



2006

Annual Report

IASMA RESEARCH CENTRE



ISTITUTO AGRARIO
DI SAN MICHELE ALL'ADIGE

Istituto Agrario di San Michele all'Adige
IASMA Research Centre

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DI SAN MICHELE ALL'ADIGE

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Giovanni Gius

Tradition and innovation, past and future are complementary notions which underlie important choices recently made at the Istituto Agrario San Michele all'Adige (IASMA). Keeping a focus on local needs while opening up internationally is not a simple equation. But this is the operating philosophy and direction that IASMA and its upcoming successor, the Edmund Mach Foundation, will have to embrace so as not to lose a heritage of knowledge and science built up over 130 years of activity. Our roots, in fact, originate in a diet of princess Contea of Tyrol, who established the institute on January 12, 1874, as "a teaching and research centre with the goal of improving the plight of agriculture in south Tyrol".

Over the last decade, IASMA research activities and initiatives have made significant strides in terms of quantity and quality. Advances made in areas such as oenology, advanced biology, and environmental studies have placed San Michele at the forefront of national and international science. To reach this level, we recently reorganized the research centre so that it could better respond to evolving research needs. In doing so, we attempted to develop the potential of available human resources, while at the same time we adopted state-of-the-art technologies and innovative approaches which make it possible to handle new scientific challenges.

Provincial law (L.P. 2 August 2005 no. 14) reorganized provincial research activities. In 2007 IASMA, and in particular the research center, will once again be restructured to be still more flexible and agile, to have greater autonomy, and to be more competitive. In this way IASMA will be better able to react to the needs of farmers and producers, but also to better able to compete with similar structures nationally and internationally. As a starting point for further evolution, it is essential that we present at least a part of the research centre's recent and ongoing activities. In this way, we can show how IASMA is working to enhance agriculture in terms of quality and human health, and how it is supports improvements to the interrelationships between agriculture and the environment. In doing so, we can also focus on the people who dedicate their energy to the research centre while providing insight to those who wish to know it better. Allow me then to thank all of those who worked on this first edition of the Annual Report for their passion and dedication in putting it together.

dr. Giovanni Gius
IASMA President





The Institute

Background

The history of the institute begins on January 12, 1874, when the regional Tyrolean Diet of Innsbruck set up an agricultural school in the Augustinian monastery of San Michele all'Adige with the aim of revitalizing regional agriculture. The first director, Edmund Mach, was an expert in the field of agricultural chemistry and oenology, and above all an excellent organiser and innovator. He established a structure where teaching and research could improve agricultural development in the surrounding territory. This fertile bond continues to distinguish the institute.

When Trentino became part of Italy after the First World War, the Istituto Agrario di San Michele all'Adige (IASMA) went to the Province of Trento in 1919, and, in 1926, it was made a part of a consortium of the Italian state.

In 1990, the institute became a working organ of the Autonomous Province of Trento, and further reorganisations completed its present features, integrating the extension service into its core activities.

Over the years, the institute has grown significantly, employing nearly 700 staff in 2006. Today, with a

school that trains experts in agriculture and oenology, a research centre that carries out studies at an international level, and an extension service that assists local farmers and stakeholders, the institution is well positioned to study solutions, give answers and support improvements in agriculture. Currently governed by a board of directors appointed by the province, the institute is once more poised for change. In 2008, the institution will become the Edmund Mach Foundation. Although funding will remain strongly public, the private aspects of foundation management will allow the institute to react faster and more efficiently to changing needs.

Organisation

IASMA is a multifaceted organisation. While its main focuses are the school, research centre, and extension service, it also includes a number of important internal and external functions. Internally, the institute relies on its administrative, financial, information technology, legal, and public relations groups.



The history of San Michele dates to around the year 1000 when many monasteries were set up or enlarged throughout Europe. In 1143, the Counts of Appiano donated the castle of San Michele all'Adige to the Prince Bishop of Trento and the Augustinian monks turned it into a monastery. Today, the castle's wine cellars, refectory and cloister convey the ancient roots of the institute.



The grounds of the ancient Augustinian monastery include two historical wine cellars from the 12th and 16th centuries. They are exquisite examples of architectural beauty and historical tradition, and proof of the ancient tradition of wine making in the area. The modern cellar produces a range of wines, as well as spumanti, grappa, and spirits typical to Trentino.

Average annual production is around 200,000 bottles of wine, 100,000 bottles of sparkling wine, and 10,000 bottles of distilled spirits.

Another function is the IASMA library, with 25,000 volumes and 1,400 periodicals.

Significant external functions include 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, qualify products, satisfy customers, and match the competition on price.

Another very public part of the institute is its farming business. 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 impacts.



IASMA centres

School

Highly versatile, IASMA has the only school in Italy that contemporaneously provides training and teaching in the agricultural, environmental and forestry sectors at a number of different levels: from those specially designed for farmers to highly specialised courses at university level. The school currently has four separate sections, each with its own distinct area of operation.

The secondary technical education unit manages technical secondary education schools for agricultural, environmental and forestry disciplines, including post-diploma courses where applicable. The vocational secondary education unit manages vocational secondary education schools for agricultural, environmental and forestry disciplines, including first and second level post-qualification courses. Both directly and in collaboration with other authorities and organisations, the vocational agricultural training unit organises and implements training, qualification and specialisation courses for development and diffusion of expertise in the fields of agriculture, forestry and the environment.

The post-secondary and university education unit provides top-level, specialised training. Its main function is to train oenologists in first-level university degree courses in viticulture and oenology in accordance with the inter-university consortium. It also handles second-level degrees and master courses, as well training and higher education, including higher technical training and education.

Extension service

The extension service provides technical and socio-economic assistance and advisory services to farms and cooperatives in all sectors of Trentino agriculture. In particular, the centre provides assistance to many economic sectors, including fruit, viticulture-oenology, strawberry and small fruits, horticulture, floriculture, zootechny and fish. It also provides socio-economic consulting services. Its objective is to assist the world of farming throughout all stages of production in order to satisfy consumer demand for high-quality, healthy products produced in accordance with “best farming practices” and with absolute respect for the environment. The centre has five offices: Fruit-Growing; Viticulture and Oenology; Vegetable, Flower and Fruit Production; Zootechny and Feed; and Socio-Economic Development and Studies. Technical consultancy is carried out by 54 technicians working in 18 units strategically located to cover the various areas of production.

The centre provides information to the Trentino farming community through direct contact with the technicians, as well as through a variety of print and electronic media.

Research centre

The research centre has a mission to promote and enhance the Trentino land-based economy through studies and innovation that improve agricultural and forestry products and enhance the quality and nutritional value of food products.

It sustains the region’s environmental resources through development and promotion of low-impact agricultural practices, study and preservation of biodiversity, and characterisation of alpine and subalpine ecosystems. The research centre also directly manages several farms where it conducts fruit and vine research.

The centre operates fully integrated research programs and state-of-the-art platform technologies to deliver innovative solutions and competitive products for our stakeholders and end-user communities. To stay relevant, our research activities are closely integrated with the up-to-date know how and technology transfer activities at IASMA. Thus, we can deliver social and economic returns to the local community.

The research centre is structured in five departments: Agricultural Resources, Agrifood Quality, Genetics and Molecular Biology, Natural Resources, and Plant Pro-

tection. Currently, the centre also hosts two externally-funded research groups. SafeCrop is a network of international research institutions that aims at the development of crop protection with low impact on the environment and consumer health. The Centre for the Study of Biodiversity (CSBT) is a joint-venture with the Max-Planck Institut für Züchtungsforschung, Köln, for the molecular characterisation of flora biodiversity in Trentino. Through intense research programs, top-flight facilities, and important collaborations, IASMA produces research results at an international level.



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



Roberto Viola

The Istituto Agrario di San Michele all'Adige (IASMA) has more than a century of tradition in providing education, technical support and innovation to its agricultural stakeholders. It is one of the earliest institutions of its kind in Italy, and it is well recognised internationally, particularly for research and teaching activities in viticulture and oenology which have represented the main fields of interest since the institute was founded.

In the course of many years of development and growth, the scope and breadth of the institute's research and experimental work has widened considerably. In particular, there has been a significant

increase in the number of research projects and experimental activities over the last few years. This coincided with a strong push in research and development funding and initiatives by the Autonomous Province of Trento, of which IASMA is a part. As a result, the size of its research centre has more than doubled in the last seven years.

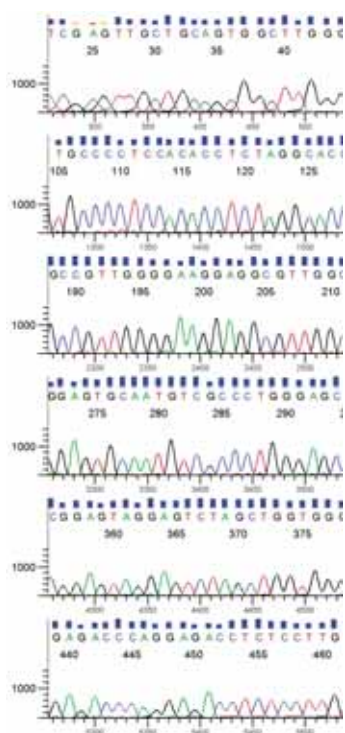
In 2005, the centre underwent a restructuring, increasing the number of research departments to five and creating a total of 17 research units. Currently, the research centre comprises 300 staff (see table page 18), including researchers, technicians and others.

The centre now has a strong international connotation, with more than five percent of its research scientists and collaborators coming from outside of Italy. In addition, a number of international research collaborations are being carried out with partners from within the EU and without, including the USA, Brazil, Chile, Israel and South Africa. Two concrete outcomes of this international approach were the activation in 2002 of the Centre for the Study of Biodiversity in Trentino (CSBT) in collaboration with the Max Planck Institut für Züchtungsforschung - Köln and of the Safecrop Center, which is a joint initiative of a number of international partners, including INRA, France; SLU, Sweden; BBA, Germany; ETH, Switzerland and ARO, Israel.

