

RESEARCH AND INNOVATION CENTRE

REPORT

2017/2018

AGRICULTURE
NUTRITION
ENVIRONMENT



FONDAZIONE
EDMUND
MACH
CENTRO RICERCA
e INNOVAZIONE

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CENTRO RICERCA
e INNOVAZIONE

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PRESENTATION

The drive towards sustainability - economic, environmental and social - is the basic ambition guiding all research, experimentation, technological transfer and education programs at the Fondazione Edmund Mach.

Unfortunately, the word 'sustainability' has been so misused that it has lost its original meaning. Put simply, the natural resources underlying our basic needs, such as pollination and irrigation, are indeed finite; thus if we exploit them we must also allow their regeneration. Such is the underlying basis of sustainability. Thus, the application of 'sustainable' practices depends on the development of systems providing the needs of the present generation without compromising those of future. In alpine areas such as Trentino, the concept of sustainability requires a particularly delicate discussion in order to balance land use and food production.

At the FEM Centre for Research and Innovation (CRI), this cycle of exploitation and renewal inherent in the concept of sustainability is mirrored in the close link between research, innovation and technology transfer. Leafing through this Report, it is clear how we are attempting to connect these components together. Page after page reveals how our researchers strive to improve the major Trentino crops, apple and grape, as well as other increasingly valuable varieties of soft fruit and walnut. Innovative projects have also been initiated in the food and health sectors, closely linked to local businesses, also thanks to traceability technologies. Environment research concerns the study of wild species in natural alpine habitats, and the impacts of change in biodiversity on ecosystem function. This interdisciplinary approach to human, animal and environmental health is a direct reference to the '*OneHealth*' concept, and a key theme of various networks and Joint Research Initiatives in which we participate, for example through the European Food Safety Authority and Climate KIC. In order for CRI itself to be sustainable, our research has also aimed at reaching several ambitious goals: maintaining a high level of scientific productivity, as confirmed by ANVUR in 2017; improving our capacity to win competitive funding from external sources for our research ideas; provide services to agri-food and environmental sectors; and last but not least, patent and market our innovations.

In addition to these achievements, we have reached another milestone in bringing together research, innovation and sustainability with teaching, beneficial to the local area. In the last edition of the CRI Report, we mentioned the imminent inauguration of the Agriculture, Food and Environment Centre (C3A) in collaboration with the University of Trento. It was early 2017, and we were finalizing negotiations. Since then the C3A has made solid progress in establishing a series of educational opportunities, from secondary school, to tertiary courses, to doctorate degrees, in another first of its kind in Italy.



Andrea Segré

President

Fondazione Edmund Mach



145 years of tradition and innovation

2019 marks the 145th anniversary of the Fondazione E. Mach, a milestone that will be proudly celebrated later in the year with official ceremonies and a public 'Open Day'. Our enlightened founder, Edmund Mach, had the intuition to establish this institute, integrating teaching with field and laboratory research, to enhance the development of local agriculture. This successful approach has been carried forward to the present day, and will remain the underlying philosophy guiding our way to future innovation.

Synergy between the three main Centres – focused on research, education, and technology transfer – make this institution unique both nationally and internationally.

At the Research and Innovation Centre, cutting-edge scientific research is carried out in collaboration with universities worldwide, as well as with private enterprise. Using the most advanced technologies, we search for innovative solutions and competitive products, for the benefit of both local and international economies. Our research must be relevant to the local economy, yet also internationally recognized and evaluated in comparison with other similar entities to guarantee the highest standards of scientific output.

Next to the Research and Innovation Centre, the Technology Transfer Centre supplies critical services to the agroforestry system in Trentino, through its expertise in applied research and consultancy, allowing the widespread dissemination of technical information to farmers. Last but not least, the various curricula offered at the Training and Education Centre are profoundly integrated with the working world, as witnessed by several national and international acknowledgements.



Sergio Menapace
General Director
Fondazione Edmund Mach

The Research and Innovation Centre



Annapaola Rizzoli
Director
of Research and Innovation Centre
Fondazione Edmund Mach

The main scientific research programs of the Fondazione Edmund Mach are developed at the Research and Innovation Centre (CRI). In recent years, CRI has attained a solid reputation in agrifood and environmental research both nationally and internationally, as confirmed by the outstanding results of the recent national evaluation of scientific output led by the *Agenzia Nazionale di Valutazione della Ricerca* (ANVUR).

CRI focuses on three macro areas of interest: agriculture, food and environment. These sectors have been chosen as particularly strategic in the current socio-economic and climatic upheaval, which poses rising challenges for modest jurisdictions like Trentino. In order to sustain long-term growth in the bioeconomy, this alpine area must define a unique and recognizable identity, based on anticipatory and resilient socio-economic models that promote its natural capital, local products, and the general well-being that such traditional rural landscapes offer. Scientific research will help support the dynamic interactions between the generation of new scientific knowledge, and transformation of the results into practical applications, including the management of natural resources. This innovation supply chain, from science to practical applications, will secure the sustainability of production systems and the protection of human health and animal welfare, as well as the preservation of characteristic natural and agricultural environments. This interdependent and interdisciplinary approach explains why CRI is considered the first “*OneHealth Centre*” in Italy, typified by the motto “*#trentinoterradellasalute*”.

One particular challenge with potentially significant long-term effects caused by the current economic downturn is the reduction in financial resources devoted to research. This has required a partial paradigm shift in the approach adopted by CRI research staff, since a greater proportion of funds must now be obtained from external sources. This requires not only increased awareness of frontier research issues, but also the ability to collaborate with local stakeholders who benefit from such research. Thus far, numerous such projects have been activated, ranging from the creation of new synergies with the members of STAR (Trentino System of Higher Education and Research), to the establishment of the new Agriculture, Food and Environment Centre (C3A) with the University of Trento, and the EPILAB with the Bruno Kessler Foundation. In addition, new international networks of excellence have been funded within the European Region Tyrol - South Tyrol – Trentino (EUREGIO), the EU strategy for the Alpine Region (EUSALP), and beyond.

This Report focuses on the most important scientific results obtained in 2017-2018, with a limited number of in depth articles on topics of general interest, followed by brief but interesting research ‘highlights’. Curious readers are invited to turn to our website for more detailed content (www.fmach.it).

As well as underlining the importance of our recent achievements, I very much hope that this Report communicates the passion and enthusiasm that the CRI community demonstrates every day in its laboratories, field sites, and experimental orchards and vineyards. This dedication is the true wealth of the Centre, unique in Italy.

STRUCTURE

Since 2008 the Experimental station has had a new organisational structure and is now known as the Research and Innovation Centre (CRI). Originally, it was organized in specialised research Areas covering the fields of Agriculture, Food and the Environment. In January 2011, a new organizational restructuring concerned the Centre, and resulted in a new arrangement based on a Research consortium (in participation with the National Research Council) and five Departments, which in turn are organized in Research Groups and Technological Platforms. CRI research focuses on: genetics and genomics of fruit plants, agri-food and nutritional quality, biodiversity and molecular ecology, sustainable agro-ecosystems and bioresources, and computational biology. These research areas were selected to meet the interests and needs of local economy. A brand new Campus with 200 m² of greenhouses, 30 hectares of breeding fields and state of the art technologies are other relevant traits.

Pioneering organisations, a young and dynamic environment, international researchers, collaborations with universities and institutions throughout the world, and the institution of high-level specialised training initiatives: all these together place the Centre in a global context, which encourages the exchange of ideas and the development of innovation and produces internationally-recognised results.



Sustainable Ecosystems and Bioresources Department (DASB)

Coordinator

Damiano Gianelle

In order to design and develop systems that can sustain human welfare and preserve natural capital for future generations, knowledge of the complex relationships and interactions in ecosystems, as well as the interpretation of their reactions to disturbances, are crucial. Therefore, the research of this Department promotes sustainable use of natural and agricultural ecosystems to link the needs of modern society and the protection of the environment. Projects focus on innovative technolo-

gies based on low impact processes to improve the quality and production of modern society. Thanks to the application of innovative approaches in chemistry, physiology and molecular biology, careful planning of resources use can be achieved and, in the case of ecosystem disturbance caused by anthropic activity, counterbalancing actions can be put on place. The understanding of the mechanisms regulating the stability of ecosystems will assist their protection and the sustainable development of agriculture.

Forest ecology and biogeochemical cycles

This research unit studies the interactions between vegetation, soil and climate in order to evaluate the effects of climate change on ecosystems and to identify the most appropriate management methods to mitigate them. We develop different biogeochemical models and innovative approaches on various scales, from ecosystem to regional mainly using remote data collection (e.g. Lidar, hyperspectral fluorescence, eddy covariance, phenocam).

Agricultural Entomology

We study the biological mechanisms of perception and processing of the sensory stimuli involved in both intra- and inter-specific communication of the most economically important insect pests, to understand how we may manipulate their behavior and provide new pest control methods, and with the long- term scope of agro-ecosystems and biodiversity protection. We make use of a multidisciplinary approach, from molecular biology and comparative genomics to neurobiology and physiology, to behavioral field observations.

Hydrobiology

Our research focuses on biodiversity and long-term ecological evolution of lakes and rivers in the Alpine and Perialpine region. In particular, we study the effects induced by climate change, eutrophication and changes in hydrological and thermal regimes on aquatic ecosystems and communities. For this purpose, in addition to traditional limnology, we also use a multidisciplinary approach based on metabolomics, molecular biology and NGS, stable isotopes and high frequency data, and for catchment level studies, eco-hydraulic analyses.

Plant pathology and applied microbiology

Our mission is reducing chemical inputs in plant protection products and fertilizers, by developing low-risk active substances and products based on natural microorganisms and their metabolites (such as biofungicides, biostimulators, and/or biofertilizers), plant antifungal molecules and plant induced resistance processes. Mechanisms of action are characterized by multidisciplinary studies of physiology, microbiology and molecular biology. Products are developed and tested in collaboration with companies, to validate efficacy and safety for human health and the environment.

Biodiversity and Molecular Ecology Department (DBEM)

A high level of 'biodiversity', defined as the number of species or total genetic variability in a population or community of plants or animals, is crucial to all species for maintaining ecosystem function, resilience in the face of global change, and resistance to pathogen and parasite invasion. In order to maintain and manage this natural resource, the Department measures diversity at all levels – genetic, species and ecosystem – to understand the basic processes of adaptation, speciation and extinction. We also study the impact of global

change on biodiversity and ecosystem function, the presence of autochthonous and invasive species, and on human and veterinary health. Using a combination of state-of-the-art molecular technologies (e.g. molecular screening, genomics, metagenomics, transcriptomics), as well as biollogging and spatial/temporal modelling and statistics, we formulate hypotheses about future scenarios, and suggest how to improve strategies for managing natural areas, to maintain genetic and species diversity, and as a consequence, also human well-being.

Ecogenomics

The mission of the Ecogenomics Unit is the characterization and study of natural plant biodiversity, with particular reference to Trentino and the alpine environment. The integration of taxonomy, phylogenetics, physiology and molecular ecology in a comparative and genomic framework allows us to elucidate the main mechanisms underlying the generation and maintenance of natural biodiversity, and to estimate how the alpine flora could evolve and adapt in the medium to long-term to environmental stress and global change, but also how changes in plant diversity can affect human health, and the production of biofuels.

Applied Ecology

The mission of the Applied Ecology Unit is to study the effects of global change and biodiversity loss on host-pathogen dynamics (especially tick, mosquito and rodent borne zoonoses), including changes in host and pathogen distribution and future scenarios of disease spread. These objectives are pursued with a multi-disciplinary approach that combines molecular epidemiology, movement ecology, spatial ecology, geostatistics, mathematical modelling, statistics and big data analysis that requires the use of innovative IT platforms.

Conservation Genetics

This research unit focuses on the study of patterns of genetic biodiversity in animal populations, including that of their microbiota, from amphibians (bio-indicators of ecosystem health) to large carnivores (umbrella species). We provide scientific support for management decisions aiming at conserving and maintaining these natural resources, as well as estimating how the distribution of genetic variability changes in time and space in relation to environmental factors and human activities. We are specialized in the analysis of non-invasive and poor quality DNA samples of non-model species, and collaborate with many local governments, as well as and private partners. Our research has recognized local and global impacts on animal conservation, sport fishing and game management, tourism, and livestock farming.

