2008

# Annual Report

## IASMA RESEARCH CENTRE

FONDAZIONE EDMUND MACH

ISTITUTO AGRARIO
DI SAN MICHELE ALL'ADIGE

Fondazione Edmund Mach
IASMA Research Centre

# Annual Report

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**FRONT COVER**

View of the Rotaliana plane from one of the micrometeorological towers active in the main ecosystems on the Trentino region. These stations enhance the understanding on the mechanisms controlling the carbon and nitrogen cycles, important information to comply with the Kyoto protocol obligations.



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**Annual Report IASMA Research Centre 2008**



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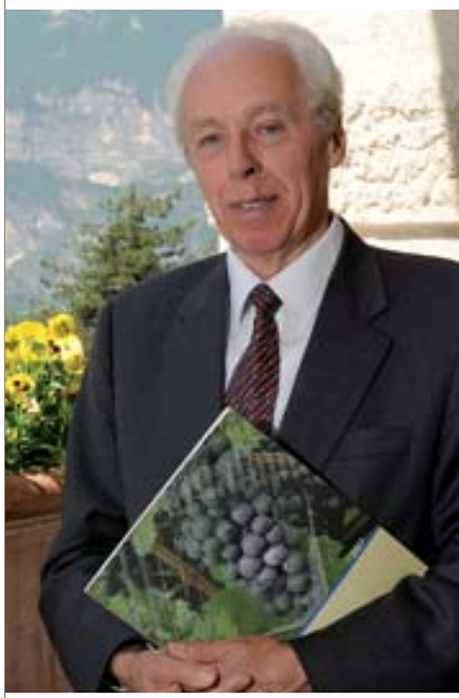
*Giovanni Gius*



*We are proud to present for the third year running the Annual Report, an extensive selection of the work and activities carried out in 2008 by the Centre for Research and Innovation of the Agrarian Institute of San Michele all'Adige.*

*This third edition is also full of scientific concepts and ideas, bearing witness to the multifarious research activities carried out by the Edmund Mach Foundation. The institute has grown and evolved over time and now comprises six hundred and sixty-nine employees and collaborators plus a complex of buildings covering a surface area of 24 thousand square metres. Important numbers and an important history.*

*This year marked a further stage on the journey that the institute began with its transformation into a private foundation. On the first of January 2009 the new organisational regulations came into operation, the most significant aspect of which was the separation of research and experimentation. The centres have changed their names and functions thus speeding up operational processes and allowing the Institute to concentrate more clearly on research, training and technical consultation in response to the province's demands. The new set-up will also see San Michele integrated into a national and international network of organisations active in these sectors, particularly with respect to research and innovation.*

*Past and future, tradition and innovation, reputations and projects: the equilibrium has, I hasten to point out, not always been easy to maintain but it has been at the heart of the important decisions recently taken by the Institute. We are committed to keeping to this path in the future: while, on the one hand, the Centre for Research and Innovation will firmly maintain its links with Trentino, it will also continue to develop and enhance the excellent standards which already distinguish its activities. It must be stressed that its research activities are a direct response to the specific demands of the Trentino territory; in other words, it is the concrete needs of agriculture in Trentino which spark the ideas and proposals which are then realised in international projects and which, developed to their full potential, lead to results of significant impact and interest on an international level.*

*The Annual Report, then, mirrors and bears witness to the team work which has brought and will continue to bring - I hope - the research activities at San Michele to the whole world through collaborations, partnerships, encounters in their various forms and exchanges of researchers.*

*Finally, I would like to congratulate all those who, in their various ways, participate in and dedicate their energies to the Centre for Research and Innovation of the Foundation-Agrarian Institute. A particular thought goes to those who have contributed to putting together this publication. To all of you my personal thanks and those of the entire administrative council.*

*Dr. Giovanni Gius*
*IASMA President*

*Roberto Viola*

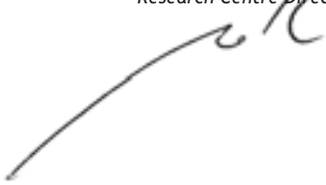
*The first year of operation for the Edmund Mach Foundation (FEM) has been particularly rich in challenges and opportunities. The transfer of IASMA activities into the Foundation, in particular, has required a considerable strategic and managerial effort and has occupied the best part of the year. In addition, the year 2008 has also seen the integration within FEM of the Centre for Alpine Ecology (CEA), until December 2007 a functional agency of the Autonomous Province of Trento. As from January 1st 2009, FEM research activities will be carried out by a newly established Research and Innovation Centre, while its experimental, consultation and service activities will be conducted by a Technology Transfer Centre.*

*In spite of these changes, the Foundation's scientific performance in 2008 has been impressive both in terms of impact and relevance. The number of publications in ISI-rated journals increased by 55% over the previous year and there has been a substantial increase in the portfolio of intellectual assets. Considerable investments have been devoted to capacity building in genomics and post-genomics through the establishment of dedicated platform technologies. Plant breeding activities have been strengthened through the establishment of a Fruit Innovation Consortium, involving FEM and the largest Trentino producers' and nurseries' associations, designed to connect firmly genetic improvement programmes to end-user needs. Cooperation between CEA and IASMA scientists has resulted in the activation of innovative large-scale research programmes in the fields of biodiversity and adaptation to climate change.*

*The results described in this report could not have been attained without the constant dedication, enthusiasm and professionalism of all personnel in the IASMA Research Centre. In particular, I would like to acknowledge the contribution of the Centre's "unstructured" community: our PhD students, postdoctoral fellows, visiting scientists and collaborators. Together they make up a large, young, dynamic and pro-active community: our true "beating heart".*

*Finally, I am grateful to all our sponsors and in particular to the Autonomous Province of Trento for its continuous financial support to our research programme.*

*Dr. Roberto Viola*
*Research Centre Director*



# The Institute

## History

On the 12th January 1874, the Tyrolean Diet in Innsbruck approved the institution of an agricultural school in the Augustinian monastery of San Michele all'Adige, the aim being to help guide the future of agriculture in what was then southern Tyrol. Activities were already under way by the autumn of the same year, according to the programme set out by its first director, Edmund Mach, from the Klosterneuburg experimental station in Vienna.

With a brilliant career in agricultural chemistry and oenology behind him, Mach was also an able organiser, valuable talents for the institute, which right from the start had an innovative constitution where teaching and research made a joint contribution to the development of agriculture in the territory.

The structure of the Agrarian Institute of San Michele (IASMA) was maintained until after the First World War when it passed to Italy and came under the authority of the Autonomous Province of Trento. In 1926 a consortium was established with the Italian State for the management of the Institute. In 1990 provincial law no. 28 formalised the transformation of IASMA into a functional agency of the Autonomous Province of Trento, again redefining its constitution, which henceforth integrated territorial services into the pre-existing structure based on training and research.

From 1st January 2008 the Institute's buildings and activities were transformed into the new legal body, the Edmund Mach Foundation (FEM-IASMA): a public agency under private law jurisdiction, in accordance with the law on the reorganisation of research (L.P.2 August 2005, n.14).

Throughout the year the new Foundation maintained its existing sub-divisions: an Educational Centre for training experts in the agricultural and oenology sectors, an Experimental Centre, which carries out studies at an international level, and a Technical Assistance Centre, providing support for businesses in the agricultural sector. Within the 3 Centres, however, systematic restructuring is taking place, which will lead to their being redefined in 2009.

The new structure will strengthen the Foundation and provide it with greater management flexibility in order to ensure prompt and efficient responses to ongoing social, economic and cultural changes.

## Organisation

Throughout 2008 the Edmund Mach Foundation has kept the existing IASMA structure based on its three main components: the Educational Centre, the Experimental Centre and the Technical Assistance Centre. Reorganisation of the 3 Centres is expected to take effect in 2009, when the restructuring process, resulting from its transformation into a foundation, will be completed.

In addition to the 3 Centres, there are numerous agencies with their own functions operating both within the institute and outside it.

Internally, the institute relies on its administrative, financial, information technology, legal, and public relations groups. Another function is the FEM-IASMA library, with 32,000 volumes and 1,100 periodicals, plus 5,000 volumes and 400 periodicals published between the end of the XIX century and the beginning of the XX century that are part of the library's historical fund. Significant external functions include the Europe Direct Carrefour Alpi office and the Agency for Quality Assurance in Agriculture (AQA). The Carrefour office we host is one of 350 centres set up by the European Commission to provide community information to rural society through individuals, farmers, associations, and public and private institutions.

AQA is an independent entity which provides certified quality assessments that help companies meet legal requirements, ensure quality products and customer satisfaction and match the competition on price.

Another very public part of the institute is its farming business. FEM-IASMA cultivates more than 100 hectares of land, growing apples, grapes and other fruits, and producing wine and distilled spirits. The farming business also functions as an advisory organisation for the research centre and the extension service, and as a teaching and training unit for the school.

The farm is committed to the concept of sustainable agriculture, adopting production principles that work from an agronomic point of view and that lessen environmental impact.

*The institute of San Michele was established around the year one thousand, at a time when various monasteries in Europe were being established or restructured in order to serve as centres for agricultural activities. In 1143 the Counts of Appiano donated the castle of San Michele all'Adige to the Prince Bishop of Trento; the castle was subsequently converted into a monastery by the Augustinian order.*
*The castle's ancient wine cellars, the refectory and the monastery's cloisters are still standing today, bearing witness to the Edmund Mach Foundation's distant origins. Indeed, the original foundations of the Augustinian monastery include two historic wine cellars which date back to the XII and XVI centuries, exquisite examples of architectural beauty and of great historic interest, testimony to an ancient wine making tradition in the territory.*
*These days the modern wine cellar has a wide range of wines and sparkling wines, and typical Trentino grappas and liqueurs. Average annual production comes to around 200,000 bottles of wine, 100,000 bottles of sparkling wine and 10,000 bottles of distillates.*

## IASMA Centres

### School

The Educational Centre of San Michele all'Adige is an unusual component of the national education-training system both for being "under the same roof" as the Experimental Centre and the Extension service, and for the wide variety of courses that it provides.
More specifically, education is grouped into four departments (known as Areas): the Technical Secondary Education area, the Professional Secondary Education area, the Professional Qualifications and Training area, and the Post-secondary and University area.
The Technical Education area provides three course options: a course leading to the title of agriculturist, a training course for agro-industrialists and a diploma course for oeno-technicians.
The Professional Education area, on the other hand, offers students a course of study for the title of Agro-technician integrated with a specialist qualification in the environmental-forestry sector.
The Professional Qualifications area deals both with professional training for young people of school age wishing to follow a practically-oriented course leading to the qualification of Agricultural Technician and Certified Agricultural Entrepreneur, and with permanent qualifications and training of adults as agricultural entrepreneurs and as technicians n the sector.
Lastly, the Post-secondary and University area runs the first level Degree course in Viticulture and Oenology in the context of the Interuniversity Consortium with the University of Trento and the Faculty of Agricultural Science of Udine. The Advanced Professional Training course for the parks sector also comes under this section. It is currently running a Masters course in Sparkling Wine Technology.

## Extension Service

The Extension Service, on behalf of the Foundation, provides socio-economic consultation and technical assistance to all the enterprises in the agricultural and forestry sectors in Trentino. In particular, the centre lends support to the fruit-growing, viticulture and oenology, and flower, fruit and vegetable sectors as well as to the animal and fish farming sectors.

Assistance and consultation is aimed at both individual entrepreneurs and organisations and associations. Support for this important section of the Trentino economy is intended to stimulate greater productivity in the agricultural and forestry sectors. It is also intended, however, that quality and respect for the environment form the basis of this productivity.

In 2008 the centre began an intensive programme to train and update its own technical staff. Most of our technicians have attended courses and seminars aimed at maintaining high, up-to-date levels of skill and professional service within the centre. We have provided support for the agricultural and forestry sectors in their various phases of production and in company management in conformity with all the latest regulations (environmental, safety, quality) and with the utmost professional attention. Technicians have been operating throughout the territory during strategically identified periods of greatest demand, guaranteeing a wide and constant coverage.

Monographs have been published and studies have been carried out covering specific issues relating to the territory with the aim of disseminating knowledge and training those involved in the agricultural and forestry sectors throughout the entire territory. Seminars and technical open-days have, on the other hand, provided them with a forum to discuss and lay the foundations for a future plan of action in the territory.

All the other information tools have been elaborated with the aim of providing continuity to an already well-consolidated information service.

All this has allowed us to provide extensive, professional support for our Province's agricultural and forestry economies.

## The Research centre

The rest of this annual report describes the Research centre and focuses on the status of its activities, we therefore only deal with some general aspects of its mission and functions in this section.

The Research centre offers its expertise to the Trentino land-based economy through research, studies and innovations that improve agricultural and forestry products and enhance the quality and nutritional value of food products. Its research programmes and advanced platform technologies are developed in order to deliver innovative solutions and competitive products for stakeholders and end-user communities. The Research centre also directly manages several farms for carrying out its studies.

Intense research programmes, top-flight facilities and important collaborations are the means with which the Research centre carries out its activities and produces results at an international level.

Research addresses prominent issues and is carried out by five departments: Agricultural Resources, Agrifood Quality, Genetics and Molecular Biology, Natural Resources and Plant Protection.

Since 1st January 2008 the Edmund Mach Foundation has also included the buildings and activities of the Centre for Alpine Ecology (CEA), a functional agency of the Autonomous Province of Trento focussed on the study and preservation of alpine ecosystems. By working together, the Experimental Centre and the CEA are able to tackle issues of paramount concern regarding the preservation of the environment and the sustainable use of natural resources in a systematic and integrated way, issues which are crucial for the territory and which are currently high on the international agenda.

**The rest of this annual report describes the research centre and focuses on the status of its activities.**

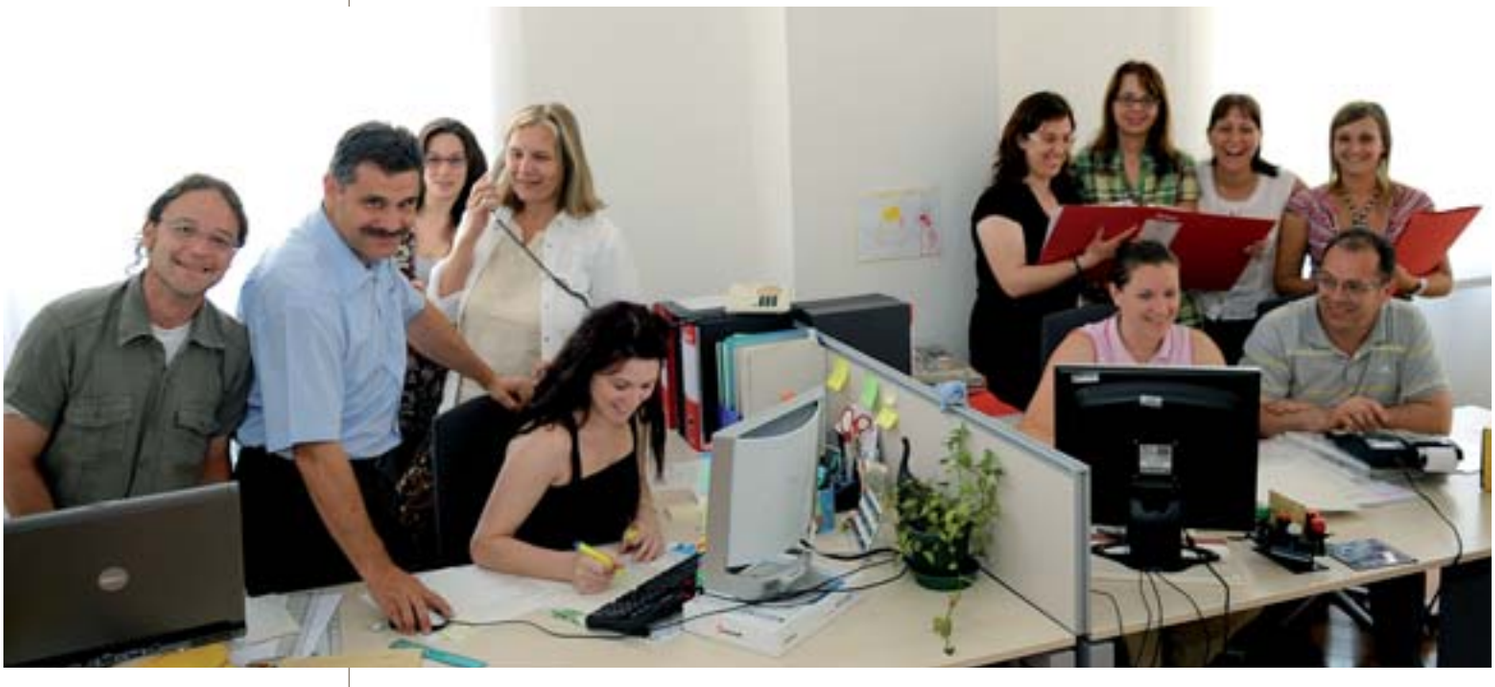
# About the Research Centre

## Mission and impact

At a local level, the Research centre's mission is to promote Trentino's land-based economy through research and innovation.

Research activities improve agricultural and forestry production and enhance the quality and nutritional value of food products. In addition, research protects the province's environmental resources by developing and promoting low-impact agricultural practices, studying and preserving biodiversity, and characterising alpine and sub-alpine ecosystems.

Over time, the centre's activities have evolved beyond its local functions. We also conduct research at an international level in collaboration with institutes and universities around the world. Our results impact the wider scientific community and the world at large.



## Organisation

Here is a brief description of the centre's departments.

- **Agrifood Quality**: Research areas include nutritional quality, sensory quality, guarantee of origin and authenticity, quality improvement in oenology, and process improvement. The department also provides analytical services.
- **Agricultural Resources**: Research areas include fruit and grape breeding, clonal selection and genetic improvement, agricultural resources management, and mountain farming systems.
- **Genetics and Molecular Biology**: Research areas include genome analysis, genetics and genomics of quality traits, plant-pathogen interaction, biodiversity of crop plants, gene transfer and functional analyses, traceability of genetically modified organisms in food and feed.
- **Natural Resources**: Research areas include ecology of the principal Trentino lakes and rivers, ecology, synecology and autoecology, genetics and biodiversity conservation, forest ecology, root rots and urban forestry, forest biodiversity, climatology, microclimatology, phenology, sustainable production and resources economy, air quality, sustainable use of natural resources.
- **Plant Protection**: Research areas include entomology, phytopathology, organic agriculture, and integrated pest management. The department also has a diagnostics and testing facility.
- **Centre for Alpine Ecology**: the Centre is aimed at promoting and developing research, information and educational programmes on alpine ecosystems in order to encourage local socio-economic development in relation to the need for a sustainable management of the territory under the pressure of global change.

## Human Resources

Approximately 92 percent of Research Centre staff are of Italian origin.

Of the remainder, about 5% come from the European Union, and the rest are from Asia, Africa, and Central America.

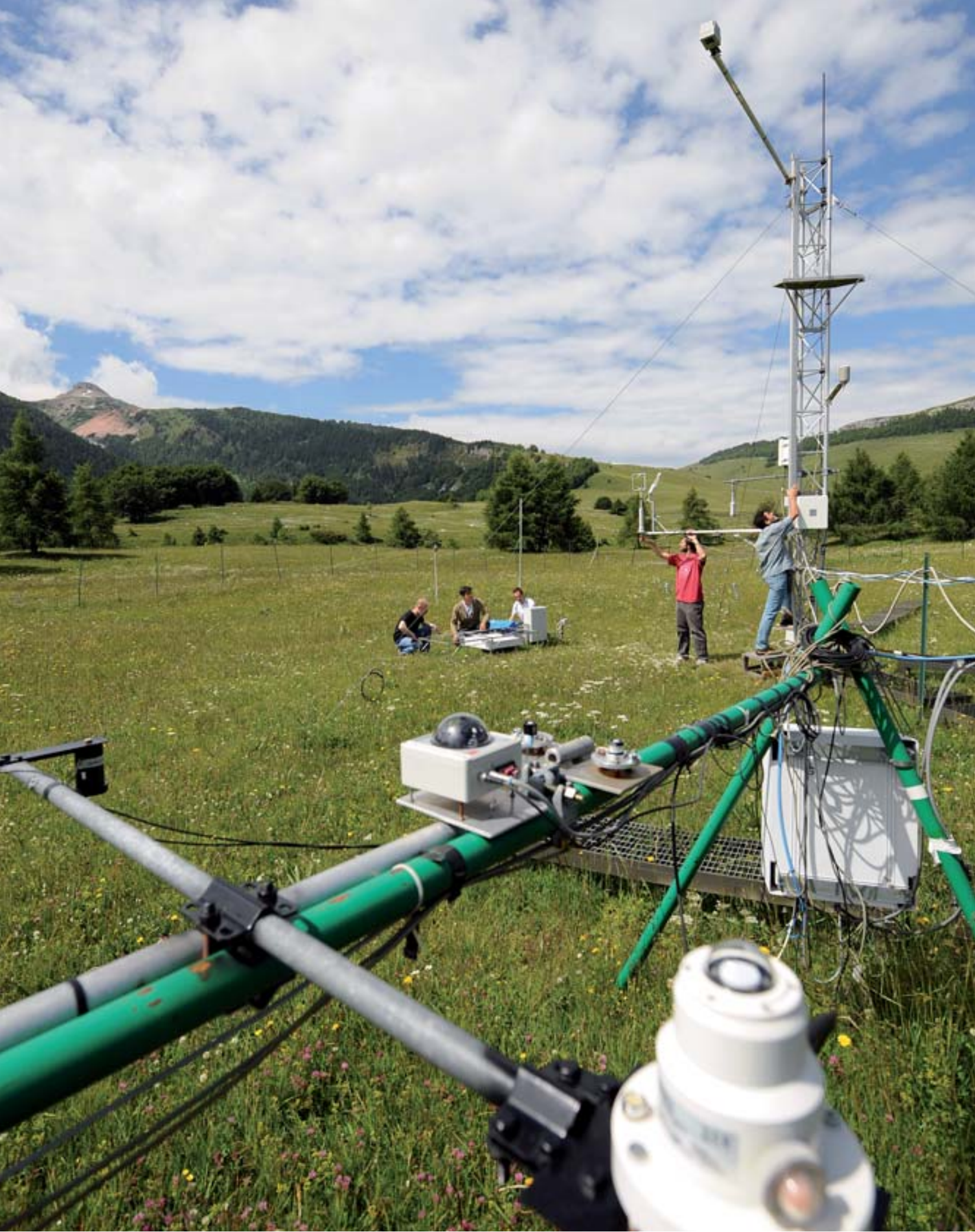
| ROLE | FEMALE | MALE | TOTAL |
|---|---|---|---|
| Researchers, permanent | 26 | 41 | 67 |
| Researchers, contract | 21 | 22 | 43 |
| Research fellows, post doc | 10 | 6 | 16 |
| Research fellows, undergraduate and graduate | 18 | 31 | 49 |
| Technical staff | 25 | 67 | 92 |
| Support staff | 9 | 22 | 31 |
| Direction and administrative staff of the Research Centre | 15 | 11 | 26 |
| TOTAL* | 124 | 200 | 324 |

* In addition, the Centre also offers internships, which are not included in these figures.

## Current international collaborations

| PROJECT | COUNTRIES INVOLVED |
|---|---|
| Molecular breeding applied to grapevine | Spain, France, Germany |
| Developing and promoting gene sequencing of the grape and apple genomes: postgenomic approaches | United States, France |
| The apple genome | United States |
| Comparative biological/biochemical analyses of anthocyanins | Germany |
| Management & Conservation of Grapevine Genetic Resources | France, Germany, Austria, Spain, Greece, Portugal, Switzerland, Hungary, Czech Republic, Cyprus, Slovakia, Moldova, Azerbaijan, Croatia, Georgia, Morocco |
| SMAP II –Programme of future research activities on apple proliferation (AP) disease at FEM-IASMA | Germany |
| QTL characterisation of aromas in grapevine (QTL A) | Spain |
| Study of the haplotypic distribution of candidate genes for the analysis of phenotypic variability in the complex agents involved in apple ripening (CANDI.HAP) | United States |
| TRACE – Traceability of foods | United Kingdom, France, Belgium, Germany, Austria, Greece, Czech Republic, Slovenia, Ireland, Norway, Poland, Iceland, Spain, China, Switzerland |
| Establishment and consolidation of a metabolomic platform for analysing determinants of quality in grape and apple | United States |
| Alpine ecosystems in a changing environment: biodiversity, sensitivity and adaptive potential | United States |
| PECC - Molecular-genetic analysis of Norwegian spruce (Picea abies): adaptive variability and evolution of the species in conditions of global climate change | United States |
| Established and emerging species of Phytophthora: growing threats to woodland and forest ecosystems in Europe | United Kingdom |


Research Centre
Plant Protection Department
Diagnostics & Testing
Entomology & Testing Facility
Organic Agriculture & Integrated Pest Managment
Natural Resources Department
Agrometeorolgy, Climatology & Aerobiology
Biomasses & Renewable Energy
Forest Ecology & Physiology
Limnology & Fish
Genetics & Molecular Biology Department
Cellular & Molecular Biology
Genomics & Bioinformatics
Molecular Genetic
Agricultural Resources Department
Fruit Production
Mountain Farming Systems
Viticulture
Agrifood Quality Department
Analytical Laboratories
Oenology & Traceability
Microbiology & Food Technology
Quality & Nutrition
Centre for Alpine Ecology
Wildlife Ecology & Biodiversity
Forest Ecology
Human Ecology



# AGRIFOOD QUALITY DEPARTMENT

*Fulvio Mattivi*

The department's mission is to improve the sensory aspects, nutritional value and health qualities of food products derived from plants grown in Trentino. We carry out basic and applied food research, aimed at improving the entire food production chain. The department develops methodologies to test and guarantee the typicality and origin of foodstuffs; it also develops innovative wine processing protocols and technologies, contributes to genetic improvement of the principal crops grown in Trentino, and provides high quality analytical support and consultation to producers through the institute's Technical Advisory Centre.

## Nutritional quality

Research conducted by the nutritional quality team is aimed at characterising the bioactive substances present in raw foods (apple, grape, strawberry and other soft fruits) and processed agrifoods (wine, juices, etc.). In collaboration with major Italian universities and research institutions we investigate the absorption, metabolism and action mechanisms of natural antioxidants (anthocyanins, cinnamic acids and resveratrols).

A further aim of our studies is to develop new products and applications (nutraceutics, cosmeceutics, pharmaceutics) of bioactive natural products, such as resveratrols. These natural hydroxystilbenes, present in grape and wine, have important anticancer and immunomodulating properties, and have been successfully investigated, resulting in Italian and European patents and one academic spin-off.

## Sensory quality

Sensory quality is one of the department's major research topics. Our aim is to characterise agrifoods using new instrumental and sensory approaches in order to identify markers of perceived quality and to provide analytical assistance and consultation to both producers and plant breeders. We support the development of innovative agrifoods through new production and stabilisation technologies based on sensory data in order to promote a more aware consumption, that is, consumption that is more sensitive to overall quality. The quality of typical agrifoods is developed through sensory characterisation and the monitoring of consumer knowledge, opinion and expectation. This task is also supported by investigation of the factors governing sensory experience and consumer appreciation, and of their correlation with biochemical and physiological parameters.

## Authenticity of origin

The department has internationally recognised experience in guaranteeing the origin and authenticity of agrifoods. We pursue this activity in the context of national and European projects, and in close cooperation with the Italian Ministry of Agriculture. This scientific collaboration was greatly expanded in 2008, due to an extended number of legal controls. We investigate new protocols and markers for the geographical, botanical and technological origin of agrifoods (e.g. stable isotopes and micro-elements). Application of this research is supported by the creation of databases and robust, validated methods for the traceability of agrifoods, as well as by the development of new instrumental methods (ICP-MS, SIRMS, etc.)

## Oenology

Quality improvement in oenology covers a variety of activities, ranging from optimising the use of techniques, technologies and fining agents to the development of rapid methods for quality control. Research is mainly focussed on nitrogen-containing compounds, volatile compounds (terpenols, sulphur-containing compounds, volatile phenols, etc.) and contaminants in grape and wines, as well as analysis of the phenolic potential of grape for the optimisation of red wine production. In collaboration with the University of Trento, the microbiology team is studying new matrices for the production of immobilised lactic bacteria. Another area of applied research in oenology is the development of methods for reducing exogenous antioxidants (sulphur dioxide) and for improving natural antioxidants in white wines. In the field of distilled beverages, the department is engaged in the improvement of distillation processes for the production of grappa and in providing scientific support for this traditional spirit so that producers in Trentino may maintain the highest quality standards.

## Industrial processes

The department's research team is also involved in improving industrial processes, in particular, the development of new techniques for stabilising juices, the exploitation of microbial biodiversity combined with the use of integrated cultures for the production of typical cheeses, and the industrial scale optimisation of processes for washing grapes.

## A new analytical platform for metabolomics

The main strategic development in the year 2008 was the implementation of a platform for metabolite profiling based on high-resolution mass-spectrometry. The first goal was to introduce into the Research Centre the know-how and instruments necessary for investigating the apple and grape metabolome. Rapid completion of this project is fundamental to improving the Foundation's ability to carry out successful research in the fields of functional and nutritional genomics.

## Analytical services

One of the department's major activities is to provide analytical services in a unique environment based on a robust analytical platform and on state-of-the-art expertise thanks to close connections with research activities. Our modern, well-equipped and efficient analytical laboratory provides high quality support and consultation to producers. The laboratory operates under the UNI CEI EN ISO/IEC 17025 with 38 SINAL-accredited methods (http://www.sinal.it/eng/) and is authorised by the Italian Ministry of Agriculture to provide viti-enological products with analytical certificates (22 different methods), which have official validity both at the national level and for export. We provide analytical services to about 300 private clients. In addition to working with the majority of regional companies and consortia and with many leading Italian wineries, we receive frequent requests from important international clients, especially from Germany and South Africa.

## Facts of the year

2008 was a particularly engaging year for both research and service in the department. This was the last year in which research, technology transfer and service were organised in a single structure. Consequently, 2008 was a year of transition, the eve of a large reorganisation planned for the following year, which delayed some of the most innovative research activities. Twenty-two externally funded projects were carried out during the year, in addition to the five internal programmes. Research activities led to consolidation of the important scientific production, with the publication of 16 scientific papers in major ISI journals, while the department's researchers were also involved in teaching ten university courses. We also performed well in the annual audit for renewal of our SINAL accreditation. Application for insertion into the national list of highly specialised laboratories recognised by the Italian Ministry of University and Research was completed. The laboratory was able to achieve a positive trend in external services, registering an 18% increase in annual turnover compared to the average of the previous three years. We handled as many as 25,000 samples for chemical or microbiological analysis, which allowed us to provide - in addition to analytical support within the research centre - analytical services to more than 300 clients, as well to the Technical Advisory Centre, the latter with around 4,000 samples.

# Geographical traceability of Italian and European extra-virgin olive oils

*Federica Camin*
*Roberto Larcher*
*Luana Bontempo*
*Matteo Perini*
*Daniela Bertoldi*
*Giorgio Nicolini*

Olive oil is one of the most important commodities produced in Italy, which is also the second largest producer in the world (630.000 tons in 2006-2007) and the largest consumer (International Olive Oil Council, http://www.internationaloliveoil.org) European law (EC Reg. N° 2568/91) provides producers with the option of indicating the geographical origin of extra-virgin olive oil according to the Protected Denomination of Origin (PDO) or the Protected Geographic Indication (PGI) and adopts from 1st July 2009 compulsory labelling of the origin of virgin and extra-virgin olive oils (EC Reg. N° 182/2009). On the other hand, no specific analytical methods are indicated for checking the authenticity of these classifications.

The ratios of stable isotopes of bioelements and the mineral profile, measured using an Isotope Ratio Mass Spectrometer (IRMS) and an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) respectively, have shown to be useful markers for tracing the geographical origin of various foods. The H, C and O stable isotope ratios (D/H, $^{13}C/^{12}C$, $^{18}O/^{16}O$) of plant material are linked to its botanical origin and to the geographical (latitude, altitude, distance from the sea) and climatic characteristics (relative humidity, temperature, precipitation, irradiance) of the growing area, whereas mineral composition is mainly related to the geological and pedoclimatic characteristics (Fig. 1).

## Italian PDO/PGI olive oils

(Camin *et al.*, Food Chem. DOI:10.1016/j.foodchem.2008.04.059; Bontempo *et al.*, RCM, 2009, 23, 1043-1048) As part of a ten-year collaboration with the Ministry of Agricultural, Food and Forestry Policy, around 100 authentic PDO and PGI extra-virgin olive oils have been sampled annually throughout Italy. This has been carried out with the aim of establishing a yearly databank of isotopic reference values, as has been done since 1987 for wine (EC Reg. No. 2676/2000).

In all the samples, the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios in the bulk oil and/or in the extracted glycerol were analysed. Since 2005, the innovative D/H ratio and mineral composition have also been measured.

The stable isotope ratios of C and O in bulk oil were significantly correlated with those in glycerol, and it would therefore seem preferable to measure the

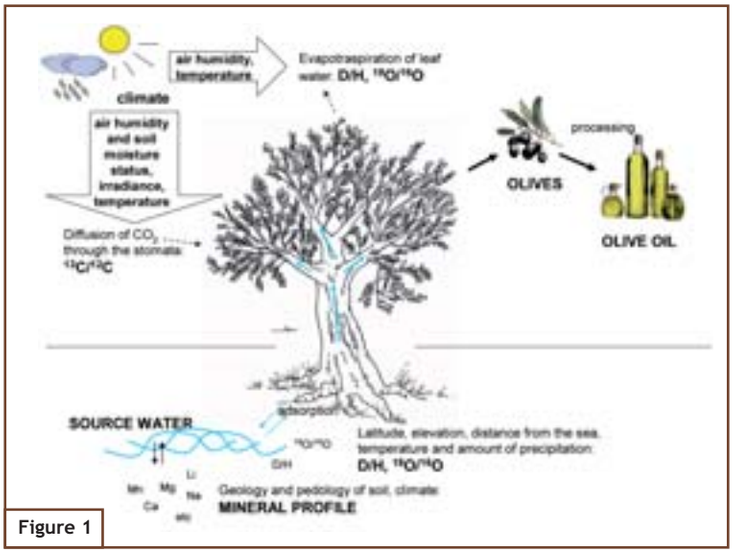



Figure 1

**Fig. 1** - The isotopic and mineral composition of olive oil records the climatic, geographical, geological and pedological characteristics of the area of provenance

isotopic ratios in bulk directly, saving time and cost. The D/H, $^{13}C/^{12}C$, $^{18}O/^{16}O$ ratios in olive oil increased from Trentino to Sicily, making it possible to distinguish Lake Garda olive oil from, at least, Sicilian and Calabrian oils in each year, and from all the central and southern regions of Italy in most years. D/H discriminated between olive oils produced on the Adriatic coast and those produced on the Tyrrhenian coast in each year. This coast effect is a consequence of the different sources and isotopic compositions of rainfall and the different climatic conditions on the two coasts. Significant differences were also found between PDOs from the same region and between harvest years. The year of production mainly influenced $^{18}O/^{16}O$, relative to the amount of rainfall and atmospheric humidity in the period of oil accumulation in the olives.

The content of 26 elements – among which Li, Rb, Cs, La, Ce and Yb, rarely reported in the literature - was measured in well-settled olive oils using ultrasound acid extraction. Taking into account the 2005 PDOs with at least 10 samples each, namely Lake Garda, Umbria and Terra di Bari, a trend towards higher Mg, Ca, Mn and Sr contents seems to characterise the Lake Garda olive oils, especially compared with the Umbrian oils. This trend may be related to the geology of the Trentino soil mainly originating from dolomitic limestone rock.