The rapid expansion of IASMA's research and development activities and its increasing international outlook were the impetus for creating this Annual Report, through which we could inform the scientific community, funding bodies, stakeholders and other interested parties about progress in our research and experimental activities. This report is structured to provide you with information about the structures and resources of research centre, and aims at providing insight into our basic and applied research portfolio in the agrifood sector and of our expanding research effort in environmental themes.

The thread that joins these activities is our mission to sustain the development of the land-based bioeconomy in Trentino through solid, science-based approaches,





building on the ever stronger interconnections between agricultural and natural resources.

The content of the annual report speaks for itself. However, I consider it particularly significant that this first issue describes one of the most important achievements in the history of the Institute. In December 2006, the IASMA genomic team, in collaboration with Myriad Genetics Inc. and 454 Life Science deposited detailed sequences of the 19 chromosomes of the grape genome (cv *Pinot noir*) in the international gene banks (www.ncbi.nlm.nih.gov) where they are available for consultation by the international scientific communi-

ty. This result came less than 18 months from the start of a very ambitious project funded by the Autonomous Province of Trento which aimed at sequencing *Pinot noir*, a commercial grape variety with a highly heterozygous genome.

Together with prior efforts of the same team, which generated genetic and physical maps of grapes (also available to the scientific community), this major result has now set the basis for the development of a smart and unique toolbox for sustaining our plant breeding efforts which aim at the development of novel varieties with enhanced quality and disease resistance traits.

In 2006, building on the success of our grape genome sequencing programme, we started a programme aimed at sequencing the 17 chromosomes of the apple genome. At the same time, through an international recruiting effort, we also started to build an interdisciplinary “translational genomics” group whose goal is to extract practical knowledge and information from our genome sequencing efforts.

The interdisciplinary nature of our activities represents an important strength, bringing scientists engaged in fundamental research together with applied scientists who carry out research and experimentation which directly affects end-users communities.

I hope that this report helps you to appreciate the wealth of research and experimental activities currently going on at IASMA. Through it, I think you will see the quality efforts and technical competences of all the scientific staff working at the institute. Now in my third year at San Michele, I can say that I feel privileged and honoured to be involved with such a dedicated and skilled group of people.

dr. Roberto Viola
Research Centre Director

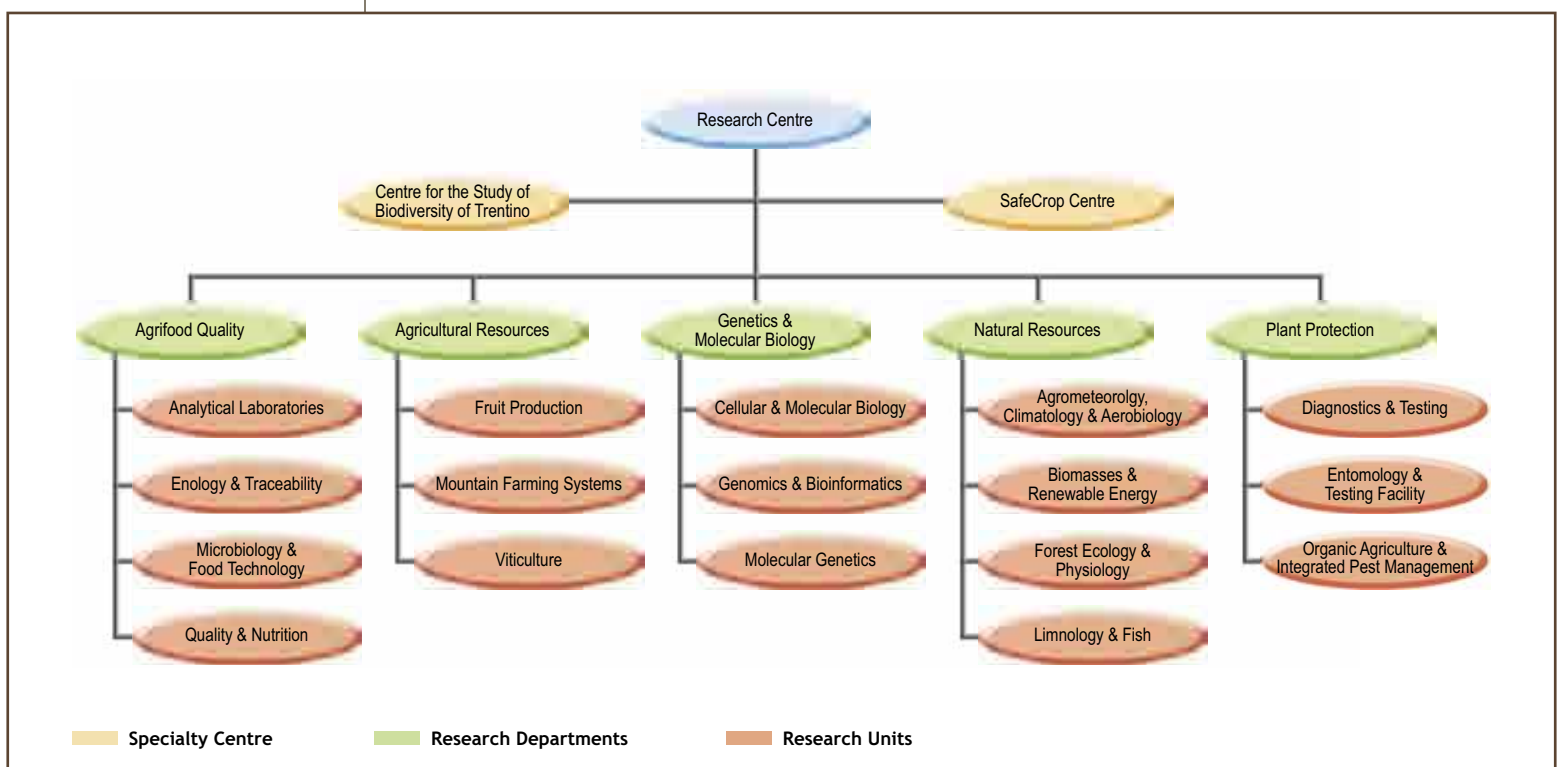
About the Research Centre

Mission and impact

At a local level, the research centre has a mission to promote the Trentino 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 preserves the province's environmental resources by developing and promoting low-impact agricultural practices, studying and preserving biodiversity, and characterising alpine and subalpine ecosystems.

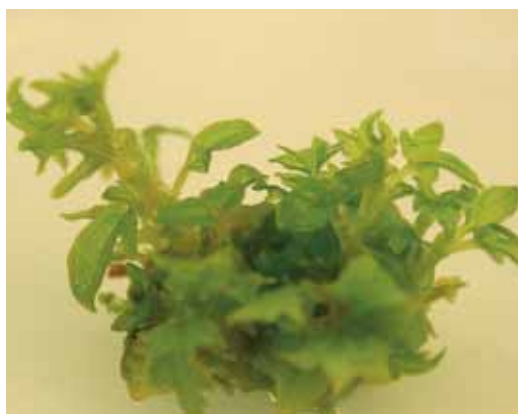
Over time, the centre's activities have evolved beyond its local functions. We conduct research at an international level, collaborating with institutes and universities from around the world. Our results impact the larger scientific community and the world at large.



Organisation

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

- **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
- **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.
- **Plant Protection:** Research areas include entomology, phytopathology, organic agriculture, and integrated pest management. The department also has a diagnostics and testing facility.
- **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.



Human Resources

ROLE	FEMALE	MALE	TOTAL
Director	0	1	1
Researchers, permanent	16	32	48
Researchers, contract	25	25	50
Research fellows, post-doc	13	8	21
Research fellows, undergraduate and graduate	19	23	42
Technicians	38	46	84
Support staff	9	33	42
Secretaries	3	0	3
Administrative staff of the Research Centre	6	4	10
TOTAL*	129	172	301

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

National origin of staff

Approximately 92 percent of Research Centre staff is of Italian origin. Of the remainder, about 5% come from the European Union, and the rest from Asia, Africa, and Central America.

Active international collaborations

PROJECT	NATION
ACCRET-E - Agriculture and climate changes: How to reduce human effects and threats	Greece, Germany, Czech Republic, Romania, Slovenia
ALPINET GHEEP - ALPINET network for sheep and goat promotion for a sustainable territory development	Germany, Austria, Slovenia
CSBT - Molecular study of biodiversity in Trentino province: From model species to applications in ecology and agriculture	Germany
Apple Genome	United States of America
Genomic Research - Assisted breeding for sustainable production of quality grapes and wine	France
HIDRAS - Sustainable agriculture, fisheries and forestry, and integrated development of rural areas including mountain areas	Belgium, Germany, United Kingdom, Poland, France, The Netherlands, Swiss
INTERBERRY - Integrated interdisciplinary project for qualitative improvement of soft fruits and study of new transformed products with high added value	Romania, Poland
REPCO - Replacement of Copper Fungicides in Organic Production of Grapevine and Apple in Europe	The Netherlands, Germany, Swiss, France, Denmark
SAFECROP - Centre for research and development of crop protection with low environmental and consumer health impact	Israel, France, Sweden, Swiss, Germany
SMAP II - Program of future research activities on apple proliferation disease at IASMA	Germany
TRACE - Tracing the origin of food	United Kingdom, France, Belgium, Germany, Austria, Greece, Czech Republic, Slovenia, Ireland, Norway, Poland, Iceland, Spain, China, Switzerland

Financing

The Autonomous Province of Trento provided core funding for 23 core projects in 2006. The province also funded 18 special projects. In addition, many other research activities were financed by Italian national and regional governments, and the European Union. The centre also derived income from contracts with private entities.







Fulvio Mattivi

AGRIFOOD QUALITY DEPARTMENT

The mission of the department 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; develops innovative wine processing protocols and technologies, contributes to the genetic improvement of the principal crops grown in Trentino, and provides high quality analytic support and consultancy to producers through collaboration with the institute's Technical Advisory Centre.