Coordinator
Heidi C. Hauffe

Genomics and Biology of Fruit Crop Department (DGBPF)

Coordinator

Claudio Moser

The Department has long-term experience in elucidating the genomics of fruit crops (grape, apple, pear, strawberry and raspberry) and marker-assisted breeding. Modern technologies have been optimized in order to lead the whole-genome sequencing of apple and grape (economically the most important Trentino crops), and to participate to other international projects on pear, raspberry, olive and strawberry). These important databases are currently being used to refine the listed genomes and resequencing varieties (genotyping and RNAseq) for various purposes. Additional "omics" technologies

(e.g. transcriptomics, metabolomics) aim to dissect biological events, with particular attention to fruit and flower development, plant-pathogen interaction, plant habitus, dormancy break, post-harvest as other processes related to plant management, plant productivity and fruit shelf-life. Holistic approaches strongly support crop breeding, and high-throughput phenotypic analysis has been set up to implement marker development. Finally, the study of small and microRNA has been recently introduced to identify the role that these molecules play in various biological processes.

Plant Biology and Physiology

We study the function of genes found in grape, apple and strawberry genomes to understand how they direct the biology and physiology of these fruit crops. The ultimate aim is to identify genes associated with resistance and quality for improving the production of new varieties in grape and Rosaceae, either by assisted breeding or by biotechnological means, as well as generating new knowledge to improve crop management in the field.

Genetics and fruit breeding

This research unit aims to understand the genetic and biological bases of the most economically interesting traits in apple and other fruit crops belonging to the Rosaceae family, such as strawberry, raspberry, blueberry, cranberry, apricot and cherry. Genomic knowledge will help identify genes for traits that may be useful in the development of new varieties and their breeding, from plant management to fruit preservation.

Grapevine genetics and breeding

This research unit aims to provide new grapevine cultivars and genetic knowledge for sustainable and smart viticultural production under various climate change scenarios. We investigate associations between genetic and phenotypic variation in segregating populations, germplasm collections and somatic variants to gain a better understanding of resistance to several economically damaging diseases such as downy and powdery mildews; resilience factors to abiotic stress (such as drought) present in *Vitis* spp.; and quality traits that increase the economic value of wine and table grapes, such as flavonoid composition, aromatic precursors and seedlessness. Functional validation of candidate genes, oriented to next-generation breeding, is performed by genetically engineering *microvine* plants. In parallel, classical breeding methods for resistance and wine quality is conducted alongside molecular-assisted programmes.

Genomics and advanced biology

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This research unit performs functional genomic studies of grape and apple to increase resistance to disease and improve fruit quality. We are committed to developing and applying 'new plant breeding technologies' in grape and apple in view of improving the classical protocols of genetic engineering routinely used for different grapevine and apple cultivars. Moreover, the unit has developed innovative analytical methods for the characterization of the genetically modified lines, for the ex-vitro cultivation, plant phenotyping (thanks to the equipment of a modern greenhouse) and the sequencing of entire genomes, the transcriptome studies, gene expression analysis and metagenomics through the most advanced technology platforms of Next Generation sequencing (Illumina, 454, Life technologies).

Food Quality and Nutrition Department (DQAN)

Research activities are carried out using new study methods, based on advanced technological platforms and infrastructures (metabolomics, stable isotopes, sensory panels, innovation incubators), and by adopting an integrated approach of a multidisciplinary nature. Innovative technical platforms and a systematic approach make it possible to carry out studies of fundamental significance, generate new study methods and obtain improved knowledge of agri-food products. The Department aims to support product and processing innovation based on

knowledge of the food and agriculture sector. It promotes the use of research in the fields of biological sciences, consumer science, metabolomics and traceability as tools for innovative production. We provide a human nutritional research nucleus for the emerging strategic area of nutrigenomics. We carry out research both in the laboratory and at the pilot project and company levels providing scientific support for the creation of products and processes with added value, based on knowledge, ideas and innovation.

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Coordinator
Kieran Tuohy

Metabolomics

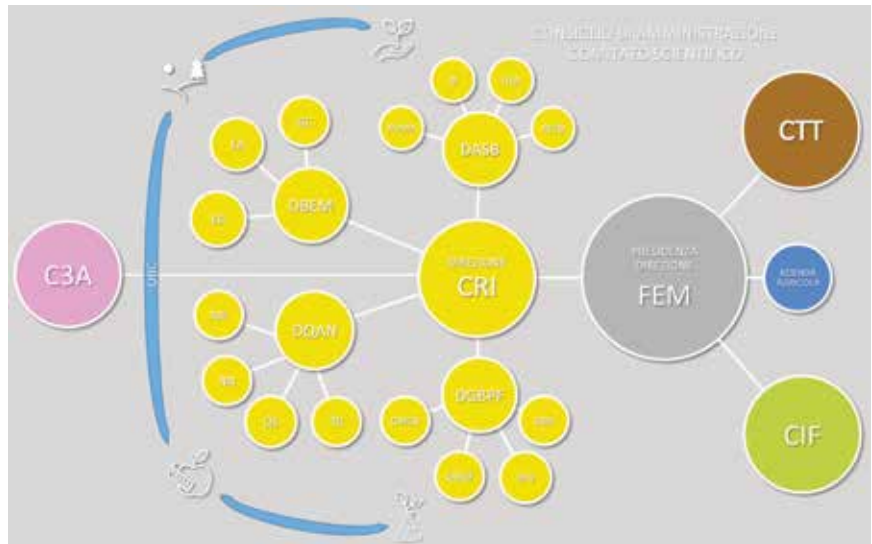
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The metabolomics unit generates new methods of study and knowledge about nutritionally and sensorially significant compounds, even participating in nutritional intervention studies, tracing the path of natural bioactive compounds inside the human body. Such information has the ultimate goal of improving the understanding of the impact of diet on human health and the production of higher value-added foods. It pursues the study of the molecular mechanisms that underlie the biosynthesis of secondary compounds in plants, knowledge of which is essential to progress in the field of biotechnology applied to plants and micro-organisms and breeding programs.

Nutrition and Nutrigenomics

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This research unit aims to measure how food and diet modulates host health and protects against chronic disease through interactions with the gut microbiota. We examine how microorganisms impact on food nutritional composition and also, how diet shapes and regulates the gut microbiome. We have a special interest in fermented foods (e.g. fermented dairy products), whole plant foods, their bioactive fractions (fibers, prebiotics and polyphenols) and probiotics, and aim to provide mechanistic insight into how these functional foods work.



Sensory quality

This research unit aims to understand the mechanisms underlying the perceived quality of food and the physiological and psychological factors that influence perception, preference development and consumer behavior. Moreover, the unit offers support to the food choices associated with health and wellness. Finally, it maintains and develops a sensory / instrumental / statistic facility to support innovation in the agroindustry.

Traceability

The unit is a recognized centre of competence for the development of research in the field of the application of stable isotope ratios analysis of bio-elements (H, C, N, O, S).

It provides fundamental scientific and technological support to national and European research programmes concerning food traceability, aiming to enhance and safeguard products of certified origin within the context of the global market. Research activity also supports projects in the fields of ecology, hydrology, plant physiology and paleo-climatology.

Computational Biology Unit (UBC)

Responsible

Claudio Donati

The advent of -omics technologies is changing biology and life sciences from descriptive disciplines into data-intensive sciences. This research unit is the reference for the analysis and statistical modeling of data, with a particular emphasis on -omics technologies. The unit brings together original methodological research in collaboration with the other CRI research groups on numerous

projects. The main research areas are: genomics (in particular of plants and bacteria), metagenomics, metabolomics, transcriptomics, statistical modeling and data integration. In addition, this unit manages the High Performance Computing infrastructure of CRI and guarantees the storage and traceability of data generated by high throughput technologies.

FEM International Doctoral Program initiative

FEM International Doctoral Program launched by the CRI – One Health Centre, hosts 46 students coming from around the world (Pakistan, India, China, Lebanon, Germany, Tunisia, Montenegro, Hungary, Spain, Romania, France, Slovenia, Great Britain and France) and studying at Universities in Italy and abroad. Research projects focus on the main sectors of interest of Fondazione Mach, Food Quality and Nutrition area, Agriculture and Environment.

FEM International Doctoral Program Initiative was born in 2012 from the merger between the collaboration network in Genomics and Molecular Physiology of Fruit (GMPF) and the training activities of other strategic sectors of the Foundation. Right from the start the School presented itself as one of the most ambitious and innovative initiatives launched by FEM-CRI in recent years, activating about 180 scholarships and creating a solid international collaboration network both with the academic world, with research institutions and private sector. It is precisely towards this last sector that over the last few months the Foundation's effort has been concentrated, which takes advantage of the

opportunity to give a direct impact on the economy of research and experimental activities. Raising funds for a dozen industrial grants is a clear example of the validity of our approach. FEM has created a solid network of international collaboration with the academic world, research institutions and the private sector. The participation of companies in the co-financing of scholarships and the availability to host the PhD student in their laboratories as hosting labs favor the exchanges between the public sector and the private sector in the research field and thus facilitate inclusion of the PhD student in the industrial world. Mobility and training experiences at partner institutions in Italy and abroad contribute to the development of an attractive skills portfolio. The success of the doctoral program is confirmed by the high employment rate for our PhD students employed. 80% of scholarship holders have been hired by prestigious institutions such as CNR IBIMET, Aarhus University (Denmark), University of Reading (Great Britain), Provincia Autonoma di Trento – Agenzia provinciale per la tutela dell'ambiente (APPA), UNESCO-IHE, (Delft, Paesi Bassi), Tech-



nische Universitaet Braunschweig (Germany) PlantEdit (Irlanda), ETH, Zurich - Department of Biology, Institute of Biochemistry (Switzerland). The new doctoral programs, already with those launched in 2018, will be enhanced in the context of the other

initiatives undertaken by FEM in recent years (see the C3A and Euregio presented below). These, in particular, will strengthen the organizational architecture (with the creation of further specific schools) and mobility opportunities in the European area.

C3A Centre

The C3A is based on an agreement signed by Fondazione Edmund Mach and the University of Trento Foundation and has become fully operational since 2017. The model on which the C3A is based represents a major novelty: the two institutions, while maintaining their own individuality and independence, share part of the resources to reach a common goal: the increase of the critical mass, the improvement of the efficiency in the use of resources and the establishment of important synergies between the research and education sectors of the two institutions. The novelty of this operating model confirms Trentino as a proficient laboratory of ideas, innovative solutions and dynamism with a strong international attitude. The areas of research and training are those of high value and smart agriculture,

focusing on quality food production, while aiming at reducing the environmental impact and sustainable use of resources.

The C3A has already activated the bachelor course in viticulture and enology, which gives the opportunity to obtain the double degree with the University of Geisenheim in Germany and the master course in environmental meteorology, activated in collaboration with the Department of physics and the Department of civil, environmental and mechanical engineering of the University of Trento, which leads to a double degree with the University of Innsbruck in Austria. The master course in Agri-food innovation management and the PhD school in Agrifood and environmental sciences are going to be activated soon.





Euregio Center on food

Since 2018 the Edmund Mach Foundation has been hosting the Euregio competence center set up as part of the international project “Environment, Food & Health” in order to tackle the socio-economic challenges related to population ageing and overweight.

Excellent public health care, a healthy lifestyle compared to the national and European context, a valuable environmental context, the presence of qualified research institutions and, last but not least, a widespread production of foods that protect against diseases linked to obesity and old age are in fact elements common to the three territories of the Euregio Trentino, South Tyrol and Tyrol. The “Environment, Food & Health” project carried out by FEM in collaboration with the Universities of Trento, Bolzano, Innsbruck and Hall, the provincial companies for the health services of Trento and Bolzano, Eurac and the Laimburg

Research Centre, starts from this awareness to promote high-level research aimed at understanding how diet and lifestyles act on the risk of metabolic diseases (in particular on obesity) and at the same time enhancing local products with high nutritional functionality, also through educational initiatives.