## European olive oils

Within the framework of the FP6 European Project TRACE (006942 "Tracing Food Commodities in Europe"), 267 authentic extra-virgin olive oils were collected during the 2005 and 2006 harvests in 8 European sites, selected on the basis of the climatic and geological variability of the olive cultivation areas: 3 in Italy (Trentino, Tuscany, Sicily); 2 in Greece (Lakonia, Chalkidiki); 1 in France (Carpentras); 1 in Spain (Barcelona); 1 in Portugal (Algarve). The H, C and O isotope ratios and the mineral profiles of the samples were analysed. The H, C and O isotopic ratios correlated significantly ($p<0.001$) with each other and with temperature, relative humidity and precipitation during the 6 months before harvest, as well as with latitude and distance from the sea. The regression lines of D/H had low $R^2$, probably because D/H is also affected by other variables such as olive cultivar/size. ANOVA and HSD for unequal N Tukey tests ($p<0.001$) of the two years data, showed that $^{13}C/^{12}C$ discriminated Trentino oils from Tuscan ones, from the group formed by Carpentras, Barcelona and Chalkidiki, and from the Algarve and Lakonia group. The $^{18}O/^{16}O$ discriminates more clearly between Algarve and Lakonia and, together with D/H, between Carpentras and Chalkidiki. By carrying out a multivariate analysis by linear canonical discriminant analysis, which maximises the differences between the groups by means of a linear combination of the variables, we obtained good differentiations between the geographical areas, with around 80% of the samples correctly reassigned to their sites using the reclassification test.

ANOVA highlighted statistically significant ($p<0.05$) differences in the content of 23 elements (Na, Mg, Al, K, Ca, V, Mn, Co, Ni, Cu, Zn, Ga, Rb, Sr, Ce, Nd, Sm, Yb, Cs, Ba, La, Eu, U) in the olive oils produced in the 3 different geological zones (limestone: Trentino, Carpentras, Algarve, Lakonia; shale/mudstone/clay/loess: Tuscany, Barcelona, Sicily; acid magmatic: Chalkidiki). The canonical multivariate discriminant analysis clearly discriminated between the 3 geological typologies, with 84% of the samples correctly reclassified.

A combination of the 3 isotopic ratios and the 23 trace elements resulted in optimal discrimination between the 8 sites (Fig. 2), with 96% of the olive oils correctly classified and cross-validated to the production site.

**Fig. 2** - Canonical Discriminant analysis of the isotopic and mineral composition of the olive oils from the 8 European sites: scatterplot of the first two canonical variables. RAD 1 is loaded mainly positively by $^{18}O/^{16}O$, Ga, Cs and negatively by Al, K and Ca; RAD 2 positively by Ni, Al, $^{13}C/^{12}C$, and negatively by Cs and Eu. The separation between Lakonia and Sicily improves when RAD3 is also considered (11%; loaded positively by Ga, Ce, D/H, and negatively by Al, La, Sr, Yb)

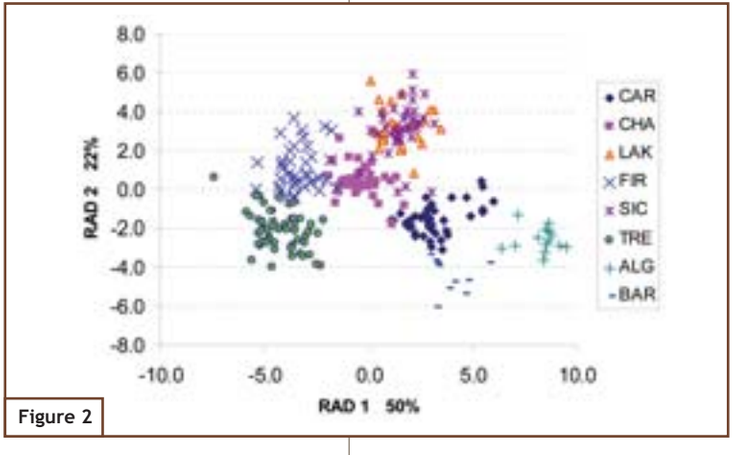



Figure 2

# Classical sensory profiling and an innovative data mining approach to typical cheese classification

*Flavia Gasperi*
*Franco Biasioli*
*Isabella Endrizzi*

Product classification for quality control or typicality assessment is mostly performed on the basis of chemical and physical data. In the case of food products, however, sensory characteristics are more and more recognised as playing a key role because of their direct relevance to consumer acceptability. This is especially the case with typical or PDO (Protected Designation of Origin) products that have characteristics linked to the territory, or when sensory marker identification is used to investigate the phenotype-genotype link, or, more generally, in the development of new products.

## Critical aspects of the usual method

In descriptive sensory analysis, the training of assessors and the validation of attributes to be chosen by a panel to describe consistently the expected sensory variability and to classify products, are critical but expensive and time-consuming phases that require organisation and skills not readily found in SME.

The physiological and cognitive processes at the root of sensory evaluation are highly dependent on individual factors and any effort to standardise and average out judgements brings with it a reduction in descriptive power. In consumer science, instead of averaging preference judgments, it is commonly preferred to divide consumers into groups with the aim of identify clusters with different characteristics. In descriptive sensory analysis, on the other hand, assessments are usually averaged and in order to maximise panel consonance, low agreement descriptors are excluded from further analyses. In addition, individual judgements are generally standardised to reduce individual variability.

A further issue arises in the case of products for which sensory analysis is already required (e.g. olive oil), as the certification of conformity indicates threshold limits for values of individual attributes. The approach is, therefore, intrinsically "univariate" and does not take into account the "multivariate" nature of sensory evaluation. Moreover, panelists' awareness of the critical values may cause psychological bias.

For all these reasons we decided to evaluate the possibility of introducing new procedures for experimental and data analyses to provide greater efficiency in sample classification on the basis of sensory evaluation, on the one hand, and, on the other hand, to avoid the intrinsic limitations of "univariate" quality definitions.

## Our proposal

With these considerations in mind, we proposed an innovative approach based on multivariate classification models of sensory profiling data, independently developed for individual judgements instead of using panel averages.

Models were developed and tested on two inde-

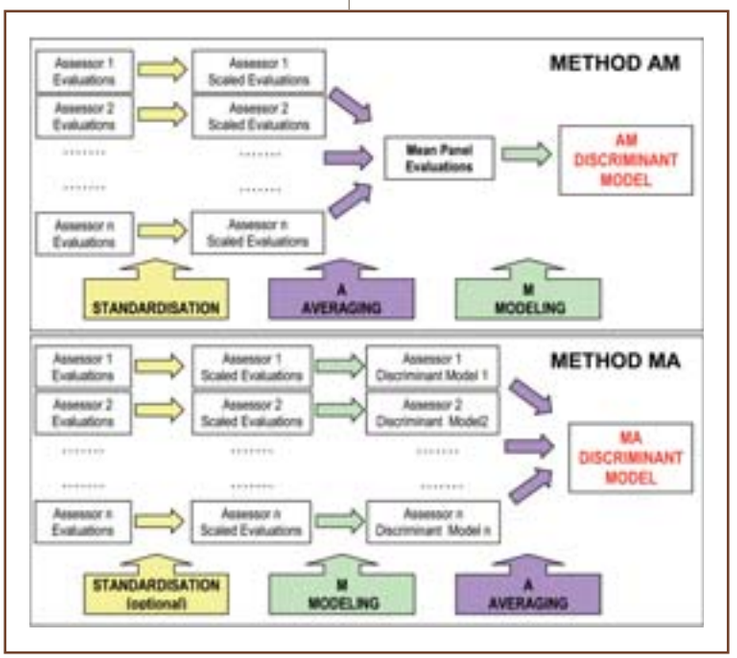



**Fig. 1** - Summary of the two methods for combining assessors' evaluations

pendent data sets obtained from previous projects aimed at sensory characterisation of dairy products from northern Italy.

The data sets were obtained by two trained panels working independently on quantitative descriptive analyses of two different kinds of cheese according to conventional sensory profiling. Sensory data were then used to discriminate different classes of products according to two different approaches (Fig. 1):

- AM (Averaging + Modelling): this is the procedure commonly adopted in sensory analysis and is based on standardisation of judgement scores, computation of panel averages and sample classification based on panel averages;
- MA (Modelling + Averaging): this is our new procedure based on a model which independently classifies each judgement. The final classification is then obtained by choosing, for each product, the class with the most votes.

## Results

Our proposal for classification using independent models for individual judges (MA) was equivalent to or better than the standard approach (AM) for all the classifiers tested: discriminant Partial Least Squares, an algorithm widely used in chemometrics and sensory analysis; Penalized Discriminant Analysis, a generalised version of the Linear Discriminant Analysis, and Random Forest, an algorithm already introduced into sensory analysis in a previous work (Granitto *et al.*, 2007, Food Quality and Preference).

The increased efficiency of MA is higher in the case of the least consonant panel, indicating that panel consonance is less relevant than sensitivity and repeatability of individual judgements.

This finding partially reduces the need for panel training and seems to indicate that, as our simulations on a set of several attributes for each judgement demonstrated, our proposal could also be used on data obtained by free choice profiling, an alternative and cheaper method that does not require panel training.

## Conclusions

We have developed a cheaper and more efficient procedure for classifying products on the basis of sensory data that avoids the limitations of "univariate" approaches and reduces costs related to panel training.

This work was made possible by bringing together expertise in sensory profiling and in data mining, which came out of a post-doc project (SAMPPA – PAT) aimed at developing innovative data mining procedures. This project initiated an ongoing collaboration between the Bruno Kessler Foundation (Povo Trento, Italy; Dr. Furlanello) and CIFASIS (Rosario – Argentina; Dr. Pablo M. Granitto).

*For further details refer to Granitto et al. Food Quality and Preference, 19(2008:) 589-595.*

# Microbial biodiversity in Trentino milk and the use of autochthonous strains in Secondary Adjunct Cultures in typical raw milk cheeses

*Elisa Poznanski*
*Elena Franciosi*
*Agostino Cavazza*

Raw milk cheeses have a more intense flavour than pasteurised milk cheeses. It is well known that non-starter lactic acid bacteria (NSLAB), originating from raw milk, play an important role in the typicality of these cheeses, so the "biodiversity" of LAB involved in cheese production is considered fundamental to the distinctive features and quality of artisan products.

The main task of dairy starter cultures is rapid acidification of the environment, because the resident LAB acidify the milk slowly. The addition of starter LAB is useful for maintaining a certain uniformity in the final product, the drawback being that the raw milk biota may be lost. This process may also eliminate bacteria with important properties for cheese-making.

The milk Natural Starter Cultures (NSC) used, for example, in most Trentino artisan cheeses, are prepared with small amounts of high quality milk scalded to 65° C and maintained at 45° C overnight, which selects the most acidifying thermophilic strains.

NSLAB resident in milk can subsequently grow and they become the main factor responsible for the typicality of cheese due to their lypolitic and proteolitic activities. The Italian Ministry of Agriculture and Forestry and the autonomous province of Trento have funded two research Projects aimed at the study of microbial biodiversity in raw milks collected in the Trentino region before being processed into cheese. 370 strains of LAB (lactic acid bacteria) were isolated from eight batches of raw cow's milk destined for artisan cheese production, in order to collect, describe and study the microbial populations found in this environment.

Isolates were subjected to molecular screening and clustered into 124 groups of strains sharing the same RAPD-PCR profiles. Strains were subsequently identified by means of different molecular techniques (species-specific PCR, multiplex PCR, amplification and separation in DGGE or sequencing of the 16S rDNA V3 region). 17 LAB species belonging to 6 genera (*Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus*) were found in the milks analysed. Almost 94% of LAB isolates were enterococci, lactococci and streptococci, while the most frequently identified species were *E. faecalis, E. durans, Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris, Lc. garvieae, St. thermophilus* and *St. macedonicus*. 63 strains dominating the microbial population in raw milk and

which are important for cheese manufacturing were further characterised for their technological aptitudes (growth at different temperatures, acidification potential, diacetyl production, autolysis, proteolytic activity, lisogeny). These strains showed high intra- and interspecific variability, thus confirming the high biodiversity of the microbial population of typical and artisan products.

Our study showed the rich biodiversity harboured in the milk samples, and therefore sources of new strains and species belonging to the LAB (enterococci, lactococci and streptococci). We characterised strains that were able to acidify and produce aromas and odours. Their performance during cheese manufacture, when used as natural starter cultures, as well as their persistence in cheese and their contribution to cheese flavour as secondary adjunct cultures will be studied in greater depth.

The research group already has experience in making cheese using secondary adjunct cultures (SACs): we have described and characterised dominant microbial populations in Puzzone di Moena cheese during production, from early curdling stages to 3-month ripened cheeses. Typically, the first species dominating the microbiota in this cheese are *S. thermophilus* and *E. faecalis*, the first one decreases during ripening, while enterococci are found at higher levels until the end of the observation period. NSLAB species *P. pentosaceus, Lb. paracasei* ssp. *paracasei* and *Lb. plantarum* grow later and dominate the microbial population during cheese maturation. Among the species isolated we have characterised and selected those strains with low acidifying and high proteolytic capacity, able to persist in dominant populations throughout the whole ripening period, and thus possessing the right traits for use in an SAC. SACs are a mix of pure strain cultures, used at low inoculum levels (contrary to starter cultures) and able to ensure the dominance of wanted strains, thus crucial for minimising microbial variability during the ripening process. The aim of our work is to obtain a "controlled typical product" using autochthonous strains which are able to drive fermentation in the desired direction, assuring that the product is always recognised by the consumer.

We have developed and used in cheese-making an SAC made with three strains belonging to the dominant species, *P. pentosaceus, Lb. paracasei* ssp. *paracasei* and *Lb. plantarum*, at two concentrations ($10^3$ e $10^4$ cfu/ml). The lower inoculum level produced a cheese with the desired traits, while the higher inoculum yielded a cheese with a flatter flavour. The use of SACs is not an easy way to modify cheese-making technology because of the intrinsic nature of the raw material, which is always variable and rich in different microbiota, but it is nevertheless a good way to sustain local production in preserving its typicality.

# In brief

## A study on benzene formation in beverages and juices with PTR-MS

*Eugenio Aprea, Franco Biasioli, Silvia Carlin, Flavia Gasperi*

There is evidence that benzene can form in beverages and juices containing preservatives such as sodium or potassium benzoate in the presence of ascorbic acid, added as an antioxidant or naturally present in fruit juices. Light and heat favour benzene formation. Surveys of benzene levels in commercial soft drinks show that diet products are more affected, suggesting a possible role of nutritional sweeteners in mitigating/reducing benzene formation. In a recent work we used Proton Transfer Reaction Mass Spectrometry for direct benzene quantification in beverages. We also investigated the role of sugars (sucrose, fructose, glucose) on benzene formation in a model system containing ascorbic acid and benzoate (Aprea *et al.*, IJMS, 275(2008): 117–121) confirming their mitigation effect.

## A Real Time PCR method for yeast detection in bottled wine

*Lorenza Conterno, Verena Scartezzini, Claudio Moser, Riccardo Velasco, Agostino Cavazza, Elisa Poznanski*

Measuring microbial stability in real time is important in order to prevent microbial wine spoilage. Carried out at bottling, this analysis will improve the commercial efficiency of the winemaking process.

The qPCR High Resolution Melting (HRM) technique currently appears to have enormous potential in the rapid quantification and analysis of differences in DNA sequences. The aim of this study was to apply qPCR HRM to count and identify microbial events in bottled wine.

The method turned out to have many advantages over traditional plating and DGGE-PCR. We successfully distinguished ten genera of yeast, including *Brettanomyces*, *Zygosaccharomyces*, *Schizosaccharomyces* and *Saccharomyces*, based on their HRM profile. Quantification of *B. bruxellensis* and *S. cerevisiae* revealed high linear correlation and efficiency. The method was applied on a culture-dependent basis, although it could be applied to direct microbial analysis post-bottling after proper sample concentration. A further study will be carried out to validate the method.

# In brief

## Differences in the amount and structure of grape tannins

*Fulvio Mattivi, Urska Vrhovsek*

Red wine is a very rich source of flavanols, which consist of catechins and oligomeric and polymeric proanthocyanidins (PAs). The structure of grape PAs was investigated by HPLC-DAD-MS analysis carried out after thioacidolysis of wine-like extracts. The grape variety determines both the amount and the structure of flavanols, and influences all their qualitative parameters: the percentage of monomers, the percentage of the building units and mean degree of polymerisation (mDP) of grape PAs. Grape extracts of the varieties Cabernet Sauvignon, Carmenere, Marzemino, Merlot, Pinot Noir, Syrah and Teroldego were mainly rich in monomers and small oligomers (mDP < 8). Biosynthesis of flavanols and PAs in the grape appears to be highly regulated at the level of the variety, leading to differences that are likely to play a significant role in the nutritional and sensorial properties of the wines. Increasing the percentages of galloylated PAs and of prodelphinidins is expected to increase the antioxidant potential of the wine. Galloylated PAs from seeds are a source of free gallic acid, the wine polyphenol with the highest bioavailability. The extent of galloylation affects both bitterness and astringency.

## A mechanistic approach to elucidate uptake of grape anthocyanins

*Urska Vrhovsek, Fulvio Mattivi*

Many fruits and vegetables in the human diet are rich in anthocyanins. In recent years, numerous studies have indicated that anthocyanins may have positive effects on human health. Any piece of knowledge concerning the absorption, tissue distribution, metabolism and excretion of dietary anthocyanins is expected to provide clues to understanding the apparent paradox between the vanishingly low concentrations of these pigments in cells and their bioactivity. The aim of this work was to investigate the renal uptake of dietary anthocyanins and the underlying molecular mechanisms. While anthocyanins in the liver were at apparent equilibrium with plasma, kidney anthocyanins were 3 times higher than in plasma. Anthocyanin uptake from blood into kidney tubular cells is likely to be mediated by the kidney isoform of organic anion membrane transporter bilitranslocase. Glomerular filtration of anthocyanins was found out to be the most plausible mechanism of anthocyanin extraction into urine.

# Facilities and Equipment

The Agrifood Quality Department has extensive experience in research and development projects using state-of-the-art technologies to deliver innovative solutions in co-operation with industry. The department's equipment is primarily aimed at supporting two research pillars of the "Food for Life" platform: "Food and Health" and "Food Quality and Manufacturing". Alongside the equipment, a panel of specialists trained to conduct sensory analyses provides the department with essential expertise. Our main equipment and facilities consist in:

- 2,000 square metres of fully equipped analytical and microbiological laboratories with SINAL certification.
- Spectrometers: GC-MS (4), UPLC-Q-TOF, GC-TOF, HPLC-MS, PTR-MS, ICP-MS, NMR, IR-MS (3).
- Analytical techniques, including: GC (15), HPLC (5), CI, FT-IR, ICP-OES, preparative HPLC, NIR spectrometer, UV-VIS spectrophotometers (2) and a differential pH-meter.
- Microbiological tools, including: autoclaves, fermenters, thermocyclers, refrigerated incubators, microscopes and stereomicroscopes, electrophoresis cells (4), laminar hoods and microcentrifuges.
- Experimental winery with a capacity for 400 test vintages per year.
- A sensory laboratory with a product preparation area, ten testing booths with serving windows, controlled light and software for data acquisition.

# AGRICULTURAL RESOURCES DEPARTMENT

*Marco Stefanini*

The Agricultural Resources Department conducts basic research and also provides laboratory services. Its principle areas of interest are:

- characterisation of the key physiological, biochemical and nutritional factors influencing productivity and quality in Trentino's major crops: grapevine, apple and soft fruit.;
- genetic improvement (breeding and clonal selection) of cultivated varieties and rootstocks, as well as continuous development of agronomic techniques and conservation methods in order to sustain the competitiveness of the local agronomic sector;
- development of "precision farming" approaches using GIS and remote sensing technologies to enhance management of agricultural resources;
- sustainable development and diversification of mountain agricultural systems through improved animal husbandry and grassland use practices;
- services and innovations that guarantee quality products through close interaction with IASMA's technical support centre and agronomy school.

The department has three research units: viticulture, fruit production and mountain farming systems. Our main areas of research are presented in the following sections.

## Breeding and genetic improvement of apple and grape

The focus is on developing new cultivars capable of meeting market needs in terms of consumer quality and economic viability. Although distinct in their final application, the viticulture and fruit production units both take a multi-disciplinary approach to agronomical and phenotypical characterisation, and to genomic research. Research issues include the agronomical and biological aspects of breeding and the evaluation of new cultivars.

While we continue to use conventional breeding techniques to create new cultivars, we make increasing use of genomic resources. In particular, we have recently begun to develop expertise in marker assisted selection, a method that greatly improves efficiency.

Our activities cover the following fields:

- maintenance and evaluation of germoplasma (grape and apple);
- conventional breeding techniques, including inter-specific crossing, development of breeding lines and progeny trials;
- vegetative propagation and nursery techniques;
- breeding strategies for disease resistance;
- selection for resistance to phytopathology and for fruit quality;
- agronomical and physiological evaluation of new genotypes.

We have an important clonal selection and genetic improvement programme for the traditional grape cultivars of the Trentino region. Clonal selection is carried out in 20 hectares of vineyards, the selection criteria being phenologic characteristics, morphological differences of leaves and grapes, productivity, vigour and cluster characteristics.

Particular emphasis is placed on grape composition and its technological potential for wine.

Phytosanitary development (virus detection and eradication) is performed in laboratories and greenhouses, while quality studies are carried out in field clone trials. Clonal selection is carried out according to procedures specified by the national committee for the homologation of grapevine material, and is officially registered and certified. Our clones are marketed under the trademark ®ISMA.

## Optimising production in grape and fruits

Our fruit and crop growth research programme capitalises on extensive experience in crops and in environmental physiology for viticulture and pomology. Regarding apple, we focus on enhancing the quality of fresh fruit to support regional fruit production and marketing. As for grape, our objective is to optimise fruit quality in order to raise the standards of regional vine production.

Compared with annual crops, perennial fruit crops offer wide flexibility in the physical arrangement of their fruiting canopies. Not only can the size and shape of the canopy be dramatically altered by tree spacing, pruning and training, but the canopy can also be physically constrained by a support structure of posts and wires.

Various tree shapes and forms can be found in orchards. In our research, we are interested in the interactions between canopy architecture and flowering, fruit development and quality. Our aim is to manage plant canopies in order to optimise the way trees intercept light and convert this energy into high yields of top quality fruit.

Apple quality benefits from regular production from year to year, which can only be achieved when trees do not overproduce. Several chemical thinning agents are currently available and we use our fields to compare their efficacy with the standard strategies for the main apple cultivars. After storage or transport comes the horticultural or harvestable maturity phase. In this developmental stage, the fruit is ready for market. To get fruit to the consumer in the best possible condition, we use several harvest indices, including SSC (Brix), acidity, dry matter, skin and flesh colour.

We have produced modelling approaches for use in estimating the maturity of grapes from various vineyards and from different cultivars. These computer models help to indicate the optimum harvest time which will consistently produce top quality grapes. We are also developing precision farming tools based on GIS and remote sensing for better management of agricultural resources.

## Sustainable land use

Trentino's productive lands and natural areas are vitally dependent on the quality of soils, mountains, water and biodiversity.

The goal of our research is to implement and assess collective action aimed at promoting sustainable agriculture and rural development in the Alps.

Trentino's agricultural systems are designed to yield premium fruit and animal products through sustainable management that exploits the natural capital of environmental resources.

Low-impact management of alpine pastures and mountain landscape protection are particularly important aspects of maintaining the natural environment of the Alps. The aim of our activities is:

- to design and implement local action plans for sustainable agriculture and which contribute to rural development in the alpine region;
- to assess the efficacy of action plans already implemented and to disseminate methods and tools that promote sustainable agriculture;
- to provide political recommendations concerning rural development.

# Bi-axis - an alternative training system for apple growing

*Alberto Dorigoni*

It is well known that, due to high surface/volume ratio, more trees of a small size provide better exposure of the crop to sunlight than fewer, large trees. This is why, in the South Tyrol and Trentino areas of the Italian Alps, for example, intensive super-spindle orchards, with 0.5 - 0.7 metres between trees in the row, achieve excellent yields and high quality. On the negative side, given the high number of trees per hectare (between 4,000 and 6,000), this system requires high capital investment and excellent cultivation management - from crop load control to summer pruning, root pruning and the use of growth regulators - in order to maintain the delicate balance between crop load and healthy vigour. As a consequence, super-spindle trained orchards usually do not last long, generally less than 15 years. To avoid loss of quality through shading, which frequently happens in bicoloured apples such as Gala, Cripps Pink and Fuji it is important to move the crop to the outer portion of the canopy, a technique known as "centrifuge pruning".

## The double-axis system

Double-axis training is carried out on trees which have been pre-formed in the nursery. After planting, the two axes are grown as small independent spindle trees).

They differ to a single tree mainly in that the distribution of vegetation over two axes induces less vigour since trees put most of their strength into forming two vertical axes instead of one. Considered an evolution from the super-spindle of the 1980s, the bi-axis system was aimed mainly at achieving the advantages of high density without the drawbacks:

- lower economic investment due to fewer trees/ha;
- better exposure of fruit to sunlight;
- exploitation of tree vigour in fertile areas;
- reduction of tree height;
- less need for growth regulators, root pruning, or limb bending in summer;
- simplification of cultivation;
- considerable speeding of harvest and pruning.

## Growth habit of bi-axis trees

The growth habit of trees trained with two axes differs significantly from that of spindle trees. While spindle-trained trees produce a strong scaffold of vigorous branches, two-axis trees usually have short limbs that tend to end in flower buds and to bend naturally under the weight of the crop.

Bi-axis trees are able to fill their allotted space more quickly than spindle trees. In addition, comparison of shoot elongation shows that vegetation is partitioned into roughly twice as many shoots in the bi-axis trees as in spindle, but these shoots are, on average, half the length of those on spindle (Fig. 1). If started with good material from the nursery, the two axes grow uniformly, provided they are grown vertically.

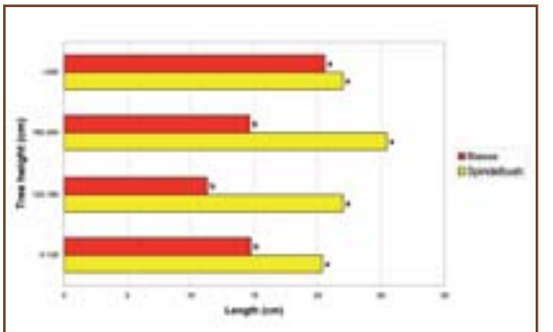


Figure 1

**Fig. 1** - Length of shoots developed on the spindle and bi-axis trained trees
**Fig. 2** - Unlike spindle, bi-axis Fuji in the sixth leaf produced apples with similar colour over the canopy and evenly on both axes
**Fig. 3** - Bi-axis Fuji in the fourth leaf produced 39 kg/tree (90 tons/ha), while spindle produced 24.6 kg/tree (68 tons/ha)

## Production and quality

The productivity of young bi-axis trees is, in general, the same or better than spindle.

In Italy's Po Valley, Fuji plantings of bi-axis trees were less prone than spindle to biennial bearing in the third year after a similarly heavy crop in the second year. In young orchards, quality in terms of fruit over-colour varied according to the production site, confirming that altitude plays a more important role in red colour development than the training system. In the case of plantings in the Po River plains area, however, there was a significant improvement of yield and red colour development in the bi-axis trees was related to better light exposure.

Differences in quality between the two training systems are more likely to emerge after three to four years. In the 6th year of a Fuji planting, for example, if we split the crop into three heights (0-1m, 1-2m and 2-3m from the ground), bi-axis had similar fruit colour over all the canopy, while spindle showed excellent quality only in the top third, with a higher percentage of poorer green-coloured fruits in the lower part of the tree (Fig. 2).

Figure 2

Figure 3

## Potential positive side effects

In addition to the clear agronomic advantages offered by bi-axis trees, mostly regarding fruit quality, several other positive effects have been found and need to be better understood and tested over time.

Changing the tree shape from spindle to bi-axis means moving from a large discontinuous sequence of trees to a thin continuous fruit wall (Fig. 3). The consequences can be of great impact since mechanisation can be introduced into such a system relatively simply.

First of all, thinning by means of a tractor-mounted rotating device (see *Australian Fruitgrower*, November 2008) can be easily carried out at bloom time. This is an important bonus since carbaryl was withdrawn from the EU market last year and some cultivars, such as Fuji and Red Delicious, are at risk of disappearing without a proper thinning strategy. Mechanical topping and hedging can also be performed on bi-axis trees both in winter and summer. In particular, the "Lorette" style of summer pruning at "12 leaves" of shoot development offers great potential for tree size control and flower bud differentiation with even less need for chemical growth regulators.

Taking this concept to the limit, the bi-axis trees are most suitable for the "Murfrutier", or "fruit wall", technique, which reduces winter pruning by hand to almost nothing. In addition, given the increased distance between trees in the row, mechanical weed control can be introduced into a double-axis orchard, which also makes this system less dependent on chemicals and more suitable for organic production. In a somewhat futuristic scenario, mechanisation of fruit harvesting would also be easier on a fruit wall. In other words, both manual labour and chemicals would be replaced to a large extent by mechanical operations.

Double-axis training could turn out to be a means of adapting the orchard system to the requirements of the machines, a bonus that cannot be overlooked. Even tree height control becomes easier and could, in some cases, result in "pedestrian" orchards comparable to the French "Murba" system. Besides the initial economic advantages linked to the lower number of trees per hectare, it is likely that orchard longevity - which is usually negatively related to tree density - will be improved in bi-axis.

In addition, the use of double-axis trees will increase the flexibility of the orchard system marking an end to the uninterrupted supremacy of M9, and paving the way for more vigorous rootstocks which have been discarded as unsuitable for spindle.

# Soft fruit and strawberry germplasm

*Lara Giongo*
*Marcella Grisenti*
*Luisa Palmieri*
*Paolo Martinatti*

Soft fruits and strawberries are historically part of the local fruit production in Trentino and the region is a leading production area in Europe. Over time, specific and ever more efficient methods of production have helped growers to build a valuable economic resource with the focus on very high quality fresh products, oriented to the fresh produce market. However, the versatility of raspberry, blackberry, blueberry and blackcurrant requires intense study of products and processes. Furthermore, these fruits are increasingly marketed and requested all year round by consumers and an awareness of superfoods and superfruits rich in disease-fighting nutrients and compounds is having a huge impact on these market sectors. Soft fruit germplasm conservation and characterisation is the first step in expanding knowledge of the variables that underlie breeding programmes and lead to new and better soft fruit varieties.

Our study was developed over three years and in three European countries, Italy, Poland and Romania, through the project Interberry. Its key factors were identification of the best soft fruit cultivars for production and processing in the three European locations, the introduction of potentially new and innovative items based on soft fruit, and the exploration of traceability techniques to guarantee authenticity for both fresh and processed products. We mention here only the results for blueberry and raspberry.

## Material and methods

72 soft fruit accessions were evaluated from 2004 to 2007 in an experimental field in Trentino, Italy, in plots of 5 plants per genotype. Most of these accessions were evaluated in Poland (Danek J., Krol K.) and in Romania (Mladin P.). Subjective descriptors and analytical data were mutually agreed and data were registered in a database. The genotypes were evaluated using a ranking scale for the traits of interest: plant vigour (range 1-5), habit, time of flowering, characteristics of the flower, ripening time, fruit size, weight, soluble solids (°Brix) measured with a DBR35 Refractometer, titratable acidity (meq/100g) measured with a Compact Titrator, firmness, pedicel and calyx sizes, suitability of harvesting, susceptibility to the major diseases and winter hardiness. Colour parameters (L*a*b*, XYZ) were also recorded. The second level trial evaluated post-harvest quality and shelf life. Tolerance to insects was examined (Grassi A.) on raspberry. Total polyphenols and total anthocyanins were measured (Vhrovsek U., Mattivi F.) on all the species. During 2005 and 2006, 12 raspberry varieties were also characterised through evaluation of the volatile profile by means of PTR-MS, coupled with the SPME/GC-MS technique (Aprea E., Carlin S., Gasperi F.) in 2006. Samples collected mainly in 2005 and 2006 were studied through stable isotope ratio investigation (Camin F., Bontempo L., Perini M.). Molecular evaluation and traceability were also carried out through DNA studies. The statistical analyses were conducted using the software STATISTICA.

## Blueberry results

In the Italian conditions, most of the accessions achieved production stability during the four years, with Duke clone 2, Elliott clone 1, Lax and Ozarkblue being the most stable and Azur the least. At this site, significant differences were found according to genotype and year.

Yield potential was considered definitive only in 2007 and Augusta was the most outstanding cv. (2683.9 g/bush), followed by cv. Brigitta (2574.5 g/bush) and the cv. Chandler (2564.2 g/bush). Production consistency within each season and from year to year was also assessed for each variety. Fruit size and fruit characteristics were best valued for the cv. Brigitta, Augusta and Chandler. Brigitta Blue clone 3 was found the have the best shelf life. In the fresh market, the best blueberry cultivar for the Polish climatic conditions was Bluecrop, followed by Duke. Ozark Blue did not fruit well during the experiment and produced fruits which were too small, confirming its unsuitability for the Polish climate. Brigitta is sensitive to winter frosts and during winter 2005-2006 (-30°C) all flower buds were destroyed. The most consistent varieties for fruit size were Duke and Bluecrop.

Chemical characteristics differed over the years. The highest RSR content (11.7 °Brix) was found in fruits from Duke in 2005, from Ozark Blue and Sunrise in 2006 (14.6; 14.6), from Simultan in 2007 (15.3). The highest level of ascorbic acid was in Duke in 2006 (6.5 mg/100 g). In the Romanian conditions, yield varied with genotype and year during the first fruiting years. The most productive cultivar was Lax, followed by Augusta, E 3/85 and Azur. Berry weight was consistent in all years and genotypes, but decreased within the same season. The selections E 3/85 and E 4/45 were outstanding with regard to berry weight (2.45 g and 2.33 g respectively), followed by Duke, Azur, Augusta, Delicia and E 4/15. Berry size also varied according to accession and year and generally decreased towards the end of the ripening season. At least 4 genotypes, Brigitta Blue, E 4/6, E 3/85 and Delicia, stood out for their large diameter while E 3/85, Augusta and Simultan were distinctive for their berry length. Simultan, E 4/15 and Azur stood out for their small pedicel scar, followed by Delicia, Duke, E 4/6, E 3/10, Lax and Brigitta. Berry firmness ranged from 0.53 N in Safir to 2.43-2.44 N in Simultan and E 4/6. In Romania, the best results for the fresh market were obtained with the cvs. Augusta, Azur and Delicia and the selections E3/85, E4/45 and E4/6, currently under State Trial Evaluation in order to be released and registered in the State Official Catalogue of Cultivars and Hybrids.

## Raspberry results

The screening of raspberry varieties confirmed a clear distinction between primocanes (PC) and floricanes (FC) for several characteristics, which could be improved by breeding. Individual fruit weights were slightly higher in FC than in PC over the three years, this being, however, the least stable characteristic over the three years and the most influenced by climatic factors. Production consistency within the same season was also investigated in the varieties as a factor potentially limiting the maintenance of standards of quality from early to late ripening periods. Caroline and Tulameen performed best, both decreasing from 5.8 g in the earliest ripening period to 3.8 g in the latest. Shelf life differed markedly as well, with Josephine performing best with a decrease of 3.9%, while Polka decreased in weight by 8.9%. Pokusa was the most tolerant to grey mould *B. cinerea* L., and all, except Polka and Benefis, had a good tolerance to yellow rust, while Josephine and Himbotop were tolerant to aphids. Open-field screening revealed different degrees of susceptibility to *T. urticae*: Josephine and Caroline gave a promising performance over the two years, though a rather large number of predators were recorded on them. The genotypes evaluated in open field showed different levels of tolerance to *R. theobaldi*, RCM. Polka appeared to be the most susceptible.

During 2005 and 2006 the above-mentioned varieties were also characterised through evaluation of their volatile profiles by means of PTR-MS, coupled with the SPME/GC-MS technique in 2006. Glen Ample, Laszka and Tulameen were clearly distinct from the other samples and from each other, while PC turned out to be more composite. Results were comparable over the two years. Gaschromatographic analysis allowed 47 compounds to be identified. There was a significant presence of terpenes and from the analysis it was possible to distinguish two main groups: low and high content. These compounds are known to be anti-herbivore defence mechanisms in the plants and have been documented in raspberry as being involved in aphid tolerance mechanisms.