Nutritional quality

Research conducted by the nutritional quality team aims to characterise bioactive substances present in raw food (apple, grape, strawberry, and other soft fruits) and processed agrifood (wine, juices, etc.). Through collaboration with major Italian universities and research institutions we investigate the absorption, metabolism and mechanism of action of natural antioxidants (anthocyanins, cinnamic acids and resveratrols). The final aim of our studies is also to develop new products and applications (nutraceuticals, cosmeceuticals, pharmaceuticals) of bioactive natural products, such as the resveratrols. These natural hydroxystilbenes, present in grape and wine, have important anticancer and immunomodulating properties, which were successfully investigated and which also resulted in Italian and European patents.

Sensory quality

Sensory quality is a major research subject of the department. We aim to characterize agrifoods through new instrumental and sensory approaches

to identify markers of perceived quality and to provide analytical assistance and consultancy to both producers and plant breeders. We support the development of innovative agrifoods through new technologies for production and stabilization based on sensory data in order to promote more conscious consumption, that is, consumption that is more sensitive to overall quality. The quality of typical agrifoods is developed through sensory characterisation and monitoring of the knowledge, opinions, and expectations of consumers. This task is also supported by investigation of factors that drive sensory experience and consumer appreciation, investigating their correlation with biochemical and physiological parameters.

Authenticity of origin

The department has internationally recognized 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. We investigate new protocols and markers



for the geographical, botanical and technological origin of agrifood (e.g. stable isotopes and microelements). The application of such 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 range of activities, from the optimal use of techniques, technologies and fining agents in oenology, to the development of rapid methods for quality control. The main topics in this area are nitrogen-containing com-

pounds, volatile compounds (terpenols, sulphur containing compounds, volatile phenols, etc.) and contaminants in grape and wines, as well as the analysis of the phenolic potential of grape for the optimisation of the production of red wines. In collaboration with the University of Trento, the microbiology team is studying new matrices for the production of immobilised lactic bacteria. Another theme 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 pursues the improvement of distilla-

tion processes for the production of grappa, providing scientific support to this traditional spirit for which the producers from Trentino aim to maintain quality standards of excellence.

Industrial processes

The department's research team is also involved in the improvement of industrial processes, and in particular in development of new techniques for the stabilisation of juices, in the exploitation of microbial biodiversity and joint use of integration cultures for production of typical cheeses and in the pilot scale optimisation of processes for production of fermented fruit drinks.

Analytical services

A major activity of the department is to provide analytical services, in a unique environment based on a robust analytical platform and on state of the art expertise thanks to the close connections with research activities. Our modern, well equipped and efficient analysis laboratory provides high quality analytical support and consulting to producers. The laboratory operates under the UNI CEI EN ISO/IEC 17025 with 38 methods accredited form SINAL (<http://www.sinal.it/eng/>), and is authorised by the Italian Ministry of Agriculture to provide analytical certificates (22 different analytical methods) on vitienological products, having official validity both at the national level and for export. We provide analytical services to about 300 private clients. Beyond working with most regional firms and consortia, and with many leading Italian wineries, we get frequent requests from important international clients in foreign countries, especially Germany and South Africa.

The year 2006 was a very intense year for both research and service in the department. Twenty-five externally funded projects were carried out during the year. The research activities lead to the publication of 14 scientific papers in major ISI journals. Moreover, researchers of the department were involved in teaching ten university courses. We hosted 16 laboratory stages and gave support to nine dissertation theses. We also turned out well in a detailed audit carried out by a new inspection team, for the renewal of our SINAL accreditation. The laboratory handled more than 21,000 samples for chemical analysis. This allowed us to provide—in addition to analytical support within the research center—analytical services to more than 300 clients, as well to the Technical Advisory Centre, the latter with about 3,700 samples.



Multi-element (H,C,N,S) stable isotope characterisation of European lamb meat

Federica Camin

Since 2005, IASMA has been involved in the Integrated Project TRACE, “Tracing Food Commodities in Europe”. The project is funded by the European Commission under the Sixth Framework Program for Food Quality and Safety Priority.

The head of scientific research was up to the date of his retirement dott. Giuseppe Versini, who has now been replaced by dott. Giorgio Nicolini. IASMA is responsible for sampling Italian foods such as mineral water, lamb, chicken, beef, honey, olive oil and wheat for analysis of the stable isotope ratios of bio-elements (coordinated by dott.ssa Federica Camin) and trace element content (coordinated by dott. Roberto Larcher) in Italian and other European products.

One of the principal aims of TRACE is to produce databases and, ultimately, prediction systems capable of linking the stable isotope composition and trace mineral content of food to climate and to geographical and geological data or maps. The first prediction model based on the stable isotope and mineral composition of European mineral waters is nearing completion.

In this article, I present the results of the project with regard to the possibility of distinguishing the geographical origin of European lamb on the basis of H, C, N and S stable isotope ratio analysis (Camin *et al.*, 2007, *Anal. Bioanal. Chem.*, <http://dx.doi.org/10.1007/s00216-007-1302-3>). The work was carried out by the IASMA stable isotope staff (Luana Bontempo, Matteo Perini and Luca Ziller) with the collaboration of several European laboratories (Isolab, LGL Bayern Dienststelle Oberschleißheim, Germany; Central Science Laboratory, Institute of Food Research, University of East Anglia, UK; Eurofins Scientific Analytics, France; ARC Seibersdorf Ges.mbH, Austria and University College Dublin, Ireland).



Isotopes in lamb

Lamb produced in 2005 in 12 European sites was investigated. Stable isotope analysis was carried out on defatted dry matter using a Isotope Ratio Mass Spectrometer (IRMS) coupled with Elemental Analysers and High Temperature Pyrolysis systems. The stable isotope ratios D/H, $^{13}\text{C}/^{12}\text{C}$, ^{15}N , ^{14}N , $^{34}\text{S}/^{32}\text{S}$ were expressed according to international conventions in ‘‰’, i.e. in $\delta\text{D}\%$, $\delta^{13}\text{C}\%$, $\delta^{15}\text{N}\%$ and $\delta^{34}\text{S}\%$. More details of the methods adopted are given in the article cited above.

Hydrogen isotope ratio

Stable hydrogen isotopes in plant and animal tissues reflect the δD of groundwater, which is in turn influenced by the average δD of precipitation water for a region. It is well known that the H isotopic ratio of water is influenced to a variable extent by geographical factors such as latitude, altitude, and

distance from the sea, as well as by the climatic conditions. The results obtained show high deuterium content for lamb from Chalkidiki with a mean value of -80‰ and from the UK and Sicily with mean values ranging from -80‰ to -90‰.

The δD values for lamb from central European regions (Carpentras, Limousin, Tuscany, Franconia) fall within the range of -90‰ to -100‰, whereas lamb reared in Alpine mountain areas (Trentino and Allgäu) and in Mühlviertel show significantly lower ($p = 0.001$) δD values, from about -100‰ to -120‰. The main variability factors result to be climate conditions, distance from the sea and the altitudes of the regions.

Carbon isotope ratio

The carbon isotope ratio of animal products is predominantly influenced by the amount of C3 and C4 plant materials in the animal diet (Piasentier *et*

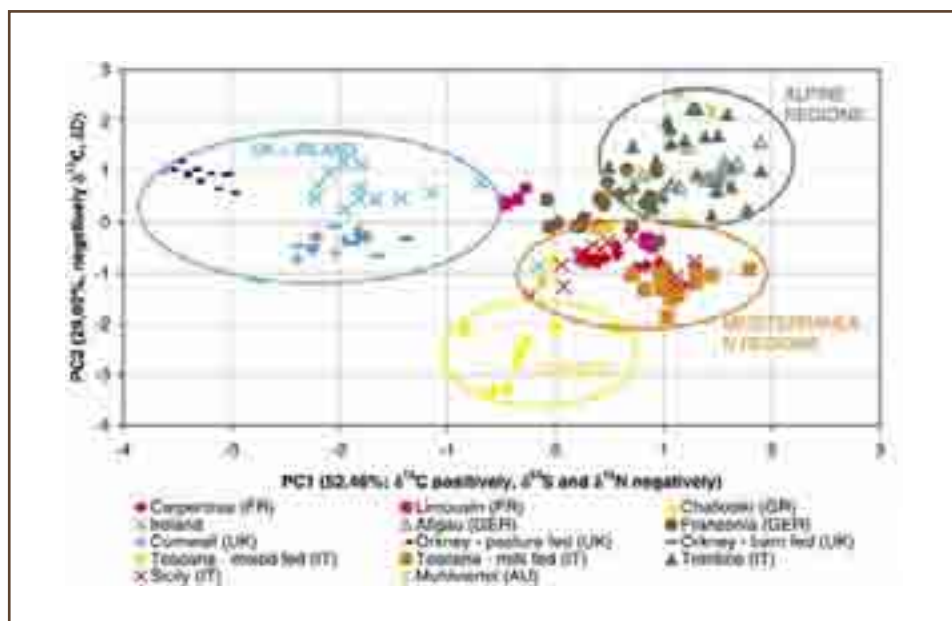
al., 2003, *Meat Science*, 64, 239-247). Maize feeding is indicated by the high $\delta^{13}\text{C}$ values measured in lamb from Chalkidiki and Carpentras, as well as some samples from Limousin, Tuscany and Mühlviertel (mean values from -21.6 to -22.3‰). UK and Irish lamb is characterised by significantly lower ($p=0.001$) $\delta^{13}\text{C}$ values (mean values from -26.2‰ to -28.5‰), due to the high humidity of those regions, which results in high C isotope fractionation during plant biosynthesis.