AGRICULTURE



The molecular mechanism of superficial scald appearance in apple is revealed by a new multi-omics approach

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NICOLA BUSATTO
BRIAN FARNETI
FRANCO BIASOLI
URSKA VRHOVSEK
FABRIZIO COSTA

In recent decades, fruit storage techniques have been substantially improved, leading to an increased apple storability and shelf life, in parallel, limiting the development of post-harvest physiopathy that can compromise fruit quality improved by the ripening process. The full understanding of the molecular mechanisms regulating the fruit ripening progression represents a key element in controlling the appearance of cold-storage-related metabolic disorders. Among them, superficial scald is one of the most important, due to the development of large brown areas on the skin of particular apple varieties (eg. Granny Smith, Pink Lady, Red Delicious). This event occurs during the cold storage, and leads to general decay and quality drop-off. Until now the accumulation of compounds deriving from the autoxidation of α -farnesene, the most abundant sesquiterpene synthesized by apple was believed as the etiological cause of superficial scald in apple. Instead, recent studies carried out by the Genetic and Fruit Breeding Unit (Department of Genomics and Biology of Fruit Crops) in collaboration with the Metabolomics and Sensor Quality Units (Department of Food Quality and Nutrition) at FEM have demonstrated that superficial scald should

be considered a typical chilling injury and that α -farnesene oxidation represents a relatively minor phenomenon resulting from the cold stress. Our research exploited an integrated strategy based on transcriptomic and metabolomic approaches shedding light on the molecular details underlying the scald resistance phenotype induced by the application of 1-methyl-cyclopropene. This ethylene competitor is widely used for prolonging the fruit storability and shelf life, due to its ability to slow down the climacteric fruit ripening process. Moreover, this treatment fosters a transcriptional reprogramming that leads to an overexpression of a consistent set of genes involved in cold acclimation processes and cold resistance in the tissues normally affected by the superficial scald appearance. Moreover, in apple skin, 1-MCP treatment leads to the synthesis and accumulation of sorbitol, a polyol with a well-known cryoprotectant role, that mitigates the effect of the cold temperature used during the storage period. This new scientific knowledge together with the availability of genetic data, will allow us to design suitable molecular markers to generate new scald-resistant varieties through a Marker Assisted Breeding approach.





First fruit from breeding: release of new improved apple and grapevine varieties

The breeding of a new type of fruit crop takes 15-20 years, and requires large plots of land and extensive manual labour, yet results in improved varieties to add to the commercial market. In 2003 the two varieties Cripps Pink and Caudle Carousel were selected here at FEM. Breeding consists in crossing mother- and father plants bearing specific and possibly complementary traits, followed by a selection of seedlings grown from the seeds of the mother plant. Among the 560 seedlings obtained from these varieties, after 5 years only one passed the evaluation of the plants in the juvenile phase. This new variety, named *FEM 16*, presented interesting characteristics; therefore, large field trials were carried out followed by the application for a

European patent. The medium-large fruit of *FEM 16* ripen around mid October, with a background color of pale green-yellow, and an upper color consisting of red stripes on a orange-red background; a large fraction of fruits are completely red. The flesh is firm, crispy and juicy, with a well balanced acidic and aromatic taste. All features are well preserved even after a long storage period even without post-harvest treatments. As well as being an attractive apple, *FEM 16* has a long shelf-life, and a storability longer than 12 months under controlled conditions.

Similarly, the Grape Breeding Unit needed 16 years to obtain and register the grapevine varieties Iasma Eco 1, 2, 3 and 4. These varieties are of great commercial interest due to their

PIERLUIGI MAGNAGO
TIZIANO TOMASI
MARCO STEFANINI

low susceptibility to Botrytis and the excellent quality of the grapes, and were registered in 2014 in the National Catalogue of wine varieties. Recently, they have also been included in the list of recognized commercial cultivars in several Italian provinces (among them Trento and Verona) and they are currently available for purchase at the Consorzio Innovazione Vite (CIVIT). The red varieties Iasma Eco 1 and Iasma Eco 2 (obtained from a cross between Teroldego and Lagre-

in) are very rich in anthocyanins and have an optimal sugar/acid ratio. The resulting wines are full bodied with a good tannin content, and pleasant flower and fruit aromas. Instead, the white varieties Iasma Eco 3 and Iasma Eco 4 (obtained from a cross between Moscato Ottonel and Malvasia di Candia) have a rather complex bouquet, which is not unexpected, since the same aromas can be perceived in the wines made from the grape varieties of origin.

The genetic control of grape aroma confirmed in the model system “microvine”

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 LAURA COSTANTINI
 LORENZA DALLA COSTA
 FRANCESCO EMANUELLI
 MASSIMILIANO TRENTI
 PAULA MORENO-SANZ
 SILVIA LORENZI
 MADDALENA SORDO
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Terpenoids are major aroma-impact compounds in grape and wine. In particular, monoterpenes confer floral and citrus notes to “Muscat” varieties and, to a lesser extent, to other aromatic non-Muscat cultivars, like Riesling and Gewürztraminer.

In a collaborative effort among several research groups in FEM, we performed the first functional characterization in grapevine of the gene VvDXS1, which has previously been associated with a regulatory role in monoterpene biosynthesis. During the same study, we also discovered new candidate genes for the missing links in monoterpene metabolism and regulation. A metabolic engineering approach was used to express distinct (muscat and neutral) VvDXS1 alleles in the model system “microvine”, which results in short generation cycles and continuous flowering, and thus, a reduction in fruit production time. Transformed microvine lines showed altered expression of several terpenoid-related genes at various development stages and a significantly higher accumulation of monoterpenes in ripe berries compared to control plants. This effect was attributed to the improved catalytic performances of the VvDXS1 enzyme coded by the muscat allele. The parallel analysis of the VvDXS1 genotype

and terpene concentration in a grapevine germplasm collection of approximately 90 cultivars confirmed for the first time that the accumulation of monoterpenes can be predicted from the VvDXS1 allele sequence; the same sequences also have an influence on sesquiterpene levels. In order to identify additional candidate genes involved in monoterpene metabolism and its regulation, the free and bound terpenoid content was integrated with genome-wide expression data in Moscato Bianco through pairwise correlation and clustering analyses. Several genes that may play a role in monoterpene skeleton biosynthesis, secondary transformations, transport and transcriptional control were discovered. Most of these candidate genes have not been reported elsewhere and will be studied further for possible applications to viticulture. For example, grape/wine flavor may be improved by limiting the reactions responsible for the depletion of key odorants through the selection of genotypes with low monoterpene glycosyltransferase or oxygenase activities. In conclusion, our collective findings contribute to the understanding of the origin of grape aromatic compounds, which is essential knowledge for the breeding of new varieties and for the management of high-quality



crops in a changing climate. In particular, VvDXS1 is an effective target for improving metabolic flux in the

monoterpene biosynthetic route that increases aroma-active metabolite levels in grapevine.

Signal exchange between grapevine and *Botrytis*: a story of knowledge, indifference and conflict

Botrytis cinerea is a fascinating pathogen as it can be necrotrophic, saprophytic or endophytic. The pathogen damages many fruit crops, including grapevine, possibly via the early quiescent infection route that affects ripe fruit. In grapevine, *Botrytis* infection is usually initiated by airborne conidia during flowering. During this quiescent infection the pathogen spends prolonged time in the host tissue asymptotically, without being aggressive, but at full maturity, it resumes to cause bunch rot.

The molecular dialogue between *B. cinerea* and grapevine inflorescence and berries from flowering until fruit

maturity is not completely elucidated although understanding this process is vital to implementing appropriate management strategies. Therefore, we performed a thorough study of this interaction integrating global gene expression profiles, metabolic analysis and microscopy, both of the pathogen and of the host. The aim was to better understand the cross talks between *Botrytis* and grapevine during the interaction at initial, quiescent and egression stages to develop new control strategies for the fungus. Open flowers from fruiting cuttings of cv. Pinot Noir were infected with GFP labelled *B. cinerea*, and samples col-

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lected at 24 and 96 hours post inoculation (hpi), at 4 weeks post inoculation (wpi), and at 12 wpi were studied. Our results indicated that penetration of the flower epidermis by *B. cinerea* at 24 hpi induced genes encoding virulence factors, representing the effort of the pathogen to invade the host. On the other hand, grapevine flowers responded rapidly, involving genes associated with the accumulation of PR proteins, stilbenoids, reactive oxygen species and cell wall reinforcement. At 96 hpi the transcriptional reaction appeared largely diminished both in the host and in the pathogen, as expected during quiescence. Infected berries continued their developmental with no

visible symptoms, although the interaction between the fungus and the unripe berries was transcriptionally active. At 12 wpi, the egressed *B. cinerea* expressed almost all virulence and growth-related genes to enable the pathogen to colonize the berries. In response, ripe berries activated several defense responses, which were ineffective.

From this study, it is evident that the initial interaction of the grapevine flower with *Botrytis* (*knowledge*) results in fungus quiescence (*indifference*), but when the berry ripens, the new conditions exacerbate the conflict, and favor *Botrytis* spread in the tissue (*conflict*).

From the grape for mass to MAS: an update on grapevine breeding for resistance to biotic stress at FEM

SILVIA VEZZULLI
PAOLA BETTINELLI
DANIELA NICOLINI
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MARCO STEFANINI

"Vinum debet esse naturale ex genimine vitis et non corruptum": Communion wine should be made of pure, uncontaminated grapes. This is how wine originated in the Augustinian monasteries, and why the genetic improvement of vines was initiated at the Istituto Agrario di San Michele all'Adige to make the cultivation of grapes more sustainable. The grapevine varieties introduced by Edmund Mach to Trentino and the new varieties later produced by R. Rigotti embraced this principle, but since the beginning of the twentieth century the main objective has been to improve quality and desirable characteristics. Plant breeding for specific varieties resistant to biotic stress began in 2010 at the Fondazione Edmund Mach and has focused on two principle research lines. The first is based on the historical varieties (*Vitis vinifera*) of Trentino such as Teroldego, Marzemino and Nosiola, using the hybrids (*Vitis* spp. × *V. vinifera*) Merzling, Bianca and Kulneany as resistance donors. Five genotypes were chosen for their resistance to downy and powdery mil-

dew and organoleptic and sensory characteristics. In particular, the red berry grapevines showed levels of diglucoside anthocyanins comparable to the levels of *V. vinifera*. Data collection on these genotypes is about to be recorded in the National Register of Grapevine Varieties.

The second line is based on characterization of both genotypic and phenotypic plants acquired from external breeding programs, both European and non-European, and of wild material. The most interesting genotypes are used as parental lines in the process of introgression and pyramidization of loci (genomic regions) of interest, with the ultimate objective being to obtain a durable resistance on the leaf and fruit. Once the crossings are planned and obtained, the evaluation of the progeny takes place every year following a Marker-Assisted Selection (MAS) process. First, phenotypic selection takes place (*greenhouse test*) based on the type of disease and the number of loci expected for the same disease. Subsequently, we proceed with mo-

lecular screening based on the specific loci expected in the parental groups. So far, there are five *Run/Ren* (Resistance to *Uncinula/Erisiphe necator*) loci associated with the resistance to powdery mildew present in the FEM program; with regard to

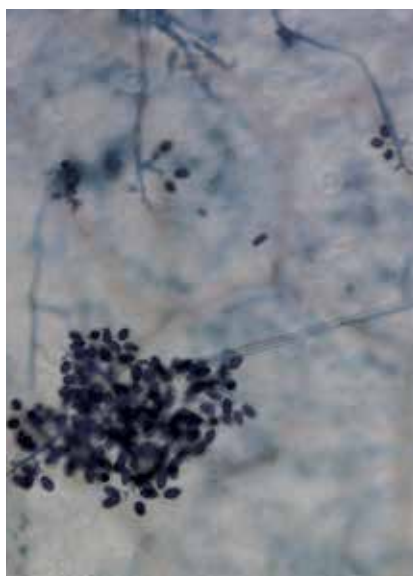
the loci associated with the downy mildew resistance, four *Rpvs* (Resistance to *Plasmopara viticola*) are well represented in the crossing plane. To date, 26% of the selections have been pyramided for four resistance loci.

A multi-omics study of the grapevine-downy mildew (*Plasmopara viticola*) pathosystem unveils a complex protein coding and noncoding-based arms race during infection

Grapevine can be infected at all growth stages by a myriad of plant pathogens and, in order to guarantee a harvest, grapevine growers use large quantities of agrochemicals. The treatments of powdery and downy mildews, including the oomycete *Plasmopara viticola*, account for almost two thirds of all synthetic fungicides sprayed in the European Union, with recognized adverse effects on the environment. The global viticulture community and many stakeholders working in the wine industry are urging researchers to find alternatives to the heavy use of fungicides to fight grapevine disease, in particular downy mildew. Therefore, we decided to use a sophisticated multi-omics approach to close this knowledge gap, including sequencing of a local downy mildew isolate (*Plasmopara viticola*). We unveiled the molecular mechanisms used by the pathogen to successfully invade its host *Vitis vinifera*. This study, consisting in both transcriptional and post-transcriptional gene regulation studies as well as a reconstruction of metabolic pathways of *P. viticola*, revealed three major findings. First, we showed that *Plasmopara viticola* infecting grapevine and *Phytophthora* species such as *P. infestans* infecting potato and tomato, are genetically related. The second important find-

ing was that *Plasmopara viticola* penetrates into the plant cell, through a structure called haustoria, an effector that leads to a resistance reaction in the wild species *Vitis riparia* but not in the cultivated grapevine. This defense mechanism is called a 'gene-for-gene interaction' suggesting that a *P. viticola* effector may interact with a grapevine resistance gene leading to a hypersensitive response. Our findings help to explain why all current cultivars of *Vitis vinifera* are susceptible, but the wild species are resistant. The third and the most interesting finding is the bi-directional exchange of small RNAs through the haustoria. The plant and the

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pathogen reciprocally silence their respective messenger RNAs using small RNAs. This is the first time that such a cross-kingdom cellular communication of nucleic acids has been demonstrated during the interaction of a grapevine pathogen with its host. It is not excluded that this small RNA-mediated gene regulation

is still evolving given that the *P. viticola*-*V. vinifera* pathosystem appeared only about 135 years ago. The winner of this arms race between *P. viticola* and *V. vinifera* is difficult to predict but our study lays the foundation for future work aiming to develop alternatives to the heavy use of chemical treatments.

