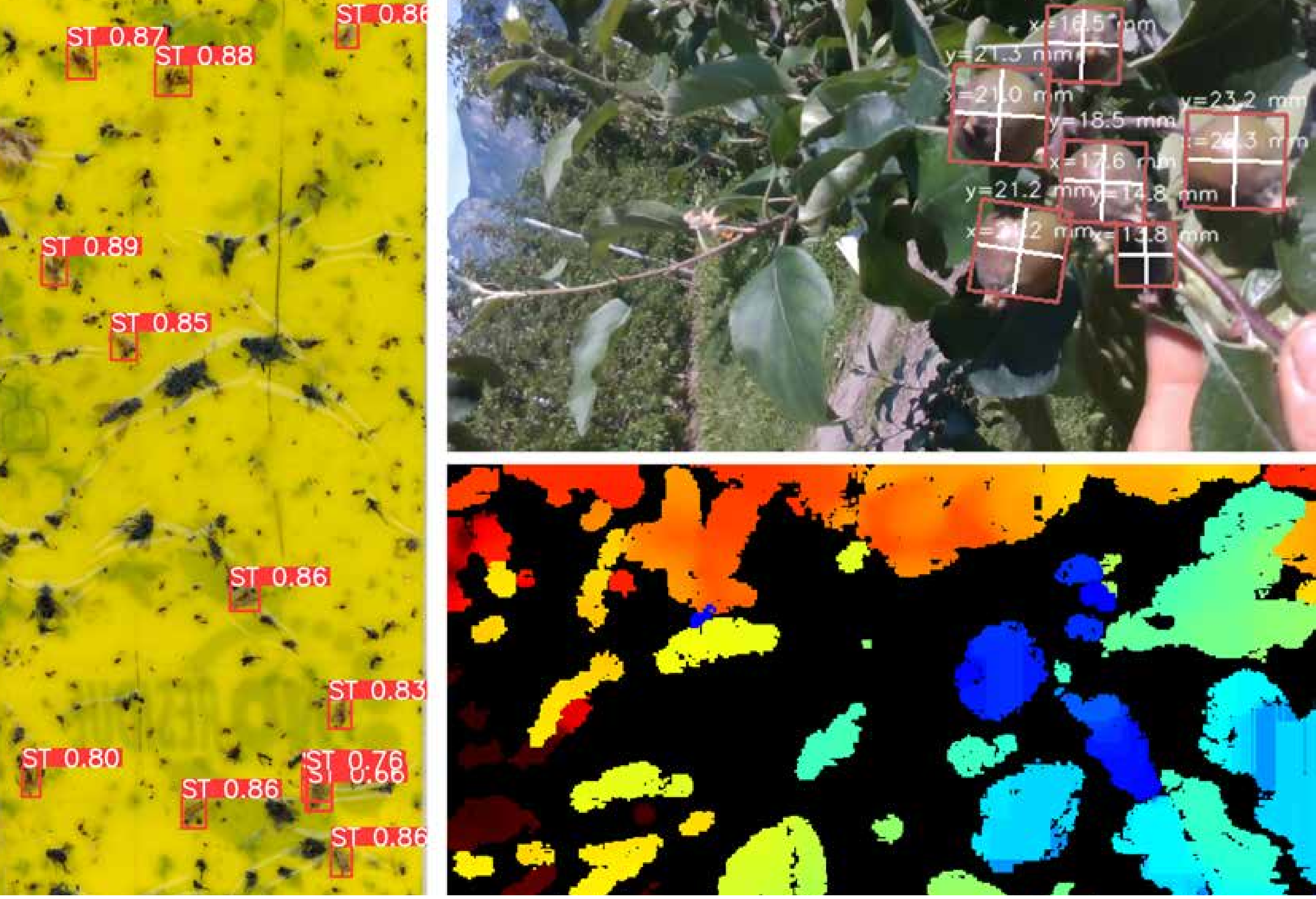


Fig. 2: Differences between the two cultivation systems for some impact categories (in percentages: the value of 100% is attributed to the most impactful system of the two)

**KEYWORDS:** life cycle assessment (LCA), sustainability, apple production systems
**SPECIES LIST:** *Malus x domestica*



# The potential of Machine Vision in agriculture: field experiences

GIORGIO CHECOLA
PAOLO SONEGO
PIETRO FRANCESCHI

Left panel: an example of a yellow trap with bounding boxes indicating insects predicted by the AI model. Right panel: a comparison between an RGB image of apple fruitlets detected and sized by the system (upper panel), and the corresponding depth image (lower panel)

Computer vision, a branch of artificial intelligence dedicated to automatic image analysis, is rapidly becoming a cornerstone of agricultural innovation. The Digital Agriculture unit has developed expertise in this area, creating cutting-edge solutions for viticulture and fruit growing.One initial application focused on developing a deep learning model for the automated detection of insect vectors of Flavescence dorée, a grapevine disease causing significant annual economic losses. Working in collaboration with entomologists Valerio Mazzoni, Alberto Gelmetti, and Franca Ghidoni, the goal was to provide a tool that simplifies the monitoring of yellow sticky traps, thereby increasing the speed and efficiency of insect identification. Training the model involved creating a comprehensive dataset of digitized trap images and meticulously labeling the target insects, *Scaphoideus titanus* and *Orientus ishidae*. Using state-of-the-art deep learning architectures, such as YOLO (You Only Look Once), a preliminary detection model was developed and subsequently integrated into the DigiAgriApp platform. This integration provides real-time support to farmers in the field and promotes citizen science through continuous dataset updates.