Nitrogen isotope ratios

The nitrogen isotopic values allow characterisation of the north-western European regions of the UK and Ireland with relatively (significantly for UK, $p=0.001$) high $\delta^{15}\text{N}$ values (mean from +7.4‰ to +9.2‰). These high values may be due to fertilisation of the grasslands with seaweed or other products from the marine ecosystem, which are known to possess high $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values (see below). The other regions show relatively homogeneous results, with those for Trentino being significantly ($p = 0.001$) lower as compared to most of the other regions (+3.8‰), and the data for Chalkidiki being slightly higher (+6.5‰). The different climatic conditions in the two areas and the presence of leguminous plants in the mountainous area of Trentino could be the reasons for this difference (Camin *et al.*, 2004, *J. Ag. Food Chem.*, 52, 6592-6601).

Sulphur isotope ratios

With regard to sulphur isotope ratios, the north-western European and Greek regions show significantly ($p = 0.001$) higher mean $\delta^{34}\text{S}$ values (mean from 7‰ to 12.9‰). The significantly ($p = 0.001$) low $\delta^{34}\text{S}$ values of Tuscany and Sicily (mean from 1.9‰ to 2.5‰) could be due to the high amounts of volcanic sulphur available in these regions, whereas for the Allgäu samples (mean $\delta^{34}\text{S} = 1.4‰$) it could be a



result of low ^{34}S content in reduced sulphur from the sediments in this region.

In order to reduce the dimensionality of the data set and to describe the full variability of the system whenever possible in two dimensions, we carried out principal component analysis. The figure shows that European lamb, on the basis of its stable isotope ratios, is distributed over the plane identified by the first two principal components in a similar way as the regions of origin.

The results show that, on the basis of four stable isotope ratios (δD , $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$), it is possible to distinguish four different groups: the north western European areas of Orkney, Ireland and Cornwall; Greece; the Mediterranean areas of Sicily, Tuscany and Carpentras and the Alpine areas of Trentino and Allgäu with Mühlviertel. The Limousin lamb, divided in two groups, and the Franconia lamb are on the borderline between the Mediterranean and Alpine areas.

Identifiable areas using stable isotope ratios

Tracing the origins of meats

The stable isotope data of hydrogen, carbon, nitrogen and sulphur could be a powerful resource for differentiating the geographical origin of lamb. Nowadays, the possibility of determining the provenance of meat is an important issue, both in terms of health (to avoid the diffusion of animal diseases or potential human epidemics, e.g. bovine spongiform encephalitis, chicken influenza, etc.) and from an economic point of view (in order to safeguard protected designations of origin products).

Future research within TRACE will include stable isotope analysis of lamb produced in 2006, as well as beef, chicken, olive oil, honey, and wheat and the measurement of other analytical data, such as the stable isotope ratio of the geo-element Sr and trace element content, which have been found to enable unambiguous geographical provenance differentiation.



Proton transfer reaction-mass spectrometry (PTR-MS) is a technique available at IASMA since 2003. Based on efficient implementation of chemical ionisation, it allows fast, direct monitoring of volatile compounds with high sensitivity.

Applications of PTR-MS in food science and technology, as well as in environmental and medical areas, have been proposed since its invention. IASMA actively contributed to exploring the potential of PTR-MS in food science. The importance of the role it plays has been recognised by the invitation to present a review paper at the most recent international PTR-MS conference (Obergrugl, Austria, January 2007). Here, we review the works we have published in the last year, and we outline the ideas leading our activity in the exploiting of PTR-MS characteristics in food-related issues and indicate per-

spectives for the future.

Almost every process that occurs in food induces the emission of volatile organic compounds (VOCs) that often strongly affect the perception of food characteristics.

Thus, the capability of PTR-MS to quantitatively monitor VOCs in real time with high sensitivity provides a tool for product and process characterisation with a direct link to perceived quality. PTR-MS studies at IASMA can be classified as follows: i) rapid product characterisation, ii) real-time detection of VOCs for process monitoring, iii) real-time VOC detection for flavour release during food consumption, and iv) measurement of fundamental parameters of gas ion chemistry such as fragmentation patterns in PTR-MS and measurement of important properties of volatile compounds such as partition coefficients.

PTR-MS in food science and technology: activities and perspectives at IASMA

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Rapid, non-invasive product characterisation

PTR-MS is often able to unequivocally identify compounds, but its strength is more in monitoring rapid changes in concentration of compounds than in compound identification. Environmental science applications rely on spectrometric peaks to identify specific compounds, but there are difficulties in extending these methods to food analysis. Nevertheless, we successfully used PTR-MS for static or semi-static headspace characterisation to classify products according to their VOC fingerprint or to associate this fingerprint to other parameters such as fruit cultivar (Granitto *et al.* 2007, *Sensor Actuat B*, 121:379-385)

and related genetic information (Carbone *et al.* 2006, *Mol Breeding*, 18:127-142); sensory attributes both in real (Biasioli *et al.* 2006, *Food Qual Prefer*, 17:63-75) and in model systems (Aprea *et al.* 2006, *Flavour Frag J*, 21:53-58); presence of defects or monitoring of degradation, post harvest storage and aging (Aprea *et al.* 2006, *Int Dairy J*, 17:226-234). PTR-MS is particularly useful because its direct inlet guarantees a measurement as close as possible to the VOC mixtures actually smelled by people, it quickly collects multiple data, and it detects trace compounds that would require pre-concentration or extraction if detected by other methods.

IASMA originated the coupling of data mining techniques with rapid PTR-MS fingerprinting. This, along with introducing the PTR-MS community to innovative methods for data analysis, has led to fundamental results on how to optimize anonymous PTR-MS fingerprinting for product classification, and how to automatically extract useful information from large spectrometric data sets (Granitto *et al.*, 2006, *Chemometr Intell Lab*, 83:83-90).

Fast process monitoring

Fast monitoring of processes is the use that best fits PTR-MS capabilities. The problem of identifying compounds has been partly overcome by the coupling of PTR-MS data with gas chromatography information. This allows for monitoring of important reactions taking place during food processing and storage. In this direction, IASMA proposed the

use of PTR-MS for online detection and monitoring of defects in olive oil, following the formation of volatile secondary oxidation products (Aprea *et al.*, 2006, *J Agric Food Chem*, 54:7635). Metabolic or catabolic pathways in plants and fruits are further examples of processes that can be successfully monitored by PTR-MS and that are under investigation at IASMA.

In vivo release of flavour compounds

Recently, in vivo release of flavour compounds during food consumption has attracted a lot of attention from investigators. The overall goal of such research is to understand the mechanisms underlying odour and aroma perception. PTR-MS is particularly well suited for this application, where the volatile organic compounds of interest are often known and present at high concentrations. PTR-MS has been used to study the influence of chemical and physical properties of food, and the influence of oral processing on the dynamic of flavour release during eating. In vivo measurements also provide information on individual consumers, allowing, on the one hand, control of the high degree of variability observed also under strict oral processing protocols and, on the other, an opening to “consumer-centric” investigation of flavour perception (Aprea *et al.* 2006, *Flavour Frag J*, 21:53-58). In this context, PTR-MS is becoming the *de facto* reference technique. We expect to demonstrate that it can also support research in other fields where objective monitoring of sensory stimuli is a requirement.

PTR-MS fundamentals

As opposed to environmental scientists who prefer ad hoc PTR-MS equipment, the food science community typically prefers to use commercial PTR-MS apparatus, although this limits potential for implementing promising technical advancement. In the near future, we hope to support the development of new commercial

type of PTR-MS systems (e.g. coupling with time-of-flight or ion trap). Currently, we are working on the measurement of relevant parameters such as PTR-MS fragmentation patterns and their dependence on instrumental settings, as well as on VOC partition coefficients (Aprea *et al.*, 2007, *Int J Mass Spectrom* 262:114-121). Early results show that PTR-MS can easily provide data on VOC properties that are often unavailable in the literature. In conclusion, we have shown that PTR-MS opens up new possibilities for food research laboratories and have provided examples of possible applications in basic and applied research. We are, however, far from complete

exploitation of its potential. Hyphenating PTR-MS with other spectrometric techniques, further developments in automatic sampling and in data analysis and visualization, and the availability of data on parameters relevant for food applications will further extend the range of scientific and industrial applications. Of particular interest is the possibility to use PTR-MS for in vivo evaluation and the effect on a broad spectrum of people of the interaction between food and consumers both from a sensory (nose space analysis) and nutritional (breath analysis) point of view. Our overall goal is to support a consumer-centric approach to sensory and nutritional studies.





Because they impart “off-flavours”, volatile phenols such as 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG), 4-vinylphenol (4-VP) and 4-vinylguaiacol (4-VG) represent a significant problem in modern wine-making. More rarely, vinylphenols can positively contribute to quality, i.e. to the spicy note of Gewürtztraminer wines, the genista-like flavour of Chardonnay and the aroma of Lambic and Weizen beers. White wines can contain vinylphenols in quantities up to several hundred µg, but usually lack ethylphenols. The contrary is true for reds, where ethylphenols can reach amounts of a few mg. In particular, even if present in quantities below the sensory threshold, 4-vinylphenol can negatively affect and mask fruity scents, conferring odours that resemble “Band-Aids” and gouache. When ethylphenols are present at higher levels and in combination with 4-vinylguaiacol, they produce phenolic, medicinal, pharmaceutical, smoky, spicy and clove-like odours.

Ethylphenols, again present in combination, confer animal-like odours. 4-ethylphenol in particular gives stable, horse-sweat and leather-like odours, while 4-ethylguaiacol is sometimes described as sweet. Vinylphenols are formed during alcoholic fermentation by *S. cerevisiae* strains that can decarboxylate cinnamic acids. Ethylphenols come from the enzymatic activities of decarboxylation of cinnamic acids and the subsequent reduction of vinylphenols by the yeasts of the *Brettanomyces/Dekkera* genera. Very small quantities are also produced by a few *Lactobacillus* spp.

Accurate quantification of volatile phenols is a basic requirement for minimising risk of off-flavours, however, the speed of the analytical methods available has been an obstacle to date. Analytically, the conventional approach is GC, equipped with flame ionisation or a mass spectrometric detector. This approach requires time-consuming extraction methods (i.e. liquid/liquid, SPE, SPME, HS-SPME, and SBSE).