Fruit quality: a primary target for blueberry and raspberry breeding

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FRANCESCO EMANUELLI
PAULA PONCETTA
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PAOLO MARTINATTI
LARA GIONGO

Berry consumption in Europe has been increasing consistently and in 2017, the value of blueberries and raspberries, reached that of strawberry, thanks to their nutraceutical properties as well as their organoleptic quality.

For the last fifteen years, the External Site of the Fondazione Edmund Mach, situated near Pergine Valsugana (Trento), has been actively developing berry research, by interacting with local and international growers and stakeholders. In particular, in the last few years, berry breeding has been funded by the Unit of Genetics and Fruit Breeding.

The breeding programs for raspberry and blueberry are highly efficient, carried out entirely hydroponically, and in collaboration with private stakeholders for the multi-environmental and horticultural trials in different Italian and European climatic areas.

The main objective of both programs is the increase of fruit quality perceived by the consumer, considering yield and storage of the product. Crucial for reaching this goal is the development of molecular and biochemical markers, in order increase the selection efficiency and the development of new high quality fruit-yielding plants that are adapted to specific environments.

Recently we have set up new validated methodologies to objectively determine fruit quality, in particular for the texture and aroma, in collab-

oration with the Sensory Quality Research Unit at FEM.

Fine phenotyping of texture and aroma has not been easy nor immediate and has required the development of *ad hoc* methodologies for the different berries, depending on their different anatomic and chemical properties. It was necessary to understand, in a detailed manner, the physiological, biochemical and genetic elements that control these qualitative aspects, considering the effects on the different genotypes in different climatic areas, and trialing systems in different ripening phases and post-harvest conditions.

Finally, it was imperative to explore the genetic variability present in available germplasm and progeny; therefore, in collaboration with the Sequencing Platform at FEM, we started a collaboration aiming at identifying genetic determinants for the major horticultural and quality traits involved in breeding.



Applied research and new technologies for monitoring and control of invasive alien insects

Invasive alien insects (IAI) are organisms intentionally or accidentally introduced by humans to areas where they otherwise would not be present. IAI can cause serious damage not only to biodiversity, but also to human health and the economy, especially in the agricultural and forestry sectors. To combat invasion risk, some actions are recommended, such as monitoring for early detection, understanding the IAI biology in the invaded area and defining control and management strategies. This is complex work that requires a multidisciplinary approach and cooperation between researchers, technicians, farmers, industry and the general public.

The smartphone application, “Bug-Map” was developed to provide a detailed study of the presence and spread of the Brown Marmorated Stinkbug, *Halyomorpha halys* in Trentino, and is an excellent example of citizen involvement in the monitoring activity of IAI.

As for post-invasion management, a well-recognized strategy is ‘integrated pest management’ (IPM) that facilitates the mechanisms of environmental regulation and promotes sustainable control methods. In this regard, genetics and evolution are studied at FEM to provide support

to applied research. Innovative techniques like the “molecular clock” have a key role in constructing predictive models both of IAI and pathogens transmitted by these insects, while comparative genomics allows the identification of genes important to improving methods of pest capture and interference. In addition, new technologies, such as the vibrational mating disruption to control grapevine leafhoppers, are being employed in collaboration with industry and farmers. FEM has a renowned laboratory of Biotremology in which the mating behavior of several insect pests is studied, including that of the Brown Marmorated Stinkbug. Based on this research, a new trap based on the combined use of aggregation pheromones and vibrational signals is being developed. Another fundamental IPM tool is biological control through the augmentative release of the native parasitoid wasp species, *Trichopria drosophilae*, to control the Spotted Wing Drosophila, *Drosophila suzukii*. Finally, the Male Sterile Technique (SIT) that is based on the massive release of gamma-ray sterilized males into the field, the target pest population density has been reduced. Pilot studies on the application of SIT to reduce local *D. suzukii* and *H. halys* populations are ongoing at FEM.

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GIANFRANCO ANFORA
OMAR ROTA STABELLI
VALERIO MAZZONI



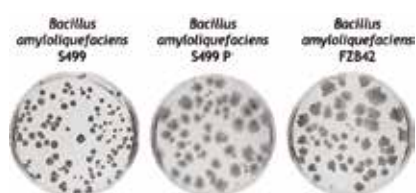
HIGHLIGHTS



Identification of biomarkers for the defense response against *Plasmopara viticola* in a resistant grape variety

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GIULIA CHITARRINI, URSKA VRHOVSEK

The use of grapevine varieties with durable resistance to downy mildew is a promising strategy to control the disease. The interaction of *Vitis* with *Plasmopara viticola* causes early changes in primary plant metabolism and thereafter a modulation of secondary metabolism. Our metabolomic approach allowing marker identification has extended knowledge on how the plant system is disrupted after pathogen infection.



Presence of a plasmid determines the differences between two *Bacillus* strains providing plant resistance to pathogenic fungi

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GIULIA MOLINATTO, PAOLO SONEGO, MARCO MORETTO, ILARIA PERTOT,
GERARDO PUOPOLO

Two biocontrol agents, strains FZB42 and S499 of *Bacillus amyloliquefaciens*, differ in their production of surfactin, a cyclic lipopeptide involved in triggering tomato resistance to pathogenic fungi. We have compared their genomes and created a mutant strain, and shown that this difference is due to the presence of a plasmid in the S499 genome, which is involved in surfactin biosynthesis.



Xylella fastidiosa: prevention is better than cure even in grapevine

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PAOLO BALDI, NICOLA LA PORTA

Xylella fastidiosa is primarily an olive tree pathogen that can also affect grapevine and many other crops. A recent FEM study, published in Frontiers in Plant Science (<http://hdl.handle.net/10449/42695>), uses the advanced molecular biotechnologies to effectively monitor the spread of the bacteria, particularly on asymptomatic plants, with the aim of providing a powerful tool for pathogen surveillance.

Tracing edited plants

LORENZA DALLA COSTA, STEFANO PIAZZA, ALESSANDRO CESTARO, MASSIMO PINDO, MICKAEL MALNOY, CLAUDIO MOSER

Recently, the EU Court of Justice ruled that organisms obtained by genome editing fall under the definition of 'genetically modified' and as such need to be traced. We are developing a method based on high-throughput sequencing to identify a short sequence left by *Agrobacterium* during gene transfer in 'edited' grapevine and apple plants. This sequence acts as a marker confirming the biotechnological origin of the organism.



Valorization of a traditional agricultural product: the walnuts of the Bleggio area

ERICA A. DI PIERRO, PIETRO FRANCESCHI, FEDERICA CAMIN, VALENTINO POLETTI, MICKAEL MALNOY, FLAVIA GASPERI, ROBERTO LARCHER, URSKA VRHOVSEK, LUCA BIANCO, MICHELA TROGGIO

In Trentino, the area around Bleggio has long been dedicated to walnut cultivation, including the selection of the indigenous variety 'Bleggiana'. The recently funded project NoBle aims to promote and fully characterize the local varieties from a genetic, isotopic/mineral and metabolic point of view. It will also include a consumer test. Micropropagation techniques will be developed and tested for a more efficient production of seedlings.

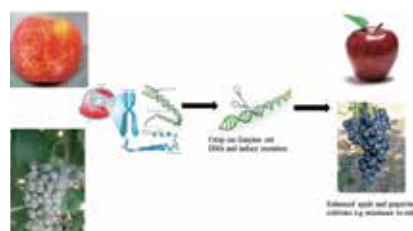


Application of the New Breeding Techniques to grapevine and apple

MICKAEL MALNOY, STEFANO PIAZZA, VALENTINO POLETTI, LORENZA DALLA COSTA, GIORGIO SORDATO, LISA GIACOMELLI, CLAUDIO MOSER

The availability of genetic information and new breeding techniques will largely impact the field of fruit biotechnology by enabling the introduction of targeted genetic changes with unprecedented accuracy.

We are currently developing different strategies of CRISPR/Cas9 editing and cis-genesis to increase the disease resistance of commercial varieties of the major crops of Trentino, apple and grapevine.

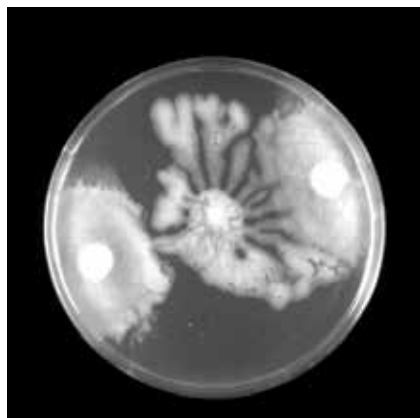


MinION technology introduces the third generation sequencing: example of application for the characterization of phytoplasma strains

MIRKO MOSER, ALESSANDRO CESTARO, DIEGO MICHELETTI, CLAUDIO DONATI

The last frontier of DNA sequencing is represented by small portable devices like the Oxford Nanopore MinION sequencer. For the first time with this technology, a group of researchers at the FEM CRI will sequence phytoplasma strains causal agents of several plant diseases in the Trentino region.





Defeating the enemy: transcriptional analysis of the interaction between a biocontrol agent and its target plant pathogen

SELENA TOMADA, PAOLO SONEGO, MARCO MORETTO, ILARIA PERTOT, MICHELE PERAZZOLLI, GERARDO PUOPOLO

We used RNA-Seq analysis to elucidate the strategy implemented by the bio-control agent *Lysobacter capsici* AZ78 to control the pathogen *Phytophthora infestans*, the causal agent of tomato and potato late blight. We showed that this agent relies on the colonization of *P. infestans* mycelium and the release of an antibiotic that induces apoptosis; the pathogen then undergoes cell death and is progressively digested by AZ78 through the action of lytic enzymes.



Genetic diversity and metabolic profile of *Salvia officinalis*

STEFAN MARTENS, LUISA PALMIERI

The genetic and metabolic diversity in seven *Salvia officinalis* populations was characterized using molecular markers and new analytical techniques. The populations were classified in two chemotypes according to their essential oil composition. Phenolic compounds could be categorized into six groups, and significant genetic differences were found between the populations. This knowledge will be used to design future breeding programs for highly valuable varieties.



Role of abscisic acid in the regulation of ripening onset genes

STEFANIA PILATI, MARCO MORETTO, PAOLO SONEGO, GRAZIANO GUELLA, CLAUDIO MOSER

Treatment of green hard berries with abscisic acid (ABA) at very low concentration for 20 hours triggered the modulation of about 1,350 genes which usually only happens during ripening, confirming the primary role of this acid in the control of the ripening process. Genes directly regulated by ABA have been identified and will be further characterized in other traits of agronomic interest, such as the response to water stress.



The systems relationships between structural genes and transcriptional regulators modulate the composition of grape berries exposed to water deficit

STEFANIA SAVOI, FULVIO MATTIVI

A detailed analysis of transcripts and both primary and secondary metabolites under water stress during grape ripening revealed a coordinated regulation of key metabolic pathways determined by the abscisic acid molecular signal, with the activation of several transcription factors. Results were published on *Frontiers in Plant Science*, 8 (2017).

Use of grapevine plants with modified kairomone emission for the control of Grapevine Moth

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UMBERTO SALVAGNIN, MICKAEL MALNOY, STEFAN MARTENS, SILVIA CARLIN,
URSKA VRHOVSEK, GIANFRANCO ANFORA

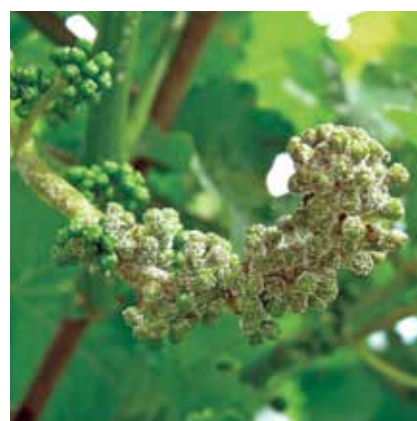
The Grapevine Moth (*L. botrana*) uses volatile compounds to locate host plants. We generated stable grapevine transgenic lines with altered (E)- β -caryophyllene and (E)- β -farnesene emission compared to natural plants. We then tested how it affected *L. botrana* behavior, discovering that the plants with the highest modification were less attractive than the natural ones under controlled conditions.