All the plant material was characterised for the genetic analysis. Molecular tools were developed, which were able to fingerprint all the berries using the RealTime platform and which could serve as a new traceability method for soft fruits in commercially processed products, thus guaranteeing authenticity.

# Application of Texture Analysis to grapevine: effects of genotype and cultivation practices

*Duilio Porro*
*Sabrina Ferrarin*
*Pierluigi Bianchedi*
*Silvano Clementi*
*Antonella Vecchione*
*Marco Stefanini*

Evaluation of the ripeness of grapevine berries is usually carried out by sampling clusters according to certain quality parameters using chemical determinants. These analytical quality parameters are not, however, entirely able to predict the enological potential of the grapes or their variability due to genetic factors.

In recent years, it has been found that the mechanical properties of grapevine berries, such as berry firmness, berry skin break force and thickness, are linked to the extractability of phenol substances and to genotype, and could therefore be useful in steering winemaking processes and as an indication of the three physiological phases of berry ripening.

In fact, during ripening, and particularly after veraison, changes in mechanical properties, ultrastructure and cell wall components in grape skin occur as a consequence both of water flow, and consequently of mineral nutrients, and of genetic diversity. So, genotype and cultivation practices can significantly affect changes in the structural epidermal properties of the berry. For this reason, knowledge of the mechanical properties of grapevine berries is very important.

In order to understand ripening, to differentiate cultivars, and to enhance knowledge in this field by investigating variation, in 2007 we started to measure the mechanical behaviour of grapes using Texture Analysis.

In 2007 and in 2008 we collected samples from a set of around 200 accessions representing the variability of the whole grapevine collection of *Vitis vinifera* (2,500 accessions from around the world) held at FEM-IASMA, and measured their mechanical properties.

In addition, similar samplings and measurements were effectuated in several trials carried out in 2007 and in 2008 with the aim of assessing the role of different cultivation practices (late water stress, calcium and silicon foliar application). Histological data were also collected in these cases.

Measurements of the mechanical properties of grapes were made by performing puncture and compression tests using a Universal Testing Machine TAxT2i Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) according to recommended instructions (Rolle L. *et al.*, 2007. Rivista di Viticoltura ed Enologia, 2: 59-70; Letaief H. *et al.*, 2008a. Journal of the Science of Food and Agriculture, 88: 1567-1575; Letaief H. *et al.*, 2008b. American Journal of Enology and Viticulture, 59/3: 323-329).

20 berries from each sample were examined with respect to each mechanical parameter. Berry skin break force was determined by needle probe, berry firmness with a 35 mm ø flat probe. Firmness was defined as the force necessary to obtain berry deformation of 50%.

For the evaluation of berry skin thickness, samples of berries were brought into contact with liquid N, a cryogenic fluid capable of causing rapid frostbite, before Texture Analyzer measurements were taken.

Frozen living tissues were then stored for three days at -20 °C.

Some frozen samples were also stored in order to confirm the berry skin values obtained using texture analysis with microscope measurements.

Tangential slices of berry skin, including the epidermis and a small amount of flesh tissue, were taken with a sharp razor blade and put into 8-10 mm of fresh internodal pieces of young apple branches. The material, with the addition of 0.1 M of sodium phosphate buffer (PBS), was then frozen at –20°C. Transverse sections (20 mm) were cut with a CM1510 cryostat (Leica Microsystems AG, Wetzlar Germany) and finally stained using a 0.1% Cellofluor solution.

Mounted sections were examined using a Nikon ECLIPSE 80i microscope (Nikon, Tokyo, Japan) equipped for epifluorescence at a final magnification of 400x. Microscope measurements were carried out using both bright-field and fluorescence (Ex 340-380) illumination.

## Results

The mechanical properties of the berries of the 200 grapevine accessions were highly dissimilar although they presented similar values for each parameter in the two years. In particular, berry skin thickness values ranged from 130 to 230 µm, as shown in figure 1, confirming that the grapevine collection individuated was a good representation of the high variability of *Vitis vinifera*.

This parameter was not linked to degree of ripeness: in fact varieties with higher Brix and lower berry firmness or berry skin break force did not necessarily have lower skin thickness values. The differences observed in skin thickness due to

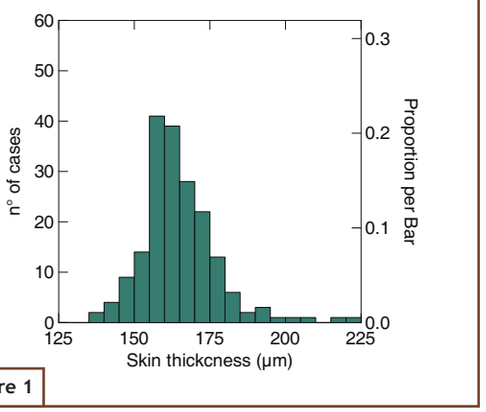



Figure 1

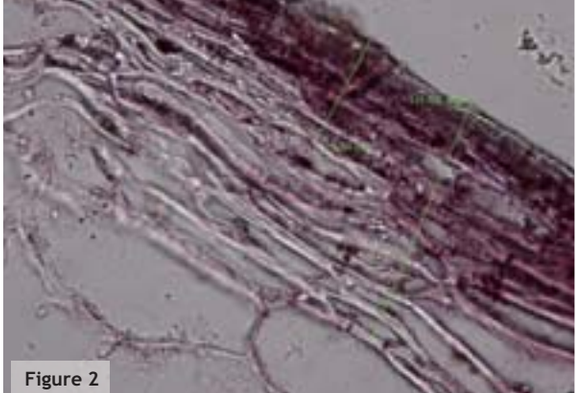
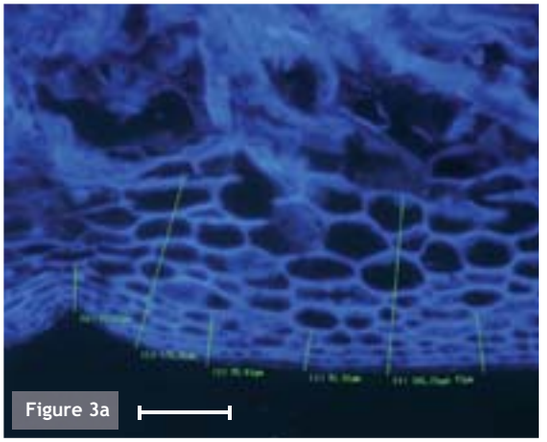


Figure 2

**Fig. 1** - Frequency distribution of berry skin thickness values observed in the 200 accessions of the FEM-IASMA grapevine collection
**Fig. 2** - Transverse section of *Cabernet franc* berry skin observed with bright-field illumination



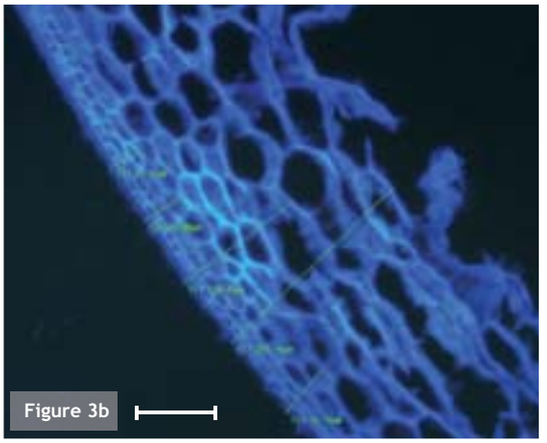




**Fig. 3** - Transverse section of berry skin observed with fluorescence illumination according to treatment: a) test; b) water stress; Bar = 100 µm; x400

the effect of genotype depended mainly on the number of skin hypodermal layers. The lowest value, recorded for cv. *Cabernet franc*, is reported in figure 2.

Greater differences, however, were recorded in experimental trials where cultivation techniques were differentiated. Late water stress imposed from pre-veraison until harvest affected berry development and the mechanical properties of the berry. This confirms that water stress may influence growth during the berry softening and ripening stages. In particular, berry firmness and berry skin thickness were significantly altered by treatment. Water reduction significantly reduced berry firmness (-22%), due to lower hydration of the berry, while greatly increasing berry skin thickness (+20%), as a physiological protection from the stress imposed.

The highest values of berry skin thickness in the stressed vines compared with the test vines, recorded using Texture Analysis, were observed with microscope measurements (Fig. 3). The number of layers of skin cells were similar between treatments and totalled 9, but different cell wall sizes were recorded: the imposed water stress promoted thicker cell walls.

The same behaviour was observed when silicon or calcium was applied foliarly. In both *Sangiovese* in 2007 and *Pinot gris* in 2007 and 2008, when silicon application was tested and compared with control plants, berry skin was found to be significantly enhanced (+5-10%). The berry skin break force values of berries of *Chardonnay* vines, to which calcium was foliarly applied during the season, were significantly higher (+10%) than controls in 2007 and 2008.

Applications of silicon and calcium both resulted in healthier grapes, which were less susceptible to rot, showing that Texture Analysis could be used to predict diseases linked to rot.

Skin thickness values recorded by microscope were similar to those obtained with the Texture Analyzer, showing the usefulness of this instrument.

# In brief

## Top-working of grapevine

*Silvano Clementi*

Top-working of grapevine is a method for substituting a variety without having to uproot the plant. This method, which has already been used for several years by the Viticulture Unit, has proved to be of fundamental importance in that it allows grapes from new varieties, obtained by crossing, to be already produced in the year following grafting, while with grafted rootings, as well as losing the year in the nursery, grapes are produced only in the 2nd or 3rd year after bedding out.

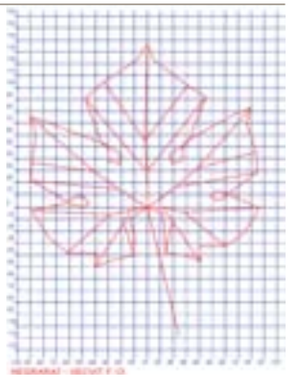
The technique of top-working consists in inserting a bud of the new variety into a shoot sprouting at the base of the old rootstock (W-GREEN method) or of grafting the bud directly onto the rootstock itself (CHIP-BUD or T-BUD method).

Using individual selected plants and with just a few buds per variety available, it is posible to obtain repetitions in only the second year after grafting with which to determine their characteristics and to obtain suffficient production for micro-vinificaton in order to evaluate the qualitative characteristics of the wines obtained from the new crosses.

This technique saves both time and space in the field in that biotypes which are considered of little interest may be substituted with others yet to be evaluated.

## SuperAmpelo, software for foliar ampelometry

*Monica Dalla Serra, Alessandra Zatelli*



The Viticulture unit uses the SuperAmpelo software to measure adult leaves of *Vitis*. The software has been mainly used to:

- characterise the Institute's varietal collection
- compare clones belonging to a single variety
- identify the phyllometric parameters which characterise the varieties, focusing on the phyllometric differences present in individuals which are part of the intra-varietal diversity.

The data thus acquired and then inserted into the SuperAmpelo database not only provide an ampelographic description of the accession, but also allow the standard profile of the leaf to be visualised and printed. It is possible to export the co-ordinates of the points of every single leaf read in a given analysis session, the calculated values (differing in Distances, Angles, Relationships, OIV Codes, GenRes Codes) of every single leaf, and the average calculated values and standard deviations of Distances, Angles, Relationships, OIV Codes and GenRes Codes.

The function known as Similarity Analysis of the Leaves in a Sample takes into account the various aspects and allows the data from two accessions to be compared in order to highlight similarities and differences.

The programme allows both ampelographic and ampelometric OIV codes inherent in the leaf to be compiled. Incomplete OIV codes may then be completed, observations made in the field being reported according to a protocol which defines the relative importance of each characteristic.

Explanatory profile - of the Pinot Bianco and Negrara leaves.

## In brief

### Breeding and genetic improvement of raspberry, blackberry, blueberry and strawberry

*Lara Giongo, Marcella Grisenti*

Conventional breeding of raspberry, blackberry and blueberry at FEM can take advantage of extensive germplasm phenotyping and characterisation, excellent skills in plant manipulation, and genetic information and tools, and is rendered particularly effective through the use of Marker Assisted Selection.
The breeding programme complements continuous implementation and evaluation of soft fruit germplasm. Phenotyping is recorded in a database.
**Raspberry** (*Rubus idaeus* **L.**) **and blackberry**: the germplasm collection includes 400 accessions. Interspecific and intraspecific crosses and backcrosses.240 preliminary selections and a few advanced selections were included in the programme at the end of 2008, while 4,700 seedlings were ready to be planted. The main sources of desirable characteristics come from *R. idaeus, R. chamaemorus, R. neglectus, R. arcticus, R. saxatilis* and *R. parvifolius*. The main objectives are better shelf life, improved primocane quality, manipulation of floricanes, suitability to soil-less conditions, biotic and abiotic resistance and bioactive compounds.
With respect to blackberry and its hybrids, there are 50 accessions in the germplasm collection and 300 seedlings were planted in 2008.
**Blueberry (*Vaccinium* spp)**: about 140 accessions of highbush, southern, halfhigh blueberry and rabbiteye. Assortative phenotype mating, interspecific hybridisation and backcross. Today we have 35 new advanced lines and about 8000 seedlings. The main goals are fruit quality, functionality, production season and storability.
**Strawberry (*Fragaria* spp)**: alpine strawberry germplasm is evaluated for its production potential in different climates. Crosses are planned in order to broaden knowledge of physiological and genetic aspects.

### Manipulation techniques for soft fruit and strawberry development cycles: carried out with maturation curves and production predictions

*Paolo Martinatti, Lara Giongo*

Mathematical models are utilised to build fruit growth curves able to mirror and define the correlation between growth accumulation factors and production. Different cultivars from different sources and grown in different environmental conditions have been tested. Plants were monitored in the nurseries, subjected to microscopic analysis and subsequently evaluated in forced conditions and finally in the farm. Evaluation in different years allows climatic limitations to be overcome and sigmoidal robust patterns to be established. The prediction models are built up from up-to-date cadastral farm data and derived production potential and maturation curves. Reliable elaboration is processed through software linked to both the meteorological stations and the programme that updates the records. The output is a weekly, multilevel production forecast, which is systematically compared with real production weights.

### Soft Fruit germplasm characterisation using molecular markers

*Luisa Palmieri, Maddalena Sordo, Stella Grando, Lara Giongo*

One of the objectives of Interberry, Sicilberry and SoftFruit is the characterisation of more than 500 accessions belonging to *Ribes spp., Rubus spp., Fragaria vesca* and *Vaccinium corimbosum* using molecular markers such as SCAR (Sequence Characterized Amplified Region), microsatellites, EST-SSR (Expressed Sequence Tag - Simple Sequence Repeat), and SNP (Single Nucleotide Polymorphism). The accessions are represented by new and old cultivars and also by advanced selections.
The results will be applied to mislabelled plants during propagation transplanting in pots or in the field. In addition, the results will be useful for MAS (marker assisted selection) in breeding programmes or for qualitative and quantitative soft fruit traceability in fresh and finished products using PCR and RealTime PCR techniques.

# Facilities and Equipment

## Ecophysiology and biochemistry laboratory

We use the following instruments for the descriptive study of plant response to ambient conditions and causal analysis of ecologically dependent physiological mechanisms:

### Leaf gas exchange

Compact Minicuvette System (Walz CMS 400)

Portable Gas Exchange System (Walz HCM 1000)

Oxygen Electrode Chamber (Hansatech LD3)

### Leaf chlorophyll fluorescence

Portable Chlorophyll Fluorometer (Walz PAM-2000)

Chlorophyll Fluorometer
(Walz PAM-210, TEACHING-PAM)

### Leaf optical property

Plant Chlorophyll Meters (Minolta SPAD 502)

Spectroradiometer UV/VIS (StellarNet EPP2000)

Proximal sensing instruments
(Skye Spectrosense 2+)

### Data loggers and micro-climatic sensors

Data loggers
(Campbell Scientific CR10, CR10X, CR1000)

Multiplexers
(Campbell Scientific AM25T, AM16/32)

Net radiation (Schenk pyrradiometer 8111)

PAR global and diffuse (Delta-T BF3)

Air temperature and humidity (Rotronic MP101A)

Soil temperature (Campbell Scientific 105T)

Soil water potential (CS 257 Gessetti Watermark)

Soil water content (CS 616 TDR)

Lisimeter for Soil Water Samples
(1900L/12 /24 /36)

### Canopy measure and structure

SunScan System (Delta-T Sunscan SS1 and BF3)

Fisheye lense (Nikon FC-E8)

Software (Gap Light Analyzer 2.0)

Software GIS (ESRI ArcGIS 9.1 Pro Edu)

### Plant water status

Plant Water Status Console
(Model 3005 Soil, Moisture Equip.)

### Biochemistry laboratory

SDS-Page e Western Blotting System (BioRad)

Nitrogen determination Kjeldahl System (FOSS)

Microfine grinder drive (IKA MF10)

Disperser (UltraTurrax T25)

Spectrophotometer (SHIMADZU UV-VIS 1601)

### Must and fruit analysis

Crison automated titratator

Digital refractometer

Pimprinnelle TR 53210

Texture analysers

### Photography equipment

Canon Powershot G7 e Nikon D80

### Breeding and production optimisation

We use the following facilities and equipment for breeding, clonal selection and production:

**Propagation and nursery techniques**
600 m2 of heated greenhouse
3 polythene greenhouse tunnels

**Cultivar improvement**
5 ha of new grape cultivars (7,000 genotypes)
10 ha of new apple cultivars (6,000 genotypes)

**Cultivation management**
20 ha of apple cultivation under direct management
50 ha of supervised private vineyards

# GENETICS AND MOLECULAR BIOLOGY DEPARTMENT

*Riccardo Velasco*

In the early 1990s the centre's biotechnology unit was set up within the chemistry department and since then competencies in genetics, molecular biology, genomics, bioinformatics and applied biotechnology have grown rapidly. In just over 15 years the number of senior and junior researchers reached 45 and this led to the creation of the Genetics and Molecular Biology (BGM) Department in 2005. Researchers, fellows and technicians work in three research units.

## Genomics and Bioinformatics (GBI) unit

The Genomics and Bioinformatics (GBI) unit has high-level competencies in various aspects of genetic research and has now completed two important genomics projects on apple and grape, economically the most valuable species in Trentino. The unit has reconstructed the 19 chromosomes of the grapevine genome and the 17 chromosomes of the apple genome, and has built several dense molecular maps. The physical map of the grapevine genome was reproduced by assembling over 50,000 BAC clones. The complete genome sequence, assembled and annotated, is associated with genetic maps and is available on the IASMA web site (http://genomics.research.iasma.it/iasma).

The annotated grapevine genome has also been deposited in international gene banks and was published in the journal PLoSONE in December 2007. A similar undertaking for the apple genome is in progress and will been publicly available since the beginning of 2009. Transcriptional profiling of differentially expressed genes during ripening and under biotic and abiotic stress are also studied by the unit. Software for genetic and genomic tasks as well as for database management and data mining are also being developed.

## Molecular Genetics (GM) unit

The Molecular Genetics (GM) unit concentrates on translating basic information on the genomes of grape, apple and soft fruits — Trentino's most important crops — into useful tools for breeding.

The development of molecular maps and markers is an essential part of the unit's molecular breeding programme. The unit is also involved in correlating markers and phenotypic traits, with particular attention focussed on: QTLs and monogenic traits related to pathogen resistance for the genera Vitis, Malus, Rubus, Vaccinum and Fragraria; quality traits such as aroma compounds, polyphenols and secondary metabolites in grape and apple; flower and seed development in grape.

In addition, molecular markers are being implemented for the characterisation and certification of plant varieties and species.

## Cellular and Molecular Biology (BCM) unit

The Cellular and Molecular Biology (BCM) unit is dedicated to gene isolation and the characterisation of gene function. The unit has various functional genomic tools, including PCR select, differential display, AFLP-TP and differential library screening, for isolating the genes involved in response to attack by apple and grape pathogens. Cell culture of grape is also used for testing elicitor induction, which mimics pathogens.

The kinetic responses of the cell are monitored biochemically and at the gene expression level. Since the early 1990s the unit has also carried out grapevine transformation work.

## Recent developments

The department has recently turned its attention to public perception of genetically modified organisms (GMOs). The BCM and GM units have together set up a GMO detection laboratory for food and feed to cover both research and service needs.

Another joint project, titled “translational genomics in apple and grape”, has the aim of defining gene function, monogenic traits and QTL position on maps as well as allelic variants. Interdisciplinary approaches are essential for the success of such programmes and in this respect the IASMA Research Centre is ideally placed. The centre can now expand on the basis of having sequenced two genomes and has the capacity to investigate other genomes such as those of pathogens or soft fruits. It is also engaged in resequencing different varieties and species of grapevine and apple. This 5-year programme exploits basic apple and grape genome research in order to create applied research tools for biotechnology and breeding purposes.

It will be funded and developed jointly, with this department and the Agricultural Resources department both playing major roles, and with the additional participation of the Agrifood Quality and Plant Protection departments.

# The foundations for successful genetic improvement: from genome to field

*Silvio Salvi*
*Michela Troggio*
*Riccardo Velasco*

Amongst FEM-IASMA's latest research objectives is an important programme of genetic improvement in apple founded on basic knowledge of the plant's functioning, its genome. Apple DNA is organised into 17 chromosomes and composed of around 750 million nucleotides. Our research began in 1999 with the work co-ordinated by Dr. Komjanc and Dr. Magnago, who carried out a lengthy series of crosses between cultivated varieties and wild species of apple. The progeny of these crosses today take up the considerable surface area of 12 hectares and are the main source of new varieties in the Institute's genetic breeding programme. New, interesting genotypes potentially presenting economically important traits or resistance to widespread diseases such as scab, oidium e phytoplasmosis, can be found in this new plant material. Our interest was focussed on the variety Golden Delicious, which is the parent of many of the crosses, given the important position that it occupies in agriculture in Trentino and as the second most widespread variety in the world. Alongside this variety we have a 'double haploid' version with a reorganised and somewhat simplified genome, obtained from Prof. Yves Lespinasse in France. This version was used in the sequencing analyses to check some of the project's results and also for basic genome studies.

A key step in the study of a genome is reconstruction of the chromosome map. To do this we developed around 2,000 genetic markers and positioned them on the chromosomes, made possible by analysis of the various populations of crosses, which included Golden Delicious and other varieties, available at FEM-IASMA or at other research Institutes associated with ours in various European and non-European countries (New Zealand, France, Spain, United States). In addition to reconstructing the chromosomes' structures, these markers were used in the sequencing project (for example, in assembly and anchoring) and will constitute the basic tools for genetic improvement (for example, in localising genes of interest on the chromosomes).

In the meantime, a programme for sequencing the genome of the variety Golden Delicious is under way in collaboration with the American companies Myriad Genetics, Amplicon Express and 454 Life Science/Roche, and numerous contacts have been made with international Institutions such as INRA (Angers, France), Plant and Food Research (Palmerston North, New Zealand) and Washington State University (Pullman, USA). The project has produced DNA sequences for a total of over 11 billion nucleotides, representing more than 15 apple genome equivalents. The sequences were assembled in the current version of the Golden Delicious genome with 742 million base pairs, chromosome by chromosome. The very first gene prediction analyses of the genetic sequences showed the apple genome to have many unusual characteristics. A wealth of genetic sequences and transponable elements (mobile segments of DNA), as well as duplication of the DNA into fairly well-defined chromosome pairs,

make the apple genome one of the most interesting sequenced so far and one that will be widely studied over the coming years. The broader studies carried out by FEM-IASMA on the basis of the sequence are for the time being focused on biological (gene annotation and content), phylogenetic (species origin and domestication) and practical aspects (comparison between the apple, strawberry and peach genomes, all three species belonging to the Rosaceae family).

Added to these basic analyses are studies on their applications aimed at discovering how many and which genes are involved in controlling characteristics affecting fruit cultivation. This knowledge is fundamental in modern genetic improvement based on molecular-assisted breeding strategies. In this approach, crossing programmes are reorganised into two steps: firstly, the parents of the cross are chosen on the basis of the genes (or rather, alleles) which may contribute to a given characteristic; secondly, the progenies of the cross undergo molecular selection in order to identify those few plants which possess favourable combinations of these genes. Of particular importance amongst these studies are those dealing with genes resistant to fungal and bacterial pathogens, such as scab and fire blight. These lines of research were previously being followed at FEM-IASMA but will now be considerably facilitated by the availability of the genome.

The genetic basis of the apple tree's habitus are also being studied. In this context, the *"Columnar"* locus, on chromosome 10, has been mapped with a precision lower than cM. This locus influences the apical bud's degree of dominance and consequently the tree's branching capacity. This information will allow for the development of new apple varieties which require less pruning. Initial results have also been obtained in genetically controlling the young fruit's propensity to self-thin, a characteristic found in few varieties. In its most favourable form the characteristic is expressed with the elimination of four of the five young fruits of the inflorescence (corimbus), while the central one is maintained. We have also started studying the inheritance of fruit quality characteristics, which include classical pomological aspects such as colour, shape, firmness and crispness, and innovative aspects such as the content of substances with highly nutritious and antioxidising properties.

Only a tiny part of the reserve of genetic variability represented by the thousands of apple cultivars and the wild species found in Europe, Asia and North-America is used in genetic improvement. Availability of the Golden Delicious sequence, in combination with the latest technology in the field of genomics, will pave the way towards the cataloguing of the genetic variation, a first step in safeguarding it and exploiting it in breeding.

In conclusion, the availability of the apple genome sequence will change genetic improvement, allowing breeders to reach their traditional objectives more quickly and making it possible to meet previously unrealistic challenges. These advances will have inevitable positive implications for fruit growing.

# Variation in the aromatic expression of two Chardonnay clones

*Juri Battilana*
*Francesco Emanuelli*
*Stella Grando*

The typical flavour of Muscat grape varieties is closely related to the presence of terpenols, which can be found in grapes and wines as free or glycosylated compounds. Based on their high concentration and low olfactory perception thresholds, linalool, geraniol and nerol, associated with an agreeable rose-like scent, and α-terpineol, associated with a camphor flavour, are considered to be the major aromatic determinants in Muscat cultivars. Moderate concentrations of monoterpenols can also be found in aromatic, non-Muscat varieties, i.e. Gewürztraminer, Riesling, Sylvaner and some Malvasias.

Variations in berry flavour as well as in berry colour or ripening times are sometimes reported within the same grapevine variety and are thought to be due to somatic mutations occurring in the buds. Vegetative propagation of derived shoots leads to the creation of a new grapevine clone. Some Chardonnay clones exhibit an interesting Muscat-like flavour, therefore comparing these variant individuals with the most widespread but neutral Chardonnay plants may help elucidate the genetic control of monoterpenol content in grapevine. Indeed, we can assume that clones derived from the same mother plant share the greater part of the genome and hence limited genetic polymorphism should account for their phenotypic differences.

## DXS gene co-localises with a major QTL for terpenol content in grapevine

Our knowledge about the genetic determinism of Muscat flavour in grape has been improved thanks to a statistical genetic analysis based on two mapping populations finely characterised for berry content of several monoterpene alcohols during three growing seasons. We

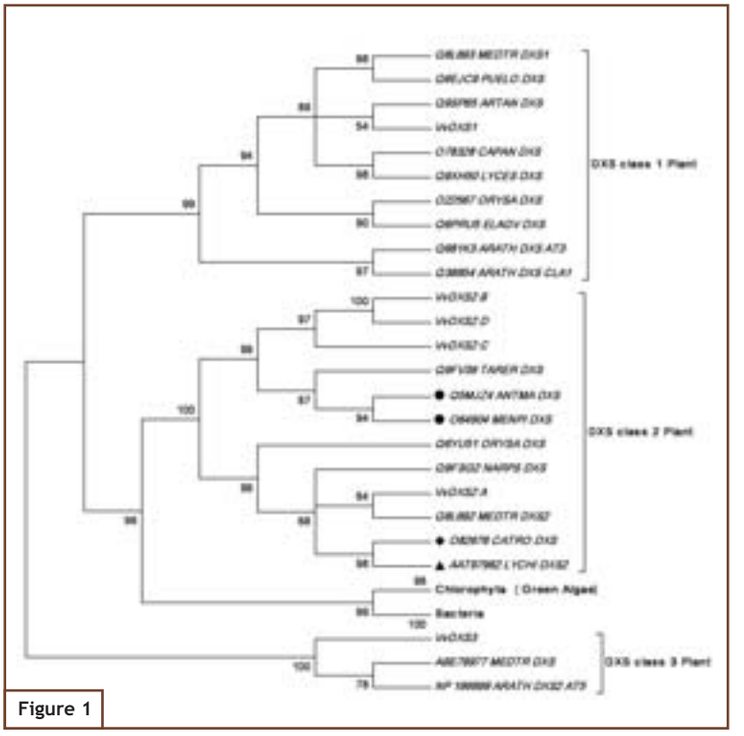



Figure 1

**Fig. 1** - Phylogenetic rooted tree inferred from the functional region of the DXS protein sequences by the UPGMA method. Numbers indicate bootstrap support for individual nodes. GenBank accession numbers and full species names are reported

detected an interesting co-localisation of the gene coding for the first enzyme of the plastidial isoprenoid biosynthesis pathway, the 1-deoxyxylulose-5-phosphate synthase class 1 (DXP-synthase 1), with a major quantitative trait locus (QTL), which explains a large amount of the total variance (Battilana *et al.*, *Theor Appl Genet*, 2009, 118(4): 653-69). This finding highlights a putative regulatory role of DXP-synthase in monoterpenoid biosynthesis during grapevine ripening, as suggested by the positive correlation between the level of *DXS* transcript and the production of specific terpenic compounds observed in other plant species.

Several studies on the expression analysis and the characterisation of the molecular function of DXS in *Arabidopsis thaliana* and *Oriza sativa* have reported the presence of three DXP-synthase proteins, although the biochemical functions have been tested and proved for only one of them so far. We identified six predicted *DXS* genes, currently assigned to plant classes 1, 2 and 3 (Fig. 1), in distinct chromosomes of the grape genome.

Different gene isoforms may encode for tightly regulated enzymes with roles specific to certain tissues, developmental stages or environmental challenges. *In silico* analysis of the *DXS* gene sequence, which in our previous study turned out to be highly associated with Muscat flavour in the berry, showed that this isoform encodes for a complete protein of class 1 with a transit peptide that specifically directs the enzyme to the chloroplast.

## Metabolic profiling of aromatic and non-aromatic Chardonnay clones

Chardonnay 809 ENTAV-INRA is a variant of the Chardonnay cultivars and is well known for the Muscat-like flavour of its berries. This clone cannot be distinguished by ampelographic methods from the most widely cultivated non-aromatic Chardonnay clones used for sparkling wine and white wine production worldwide. We proved that Chardonnay 809 (CH809) shares the same profile of SSR markers with the neutral clone Chardonnay SMA130 (CH130) at more than a hundred loci, therefore this pair of clones may be considered well-suited for additional studies on the genetic control of terpenol biosynthesis.

CH809 and CH130 plants grown in the same field were finely characterised during ripening by analysing the pH, total acidity, sugar, malic and tartaric acid content of the berries (Figures 2 and 3). In order to determine the metabolic situation, free and bound fractions of monoterpenoid and derivate compounds were also extracted from the grapes and quantified, with the support of the Quality and Nutrition Research Unit. Berries were sampled from the pre-veraison stage up to the late ripening stage, thus covering a period of three months, during the

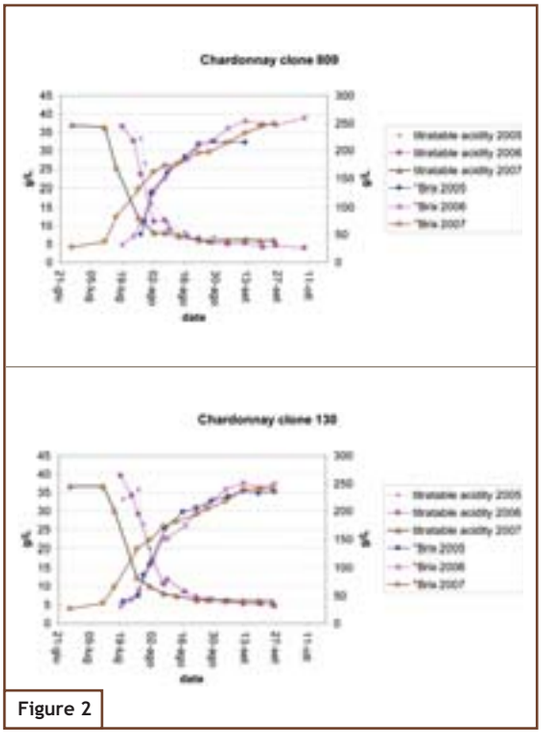



Figure 2

**Fig. 2** - Titratable acidity content and °Brix concentration (secondary Y-axes) of grapes sampled from berry onset to over-ripening stages measured in grams per litre of must

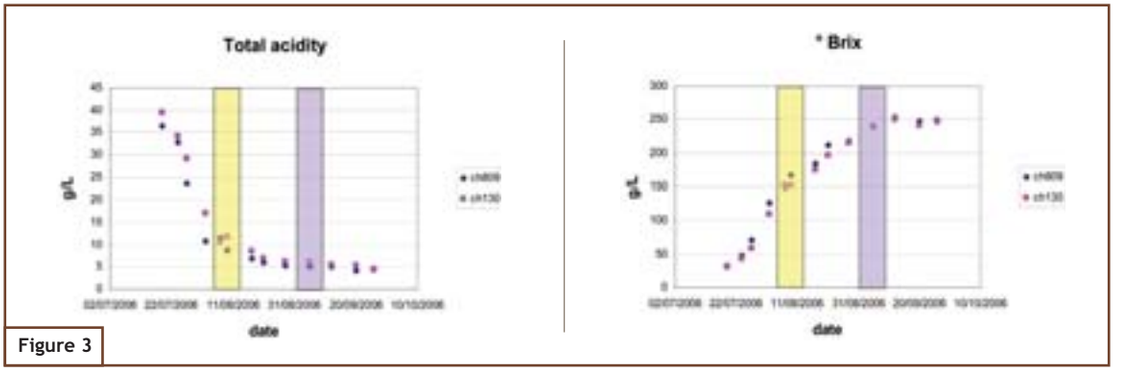



Figure 3

**Fig. 3** - Changes in the physiological parameters titratable acidity and sugar content in CH809 and CH130 measured in correspondence to the main phases of berry development and ripening. Yellow and violet bars correspond to veraison and ripening stages respectively

**Fig. 4** - Free monoterpenoid content (µg/Kg) quantified in berries of CH130 and CH809 at veraison and ripening in 2006

growing seasons 2005, 2006 and 2007.

In figures 2 and 3, the maturation curves of CH809 and CH130 clones are reported and compared in terms of titratable acidity and sugar concentration of the grape berries. With respect to these parameters, the two clones showed very similar behaviour in 2005 and 2006, and also in 2007 when veraison occurred earlier than in the previous years.

The concentration of monotepenoids and their derivates in the berries were, instead, very different in the two clones, especially at the veraison and ripening stages. Free-linalool was the most abundant volatile monoterpene accumulated in the berries of the aromatic clone CH809, its content being fourteen times higher in CH809 than in the neutral clone CH130 at ripening time (Fig. 4).

Differences were found not only between the absolute concentrations of individual monoterpenol. Clone-specific as well as season-dependent features also emerged by evaluating the kinetics of terpenoid compound accumulation.

The relationship between the transcription profile of *DXS1* and monoterpenoid accumulation in the grape berry was investigated in our study by targeting the expression of *DXS1* over an extended period of time in fruit development.