Subsequently, research expanded to apple growing, by applying computer vision techniques to apple thinning practices. Monitoring early fruit development is essential for understanding plant physiology, improving fruit quality, and optimizing fruit thinning. Traditional methods, such as manual fruit diameter measurement using calipers, are labor-inten-

Smart Trap prototype (on the left) for the automated acquisition of images from yellow sticky traps in the field. On the right, the Intel® RealSense™ D435i depth camera used for estimating the diameters of apple fruitlets

sive and require high precision. To address this, the team collaborated with the Fruit Growing Experimental unit (represented by Franco Micheli, Damiano Moser, and Cristian Iob) to develop a smart vision system for field support. This methodology uses deep learning algorithms to extract fruitlet data, including size and count, from videos captured by a depth camera. This device, commonly used in robotics, employs stereo vision to calculate object distance, a technique known as depth measurement. The results are highly promising, demonstrating the potential of this data acquisition tool to complement traditional practices without sacrificing reliability. This approach may facilitate future applications, including the evaluation of plant growth regulators and the development of predictive models for yield and productivity optimization.

**KEYWORDS:** computer vision, insect detection, apple fruitlet sizing, artificial intelligence
**SPECIES LIST:** *Scaphoideus titanus*

Selection of fruit for the storage trial

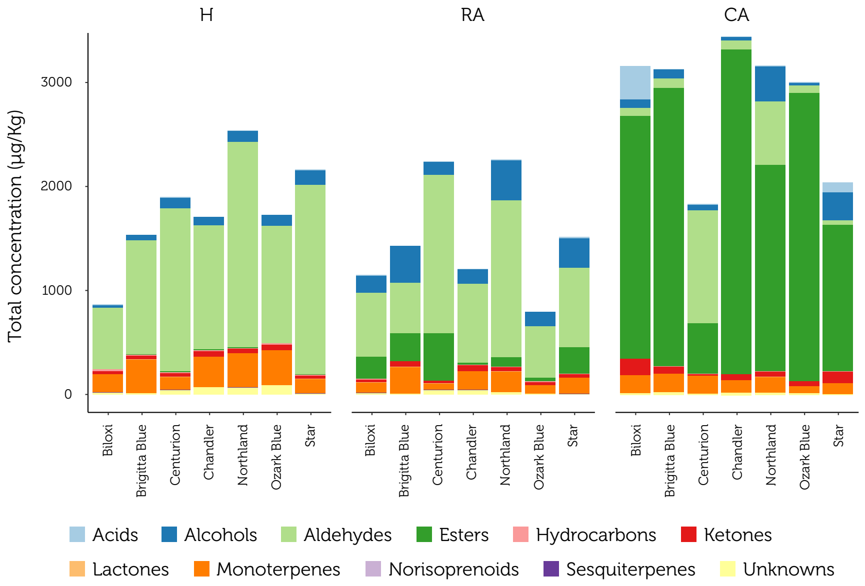
# Improving blueberry quality through tailored management of controlled atmosphere storage

BRIAN FARNETI
MATTEO AJELLI
LARA GIONGO

This research investigates the effects of high-$CO_2$ (16 kPa) controlled atmosphere (CA) storage on the shelf life and quality of blueberry fruits (*Vaccinium* spp.), examining the broad genetic variability present in the FEM germplasm collection. The study particularly focused on how storage conditions affect the volatile organic compound (VOC) profile, with special attention to the de novo biosynthesis of esters and their correlation with VOCs associated with fermentative processes. The aromatic profile analysis was conducted using two complementary analytical techniques: PTR-ToF-MS (Proton Transfer Reaction - Time of Flight - Mass Spectrometer) and GC-MS (Gas Chromatography-Mass Spectrometry) with Solid Phase Microextraction (SPME).

The research comprised three distinct experiments. The first analyzed seven cultivars, comparing regular atmosphere (RA) and controlled atmosphere (CA) storage over 42 days at 2°C. The second involved 39 cultivars to assess genetic variability in response to CA storage. The third examined the effect of varying oxygen concentrations (1,7, and 12 kPa $O_2$) on 4 selected cultivars.

Results revealed significant genotypic variability in quality parameters, which became more pronounced during storage, particularly under modified atmosphere conditions. CA storage demonstrated substantial advantages over refrigeration alone, limiting texture deterioration and dehydration while enhancing the aromatic profile. Under low oxygen con-



Changes in VOCs concentration, expressed as µg/L of 2-octanol, assessed by SPME/GC-MS, for each blueberry cultivars ("Biloxi", "Brigitta Blue", "Centurion", "Chandler", "Northland", "Ozark Blue", and "Star") at harvest time ("H") and after 42 days of storage under Regular Atmosphere ("RA": 2°C, 90% RH, 20.9 kPa $O_2$, and 0.03 kPa $CO_2$) and Controlled Atmosphere ("CA": 2°C, 90% RH, 7 kPa $O_2$, and 16 kPa $CO_2$) conditions

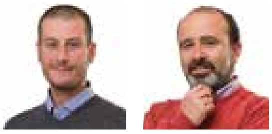
ditions, researchers observed the de novo synthesis of specific esters (ethyl propanoate, ethyl (2E)-2-butanoate, methyl isovalerate, ethyl isovalerate), likely representing an adaptive response to hypoxic stress.

The research demonstrates that CA storage not only extends shelf life but can enhance post-harvest quality through increased production of aromatic compounds that impart fruity and sweet notes. However, the variable responses among cultivars suggest the need for customized storage protocols. These findings provide valuable insights for market segmentation and blueberry breeding programs, while also confirming PTR-ToF-MS as an efficient tool for rapid aromatic phenotyping of fruits.

Acknowledgments: We thank the Sensory Quality unit, particularly Iuliia Khomenko, Emanuela Betta, and Franco Biasioli, for their support in the biochemical analysis of volatile compounds.

**KEYWORDS:** VOCs, texture, germplasm
**SPECIES LIST:** *Vaccinium* spp.

# Innovation in the sustainable control of alien insects



MARCO VALERIO ROSSI STACCONI
VALERIO MAZZONI

Invasive alien species (IAS) are organisms introduced outside their natural range that harm the environment, economy, and society. Species such as the fruit fly (*Drosophila suzukii*), the brown marmorated stink bug *(Halyomorpha halys*), and the painted bug (*Bagrada hilaris*) have a significant negative impact on agricultural crops. To control them, FEM develops innovative and sustainable approaches that combine behavioral manipulation and biological control.