Grapevine defence mechanisms mediated by volatile compounds

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MICHELE PERAZZOLLI, VALENTINA LAZAZZARA, ILARIA PERTOT

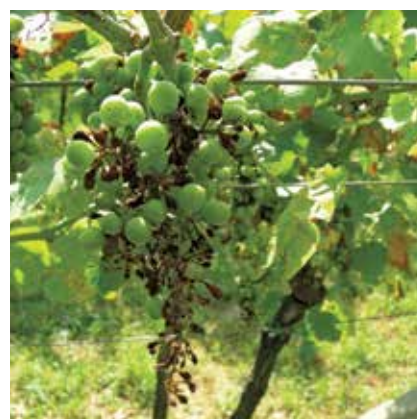
Volatile organic compounds (VOCs) play crucial roles in the plant communication with other organisms. We identified VOCs produced by resistant grapevines in response to *Plasmopara viticola*, and four of them reduced downy mildew symptoms on susceptible grapevines. Thus, VOC-mediated communication between resistant and susceptible plants is responsible for a social defence reaction.



Microbiome engineering for plant health

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MICHELE PERAZZOLLI, ANDREA NESLER, MARTINA CAPPELLETTI, CARMELA SICHER,
OSCAR GIOVANNINI, GERARDO PUOPOLO, ILARIA PERTOT

Plants are colonised by a wide range of microorganisms that can have beneficial interactions with their host. In the research on grapevine microbiome carried out at FEM, we have developed a protein-based method to stimulate beneficial microorganisms and protect plants against diseases. This microbiome engineering approach is based on using indigenous microorganisms to activate plant resistance against pathogens.







NUTRITION



Colourful cell factories - from engineering to colourants

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STEFAN MARTENS
SASCHA HEIN
LUISA PALMIERI

Anthocyanins are widely distributed, glycosylated, water-soluble plant pigments, which give many fruits and flowers red, purple or blue colouration. Their beneficial dietary effects have encouraged increased use of anthocyanins as natural colourants in the food and cosmetic industries and also as health-promoting substances. However, the limited commercial availability and the natural diversity of anthocyanins have initiated searches for alternative sources of these colourants. Furthermore, plant production of anthocyanins cannot always meet the above-mentioned demands, due to low productivity and the complexity of the plant extracts.

High-level production of secondary metabolites in plants, including anthocyanins, can be achieved by engineering of regulatory genes as

well as genes encoding biosynthetic enzymes. Such engineered plant cell lines are stable in their production rates and superior to the conventional cultures. Scale-up of anthocyanin production in bioreactors has been demonstrated with the ERA-IB project "ANTHOplus". Additionally, this strategy for anthocyanin production is transferable to other plant species, such as *Arabidopsis thaliana*, demonstrating the potential of this approach for making a wide range of highly-decorated anthocyanins. Therefore, plant cell cultures represent a customizable and sustainable alternative to conventional anthocyanin production platforms (transformed plants, bacteria, chemical synthesis) (Appelhaugen et al., 2018, *Metabolic Engineering* 48, 218-232). The plant cell system microbial cell

factories, such as baker's yeast (*Saccharomyces cerevisiae*), have been successfully established to produce a number of plant polyphenols, including anthocyanins. In this case, yeast was here engineered to produce pelargonidin and its glucoside, which are known to occur in for instance strawberries, from glucose. To this end, specific anthocyanin biosynthetic genes from *Arabidopsis thaliana* and *Gerbera hybrida* were introduced in a strain which had previously been engineered to produce naringenin, a polyphenolic precursor

of anthocyanins. Upon culturing in synthetic medium, pelargonidin and its glucoside could be detected, albeit at low concentrations. To optimize titres of pelargonidin, biosynthetic genes were stably integrated in the genome (Lewison et al., 2018, *Microbial Cell Factories* 17, 103).

In summary, these two systems lay the basis for further improvement of productivity, upscaling and downstream processing for biofactories producing certain anthocyanins and have considerable potential for use in industrial and medical applications.

CABALA_Diet&Health: bile acids as biomarkers connecting diet, intestinal microbiota and metabolism

Bile acids (BA) through G protein-coupled bile acid receptor 1 (GPBAR1 or TGR5) and nuclear receptors like Farnesoid X receptor (FXR) regulate mammalian inflammation, and lipid, glucose, energy, and xenobiotic metabolism. The gut microbiota modulates the enterohepatic circulation of BA and microbially produced secondary BA appear to be more potent receptor agonists than primary BA, providing a mechanistic link between microbiome structure/function and regulation of host physiology. Evidence mainly from animal studies shows that dietary fibers and polyphenols can bind BA driving them into the colon and modifying their absorption and/or excretion and importantly, that exercise can impact on BA metabolism. Similarly, certain bile salt hydrolyzing (BSH) probiotic bacteria modulate circulating bile acid (CBA) profiles and strongly influence cholesterol uptake. However, human data confirming dietary modulation of CBA profiles and subsequent regulation of physiological homeostasis remains elusive. CABALA_Diet&Health aims to establish circulating BA profiles as biomarkers of health, modulated by diet and exercise which reflect

a change in metabolic health. This project will establish whether dietary modulation of BA profiles indicate a change in health status. Using samples from the ROCAV cohort (Risk Of Cardiovascular diseases and abdominal aortic Aneurism in Varese - University of Insubria) and existing randomised control trials (RCT), we will correlate CBA profiles with adherence to the Mediterranean diet, intake of fiber/polyphenol rich foods and measures of metabolic health (BMI, insulin/glucose and lipid homeostasis). In a bespoke short-term RCT we will measure the ability of probiotics, prebiotics and polyphenols to modulate post-prandial BA profiles and in a long term (18 month), large-scale (n=300) existing dietary and lifestyle intervention, we will measure how polyphenol rich whole foods and exercise promote metabolic health in susceptible individuals through modulation of BA signalling. Finally, we will link BA profiles or metabotype with microbiome signatures and BA biotransformation potential using high-resolution metagenomics and establish the molecular basis of BA regulation of immune and metabolic homeostasis by measuring the rela-

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tive BA-metabotype receptor activation potential. CABALA_Diet&Health will provide direct evidence in humans that diet:gut microbiota interactions, as indicated by CBA profiles,

determine metabolic disease risk providing at once a validated biomarker of health and a unifying molecular basis for efficacious probiotics, prebiotics and polyphenols.

The factors that influence the food preferences of Italians: the Italian Taste project

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JESSICA ZAMBANINI
FLAVIA GASPERI

The Italian Taste project involved the main Italian universities and research centers including FEM, with the participation of over three thousand individuals over three years, from whom information regarding food preferences, sensitivity to tastes, socio-cultural, psychological and personality traits and genetic information was collected.

The results of this study are of international concern because for the first time it has been possible to create a large database, complete both from the point of view of the multidisciplinary of the information collected and the sampling representativeness. This will allow us to study the

link between sensitivity, liking and food choices of Italians and to understand the mechanisms underlying the development of preferences that strongly affect our eating habits and therefore our health.

The sensory FEM laboratory was one of the main partners of the project: it contributed to the project design being responsible for the development of the products to be tested and of the data analysis. It also provided the data collected in the FEM laboratory for 256 consumers from Trentino region.

The first published works illustrate the overall experimental design of the study and the potential to inves-



tigate the responses of Italian consumers in relation to the different collected variables. For example, the physiological indices of taste sensitivity, such as the number of fungiform papillae and bitter sensitiveness, vary according to age and gender and are indicators of different levels of perception in real products. Furthermore food neophobia, a personality trait that measures the reluctance to taste and eat foods never tried before, differentiates responses to critical sensations like bitterness and astringency, or like some personality traits are associated with a different preference and sensitivity for spicy foods

. FEM researchers explored also the association between liking and sensory perception in the four tasted products: pear juice, chocolate pudding bean purée and tomato juice each of them proposed in 4 variations related to 4 intensity levels of the target sensation (sourness, sweetness, saltiness and pungency). The data show how the responses to products vary according to gender, age and region of residence and how it is also possible to identify consumer segments that show different preference directions according to the intensity of the perceived target sensation (Endrizzi et al., Eurosense 2018).

TrentinCLA: a project to evaluate the beneficial effects of traditional alpeggio on milk

Between mid-June and mid-September, dairy cows may have the opportunity to go to the alpine pasture, where they are free to graze and feed themselves in the rich alpine pastures, constituted by hundreds of different grass species that are not present in the valley.

The aim of the TrentinCLA project (written by FEM and co-funded in collaboration with Caritro Foundation) was to evaluate the influence of mountain pasture on the rumen, milk and cheese microbiota and to understand how bacteria could increase the level of conjugated linoleic acids (CLA) in milk. For this project, 12 dairy cows were divided into two groups: the first group was transferred from July to September 2017 to Malga Juribello, the second remained in the farm downstream at the valley. For 5 consecutive months, before, during and after the summer pasture, samples of milk and rumen were taken for a total of 120 samples, and 60 experimental cheeses were produced in the laboratory by single cow milk.

The result was a picture of the milk microbial ecology strongly and posi-

tively affected by the mountain pasture. During the pasture, the milk was characterized for about 40% by lactic acid bacteria species desired for their good dairy technological properties and for more than 25% by bacterial species, such as bifidobacteria, commonly used as probiotic and known to have interesting healthy properties including the ability to produce CLAs. When the cows were kept in the valley, the milk microbiota significantly changed and was mainly constituted by *Enterococcus faecalis* that could carry antibiotics resistances, while the lactic acid bacteria species were halved. Probiotic bacterial species were found in traces in the valley milk and the presence of *Staphylococcus aureus*, a mastitis agent, was recorded at 10%. From these data we could conclude that the alpine pasture practice contributes to the production of a good quality milk not only for the improvement of the lipid fraction but also for the high microbial biodiversity, which can be delivered to the cheese spontaneously. So the traditional practice of the mountain pasture should be preserved as a guaran-

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ELENA FRANCIOSI
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tee for the landscape preservation, for a good quality of animal welfare, but also for a milk with improved chemical and microbiological characteristics. The practice of mountain pasture is

doubtless difficult and expertise demanding but, if wisely conducted and controlled, can lead to the production of a raw milk of quality that should be encouraged and enhanced.

One step closer to precision wine-making

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PANAGIOTIS ARAPITSAS
FULVIO MATTIVI

Sulfur dioxide (SO_2) is the most widely used additive in winemaking. Today oenologists and winemakers recognise SO_2 as an indispensable additive in winemaking, able to protect wine from various unwanted reactions. The use of this additive is controlled by legal limitations, but the recommended doses are imprecise and it is still not known why very similar wines consume the added SO_2 differently.

With the scope of generating knowledge for better targeted use of SO_2 , and based on our earlier findings

regarding new SO_2 -binders in wine, the research team first developed an analytical method able to monitor 32 metabolites and then use it to measure 195 commercial wines from the 1986-2016 vintages.

The general finding was that SO_2 influences wine quality in more ways than is currently known. The quality of aged premium wines, as we know them today, is strongly characterised by the addition of SO_2 . A very slow reaction between SO_2 and wine tannins (epicatechin, procyanidin B2), thus the major responsible metabo-



lites of wine body, leads to products that highly characterise the chemical fingerprint of red wines aged at length. This reaction could also be partly responsible for alleviating the aggressiveness of young red wines, although this remains to be proved in the future. On the other hand, white and sparkling wines were found to be characterised by a relatively fast reaction between SO_2 and catabolites of the amino acid tryptophan pathway (a.k.a. indoles). These metabolites, with high concentrations in wines, could be responsible for the

phenomenon (well known among winemakers) of unexpectedly large doses required by some wines. The same sulfonated metabolites could also influence the aromatic characteristics of wines; since, for example, the sulfonation of indole-3-acetic acid could deliver 2-aminoacetophenone, a volatile compound responsible for the wine fault of atypical aging. These new findings could enable better understanding of the chemical changes occurring in wine during ageing and offer new prospects for more precise use of SO_2 in winemaking.