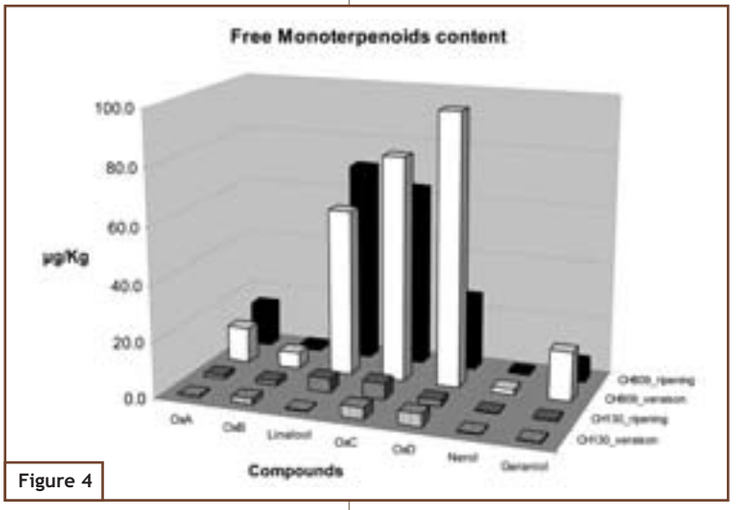



Figure 4

In addition, a microarray analysis of CH809 and CH130 developing berries was also performed in collaboration with Dr. Claudio Bonghi (University of Padua, Italy), and comparison of RNA expression at veraison was undertaken.

We found that *DXS1* was expressed with distinctive profiles in the two clones during berry development. Moreover, we identified a set of cDNAs composed of a diverse range of genes differentially expressed between the aromatic and non-aromatic clones, which we are now investigating further.

# Development of a reliable method for quantifying exogenous genes in grape *plantlets*

*Lucia Martinelli*
*Lorenza Dalla Costa*
*Valentino Poletti*
*Marco Mandolini*

## The need for a reliable technique for quantifying exogenous genes

Molecular characterisation is a major step in the overall process of establishing a genetically modified plant when transgene expression is assessed *in planta* for advanced biological studies and when deliberate *in vivo* release has to be authorised (European regulation EC 18/2001). Confirmation of transgene integration in the host genome and quantification of its copy number require reliable assays.

Southern Blot is the one most commonly applied. However, for DNA quantification Real-time PCR has proven to be a suitable alternative, being less laborious and time-consuming. For a given target exogene, different quantification results can be obtained with these techniques. Real-time PCR estimation is the result of the ratio between the total number of both foreign and endogenous reference genes present in the cell pool of the sample. Thus, a potential cell mixture with different patterns of exogene integration would be reflected in the copy number estimation. This is a frequent outcome of exogene instability or chimerical integration of the exogene occurring during the several morphogenic steps leading from embryogenic callus to plantlet regeneration, when foreign gene deletion or ineffective selection between transgenic and non-transgenic cells may occur. Southern Blot, instead, gives the uppermost quantifiable integration pattern present in the DNA extracted from the sampled plant tissues, thus concealing the possible presence of non-transgenic cells. As detailed in our publication (Dalla Costa & Martinelli, 2009, J. Agric. Food Chem. 57: 2668–2677), we have developed an effective Real-time PCR assay to analyse *V. vinifera* plantlets following gene transfer. We concentrated on quantification of the neomycinphosphotransferase gene *(nptII)*, a marker gene typically used for discriminating between cells that have inserted the foreign gene and those that have not. This exogene was transferred to *Vitis vinifera* Brachetto plantlets by co-cultures of embryogenic calli with *Agrobacterium tumefaciens*.

## Development of a Real time-based analysis for exogenous gene quantification

On the basis of our experience in the development of advanced techniques for the traceability of genetically modified organisms in food and feed (Dalla Costa & Martinelli, 2007, J. Agric. Food Chem. 55: 1264-1273), we exploited an *ad hoc* assay for quantifying the exogene copy number in the *Vitis* genus. This assay applies the relative quantification method based on standard curves for the foreign and the endogenous reference genes. The latter needs to be a species-specific gene which is highly conserved in the species and possibly present in single copy in the genome (prEN ISO 2426 and

21569). Data base research and Southern blot assays on a consistent number of *Vitis* cultivars and genotypes revealed *nced2* (9-cis epoxycarotenoid dioxygenase2) and *chi* (chalcone isomerase) as suitable reference endogenes, which were also present in single copy number in the grape genome. They are coding for two enzymes involved, respectively, in the biosynthesis of abscisic acid (ABA) and in the isoflavonoid pathway.

The most innovative aspect of our analysis concerns the construction of a synthetic plasmid containing both the endogene and the exogene in singly copy, to be used as a calibrator for building the standard curve. This plasmid simulates an ideal transgenic grape plant containing a single homozygote copy of the foreign gene in all its cells. Reproducibility, stability and low cost are additional decisive attributes, making this plasmid the preferred calibrator compared with the usually applied reference calibrator. The latter is the DNA extracted from a real transgenic plant at a known exogene copy number insertion, as occurs with Southern blot assay. Two plasmids have been evaluated, both containing the sequence of the *nptII* exogene and linked respectively with *nced2 (p-nptII:nced2)* or *chi (p-nptII:chi)*.

## Evaluation of our method on transgenic grape samples

The effectiveness of our method was evaluated on Brachetto transgenic plants: the nptII marker gene was transferred after co-culture with agrobacterium carrying the chemical-inducible site-specific *cre/loxP* pX6 vector with *nptII* removal induced by 17-ß-estradiol (Zuo *et al.*, 2001, Nature Biotechnol. 19, 157-161, concession by Nam-Hai Chua, Rockefeller University, New York). In this construct, the *gfp* gene was replaced with a coat protein sequence of the Grapevine Virus A coding for a hairpin RNA (as part of a collaboration with P. Saldarelli, Institute of Plant Virology, CNR, Bari). We quantified the copy number of *nptII* and the effectiveness of its further removal following estradiol induction. Plantlets were selected for *nptII* copy number quantification and the following formula was applied: exogene copy number insertion = (exogene copy number/reference gene copy number) x 2. The ratio between the two genes has to be doubled since a single-copy exogene insertion in a diploid T0 plant is expected to have exogene hemizygosity and endogene homozigosity. Quantifications obtained with both p-*nptII:nced2* and p-*nptII:chi* plasmids were found to be very similar and both plasmids showed precise quantification. Stability of the exogene was assessed on a selected transgenic line after 1 year micropropagation on DNA extracted from three different parts of the plants, i.e. leaves of the apical bud, two superior internodes and roots.

Further evaluation of the efficiency of *nptII* marker gene removal with 17-ß-estradiol induction was quantified on this plant line after hormone induction on the buds during micropropagation. Gene removal occurred with different efficiencies depending on the plant tissue, with the highest level in the roots, according to the lowest mean copy numbers quantified. Since different concentrations, supply strategies and application times of the hormone were not significant when buds induced to micropropagate were used, we believe that penetration of the hormone in the tissues is a key point in the overall strategy.

In conclusion, the Real-time PCR strategy we elaborated was found to be a valuable tool for foreign gene analysis in grapevine. Our assay may be adopted for use with a wide range of crops since specific calibrator plasmids can be designed once proper endogenes have been identified.

# Ethylene synthesis and signalling in grapevine: its role in berry development

*Antonio Dal Ri*
*Stefania Pilati*
*Emanuela Coller*
*Vadim Goremykin*
*Claudio Moser*

Grape berry development is characterised by extensive morphological and biochemical changes and is normally divided into three main phases: two phases of rapid berry growth separated by a transition phase of reduced size increase. During the last growing phase, known as ripening, the berry undergoes a fruit-specialised metabolism consisting of many processes, such as berry softening, sugar accumulation, acid degradation, and synthesis of amino acids, aroma compounds and polyphenols.

For several years, our research has been mainly focusing on characterisation of the berry ripening process from a transcriptional and functional point of view, in order to elucidate its molecular mechanisms and regulation. Using a grape-specific microarray we carried out a genome-wide study of the berry transcriptome and identified a set of about 2,000 genes that were significantly modulated during the ripening of Pinot Noir berries *(Pilati et al. BMC Genomics 2007)*.

Annotation of these genes into functional classes highlighted a large modulation of the transcripts involved in transcriptional regulation, signal transduction and hormone metabolism before véraison, pointing to a re-programming of cellular metabolism during this phase. In the post-véraison phase, on the other hand, genes linked to ripening-specific processes come into play.

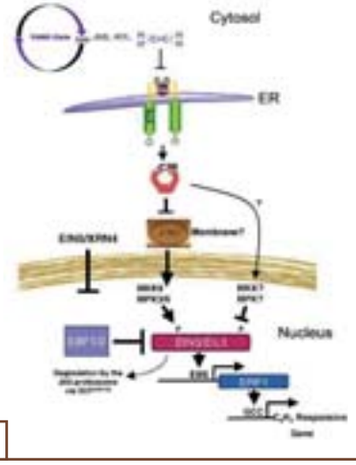



**Figure 1**

In climacteric fruits the phytohormone ethylene plays a key role in the ripening process, determining its onset and duration. Although grapes are considered a non-climacteric fruit, there is new evidence that the differences between these two categories are not so marked and that ethylene might also be important in the ripening of non-climacteric fruits *(Chervin et al. Physiologia Plantarum 2008)*. Ethylene synthesis and ethylene signal transduction have been thoroughly characterised in the model species Arabidopsis as well as in tomato.

The availability of mutants impaired in the synthesis, perception and transduction of this hormone has allowed the action of ethylene in these two species to be described and the schema in figure 1 to be drawn up *(Kendrick et al. Current Opinion in Plant Biology 2008; Cara et al. Plant Science 2008)*.

Following publication of the grapevine genome sequence *(Jaillon et al. Nature 2007; Velasco et al. PLoS ONE 2007)* it has become possible to search for the grape homologs of these genes and to study their expression in different plant tissues and during berry development.

The relevance of this is that ethylene response is also known to be controlled at the transcriptional level by regulating the expression of the genes involved in its synthesis and in the transduction of its signal. We therefore used quantitative PCR to assess which ethylene-related genes are expressed during berry development and in the different plant tissues.

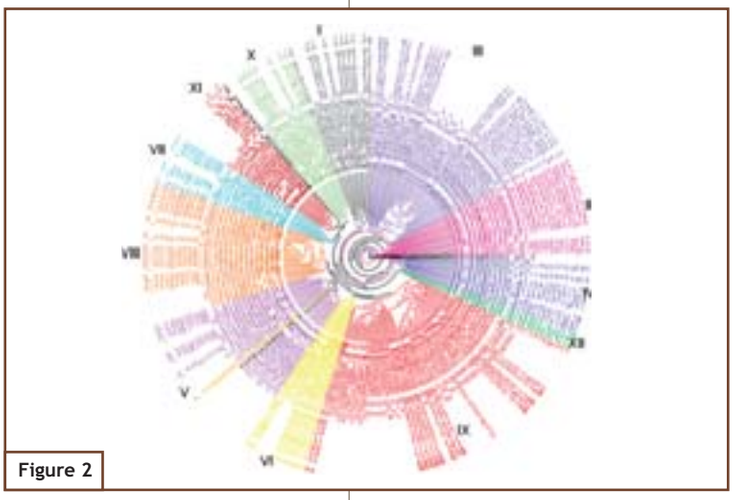


Figure 2

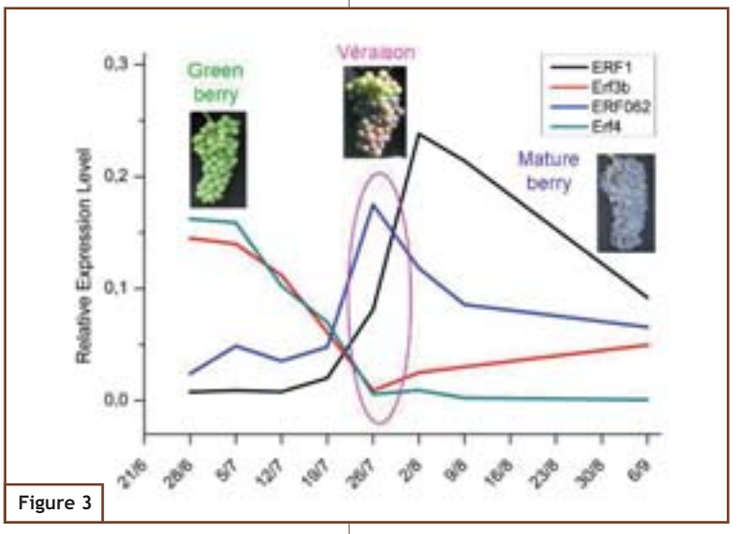



Figure 3

## Identification and expression analysis of the grapevine genes involved in ethylene synthesis and signalling cascade

In several plant species, the key enzymes for ethylene synthesis, ACS and ACO, are encoded by gene families. The same is true for grape, since we identified eight *ACS* and four *ACO* isogenes in the Pinot Noir genome. In the case of the ethylene receptors *(ETRs)*, we also found five sequences which are putative orthologs of the Arabidopsis and tomato *ETRs*. We then studied the expression pattern of all these genes in different tissues and during berry development.

In addition, we identified putative homologs of all the genes involved in the signalling cascade *(CTR, EIN, EBF)*, including the ethylene responsive factors *(ERFs)*. *ERFs* belong to a large gene family sharing a common DNA binding domain of 58-59 amino acids (AP2 domain). In the Vitis genome we were able to locate about 160 sequences encoding for ERF proteins. Phylogenetic analysis has allowed them to be divided into 11 main groups (Fig. 2).

As an example of the results of the gene expression analysis, we report here the profiles of four *ERFs* which appeared to be modulated during berry ripening (Fig. 3). *ERF1* and *ERF062* transcript abundance increases rapidly at véraison and declines afterwards, especially in the case of *ERF062*. *ERF4* and *ERF3b* are instead highly expressed only in the green berry, until véraison. These data are even more significant when integrated with those obtained from the quantitative analyses carried out on the other plant tissues: *ERF1* is expressed almost exclusively in the ripe berry, *ERF4* in the green berry. These two ERFs, therefore, appear to be specifically linked to the berry development process, while *ERF062* and *ERF3b* seem to be involved in more general mechanisms.

## Conclusions and future directions

By searching the Pinot Noir genome sequence we were able to identify the grapevine homologs of the genes responsible for ethylene synthesis and signal transduction in Arabidopsis and tomato. Expression analysis of some of them in several tissues and during berry development, has led to the conclusion that some isogenes are expressed specifically in the berry or in a specific phase of its development while others are expressed in a more general manner. These results will help in understanding the regulatory mechanisms governing berry ripening but they could also contribute to field management practices and breeding activities by providing new functional markers.

# In brief

## Epigenetics in fruit tree crops

*Marina Cavaiuolo, Azeddine Si-Ammour*

The availability of grape and apple genomes recently sequenced at FEM allows comprehensive studies of gene regulation in fruit crops to be carried out. Our work aims in particular to identify genes controlled at the transcriptional (DNA and histone methylation) or post-transcriptional (small RNAs) level during fruit development and under environmental stresses. Novel small RNAs were discovered by deep sequencing (Solexa) and validation of their target genes is underway. DNA fragments associated with methylated DNA and marked histones were isolated from different tissues and will be sequenced by 454. Our ultimate goal is to identify epigenetic target genes controlled by environmental factors and crucial for fruit development and maturation. A better understanding of the molecular pathways repressing genes during fruit growth will provide new tools required by agriculture to face new environmental challenges.

## Localisation of the genes controlling grapevine phenology

*Laura Costantini*

Phenology, that is, the study of developmental stages, is a series of complex traits underlying optimal plant adaptation to environmental conditions. Defining the genetic mechanisms which regulate phenological phases is the first step towards the development of molecular tools to predict the earliness or lateness of a specific genotype.

To this purpose, we analysed the segregation of genotypic (DNA) and phenotypic (dates of flowering, véraison and ripening) variability in an experimental progeny obtained from the cross of two cultivars with distinct features. By integrating this information we were able to identify the grapevine genomic regions having a high probability of controlling variation of the traits under study. These regions were further explored in the light of the recent sequencing of Pinot Noir. Some interesting candidate genes were detected, and are currently under validation.

## Apple Scab: understanding the resistance mechanisms of some accessions

*Valentina Cova, Elena Zini, Matteo Komjanc, Mickael Malnoy*

Apple scab *(Venturia inaequalis)* is the major fungal disease affecting apple, especially in temperate areas. Its control in commercial orchards can require up to 15 fungicide treatments per year. The breakdown of *Vf* resistance, the most frequently used source of resistance in breeding programmes against apple scab, underlines the importance of diversifying the resistance basis. FEM-IASMA is therefore focusing on studying and characterising different R genes whose mechanisms are still unclear: *Vm, Va* and *Vg*.

*Vm* (from *M. micromalus*), which provokes hypersensitive reaction, is in the process of being isolated using a chromosome walking approach performed on LG17. The regions carrying *Va* (from cv Freedom) and *Vg* (from cv Golden Delicious), located in LG 1 and 12, respectively, are in the process of being fine mapped. It will be especially easy to isolate *Vg* since the Apple Genome Project is producing the entire DNA sequences of cv G.Delicious. These three scab R-genes will soon be cloned and characterised.

# In brief

## Rapid Annotation of Anonymous Sequences using Gene Ontology

*Paolo Fontana, Alessandro Cestaro*

Large-scale sequencing projects have now become routine lab practice and this has led to the development of a new generation of tools involving function prediction methods, bringing the latter back to the fore. The advent of Gene Ontology (GO), with its structured vocabulary and paradigm, has provided computational biologists with an appropriate means for this task.

We have developed a novel method called ARGOT (Annotation Retrieval of Gene Ontology Terms) that is able to process thousands of sequences for functional inference. The tool exploits, for the first time, an integrated approach which combines clustering of GO terms, based on their semantic similarities, with a weighting scheme which assesses retrieved hits sharing a certain number of biological features with the sequence to be annotated.

## Improvement of techniques for gene transfer in grape: the opinions of European researchers

*Lucia Martinelli*

Within the framework of the project *EcoGenEitc.Com*, supported by the Autonomous Province of Trento and in collaboration with the Society Observa (G. Pellegrini and G. Sonda), we investigated scientists' views on various aspects of exogenous gene transfer. According to the literature and a personal network, we identified 24 research groups in 8 European Countries involved in gene transfer into grape. The results of our investigation show that marker gene avoidance is not considered a priority. Improvement of the gene transfer technique, i.e. the identification of promoters and genes belonging to the same *taxa* and the avoidance of random exogene insertions in the host plant genomes, is considered to be more relevant. Bioethical and sociological issues seem to be less important than technical ones. The reduction in public funding for biotechnology in recent years is considered a limitation on research progress, in particular the development of improved and safer techniques for gene transfer.

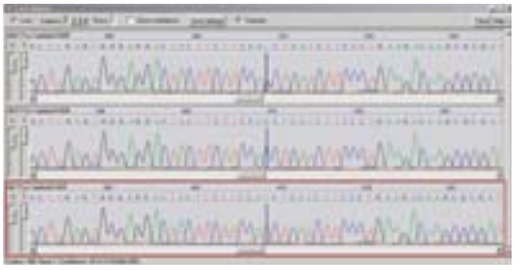
## Tackling Pinot Noir genome heterozygosity

*Michela Troggio, Silvia Vezzulli, Marco Moretto*

The genome sequence data from the cultivated Pinot Noir grape variety provide unprecedented insight into the structural nature of heterozygosity in an outcrossing species.

A total of 2 million single-nucleotide polymorphisms (SNPs) were discovered along the grapevine genome together with more than a million insertion/deletion events. Of these, one or more SNPs were found in 86.7% of genes, while 71.4% of genes had more than four SNPs. Our data allow us to extend the evaluation of nucleotide variation to the entire genome rather than to limited resequenced DNA regions, and thus represent a valuable resource for developing new markers for breeding.

A considerable number of haplotype-specific regions (sequences present in one chromosome but not in the other) totalling 114 million bases were also identified. Altogether, these data allowed us to conclude that the homologous chromosomes of Pinot Noir differed by an average of 11.2% of their DNA sequences.



# Facilities and Equipment

The Genetics and Molecular Biology department has all the necessary instrumental platforms to conduct molecular research on grape and apple biology.

## Structural genomics platform

The structural genomics platform is composed of two tissue lyophilisers, an automatic DNA extractor, a Perkin Elmer Multiprobe IIX expanded automatic liquid handler, two automatic TECAN platforms, six ABI 9700 thermocyclers of 96 and 384 wells, five Applied Biosystem DNA Sequencers (one ABI 310 with a single capillary, three ABI 3130xl with 16 capillaries and two ABI 3730xl with 96 capillaries) and one 454 Life Science/Roche sequencer. These instruments are commonly used for DNA sequencing projects, molecular marker and genetic map development, and marker screening and genotyping.

The platform was set up for the grape and apple sequencing projects and will be expanded next year by the addition of a second ABI 3730xl with 96 capillaries and a high throughput sequencing system such as the 454 Life Science or an ABI SOLID system.

## Functional genomics platform

The functional genomics platform is composed of six sterile benches for cell and tissue culture (where transgenic assays for gene function are carried out in grape, apple, tobacco and Arabidopsis), four real-time thermocyclers (one ABI 7000, two iCycler iQ Thermocyclers (Biorad) for gene expression assays, one Amersham Pharmacia Typhoon 9210 plus phosphoimager for DNA analysis), and one radioisotope laboratory.

The functional genomics unit specialises in automatic RNA extraction from grape and apple tissue for gene expression analysis of single genes as well as the entire transcriptome (Affimetrix and Combimatrix assays). An additional eight thermocyclers are distributed throughout the 800 square metres of the recently equipped molecular biology laboratories.

A fluorescent stereomicroscope (Leica MZ16FA) was recently set up for non-invasive GFP reporter gene detection, adding GUS and NPTII transient and stable transformation analysis to the department's capabilities. An area of growth chambers completes the facilities of the functional genomics unit. In 2008, 250 square meters of greenhouses and three additional growth chambers allowed for an increase in the number of stable transformation assays in grape, apple and model systems.

## Bioinformatics platform

The core prerequisite of any structural and functional genomics assay is a strong bioinformatics platform.

The bioinformatics unit runs a terabyte-sized server and a large client PC network. This unit manages the grapevine and apple genome data bank, which provides data through a Web interface and through a dedicated private virtual network. The data bank provides an overview of the genomes and their functional fractions by integrating genetic maps, physical maps, genomes and transcriptome assays. Bioinformatic expertise includes semi-automatic genome annotation using novel algorithms based on gene ontology.

# NATURAL RESOURCES DEPARTMENT

*Nico Salmaso*

The mission of the Natural Resources Department is to advance ecological knowledge for the purposes of understanding, managing and conserving biological resources for their natural, aesthetic, recreational and economic values. This task is accomplished through multi-disciplinary approaches, which comprise both classic ecological procedures and advanced methodologies, including modern genetic analyses, molecular chemistry, information technology and geomatics. Considerable importance is given to communicating knowledge of conservation and management to young researchers undertaking PhD studies.

Research issues initiated in 2008 include studies on the ecology of inland waters and forests, Agrometeorology/Climatology and Aerobiology, and biomass as a renewable energy source. Several papers on the above-mentioned activities have been published in scientific and technical journals (http://www.iasma.it/UploadDocs/4003_2008.pdf).

## Limnology

The Limnology and Fish Research unit carries out studies on the ecology of lakes and rivers, long-term research on selected sites and genetic studies on freshwater species. Within this context, studies are being conducted on the trophic evolution of lake ecosystems in the Province of Trentino, and include: long-term research on the physical, chemical and biological characteristics of Lake Garda (which has recently been included in the International Long Term Ecological Research (ILTER) network); limnological investigations for the restoration and rehabilitation of certain Trentino reservoirs; detailed research on the mechanisms controlling the development and seasonality of potamoplankton in large rivers. Further studies are being carried out on the autoecology and biogeography of planktonic taxa (dinoflagellates and rotifers) in small lakes, and on the application and development of biological and functional indices in freshwater ecosystems based on the study of epilithic and epiphitic diatoms and phytoplankton. Ichthyological research focuses on aspects of genetic diversity and restoration ecology. In particular, specific studies have examined the sustainable management of marble trout *(Salmo trutta marmoratus)* aimed at its conservation in the Adige basin through both genetic and ecological characterisation. In 2008 research has also been carried out in other areas, including paleolimnology (with focus on the secular variations of the trophic state and on the definition of ecological reference conditions) and molecular chemistry (with focus on the determination of secondary toxic metabolites in aquatic cyanobacteria).

## Forest Ecology and Physiology

This research group carries out investigations on forest physiology and genetics, biodiversity conservation, and the effects of climate changes on forest ecosystems (including anthropogenic pressure) through the Integrated Monitoring Programme, which is part of the International Long Term Ecological Research (ILTER) network and the national CONECOFOR project.

The principal work carried out in recent years includes studies on the incidence, epidemiology and biological control of root-rot fungi in conifers, Chestnut canker, and Black pine and Alder decline. Also underway are investigations on the bioethology, ecology and biological control of phytophagous species, and taxonomic identification and biodiversity monitoring of Orthoptera and Macromycetes communities with the goal of identifying bioindicators of environmental changes. Other activities have included detailed examination of the genetic variability of Norway spruce in the Alps in relation to its ecological conservation, high stability and quality timber yield. In addition, a multi-disciplinary project concerning the ecological evaluation of Italian cypress in the pre-alpine landscape is aimed at developing the sustainable introduction of this species, its potential diffusion to vocational areas and its exploitation in landscape amenity. Extension activity has also been carried out for local and national institutions.

## Agrometeorology, Climatology and Aerobiology

The Agrometeorology, Climatology and Aerobiology unit carries out studies of agrometeorology, microscale climatology and aerobiology. The unit's agrometeorology researchers have recently concluded a project that studied frost dynamics and climate in alpine areas (the most suitable forecasting, alarm and protection techniques have been established). Other investigations have dealt with the possibility of using purified waste-water in Trentino for irrigation purposes. A dissemination project has promoted the diffusion of knowledge on global climate change to the agricultural world. The research group is also involved in: service and monitoring activities, including maintenance of a network of stations to monitor agrometeorology and alpine ecosystems; testing innovative irrigation technologies aimed at optimising the use of water resources; pre-processing of meteorological data for infection modelling; application of geomatic techniques for territory analysis of meteorological data, and, more generally, of data concerning agrometeorological issues (water balance, meteorological downscaling, high-resolution climatology, etc.); local climatology investigations and the consequences of climate change on the environment and on the local agricultural system; improvement of weather forecasting techniques for agricultural use; phenological surveys and modelling of crop and forest species.

Recent activities in the field of aerobiology include: the collection of information on atmospheric pollen content for diagnosis and prevention of respiratory allergopathy; monitoring of the effects of air pollutants using different bio-monitors such as lichens and pollen; testing innovative methods for bioaerosol identification.

## Biomass and Renewable Energy

The study of the biological processes underlying the energy output of different biomasses and the development and implementation of related technologies is the main focus of this research group. One of the most interesting processes, anaerobic digestion (AD), is being studied from both microbiological and technological perspectives. In particular, the quality of the output material and its possible uses are being investigated, and new systems to increase the amount of energy produced (power and thermal) are being tested and introduced. Integrated management systems, based on AD, for the treatment of animal waste (husbandry) in mountain areas with a high level of tourism have been specifically defined (odour reduction, soil and water protection, energy

recovery). Regarding wood biomass from forests and agriculture, attention is mainly focussed on collection systems, efficient use of energy by direct combustion, and new technologies such as gasification; environmental implications and related costs are also studied.

Laboratory facilities have allowed for the development of modern parameters to assess compost stability (static and dynamic respiration index), and for the identification and measurement of environmental impact through the application of new techniques such as olfactometry and electronic nose. A laboratory scale tool and a pilot plant for advanced research on the AD process are being installed. Other activities concern the mechanical-biological treatment of solid waste before landfill disposal and technical support to local authorities and/or private companies in the sector.

## Molecular Ecology

This group brings together various elements which continue the work of a group established through the CSBT special project. It deals with the long-term study of the different levels of plant biodiversity with the aim of understanding the mechanisms underlying how it is generated and maintained in natural ecosystems.

Our analyses are carried out on a range of subjects from populations to species and ecosystems, using approaches ranging from phylogeny, ecology, population genetics and molecular biology. At the population level, the group is mainly involved in study of the correlation between genetic variation in selected natural plant populations and the topography of the environment hosting them, with the aim of arriving at a better understanding of the extent to which genetic variation depends on environmental variables such as temperature or forest coverage. Still at the intraspecific level, the group is involved in identification of the genes responsible for phenotypic (e.g. corolla pigmentation) and adaptive (e.g. cold resistance) variation observed in natural populations using a candidate gene approach.

Concerning comparison among species, the group is focussing on two main research lines: the development of universal molecular markers for phylogeny and population genetics studies, and the comparison among species of genomic regions under selective pressure. In particular, we have developed new methods for efficient isolation of orthologs from non-model species. We are currently applying these techniques to the characterisation of the regulatory regions of genes involved in adaptation.

# Modelling the impact of global warming on the phenology of grapevines in Trentino

*Amelia Caffarra*
*Emanuele Eccel*

Phenological models are useful tools in viticulture, providing important information for crop management and for evaluating the potential suitability of a cultivar for areas with a defined climate. In fact, the timing of phenophases is closely linked with climate, with warmer temperatures advancing the mean dates of budburst, flowering and ripening. For this reason, phenological models are particularly effective in projecting the impact of a changing climate on grapevine. At present, the most widely used phenological models for *Vitis vinifera* are based on bioclimatic indices, such as those of Winkler (Amerine and Winkler, Hilgardia (1944): 493-675) and Huglin (Comptes rendus de l'Académie d'Agriculture (1978): 117-126), which hypothesize the linear accumulation of degree-days up to a thermal threshold defined for each phenophase. The drawback of these approaches is that they usually have only local validity, and hence are applicable to only a narrow range of climatic conditions, similar to those used for calibration. In addition, poor knowledge of the physiology of some aspects of the phenological cycle (such as dormancy) hampers the development of biologically sound models. Thus, phenological models generally need to be recalibrated to the actual climatic conditions of the sites they are applied to, in the event of the unavailability of phenological data at these sites.

During a two-year study funded by the CARITRO Foundation, we developed the phenological models that best simulated and predicted the key phenophases (budburst, flowering, veraison) of *Vitis vinifera* cv. Chardonnay, a variety of foremost interest to the Trentino region. The three phenophases were selected in order to cover most of the active growth cycle of the grapevine and to exploit the availability of data for calibrating the models. Our approach involved the calibration and testing of models based on different hypotheses using data from different sites, and the selection of the best and most general model for each phenophase of interest.

There were a total of 5 sites in the study, 3 of which were in Trentino (San Michele all'Adige, Mezzocorona and Cembra), 1 in Piedmont (Carpeneto) and 1 in Veneto (Conegliano). The phenological data series ranged between 7 and 39 years. Prior to modelling, exploratory regression analyses were performed to select the most important meteorological agents affecting phenological development, for both main and interaction effects between predictors, and simplified to their significant terms by a stepwise selection. Multiple regression analyses tested the significance of models based on the interaction of solar radiation, precipitation and

temperature. However, minor effects could be attributed to factors other than temperature, so we only considered temperature-driven phenological models.

Thus, a set of candidate models based on temperature and photoperiod (in terms of the start date for forcing unit accumulation) was selected for each phenophase and applied to the Chardonnay phenological series pertaining to the different sites. The models, 7 in total, differed in their responses to temperature (linear or sigmoidal) and the periods in which temperature acted on phenological development. The model that best explained the dates of budburst in the sites under study was based on the action of chilling temperatures for endodormancy release, which results in the observable stage of budburst. The best model for flowering and veraison simulates phenophase attainment as the result of the accumulation of heat and calculates forcing units with a sigmoidal function. The models were fitted to 4 of the data series by minimizing the sum of squared residuals by a simulated annealing algorithm, already successfully used for the calibration of phenological models. In order to increase the amount of information for model calibration and to obtain a more general and widely applicable predictive model, we searched the literature for parameter values defining the responses of phenological development to temperature.

A study by Pouget (Vitis (1968): 201-205) offered some information on the rates of budburst of different *Vitis vinifera* cultivars at different forcing temperatures. These responses were normalised and fitted with a sigmoidal curve, which was then used in the previously selected models as the "forcing curve". The phenological models were then calibrated on the longest dataset available, Conegliano (39 years). The "external validity" of the resulting models was then tested by applying them to the data sets from the remaining stations. The results indicated good predictive power for the phenological models and supported the adopted "informed approach" to model calibration.

Trends of future phenology for Chardonnay in Trentino were then obtained, using climate projections for the next century. Output from the Atmosphere-Ocean General Circulation Model HADCM3 according to two IPCC atmospheric scenarios ("A2" and "B2") were downscaled to two key locations in Trentino, one in the Adige Valley bottom (Piana Rotaliana) and another for a hill site (Cembra Valley), and used for the simulation. The projections forecast a significant advance of budburst, flowering and veraison in the next century, with an increasing trend from the second half of the 21st century (an advance of up to 20 days compared to the present). The advance is greater for the phases that do not need a chilling requirement (flowering and veraison), probably because the relevant heat summation for these phases, as opposed to budburst, does not display the lengthening of the chill accumulation stage due to higher temperatures in autumn and winter. This behaviour may have practical consequences on the timing of agricultural practices, as well as on the synchrony between the developmental rates in grapevine and in its parasites.

As a concluding remark, some steps toward a greater robustness of phenological models for grapevine would increase their "portability", even for climatic simulations: a current, general limitation in such models is the limited accuracy when models are applied to series different from the ones they have been calibrated on. For this reason, the results of applications of phenological models to climate change scenarios have to be considered with caution.

# Tropospheric ozone in Trentino: a danger to forests?

*Elena Gottardini*
*Fabiana Cristofolini*
*Antonella Cristofori*
*Maria Cristina Viola*

Ozone is a gaseous pollutant formed by three oxygen atoms ($O_3$), with high oxidative power. In the upper atmosphere, its function is to protect against UV radiation emitted by the sun and a danger to living beings. In the troposphere, the lower atmospheric layer, ozone mostly originates from the photochemical transformation of primary pollutants such as nitrogen oxides, hydrocarbons and volatile organic compounds.

Global ozone concentrations have doubled since industrialisation and several studies forecast a further increase in the future. Widely diffused in rural and forested areas, $O_3$ is considered potentially the most dangerous pollutant for vegetation: prolonged exposure may lead to a decrease in photosynthetic activity causing a decrease in plant growth and lower $CO_2$ sequestration from the atmosphere.

Changes in yield quantity and quality may also occur. A reduction of plant vitality and of its resistance to insects and other pathogens may also impact on biodiversity.

Standard community regulations (Directive 2008/50/CE; UN/ECE 2004 and revisions) have set long-term $O_3$ objectives for the protection of vegetation and this necessitates the measurement of ozone concentrations in order to evaluate the potential risk to plants.

Ozone measurements are commonly performed in urban areas, where gauges for monitoring air pollutants are usually installed; this means a lack of data for semi-natural and forested areas.

Previous studies carried out in forested sites in Trentino showed such high ozone concentrations as to present a potential risk to the vegetation.

The need for a better understanding of the local situation led to a study being initiated - in collaboration with the Forests and Fauna Service of the Autonomous Province of Trento - aimed at obtaining reliable data necessary for answering the following questions:

1. Are the ozone levels in Trentino high enough to be a potential risk to vegetation?
2. If yes, are there measurable effects on vegetation?

## Ozone levels in Trentino

In order to carry out realistic estimates of the potential risk to vegetation, systematic measurements of ozone concentrations were initially carried out in the provincial territory. For this purpose, 15 stations for ozone measurement were installed in Trentino, on the basis of the EU and UN/ECE Level I grid for forest monitoring.