Biotremology and chemical ecology provide advanced tools for analyzing communication signals and employing them to manipulate the behavior of harmful insects. In synergy with biological control, these methods enable more targeted strategies that reduce dependency on pesticides, enhancing agricultural sustainability.

In recent years, biotremology has paved new paths for IAS control; for example, bimodal traps for *H. halys* combine chemical and vibrational signals to increase capture rates, and the study of *B. hilaris* mating signals has enabled the development of innovative sexual interference techniques; and the vibrations produced by *D. suzukii* larvae inside fruits have been characterized to detect ongoing infestations. Similarly, in chemical ecology, volatile compounds used by natural enemies of *D. suzukii* to locate larvae in infested fruits have been identified. These discoveries open new opportunities to optimize biological control by developing specific attractants or planning synchronized releases timed with infestation stages.

The FEM initiative to integrate biotremology and chemical ecology has resulted in the proposal of a new scientific lexicon to harmonize the two disciplines. In 2024, an article co-authored by leading experts in both fields introduced a shared terminology, including terms such as ferodons and allelodons to refer to intra- and interspecific vibrational signals, respectively. This standardization is crucial for improving scientific communication and facilitating the regulation and commercialization of new products based on these technologies.

Finally, in biological control, FEM has participated in two national programs for releasing non-native natural enemies. *Trissolcus japonicus*, a parasitoid of *H. halys* eggs, and *Ganaspis brasiliensis*, a parasitoid of *D. suzukii* larvae, were released in several areas of Trentino in 2020 and 2021, respectively. Results have shown a good establishment and spread of these natural enemies, with visible effects already observed in the case of *T. japonicus*, which has significantly reduced the population of *H. halys*.

The authors wish to thank Prof. Gianfranco Anfora (University of Trento) for his valuable contribution.

Adult specimens of *Ganaspis brasiliensis* (a parasitoid of *D. suzukii* larvae) in a vial shortly before release

**KEYWORDS**: biotremology, biocontrol, crop protection
**SPECIES LIST**: *Drosophila suzukii, Halyomorpha halys, Bagrada hilaris*

# Improving grapevine resistance to downy mildew using New Genomics Techniques

LISA GIACOMELLI
UMBERTO SALVAGNIN

The use of pesticides to control downy mildew is becoming unsustainable due to high costs and negative impacts on human health and the environment. Thus, there is a pressing need to develop resistant grapevine plants by introducing resistance loci derived from wild grapevines– into the genome of susceptible varieties through long-term breeding programs. However, this approach has limitations, as rapidly evolving pathogens can overcome resistance mechanisms in a relatively short time. Furthermore, there is reluctance in the wine industry to replace traditional grape varieties.

New Genomic Techniques (NGTs), such as *CRISPR/Cas9*-mediated gene editing, offer a promising alternative by enabling precise and targeted mutations. Editing susceptibility genes, such as *DMR6*, in elite varieties could create clones with more durable resistance while preserving their original genetic background.

*DMR6* genes encode enzymes involved in the catabolism of salicylic acid, a key regulator of the plant's immune response. Recent studies have shown that gene editing of *DMR6* genes reduces susceptibility to downy mildew and other diseases in various species. Grapevine has two *DMR6* genes and three *DMR6-like* genes, so that the contribution of each gene to resistance and the phenotypic effects of their mutations must be analysed separately. In our laboratory, we generated transgenic grapevine plants with mutations in the *DMR6* and *DMR6-like* genes using CRISPR/Cas9. Our results indicate that only the simultaneous silencing of two *DMR6* genes effectively reduces downy mildew susceptibility by increasing endogenous salicylic acid levels. Mutations in only one of the two genes showed no significant effect under greenhouse conditions.

To assess the actual effectiveness of these plants to resist downy mildew, and to verify any potential unwanted effect caused by the mutations, further research is needed, including tests in experimental vineyards.

To this end, we are working to produce grapevines of locally relevant varieties that are more tolerant to diseases using the current NGTs based on protoplasts. This approach allows the generation of plants with the desired mutations without the addition of any exogenous DNA.

Gene-edited grapevine plants. From the left: unmodified plant, grapevine plant edited in *DMR6-1*, plant edited in *DMR6-2*, and double mutant edited in both *DMR6-1* and *DMR6-2*