New disease tolerant grape varieties: characterization of the chemical composition of wine

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The continuous use of plant protection products in viticulture and the increasing awareness of their negative consequences on environment and human health are a very discussed topic that have led to search for alternative and low impact strategies in order to control the major grapevine pathogens. In this context, the new disease tolerant grape varieties, which combine the resistance to pathogens of the American varieties and the quality of the wine of traditional *Vitis vinifera* varieties, have the potential to significantly reduce the application of chemical products as well as to achieve a significant energy and economic savings. However, if on the one hand these new varieties are one of the most promising tool for a more sustainable viticulture, on the

other they still suffer from negative prejudice related to the poor wine quality of the first hybrid varieties developed at the beginning of the 20th century. To promote the cultivation and use of disease tolerant varieties for the production of high quality wines, it is necessary to obtain more information by identifying the peculiar aspects of their composition and measuring their positive and negative quality traits. So far, comparative studies of the overall chemical composition of wine produced from a wide selection of modern disease tolerant varieties, grown in climatically different regions in different years, have not yet been reported in the literature.

For this purpose, the chemical profile of the wine obtained from a wide

selection of the most promising disease tolerant varieties, grown in Italy and Germany in three different years, has been studied and compared with a group of high quality *V. vinifera* ones. A total of 92 wines produced under standardized protocols were analyzed using a multi-targeted approach in order to investigate the main classes of volatile and non-volatile compounds responsible for the organoleptic properties of wine. This study provides a clear picture of the metabolite profile of wines made from new disease tolerant varieties

highlighting that, with the exception of anthocyanins, wines produced from these varieties have a general composition closely resembling that of well-known *V. vinifera* ones. Therefore, these modern varieties are equally valuable and promising for the production of high quality wines. The information gained can be used for further breeding programs as well as to suggest the most appropriate wine-making methods allowing the improvement of the wine quality and the valorization of the characteristics of each grapevine variety.

Authenticity of nutraceutical products

Nutraceutical is a food or part of a food which provides medical or health benefits, encompassing prevention and treatment of disease. Nutraceutical products have normally premium value and can be subjected to adulteration. Recently in studies performed in FEM, stable isotope ratios analysis has proved to be effective in detecting the authenticity of some of these products.

For example, red yeast rice is a dietary supplement obtained from rice fermented with the mould *Monascus purpureus*. It contains Monacolin K which is a hypocholesterolemic statin used to prevent cardiovascular diseases. The homologous prescription biosynthetic statin, lovastatin, is not chemically distinguishable from monacolin K. Because red yeast rice has very high cost of production, there is a suspicion that it could be fraudulently spiked with purified lovastatin without declaration. 18 samples of red yeast rice powder, covering the main producers, and 18 samples of synthetic lovastatin were considered. Monacolin K, once isolated and lovastatin were subjected to the analysis of stable isotope ratios. Thanks to the different photosynthetic cycles of the matrices used for their synthesis, monacolin K and lovastatin have different $\delta^{13}\text{C}$ values ($-29.6\text{‰} \pm 0.6$ and -16.7‰

± 2.6 , respectively). By defining a $\delta^{13}\text{C}$ threshold value of -28.3‰ for monacolin K, addition of lovastatin from a minimum of 10% can be identified.

Another example is saw palmetto extract, which is the most expensive oil source of the pharmaceutical and health food market, and its high cost and recurrent shortages have spurred the development of designed blends of animal fatty acids that mimic its phytochemical profile and fraudulently comply with the current authentication assays of the oil. To detect this adulteration, stable isotope ratio technique has been applied. 20 samples of authentic saw palmetto extract were considered, covering the main producers, different harvest years and extraction techniques. 12 samples of lipids from different types of meat (beef, lamb and chicken) and 4 different pure fatty acids (caprylic,



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capric, lauric and myristic acid) were considered as possible adulterants. Authentic saw palmetto extracts are characterized by characteristic values of $\delta^{18}\text{C}$ (between +27.2‰ and +40.7‰), significantly higher than those reported in the literature for vegetable oil and than those of animal fatty acids (from 12.2‰ and

18.9‰). By analyzing $\delta^{18}\text{C}$ in the samples of the market, it is possible to verify if they are authentic saw palmetto extracts or they have been adulterated with mixtures of animal fatty acids.

References: Perini et al., Food Chemistry 274(2019), 26-34 - Perini et al., Talanta 174 (2017), 228-233.

Unravelling the chemical composition and the microbiological populations characterizing the traditional grass baths of Garniga Terme

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"Grass baths", a traditional natural treatment for rheumatic diseases, is a symbol in the Trentinian culture. The fondazione Mach, in collaboration with Trento facility, applied its resources and its knowledge to study the fermentation process of the grass baths, involving also known researchers in the study, like prof. Franco Pedrotti and Dr. Alberto Beretta. Indeed,

despite several scientific studies and efficacy evidences, grass baths still remain a treatment not fully understood both in their fermentation process and their mechanisms of action. Researchers from Fondazione Edmund Mach replicated the grass bathing process in the original Garniga Terme's baths to study the chemical and microbiological variation during



a whole treatments cycle. The studies highlighted performance limits of the process, mostly related to the uncontrolled spontaneous fermentation; indeed, without assuring the optimal conditions for the grass fermentation, grass baths might fail to reach the desired temperatures, develop an unintended high amount of molds, and a consequent unpleasant odor.

The use of mass spectrometry technologies coupled with bi-dimensional chromatography allowed to profile the volatiles released during the grass bathing cycles. The profile can be used to evaluate the fermentation quality and prevent spoilage. Furthermore, this technique highlighted the presence of volatile compounds possibly involved in the anti-rheumatic effects of the grass baths. For example, the chemical analysis evidenced the presence of methyl-salicylate, a compound already used in modern medicine to treat joint inflammations; menthol and thymol, natural mio-relaxants; gamma-butyro-lactone, a known anesthetic; and Thujone and

its derivatives, which at low dosages have anti-nociceptive effects.

The microbiological studies found the presence of two main actors in the fermentation process: *Erwinia* bacteria which are involved in the free sugars consumption and the consequent rapid temperature growth to reach a maximum of 55-65 °C. *Aspergillus* molds, involved in the grass spoilage, with consequent changes in the odor profile and the worsening of the bathing conditions.

Through the acquired knowledge on the grass bathing process, the researchers developed several technical improvements aimed to improve the process stability, replicability and to limit grass' spoilage. These results have been submitted to an independent consumers' test, which confirmed the quality of the results. Applying these new methods on the real process, they increased the grass bathing efficacy against rheumatic diseases, while allowing to perform better and deeper studies to determine its mechanisms of action.

HIGHLIGHTS

Two apples a day keeps the doctor away!

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PIETRO FRANCESCHI, DANIELE PERENZONI, FULVIO MATTIVI, KIERAN M. TUOHY

Together with partners at the University of Reading, we found that feeding 2 apples (Renetta Canada) a day for 8 weeks to people with hypercholesterolemia reduces cardiovascular disease risk by decreasing cholesterol and improving vascular function, activities linked to gut microbial metabolites in blood. Results will be published soon; a description can be read in "Clinical Trials" (NCT01988389).





Extracts from *Hypericum hircinum* subsp. *majus* exert antifungal activity against a panel of sensitive and drug-resistant clinical strains

NOEMI TOCCI, DANIELE PERENZONI, FRANCESCA FAVA, TOBIAS WEIL, FULVIO MATTIVI

Fungal infections are increasing and the urgent need for novel antimycotics calls attention to the study of natural products. This study unveiled that *Hypericum hircinum* is a source of antifungal principles. The antifungal properties of the infusion against a panel of clinical strains paves the way for further investigations aimed at developing novel antifungal therapies.



Nutrimetabolomics: an integrative action in human nutritional studies

MARIA M. ULASZEWSKA, PIETRO FRANCESCHI, MAR GARCIA-ALOY, FULVIO MATTIVI, SAMANTHA RICCADONNA

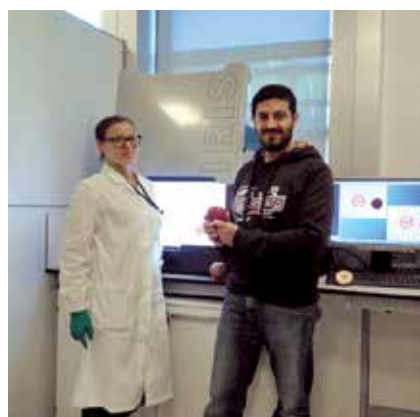
FEM researchers, in the frame of the Food Biomarker Alliance, contributed to a project in JPI-HDHL (Joint Programming Initiative "A Healthy Diet for a Healthy Life"), writing a description of state-of-the-art techniques applied in nutritional metabolomics. Even though not exhaustive, it presents a pragmatic guidance on metabolomic methodologies, providing useful "tips and tricks" along the analytical workflow.



Colourful cooking - red and blue potatoes

STEFAN MARTENS, LUISA PALMIERI, PANAGIOTIS ARAPITSAS

Phytochemical elucidation and characterization in red and violet potato cultivars by liquid chromatography / mass spectrophotometry was used to assign groups differing in their anthocyanin profiles, among which 21 correlated with their specific tissue coloration. The characterized cultivars represent a valuable resource for analysis of the genetic background and the regulation of anthocyanin side-chain modification.



Use of e-eye for the classification of Gala and Fuji apples according to the skin pattern

EUGENIO APREA, JESSICA ZAMBANINI, FLAVIA GASPERI

Stripes' pattern in Fuji and Gala skin apples is a key factor determining consumer acceptability, sale price and, in South Tyrol, it is used when paying the producers. An automatic method to grade apples into classes defined by experts was developed using features of images acquired by a camera-based imaging system. This objective and automatic method permits the apple classification with accuracy similar to that of the experts.

Effect of Low-FODMAP diet and probiotic supplementation on the intestinal microbiota of patients with irritable bowel syndrome

FRANCESCA FAVA, ELENA FRANCIOSI, MATTHIAS SCHOLZ, KIERAN M. TUOHY

Low FODMAP diet (i.e. a diet restricted in fermentable carbohydrates) adequately relieves IBS symptoms and administration of a multi-strain probiotic restores numbers of *Bifidobacterium* species in IBS patients following a low FODMAP diet in a randomized, placebo-controlled study carried out at King's College (London, U.K.).



Geographical and botanical traceability of Italian honey

LUANA BONTEMPO, FEDERICA CAMIN

According to EU law, the country of origin and the botanical origin of honey must be declared on the label but the conventional honey analyses are not applicable and effective for the verification of this information. The determination of stable isotope ratios and the elemental composition were tested. The approach resulted effective and can be useful as support of the official methods.



Geographical traceability of soft fruits from Trentino

FEDERICA CAMIN, LUANA BONTEMPO, LARA GIONGO, MATTEO PERINI

For the first time stable isotope ratios of soft fruits were extensively explored. The method was applied to different soft fruits produced in Trentino, Romania and Poland. The different soft fruits showed a typical range for one or more parameters that can be used to verify the authenticity of the fruit composition or other information (e.g. geographical origin) declared on the label.



Geographical traceability of Italian rainbow trout (*Oncorhynchus mykiss*)

FEDERICA CAMIN, LUANA BONTEMPO, MATTEO PERINI

Stable isotope ratios were determined in rainbow trouts reared in different Italian farms, focusing on Friuli-Venezia Giulia and Trentino. This study provided effective evaluation of this traceability model in the trout industry, quantifying the contribution of geographical and trophic signatures. The developed approach allowed a 91% accuracy determination for Trentino and Friuli trouts.







ENVIRONMENT



Dust storms transport entire Saharan microbial communities to the Alps

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To move from the Saharan desert to the Dolomites would not be an easy task for anyone. However, due to climate change and land use we are witnessing increasingly frequent episodes of microbial migrations from the Sahara to areas with very different climatic conditions than those in their place of origin. In February 2014, an extremely rare event occurred, known as a 'pink snowfall', when large amounts of snow containing Saharan sand fell on the Alps, leaving summits 'painted in pink'. Thanks to successive snowfalls, the deposited sand remained sealed and frozen for months in layers of clean snow. However, this sand is not inanimate. The peculiarity of the event and the natural preservation of the samples provided researchers with a unique opportunity to study the microbiological load of

the sand using advanced genetic analyses. In the samples collected on the Marmolada and Latemar, a multidisciplinary team of microbiologists, geologists, chemists and bioclimatologists from the Fondazione Edmund Mach, Institute of Biometeorology of the National Research Council, Universities of Florence, Venice and Innsbruck discovered that large dust storms can move entire microbial communities from the Saharan areas to Europe, and that this microbiota is rich in many extremely resistant organisms capable of surviving in different environments. By comparing the DNA sequences from the frozen bacteria and fungi, to those from local soil microbial communities, the researchers also found that some of the Saharan microbes can persist in the alpine soil even after the snow has melted. This

suggests that, with climate change and the subsequent increasing frequency of events like the one studied, the microbial communities of our soils could significantly change. The study published in the prestigious journal *Microbiome* paves the way for

new research areas in the field of microbiology and has been listed as a 'success' by Research Italy (MIUR) and highlighted by the World Meteorological Organization (WMO), in national and international news bulletins, and widely read internet platforms.