Mean hourly ozone concentrations obtained by means of passive samplers (PASSAM AG) in

**Table 1** - Mean ozone concentrations (± standard deviation) from 6 June to 19 September 2007 and from 30 April to 30 July 2008 in Trentino

| Stazione | Quota (m s.l.m.) | 2007 [$O_3$], media ($\mu g*m^{-3}$) | 2008 [$O_3$], media ($\mu g*m^{-3}$) |
|---|---|---|---|
| Bedollo | 1400 | 70.0 ± 19.2 | 88.0 ± 20.9 |
| Canazei | 1588 | 89.8 ± 18.2 | 99.1 ± 24.1 |
| Castello Tesino | 1549 | 66.0 ± 18.5 | 84.4 ± 21.7 |
| Cles | 1698 | 93.8 ± 21.7 | 113.1 ± 21.6 |
| Concei | 1309 | 122.0 ± 20.3 | 128.4 ± 32.2 |
| Condino | 1856 | 130.6 ± 22.3 | 176.1 ± 34.2 |
| Coredo | 1389 | 87.9 ± 24.6 | 93.8 ± 19.4 |
| Faedo | 306 | 63.9 ± 16.7 | 99.9 ± 32.8 |
| Folgaria | 1210 | 87.3 ± 12.4 | 104.4 ± 18.3 |
| Fondo | 1375 | 89.5 ± 17.1 | 89.9 ± 11.6 |
| Levico Terme | 1105 | 70.0 ± 20.2 | 87.2 ± 21.9 |
| Peio | 1675 | 74.2 ± 20.2 | 94.6 ± 16.8 |
| Predazzo | 1464 | 90.7 ± 16.3 | 94.9 ± 19.9 |
| Tonadico | 1854 | 121.1 ± 20.0 | 130.2 ± 24.4 |
| Trento | 359 | 62.4 ± 12.4 | 89.0 ± 22.2 |

2007 and 2008 are reported in table 1.

It can be seen that the higher concentrations occur mostly at higher altitudes; 2008 ozone values are higher than in the previous year in almost every site monitored. In terms of AOT40 – the exposure parameter adopted to protect vegetation – the 2007 data show that in 10 out of 15 sites, values exceeded the critical level fixed by the UN/ECE (Fig. 1).

## Effects on vegetation

After verifying the presence of a potential risk to the vegetation, the second step undertaken was to assess whether the $O_3$ levels surveyed may lead to plant damage. A preliminary study was carried out to assess the development of leaf injuries to plants of well-known sensitivity to ozone; with this aim, plants of *Nicotiana tabacum* L. cv Bel-W3 were exposed to ambient air in areas of differing $O_3$ levels, following an experimental design developed *ad hoc*.

The tobacco plants showed typical ozone leaf symptoms and damage, as described by the Leaf Injury

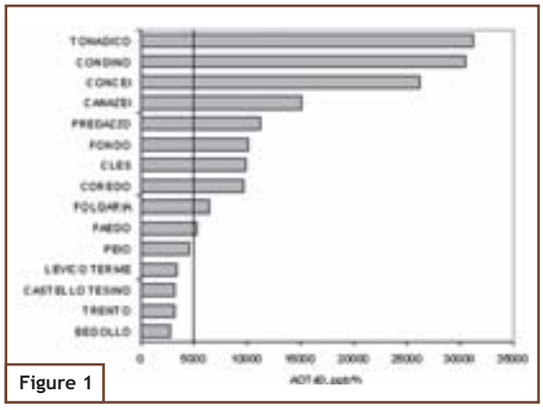



Figure 1

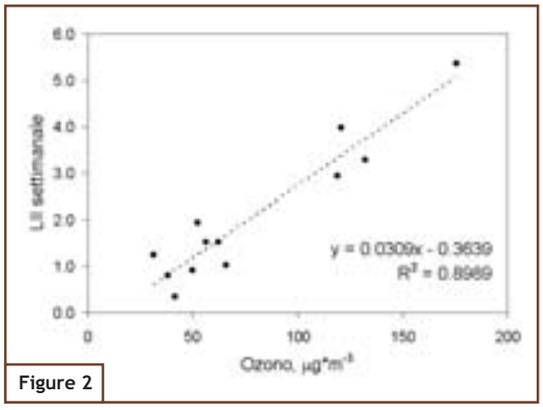



Figure 2

**Fig. 1** - AOT40 in the Level I sites for the period 13 June - 12 September 2007. The vertical line represents the critical level fixed by the UN/ECE for vegetation protection

**Fig. 2** - Linear regression between the weekly mean Leaf Injury Index (LII) values (y) and ozone concentrations (x), expressed as $\mu g*m^{-3}$

Index (LII), highly correlated with ozone concentrations (Fig. 2).

The results of this preliminary study showed the need to extend the survey to spontaneous vegetation. The study was thus oriented to detecting the presence of visible symptoms on trees and shrubs; observations were performed in Level I sites, in the Level II site of Lavazè Pass and in areas where tobacco plants had been exposed during the preliminary study.

The results showed a general absence of symptoms due to ozone in forested sites, although symptoms due to other agents were prevalent in sites with high levels of $O_3$. Ozone symptoms were found on some shrub species *(Acer campestre, Viburnum lantana, Rhamnus catartica, Cornus mas)* (Fig. 3) in the areas where specific symptoms had been found on ozone-sensitive vegetation (*Nicotiana tabacum* cv. Bel-W3).

## Concluding remarks

In this study, ozone values were found to exceed long-term objectives for vegetation protection in the majority of sites, evidence of a potential risk to vegetation in Trentino. This risk turned out to be real for ozone-sensitive vegetation, as confirmed by the development of specific leaf injuries. It was not possible to ascertain so clearly the ozone effect on forest species, though we may hypothesise that ozone is responsible for the decrease in plant resistance to pathogens. It is important to point out that microclimatic conditions do not always permit sufficient stomatal ozone uptake to cause visible damage to the plants.

There is a clear need to extend research on this issue, both with respect to risk estimation – continuing and intensifying the measurement campaign - and with respect to the effects on vegetation.

It is worth while remembering that ozone has a significant influence on a plant's ability to sequester carbon ($CO_2$) from the atmosphere: given that $CO_2$ is the main factor responsible for the greenhouse effect, it is easy to understand the necessity for broader knowledge of the relationship between ozone and climate change.

**Fig. 3** - Ozone injuries on the leaves of two sensitive species

# The interaction between forest pathogens and climate change

*Nicola La Porta*

Field crops and other annual or short-lived vegetation are generally considered to reflect weather changes, whereas trees and other perennial flora reflect, in addition, climate changes. A severe outbreak of disease occurs, rarely, when the weather removes the barriers that ordinarily restrain the pathogen (Hepting, G.H. 1963. Climate and Forest Diseases. Annu. Rev. Phytopath. 1: 31-50). Most atmospheric scientists agree that climate changes will mean an increase in average temperatures in Europe bringing with it increasingly frequent extremes of climate such as drought, floods and storms. Average precipitation should decrease, but more rain is expected to fall in winter while summers will become drier.

Storms may become more frequent and the consequences for the main European forest tree species are expected to be quite severe. In recent years, several studies have been carried out to predict and estimate the effects of globally rising $CO_2$ emissions and temperatures on the phenology, biochemistry, photosynthesis and other physiological traits of European forest trees. Less effort has been directed to identifying changes in systems of host-pathogen interaction brought about by the new global environmental conditions forecasted. Several plant diseases have been observed to be more severe after mild winters or during periods of warmer temperatures, which suggests that climate warming alters the severity of plant disease. In these conditions, there is a high probability that forests will be subject to increased frequency and intensity of stress due to extremes of climate. The impact of climate change on forest health should, therefore, be carefully evaluated.

Given these assumptions, several fungal diseases on trees may become more devastating due to the following factors:

1) abiotic stresses such as drought and flood are known to predispose trees to several pathogens;
2) temperature and moisture affect pathogen sporulation and dispersal, and changes in climatic conditions are likely to favour certain pathogens;
3) migration of pathogens triggered by climate change may increase the incidence of disease or its geographical range as pathogens encounter new hosts and/or new potential vectors;
4) new threats may appear, either because of a change in the composition of tree species or due to the arrival of invasive species.

If successful infection is dependent on temperature, higher average temperatures may lead to more attacks. Pathogens which have been prevalent in southern Europe may spread northwards and also upwards to the mountains. Pathogens with evolutionary potential for greater damage have been identified in order to estimate the magnitude of the threat and to prepare for the changing conditions (La Porta *et al.*, 2008. Canadian Journal of Plant Pathology 30 (2): 177-195). In this broad phytopathological review the main European forest diseases and their causal agents that may take advantage of one or more of the above mentioned

factors have been highlighted. The following fifteen diseases may became serious threats to European forests suffering the effects of climate change, due to their particular biological traits: root and butt rot in conifers (*Heterobasidion* spp.) and broad-leaves (*Armillaria* spp.); Dutch elm disease (*Ophiostoma novo-ulmi*); cypress canker (*Seiridium cardinale*); chestnut blight (*Cryphonectria parasitica*); shoot blight in pines (*Sphaeropsis sapinea*); oak decline (*Phytophthora* spp. and others fungi); tea break fungus in conifers (*Rhizina undulata*); charcoal disease in oak and beech (*Biscogniauxia* spp.); alder decline (*Phytophthora* spp.); canker stain in plane (*Ceratocystis platani*); silver fir decline (several pathogens); *Brunchorstia* die-back (*Gremmeniella abietina*); red and brown band needle blight in pines (*Dothistroma* spp.); Phytoplasmas.


Figure 3

The work carried out by the Forest Physiology and Ecology Research Unit in 2008 focused on some of the above-mentioned pathogens in relation to climate change. The incidence of *Heterobasidion* spp. was studied in Norway spruce forests in Trentino to obtain useful data for epidemiological investigations (Fig. 1). The results were shown to the 3rd National Congress of Silviculture in September 2008. *Armillaria* spp. was investigated both as a pathogen and for its capacity to compete with *Heterobasidion* spp. on the stumps of several conifer species (Fig. 2). The results of the study were published in the proceedings of an international conference in California (ISBN 9780615230764). The world's most serious tree disease, Dutch elm disease, which caused the near extinction of elm species in Europe and North America last century, was investigated in Italy and a breeding programme was set up with the aim of finding resistant clones to be planted in Mediterranean-like climates (Santini *et al*. Euphitica 163 (1): 45-56). The ecological behaviour of cypress in the new climatic conditions was explored at the northernmost border of cultivation of this species to evaluate its reaction to its main limiting factor, the canker fungus *Seiridium cardinale* (Zocca *et al.*, Acta Oecologica. 33: 307-313). Interesting results regarding the persistence of hypovirulent strains of *Cryphonectria parasitica* (chestnut blight) on chestnut coppices were published (Turchetti *et al.*, Forest Pathology 38 227–243). Investigations were also carried out on *Sphaeropsis sapinea*, an opportunistic pathogen of shoot blight in pines, that caused a serious epidemic in the Alps following years of severe drought (Salvadori and Maresi, 2008. Forstschutz Aktuell 44: 21-22). In addition, the Research Unit is participating in an EU project, which started in 2008, dealing with several *Phytophthora* spp. that have shown devastating potential in North America, in particular in oak (Fig. 3) but also in *Alnus* spp. and chestnut.

However, even though all the above-mentioned pathogens are predicted to be increasingly virulent, the general assumption is that the effects of climate change will vary for each pathosystem and location, so it is difficult to generalise. Climate change is likely to have a powerful impact on plant–pathogen interactions and it will probably be one of the biggest challenges for the ecological and functional stability of forests in the coming years. Future challenges in this emerging sector of research will include spatial and temporal variations in changes to the environment and to plant pathosystems, co-evolutionary interactions between hosts and pathogens, and latency effects not only in the responses of pathosystem dynamics but also in the ability of land managers to adapt to changing conditions.


Figure 1


Figure 2

**Fig. 1** - Fruit body of Heterobasidion annosum s.l. main causal agent of root and stem rots of conifers
**Fig. 2** - "Oil spot" growth of Armillaria infection
**Fig. 3** - Sudden Oak Death caused by infection of Phytophthora ramorum

# Energy and environmental sustainability on a mountain farm

*Daniela Bona*
*Silvia Silvestri*

## Introduction

There is potential for agriculture on the whole to be directly involved in issues of renewable energy, both as users and even more so as prime movers in a new and developing sector, i.e. agro-energy. Promoting wider use of alternative energy sources involves the creation of models that can be reproduced in the territory, thus facilitating diffusion of actual working prototypes. The final aim, in this case, is to cover individual energy needs and to increase the farm's profits by selling surplus power.

In the present study, an innovative approach was adopted based on enhancing potential sources of energy produced within the farm while seeking technical and economic solutions to several environmental problems, in line with the main aims of sustainability.

The study was funded by the Italian Ministry for Agriculture (GU n.188 of 23 May 2007) and was carried out with technical advice from the Centre for Theoretical and Applied Ecology of Gorizia (CETA).

## Materials and Methods

The farm studied (the model used) is a mountain farm located 900 meters above sea level. Animal husbandry is the main activity with 100 dairy cows for cheese production and 30 pigs (100 kg) for the processing of salami, both directly marketed by the farmer.

The first step was to analyse the supply and demand of energy by collecting data on consumption (electricity and fuels) and the amounts of renewable sources produced by the farm. Appropriate solutions for enhancing the available biomass were then proposed, in particular anaerobic digestion (AD), whereby biogas is produced and then converted into electricity and heat. This technique has a positive environmental impact as it also improves the characteristics of the slurry (Fig. 1).

An integrated option, in addition to AD, was also considered, which involved post-treatment of the digestate through solid-liquid separation with subsequent composting of the solid part and phytoremediation of the liquid part. Evaluation of energy, environmental and economic issues completed the feasibility study.

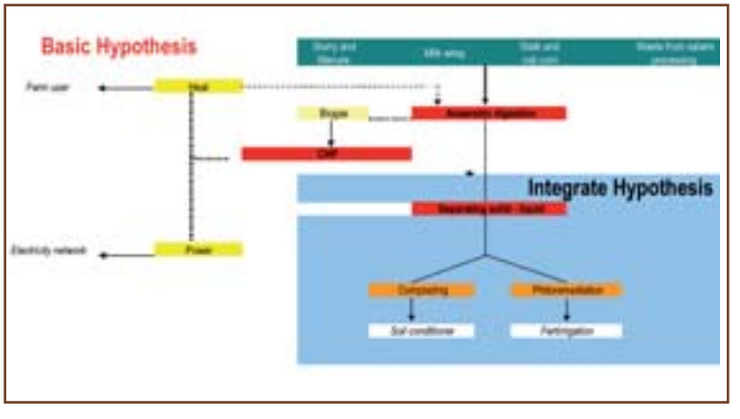



**Fig. 1** - Schema of the proposed treatment chain

## Energy analysis of the agro-energy chain

The biomass produced by farm activities is 80% animal waste and 20% organic residues, such as milk whey, waste from salami processing and residues from corn growing (corn stalks and cobs), the latter being useful for increasing the energy yield of the animal waste, which is not very high. Gas production is about 300 $m^3$/day with a $CH_4$ content of 55% and a calorific value of 5.3 kWh/$m^3$. The installation of a co-generator makes it possible to con-

vert biogas into power (189,000 $kWh_{el}$/year) and heat (349,700 $kWh_{thermic}$/year), part of which is used to cover own demand (11% power and 45% thermal energy) (Fig. 2). Thorough assessment of the farm's heating requirements, both existing and foreseeable in the near future, seasonal consumption and daily peaks in power use, show that there is a surplus of energy with respect to demand. However, further study needs to be made of peak power supply in milk processing, for example, the possibility of heating and storing hot water overnight, or of using a 9-10 $m^3$ capacity tank for storing steam at a pressure of 10 atmospheres (for ricotta). The economic implications and other technical details of these potential solutions also need to be carefully studied.

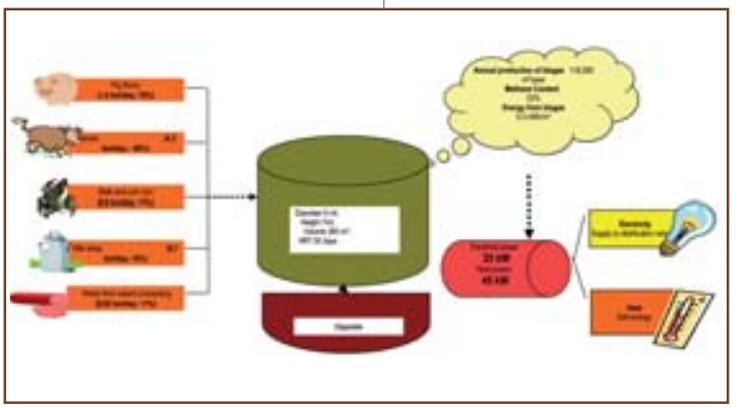


**Fig. 2** - Dimensions of the anaerobic digestion plant

## Environmental analysis

Apart from the environmental benefits arising from the improvement of animal waste quality after AD treatment (mineralisation of organic matter, reduction of pathogens, odour abatement, reduction of phytotoxic substances, improvement of soil quality and fertility), improvements in the balance of greenhouse gases and nitrogen emissions are also very important. In the first case, estimates are based on emissions and indicators of activity. With respect to greenhouse gases, or changing climate gases, the relevant methodology is provided by the Intergovernmental Panel on Climate Change (IPCC). In the case studied, it was estimated that AD and the transformation of organic matter into biogas results in a reduction in $CO_2$ of 11 tons/year, against the 280 tons/year of $CO_2$ produced without any kind of treatment. The emissions "saved" due to avoiding the use of fossil fuels have to be added. The nitrogen balance shows that the maximum amount of digestate per hectare does not exceed the limit of 170KgN stated by the national law. Systems for N abatement are not "strictly necessary", while phytoremediation for the treatment of wastewater from the milking room and the dairy processing stages is still required.

## Economic analysis

The following costs were taken into account: maintenance of the plant (€2.5/$kWh_e$), labour (€15/hour), purchase of corn stalk and cob residues (€15/ton), power consumption (€0.18/kWh), insurance (0.5% of the value of the facility), financial costs (6% rate of interest on the entire funding), depreciation (8 years for electro-mechanical tools, 20 years for installation costs, with additional costs of €2.5/$m^3$ for spreading the digestate). Revenues are the averted heating costs and the sale of the power produced.

The critical issues are primarily the definition of the minimum plant size for economic viability and the spreading of costs over around 7-8 years. Installing a power generator of 50 kW instead of 25 kW involves treatment of a greater quantity of biomass (which could exceed the farm's available resources) but would certainly result in better performance in terms of conversion into power and heat.

Finally, the lack of specific rules and regulations and resulting uncertainties with respect to the value of the all-inclusive rate and to green certificates, has given rise to market speculation and to the current standstill in business initiatives.

# In brief

## 20 years of aerobiological monitoring: what is changing?

*Antonella Cristofori, Fabiana Cristofolini, Maria Cristina Viola, Elena Gottardini*

Aerobiological monitoring data, as well as playing a major role in the development of alert systems for allergic patients, may be exploited in the field of environmental research. The assessment of pollen dispersion in the atmosphere can, indeed, be an effective indicator of plant distribution and phenological stage. Due to the high quality of the input data, the time series collected by the IASMA Research and Innovation Centre of the Fondazione Edmund Mach over a period of 20 years provides the basis for developing research through the study of spatial and temporal changes in the spread of pollens. First explorative analyses on the data collected in Trentino show changes in the spectrum of pollen composition with a tendency to an increase in pollen production and a lengthening of the pollen seasons. These modifications could be attributed both to anthropic activities and to the rise in mean temperatures as an effect of ongoing climate changes.

## Recovery of agricultural biomass for energy

*Silvia Silvestri, Andrea Cristoforetti, Enzo Mescalchin*

Pruning waste from orchards and vineyards represents an energy resource for farmers. Wood waste is usually left in the vineyard after cutting since the practice of burning in the field is forbidden in Trentino, due to environmental problems caused by short-term concentrations of smoke, dust and odours.

A feasibility study was carried out in 2008 for the Avio Wine Co-operative (Southern Trentino) with the following goals: to plan a chain of operation for the collection of pruning wood and for its mechanical treatment by comparing different techniques tested in local vineyards; to ascertain the quality of pruning waste as biofuel with regard to the main parameters of moisture, ash content, possible presence of organic pollutants on the wood surface (pesticide residues) and abatement levels over time; to monitor air quality at the chimney after combustion in a small scale boiler and a central heating plant.

# Facilities and Equipment

The department has facilities and equipment for conducting research on forest ecology and biology, limnology and fisheries, biomass and renewable energy, as well as agro-meteorology, climatology and aerobiology.

## Forest ecology and physiology

This unit has all the classical dendrological instruments, including a Haga Ipsometer, a Clinometer, a Bitterlich Relascope Optical distance meter, tele¬meters, binoculars, Pressler's increment borers, an Incremental Hammer and dendrometric callipers. Laboratory work is supported by optical microscopes, stereo microscopes, sterile laminar flux cabinets, thermostats and incubators for microbiological cultures. Tree stability tools include a Resistograph and a Picus Sonic Tomograph.

Molecular laboratory facilities include a Gradient thermocycler, a Gradient multiple robotisable thermocycler, microcentrifuges, a fluorimeter, horizontal and vertical electrophoretic chambers, a Phytotrone for tissue and plant growth, as well as freezers and refrigerators.

## Limnology and fish research unit

The field facilities of the limnological research group include 2 rubber dinghies, 2 multi-probe sondes, a FlowTracker Handheld ADV Sonntek, an underwater Quantum sensor LI-192 SA, a Kajak corer and an Eckman dredge for sediment sampling. The unit also has several laboratories.

These include the hydrobiology and microscopy laboratory, equipped for analyses of basic limnological variables (photosynthetic pigments, dry weight, dissolved oxygen, water turbidity, macrophytes) and of phytoplankton, picoplankton and periphyton samples (microscopes, invertoscopes, fluorescent microscopy, image analysis).

The analytical and environmental chemistry laboratory is equipped for analysis of the major ions and nutrients (phosphorus, nitrogen and silica), as well as organic molecules (with focus on cyanotoxins) by means of spectrophotometry, ion chromatography and Liquid Chromatography-Mass Spectrometry (LC-MS).

The genetics laboratory has the principal analytical tools, i.e. PCR, horizontal and vertical electrophoresis, sequencing (capillar electrophoresis), and software for data analysis.

## Biomass and renewable energy

The biomass laboratory is equipped with three dynamic respirometers for assessing the biological stability of samples collected during composting and after the mechanical biological pre-treatment of residual solid waste.

An olfactometer ECOMA (mod.TO7) and an electronic nose are used to monitor odour release and the environmental impact of different waste treatment plants. Respirometric tests and air analyses follow standardised methodologies.

A complete pilot-scale composting plant is available, consisting of all the sections present in a full-scale treatment facility: a waste pre-treatment area, an intensive bio-tunnel phase with air treatment using a biofilter, a curing section and a compost sieving area. In the near future (2009) new tools for the study of the anaerobic digestion process in the laboratory and at pilot scale will be available.

## Agrometeorology, climatology and aerobiology

The meteorological centre manages a network of 80 real-time meteorological stations spread over the main agricultural and forest areas of the Province of Trentino. Data are collected by a three-tiered information system consisting of a database server, an application server and a Web server.

The electronic meteorology laboratory is equipped with calibration instruments for temperature, radiation and moisture sensors, oscilloscopes, function generators and precision digital multimeters. Other meteorological instruments include: a disdrometer, sonic anemometers, an IR video camera, a TDR reflectometer, a data logger for temperature and humidity, multichannel data loggers, and other sensors. The aerobiology sector has a set of five automatic pollen samplers, and the geomatics sector is equipped with four workstations, an A0 plotter and precision GPS devices.

## Molecular Ecology

The Molecular Ecology group carries out field data collection using hand-held tablet PCs that are specially designed to operate in environmental conditions normally not suitable for computers.

The screen is designed to be readable in full sunlight; the computer casing is moisture- and water-proof; weight is reduced to a minimum to allow ease of transportation in the field; the use of a touch-screen and standard OCR software allows the user to hand-write notes that are automatically acquired as text files and to quickly select options from pre-compiled lists.

In this way data are recorded directly in digital format without the need for transcription. The computers are equipped with GIS software for direct acquisition of georeferentiated data through external GPS systems.

Facilities and instruments for high throughput DNA extraction, amplification and sequencing and for fingerprinting are shared with the Genetics and Molecular Biology department. The group manages one DNA sequencer ABI 3730xl for the department.

Highly standardised, quantitative and semi-automatic scoring of AFLP fingerprinting is carried out by means of the AFLP-quantar software. Additional software developed in-house allows for automation of the repetitive tasks associated with sequence validation, reformatting and screening for homology in comparison with public databases.

# PLANT PROTECTION DEPARTMENT

*Claudio Ioriatti*

The department's mission is to provide support and improved control strategies for integrated plant protection based on new knowledge and new technologies. Most of our work is dedicated to apple, grape and soft fruits, the main crops of the Province of Trento. The department handles a range of services from plant-disease diagnostics to pesticide registration, as well as research on sustainable crop protection strategies and the introduction of new technologies to farmers and industry.

Our plant diagnostic services include insect identification, nematode analysis, and fungal and bacterial disease diagnosis. Foliar and root diseases caused by fungi, bacteria, viruses and nematodes can be difficult to identify in the field and often require laboratory confirmation through sophisticated instrumental analyses (PCR, DAPI and ELISA). Prompt identification of the etiological agent contributes to improving the efficacy of the control measures suggested by the Extension Service, and also helps in identifying quarantine diseases and pests.

The department provides research and technical services to assist the agrochemical industry in registering pesticide compounds for agricultural application. In particular, we conduct a wide range of laboratory and field studies that support pesticide registration under Italian and European legislation. All studies are conducted in full compliance with good experimental practices (GEPs) and in conformity with European Plant Protection Organisation (EPPO) guidelines. This allows a vital link between researchers and Extension Service consultants to be maintained.

The department promotes research which enhances the sustainability of fruit production and is thus beneficial to the region. We are dedicated to discovering and accessing knowledge that contributes to safe food production, the maintenance of environmental quality and the preservation of natural resources and ecological systems.

The research areas we are currently focusing on are detailed below.

## Pesticide side effects on beneficial organisms

The implementation of integrated pest management (IPM) relies heavily on knowledge of the effects of pesticides on important natural enemies present in the crop. As a consequence, the assessment of pesticide side effects on natural enemies, such as predatory mites, is important in selecting IPM-compatible products.

## Innovative semiochemicals and biocontrol agents for pest and disease control

Mating disruption, as currently applied to insects, is the practice of continuously dispensing synthetic sex attractants onto a crop for extended periods of time in order to suppress pest reproduction by interfering with mate finding. This pest management strategy is particularly promising in the management of moths and other pests that rely on long-distance chemical communication.

The Trentino region is recognised as a pioneer in Italy in the application of behavioural modification strategies for pest control in both apple and grape. Improvements to this pest control tool may be made through the study of volatile metabolites in plants that guide insect herbivores to their host plant for oviposition. These chemicals are of interest from an ecological and evolutionary perspective.

Co-occurrence of volatile plant compounds has been shown to play a role in host plant shifts and is probably also at work in sympatric speciation. Information about the chemical signal which attracts gravid females is essential in plant breeding for insect resistance and can also be used directly to improve insect monitoring and control.

## Pesticide resistance in apple pests

Over the last 20 years, the department has developed integrated pest management programmes for conventional apple orchards in Trentino.

The approach is based on the use of a restricted number of selective insecticides for controlling key pests. The programme is now threatened by the development of insecticide resistance in codling moth *Cydia pomonella*. Early detection of insecticide-resistant populations is of paramount importance in implementing effective integrated resistance management programmes. We are conducting studies on the interrelations between the resistance spectrum and resistance mechanisms.

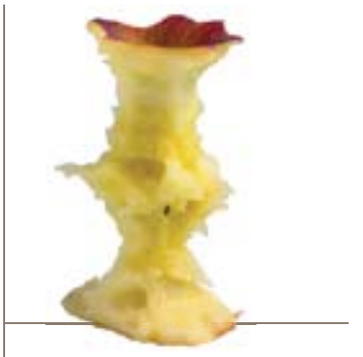
The aim is to set up an efficient method for assessing insecticide resistance risk and for designing resistance management programmes for field use.

## Apple proliferation disease: short-term control strategies and resistant rootstock breeding

Apple proliferation disease, a serious affliction of apple, was first reported in Trentino in the early 1950s. However, a serious epidemic broke out only in the late 1990s. The disease led to important economic losses due to undersized fruits with poor taste.

A specific phytoplasma, the *Candidatus* Phytoplasma mali, is associated with the disease. Non-cultured, phloem-restricted phytoplasmas are transmitted by man through grafting, and latently infect planting material. The phytoplasmas are spread naturally by psyllid vectors and two Cacopsylla species, *C. picta* and *C. melanoneura*, which have been identified as *Candidatus* Phytoplasma mali vectors in northern Italy and in Germany.

As no curative treatments are available, control of the insect vectors is the most promising short-term way to prevent further spread of the disease. To develop efficient control strategies, it was necessary to identify the vectors in Trentino, study their biology and understand their transmission parameters. We are also seeking a more durable solution through an ongoing breeding programme to develop resistant plant material.



# Morpho-functional studies on olfactory communication in *Apis mellifera* L. and other Apoidea species

*Gianfranco Anfora*
*Valerio Mazzoni*
*Marco Tasin*
*Federica Trona*

The honey bee, *Apis mellifera L.* (Hymenoptera Apidae) (figure 1), is the most common and the most important bee species worldwide. It provides an essential contribution to pollination in about 80% of crops. A serious decrease in the bee population could cause dramatic reduction in crop production with consequent damage to the agricultural economy.

Honey bee colonies are declining all over the world, with losses of up to 90% in some areas of the USA. This phenomenon, called Colony Collapse Disorder (CCD), occurs when the inhabitants of a bee hive suddenly disappear.

Scientists have not yet found a reliable cause of bee decline, which is likely to be caused by several factors (pathogens and parasites, neurotoxic insecticides, genetically modified plants, changes in climate conditions and in the natural habitat, electromagnetism, etc.). However, one of the main factors responsible for CCD has been found in neonicotinoids, systemic insecticides acting on the transmission of nervous impulses.

Sublethal doses of neonicotinoids have been documented to affect the homing and foraging activities of honey bees. In particular, it has been suggested that imidacloprid impairs the bees's memory and its ability to learn, causing foragers to get lost or disoriented. However, the mechanisms and the doses that cause neonicotinoids to interact with the bee's nervous system are still a matter of conjecture.

Understanding the cognitive and behavioural processes in honey bees is, therefore, of fundamental importance.

The main aim of our project is to understand the neurophysiological and behavioural mechanisms involved in recognition, transduction and processing of the olfactory stimuli in *A. mellifera*, in both the peripheral and the central nervous systems.

More specifically, we aim to analyse the asymmetries in olfactory perception and learning. The present project is a collaboration with the Center for Mind/Brain Sciences and the Department of Physics of the University of Trento, and the Center for Agriculture and the Environment in Crevalcore (BO).

The phenomenon of right-left asymmetry in neural structures is associated with cognitive skills, and is common and well studied in vertebrates. Brain asymmetries in insects seem to be correlated with long-term memory.

Recent studies have shown functional asymmetries in *A. mellifera* between the right and left sides of the nervous system, both in vision and in olfaction. Moreover, the recall of olfactory memory exhibited a time-dependent shift involving first the right and then the left antenna. We are verifying whether lateralisation in honey bees can also be seen in the olfactory receptors on the antenna through electrophysiological, morphological and behavioural experiments.

**Fig. 1**

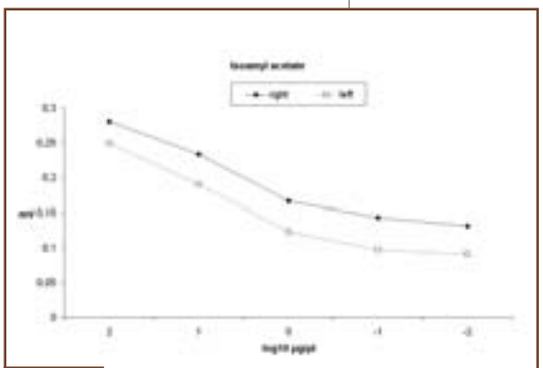


Figure 2

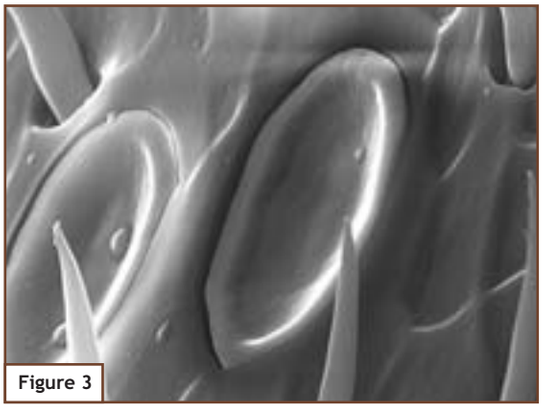


Figure 3

Antennal responses of *A. mellifera* foragers to linalool, a common floral odour, and to isoamylacetate, the alarm pheromone (5 doses increasing in decadic steps, from 0,01 to 100 µg/µl) have been recorded using electroantennography (EAG). Behavioural bioassays in wind tunnel have been carried out to test the responses of individual bees to the odour sources of isoamylacetate after the removal of one antenna.

The volatile compounds tested elicited, at each dosage, higher EAG responses (mV) on the right antenna than on the left antenna (figure 2). Sensilla placoidea (figure 3), involved in olfaction, have been found to be more abundant on the surface of the right antenna than on the left one. Bees having only the right antenna are better able to respond to sources of alarm pheromone.

Our results reveal, for the first time, the existence of lateralisation in the peripheral perception of odours in *A. mellifera*. Lateralised olfactory mechanisms can affect both intra- and inter-specific communication in insects and, in addition, the sublethal effects of different pesticides, such as the neonicotinoids, can affect structures involved in the processes of memory formation and recall.

The same experiments are being carried out on *Osmia rufa* L. (Hymenoptera Megachilidae), a solitary bee species, with the aim of comparing its responses with those of the eusocial honey bee species. The preliminary results of the electrophysiological analyses indicate a lack of preferential asymmetry within the population of *O. rufa* tested, although it may be present differently in individual examples.

Further studies will use innovative techniques able to integrate analyses of peripheral olfactory perception with brain responses (optical imaging). The existing high-resolution, multiphoton imaging platform at the University of Trento will be adapted for small insect imaging, providing extremely high temporal and spatial resolution within the bee's brain. This technique will allow a three-dimensional time mapping of neurofunctional signals to be made providing deep insight into behavioural and odour-evoked signals and, as a consequence, the potential existence of physiological and functional asymmetries between the right and left side of the bee brain.

In addition, field surveys and laboratory experiments are being conducted in collaboration with colleagues at the IASMA/FEM Center for Technology Transfer to explore how different environmental factors may influence the behaviour of bees.

In conclusion, we are using a multi-disciplinary approach to fill the gap in the knowledge of the bee's biology, taking into consideration the sequence of steps ranging from environmental input to receptor structures in the bee antenna and to the relevant brain centres and how this neurophysiological activity is linked with behaviour. This work will serve for the protection of pollinators.

# *In vitro* screening for resistance to apple proliferation in *Malus* species

*Claudia Bisognin*
*Anna Maria Ciccotti*
*Wolfgang Jarausch*

## Introduction

Apple proliferation (AP) is one of the most serious phytoplasmoses in Europe, causing considerable economic loss. As there is no cure for phytoplasma infection, the most promising approach to combatting AP appears to be the use of resistant plant material. Natural resistance to AP has been discovered only in wild, apomictic *Malus* species, namely *M. sieboldii*. Crosses of these wild *Malus* species with *M. domestica* have been carried out in the past and attempts have been made to exploit this resistance in apple rootstocks. Studies on the colonisation behaviour of phytoplasmas in apple revealed that they were eliminated once a year in the aerial parts of the tree during phloem renewal in late winter / early spring. Long year's field trials further demonstrated that the use of resistant rootstocks can prevent the disease and is sufficient to establish field resistance in the whole tree (Bisognin *et al.*, 2008, *Phytopathology* 98:153-158). Resistance to AP in the field has been classified as reduced phytoplasma titre in the roots and absence of symptoms in the aerial parts of the tree. In the terminology proposed by Cooper & Jones (Phytopathology 73(1983):127-8) the tested genotypes were resistant to '*Ca.* P. mali' and tolerant to the disease. Unfortunately, all the *M. sieboldii* hybrids examined turned out to be too vigorous to be used as rootstocks in modern apple culture.