**KEYWORDS:** grapevine, NGTs, downy mildew
**SPECIES LIST:** *Vitis vinifera, Plasmopara viticola*

# New genomic technique to mitigate water stress in grapevine

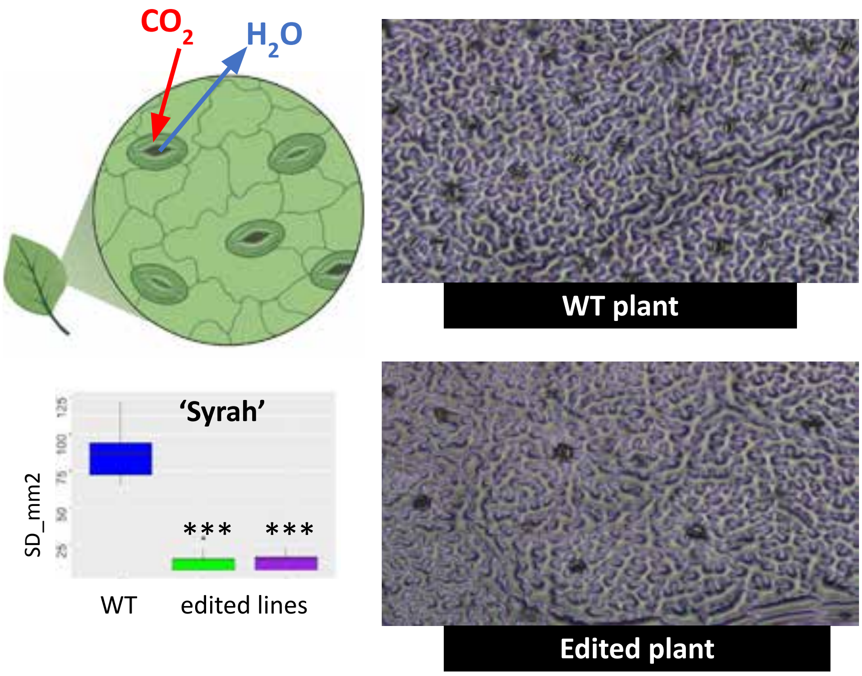
LORENZA DALLA COSTA
MICKAEL MALNOY

Plants in the greenhouse and PhD student Umar Shahbaz

In the context of climate change, increasing the resilience of fruit crops to reduced water availability is essential to safeguard fruit quality and production yields. While new genomic technologies (NGTs) are constantly advancing plant genetic improvement, a limiting factor for their effective application is the knowledge of the molecular mechanisms underlying specific traits and the key genes regulating them. Using complementary experimental approaches, two PhD projects identified and functionally characterized candidate genes for mitigating the adverse effects of water stress in grapevine.

Umar Shahbaz, within the FEM-PhD project Stomalter, co-funded by FEM and the French group Mercier (one of the largest nurseries of *Vitis spp.* in Europe), elucidated the specific role of the two paralogs of the *VviEPFL9* gene in controlling stomata formation in grapevine. This study highlighted the major role of *VviEPFL9-2* in modulating stomatal density in mature leaves, demonstrating that reducing stomatal density effectively decreases transpiration and enhances drought tolerance without significantly compromising photosynthesis. In addition, plants edited for *VviEPFL9-2* exhibited altered root architecture compared to controls suggesting a role for this gene, not only in leaf stomatal regulation but also in root and stem vascular development.

As part of another PhD project conducted in FEM, GrapeSyStress, co-funded by FEM and Concha Y Toro (the largest wine-producing company in Chile), Alvaro Vidal developed a bioinformatic tool to explore publicly



Top left: Schematic representation of stomata, small pores in the leaf epidermis through which gas exchange with the atmosphere takes place, enabling the processes of photosynthesis and transpiration. Bottom left: Stomatal density plot showing how knock-out of the *EPFL9-2* gene in edited plants induces a significant reduction in stomatal density. Right: Images of leaf prints from WT and edited plants

available transcriptomic datasets. This tool analyzed RNA-seq data from 997 experiments, encompassing four tissues (leaf, root, berry, and shoot), various levels of water stress, and diverse grapevine cultivars and rootstocks. The analysis identified the raffinose synthase gene (*VviRAFS*), encoding an enzyme that synthesizes the known osmotic protectant raffinose, and its controlling transcription factor as promising candidates for enhancing grapevine drought tolerance.

Phenotypic characterization and greenhouse water stress experiments on the edited and cisgenic plants generated in these projects confirmed that, through advanced NGTs, the selected genes represent promising targets for developing grapevine varieties and rootstocks with enhanced resilience to projected water scarcity.

**KEYWORDS:** water stress, gene editing, cisgenesis
**SPECIES LIST:** *Vitis* spp., *Vitis vinifera, Vitis riparia*

# Green gold: the viticultural biodiversity heritage preserved at the Fondazione Edmund Mach

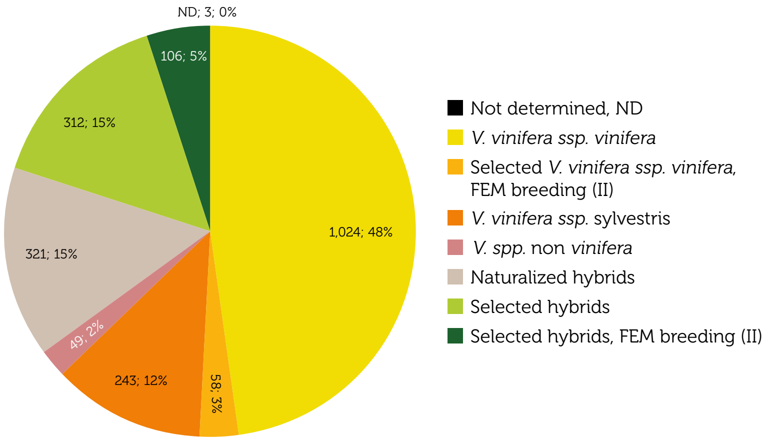
PAOLA BETTINELLI
LAURA COSTANTINI
SILVIA VEZZULLI

View of the germplasm collection of the Fondazione Edmund Mach at the Giaroni location

For the past 40 years, the Grapevine Genetics and Breeding unit, led by Marco Stefanini, has been dedicated to establishing and maintaining a valuable collection of grapevine germplasm, currently comprising over 3000 accessions conserved across 4.5 hectares of vineyard. Preserving grapevine genetic diversity is essential for ensuring a sustainable future for viticulture. With rising global temperatures and the emergence of novel diseases, the adaptive capacity of grapevines becomes increasingly critical. Fondazione Edmund Mach plays a key role in safeguarding this genetic diversity, providing access to resilient and disease-resistant varieties for winegrowers.

This valuable germplasm collection contributed to FEM's inclusion in the Italian National Recovery and Resilience Plan (PNRR) funding for the National Center AGRITECH, enabling comprehensive genotyping of the collection. Using nine universal microsatellite markers recommended by the scientific community for varietal identification, 2,116 unique genetic profiles were identified, along with 57 somatic variants within 30 different varieties. Notably, 928 of these profiles were previously undocumented, representing a unique resource that significantly enriches the viticultural community and underscores the value of FEM's research and conservation efforts in grapevine biodiversity.