Geographical traceability of *Picea abies* in Trentino

Exploitation of local timber is of central importance to mountain communities and local economies. As markets have expanded from local to global, many timber species have now been superseded on the local market by cheaper, imported timber species. Recognising the added value of local timber, consumers are often prepared to meet the higher costs of these products for ecological, social and ethical reasons. This is particularly significant for an Alpine region like Trentino, which boasts 60% forest cover.

The development of a suitable method for identifying the geographical provenance of timber would be a very useful tool for protecting the local economy. Of the various available techniques, stable isotope ratio analysis has proved to be a powerful method for identifying the origin of many agricultural products.

In this study, we used kriging procedures to study the spatial distribution of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in Norway spruce (*Picea abies*) with samples from 151 sites in Trentino. We tested the accuracy of four spatial interpolation methods (ordinary kriging, two universal kriging methods with spatial trends, cokriging) using an annual resolution of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measured in the tree rings formed in 2013 and 2014.

Statistical analysis revealed that the cokriging model is the best method for this species due to its low error rates in the prediction of area of origin for timber. Altitude proved to be the most appropriate covariate for along with mean summer tempera-

ture, longitude and latitude.

Regional maps of interpolated $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in *P. abies* wood together with the 95% confidence intervals were created. The isoscapes revealed the $\delta^2\text{H}$ values to have a strong latitudinal dependency, with the northern area having lower $\delta^2\text{H}$ values. For $\delta^{18}\text{O}$ there was a slight separation between east (higher $\delta^{18}\text{O}$) and west (more depleted). The spatial structures of both the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values are quite stable and are mainly influenced by altitude, although there is a certain inter-annual variability. Analyses of the two superimposed stable isotope maps reveals that five main areas can be identified: the north, centre and south reflecting the $\delta^2\text{H}$ north-south gradient (the latitude effect), and the east and west, reflecting the relationship between $\delta^{18}\text{O}$ and longitude. The strong spatial structure of the data demonstrates the potential of multivariate spatial interpolation, even in a highly heterogeneous area such as Trentino. We believe that this geospatial approach can be successfully applied on a wider scale in order to combat illegal logging.

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Mosquitoes and Public Health

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One important research topic of the Research and Innovation Centre (CRI) of Fondazione Edmund Mach (FEM) is the study of mosquito species acting as vectors of pathogens, especially in Trentino and northern Italy. In particular, we are currently studying various mosquito species: a native species (*Culex pipiens*), two invasive species (*Aedes albopictus* and *Aedes koreicus*), and potential malaria vectors (*Anopheles* spp.).

Cx. pipiens is an endemic mosquito to Italy and carries the zoonotic West Nile Virus (WNV), which can have serious or even fatal outcomes in humans and domestic livestock. This mosquito recently hit the headlines due to the high number of WNV cases last summer (more than 500, mainly in northern Italy, although not Trentino). Given the relevance for public and animal health, CRI has a longstanding commitment to the

study of the ecology of this mosquito species and the pathogens it carries. Our most important recent results are summarized in three scientific papers published in prestigious peer reviewed journals, and present methods for WNV risk assessments using quantitative models to generate estimates of both the risk of transmission and the annual re-occurrence of infections.

The ecological interaction between the native *Cx. pipiens* with the invasive mosquito species *Ae. albopictus* has also been investigated. *Ae. albopictus*, better known as Tiger mosquito, was introduced to Europe and Italy several decades ago, and is particularly annoying due to its daytime biting behavior. This species represents a new potential health risk, as it is a competent vector of arboviruses such as dengue, chikungunya e zika. Indeed, two outbreaks

of chikungunya virus have already occurred in Italy, one in 2007 in Castiglione di Cervia (Emilia Romagna) and one in 2017 in Anzio (Lazio), resulting in about 200 and 400 cases, respectively. Following the last outbreak, CRI carried out a study on the risk of chikungunya transmission, clearly placing CRI researchers among the top European experts in the field of eco-epidemiology of *Ae. albopictus*. We are currently developing extend-

ing these quantitative and predictive models to the biology, future colonization and establishment of the invasive species, *Ae. koreicus*.

Thanks to CRI expertise, FEM is one of the leading institutions developing guidelines for the monitoring and control of *Ae. albopictus* in the Province of Trento (in collaborazione con FBK, Azienda Provinciale per i Servizi Sanitari, Fondazione Museo Civico di Rovereto, e MUSE).

BEARFENCE - A dissuasion system to re-educate confident bears and promote coexistence

The population of reintroduced brown bear (*Ursus arctos*) in the Central Alps (Trentino, Italy) has successfully expanded to 50–60 individuals. However, brown bear presence poses significant management challenges to promoting human-bear coexistence

in a human dominated landscape, where some confident bears may approach and use anthropic resources. Prevention measures are crucial to reducing conflict with local stakeholders, and to ensure the success of the reintroduction project. Within this

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FEDERICO OSSI
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context, in partnership with Trento University, Fondazione Bruno Kessler and two enterprises, we have developed 'BEARFENCE', an innovative prevention system that aims at deterring bears and re-educating confident individuals. BEARFENCE is built from several highly technological components that detect collared individuals when they approach a resource that humans want to protect (e.g. beehive, livestock, etc.). Specifically-designed sensors on the bear collars activate a sequence of deterrents, which are randomized to minimize the risk of habituating the bears to the prevention system. After a first stage of technological development, the team has extensively tested the performance

of BEARFENCE under different environmental conditions and application scenarios. Long-term tests of functionality were performed, which confirmed a high detection reliability of the system. BEARFENCE is now ready to be deployed on confident bears, as defined by the Forestry and Wildlife Service of the Autonomous Province of Trento, in order to test its efficacy. From what we know of brown bear behaviour, it is likely that the response to the system will differ from bear to another. Nonetheless, the BEARFENCE team is convinced that the application of this or similar hi-tech systems can represent a crucial step in ensuring a long-term coexistence of bears and humans.

Alpine permafrost, water sources and biodiversity hotspots for the future of mountain aquatic ecosystems

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 DAVIDE ALBANESE
 STEFANO BRIGHENTI
 LEONARDO CERASINO
 CLAUDIO DONATI
 MASSIMO PINDO
 MARIA CRISTINA BRUNO

The European Alps are among the areas most impacted by current global warming. In this region, glaciers still represent the main hydrology drivers, as summer ice thaw provides water to lowland areas. However, this water provisioning capacity is strongly decreasing due to glacier retreat and is destined to become negligible within the next few decades, when most of the Alpine glaciers are predicted to disappear. However, an alternative water source for Alpine headwaters is represented by permafrost (i.e. ground that remains at $\leq 0^\circ\text{C}$ for at least two consecutive years), whose extent has been estimated to exceed that of the glaciers. Rock glaciers, i.e. slowly sliding rock debris containing ice, are among the most evident permafrost forms in the Alps. As their ice is insulated by a thick ground layer, it thaws at a lower rate with respect to glacier ice, so that rock glacier may represent an important water reservoir in a warmer future. Although the permafrost contribution to the Alpine hydrological cycle is expected to in-

crease considerably over the next decades, its ecological role is still poorly understood.

Our recent research has outlined how permafrost-fed headwaters are enriched in solutes (including heavy metals), and have unique biological communities (bacteria, diatoms, zoobenthos) that withstand harsh physical conditions typical of glacier-fed waters (e.g. low temperature, prolonged snow cover, high UV penetration), yet in a more benign environment characterized by e.g. stable channel and slow mineral turbidity. In collaboration with DICAM-UniTn, we have expanded the study of deglaciation effects, and of biodiversity and ecology of permafrost-fed waters in three sub-catchments of the Ortles-Cevedale mountain group (Solda, Zai, and de la Mare Valleys). The study combined a space-by-time substitution approach, where distance from the glacier snout is used as a proxy of established deglaciated conditions, with a paleolimnological approach. The latter was aimed



at reconstructing the relationships between lake chemistry and biology, and past changes in glacier and permafrost cover in the surrounding catchment. The results of the research confirm the chemical and biological peculiarity of rock glacial streams, and illustrate the double

ecological role of these habitats, that function as biodiversity hot-spots, where cold-stenothermal species could find refuge in a warmer future without glaciers, and as “stepping stones” for the colonization by downstream communities of upstream reaches created by glacier retreat.

Mechanisms of isoprene generation in fast-growing plants

Isoprene (2-methyl-1,3-butadiene, C_5H_8) is the most abundant non methane volatile hydrocarbon compound naturally emitted by vegetation into the atmosphere. It has been known for 50 years that many plants, especially perennial, fast-growing forest species like oaks and poplars, normally emit large amounts of isoprene into the atmosphere, mainly from green leaves. Due to its large emission quantity and rapid oxidation in

the atmosphere, isoprene plays a major role in atmospheric chemistry, as it can contribute to the formation of ozone and haze. A series of experiments have demonstrated that isoprene plays at the same time fundamental roles in protecting plants from environmental stresses.

In plants, isoprene biosynthesis is regulated by the presence and activity of a single enzyme called isoprene synthase (IspS), which catalyzes the

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conversion of IspS substrate to isoprene in chloroplasts. Up to now the origin of this important enzyme and the reasons for its highly stochastic occurrence in the plant kingdom were not known. By isolating AdolspS, the first isoprene synthase gene from a monocotyledonous species (*Arun-
do donax* L., Poaceae – giant cane), and validating its function, we found phylogenetic evidence that AdolspS and dicots isoprene synthases have likely originated independently from different classes of closely related plant enzymes, called monoterpene synthases. This led us to investigate the function of four amino acids specific to isoprene synthases in detail. Surprisingly, mutation of a single amino acid close to the catalytic site changed the substrate specificity of AdolspS, leading to emission of large amounts of ocimene and strongly in-

dicating that isoprene synthases have evolved from ocimene synthases. The mechanism responsible for this dramatic change in enzymatic activity through active site size modulation by a single amino acid mutation seems to be generalized, as the very same amino acidic position is implicated in the parallel evolution of several other terpene synthases from highly divergent plant lineages. In conclusion, we found that the ability of plants to emit about one-million tonnes of isoprene per year worldwide can be pinned down to a single amino acid regulating the specificity of the IspS enzymes. For the first time, this discovery links an ecological trait with global relevance like isoprene emission to a molecular mechanism which can be exploited to improve the sustainable production of this high added value molecule of plant origin.

Innovative approaches for the study of biodiversity and water quality assessment in the Alpine Region: The Interreg project Eco-AlpsWater

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Lakes and rivers represent one of the most important natural and economic resources in the Alpine region. Their waters are intensively used in agriculture and industry, becoming a life-sustaining element for the economy of one of the most densely populated and productive European areas. In addition, water bodies are one of the key elements for the tourist economy, attracting millions of people every year (e.g. over 20 million per year in Lake Garda) and contributing to the gross domestic product of the Alpine Region. The services offered by lakes and rivers are, however, strongly dependent from their water quality. The ecological status of water bodies is defined by the criteria provided by the Water Framework Directive (WFD, Directive 2000/60/EC). The results

obtained by the monitoring activities have important economic implications, being used to identify action plans for the recovery of lakes and rivers, based on the identification of target quality objectives. Nevertheless, the criteria and basic methods used in the application of WFD can be quite different from country to country, and are often based on the time-consuming expert identification of biological elements. These limits have been challenged by Eco-AlpsWater (2018-2021), a new European project co-financed by the Interreg Alpine Space programme and coordinated by FEM, including 12 partners from Austria, France, Germany, Italy, Slovenia, and Switzerland. The main aim of this European initiative is to integrate the traditional monitoring approaches with the anal-



ysis of environmental DNA (eDNA), which will provide detailed information on the composition and quantity (relative abundances) of aquatic biota. The piloting activities will allow: 1) preparing government agencies in the adoption of next generation monitoring tools; 2) integrating new innovative Next Generation Sequencing (NGS) technologies in the monitoring plans of the whole Alpine Region; 3) improving the management of water basins based on solid and innovative technologies and approaches at the local and Alpine Region scales. Besides contributing to the upgrade of monitoring approaches in Euro-

pean regions, the new NGS technologies will provide the census of lake and river biodiversity in the Alpine region at an unprecedented level, based on the analysis of hundreds of samples collected in over 50 bodies of water. The data will provide information on microbial, microalgal and fish communities, identifying the areas most at risk due to the presence of toxigenic cyanobacteria, waterborne pathogens, and invasive organisms.