New, faster HPLC methods

The Analytical Laboratories and the Oenology and Traceability research units developed two new analytical HPLC methods. Through the use of electrochemical (ED) and fluorimetric (FLD) detectors, it is now easier to control volatile phenols during the winemaking process. To be useful for quality control laboratories, the methods were designed to have accuracy, precision, and detection limits comparable to the most widespread reference methods, but are significantly faster and eliminate the need for sample preparation.

The operative procedures of the new methods are described below (Larcher *et al.*, 2007, *Anal. Chim. Acta* 582: 55-60; Larcher *et al.*, 2007, *Proc. Enoforum* 2007: 70).

HPLC- ED method

Two mL of wine are filtered with a 25mm x 0.45µm PTFE syringe filter and transferred into a 2mL glass screw-top vial. A HPLC equipped with an 8-electrode coulometric array electrochemical detector 5600A and CoulArray (ESA, Bedford, MA) is used. Isocratic separation is carried out (50mM NaH₂PO₄ buffer adjusted to pH 3.40 with phosphoric acid: ACN:MeOH, 65:30:5, by volume; flow rate 0.6mL min⁻¹) with a LiChroCART RP-18e Purospher column (125 x 3mm, 5µm particle size) kept

Reduction of off-flavours in wine through rapid measurement of volatile phenols

Roberto Larcher
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at 25 °C. The injection volume is 10µL. Routinely, only 4 electrodes are used, specifically at 340, 380, 550 and 800mV versus Pd/H₂ reference electrode, the dominant channels being at 380mV for 4-VG and 4-EG, and at 550mV for 4-VP and 4-EP. The use of eight electrodes (280, 340, 380, 430, 490, 550, 650, 800mV) makes it possible to resolve the few cases where 4-VG can partially co-elute.

HPLC- FLD method

The wine sample is filtered as reported above. Analysis is carried out with HPLC equipped with a fluorimetric detector (excitation at 225nm; emission at 320nm). Isocratic separation is performed with the aforementioned eluent, on a Zorbax Eclipse Plus C18 column (4.6mm x 50mm, 1.8µm particle size; 25 °C), with a flow rate of 1.5mL min⁻¹. The injection volume is 10µL.

Assessing volatile phenol risks in wine

In order to assess the actual extent of volatile phenol-related risk in the wines available on the market, the methods were applied to the analysis of

105 white, and 720 DOC and table red wines that pass through IASMA's chemical laboratory for various analyses. Vinylphenols were measured using HPLC-ED and 4-ethylphenol using HPLC-FLD, taking advantage of the particular rapidity of the latter analytical procedure.

Only 3% of the whites had vinylphenol content (4-vinylphenol + 4-vinylguaiacol) higher than the sensory threshold (720 µg), spoiling aroma quality.

The measurements carried out for 4-ethylphenol in the reds revealed values of 25th percentile, median and 75th percentile at 140, 325 and 860µg, respectively; meaning that a considerable percentage of them had a content (>426µg) which could potentially affect wine aroma.

We observed a trend towards higher concentrations in DOC wines (median = 400µg) than in table wines (230µg). This apparent contradiction with the typically higher quality image of the former must be traced back to the limited use of barrique ageing (and relative "Brett" pollution) for lower priced wines.

Advantages of the HPLC-ED method

The HPLC-ED method accurately analyses 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol, and 4-vinylguaiacol in wine as well as in beer. Compared to the previously published methods, the proposed procedure offers important advantages as it does not require sample preparation and performs chromatographic separation in roughly 15 minutes.

The method is linear up to concentrations of 2000µg L⁻¹ and precise (RSD < 3%). Limits of detection are low (1-3µg L⁻¹) and suitable for analytical requirements in the oenological field. In contrast to the electrochemical approach above, the HPLC-FLD method specifically quantifies 4-ethylphenol, carrying out chromatographic separation in less than 5 minutes. It is linear up to 2000µg L⁻¹, with RSD <3% over 20µg L⁻¹, and has a detection limit of 4.0µg L⁻¹. Such values satisfy oenological needs, accurately covering the concentration range within which 4-ethylphenol plays a negative sensory role.

The availability of new, rapid and sensitive methods now makes it easier for our research team to investigate options with regard to treatment with specific fining agents and to characterise yeasts and bacteria strains.

The Agrifood Quality Department has extensive experience in cooperating with industry in research and development projects, operating with state-of-the-art technologies to deliver innovative solutions. The department's instrumentation is primarily aimed at the two research pillars of the "Food for Life" platform: "Food and Health" and "Food Quality and Manufacturing". Along with the equipment, an essential expertise available to the department is a panel of experts trained to conduct sensory analyses. Our primary equipment and facilities are:

- 2,000 square meters of fully equipped analytical and microbiological laboratories with SINAL certification.
- Spectrometers: GC-MS (3),

HPLC-MS, PTR-MS, ICP-MS, NMR, IR-MS (3).

- Analytical techniques include: GC (15), HPLC (4), CI, FT-IR, ICP-OES, preparative HPLC, NIR spectrometer, UV-VIS spectrophotometers (2), and differential pH-meter.
- Microbiological tools include: autoclaves, fermenters, thermocyclers, refrigerated incubators, microscopes and stereomicroscopes, electrophoresis cells (4), laminar hoods, and microcentrifuges.
- Experimental winery with the capacity for 400 test vintages per year.
- A sensory laboratory with product preparation area, ten testing booths with serving windows, controlled light and software for data acquisition.

Facilities and Equipment





Massimo Bertamini

AGRICULTURAL RESOURCES DEPARTMENT

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

- Characterisation of key physiological, biochemical and nutritional factors involved in productivity and quality for Trentino's major crops: grapevine, apple and soft fruit.
- Genetic improvement (breeding and clonal selection) of cultivated varieties and rootstocks to sustain competitiveness of the local agronomic sector and through the continuous improvement of agronomic techniques and of conservation methods.

- Development of "precision farming" approaches using GIS and remote sensing technologies to better manage 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. The following sections present our main areas of research.

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 apply a multidisciplinary approach to agronomical and phenotypical characterisation, as well as to genomics research. Activities include agronomical and biological aspects of breeding and evaluation of new cultivars. While we continue to use conventional breeding techniques to create new cultivars, we increasingly apply genomics 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 a clone selection and genetic improvement program for the traditional grape cultivars of the Trentino region. Clonal selection is carried out in old vineyards. Selection criteria concerns 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 virus eradication) is performed in laboratories and greenhouses, while quality studies are carried out in field clone trials. In the end, using procedures specified by the national committee for the homologation of grapevine material, the clonal selection is officially registered and certified. Our clones are marketed under the trademarked SMA.





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 quality enhancement of fresh fruit to support regional fruit production and marketing. Regarding grape, our objective is to optimize the quality of fruit to maximize the quality of regional vine production.

Compared with annual crops, perennial fruit crops offer wide flexibility in the physical arrangement of their fruiting canopies in space. Not only can the size and shape of the canopy be dramatically altered by tree spacing, pruning and training, but the canopy can 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. We aim to manage plant canopies to optimise the way trees intercept light and convert this energy into optimal yields of top quality fruit.

Apple quality benefits from regular production from year to year, this can only be achieved when trees do not overproduce. Several chemical thinning agents are currently available. We use our fields to compare thinning efficacy of various agents to standard strategies for the main apple cultivars.

Following storage or transport comes the horticultural or harvestable maturity phase. In this development stage, 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 produced modelling approaches for use in estimating the maturity of grapes coming from various vineyards and from different cultivars. Such computer models provide help to indicate the optimum harvest time that 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 landscapes 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 actions aimed at promoting sustainable agriculture and rural development in the Alps.

Trentino's agricultural systems seek to create premium fruit and animal products through sustainable production that exploits the natural capital of environmental resources.

In particular, the low-impact management of alpine

pasture and mountain landscape protection is an important part of maintaining the natural environment of the Alps.

The aim of our activity is:

- To design and implement local action plans for sustainable agriculture 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.



Automation of grapevine accession management at IASMA

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Marco Stefanini

Information technology is having wide impact on germplasm research, natural resource management and plant genetic resources conservation. New tools offer opportunities for developing more efficient and effective systems for characterisation and assessment accessions, as well as for plant genetic resources management. Genetic resource evaluation has always been a prerequisite for increasing breeder use. The ability to identify phenological, morphological and genetic variation is indispensable to effective management and use of genetic resources.

With regard to *Vitis*, knowledge of the phenological stages is important for timing pest-control treatment, managing vines, and predicting key developmental events. Consequentially, *Vitis* phenological reliability data is important for improving vine use in different environmental conditions. Precision and speed in collecting phenological data thus assume a decisive role in accession assessment that aims to use the genetic diversity present in germplasm. Indeed, the ability to rapidly collect phenological phases is indispensable for improved characterisation and more effective use of data. Thanks in part to provincial funding of the GERECA project, we were able to assess an integrated RFID system in collection management and surveys. The example we discuss here involved the phenological characterisation of IASMA's core collection.

Accession method

The integrated system includes a hand-held computer with RFID reader, a global positioning system (GPS) receiver, RFID transponder read/write tags for accession identification, hand-held computers with data acquisition tools, and PC software, including the main database and a synchronisation tool.

The IASMA *Vitis* germplasm collection includes 2,688 accessions located in the 5 hectare Giaroni vineyard in San Michele all'Adige in Trentino province. Each accession of five established replicates was tagged with a radio frequency identification (RFID) transponder. More than 2,700 tags were inserted in the wooden poles in the vineyard of the IASMA collection.