The collection encompasses common and rare cultivated varieties (*Vitis vinifera*), wild *Vitis* species from Europe,



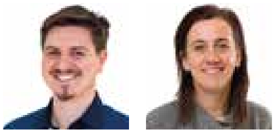
Genetic analyses identified 2,116 unique profiles belonging to the different classes described in the legend. *V.* = *Vitis*, genus to which the grapevine species belong; *ssp.* = *subspecies*, subspecies; *spp.* = *species pluralis*, plural of species, indicates more species within the genus

America, and Asia, and a diverse array of interspecific hybrids. These hybrids are utilized both as rootstocks and for introgressing resistance to fungal diseases into elite cultivars. A further significant outcome of this project is the development of the FAIR FEM Vitis Database, created in collaboration with the Computational Biology unit. Following meticulous standardization and digitization of phenotypic data collected over the past decade, this information, along with microsatellite data, has been integrated into the database. This resource, coupled with a second ongoing genotyping effort using SNP array technology, will facilitate the identification of genetic determinants underlying traits of interest, informing breeding strategies and opening new avenues of research into the underlying biological mechanisms.

We acknowledge the colleagues Daniela Nicolini and Giulia Betta for their work on the production of genotypic data and the digitization of information for the database, respectively.

**KEYWORDS:** germplasm, database, grapevine
**SPECIES LIST:** *Vitis vinifera* ssp. vinifera, *Vitis vinifera* ssp. *sylvestris*, *Vitis* spp. *non vinifera*

# Simulations of local bioeconomy models for optimal management of agri-industrial waste



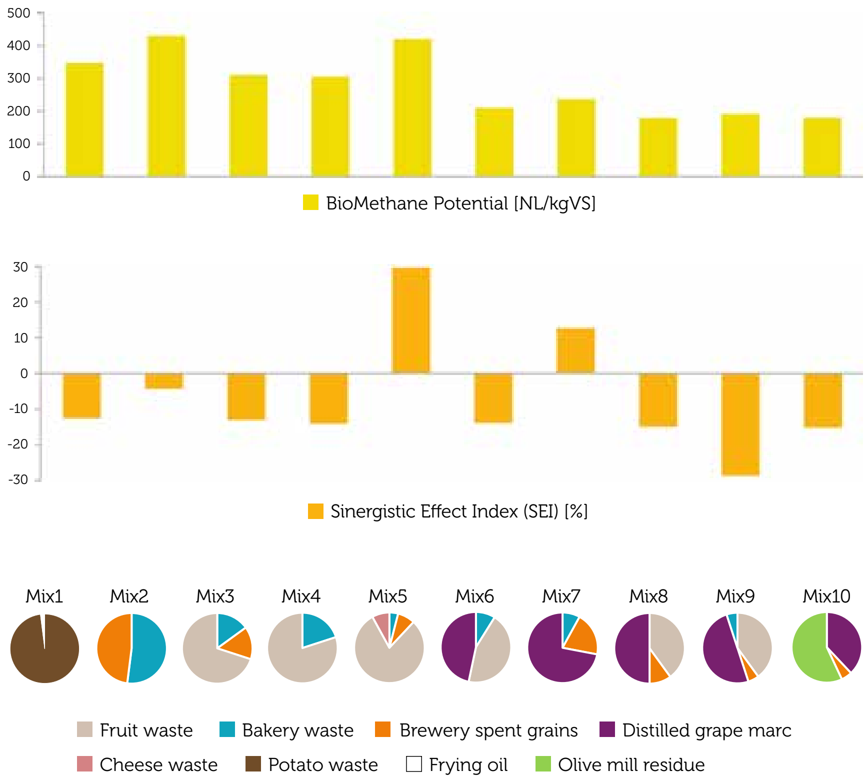
DONATO SCRINZI
DANIELA BONA

About 35% of the agro-industrial waste produced in Trentino is transported outside the Province, resulting in significant costs and climate-impacting emissions. A possible solution to optimize their on-site management is to integrate this waste into the livestock anaerobic digestion plants already present in many valleys. These plants produce renewable energy from biogas, a mixture of methane and $CO_2$, and the residue obtained, called digestate, is an excellent fertilizer and is characterized by a reduced impact on odours and greenhouse gas emissions after soil application. The co-digestion of agro-industrial waste with livestock effluents can have many benefits, both from an energetic and an agronomic point of view, but it is necessary to analyse the behaviour of the matrices, alone and mixed with each other.

For this reason, we have characterized the main types of agro-industrial waste alone and mixed in different proportions, tracing the representative quantities of some local realities, based on the previous studies by APRIE (Provincial Agency for Water Resources and Energy). Waste from the processing of fruit, beer, potatoes, the dairy industry, bakery, winemaking and distillation were tested in biomethanation trials to improve the estimates of biogas production in co-digestion with livestock slurry.

The specific biomethane production of these biomasses is relatively high, that is in the range of 270-850 compared to about 170 NL/kgVS for slurry, where NL = Normal Liters and kgVS = kg of volatile substance. However, it seems to be influenced by the type and relative percentages of mixed biomasses. This synergistic effect visible in the graph is of particular interest in order to plan the yields

Laboratory instrumentation used for Biomethane Potential tests (Gas Endeavour, BPC Instruments)



Specific biomethane production and synergistic effect of the waste mixes tested

and efficiency of co-digestion plants. These results constitute an important basis for future feasibility studies. Within the PNRR-INEST project, biomethane production scenarios in which waste flows are treated in local plants in co-digestion with livestock slurry will be modelled, as an alternative to delivery outside the Province.

In this way, biomethane production can be increased by up to 62%, the recovery of contained nitrogen and phosphorus could replace up to 12% of the synthetic fertilizers currently used in the province (ISTAT 2022 data), and up to 96% of emissions due to transportation out of the province can be avoided.