A new breeding programme was therefore started within the SMAP project aimed at developing a rootstock comparable to the standard stock cv. M9, containing features of both dwarfing and resistance to AP. Several different cross combinations were made between apomictic-resistant accessions and susceptible *M.* x *domestica* parents in order to find the best selection. Resistance screening of the progeny is currently done by graft inoculation of the seedlings, which are then observed in the nursery for symptom expression over a period of 2-5 years. As an alternative to this time-consuming and labour-intensive screening, an *in vitro* resistance screening system based on micrografting has been developed (Bisognin *et al.*, *Plant Pathology* 57(2008): 1163-1171). It allows analyses of response to infection of resistant selections compared with susceptible controls to be made under standardised conditions.

## The *in vitro* resistance screening system

The system requires the establishment of *in vitro* shoot cultures of the genotype to be analysed and the maintenance of '*Ca.* P. mali' in micropropagated apple. Healthy *in vitro* shoot cultures of AP-resistant genotypes *M. sieboldii*, the donor of resistance, D2212, considered as highly resistant, H0909, already a cross with the standard apple rootstock M9, and susceptible genotypes GD and M9, were successfully established and propagated as well as infected cultures of cv. GD (Ciccotti *et al.*, *Agronomy Research* 6/2(2008): 445-458). For this study, two different strains were selected: strain PM6, and strain PM4. Healthy *in vitro* shoots were inoculated by micrografting with infected shoots used as graft tips. Micrografting was done in 10 repetitions for each combination of genotype x strain. Graft contact was maintained for 1.5 months.

**Fig. 1** - Specific symptoms of AP in vitro: enlarged stipules

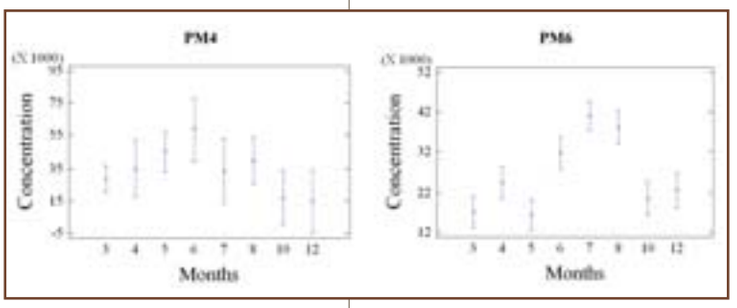



**Fig. 2** - Dynamics of infection with 'Ca. Phytoplasma mali' strain PM4 or PM6 in all in vitro shoots inoculated with the respective strain. Data were recorded at 8 time points and are expressed as means of phytoplasma concentration (nb of copies of phytoplasma/ng DNA of apple) in an ANOVA analysis

Only grafts where a good phloem connection between the two bions was ensured were considered successful grafts, and were then subcultured and analysed for the presence of '*Ca.* P. mali'. Parameters such as quality of the grafts, transmission rates, and mortality 3 months post-inoculation (m.p.i.) were included in the study. Phytoplasma titre in the inoculated material was determined by quantitative PCR, based on a TaqMan™ assay, each month until 12 m.p.i.. At 6 and 12 m.p.i. survival, growth habits and symptoms were recorded and compared with the healthy genotype. A disease index was developed for this analysis (table 1).

## Results

Both in the field and *in vitro*, a reduced phytoplasma concentration and an absence or slight expression of disease symptoms are sufficient to assess resistance to AP. Infected *M.* x *domestica* genotypes were severely affected by the disease *in vitro* and showed stunted growth with proliferation of shoots, small leaves and enlarged stipules (Fig. 1). On the other hand, the highly resistant genotype D2212 was almost unaffected by the disease both *in vitro* and *in vivo*. *M. sieboldii* was only slightly affected and could still be classified as resistant, while the genotype H0909 showed an intermediate behaviour as revealed by the higher concentration of phytoplasmas measured in this genotype (table 1). Mean values of phytoplasma concentration measured for the two strains differed significantly, with a concentration of 54528 copies of phytoplasma/ng DNA of apple for strain PM4 vs 33292 copies of phytoplasma/ng DNA of apple for strain PM6. This might be an indication of varying strain virulence. The multiplication efficiency of strain PM4 was much higher, especially in GD. Thus, the *in vitro* system is also suitable for evaluating virulence variation in phytoplasma strains and for studying their host-pathogen interactions. These interactions also became evident during the analysis of phytoplasma infection dynamics after *in vitro* graft inoculation: for both strains an increase in the phytoplasma concentration was measured for the first 6-8 m.p.i. (Fig. 2). Differences in phytoplasma concentrations between resistant and susceptible genotypes were kept stable and followed the same dynamics. After an initial period, plant defence reaction became active and the phytoplasma concentration decreased. After 10-12 m.p.i., a steady-state was reached, similar to phytoplasma concentration at 3 m.p.i.. Therefore, 3 m.p.i is already an appropriate time point at which to analyse phytoplasma titre and the habits of infected genotypes and, hence, to evaluate AP resistance *in vitro*.

**Table 1** - Phytoplasma concentration and growth habit of genotypes infected with strain PM6

| Genotipo | Ceppo | Concentrazione del fitoplasma [a] 3 m.p.i. | Indice di malattia (D.I.) [b] 12 m.p.i. |
|---|---|---|---|
| *M. sieboldii* | PM6 (AT2 subtype) | 11289±4905 ab | 1 |
| D2212 | PM6 (AT2 subtype) | 6955±4477 a | 0 |
| H0909 | PM6 (AT2 subtype) | 16481±4905 ab | 2 |
| GD | PM6 (AT2 subtype) | 24702±6332 b | 3 |
| M 9 | PM6 (AT2 subtype) | 18162±7755 ab | 2,5 |

[a] expressed as nb of copies of phytoplasma/ng DNA of apple
[b] Disease index 12 m. p. i.: 0 = like healthy control; 1 = weak growth reduction, smaller leaves than healthy control, 2 = growth reduction, small leaves; 3 = stunted, proliferation, enlarged stipules, small leaves

## Conclusions and prospects

The results obtained in this study indicate that resistance to '*Ca.* P. mali' can be observed *in vitro* in a similar way to *in vivo*, speeding up the resistance screening procedure. The *in vitro* system established can, therefore, be a valuable component of a resistance-screening programme when only a single plant of a given genotype is available, as in a breeding progeny. The method is currently employed for testing individual genotypes of different breeding progenies. Resistance evaluation of several hybrids is under way. Moreover, the *in vitro* system offers the possibility of studying specific host-pathogen interactions in standardised conditions and of simultaneously evaluating the virulence of different strains.

# An innovative method based on nicotinic acid degradation for the selection of microbial antagonists of fire blight

*Thomas Paternoster*
*Ilaria Pertot*

Fire blight, caused by the gram-negative bacterium *Erwinia amylovora*, is a serious disease of important crops, such as apple and pear, causing significant losses throughout most of the world. The disease can affect all aerial parts of the plant, although blossoms are the main site of infection (Fig. 1). After an epiphytic phase on the stigma, *E. amylovora* reaches the floral cup (hypanthium), where, given a high population and suitable environmental conditions (high relative humidity and/or rain), the infection generally starts. The spread of fire blight to disease-free areas is prevented by the adoption of expensive quarantine measures. When initial symptoms of the disease are found , infected and potentially contaminated material is promptly removed and destroyed. If quarantine measures are unsuccessful and the pathogen establishes itself, disease control can only be achieved with the use of antibiotics (not allowed in some countries) or to partially effective control agents (i.e. copper or resistance inducers). In this case, the use of microbial antagonists of E. amylovora can be an alternative or complementary measure to antibiotics. Several species, mainly *Pseudomonas fluorescens, Pantoea agglomerans* and *Bacillus subtilis*, are active against *E. amylovora* and a few isolates have been developed as commercial products.

**Fig. 1** - Apple blossoms

The selection of new biocontrol agents (BCAs) is usually based on evaluation of biocontrol activity in plants, which is extremely expensive and time consuming. *E. amylovora* requires nicotinic acid (NiAc) as a growth factor in laboratory culture media. This is not a requirement in the *Erwinia* genus and has been proposed as a biochemical test for identifying *E. amylovora*. Bearing in mind this particular requirement of the pathogen, we have developed a new, streamlined approach based on the hypothesis that an organism able to eliminate or reduce the availability of NiAc could effectively inhibit the growth of *E. amylovora* and therefore control fire blight. This approach selects only NiAc degraders (in our experiments about 10% of the initial 2,000 isolates collected from apple and pear blossoms), dramatically decreasing the number of microorganisms to be further tested as potential BCAs. A second step was identification of the fast growing NiAc degraders. Only six isolates were considered for *in vitro* evaluation and among these JAN, which showed the greatest biocontrol efficacy, was chosen as the best candidate BCA.

Based on 16S rDNA sequence analysis, JAN has high

**Figure 2**

homology with *Pseudomonas rhizosphaerae* IH5. *P. rhizosphaerae* JAN did not produce *in vitro* antimicrobial compounds against *E. amylovora* and was not able to survive at 35°C and 37°C (human body temperature), suggesting an absence of pathogenicity for humans. These proprieties could speed up its registration as a biofungicide, avoiding otherwise necessary costly studies (i.e. for *Pantoea agglomerans*). Biocontrol efficacy of *P. rhizosphaerae* JAN against *E. amylovora* was compared to *P. fluorescens* A506, a commercially available fire blight biocontrol agent (BlightBan A506®) unable to degrade NiAc, and to *P. fluorescens* TN5, a NiAc degrading bacteria which has never been tested against fire blight. *P. rhizosphaerae* JAN was first tested on immature pear fruit slices (Fig. 2). The BCAs were inoculated 24 h before the pathogen and tested at three different concentrations ($10^7$, $10^6$, or $10^5$ cfu/ml). *P. rhizosphaerae* JAN was highly effective (90% efficacy) in controlling fire blight after 7 days of incubation (in the untreated control, 86.7% of slices were infected). No significant differences were found between the three different concentrations of *P. rhizosphaerae* JAN. *P. fluorescens* A506 and *P. fluorescens* TN5 were as effective as JAN when used at the two highest concentrations, but less effective when used at the lowest concentration. Even when used at a low concentration, the efficacy of *P. rhizosphaerae* JAN did not change from 7 to 12 days of incubation, indicating persistent biocontrol activity over time, whereas the efficacy of *P. fluorescens* A506 and *P. fluorescens* TN5 decreased over time. The three BCAs were able to colonize efficiently detached apple blossoms. The population increased exponentially in the first 24 h and reached its maximum after 48 h. The presence of *E. amylovora* did not alter the population sizes of the three BCAs to a statistically significant degree (Fig. 3). When inoculated 24 h before the pathogen, *P. rhizosphaerae* JAN and *P. fluorescens* A506 were able to control *E. amylovora* growth in apple hypan-

**Fig. 2** - Pear slices inoculated with *Erwinia amylovora* (A) and pre-treated with *P. rhizosphaerae* JAN (B)
**Fig. 3** - *Pseudomonas rhizosphaerae* JAN, *P. fluorescens* A506 and *P. fluorescens* TN5 populations on apple blossoms
**Fig. 4** - *Erwinia amylovora* population in apple blossoms after treatment with *Pseudomonas rhizosphaerae* JAN, *P. fluorescens* A506 and *P. fluorescens* TN5
**Fig. 5** - Comparison of biocontrol activity against fire blight of *Pseudomonas rhizosphaerae* JAN and *P. fluorescens* A506 (standard) in controlled greenhouse conditions

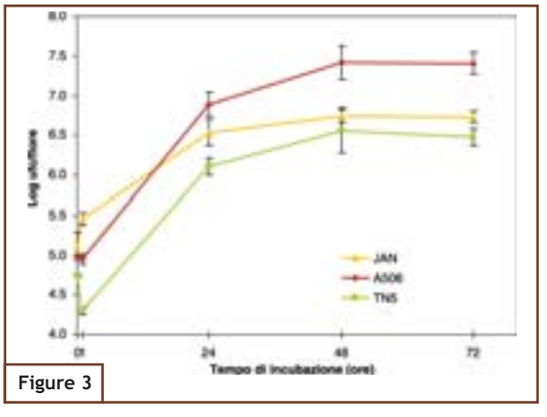



**Figure 3**

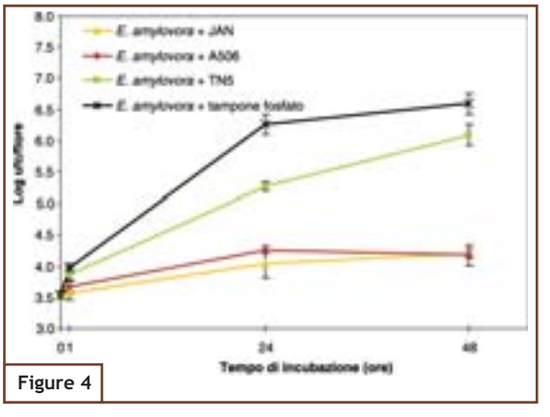



**Figure 4**

thium, while in the untreated blossoms the pathogen population increased rapidly to reach 3.9 × $10^6$ cfu/blossom after 48 h. *P. fluorescens* TN5 showed an ability to suppress *E. amylovora* growth, but less efficiently than P. rhizosphaerae JAN and P. fluorescens A506 (Fig. 4). The ability of *P. rhizosphaerae* JAN and *P. fluorescens* A506 to control fire blight was subsequently assessed on blooming apple trees (cv. Gala) in a quarantine greenhouse. Three experiments were performed at temperatures of 18 ± 6° C, corresponding to the average temperature fluctuations normally encountered during pear and apple bloom in Trentino and Switzerland. *P. rhizosphaerae* JAN showed consistent protection in all experiments (Fig. 5), indicating its validity as a BCA against fire blight in areas such as Trentino (northern Italy) and Switzerland.

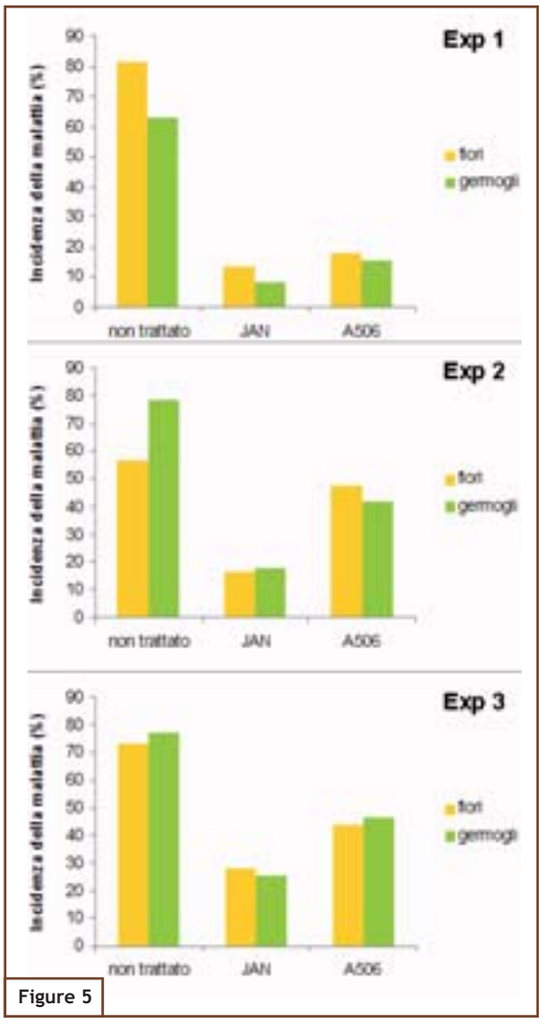



Figure 5

The efficacy of *P. rhizosphaerae* JAN in controlling fire blight in different apple and pear varieties may be directly correlated with the NiAc concentration in the hypanthium. Similarly, fire blight susceptibility between hosts and non-hosts as well as the varying sensitivity to the disease observed in different apple and pear varieties could depend on the presence and/or differing concentrations of NiAc in the hypanthium. At the moment, no methods are available for the evaluation of NiAc content in apple and pear blossoms, and further studies will therefore focus on this issue. The next step in the commercial development of JAN will be to confirm its efficacy in field conditions with several repeated trials in different locations and under different environmental conditions. This research is a collaboration between FEM, Agroscope Wädenswil and ETH Zürich (Switzerland).

# In brief

## *Salvia officinalis* metabolites actively protect grape against *Plasmopara viticola*

*Silvia Dagostin, Ilaria Pertot*

A Peronospora infections on a grapevine leaf

Some plants are resistant to diseases caused by certain pathogens due to constitutively produced inhibitory antimicrobial compounds present before infection. *Salvia officinalis* is known to have a broad range of antimicrobial properties. We found alcoholic extract of *S. officinalis* to be active against *Plasmopara viticola*, one of the most serious grape pathogens. After separation with liquid chromatography the active compounds are found in the less polar fraction of the extract. In-depth characterisation of the active molecules, such as molecular and enzymatic identification of biosynthesis pathways, could pave the way for the development of bioreactors, thus maximising the production of active molecules for low-cost production of a biodegradable biocide. These molecules could be a natural, safe and biodegradable alternative to copper fungicide for the control of grapevine downy mildew.

## Risk analysis of *Trichoderma atroviride* SC1 in soil microbial communities

*Claudia Maria Longa, Ilaria Pertot, Federica Savazzini*

*Trichoderma atroviride* SC1 is a biocontrol agent of Armillaria root rot, an emerging grapevine disease. After application on vineyard soil surface, it survives well and becomes an integral part of the local microbial community. This behaviour is a positive characteristic for its future use as a biofungicide against soil-borne pathogens. However, a long survival could have negative side effects on non-target soil micro-organisms. Changes in the genetic structure of the indigenous bacterial and fungal soil community were evaluated using automated ribosomal intergenic spacer analysis (ARISA). A principal component of variance analysis demonstrated that *T. atroviride* SC1 had an effect on the soil microflora for two weeks following inoculation. However, at later dates environmental conditions had a greater influence on soil microbial communities than *T. atroviride* SC1 (Shannon index of biodiversity). Therefore *T. atroviride* SC1 can be considered safe for soil application.

# In brief

## Differences among *Cacopsylla melanoneura* Förster (Hemiptera: Psyllidae): insight from molecular markers

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The psyllid *Cacopsylla melanoneura* (Föster) is one of the vectors of '*Candidatus* Phytoplasma mali', the causal agent of apple proliferation disease (AP).

In northern Italy, overwintering adults of *C. melanoneura* can be found on both apple and hawthorn from the end of January. After the development of juvenile instars, the new generation of adults move to shelter plants around mid-June.

These adults can be found on conifers at high altitudes from the end of summer to winter. Different populations of *C. melanoneura* collected from different host plants and localities were tested by PCR in order to assess the presence of AP phytoplasma. In addition, the genetic variations among these populations were analysed using microsatellite markers and COI sequences.

'*Ca.* P. mali' was found in most of the *C. melanoneura* populations in different percentages and titre.

Genetic data indicate differences among the populations, which could explain the differences in the efficiency of acquisition and transmission of AP phytoplasma by these different populations.

This work was carried out in collaboration with Dr. Chiara Papetti (University of Padua).

## *Ampelomyces quisqualis* recognises powdery mildew and activates the germination process

*Lorenzo Tosi, Ilaria Pertot, Monika Maurhofer*

*Ampelomyces quisqualis* is a naturally occurring mycoparasite of several powdery mildew species.

The anatomy of the mycoparasitic relationship between *A. quisqualis* and its hosts has been widely investigated, but the interaction at the molecular level is poorly understood. After a recognition phase, during which a water-soluble, heat-resistant molecule of approximately 3,000 Da produced by powdery mildew enhances germination of *A. quisqualis* conidia, the mycoparasite actively penetrates the host cell wall and invades the cytoplasm causing the death of the cell. *A. quisqualis* never completely kills the fungal host and its aggressiveness in the last phase of mycoparasitism is generally reduced. Powdery mildews are obligate parasites of plants; therefore gene expression during mycoparasitism was studied in a tritrophic system (*A. quisqualis*, *Podosphaera xanthii* and *Cucurbita pepo*). Gene identification was carried out in collaboration with Prof. Delledonne (Verona University, Department of Biotechnology).

# Facilities and Equipment

The department laboratories equipment includes current versions of instruments necessary for:

- genetic analysis of plants and micro-organisms;
- olfactory reaction analysis of living insects and antennas;
- efficacy trials of plant protection agents in the laboratory and in small and large field plots;
- climatic data collection at the plot level;
- pest and disease diagnostics;
- micro-propagation of plants.

## TECAN Freedom EVO® workstation

A TECAN Freedom EVO® workstation (automated multi-channel pipeting robot) supplemented by a versatile vacuum system for automate filtration processes allows for the processing of 96-well plate from cell lyses, soil extraction or any other sample material to DNA elution in less than 3 hours.

DNA samples are further processed automatically with various reagents until specific amplification in real-time PCR-cyclers and sequenced or fragment length determined in a AB3130XL genetic analyser.

Other detection tools, such as the electrophoretic apparatus, are also available.

High throughput mechanical extraction equipment and appropriate centrifuges complete the range of facilities.

## Wind tunnel with flight section and electroantennal detector

The department has a custom-designed wind tunnel with a flight section of 63 x 90 x 200 cm equipped with activated charcoal filters, and a volatilisation system for test compounds consisting of a glass capillary with an elongated tip. The tip vibrates at ultrasonic frequency (about 100 kHz) by means of a piezo-ceramic disc and a motor-driven syringe. This ensures constant delivery of the solution for testing the reaction of insects to olfactory stimuli. Antennography (EAG) joined to an electroantennal detector EAD-GC allows the reaction of single antennas to specific volatile metabolites to be recorded with real-time identification of the substance in question. The equipment is complemented by an instrument for single sensillum recording and single-cell recording with similar characteristics.

## Field equipment

Field equipment includes:

- Motorised sprayers, from single knapsack to multi-tank airblast sprayers, ranging in size from self-driving to tractor-pulled, which allow experimental products to be applied on variously sized plots on IASMA farms. A tractor-carried tunnel spray system is employed specifically to avoid drift in row grown grapes. A laboratory and greenhouses with the requisite infection towers, sprayers and safety equipment, are available.
- A large number of micro-climate sensors and field data entry stations for collecting climate data at field level. The stations are interlinked with a remote communication system.
- Microscopes of the most advanced generation are available for classical diagnostics. The diagnostic lab also contains the equipment required for ELISA DAPI, which is also used for genetic analysis.
- New climatic chambers and appropriate sterile benches are available for producing plant stocks under IASMA's trademarked SMA® label. The chambers maintain sanitised lines used in meristem culture of progeny genotypes used in breeding programmes for Apple Proliferation resistance and for new rootstock genotypes.

# THE CENTRE FOR ALPINE ECOLOGY

*Annapaola Rizzoli*
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The Centre for Alpine Ecology was established in 1992 as a regional research institute of the Autonomous Province of Trento. Its aim is to promote and develop research, information and educational programmes on alpine ecosystems in order to encourage local socio-economic development in relation to the need for a sustainable management of the territory under the pressure of global change.

The Centre, located at Viote on Monte Bondone (1500 m a.s.l.), is housed in buildings erected at the beginning of the 1900s by the Austro-Hungarian army and recently restored by the Autonomous Province of Trento. It is surrounded by coniferous forests interspersed with well-maintained meadows and pastures which represent an ideal "open air laboratory" for environmental research.

The facilities include a residential building and two conference rooms which make the centre an ideal location for training courses, scientific conferences, seminars, workshops, and public meetings. There is also an interpretation area, which includes a nature trail, a trail dedicated to the legends of the Alps, three large aviaries housing endangered alpine birds, and a pond with an underwater viewing window. Exhibitions are also being designed to explain the history of the buildings hosting the Centre for Alpine Ecology.

Until the new administrative reorganisation, which brought it within the research centre of the Edmund Mach Foundation, the Centre for Alpine Ecology had its own Board of Directors, composed of representatives of the Administrative offices of the Autonomous Province of Trento, the City of Trento, universities, natural history museums and environmental associations. An international scientific committee was engaged in the evaluation of research outcomes and in planning research priorities.

The Centre had a few permanent staff, mostly engaged in administration and support activities, and a number of researchers employed on a contractual basis. The general management and organisation were entrusted to the Director in collaboration with the Scientific Co-ordinator and the Senior Accountant.

## Scientific research

During its 15 years of activity, the Centre has promoted a series of multidisciplinary research programmes on various issues encompassing the main themes of European mountain research. The research groups maintained within the Edmund Mach Foundation are currently those working on forest ecology, wildlife ecology and epidemiology, molecular ecology, conservation genetics and sustainable development.

Research into forest ecology is mainly focussed on vegetation-atmosphere interactions and on soil structure and functionality. In particular, energy and gas exchanges (C, N and water) between terrestrial ecosystems and the atmosphere are being analysed, and spatial biogeochemical models are being developed. Upscaling of the carbon budget, using forest inventory data and remote sensing images is carried out on different scales (Kyoto protocol). The Centre planned and carried out a

regional forest inventory and has collected field data for the National Inventory of Forest and Carbon Pools. It collaborates with the National Centre for the Study and Conservation of Forest Biodiversity of Verona-Bosco della Fontana on research projects dealing with remote sensing, LAI measurements and biodiversity. The group has taken part in different EU projects (IV, V and VI Frameworks) dealing with the effects of land and climate changes on carbon and nitrogen balance (ECOMONT, CARBOMONT, CARBOEUROPE-IP, NITROEUROPE-IP). The institute is also a partner in the national project for carbon balance (CARBOITALY).

Research into wildlife ecology and epidemiology is focussed in particular on alpine wildlife population demographics and behaviour, the ecology and epidemiology of emerging diseases and host parasite interactions in natural populations. The interdisciplinary research group includes veterinarians, biologists, molecular biologists, mathematicians and foresters. The group has carried out research on the behaviour of wildlife species, host-parasite dynamics and modelling in alpine species, the epidemiology of zoonotic diseases, and how the functional role of biodiversity affects the risk of the introduction and spread of new and emerging pathogens into wildlife populations and humans living in the Alps. The group is part of an international network of research in these fields. A highly successful outcome of this cooperation was the publication of the book "The Ecology of Wildlife Disease", Eds. Hudson P.J., Rizzoli A., Grenfell B.T., Heesterbeek H. and Dobson A.P., 2002, published by the Oxford University Press (http://www.oup.co.uk/isbn/0-19-850619-8). The group is also a core partner in the Integrated Project of the European Community "EDEN" (Emerging Diseases in a Changing European Environment: (http://www.eden-fp6project.net/).

The genetics group has set up several molecular ecology projects aimed at understanding which biotic and abiotic factors affect the current distribution of genetic variability and structure in natural populations, at both the population and community levels.

This group's interests range from inferences from evolutionary and demographic history to the study of the processes of adaptation. Being interested in the processes driving the current biodiversity crisis, they also run projects aimed at reconstructing phylogenetic and phylogeographic relationships at the molecular level in key taxa living in one of the world's most important biodiversity hotspots, the Eastern Arc of Tanzania.

Tackling the biodiversity crisis also requires evaluation of how genetic diversity has changed over time: to this end, we have recently set up a laboratory specifically devoted to the analyis of DNA retrieved from ancient specimens. In 2003 the group organised the international workshop 'Population Genetics for Animal Conservation'. The outcomes of the meeting were published by the Cambridge University Press.

The research Group "Human Ecology and Territorial Development" has long-standing experience going back to the creation of the Centre itself. The growth of the research team has led the CEA to focus attention on specific activities within multi-year projects, supported by the institutions and directives of the Autonomous Province of Trento, the Italian Government and the European Union. In particular, since 2003 the Institutional project "Patterns of sustainable development for the marginal communities of Trentino" has focussed on issues related to the application of socio-anthropological research to economic development, and has produced an archive of data and information related to the specific culture and socio-economics of people living in the Alps.

# Humans and the Environment: social-ecological systems

*Alessandro Gretter*

A further aspect of ecology to be considered is that known as "Human Ecology". It deals not only with the influence of humans on their environment but also the influence of the environment on human behaviour, and the adaptive strategies people adopt as they come to understand those influences better. Actively pursued since the 1920s, human ecology presents itself "as a study of the spatial and temporal relations of human beings as affected by the selective, distributive, and accommodative forces of the environment." (McKenzie, 1925). More recently, its aims have been encompassed by "social-ecological systems" (Berkes and Folke, 1998).


Conceptual diagram of the elements in a social-ecological system

Drawing on a holistic understanding of environmental and social systems, this approach fosters the development of practical solutions and influences new thinking in tackling the root causes of global issues. In the field of sustainable development, social-ecological resilience is a major research area and is of special interest for natural resource management. It can be defined as potentially absorbing disruption in the social-ecological system before it changes its structure and its key-variables, by changing the patterns and processes that control the behaviour of the system. In a broader sense, it can also be characterised as a support for continued stable development by incorporating change and disruption.

A crucial issue in this research area is that of adaptive capacity (Gunderson and Holling, 2002), which, in the general sense, is the design of social-ecological elements, such as institutions or management policies, and their potential for change and adaptation in response to the altered conditions and unpredictable effects of (co)evolutionary dynamics. Given that there is neither a linear path towards any social vision, nor the possibility of eliminating surprise and uncertainty, we must seek the elements and functions that maintain the adaptive capacity of social-ecological systems, which involves identification of critical thresholds and the nature of the connections between the different interacting sub-systems.

## Adaptive Governance in practice: Common Land in Trentino

In the Italian Alps[1] the role of land and goods of common property has been revitalised in recent years in line with the increasing importance of the environmental functions embedded within them. Of particular interest are the management processes adopted, which differ from the most efficient solutions typically embedded within public and private goods (Ostrom, 1990).

While the supply of timber is still important for communities living in the mountain areas of the

[1] Most of the Common Lands in Italy (estimated at 1.5 million hectares) are located in mountain areas, namely the Alps and Apennines.

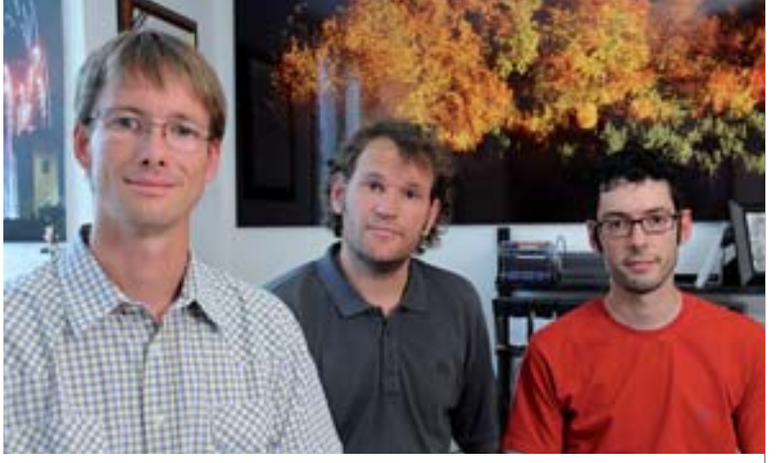
Southern Alps, from a strictly economic point of view other functions of multiple-use within the forest are also noteworthy. These derive not only from changes in the socio-economic scenario, but also from the growing influence of human activity on the ecosystems and an increased understanding of the role played by the forest itself. Biological and cultural diversity in the Alps has come about through adaptation and exploitation: in other words, traditional forest use has generated expansion of natural diversity (CIPRA, 2008). This diversity must, today, be considered as a sort of capital, from environmental, economic and social perspectives. It leads to social-ecological systems playing a very important role: biodiversity, in fact, has to be considered a part of the cultural context. Transformations occurring in the social arena have led to changes in the exploitation of biodiversity; the focus has recently been on recreation, resulting in many cases in a landscape intentionally created by man (UNESCO, 2005).

Almost 60% of the overall surface of Trentino is common property, with designated rights of "uso civico" (customary rights of use granted to member of community), and has been managed for over a hundred of participatory bodies. Most of these lands are located in mountain and upland areas covered by forest and grassland and have a high natural value. Ecological functions have been conserved on Common Land thanks to two favourable circumstances. Traditional extensive use has not made a large impact, resulting in a stable equilibrium or, at least, one guaranteed by human intervention. Secondly, the necessity for a large consensus (sometimes unanimous) within the community on rules of management has halted the introduction of wide-scale changes (with unpredictable consequences).

Changes in the socio-economic structure and the lesser importance of traditional production have weakened the role of common property management. This has led to a clear collapse of the centuries-old equilibrium and created the opportunity for high-impact intervention (tourist accommodation, winter sports infrastructures). In the first case, the return to a state of wilderness could cause a reduction in many of the non-market functions in the areas in question. For example, abandoning the traditional activities of grazing and forestry in the Alps creates a less attractive landscape, which is unfavourable to animal habitats and has a lower biodiversity and a higher risk of fire.

The greater importance of market-based functions and the presence of characteristics typical of mixed goods (partial rivalry and partial excludability, frequent in managing the Common Lands) necessitate a focus on the latter. New models and forms of organisation, able to safeguard the principles of Common Lands, rather than conserving the traditional system of management, could lead to effective solutions to new needs. Innovation on the organisational and managerial levels is thus necessary, although not easy to find and implement.

Since 1st October 2008, the research project "Public Policies and Local Development: innovation policy and its effects on locally embedded global dynamics" (OPENLOC) has been under way, its main objective being to spread new paradigms of development at the local level. FEM is involved in the work-package "Social and Environmental Capital: the possible contribution to local development in a global context". In collaboration with the Natural History Museum of Trento and the University of Trento, the aim of this project is to learn from the interrelationships within the territory. Research will focus on two areas in Trentino and on a benchmark territory in another European mountain region.



# Remote Sensing and Environmental Research

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The GIS (Geographic Information System) and Remote Sensing studies include scientific data elaboration and management of physical, ecological and geographical data originating from ground measurements as well as from aerial and satellite observations. We process massive amounts of data in order to extract ecological indicators, identify carbon fluxes and to create maps. Quantitative and qualitative analyses help to provide a better understanding of biological and ecological processes.

## Eco-Health Applications

GIS, remote sensing and meteorological data analyses are used to study the dynamics of certain emerging zoonotic diseases transmissible to humans. These diseases include tick-borne encephalitis (TBE) and Lyme borreliosis, mosquito-borne West Nile virus, and rodent-borne Hantaviruses (Arena virus and Cowpox). For a recently published study on forest structure and roe deer profusion in order to predict the TBE risk in Northern Italy, we analysed a long-term series of daily minimum, mean and maximum temperatures and total precipitation over the last 50 years. The climatic trends show that climate change is occurring uniformly on a much larger scale than the provincial. Hence, the best model for explaining the increase in TBE incidence in humans in this area includes changes in forest structure, in particular the ratio of coppice to high stand forest, and the density of roe deer.

Besides performing geostatistical data analyses, we are also co-developing our GIS software framework. We believe in the developmental approach of Open Source software, which is in line with academic research and scientific production as it involves peer-review of code style, functionality and quality. We are a

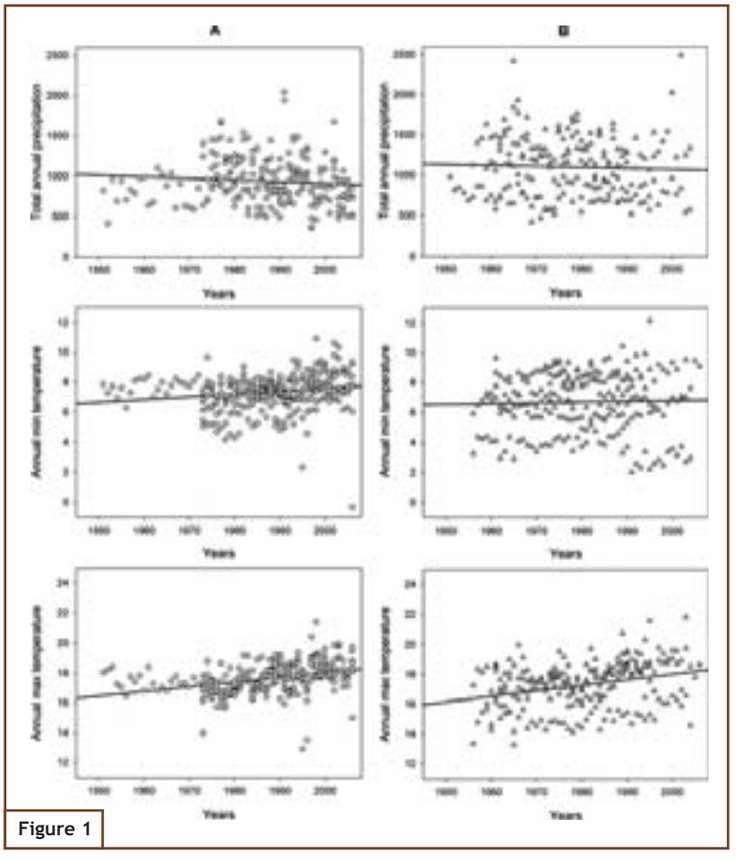


Figure 1

**Fig. 1** - Trends in climatic variables.
http://www.plosone.org

co-developer of the Open Source software GRASS GIS and contribute to further GIS libraries.