Phenological growth stage data were collected on 189 accessions by means of a hand-held application based on BBCH-identification keys with the addition of two phenological stages, "end veraison-code 84" and ripening at "16 ° Brix-code 88". The selected accessions belong to IASMA core collection, established as the most representative samples of genetic and agro-morphological diversity. The phenological dates were registered three times a week from May to October of 2005 and 2006 for the following phases: start flowering (code 60), end flowering (code 69), start veraison (code 81), end veraison (code 84), and bunch grape reaching of 16 ° Brix (code 88). The Giaroni map associates the position of single vines to a unique

code composed of the name of the vineyard, name of the field, row number, block number and plant number (for example Giaroni/A F1-1-P1) and the geographic coordinates. Identification of the accessions on which the phenological phases were surveyed was assisted by a hand-held PC through RFID transponder reading. We confirmed each accession by going to the indicated field, row, and block of the vineyard. Next, we carried out plant selection by choosing the most representative from among the five available replicates. We did this by opening a template on the hand-held PC and inserting the registered phenological data using menus. Afterwards, data was transferred to the project database.



The dates of each phenological stage were converted in growing degree days (GDD) to compare 2005 and 2006 data. GDD were calculated as the sum of the differences between the mean daily air temperature and 10 °C threshold temperature from 1 April to 31 October. Mean values per accession and phenological traits were subjected to multivariate analyses by K-means clustering. Clustering was computed using normalized Euclidean distance by complete linkage method (farthest neighbour).

RFID: efficient data collection

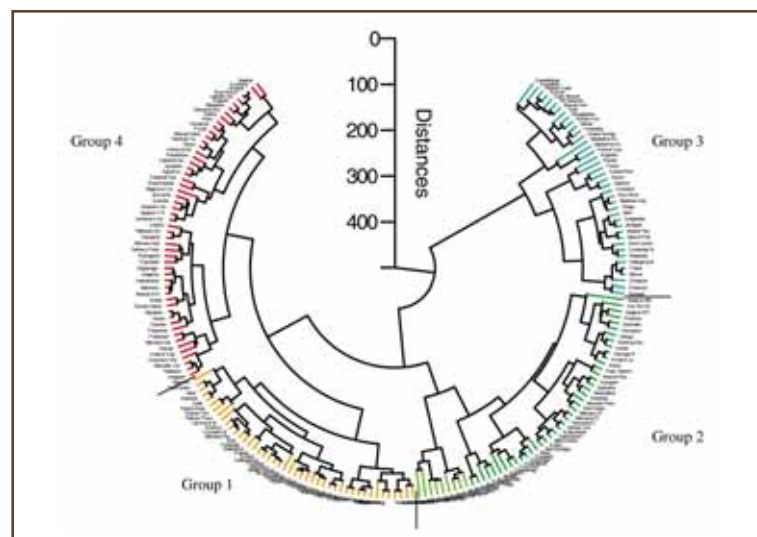
The integrated RFID system permits fast and sure field identification of accessions and allows for immediate insertion the data. The process reduced coding errors that occur in phenological phases from poor data insertion. Moreover, the survey data are immediately available for use due to the automated transfer to main project data base, speeding production of phenological maps and models. The integrated system RFID transponder worked efficiently for characterising accessions that were part of the phenological surveys. Differences in flowering time, veraison time and days to maturity reflected the consider-

able genetic variation present in the core collection. The results indicated that in the core collection the flowering time during 2005 and 2006 reached 26 and 27 days respectively, complete veraison occurred in 85 and 86 days, respectively, and the days the bunch grape needed to reach 16 °Brix after veraison were 65 and 61, respectively, for the two years. The flowering started at 358 and 352 GDD in 2005 and 2006, respectively. Similarly, complete flowering finished at 469 and 453 GDD; veraison started at 1125 and 1189 GDD, and it ended at 1352 and 1413 GDD. Finally, 16 °Brix ripening was reached with 1401 and 1514 GDD. Multivariate analyses by K-means clustering found four different classes with similar phenological behaviour in terms of precocity, namely classes 3, 2, 4, and 1 with 40, 51, 54, and 44 accessions respectively. The GDD trend significantly differed after flowering time. The figure offers a complete cluster tree representation of the accessions determined within the four groups. The nodes or clusters represent groups of accessions which are linked to each other by their degree of phenological similarity. *Muller Thurgau* accession resulted in the earliest, group 3, *Pinot Noir* and *Chardonnay* are represented in group 2, *Cabernet Sauvignon* in group 4, and, finally, the late ripening *Frappato* in group 1.

Germplasm evaluation

The integrated RFID system proved particularly efficient because it increased precision and speed of phenological data collection for characterising the accessions present in the core collection. It was especially useful in germplasm evaluation which

Cluster tree of the core collection with four groups of precocity



involves simultaneous examination of a large number of accessions of several characteristics, the first step in plant breeding programs. Our integrated system did not identify individual plants. (Placing an RFID transponder for each plant was too expensive.) Cluster analysis allowed us to quantify diversity in germplasm collections and to group accessions with similar behaviour in terms of GDD requirements. This information could be used in predicting phenological stages on a landscape scale, and in improving the vegetative and productivity output in different environmental conditions.

RFID transponders offer opportunities for developing a more efficient data collection system for germplasm. This integrated system proved is useful for plant collectors for its increased precision and speed in germplasm data collection, especially when variation patterns change quickly. Determining the presence of diversity within the core collection as well as clear ecotype differentiation is of great interest to breeding programs.





Implementing sustainable agriculture in mountain regions through collective action: IMALP Results

Giorgio De Ros

To promote sustainable agriculture, the European Union mainly applies a set of standard measures that focus largely on the farm, such as agri-environmental schemes, farm investment support, compensatory payments for less favoured areas, etc. The EU also supports area-based collective actions such as LEADER (“Liaisons Entre Actions de Développement de l’Économie Rurale”) initiatives. Although various expectations were placed on these actions, their effectiveness in finding locally adapted solutions to multidimensional sustainability problems is still questioned. To get deeper knowledge about that, a combined demonstration and research project was promoted by IASMA and three other European scientific Institutions (Service d’Utilité Agricole à Compétence



Interdipartimentale/Groupement d’Interêt Scientifique Alpes du Nord, the Department of Sociology of Innsbruck University, and the Institute of Agricultural Economics of the Swiss Federal Institute of Technology) and later funded by the European Commission in the Fifth Framework Programme. The Implementation of Sustainable Agriculture and Rural Development in Alpine Mountain

(IMALP) project ran from 2003 to 2006, articulated in a demonstrative phase and a research phase. Its demonstration phase involved four pilot areas: Moyenne-Tarentaise, France, Val d’Hérens, Switzerland, Murau, Austria and Val di Sole, Italy.

Rural development using participative approaches

The first step was the constitution of local groups representing the different actors concerned with the rural development of the areas (in particular farmers, elected officials and NGO members). Through the use of a participative, bottom-up approach and with the help of an activator, the groups elaborated “action plans” consisting in concrete innovative actions addressing the sustainability of local agriculture on three levels: farm and farming practices, farmer collective organisations, and the local level. In each area, an action group was responsible from four to eight

Sustainability action plan in Val di Sole

ACTION	OBJECTIVE
Recovering grassland from abandoned meadows	<ul style="list-style-type: none"> - Improve protection of landscapes - Improve use of local forage
Manure curing using a collective turning machine	<ul style="list-style-type: none"> - Adapt manure distribution to season - Correct input of fertiliser according to local conditions - Develop use of grasslands - Improve quality of sward to prevent soil erosion
Cooperative management of small acreage orchards	<ul style="list-style-type: none"> - Creating an additional income for farmers - Promoting collective organisation and self-help of part time farmers
Development of local products (“Casolet” cheese, sheep meat)	<ul style="list-style-type: none"> - Improve income opportunities for farmers - Improve local supply and image of typical valley products - Support traditional valley breeding activity and other agricultural activities
Recovering old apple cultivars	<ul style="list-style-type: none"> - Conserve local genetic variability - Reclaim unproductive orchards - Create income opportunity through innovative products (fruit juices or/and liquors)
In-farm educational activities	<ul style="list-style-type: none"> - Increase social recognition of farmers among local young people in the valley - Stimulate co-operation between different actors and institutions in the area in order to support agriculture and local traditions



actions. Stakeholder empowerment was not limited to the preparation phase of an action, but extended to implementation.

Evaluating demonstrative actions

A crucial part of the project consisted in the scientific evaluation of the participative approach adopted to develop sustainable agriculture. In contrast to other studies on partnerships in rural development, the scientific evaluation in IMALP was not an ex post analysis, but accompanied the demonstrative actions during all the phases of the project.

Two actions, preservation of old apple cultivars and co-operative field management of small-acreage orchards were aborted during the first implementation phase. The other actions had varying degree of success.

What outcomes can be highlighted by the carrying out of the action plan in the Italian pilot area?

As a preliminary conclusion, the actions promoted by IMALP in the Italian pilot did not seem to substantially influence the economic aspect of sustainability.

In this regard, broader national and international processes probably have greater influence. In contrast, change was seen in the social aspects of sustainability, i.e. in promoting interaction among the different productive and non-productive sectors (public institutions) of the local society.

The interest showed by some farmers towards the adoption of an environmentally friendly technique in manure management also indicates an opportunity for local participation projects in handling environmental aspects of sustainability.

Finally, some remarks can be made about three key factors:

- **Time frame.** Working on a territorial scale requires cognitive and cultural changes both for farmers and other actors. Thus, the whole implementation process can be considered to be a learning process. A short time frame facilitates people and groups which already have the “know-how”, but it turns into an exclusion factor for people and groups with less social resources. This is clearly evident in Val di Sole, where the participating farms are characterised by existing ties with nonagricultural institutions and bodies.
- **Legitimisation of the local group.** The Val di Sole group was created specifically for the IMALP demonstration phase, because there was no existing institution suitable for carrying out a multiactor participative project. Given the project’s duration and the available budget for the demonstrative actions, no formal institutional recognition was sought for the local group. This situation did not hinder the debate which, on the contrary, increased in vivacity and participation as the project progressed. But this was a problem for conflict resolution and for contacts with external, nonparticipating institutions.
- **Participative monitoring.** The self-evaluation made at mid-term of the project was an important step in the process of taking charge of the various actions by group members. In general, it had a positive effect on group cohesion and motivation. Consequently, we suggest that initiatives based on a bottom up approach also include participative monitoring.