**KEYWORDS:** bioeconomy, agri-food, anaerobic digestion

# Gene functional studies and NGTs for next generation viticulture

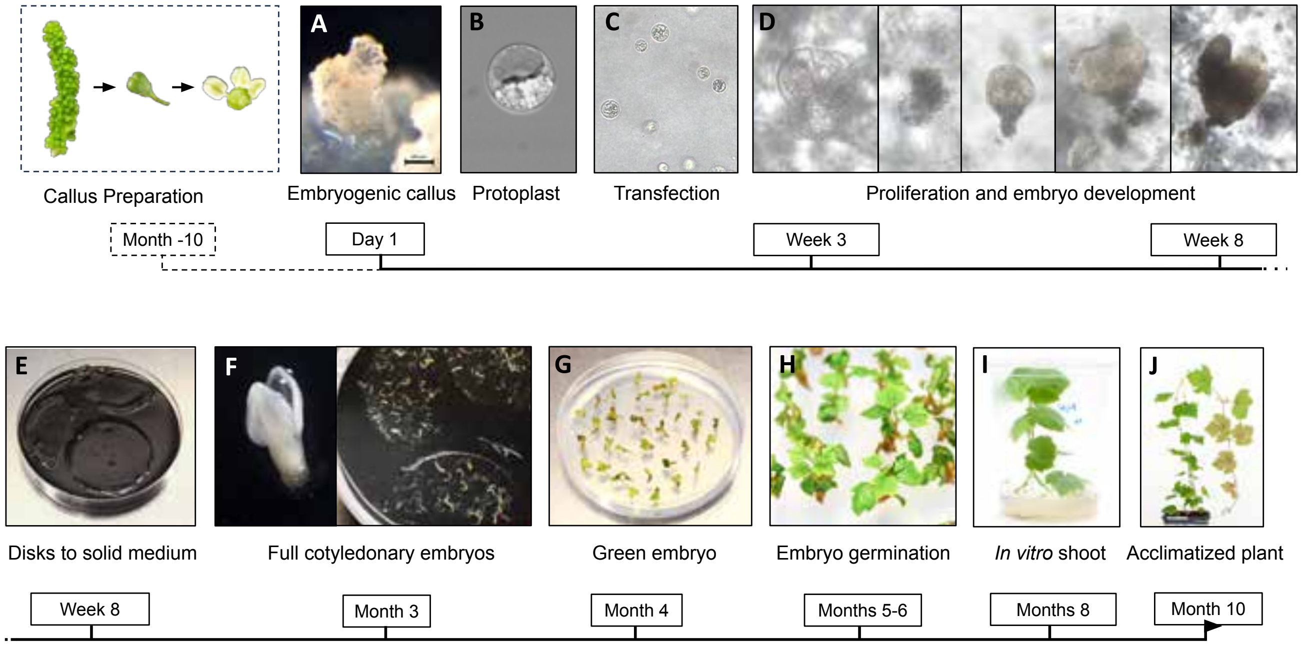
UMBERTO SALVAGNIN
LISA GIACOMELLI
LORENZA DALLA COSTA

*In vitro* propagation of grapevine plants regenerated from a single cell with the desired mutations

New Genomic Techniques (NGTs), including *CRISPR/Cas* gene editing, offer new opportunities to improve crops of agricultural interest. In viticulture, these technologies are invaluable tools for validating gene function and developing resilient grapevines, contributing to more sustainable viticulture practices capable of addressing the challenges posed by climate change and emerging pests. NGTs mimic natural mechanisms of generating genetic variation by introducing small, targeted mutations into the genome of cultivated grapevines. However, unlike natural processes, NGTs enable precise and specific genome modification.

To improve grapevines using gene editing, the first step is the identification of the genes that regulate the specific traits of interest. In our laboratories, these techniques have been used for example to study the function of the *EPFL9* gene. It has been demonstrated that silencing this gene significantly reduces stomatal density and leaf transpiration without a notable impact on photosynthesis, making plants more resilient under water stress conditions.

Another example involves the pectin methylesterase (*PME*) gene family, members of which are involved in cell wall modification and, consequently, in determining berry texture. Studies conducted at Fondazione Edmund Mach (FEM) by Jorge Lagreze and Giulia Malacarne have demonstrated that increased *VviPME10* expression, significantly induced in mature berries by *Botrytis cinerea* infection, enhances grapevine resistance to this fungal pathogen.



Scheme showing the NGT process from protoplasts in grapevine for functional genomic studies

Following validation of key candidate gene function, advanced genome editing technologies become powerful tools for modifying target varieties. According to the criteria outlined in the European Commission proposal on NGTs, these modified varieties could be considered equivalent to conventionally bred counterparts. To this end, our laboratories have developed a genome editing protocol that delivers the *CRISPR/Cas9* system as a ribonucleoprotein (RNP) complex - thus avoiding the use of exogenous DNA - into protoplasts (grapevine cells devoid of cell walls), from which whole plants can then be regenerated.

Researchers at FEM are applying these cutting-edge technologies to enhance resistance to diseases (e.g., powdery mildew and downy mildew) and tolerance to environmental stresses (e.g., water stress) in grapevine varieties of national and local importance.