In order to provide better support for studies on disease vectors in regions with complex terrains, we use new remote sensing technologies to derive ecological indicators from satellite data time series with high temporal resolution. In particular, the Moderate Resolution Imaging Spectroradiometers (MODIS) aboard two US satellites deliver an almost complete Earth coverage four times a day. We process land surface temperature maps (LST), snow coverage, leaf area index (LAI), and vegetation indices (NDVI, EVI). For an ongoing study on the current and potential distribution of *Aedes albopictus* in Trentino, Northern Italy, we generated a set of temperature based ecological indicators from 11,000 reconstructed LST maps.

## Forest Applications

Light Detection and Ranging (LIDAR) sensors provide detailed information on the vertical structure of forests. In particular, the most recent sensors provide information not only on canopy height and on ground elevation, but also on the different layers of the forest, canopy density, canopy structure, etc. In this context, we have carried out several studies on two scale levels: plot and single-tree. Concerning the plot level, several biophysical parameters have been estimated, such as biomass volume, basal area, number of trees, etc. In addition, a study on 3D forest structure has been carried out on the basis of the vertical information contained in multiple return LIDAR data. Regarding the single-tree level, we have developed a system that identifies the individual trees through a segmentation process. It comprises a complex estimation phase, based on advanced machine learning techniques (e.g., Support Vector Regression), which estimate the diameter and stem volume of each tree.

An important step in the analysis of forested areas is identification of the tree species that characterise the environment being studied. This process is particularly complex when the analysed area is composed of natural forest with a mixture of many different tree species. In this situation, hyperspectral data from remote sensing platforms provide a huge amount of information thanks to their dense sampling of the spectral signatures. They allow the user to separate classes characterised by very similar spectral signatures. So far, three main investigations have been carried out on this topic. Firstly, the discriminatory abilities of these data have been tested in several study areas, char-


**Fig. 2** - Multiple returns Light Detection and Ranging (LIDAR) data of a coniferous forest
**Fig. 3** - A multispectral sensor used to monitor vegetation reflectance on a continuous basis

acterised by different morphologies, tree species compositions and altitude. Secondly, the integration of hyperspectral and LiDAR data has been studied in order to understand whether a combination of the two may be useful. Finally, an analysis of the relationship between spectral resolution and classifier complexity has been carried out.

**Fig. 4** - Spectroradiometric measurements campaign to obtain high spectral resolution vegetation reflectance data

## Carbon flux studies

Linking eddy-covariance with spectral ecosystem-scale measurements can provide a better understanding of the ecological mechanisms controlling carbon fluxes. On the large scale, satellite and aircraft sensors allow spatial patterns to be described with regional or global coverage. Our work focuses on investigating the relationships between NEE-GPP and vegetation indices calculated from remote sensing platforms, using the bands available on MODIS and CHRIS-Proba sensors.

The optical sampling approach is based on ground spectroradiometric measurements in order to avoid spatial resolution limitations (typical of satellite platforms). Biophysical parameters (such as LAI, plant water content, nitrogen content, biomass, etc) are measured with scale-appropriate direct methods in order to compare consistent optical and biophysical data. With optical sampling, high-spectral resolution spectroradiometer data (acquired episodically) or simpler multispectral sensor data (acquired on a continuous basis) were used, in order to investigate the relationships between spectral data and ecosystem parameters/fluxes. A SPECNET Europe meeting on this topic was held on Monte Bondone to develop a European network of optical sampling sites.

Starting with ground-truth measurements, these parameters can be upscaled to the airborne and satellite platforms to obtain detailed maps. Upscaling has been used successfully both for grasslands and for forests. In grasslands, significant relationships were found between airborne optical data (AISA Eagle hyperspectral sensor) and the vegetation biophysical parameters. Optical data showed their potential in monitoring grassland productivity, curing ratio and fire risk. In forests, significant relationships were found between LANDSAT satellite optical data and biomass.

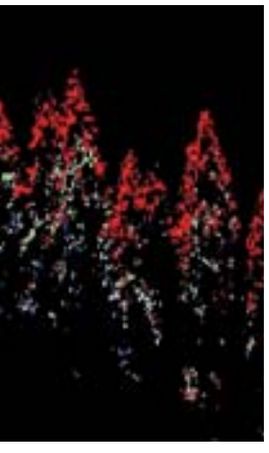

Figure 3

# Global changes and the risk posed by emerging wildlife diseases to human health in the Alps

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In addition to affecting ecosystem function and the rate of biodiversity loss across the European Union (EU), the combination of changes in climate, land use and land management may have serious consequences for human health that are difficult to recognise and predict. This is especially the case for diseases that usually circulate between wild animals and their parasites, but are occasionally transmitted to humans (known as "emerging wildlife zoonotic diseases").

The emergence of these diseases is not uncommon in the Alpine areas; for example, the spread of rabies by foxes is just one dramatic historical example. However, many of the microorganisms responsible for other diseases are insufficiently studied or even unidentified, and most of these are not easily controlled by depopulation or vaccination of wildlife species, as was possible for rabies.

This is the case for diseases transmitted by ticks or mosquitoes and/or maintained by wild rodent populations. Because this issue is of critical importance at both a European and Regional level, during the last 10 years we have been investigating several zoonoses with funds provided by the Autonomous Province of Trento and the European Union.

All of our research is multi-disciplinary, combining traditional wildlife and ecological research with the most advanced technologies, such as molecular ecology and genomics, GIS and Remote Sensing applications, and mathematical modelling.

The first two diseases we are studying, Tick Borne Encephalitis (TBE) and Lyme disease (or Lyme borreliosis, LB), are transmitted by the wood tick *Ixodes ricinus*. TBE is caused by a virus that induces flu-like symptoms, sometimes followed by meningoencephalitis and/or myelitis. Up to one third of patients have long-lasting symptoms, frequently accompanied by cognitive dysfunction and a substantial reduction in quality of life; consequently, the most severely affected TBE cases have high healthcare and social costs.

The TBE virus found in Europe is most often transmitted to humans by ticks that have acquired the infection while feeding on forest-dwelling rodents, especially the yellow-necked mouse (*Apodemus flavicollis*), which is widespread throughout the continent. In the EU, the number of cases of TBE has risen by an average of 400% over the last 30 years, and in Italy the disease has now been recorded in all the north eastern Regions of the peninsula (Trentino Alto Adige, Veneto, Friuli Venezia Giulia).

A total of 198 cases have been recorded from 1992 to 2006 with the annual number of cases increasing each year. The disease is preventable by means of vaccination, but so far only Austria has successfully initiated a vaccination programme.

Instead, LB is caused by the spirochete *Borrelia burgdorferi*. The clinical presentation of LB ranges from asymptomatic infection to serious

chronic illness, usually affecting the skin, nervous and musculoskeletal systems and, rarely, the heart. However, very few deaths associated with LB have been reported in the medical literature. Differences in clinical manifestations of LB in different individuals can be attributed to the different types of *B. burgdorferi,* called genospecies, causing LB. In the EU, many different genospecies circulate between ticks and vertebrate hosts, including many species of small mammals, birds and reptiles, attesting to the complexity of the ecology of this microorganism and the variety of ecological niches it can occupy.

At least 85,000 new cases of LB are officially reported each year across most member states of the EU, with an annual incidence as high as 135-200 cases/100,000 in parts of central Europe. In Italy the disease is endemic in most of the central-northern region, with an average of about 20 cases every 100,000 inhabitants per year, but physicians agree that this is almost certainly underestimated. No vaccine exists for LB, but it can be treated successfully with an antibiotic therapy if diagnosed at an early stage.

Recent studies, including our own, have shown that the number of ticks is increasing in many parts of the EU as a result of changes in climate, forest composition and abundance of wild ungulate species, especially roe deer and red deer. The abandonment of pastures and marginal areas has favoured not only the creation of suitable habitat for ticks, but also a greater risk of contact between ticks, their hosts and humans, increasing the circulation of certain tick borne diseases.

Other studies have also shown that the strains of TBE virus and LB found in Trentino are both endemic and introduced.

Genetic analysis has shown that local genospecies of *Borrelia* and TBE virus are distinct from other EU strains, while others appear to be closely related to those isolated in different parts of the EU. Our results also indicate that the circulation of endemic strains is enhanced by the environmental changes mentioned above, but also that new strains are continuously being introduced, probably by means of birds or terrestrial mammal migration. Our results clearly indicate the importance of maintaining our research efforts in order to guarantee early detection of these pathogens and to constantly monitor the evolution of their potential to cause serious diseases in humans. Our results are fundamental to physicians who must be adequately prepared to recognise and treat diseases transmitted to human from wild animals.

More recently, we have begun to monitor diseases transmitted to humans by migratory birds and mosquitoes (both common mosquito species and the tiger mosquito, which is spreading into the Alps), and diseases maintained by wild rodents living in forests and agricultural areas. Our results are crucial to the welfare of the people of Trentino, and provide constant improvements to our knowledge of the ecology of wildlife and emerging wildlife diseases. However, our research has also been recognised at an international level by the scientific community, as shown by their reactions to our presentations at conferences and publications in books and prestigious scientific journals.

Our research group also actively participates in networks involving many EU countries and several North American Universities.

# GHG fluxes in forest ecosystems

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Water and carbon cycles are the two most important biogeochemical cycles on the planet. Both cycles are extremely vulnerable to human disturbance and can induce changes in the climate. In the thousand years up to the beginning of the industrial age, carbon fluxes between the atmosphere, hydrosphere and geosphere were in a state of equilibrium. It is estimated that at the beginning of the 1800s, at the dawn of the industrial revolution, the atmospheric concentration of $CO_2$ was 290 parts per million (ppm), i.e. 0.029%. In 1958, when the first analytical measurements were made, $CO_2$ concentration was between 310 and 320 ppm. Although photosynthesis in aquatic and terrestrial ecosystems and carbonate dissolution in the sea tend to stabilise the atmospheric concentration of carbon dioxide, the enormous increase in the use of fossil fuels is causing an increase in concentration of around 1.5-2 ppm per year. High carbon dioxide emissions also derive from the intensification of agricultural practices (ploughing, mineral fertilization), which has led to a progressive depletion of carbon in agricultural soils and a change in the biogeochemical cycles of other elements such as nitrogen. At the same time, deforestation of large areas of the Earth has caused the release of the carbon contained in the wood and the rapid oxidation of organic soil.

Forests are one of the major terrestrial sinks of carbon. Carbon in forest ecosystems is stored in several components: leaves, branches, roots, litter, soil, etc. Distribution between these different pools is driven by carbon fluxes between the atmosphere, the various components of the biosphere and the soil (Schimel, *Global Change Biol.*, 1(1995): 77-91). Study of the carbon balance of an ecosystem requires accurate quantification of its components. However, this raises substantial difficulties due to the number and complexity of the processes involved and is carried out according to different methodologies depending on the spatial and temporal scales of the investigation. At the global level, carbon fluxes originating from different continents are quantified by atmospheric inversion models from data on $CO_2$ concentrations in the troposphere (Ciais *et al.*, *Science* 269(1995):1098-1102; Fan *et al.*, *Science*, 282(1998): 442-446), or are estimated on the basis of vegetational models parameterised with remote sensing data (Running *et al.*, *Remote Sens. Environ.*, 70(1999): 108-127). At a regional level, carbon fluxes are estimated either by assessing the carbon balance of the boundary layer (Denmead *et al.*, *Global Change Biol.*, 2(1996): 255-264) or through direct measurements of the turbulent fluxes by aerial platforms (Crawford *et al.*, *Global Change Biol.* 2(1996): 275-285). Information on a regional scale may also

be obtained from comparison over time of carbon sink inventories (Kauppi *et al.*, *Science* 256(1992): 70-74; Gower *et al.*, *Remote Sens. Environ.* 70(1999): 29-51).

On an ecosystem scale, the eddy covariance methodology is widely used for aerodynamic flow measurements of carbon and energy. The eddy covariance system directly measures the net ecosystem exchange of carbon, while most of the other techniques calculate the flow indirectly from measures of other variables. The eddy covariance system allows for continuous non-invasive measurements and is based on relatively simple tools (an ultrasonic anemometer, a $CO_2$ analyser, sensors for temperature and solar radiation).

Eddy covariance measurements have been carried out for several years in the Trentino region in three different ecosystems: an alpine meadow (Viote del Monte Bondone), a vineyard (Mezzolombardo) and a mixed forest with white spruce dominance (Lavarone). The three micrometeorological stations are included in the international FLUXNET network, which comprises about 500 stations in different plant ecosystems in every continent. Important information relating to carbon sequestration by the ecosystems investigated is gathered from analyses of the data collected.

In the alpine meadow, photosynthetic activity is concentrated in the spring-summer period from mid-April to mid-August (Fig. 1; by convention, negative values indicate absorption of $CO_2$, while positive values indicate emission, i.e., respiration). In autumn and winter, respiration processes prevail in this ecosystem because the plants are in a state of vegetative rest.

The dynamics of carbon absorption are very different in the forest ecosystem where photosynthetic activity continues virtually throughout the year except for a brief period during the winter (Fig. 2). In these climatic conditions conifers are able to absorb $CO_2$ even during the winter when the temperatures rise during the hottest hours of the day.

The vineyard site represents an intermediate situation between the two other ecosystems. Vegetative activity begins much earlier than in the alpine meadow and continues until leaf senescence in the autumn (Fig. 3).

The first draft analyses carried out in the three ecosystems showed that the forest site absorbs, on average, 8.5 tonnes of carbon per hectare per year, the vineyard absorption rate is about 1.55 t C / ha, while the alpine meadow about 0.8 t C / ha. This study therefore emphasises the importance of forests as atmospheric $CO_2$ sinks.

**Fig. 1** - $CO_2$ flux in the Bondone grassland
**Fig. 2** - $CO_2$ flux in the Lavarone forest
**Fig. 3** - $CO_2$ flux in the Mezzolombardo Vineyard

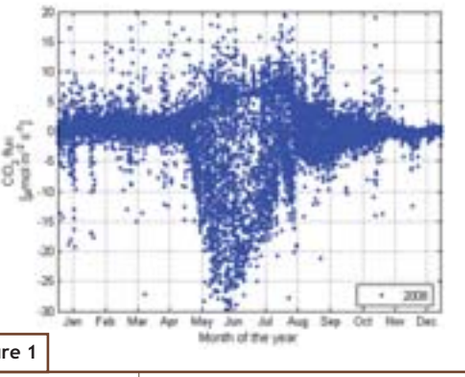

Figure 1

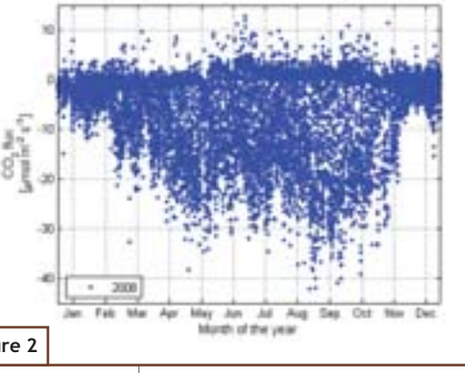

Figure 2

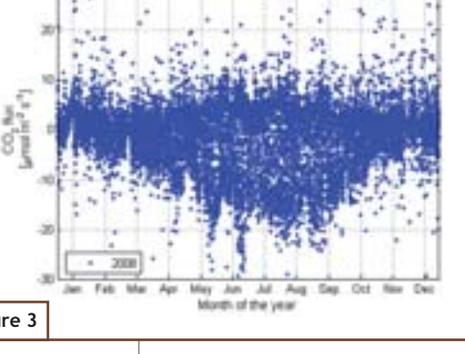

Figure 3

# Conservation Genetics

*Cristiano Vernesi*
*Elena Pecchioli*

## Introduction

This discipline aims at applying genetic analyses for preserving species as dynamic entities so that they can effectively cope with environmental change. Resolution of taxonomic uncertainties, genetic management of small populations, definition of significant evolutionary units within species, understanding the biology of species and the use of molecular tools to solve forensic cases are typical issues addressed by conservation genetics (Frankham *et al.*, 2002).

As part of the Alpine environment, Trentino is particularly exposed to the multifaceted risks posed by climate and land use change such as habitat fragmentation and degradation and increases in anthropogenic pressure. Assessing levels of genetic diversity and differentiation leads to an understanding of how evolutionary trajectories of natural populations are affected by these factors.

When applied to a restricted geographical area, conservation genetics approaches are of the utmost importance for providing information, in conjunction with that from other 'classical' disciplines, such as ecology and ethology, which can be useful for implementing more informed management and conservation policies.

Conservation genetics research at the former Centre for Alpine Ecology, now incorporated into the Research and Innovation Centre, has been mainly focussed on wild mammals species. Quite recently we also included birds, amphibians and reptiles, such as black grouse, common frog and viviparous lizard. Furthermore, in collaboration with researchers from the Trento Museum of Natural History we are also engaged in genetic analyses of *taxa* from one of the world's most important biodiversity hotspots, the Udzwunga Mountains of the Eastern Arc, Tanzania, Africa.

Thanks to an exclusively dedicated and fully equipped laboratory, we also analyse DNA extracted from ancient biological samples such as bones, teeth, skins, seeds, etc., which makes it possible to evaluate whether and how genetic diversity and differentiation has changed over time.

## Results

The completion of a multilocus project has allowed us to infer a peculiar phylogeographical pattern in Trentino shared by five mammal species, namely roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), chamois (*Rupicapra rupicapra*), mountain hare (*Lepus timidus*) and brown hare (*Lepus europaeus*). Populations located east of the Adige Valley are genetically different from those on the western side of the valley. The degree of genetic differentiation ranges from moderate to strong, according to the kind of marker analysed. At any rate, considering the short distances between the different sampling sites (usually less than 100 km), the pattern of geographic structure found is rather astonishing and unexpected. It is likely that biogeographical events related to the last glaciation (which ended around 15,000 years ago in Trentino) gave rise to the observed genetic diversification between the eastern and western sides of the Adige Valley. Furthermore, this valley nowadays acts as a major geographic barrier due to the presence of several human settlements, a railway, a motorway and other infrastructures, which make crossing it very difficult for most of the species, thus reinforcing the ancient genetic differentiation.

A far as genetic diversity within populations is concerned, we recorded a substantially good situation across all five species.

In our opinion, the results of this

research have a relevance that can be immediately transferred beyond the scientific field. We can no longer treat the different populations as though they were just single homogeneous entities. The two groups of populations identified in the five species studied should, at least, be considered as two distinct evolutionary units, deserving adequate recognition in future management interventions. Aside from the more general results, this study supports specific findings such as those related to chamois and mountain hare. In the chamois population of Caldonazzo we recorded a surprisingly high level of genetic diversity in mitochondrial DNA (mtDNA), a uni-parentally female transmitted marker. After broadening the genetic analyses to other *taxa* of the *genus*, it emerged that some of the Caldonazzo mtDNA alleles can indeed be assigned to the species *Rupicapra pyrenaica*, that is, the chamois species from the Pyrenees. We argue that natural dispersal of individuals from the Pyrenees to the Alps is highly unlikely. For the time being, human-mediated movement of animals between the two areas appears the most parsimonious explanation. While it is common to use high genetic diversity as an indication of population health, our results clearly call for detailed consideration of the phylogeographic aspects of this diversity as well.

The concomitant molecular analyses on mountain and brown hares allowed us to detect the first-ever documented events of hybridisation between these two species in the Alps. This phenomenon can have serious consequences for the survival of mountain hare populations. In fact, the literature shows that interspecific competition might negatively affect mountain hare. This hybridisation could be one of the side effects of climate change: due to the increase in temperatures and the decrease in precipitation (especially during the winter) brown hare now has the opportunity to colonise areas at higher altitudes, so far occupied only by mountain hare. The two species can, therefore, come into contact much more frequently than in the past. This result also calls for more informed management policies.

Our desire to promote conservation genetics as an effective tool for biodiversity conservation led us to organise an international workshop in which researchers from 18 different countries agreed to create a European consortium. This group will set up shared procedures, protocols and guidelines for making genetic analyses more easily understandable and accessible to all stakeholders in conservation and management policies.

Our ancient DNA laboratory was involved in collaborative research on mitochondrial sequence variation in specimens belonging to the first *Homo sapiens* to inhabit Europe, the Cro-Magnons. The results confirmed the sharp genetic discontinuity between these and late Neanderthals, excluding, at the same time, any confounding effect of modern DNA contamination, thus corroborating the hypothesis of taxonomic separation between *H. sapiens* and Neanderthal.

# In brief

## Animals from a new perspective: the use of GPS telemetry in animal ecology

*Francesca Cagnacci, Annapaola Rizzoli*

Knowledge of animal position in space and time obviously represents baseline information for studying the principal determinants of animal ecology and behaviour, including intra- and inter-specific relationships, the interaction between individuals and their habitat and their susceptibility to alterations in it. Recent technological advances have made continuous time-series of location data available, such those derived from Global Positioning System (GPS). This is a new area of research that heralds a revolution in behavioural ecology studies with improved opportunities for observing animals from their own perspective. In recent years, we have used this methodology to study roe deer, an ideal reference species for monitoring the effects of climate and habitat changes on large species and cascade consequences on ecosystems. In September 2008, we organised the workshop "GPS-Telemetry data: challenges and opportunities for behavioural ecology studies", an international gathering of eminent scientists aimed at defining the state-of-the-art of the field.

The male roe deer individual "M9" in Aldeno, marked with a GPS collar and an ear tag (photo: S. Gottardi)

## The Risktiger project: risk assessment of the emergence of arbovirus diseases transmitted by the tiger mosquito *Aedes albopictus* (Diptera: Culicidae) in the Autonomous Province of Trento

*David Roiz*

The invasive tiger mosquito *Ae. albopictus* is a vector of several viruses, such as dengue, yellow fever, West Nile, Chikungunya and other types of encephalitis. Native to Asia, it is a paradigm of the spreading of species worldwide caused by global climate change. It has spread to all continents in shipments of used tyres, arriving in Padua in 1991 and detected in almost all the regions of Italy. *Ae. albopictus* was involved in an outbreak of Chikungunya virus in 2007 in Emilia-Romagna, affecting 300 people. Optimal areas for its establishment and its current distribution in Trentino, assessed with GIS/GRASS methodologies in ovitraps, detected a presence in the northern Sarca and Adige Valleys, including the city of Trento. Seasonal dynamics and the influence of climate (temperature, rainfall) on the abundance and activity of females were assessed with BG-traps in the Upper Garda (Arco, Riva). Screening of arboviruses was performed and did not detect viruses of interest to human health. However, a novel insect flavivirus was detected and characterized.

## In brief

### A forest inventory of provincial and national carbon

*Sergio Tonolli and Lorenzo Frizzera*

With the signing of the Kyoto Protocol (2007), the importance of the ability of forests to reduce carbon in the atmosphere by storing it in biomass and soil was officially recognised. Application of the protocol requires the development of new knowledge to contribute to the understanding of the processes involved in the carbon cycle and to quantify the role played by natural ecosystems in that cycle. To assess the amount of carbon stored in forestal ecosystems, the approach chosen has been to create an inventory based on test areas, in which the different "pools" involved in the carbon cycle are measured. The research led to the creation of the Forest Carbon Inventory in the territory of the Autonomous Province of Trento, the methods serving as a model for the *National Inventory of Forests and Carbon Sinks* (www.infc.it), promoted by MIPAAF and coordinated by the CRA. Phase 3 + is currently being carried out, in which the acquisition of data will be completed and analyses of all forestry sinks as demanded by the Kyoto Protocol will be performed.

### The global flux tower network

*Roberto Zampedri e Mauro Cavagna*

As one of the projects begun after the Kyoto international agreement (1997), the micrometeorological towers set up in the Province of Trento have the aim of studying the role of plant ecosystems in the carbon-nitrogen cycle, and of quantifying the exchanges of carbon dioxide, water vapour and energy between the biosphere and the atmosphere using the eddy covariance technique. Site vegetation, soil, hydrological and meteorological characteristics are also analysed. The ecosystems involved are: a mixed forest in Lavarone, an alpine grassland on Monte Bondone and a vineyard in the Piana Rotaliana. The three sites are part of the Fluxnet global network (http://www.fluxnet.ornl.gov/) encompassing more than 540 stations operating on a long-term and continuous basis distributed over five continents in the most widespread ecosystems. On a continental scale, the sites participate in two European Projects, CarboEurope IP and NitroEurope IP, and in a national project, CarboItaly.

# Facilities and Equipment

## Ecophysiology laboratory

The laboratory is equipped with state-of-the-art instruments for measuring the radiative field of intercepted radiation (Li-cor LAI 2000, SunScan ceptometer (Delta-T Devices) and digital equipment for hemispherical pictures), and of incident radiation (quantum sensors, global and net radiometers, quantum sensors of direct/diffuse radiation). Equipment also includes portable spectroradiometers (Analytical Spectral Devices) for retrieving optical properties at high spectral resolution (3nm), as well as two portable LiCOR 6400s for gas exchange, fluorescence and soil respiration. The laboratory has a CHN analyser, atomic absorption and UV-Visible spectrophotometers and a Kjeldhal elementary analyser for biochemical analyses.

### Structures and equipment

| | |
|---|---|
| SpectroRadiometer ASD, FieldSpec HandHeld | 400-1100 nm |
| SpectroRadiometer ASD, FieldSpec Pro | 350-2500 nm |

Integration sphere LiCOR 1800 plus lamp
Gap Light Analyser LiCOR 2000
Portable gas analyser LiCOR 6400, fluorimeter and soil respiration system
Ceptometric Bar SunScan, Delta-T Devices
Digital Reflex Nikon D1 with Sigma 8mm fish-eye lens
Elementary Analyser Perkin Elmer series II CHNS/O Analyzer 2400
Atomic Absorption Spectrophotometer
UV-Visible Spectrophotometer

## Ecopathology Laboratory

The ecopathology lab is divided into 3 different rooms: an RNA extraction room, a room for manipulating biological samples (blood, tissues, organs and faeces) and a microscopy room.

The RNA extraction room has UV lights for sterilising the bench and floors. In this area RNA extractions from several samples are carried out in a biological safety cabinet (BSL-2) (Top Safe). This ventilated device protects the user from direct contact with potentially infectious samples (e.g. positive single-strand RNA viruses). Other safety devices and instruments used in the RNA extraction room include a chemical fume hood (Heron), a refrigerated centrifuge (Sigma), a Tissue Lyser (Qiagen), an autoclave and a dry stove.

In the second room (*dirty area*), which is physically separated from the RNA room, biological samples isolated from animals are manipulated in a biological safety cabinet (BSL-2) (Steril VBH). An optical microscope (Leica) connected to a CCD camera is used to examine tissues or parasites directly on a computer screen (Sony).

The microscopy room has an optical microscope, a stereomicroscope and a refrigerator for the conservation of biological samples. The ecopathology lab is also supplied with two freezers (- 20 C and - 80C) for the storage of biological samples.

## Genetics laboratory

The genetics lab is dedicated to conservation genetics research activities mainly through determination of nucleotide sequences of informative DNA stretches and sizing of microsatellite alleles. Various collaborations have also given us the opportunity to work on determining DNA sequences of

new species, on analysing plants and soil samples, and, sporadically, on forensic cases, on behalf of the Corpo Forestale dello Stato (State Forestry Commission). DNA is usually extracted from small tissue fragments, although we sometimes also use hair, blood, leaves and, much more rarely, faeces.

### Structures and equipment

The first step is DNA extraction, performed on a dedicated bench, which is always clean and prepared for use and is equipped with a microcentrifuge and a temperature-controlled heater.

When protocols with phenol and chloroform are to be used, DNA extraction is performed under a chemical hood.

The next step is setting up the PCR: this is done at a distance from the extraction bench and under a laminar flow hood where all reagents for DNA amplification are mixed. Amplification: close to the PCR hood there are three thermal cyclers, a real-time PCR machine and a DNA bioanalyzer, each connected to a dedicated PC. The last part of the lab is used for analysing amplified DNA. This post-PCR room is well separated from the pre-PCR space, as amplified DNA is highly concentrated and mobile and represents a major source of contamination. Located in this room are two automated sequencers for DNA sequence determination and microsatellite sizing. There are also power supplies and supports for horizontal agarose gel electrophoresis.

In addition, the lab has a biological hood and temperature-controlled agitating heating systems for cloning reactions. Reagents are stocked in freezers and refrigerators while flammable, acid and basic substances are preserved in their respective dedicated cabinets, in accordance with safety regulations.

## Ancient DNA (aDNA)

The FEM buildings at Viote del Monte Bondone have a laboratory exclusively dedicated to ancient DNA research, the only one in the region.

DNA is mainly extracted from bones, teeth and skin, up to thousands of years old; these highly degraded tissues are particularly sensitive to contamination, so DNA extraction can be successfully performed only in specially designed labs.

### Structures and equipments

The lab has been installed in a building exclusively used for this kind of activity and is separated by several tens of metres from the building where research activities on modern DNA are carried out.

A range of precautions regarding cleaning and the researchers' clothing, make this lab a highly sterile environment. It is subdivided into three consecutively arranged rooms separated by doors. In the first room there is a small dressing room, a freezer for storing samples, a liquid nitrogen container and a cabinet for stocking reagents.

In the second room all the solutions used for DNA extraction are set up and the cleaning of samples is carried out. In the case of bones and teeth, a tiny layer of their outer surface is removed by abrasion in order to avoid potential extraction of DNA from the persons manipulating the samples. All these operations are carried out under a chemical hood. Particular attention to efficiency and cleanliness is given to the equipment used for grinding specimens, that is, the 'Freezer Mill', a magnetic liquid nitrogen grinder.

In the third and last room there is a laminar flow hood where the PCR reaction is set up. This room must conform to the highest standards of sterility as it has been demonstrated that modern DNA contamination occurs mainly during PCR. For this reason also, this room has been located at the end of the path to prevent through traffic and to limit the number of operators entering it exclusively to those performing PCR. Overall sterility in the extraction and PCR rooms is guaranteed by UV light (254 nm) irradiation.

# Services and Support to Stakeholders

Until December 2008 our laboratories continued to carry out both basic and highly specialised analytical services. The purpose of these services, developed directly by the research staff, is to support, supervise and promote the quality of products and processes. Particular attention is given to contextualising the data analysed, with the aim of supplying integrated information which is not limited to just the analytical results. These services, therefore, allow a constant monitoring of issues of interest, which in turn provide the stimuli and intuitions for new research lines and projects. In fact the services offered cover a multitude of fields in the agrifoods sector and are available to both institutions and the private sector.

## Chemical, biochemical, and microbiological analyses

The Agrifood Quality department and its units carry out chemical, biochemical, and microbiological analyses. The most common analyses are in oenology (grapes, must, wine, grappa, and distillates). Other common matrices are fruit (especially apples and berries), leaves, and soil. The most relevant equipment for these studies are the high resolution gas chromatograph mass spectrometer (HRGC-MS), the isotopic mass spectrometer, the nuclear magnetic resonance (NMR), the Fourier transform infrared spectrometer (FT-IR), as well as several high performance liquid chromatograph (HPLC), a high performance liquid mass chromatograph spectrometer (HPLC-MS) and inductive coupled plasma (ICP-OES/MS).

## Characterisations of specific territories

The Agricultural Resources department handles studies aimed to the botanical and zoological characterisation of alpine pastures and the economic analysis of mountain farming, particularly for cost analysis of milk production.

## GMO detection

The Genetics and Molecular Biology department performs analysis based on the real-time PCR for detecting genetically modified organisms (GMO) in food and feed.

## Phytosanitary analyses

The Plant Protection department provides phytosanitary analyses including: plant-disease diagnostic services, sanitation of plant materials, and test facilities.

These analyses are possible thanks to laboratories and equipment for analysis of phytoplasm, virus, bacteria, arthropods, and fungus carried out in greenhouses or in the field.

The department also has the equipment for studying agropharmaceuticals in the laboratory, greenhouse or field, and greenhouse facilities and equipment for evaluating the efficacy of biocontrol agents and low environmental impact compounds.

## Accreditation

The Analysis and Research laboratory at San Michele all'Adige obtained SINAL accreditation in 1998.

The Accreditation scheme was put into place by the European Union in order to allow free circulation of goods and produce throughout the union's territory without the necessity of repeating controls in individual countries.

The body responsible for national accreditation is SINAL (Sistema nazionale per l'accreditamento dei laboratory - National system for the accreditation of laboratories), which was formed in April 1988. It ensures that Laboratories conform to UNI CEI EN ISO/IEC regulation 17025 "General Requisites for the competence of testing and calibration laboratories"and to SINAL's own regulations, and carries out periodic controls. Clients are therefore guaranteed that the laboratories carry out accredited tests competently and impartially.

The FEM Experimental Centre's List of Tests, dated 18th June 2008, provides official recognition of 42 accredited tests in three departments (40 in the Department for Agrifood Quality; 1 in the Molecular Biology and Genetics Department; 1 in the Department for the Improvement of Productive Resources). Of these 42 test methods, 27 are official and 15 internal, i.e. developed by the laboratory itself. The SINAL inspection team of the third cycle of accreditation currently operational includes a chemist, Dr E. Cucchiarelli, a biologist, Dr F. Zuccon and a system administrator, Dr T. Miccoli. An up-to-date list of accredited laboratories and their respective accredited tests is available on the website www.sinal.it.

**In addition,** documentation is currently being prepared which will allow the laboratories of the Edmund Mach Foundation to be added to the MIUR List of highly specialised laboratories.

The director of FEM-IASMA's Quality System is Anita Dalla Serra, +39.0461.615346, anita.dallaserra@iasma.it.