Acknowledgements

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Chemical thinning of apple with new compounds

Alberto Dorigoni
Paolo Lezzer
Nicola Dallabetta

While at present the strategies for thinning the main apple cultivars are based on carbaryl, from 2008 onwards growers will have a hard time achieving adequate crop load without this well known compound. The tank-mix NAA+benzyladenine proved to be an option for thinning difficult cultivars like Fuji by using active ingredients that will still be available after 2008. Intensive but selective thinning occurred using metamitron, an a.i. currently registered as herbicide that has been recently proposed by the Eufirin Thinning Group as a high potential molecule for crop load control.

The apple industry benefits from regular production of high internal and external quality. A prerequisite for an annual crop load is a sufficient flower-bud formation in the preceding year. This can only be achieved when there are not too many fruits per tree, which through their seeds, negatively affect flower-bud growth. Thinning large number of trees by hand is neither practical nor economic and therefore thinning fruits by chemicals is customary. Several chemical thinning agents are currently available, but some may not remain on the market: at present carbaryl is the main chemical thinner used in Italy in combination with several others like NAD, NAA and benzyladenine, but its withdrawal from the European market has been scheduled for 2008. Back in 1995, IASMA joined the Eufirin Thinning Group, whose aim is testing new chemical thinners through common protocols.

A carbaryl-free alternative

One carbaryl-free alternative is based on the tank-mix NAA/BA, but its effectiveness strongly varies by cultivar.

Thus, a thinning program, optimal for one apple cultivar, can be unsafe for another one. For apple cultivars like Fuji, a double application of carbaryl reinforced by BA is usually the best thinning

technique.

One potential candidate, metamitron, was suggested by the Jork Obstbau Center, Germany, and was tested in 2006 by most of the Eufirin members. Metamitron is a commonly used herbicide which at low dosage reduces photosynthesis and consequently enhances fruit drop. In principle, it acts in a similar way to shading, by stress-

ing competition between shoots and smaller fruitlets, thus causing their starvation and drop. In 2006 metamitron thinning efficacy was compared to the standard strategies on the main apple cultivars and even on pear Conference. The IASMA pomology group participated in these studies and we present highlights of our research here.

Circumstances

TREATMENTS	DATE	FRUITLET DIAM.	TRADE PRODUCT	% ACTIVE INGREDIENT	CONCENTRATION		WETTER
					PPM A.I	CC/HL T.P	
3 e 5	4/5	6	Goltix	50% metamitron	350	70	---
4	8/5	9	Sevin	47% carbaryl	237	50	mineral oil
2	11/5	11	Dirager	3,3% Naftalenacetic acid (NAA)	10	30	mineral oil
			Brancher	10% benzyladenine	100	100	
4	11/5	11	Sevin	47% carbaryl	237	50	mineral oil
			Brancher	10% benzyladenine	100	100	
5	12/5	12	Goltix	50% metamitron	350	70	---

The trial was carried out in an 8-year old Fuji orchard in the Adige valley, spindlebush trained, at a spacing of 3.8x1.2 meters, grafted on M9.

The fruit set was measured on four branches per tree as the ratio between fruit number after June drop and the initial flower cluster number on the same branches.

Yield and external quality of each tested tree was measured at harvest by means of a Greefa grading machine. Data were analysed by means of the analysis of variance and Duncan test of the S.A.S. package.

The standard technique of carbaryl at 8-9 mm followed by a second application at 11-12 mm in tank-mix with BA resulted in adequate thinning and excellent quality in terms of fruit size and colour. The presence of BA enhanced fruit size beyond the simple effect of reduced competition among the left fruits on tree. On the contrary, the thinning effect of BA+NAA was satisfactory but, as often happens when using NAA, fruit size was less than expected with respect to sheer fruit number reduction. This is a well known consequence of the stress caused by NAA on trees after application. As for

metamitron, two weeks after treatment a slight chlorosis and stunted growth confirmed that this active ingredient temporarily hinders the photosynthetic system.

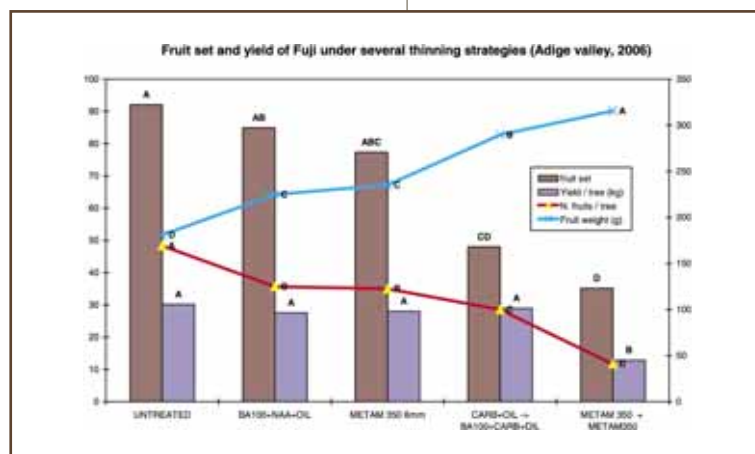
At harvest metamitron thinning efficacy was surprising: the single application at 6 mm was close to the crop target of 100 fruits per tree with fruit size of at least 250 grams. The double treatment at 6 and 12 mm fruitlet diameter strongly overthinned Fuji. This fact has to be considered an interesting achievement, since Fuji is the least responsive apple cultivar to all thinners and is often regarded as practically impossible to overthin using the standard chemicals, even in tank-mix.

The amazing fruit size, averaging 300 grams and without apples smaller than 70 mm in diameter, shows that despite transitory caustic action, this active ingredient acted in a very selective way, hitting the small fruitlets without affecting the ones with big growth potential.

At the same time this suggests that the clearly visible phytotoxicity was in the end less damaging than the NAA, which was apparently gentle, but in fact had strong growth inhibition.

Thinning potential of metamitron

From a pure technical point of view metamitron, at present quite far from being registered in Europe as thinner, showed great potential for crop load control. One of its key features is the dose-response, since double amounts of active ingredient strongly increased efficacy. Another important aspect is the flexibility of use, since it thins several cultivars, from the relatively easy ones like Golden Delicious to the most difficult ones, like Fuji. Its simple and “universally effective” mode of action suggests that unlike hormone-based thinners, whose activity is strongly related to specific cultivars, it can cover a wide spectrum of apple cultivars as well as other species. Indeed in 2006 the double application in similar phenological phases as the ones reported for Fuji was able to overthin Conference pear, with similar selectivity and enhancement of fruit size, despite phytotoxicity was stronger on pear than on apple.



Values in column followed by the same letter(s) do not differ significantly at the Duncan test ($p = 0,05$). ns: not significant at the analysis of variance

Fruit colour distribution also confirmed the negative correlation between fruit-set and presence of red overcolour. Again the double treatment with metamitron outperformed all other treatments.

In conclusion, when compared to the standard practice, NAA+BA represents a potential alternative for the years to come, when carbaryl will be withdrawn from the EU market. This tank-mix will be available to the growers for a reasonable period of time since both compounds have passed the EU test and have been placed in annex 1. On the other hand, it is not as effective as the standard strategies based on double carbaryl application reinforced by BA.

The moderate phytotoxicity shown on leaves 15 days after metamitron treatment (photo) disappears by harvest time



Facilities and Equipment

Ecophysiology and biochemistry laboratory

We use the following instruments for descriptive study of plant response to ambient conditions and causal analysis 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 (TEACHING-PAM)	Walz PAM-210

Leaf optical property

Plant Chlorophyll Meters	Minolta SPAD 502
Spectroradiometer UV/VIS	StellarNet EPP2000
Proximal sensing instruments	Skye Spectrosense 2+

Data loggers and microclimatic sensors

Dataloggers	Campbell Scientific CR10, CR10X, CR1000
Multiplexers	Campbell Scientific AM25T, AM16/32
Net radiation	Schenk pyrriadiometer 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.
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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 titrator
Digital refractometer
Pimprinnelle TR 53210
Texture analyzers

Breeding and production optimization

We use the following facilities and equipment are available for breeding 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 private vineyards under supervision



Riccardo Velasco

GENETICS AND MOLECULAR BIOLOGY DEPARTMENT

In the early 1990s, the centre's biotechnology unit got its start within the chemistry department, and competencies in genetics, molecular biology, genomics and applied biotechnology grew rapidly. This led to the creation of the Genetics and Molecular Biology (BGM) department in 2005. The 45 researchers, fellows, and technicians of the department work in three research units.

Genomics and Bioinformatics unit

With important competencies in various aspects of genetics

research, the Genomics and Bioinformatics unit is currently driving two significant genomics projects. The unit has reconstructed the 19 chromosomes of grapevine genome and the 17 chromosomes of apple genome, building several dense molecular maps. For the grapevine genome, the physical map was reproduced by assembling over 50,000 BAC clones. In addition, the genome was associated with genetic maps. These are viewable on the IASMA web site (<http://genomics.research.iasma.it/iasma>).

The decoding of the entire

genome was completed in December 2006, and the first draft assembled. The annotated grapevine genome has been deposited in international gene banks. A similar effort is currently ongoing for the apple genome, which is scheduled for completion in early 2008. Transcriptional profiling of differentially expressed genes during ripening, and biotic and abiotic stress are further objects of study by the unit. The unit also develops software for genetics and genomics tasks, as well as for database management and data mining.