For further information: <http://www.alpine-space.eu/projects/eco-alpswater/en/home>

HIGHLIGHTS



Conservation genetics of alpine grouse

BARBARA CRESTANELLO, ALICE FRASER, HEIDI C. HAUFFE

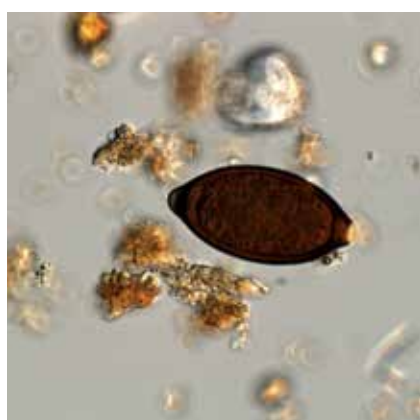
Grouse are well-known inhabitants of Alpine habitats, but endangered by climate change and human disturbance. DNA analysis of feathers shed by the birds is proving crucial for conservation decisions. We have found that both male and female birds successfully move between groups and find mates, indicating that, if managed carefully, these species should be able to increase and form new populations.



Moving in the Anthropocene: Global reductions in terrestrial mammalian movements

FRANCESCA CAGNACCI

In the past, the movement of wild animals was relatively unrestricted, and their travels contributed substantially to ecological processes. As humans have increasingly altered natural habitats, wild animal movements have also been restricted. This has been shown for the first time for terrestrial mammals in a metanalysis published in the prestigious journal *Science*, including the contribution of CRI researchers who coordinated 'Euroungulates' collaborative research network.



Gut biota indices: new tools for conservation

CLAUDIA BARELLI, HEIDI C. HAUFFE

The project WILDGUT, in collaboration with the University of Illinois (USA), brings together ecology and genomics to identify biodiversity indices useful for improving conservation strategies of wild threatened species. By measuring variation in micro-diversity (gut bacteria and helminths), we aim to apply ecological concepts normally used to detect biodiversity loss of larger species. The project has received prestigious funding from the EU H2020 Marie Skłodowska-Curie Actions (Fellowship n. 752399).



Forest Soil Respiration in a Changing Climate

MIRCO RODEGHIERO

Soil respiration is a key process for understanding the carbon cycle in forest ecosystems and the role of forests in climate change mitigation. This topic is extensively discussed in a book edited by Robert Jandl (Austrian Forest Research Center, BFW, Vienna) and Mirco Rodeghiero (FEM), collecting eleven articles from a Special Issue of the open access journal *Forests*.

SMARTsurveillance: aerobiological approaches for the early detection of emerging fungal pathogens

ELENA GOTTARDINI, MINGAI LI, ANTONELLA CRISTOFORI, FABIANA CRISTOFOLINI, CLAUDIO VAROTTO

Researchers from the Ecogenomics and Environmental Botany Unit have been appointed to coordinate one Working Package of SMARTsurveillance, a European project led by Fera Science Limited (UK) involving partners from 5 European countries. The project has the goal of developing novel approaches for the early detection of fungal pathogens of plants, which is key to their successful containment and eradication.



Applying metagenomics to the prevention of mastitis in dairy cows

FRANCESCA ALBONICO, ERIKA PARTEL, MASSIMILIANO MAZZUCCHI, MATTIA MANICA, ROBERTO ROSÀ, DAVIDE ALBANESE, CLAUDIO DONATI, MASSIMO PINDO, HEIDI C. HAUFFE

The MASTIRISK project, funded by Fondazione Caritro, and the Federazione Allevatori Provinciale, is using modern molecular techniques to analyze changes in milk microflora during the development of subclinical mastitis. Microbial indicators of disease risk are being used to develop a statistical model of risk assessment that farmers can use to make decisions about herd management to prevent mastitis and decrease reliance on antibiotics.



Rapid-E, a real-time pollen detection device in FEM

ANTONELLA CRISTOFORI, FABIANA CRISTOFOLINI, CLAUDIO VAROTTO, ELENA GOTTARDINI

Effective and timely allergenic pollen warning alerts, detection of fungal crop pathogens and monitoring of pollutants: thanks to the Rapid-E device, designed by a spin-off of Geneva University and installed in the San Michele all'Adige Multi-equipped Aerobiological Facility, CRI palynologists will join an international network for the automatic and real-time detection of pollen.



News from the rotifer *Keratella cochlearis*

ULRIKE OBERTEGGER, ADAM CIEPLINSKI

The rotifer *Keratella cochlearis* is occurring world-wide. Within the context of integrative taxonomy the existence of cryptic species (i.e. species impossible or difficult to distinguish based on their morphology but satisfy the biological species concept) was shown. The analysis of rotifer swimming patterns set the basis for automatic analysis of rotifer trajectories with open-source software.



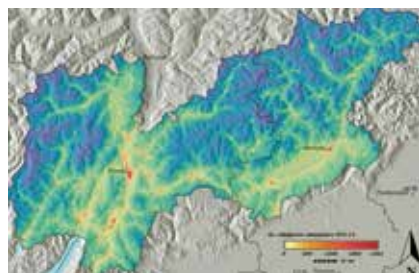
Networks and new joint research initiatives

ROBERTO CHINCARINI, GIAN ANTONIO BATTISTEL



FEM has become a “competent body” of the European Food Safety Authority – EFSA

During 2017 FEM was recognized, along with 300 other institutions, universities and research centers at European level - as a “competent body” of the European Food Safety Authority (as defined in Article 36 of EFSA’s establishing treaty). FEM can now be called by EFSA to provide technical-scientific support in the areas of food and nutrition as well as more specifically in the area of food production, additives used in preparation and processing, food traceability, food effects on human health and well-being. It can provide expertise in plant pests and diseases, including management strategies. In addition, it will provide knowledge in the field of nutrition, food safety and livestock farming, and the risk of diseases important for animal and human health.



Joint Research Unit - EPILAB-JRU

EPILAB is a Joint Research Unit (JRU) between Fondazione Bruno Kessler and Fondazione Edmund Mach for concerted research actions in quantitative epidemiology of emerging diseases by sharing of scientific resources and expertise, as well as participation in national and international research projects. EPILAB aims to improve the understanding of epidemiology, evolution and control of infectious disease agents. EPILAB also proposes the development and implementation of new techniques to inform decision-making regarding the prevention and control of these diseases in order to limit their effects on human well-being.



Plant Phenotyping Network - PHEN-ITALY-JRU

Since 2018, FEM has been a member of the “National Network of Plant Phenotyping” as a Joint Research Unit (JRU), coordinated by the Department of Bio-Agri-Food Sciences of the National Research Council and by the Lucana Agency for Development and Innovation in Agriculture (ALSIA), under the name PHEN-ITALY. PHEN- ITALY is based on the ESFRI-EMPHASIS-PREP project “European Infrastructure for Multi-scale Plant Phenotyping and Simulation for Food and Security in Changing Climate” which aims to develop and implement an infrastructure accessible to other institutions interested in this topic. PHEN-ITALY acts as a node by promoting, coordinating and facilitating the participation of the national scientific community in related research activities.

International Joint Center for Apple Research

ANNAPAOLA RIZZOLI, MICKAEL MALNOY, STEFAN MARTENS

In 2018, Northwest Agricultural and Forestry University (NAFU) and Fondazione Edmund Mach officially signed a cooperation framework agreement to set up a joint Apple Research Center between the two institutes. According to the agreements, NAFU will carry out extensive and in-depth cooperation in basic research, joint training of students or young faculties, and international project application with the partners. In October 2018, the International Joint Center for Apple Research was inaugurated at NAFU in the presence of a delegation from Cornell University (USA), Bologna University, FEM, and Plant and Food Research (New Zealand). The International Joint Center for Apple Research will be an open sharing, cooperation and win-win apple research platform for our scientists, contributing to the development of the apple industry in the global community.



CLIMATE KIC and EUSALP

ALESSANDRO GRETTER

During the last two years, CRI has extended its participation in international networks of strategic relevance for its research. Within the Alpine Macro Strategy (EUSALP), as representatives of PAT, we have been involved in the working groups on ecological connectivity, agro-forestry resources and education. Since spring 2017 FEM is also a member of the Climate KIC, the knowledge and innovation community created by the European Institute of Technology challenging climate change. In both of these initiatives, our scientific competence and expertise has allowed us to participate and coordinate 12 externally funded initiatives.



Project E-STaR: science communication and public engagement

ANNA ERIKSSON

E-STaR is a project financed by the Autonomous Province of Trento through the call "I comunicatori STAR della Scienza" with the purpose of stimulating the acquisition of new skills and designing innovative methodologies to relate science and society. E-STaR aims to bring science closer to society by engaging the general public using a citizen science approach, educating young people in actions to safeguard our environment and improve citizen and consumer awareness. The E-STaR project also works to strengthen the collaborations of the research institutes in Trentino through the organization of public events such as science cafés and workshops.



Storm postcards: a piece of history of Trentino meteorology

EMANUELE ECCEL

Co-funded by Fondazione CARITRO, the project ASTRO2 has enabled the creation of a digital file of 1618 "Storm Postcards" mailed from Trentino in the period 1951-1981, and maintained at the CREA archives in Rome. The postcards also yield autograph information of high interest to the historic and epistemological framework of hailstorm defence in Trentino in that period.







FACTS AND FIGURES

SELF-FUNDING AND IMPACT-FACTOR PUBLICATIONS

Gross self-funding capacity 2017-2018

During the 2017-2018 period, the self-financing deriving from competitive projects and CRI contracts was **11,842,265** euros. Competitive projects include funding on calls for proposals H2020, COST Action, EFSA, EUREGIO Science Fund, KIC-Climate, PRIMA, CARITRO, MAB-UNESCO, PSR PEI, PSR-POR, Interreg Alpine Space.

Articles published in scientific journals 2017-2018

383



STAFF

AT 31/12/2018

TOTAL **182**



43%
FEMALE



57%
MALE

ITALIAN **86%**

FOREIGN **14%**



FROM 16 DIFFERENT COUNTRIES

POSITION



PERMANENT
76%

TEMPORARY
24%

37% HOLDING
A PhD



AVERAGE AGE

42.1 YEARS OLD

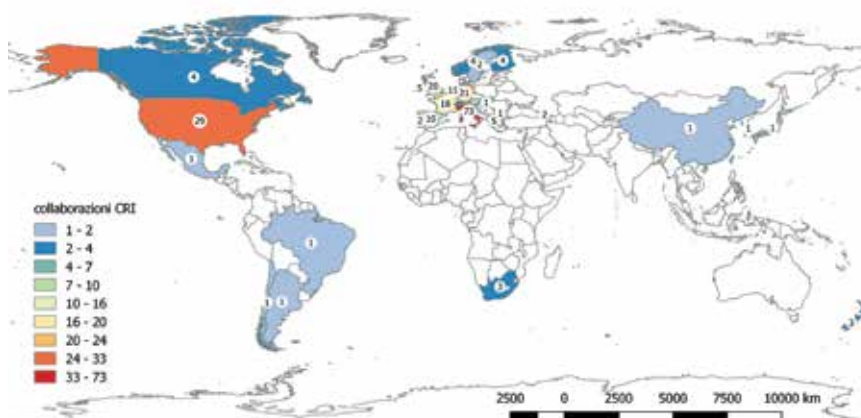
<30	6%
31-40	33%
41-50	35%
>50	26%

COLLABORATIONS

IN THE WORLD

Globally, FEM Research and Innovation Centre is involved in more than 200 research collaborations and contracts

.....



IN EUROPE

We cooperate with institutions and universities in almost all the EU member states and also in some extra EU Countries

.....



IN ITALY

In Italy, our research collaborations are mainly located in the central-northern area

.....





MAIN EVENTS ORGANIZED BY CRI

2017-2018

.....

6 big events

1.000 people involved

Abstract submitted

More than 700

50 seminars

11 educationals

(per month on average)

13 dissemination events

20.000 people involved

In 2017 and 2018, about 60 registered events were organized by CRI alone or in collaboration with partner institutions. Among them, six big international conferences with an overall participation of 1.000 people.

Moreover, an amount of about 50 seminars and 11 educational initiatives dedicated to PhD students and researchers' careers and expertise: specialist courses, workshops and summer/winter schools. Finally, 13 dissemination events that engaged the non-expert public and citizens and saw the participation of about 20.000 people. Kudos to all our supporters, staff and volunteers who helped us last two years!

CREDITS

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