**KEYWORDS:** grapevine, NGTs, sustainability
**SPECIES LIST:** *Vitis vinifera, Erysiphe necator, Plasmopara viticola, Botrytis cinerea*

# Apple pomace residues offer opportunities for a circular economy

SARA BERTOLINI
SILVIA SILVESTRI

Soil samples in a climate chamber

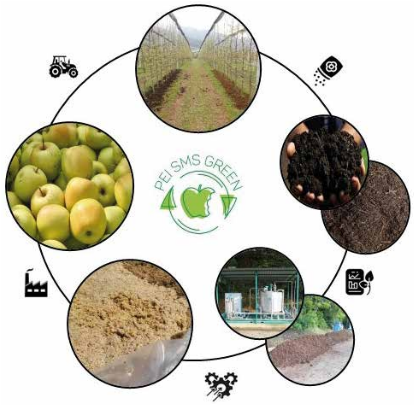
Within the growing focus on circularity and ecological transition, agriculture plays a crucial role due to its strong connection to local areas and its potential for creating integrated local production systems. The project *SMS Green-Bioeconomia circolare: sostenibilità melo-suolo*, started in 2022 and currently in progress, aims to close the apple cultivation, production and transformation chain through the recovery of apple pomace, the residue generated from industrial apple processing. Apple pomace constitutes approximately 20–30% of the fresh fruit weight, and chemical and physical properties make it suitable for cascading various technological processes. Specifically, the total solids content of apple pomace (approximately 28%, of which 98% are volatile solids), the presence of cellulose and hemicellulose (approximately 47% of total fibers), and its specific methane production (314 Nm3/kgSV) make it a suitable feedstock for biological processes such as composting and anaerobic digestion. It can also be used for hydrothermal carbonization, a thermochemical process to produce a high-carbon material tested by the Green Processes Engineering Group at the University of Trento.

Four soil types have been obtained from the processes tested: compost, digestate, hydrochar, and hydrochar co-compost. Characterization tests are underway to assess the agro-environmental properties of these soils and to identify their effective and efficient use for managing the fertility of Trentino's orchards.

The processes considered in the

project can be considered effective strategies for reducing the significant volumes of apple residues. For example, co-digestion produces high quality biogas that can be used in Trentino. In addition, by addressing apple pomace to the local anaerobic digestion plants, farmers avoid high transportation costs, as this pomace is currently treated outside Trentino. The use of other products such as digestate, compost, and co-compost in local agriculture can also contribute to a better management of soil organic matter in mountain orchards, creating a valuable and sustainable local supply chain in line with European policies.

Our next experiments will focus on evaluating the effects of the amendments on soil microbial communities and nutrient dynamics to better understand the benefits of these quality organic fertilizers as valid substitutes for synthetic fertilizers.



"SMS Green" project synthesis

On the left, the biomass being tested (apple pomace) and on the right, a compost pile (one of the processes tested)

**KEYWORDS**: bioeconomy, transformation residues, organic amendments
**SPECIES LIST**: *Malus x domestica*

Non-destructive analysis of fruit volatile organic compounds

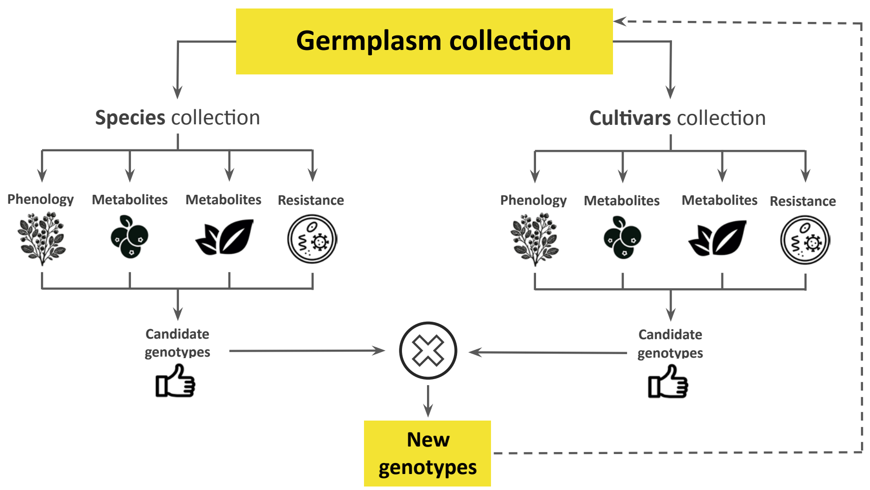
# Multi-omic assessment of phenotypic diversity within berry fruit germplasm collections

BRIAN FARNETI
MARTA DEGASPERI
LARA GIONGO

This research focuses on characterizing the qualitative and nutraceutical attributes of genetic resources in two important berry crops: blueberry and raspberry, with two primary objectives: obtaining detailed metabolomic profiles for future gene association studies, and identifying optimal genotypes for use as parental lines in future breeding programs.

For raspberry (*Rubus idaeus*), we investigated how ripening and storage processes affect the composition of volatile organic compounds (VOCs). The comprehensive aromatic profile characterization employed two complementary analytical techniques: PTR-ToF-MS (Proton Transfer Reaction - Time of Flight - Mass Spectrometer) and GC-MS (Gas Chromatography-Mass Spectrometry) with Solid Phase Microextraction (SPME). Results revealed substantial qualitative variability within the germplasm collection, and identified promising cultivars for genetic improvement of fruit organoleptic characteristics. A particularly significant finding concerns the commercial practice of early fruit harvesting: while this technique enhances storability, it irreversibly compromises the fruit's aromatic profile, leading to a substantial reduction in monoterpenes and norisoprenoids, two volatile compound classes crucial for raspberry's characteristic aroma.

For blueberry (*Vaccinium* spp.), we analyzed 77 cultivars representing the genetic diversity of the collection. The study involved systematic analysis of primary and secondary metabolites and VOCs in both leaves



Schematic illustration of the phenological and multi-omic evaluation process used to identify and generate new genetic variability in the blueberry germplasm collection

and fruits, evaluated at harvest and after cold storage. The metabolomic characterization utilized state-of-the-art analytical techniques: PTR-ToF-MS and GC-MS for VOCs, UPLC-MS/MS for polyphenols and lipids, and NMR spectroscopy for primary metabolites. This integrated approach generated a comprehensive metabolomic dataset that we believe will be instrumental in optimizing future breeding programs aimed at improving both organoleptic qualities and resistance to abiotic and biotic stresses.