# Research Projects

## Activities

### The Autonomous Province of Trento (PAT) supports the following core projects

| PROJECT | COORDINATOR | DEPARTMENT |
|---|---|---|
| Agrometeorology, climatology and aerobiology | Giambattista Toller<br>Nico Salmaso | Natural Resources |
| Alpine ecosystems in a Changing Environment: biodiversity Sensitivity and Adaptive Potential | Cristiano Vernesi<br>Annapaola Rizzoli | Centre for Alpine Ecology |
| Ampelography, management of germplasm bank and grapevine genetic improvement | Marco Stefanini | Agricultural Resources |
| Apple genome sequencing | Riccardo Velasco | Genetics and Molecular Biology |
| Clonal and sanitary selection of winegrape cultivars | Umberto Malossini<br>Marco Stefanini | Agricultural Resources |
| Communication distruption of vine pests | Claudio Ioriatti | Plant Protection |
| Constitution and valuation of new apple genotypes | Pierluigi Magnago<br>Marco Stefanini | Agricultural Resources |
| Cultural practices in integrated apple production: less chemistry and more mechanics | Alberto Dorigoni<br>Marco Stefanini | Agricultural Resources |
| Epigenetics and natural variation | Claudio Varotto<br>Nico Salmaso | Natural Resources |
| Food processing and technology | Agostino Cavazza<br>Fulvio Mattivi | Agri Food Quality |
| Food safety and traceability | Roberto Larcher<br>Fulvio Mattivi | Agri Food Quality |
| Genomics and bioinformatics of grape berry ripening | Claudio Moser<br>Riccardo Velasco | Genetics and Molecular Biology |
| Human ecology | Alessandro Gretter<br>Annapaola Rizzoli | Centre for Alpine Ecology |
| Integrated ecological investigations of aquatic ecosystems | Nico Salmaso | Natural Resources |
| Molecular breeding of grapevine | M. Stella Grando<br>Riccardo Velasco | Genetics and Molecular Biology |
| Multi-trophic interaction | Ilaria Pertot<br>Claudio Ioriatti | Plant Protection |
| New approches for pest control | Gino Angeli<br>Claudio Ioriatti | Plant Protection |
| Oenology | Giorgio Nicolini<br>Fulvio Mattivi | Agri Food Quality |
| Plant ecology, biometeorology e remote sensing | Damiano Gianelle<br>Annapaola Rizzoli | Centre for Alpine Ecology |
| Plant gene expression | Lucia Martinelli<br>Riccardo Velasco | Genetics and Molecular Biology |
| Post-harvesting, quality, and conservation problems of fruit and vegetable products | Livio Fadanelli<br>Marco Stefanini | Agricultural Resources |
| Public Policies and Local Development: innovation policy and its effects on locally embedded global dynamics | Alessandro Gretter<br>Annapaola Rizzoli | Centre for Alpine Ecology |
| Quality, health and nutrition | Flavia Gasperi<br>Fulvio Mattivi | Agri Food Quality |
| Set up of a new metabolomic platform | Fulvio Mattivi | Agri Food Quality |
| SMAP II: Apple proliferation | Wolfgang Jarausch<br>Claudio Ioriatti | Plant Protection |

| PROJECT | COORDINATOR | DEPARTMENT |
|---|---|---|
| Soft fruit | Lara Giongo<br>Marco Stefanini | Agricultural Resources |
| Soft fruits protection strategies | Claudio Ioriatti | Plant Protection |
| Study of the processes and technologies to exploit biomasses for renewable energy production and materials recovery | Silvia Silvestri<br>Nico Salmaso | Natural Resources |
| Study of Trento province forest ecosystems | Nicola La Porta<br>Nico Salmaso | Natural Resources |
| Survey of farm incomes in Trentino | Giorgio De Ros<br>Marco Stefanini | Agricultural Resources |
| Sustainable plant protection and global change | Ilaria Pertot<br>Claudio Ioriatti | Plant Protection |
| Translational plant genomics | Silvio Salvi<br>Riccardo Velasco | Genetics and Molecular Biology |
| Crop physiology and management: effects of exogenous factors and crop management on grapevine physiology (ripening, senescence, and nutrition) and evaluation of viticultural sites | Roberto Zorer<br>Marco Stefanini | Agricultural Resources |
| Wildlife ecology and biodiversity | Annapaola Rizzoli | Centre for Alpine Ecology |

## Other projects

### The following projects are supported by various sources

| PROJECT | FUNDING | COORDINATOR |
|---|---|---|
| ACCRETE<br>Agriculture and Climate Changes: how to Reduce Human Effects and Threats | European Union | Giambattista Toller |
| AVirVENT-STSM<br>Identification of avirulence/virulence genes of Venturia inaequalis | European Union | Ilaria Pertot |
| Carboeurope-ip<br>Assessment of the European Terrestrial Carbon Balance | European Union | Damiano Gianelle |
| COST 858<br>Viticulture: biotic and abiotic stress, grapevine defence mechanism and grape development | European Union | Stella Grando |
| COST 864 - Pome fruit health | European Union | Ilaria Pertot |
| ECDC Tender Call.<br>Assessing the magnitude of vector borne diseases in Europe | European Union, European Centre for Diseases Control | Annapaola Rizzoli |
| EDEN-IP<br>Emerging diseases in a changing European environment | European Union | Annapaola Rizzoli |
| ENDURE<br>European Network for the Durable Exploitation of Crop Protection Strategies | European Union | Ilaria Pertot |
| First Meeting of the EU ConGen Consortium: development of the CGToolBox and exploring the genomic approach to conservation genetics | European Union, European Science Foundation | Heidi Hauffe |
| GRAPEGEN06<br>Management and conservation of genetic resources | European Union | Stella Grando |
| NiTROEUROPE-ip<br>The Nitrogen cycle and its influence on the European greenhouse gas balance | European Union | Damiano Gianelle |

| PROJECT | FUNDING | COORDINATOR |
|---|---|---|
| TRACE<br>Tracing food commodities in Europe | European Union | Giorgio Nicolini |
| BLAIM<br>Immobilized lactic bacteria | Denmark, Danstar ferment | Agostino Cavazza |
| Kerry<br>R&D convention | Ireland, Kerry Bioscience | Giorgio Nicolini |
| SA wine data bank<br>Isotopes Database of southafrican wines and distilled drinks | South Africa, ARC Stellenbosch | Federica Camin |
| ABTM<br>Database of balsamic vinegars | Italy, Region Emilia Romagna | Fulvio Mattivi |
| ASSAGRARIA RIVA<br>Effect of terroir on the quality of Merlot wines | Italy, Associazione Agraria, Riva del Garda | Giorgio Nicolini |
| BIOMARKERS<br>New markers for tracing organic fruits | Italy, Ministry for Agriculture and Forestry Policies | Federica Camin |
| CarboITALY | Italy, FIRS, Ministry of Education, University and Research | Damiano Gianelle |
| CARITRO<br>Molecular modelling of polyphenols biosynthetic pathways and its application in gene discovery and gene modulation in living cells for pharmacogenomics purposes | Italy, CARITRO Foundation | Riccardo Velasco |
| CARPOL<br>Development of innovative methods for identifying and quantifying allergenic pollens | Italy, CARITRO Foundation | Elena Gottardini |
| Castellani<br>R&D convention | Italy, Castellani company | Roberto Larcher |
| CAVIT<br>Chemistry, technology and microbiology in winemaking | Italy, Cavit | Giorgio Nicolini |
| CAVIT<br>Maso Romani | Italy, Cavit | Umberto Malossini |
| COLLI di PARMA<br>Improvement of the quality of Malvasia & Sauvignon Blanc DOC Wines | Italy, Consorzio Colli di Parma | Giorgio Nicolini |
| CuDR<br>Plant extracts and low copper content products against Plasmopara viticola | Italy, Piedmont region | Ilaria Pertot |
| DEFENSINE<br>Defensins as mediators of the innate immune response: mechanism and applications for plant and human defense | Italy, CARITRO Foundation | Claudio Moser |
| Effects of pesticides on mites inhabiting orchards | Italy, Miur-PRIN | Gino Angeli |
| Ever<br>R&D convention | Italy, Ever | Agostino Cavazza |
| Food Quality<br>The role of red wine and its minor components in the prevention of chronic degenerative diseases | Italy, Ministry for Agricultural and forest policy | Fulvio Mattivi |
| GAME<br>Sustainable management of marble trout (S. t. marmoratus) in the Adige basin: genetic, phenotypical and ecological characterisation for conservation purposes | Italy, Authority of Basin of river Adige | Andrea Gandolfi |
| GARDA<br>Long term development of the physical, chemical and biological characteristics of Lake Garda | Italy, Agenzia regionale per la prevenzione e protezione ambientale del Veneto | Nico Salmaso |

| PROJECT | FUNDING | COORDINATOR |
|---|---|---|
| GOEMAR<br>Evaluation of weed leaf application on the quali-quantitative performance of grapevine | Italy, GOEMAR | Duilio Porro |
| GRANA PADANO<br>Isotope and elemental characterisation of Grana Padano cheese and relevant data bank | Italy, Consorzio Grana Padano | Federica Camin |
| ICQ-MIPAAF<br>Research and investigations to upgrade the national isotopic data bank of food and beverages | Italy, Ministry for Agricultural and forest policy | Federica Camin |
| ILSA<br>Evaluation of silicon products (Siliforce) and dehydrated healing herbs (EM23) leaf pplication on the quali-quantitative performance of grapevine | Italy, Ilsa Spa | Duilio Porro |
| INFC3+ Forest and Carbon pools National inventory<br>Soil, litter and necromass | Italy, Ministry of Food and Forestry, State Forestry | Damiano Gianelle<br>Mirco Rodeghiero |
| K+S Kali<br>Evaluation of the contribution of magnesium products against precocious Philloptosys on apple trees | Italy, K+S Kali | Duilio Porro |
| MESVIT<br>Basic and applied research for the control of grapevine esca disease | Italy, ARSIA Toscana | Ilaria Pertot |
| Mechanisms of host-plant location and host-plant preferences in two leafhoppers (Hemiptera: Auchenorrhyncha) vectors of grapevine yellow diseases | Italy, Miur-PRIN | Claudio Ioriatti |
| Parallelomics<br>Metabolomic and genomic high parallel profiling of typical agri-food products | Italy, Ministry for Research and Higher Education | Riccardo Velasco |
| PARMIGIANO REGGIANO<br>Traceability model of Parmigiano Reggiano cheese using isotopic and mineral analyisi | Italy, Parmigiano Reggiano, Emilia Romagna Region, Consorzio Grana Padano | Federica Camin |
| PARMIPS<br>Assessment of the ecological impact of the Ips typographus attacks | Italy, Regional Park of Cedra and Parma valleys | Cristina Salvadori |
| PICEA-ARMILLARIA<br>Study on genetic expression of the forest pathosystem Picea abies/Armillaria abies/Armillaria | Italy, CARITRO Foundation | Nicola La Porta |
| PLANADIGE<br>The river plankton as a tool to investigate the ecological quality and the management of waters of the river Adige | Italy, Authority of Basin of river Adige | Nico Salmaso |
| PROALPE<br>Investigation of alpine terroirs for the characterisation and protection of mountain dairy products | Italy, Ministry for Research and Higher Education, MIUR-FIRS | Luana Bontempo |
| ProPlan<br>Growth cycle of soft fruit and strawberry: maturation curves and production forecasts. | Italy, Sant'Orsola sca | Lara Giongo |
| Quargentan -<br>R&D convention | Italy, Quargentan | Roberto Larcher |
| RAME<br>Studies to comply with limits on copper residue with low-dosage formulations or alternatives | Italy, Ministry of agriculture and forestry | Ilaria Pertot |
| Ruffino<br>R&D convention | Italy, Ruffino | Giorgio Nicolini |

| PROJECT | FUNDING | COORDINATOR |
|---|---|---|
| SicilBerry<br>Project for the improvement of the quality of berries and development of their nutritional and antioxidant potential | Italy, Sicily Region | Lara Giongo |
| SICILIA_GEN<br>Gene-based traceability of the grape production chain in Sicily | Italy, Siciliy Region | Stella Grando |
| Syngenta<br>Integrated control of the major phytophaga and pathogens of apple and grapevine | Italy, Syngenta | Gino Angeli |
| Tuscania<br>Integrated experimentation in viticulture through different ways of managing the canopy | Italy, Cavit | Duilio Porro<br>Roberto Zorer |
| TUTZER<br>Evaluation of plants obtained from seeds or harvested in various wine growing areas at international level | Italy, Tutzer Vivai | Marco Stefanini |
| Università di Piacenza<br>R&D convention | Italy, University of Piacenza | Agostino Cavazza |
| VITIS<br>Development and application of analytical methods in support of clonal selection | Italy, Vitis Rauscedo Scarl | Fulvio Mattivi |
| AMPELO<br>Opening new frontiers in plant protection by understanding the molecular language between a plant pathogen and its mycoparasite | Autonomous Province of Trento | Monika Maurhofer |
| ArAquae<br>Study of Irrigation needs of crops in Trentino | Autonomous Province of Trento | Giambattista Toller |
| CANZOLINO<br>Limnological research for restoration of Lake Canzolino | Autonomous Province of Trento | Monica Tolotti |
| CERCA<br>Effects of global change on Trentino lakes: temperature and UV radiation impacts on plankton, environmental trends, and dissemination | Autonomous Province of Trento | Giovanna Flaim |
| ECOCYPRE<br>Ecological assessment and sustainable management of cypress in the landscape of Trentino | Autonomous Province of Trento | Nicola La Porta |
| EcoGenEtic.Com<br>Eco- friendly genes: from scientific research to risk management, ethical issues and communication | Autonomous Province of Trento | Lucia Martinelli |
| ECOPLAN<br>Ecology and biogreography of two planktonic taxa in small lakes of Trentino | Autonomous Province of Trento | Giovanna Flaim |
| ENVIROCHANGE<br>Global change and sustainable management of agriculture in highly developed mountain environment. | Autonomous Province of Trento | Ilaria Pertot |
| FLAVONOIDI<br>Study of the genetic determinants of the flavonoid content in the grape berry: integration of gene expression and metabolic data | Autonomous Province of Trento | Claudio Moser |
| GeReCa<br>Subtle characterisation of the Vitis genetic resources in the IASMA collection | Autonomous Province of Trento | Marco Stefanini |

| PROJECT | FUNDING | COORDINATOR |
|---|---|---|
| HOST<br>Study of host-plant volatile compounds (kairomones) for the control of the insect vectors of the grapevine yellows diseases | Autonomous Province of Trento | Gianfranco Anfora |
| INTERPLAY<br>Are phytophagous insects using volatile cues as an indicator for resource quality? | Autonomous Province of Trento | Marco Tasin |
| IRRI WEB<br>Development of telecontrol systems for irrigation, fertilization and climatization plants in Trentino | Autonomous Province of Trento | Giambattista Toller |
| METAQUALITY<br>Establishment and validation of a metabolomic platform towards the discovery of grape and apple quality determinants | Autonomous Province of Trento | Urska Vrhovsek |
| MIGLIORFILETTO<br>Integrated research on the improvement of Trentino commercial fishery production in terms of yield of the proceeded products of the species Oncorhyncus mykiss (Rainbow trout) | Autonomous Province of Trento | Fernando Lunelli |
| Monitoring of the MBT of solid waste before landfill disposal | Autonomous Province of Trento | Silvia Silvestri |
| O3-NTO<br>Ozone in Trentino: levels and effects on vegetation | Autonomous Province of Trento | Elena Gottardini |
| PARMA<br>Edible, aromatic and medicinal plants of the Alps: a resource to value | Autonomous Province of Trento | Matteo Komjanc |
| PECC<br>PostDoc project Genetic and molecular analyses of Norway spruce (Picea abies Karst.): adaptative variability and species evolution under global change conditions | Autonomous Province of Trento | Nicola La Porta |
| RESISTEVITE<br>Functional and molecular characterization of the induced systemic resistance activated in grapevine by beneficial microorganisms. | Autonomous Province of Trento | Michele Perrazzolli |
| Risk Assessment of the Emergence of New Arboviruses Diseases trasmitted by the Tiger Mosquito Aedes Albopictus (Diptera: Culicidae) in the Autonomous Province of Trento | Autonomous Province of Trento | David Roiz<br>Annapaola Rizzoli |
| SAMPLEVITIS<br>Molecular approaches for clonal characterization in grapevine | Autonomous Province of Trento | Claudio Moser |
| SyrTox<br>Mechanism of action of Pseudomonas spp. metabolites and their potentiality in biocontrol | Autonomous Province of Trento | Ilaria Pertot |
| TRENTINGRANA<br>Quality aspects of the Grana Trentino supply chain | Autonomous Province of Trento | Giorgio De Ros |

# Affiliations

| SOCIETY | WEBSITE | PERSON |
|---|---|---|
| American Phytopathological Society (APS) | http://www.apsnet.org | Nicola La Porta |
| American society for enology and viticulture | http://www.asev.org | Marco Stefanini |
| Associazione Italiana Ittiologi di Acqua Dolce (AIIAD) | http://www. aiiad.it | Francesca Ciutti<br>Andrea Gandolfi |
| Associazione teriologica italiana (A.T.It.) | http://biocenosi.dipbsf.uninsubria.it/atit/ | Francesca Cagnacci |
| Centro Italiano Studi di Biologia Ambientale (CISBA) | http://www.cisba.it | Francesca Ciutti<br>Silvia Silvestri |
| COST Action 858, member of management committee | http://www.bordeaux-aquitaine.inra.fr/cost858_eng | Stella Grando |
| European Society for Mathematical and Theoretical Biology | http://www.esmtb.org | Roberto Rosà |
| Fifth International Workshop on Grapewine Downy and Powdery Mildew, member of the scientific committee | http://www.safecrop.org | Ilaria Pertot |
| Polyphenol Group, member of steering committee | http://www.groupepolyphenols.com/ | Fulvio Mattivi |
| Integrate Pest Management Europe, Italian delegate and steering committee member | http://www.ipmeurope.org/ | Nicola La Porta |
| International Association for Plant Biotechnology (IAPB) | http://www.IAPB-STL.org (http://www.ibba.cnr.it/APTCCB) | Lucia Martinelli |
| International Grape Genome Program-IGGP, member of steering committee | http://www.vitaceae.org | Stella Grando<br>Riccardo Velasco |
| International Organisation for biological and integrated control of noxious animals and plants (IOBC) | http://www.iobc-wprs.org/wg_sg/index.html | Claudio Ioriatti |
| International Society for Diatom Research (ISDR) | http://www.isdr.org | Cristina Cappelletti |
| Italian Association for Oceanology and Limnology (AIOL), components of directive committee | http://www.aiol.info/home.htm | Giovanna Flaim<br>Monica Tolotti<br>Nico Salmaso |
| Italian Biotechnologists Association (ANBI) | http://www.biotecnologi.org/public/portale/html/index.php | Lorenza Dalla Costa |
| Italian Professional Order of Biologists, (Provincial Delegate) | http://www.onb.it/ | Lucia Martinelli |
| Italian Society for Agricultural Genetics (SIGA) | http://www.siga.unina.it | Stella Grando<br>Matteo Komjanc<br>Lucia Martinelli<br>Claudio Moser<br>Riccardo Velasco |
| Italian Society for Horticulture | http://www.soihs.it | Stella Grando<br>Marco Stefanini<br>Duilio Porro |
| Italian Society for Vegetal Physiology | http://www.sifv.it | Riccardo Velasco |
| Marie Curie Fellows Association | http://mcfa.eu/ | Nicola La Porta |
| Open Source Geospatial Foundation | http://www.osgeo.org/ | Markus Neteler |
| Rete dei demani civici | http://www.jus.unitn.it/usi_civici/esperti/home.html | Alessandro Gretter |
| Società Italiana di Biologia Evoluzionistica (SIBE) | http://www.sibe-iseb.it/ | Andrea Gandolfi |
| Società Italiana di Ecologia (SITE) | http://www.societaitalianaecologia.org/ | Nico Salmaso |

| SOCIETY | WEBSITE | PERSON |
|---|---|---|
| Società Italiana di Orticoltura | http://www.soihs.it | Anna Maria Ciccotti<br>Stella Grando<br>Umberto Malossini<br>Marco Stefanini<br>Duilio Porro<br>Luca Zulini |
| Società Italiana di Patologia Vegetale | http://www.agr.unipi.it/sipav/ | Giorgio Maresi |
| Società Italiana di Scienze Sensoriali, founder and scientific committee member | http://www.scienzesensoriali.it | Flavia Gasperi |
| Società Italiana di Selvicoltura e Ecologia Forestale (SISEF) | http://www.sisef.it | Nicola La Porta<br>Giorgio Maresi |
| Societas Internationalis Limnologiae (SIL) | http://www.limnology.org | Giovanna Flaim<br>Monica Tolotti<br>Nico Salmaso |
| SOIPA, Società Italiana di Parassitologia | http://www.soipa.it | Annapaola Rizzoli |
| Wildlife Diseases Association | http://www.wda.org<br>Duilio Porro | Annapaola Rizzoli<br><br>Zulini Luca |
| Società Italiana di Patologia Vegetale | http://www.agr.unipi.it/sipav/ | Giorgio Maresi |
| Società Italiana di Scienze Sensoriali, founder and scientific committee member | http://www.scienzesensoriali.it | Flavia Gasperi |
| Società Italiana di Selvicoltura e Ecologia Forestale (SISEF) | http://www.sisef.it | Nicola La Porta<br>Giorgio Maresi |
| Societas Internationalis Limnologiae (SIL) | http://www.limnology.org | Giovanna Flaim<br>Monica Tolotti<br>Nico Salmaso |
| SOIPA, Società Italiana di Parassitologia | http://www.soipa.it | Annapaola Rizzoli |
| Wildlife Diseases Association | http://www.wda.org | Annapaola Rizzoli |

# Editorial Board Participation

| JOURNAL | WEBSITE | PERSON |
|---|---|---|
| American Journal of Enology and Viticulture | http://www. ajevonline.org | Riccardo Velasco |
| ISHS Acta Horticulturae 810 | http://www.actahort.org | Lara Giongo |
| Journal of Food, Agriculture & Environment (JFAE) | http://www.isfae.org/scientific journal.php | Lucia Martinelli |
| Journal of Limnology | http://www.iii.to.cnr.it/ | Nico Salmaso |
| Mycology | http://www.mscfungi.org/ | Nicola La Porta |
| Molecular Breeding | http://www.springer.org | Silvio Salvi |
| Phytopathology Informer | http://www.edagricole.it/r_ 22_dett.asp | Cesare Gessler |
| Plant Molecular Biology Reporter | http://www.springer.org | Riccardo Velasco |
| SITIS 2008, Fourth international conference on signal-image technology IEEE, Track Open Source Software Development and Solutions (OSSDS) | http://www.u-bourgogne.fr/SITIS/ 08/Track4.htm | Markus Neteler |
| South African Journal of Oenology and Viticulture | http://www.sasev.org/journal | Lucia Martinelli<br>Fulvio Mattivi<br>Giorgio Nicolini |
| Tree Genetics and Genomes | http://www.springer.org | Riccardo Velasco |
| Vite, vino e qualità | http://www.rivistedigitali.com/ Vite_Vino_e_Qualita/ | Stella Grando<br>Riccardo Velasco<br>Fulvio Mattivi |
| VITIS - Journal of Grapevine research | http://www.bafz.de | Fulvio Mattivi<br>Lucia Martinelli |
| Mitteilungen Klosterneuburg | http://bundesamt.weinobstklostern euburg.at/seiten/index.php/ view.84/service.true/ | Fulvio Mattivi<br>Urska Vrhovsek |
| Rivista di Viticoltura ed Enologia | http://www.inea.it/isv/Rivista ViticEnol.htm | Fulvio Mattivi |
| VQ - In vite qualitas, in vino excellentia | http://www.tecnichenuove.com/ep ages/Store.sf/?ObjectPath=/Shops/ TN/Products/VQ/SubProducts/VQ-0001 | Stella Grando<br>Fulvio Mattivi |

# Recognition

| PRIZE | INSTITUTION | PERSON |
|---|---|---|
| Appointment as convenor of the international working group "Biological Control of Fungal and Bacterial Plant Pathogens" | International Organisation for Biological Control | Ilaria Pertot |
| Appointment as convenor of the international working group "Pheromones and other semio-chemicals in integrated production" | International Organisation for Biological Control | Marco Tasin |
| Best research presented in a poster | Associazione Italiana tecnici del latte (AITeL) | Federica Camin<br>Matteo Perini<br>Gianni Colombari<br>Luana Bontempo<br>Giuseppe Versini |
| 2nd best poster | 4th International TRACE Meeting | Federica Camin<br>Matteo Perini<br>Luana Bontempo<br>Roberto Larcher<br>Giorgio Nicolini |
| Corresponding academician | Accademia dei Georgofili | Claudio Ioriatti |
| Italian Society for Agricultural Genetics (SIGA) 2008 | Italian Society for Agricultural Genetics (SIGA) | Valentina Cova |
| Recognition for the constant activity in the field of aerobiology | Società Italiana di Aerobiologia | Elena Gottardini<br>Fabiana Cristofolini |

# Academic Theses at IASMA in 2008

## Doctoral theses

| PERSON | UNIVERSITY | DEPARTMENT | FEM-IASMA ADVISOR |
|---|---|---|---|
| Endrizzi Isabella | University of Bologna | Agri Food Quality | Flavia Gasperi |
| Pedron Luca | Firenze | Valorizzazione Risorse Naturali | Nicola La Porta |
| Stradiotto Anna | University of Parma | Centre for Alpine Ecology | Francesca Cagnacci |

## Five-year theses

| PERSON | UNIVERSITY | DEPARTMENT | FEM-IASMA ADVISOR |
|---|---|---|---|
| Bocedi Greta | Università di Parma | Centre for Alpine Ecology | Anna Stradiotto |
| Coller Emanuela | University of Verona | Genetics and Molecular Biology | Stefania Pilati<br>Claudio Moser |
| De Vigili Mauro | University of Verona | Genetics and Molecular Biology | Claudio Moser |
| Ghidoni Franca | University of Milan | Genetics and Molecular Biology | Stella Grando |
| Monfredini Luca | University of Bologna | Agri Food Quality | Elisa Poznanski |
| Peripoli Giorgio | University of Padua | Natural Resources | Nico Salmaso |

## Three-year theses

| PERSON | UNIVERSITY | DEPARTMENT | FEM-IASMA ADVISOR |
|---|---|---|---|
| Amaldi Luca | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agri Food Quality | Roberto Larcher Giorgio Nicolini |
| Angeli Paolo | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Luca Zulini |
| Antonelli Luigi | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Luca Zulini |
| Bartoli Francesco | Università di Verona | Natural Resources | Nicola La Porta |
| Bertola Federica | University of Padua | Centre for Alpine Ecology | Anna Stradiotto |
| Brugnara Mirko | University of Udine | Agri Food Quality | Agostino Cavazza |
| Brunelli Alberto | FEM-Iasma, Trento Udine, Geisenheim Consortium | Genetics and Molecular Biology | Flavia Moreira Stella Grando |
| Campei Ivan | University of Udine | Agri Food Quality | Agostino Cavazza |
| Cassandro Elisa | University of Padua | Natural Resources | Silvia Silvestri |
| Chini Silvia | University of Padua | Agricultural Resources | Roberto Zorer |
| Collini Margherita | University of Padua | Natural Resources | Nico Salmaso |
| Dallabetta Luca | University of Trento | Plant protection | Gino Angeli |
| Decarli Elisa | University of Padua | Agricultural Resources | Giorgio De Ros |
| Faitelli Simone | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agri Food Quality | Giorgio Nicolini Roberto Zorer |
| Gamper Jakob | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Marco Stefanini |
| Giacomoni Matteo | University of Trento | Plant protection | Claudio Ioriatti |
| Lorenzi Silvia | FEM-Iasma, Trento Udine, Geisenheim Consortium | Genetics and Molecular Biology | Laura Costantini Stella Grando |
| Manuela Kerschbaumer | University of Bologna | Genetics and Molecular Biology | Massimo Pindo Riccardo Velasco |
| Marocchi Laura | University of Udine | Agri Food Quality | Agostino Cavazza |
| Martinelli Jessica | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Marco Stefanini |
| Martinelli Andrea | University of Udine | Agri Food Quality | Agostino Cavazza |
| Michael Pancher | University of Verona | Genetics and Molecular Biology | Pamela Gatto |
| Pedrini Erika | University of Udine | Agri Food Quality | Agostino Cavazza |
| Pedrini Martino | FEM-Iasma, Trento Udine, Geisenheim Consortium | Genetics and Molecular Biology | Stella Grando |
| Pellegrini Anita | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Marco Stefanini |
| Rohregger Hannes | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agricultural Resources | Luca Zulini |
| Stefani Erika | University of Verona | Genetics and Molecular Biology | Massimo Pindo Riccardo Velasco |
| Struffi Irene | FEM-Iasma, Trento Udine, Geisenheim Consortium | Agri Food Quality | Flavia Gasperi |

# Publications 2008

The results of research activities carried out at FEM-IASMA are published in scientific papers and presented at conferences and lectures for experts as well as for the general public. Below is a selection of the centre's major research papers published in the international press (J) and in various proceedings (P). Given the international audience of this book, we have omitted publications in Italian.

## Publications in ISI Journals


Alaphilippe A., Elad Y., David D.R., Derridj S., Gessler C. (2008). Effects of a biocontrol agent of apple powdery mildew (*Podosphaera leucotricha*) on the host plant and on non-target organisms: an insect pest (*Cydia pomonella*) and a pathogen (*Venturia inaequalis*). *Biocontrol science and technology*, 18, (2): 121-138. http://dx.doi.org/10.1080/09583150701818964. (J)

Anfora G., Baldessari M., De Cristofaro A., Germinara G.S., Ioriatti C., Reggiori F., Vitagliano S., Angeli G. (2008). Control of *Lobesia botrana* (Lepidoptera: Tortricidae) by biodegradable ecodian sex pheromone dispensers. *Journal of economic entomology*, 101, (2): 444-450. (J)

Anfora G., Isidoro N., De Cristofaro A., Ioriatti C. (2008). Demographic interactions between the inquiline midge *Macrolabis mali* (Diptera: Cecidomyiidae) and its gall-inducer host *Dasineura mali* (Diptera: Cecidomyiidae). *Entomologia generalis*, 31, (1): 75-82. (J)

Aprea E., Biasioli F., Carlin S., Märk T.D., Gasperi F. (2008). Monitoring benzene formation from benzoate in model systems by proton transfer reaction-mass spectrometry. *International journal of mass spectrometry*, 275, (1-3): 117-121. http://dx.doi.org/10.1016/j.ijms.2008.06.002. (J)

Aprea E., Biasioli F., Sani G., Cantini C., Märk T.D., Gasperi F. (2008). Monitoraggio in linea dello spazio di testa di oli di oliva tramite proton transfer reaction-mass spectrometry. *La rivista italiana delle sostanze grasse*, 82, (2): 92-97. (J)

Bahn M., Rodeghiero M., Anderson-Dunn M., Dore S., Gimeno C., Drösler M., Williams M., Ammann C., Berninger F., Flechard C., Jones S., Balzarolo M., Kumar S., Newesely C., Priwitzer T., Raschi A., Siegwolf R., Susiluoto S., Tenhunen J., Wohlfahrt G., Cernusca A. (2008). Soil respiration in European grasslands in relation to climate and assimilate supply. *Ecosystems*, 11, (8): 1352-1367. http://dx.doi.org/10.1007/s10021-008-9197-1. (J)

Bavaresco L., Gatti M., Pezzutto S., Fregoni M., Mattivi F. (2008). Effect of leaf removal on grape yield, berry composition, and stilbene concentration. *American journal of enology and viticolture*, 59, (3): 292-298. (J)

Bayfield N., Barancok P., Furger M., Sebastiń M.T., Dominguez G., Miloslav L., Cudlinova E., Vescovo L., Gianelle D., Cernusca A., Tappeiner U., Drösler M. (2008). Stakeholder perceptions of the impacts of rural funding scenarios on mountain landscapes across Europe. *Ecosystems*, 11, (8): 1368-1382. http://dx.doi.org/10.1007/s10021-008-9197-1. (J)

Beltrami M.E., Blanco S., Ciutti F., Cappelletti C., Monauni C., Monauni C., Pozzi S., Rimet F., Ector L. (2008). Distribution and ecology of *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) in Trentino watercourses (Northern Italy). *Cryptogamie algologie*, 29, (2): 141-160. (J)

Beltrami M.E., Cappelletti C., Ciutti F. (2008). *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) in the Danube basin: new data from the Drava river (northern Italy). *Plant biosystems*, 142, (1): 126-129. http://dx.doi.org/10.1080/11263500701872788. (J)

Bisognin C., Ciccotti A.M., Salvadori A., Moser M., Grando M.S., Jarausch W. (2008). *In vitro* screening for resistance to apple proliferation in *Malus* species. *Plant pathology*, 57, (6): 1163-1171. http://dx.doi.org/10.1111/j.1365-3059.2008.01890.x. (J)

Bisognin C., Schneider H., Salm H., Grando M.S., Jarausch W., Moll E., Seemüller E. (2008). Apple proliferation resistance in apomictic rootstocks and its relationship to phytoplasma concentration and simple sequence repeat genotypes. *Phytopathology*, 98, (2): 153-158. http://dx.doi.org/10.1094/PHYTO-98-2-0153. (J)

Botton A., Lezzer P., Dorigoni A., Barcaccia G., Ruperti B., Ramina A. (2008). Genetic and environmental factors affecting allergen-related gene expression in apple fruit (*Malus domestica* L. Borkh). *Journal of agricultural and food chemistry*, 56, (15): 6707-6716. http://dx.doi.org/10.1021/jf800813d. (J)

Bovolenta S., Sacca E., Corazzin M., Gasperi F., Biasioli F., Ventura W. (2008). Effects of stocking density and supplement level on milk production and cheese characteristics in Brown cows grazing on mountain pasture. *Journal of dairy research*, 75, (3): 357-364. http://dx.doi.org/10.1017/S0022029908003531. (J)

Cadavid-Labrada A., Medina C., Martinelli L., Arce-Johnson P. (2008). Somatic embryogenesis and efficient regeneration of *Vitis vinifera* L. 'carmenere' plants. *Vitis*, 47, (1): 73-74. (J)

Cagnacci F., Massei G. (2008). Monitoring bait uptake in badgers: a pilot study using PIT-tag system and time-lapse video surveillance. *Ethology ecology & evolution*, 20, (2): 165-171. (J)


Cagnacci F., Urbano F. (2008). Managing wildlife: a spatial information system for GPS collars data. *Environmental modelling & software*, 23, (7): 957-959. http://dx.doi.org/10.1016/j.envsoft.2008.01.003. (J)

Callone E., Campostrini R., Carturan G., Cavazza A., Guzzon R. (2008). Immobilization of yeast and bacteria cells in alginate microbeads coated with silica membranes: procedures, physico-chemical features and bioactivity. *Journal of materials chemistry*, 18, (40): 4839-4848. http://dx.doi.org/10.1039/b807301e. (J)

Camin F., Larcher R., Perini M., Bontempo L., Bertoldi D., Gagliano G., Nicolini G., Versini G. (2008). Characterisation of authentic Italian extra-virgin olive oils by stable isotope ratios of C, O and H and mineral composition. *Food chemistry*, http://dx.doi.org/10.1016/j.foodchem.2008.04.059. (J)

Camin F., Perini M., Colombari G., Bontempo L., Versini G. (2008). Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope ratios of milk. *Rapid communications in mass spectrometry*, 22, (11): 1690-1696. http://dx.doi.org/10.1002/rcm.3506. (J)

Caramelli D., Dilani L., Vai S., Modi A., Pecchioli E., Girardi M. et al. 2008. A 28,000 years old Cro-Magnon mtDNA sequence differs from all potentially contaminating modern sequences. PloS One 3 (7): e2700. (J)

Carpi G., Cagnacci F., Neteler M., Rizzoli A. (2008). Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. *Epidemiology and infection*, 136, (10): 1416-1424. http://dx.doi.org/10.1017/S0950268807000039. (J)

Consolandi C., Palmieri L., Severgnini M., Maestri E., Marmiroli N., Agrimonti C., Baldoni L., Donini P., De Bellis G., Castiglioni B. (2008). A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR-universal array analysis. *European food and research technology*, 227, (5): 1429-1438. http://dx.doi.org/10.1007/s00217-008-0863-5. (J)

Costa F., Van De Weg W.E., Stella S., Dondini L., Pratesi D. , Musacchi S., Sansavini S. (2008). Map position and functional allelic diversity of *Md-Exp7*, a new putative expansin gene associated with fruit softening in apple (*Malus x domestica* Borkh.) and pear (*Pyrus communis*). *Tree genetics & genomes*, 4, (3): 575-586. http://dx.doi.org/10.1007/s11295-008-0133-5. (J)

Costantini L., Battilana J., Lamaj F., Fanizza G., Grando M.S. (2008). Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. *BMC Plant biology*, 8, (38) http://dx.doi.org/10.1186/1471-2229-8-38. (J)

Cristofolini F., Giordani P., Gottardini E., Modenesi P. (2008). The response of epiphytic lichens to air pollution and subsets of ecological predictors: a case study from the Italian Prealps. *Environmental pollution*, 151, (2): 308-317. http://dx.doi.org/10.1016/j.envpol.2007.06.040. (J)

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## GETTING TO SAN MICHELE ALL'ADIGE

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