Acknowledgements: Iuliia Khomenko and Emanuela Betta (Sensory Quality unit), Domenico Masuero (Metabolomics unit), and Pavel Solovyev (Traceability unit) for their support in the biochemical analysis of fruits.

**KEYWORDS:** primary metabolites, secondary metabolites, VOCs
**SPECIES LIST:** *Rubus idaeus, Vaccinium* spp.

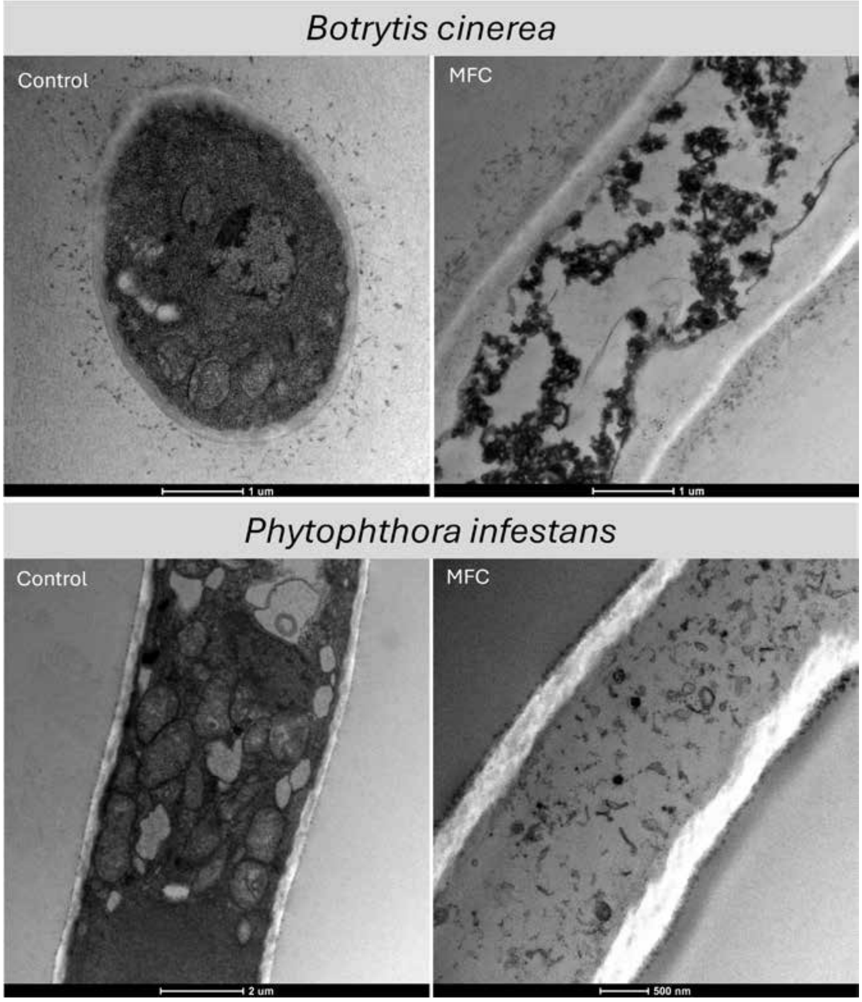
# Towards a more sustainable agriculture: investigating the action of an innovative fungicide

SOFIA MONTANARI
CLAUDIA MARIA OLIVEIRA LONGA

Leaf affected by Grapevine Downy Mildew

The use of traditional synthetic pesticides to control plant diseases is often associated with risks to human health and the environment. Consequently, research is now focusing on developing more sustainable alternatives. New fungicides based on natural substances derived from plants, animals, and microorganisms are emerging as valid alternatives to chemical pesticides, responding to the growing demand for healthier and safer food. Knowing how fungicides act at the cellular level is crucial to preventing the development of resistance, limiting the selection of more aggressive pathogen strains. Furthermore, this understanding allows for the optimization of treatment effectiveness, reducing excessive use of chemicals and ensuring safe protection for the environment and human health.

One example of a fungicide under development is TEMAP (patent BE1026779B), in collaboration with BI-PA, a Belgian SME that develops innovative solutions to crop protection. This fungicide is based on tri-methyl-ethanolamine pelargonate (CP), a molecule derived from pelargonic acid neutralized with choline, which is free from phytotoxicity to plants but maintains effective fungicidal activity. To investigate the mechanism of action of this new active ingredient, targeted experiments were designed to evaluate its effectiveness in different stages of the life cycle of *Botrytis cinerea* and *Phytophthora infestans*. Specifically, two crucial aspects were examined: mycelial growth and spore



Effect of choline pelargonate on *Botrytis cinerea* and *Phytophthora infestans* ultrastructure, showing evidence of damage to cells

germination. The mycelial growth experiment assessed how CP affects the pathogen's proliferation during the active developmental phase, while the spore germination analysis tested its effectiveness in blocking the pathogen's spread.

These experiments were followed by more in-depth investigations to analyze the cellular damage induced by CP. Electrolyte release, an indicator of plasma membrane damage, was studied, and electron microscopy tools were used to observe any morphological changes in the treated cells, such as plasmolysis or vacuolization. Additionally, to understand how CP modifies the structure of cellular membranes, NMR spectroscopy was employed to analyze changes in the lipid composition of the membranes. These experiments aim to thoroughly explore the mechanism of action of CP and determine whether there are differences in the responses of fungi versus oomycetes, to optimize the fungicide's effectiveness.

Our results will allow CP to be used in a targeted and efficient manner, promoting sustainable agricultural practices that protect the environment and human health, while reducing dependence on chemical pesticides.

The authors wish to thank Prof. Michele Perazzolli (University of Trento) for his valuable contribution.

**KEYWORDS:** choline pelargonate, biofungicide, phytopathogens
**SPECIES LIST:** *Botrytis cinerea, Phytophthora infestans, Vitis vinifera*

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