Molecular Genetics (GM) unit

The Molecular Genetics (GM) unit concentrates on translating basic knowledge of the genomes of grape, apple and soft fruits – Trentino's most important crops – into tools useful for breeding. Development of molecular maps and markers is an essential part of the unit's molecular breeding program. In addition, molecular markers are being implemented for characterisation and certification of plant varieties and species. The unit is also involved in the correlation of markers and phenotypic traits, with particular attention on QTLs and monogenic traits related to pathogen resistance for *Rubus*, *Vaccinium*, and *Fragaria*; quality traits such as aroma compounds, polyphenols and secondary metabolites in grape and apple; and flower and seed development in grape.

Cellular and Molecular Biology (BCM) unit

The Cellular and Molecular Biology (BCM) unit is dedicated to gene isolation and characterisation



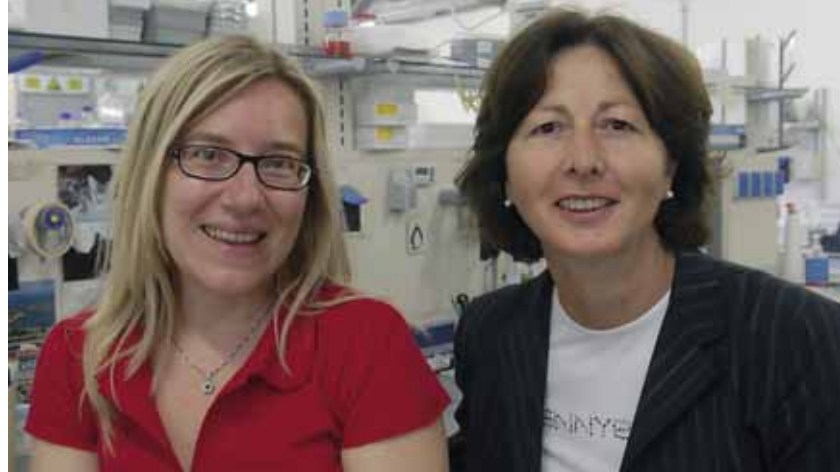


of gene function. The unit employs various functional genomics tools, including PCR select, differential display, AFLP-TP, and differential library screening to isolate the genes involved in apple and grape pathogen attack response. Cell culture of grape is also used for testing elicitor induction which 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

A recent departmental focus is on public perception of genetically modified organisms (GMOs). Working together, the BCM and GM units set up a GMO detection laboratory for food and feed to cover both research and service needs. Another joint project is the definition of gene function, monogenic traits and QTL position on maps as well as allelic variants. For this, the units work together with breeders, chemists and pathologist from other departments of the centre to define markers useful for trait selection in the centre's grapevine and apple breeding program.

Interdisciplinary approaches are essential for the success of such programs and, in this regard, the IASMA Research Centre is ideally positioned. The centre can now expand on the experience of having sequenced two genomes, and has the capacity to approach other genomes such as for pathogens or soft fruits. It also has an interest in the resequencing of varieties and species of grapevine and apple. A significant development for the department is a provincially financed, 5-year program to exploit basic apple and grape genome research to find applied research tools for biotechnology and breeding purposes. The program will be developed jointly, with this department and the Agricultural Resources departments playing major roles, but with the Agrifood Quality and Plant Protection departments also taking part.



Aroma is one of the traits consumers value the most in wines and in table grapes. Although wine quality is significantly shaped by winemaking practices, it derives primarily from berry flavour components, which are in turn determined by the complex interplay of vine genotypes, environment and cultivation methods (Lund and Bohlmann, 2006, *Science* 311:804-805).

The main determinants of grapevine aroma are volatile compounds (terpenes, norisoprenoids, thiols, methoxy-pyrazines), non-specific precursors (aromatic amino acids, organic acids) and non-odorant precursors in the form of sugar or amino acid conjugates of terpenes, norisoprenoids and thiols, which contribute to flavour upon hydrolysis. It is widely recognized that the

characteristic taste and aroma of muscat grape varieties are associated with the presence of several monoterpenes, the most important being linalool, geraniol, nerol, α -terpineol and various forms of linalool oxides (Ribéreau-Gayon *et al.*, 1975, *J. Agric. Food Chem.* 23:1042-1047).

These findings have been a major stimulus to research on free and bound grape monoterpenes, especially on their structure elucidation, their distribution within the plant, and changes in their levels during grape maturation and vinification.

At this time, relatively little is known about the molecular mechanisms underlying muscat flavour (the nature of the genes and enzymes involved and their regulation).

The reason for the lack of cer-

tainty lies in the fact that aroma presents continuous variation which is strongly influenced by environment and attributed to the joint action of many genes. This joint interplay is known as quantitative trait loci (QTL).

In order to identify the genomic determinants of muscat flavour, we mapped QTLs with molecular markers and characterised them through the candidate gene method, a promising approach for finding the associations between trait variation and genes involved in relevant pathways.

Its working hypothesis assumes that a polymorphism within the candidate gene sequence is correlated with phenotypic variation (Pflieger *et al.*, 2001, *Mol. Breed.* 7:275-291).

Here we report the main results of our research.

Genetic determination of Muscat aroma in grapevine (*Vitis vinifera* L.)

M. Stella Grando
Laura Costantini

Fig. 1 - Italia x Big Perlon progeny



Analysing the candidate genes

We analysed two F_1 grapevine progenies, segregating for berry aromatic composition. The first (163 individuals) was obtained from the cross between the table grape cultivars Italia and Big Perlon (Fig. 1), the second (175 individuals) derived from the variety Moscato bianco and an accession of *Vitis riparia*.

Following the segregation of molecular markers (mainly simple sequence repeats and amplified length polymorphisms) in the two populations, we obtained a linkage map representing the 19 chromosomes of *Vitis* for each parental variety. These maps were enriched by locating about 40 EST-based markers for candidate genes, which we selected according to predicted functions. Most of them are involved in terpene biosynthesis, which in higher plants takes place in two distinct pathways: the cytosolic acetate/mevalonate (MVA) pathway and the recently discovered plastidial 2C-methyl-D-erythritol-4-phosphate (MEP) pathway (Lichtenthaler, 1999, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:47-65). Other candidate genes encode enzymes involved in amino acid metabolism, stress-induced proteins and regulatory proteins of chloroplast gene expression.

In parallel, we conducted a phenotypic evaluation of all the progeny individuals at the same ripening stage, quantifying the compounds mainly responsible for muscat flavour. Working in collaboration with the Quality and Nutrition Research unit of the Agro-Food Quality department, we used the combined approach of gas chromatography and mass spectrometry.

In IxBP progeny, we analysed the volatile forms of linalool, nerol and geraniol for three growing seasons. Regarding MxR progeny, we measured for two seasons the content of the free and glycosidically bound forms of 18 additional compounds which contribute to aroma.

To identify the genomic regions responsible for trait variation, we combined genotypic and phenotypic data in QTL analysis, using a single marker and interval mapping methods. QTLs were detected for all the investigated traits, a number of which presented strong and reproducible effects across different years and genetic backgrounds. Clusters of QTLs, namely common QTLs controlling different traits, were also recognized on two linkage groups, probably reflecting the chemical relationships among traits (Versini *et al.*,

1993, *Proc. of Intern. Symp. "Connaissance aromatique des cepages et qualité des vins"*, Montpellier, France).

An interesting map colocalisation between candidate gene markers and QTLs was observed in some cases, which provides clues regarding the possible role of these genes in the regulation of aroma traits. The most interesting case concerns 1-deoxy-D-xylulose-5-phosphate synthase (DXS), which co-maps with QTLs for the content of several aromatic compounds (Fig. 2). It encodes the first enzyme in the MEP pathway, which is the dominant metabolic route for monoterpene biosynthesis in grapevine (Luan and Wüst, 2002, *Phytochemistry* 60:451-459). This suggests that DXS is a limiting enzyme for plastidic isoprenoid biosynthesis in this species, as already demonstrated in *Arabidopsis* (Estévez *et al.*, 2001, *J. Biol. Chem.* 276:22901-22909) and tomato (Lois *et al.*, 2000, *Plant J.* 22:503-513).

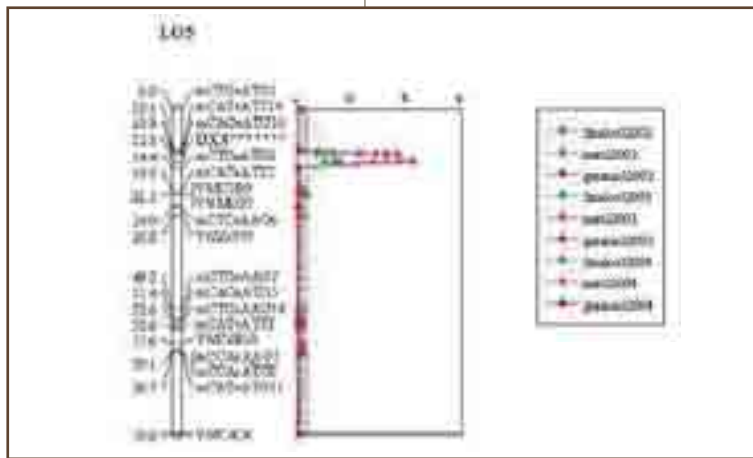


Fig. 2 - Co-mapping of the DXS marker and the QTLs for linalool, nerol and geraniol content on Italia LG5

Successful example of “genetic metabolomics”

This work represents a successful example of “genetic metabolomics”, that is, metabolite profiling combined with QTL analysis to detect the loci which control metabolite abundances. We identified the main genomic regions responsible for aromatic compound content and confirmed their reliability in terms of stability over time and genetic makeup. The application of the candidate gene approach allowed us to increase the resolution of our analysis and close in on the assignment of trait variation to the effect of specific genes.

We are now undertaking the study

of the candidate genes underlying QTLs in order to characterise them and validate their role in trait control.

The availability of the genomic sequence of Pinot noir has made it possible to characterise these candidate genes *in silico*, as well as to identify new positional candidate genes. Their functional characterisation is being performed through measurement of expression using real time PCR with contrasting phenotypes (including two clones of the same varieties) at different ripening stages.

This will allow us to verify the existence of developmental regulation of mRNA accumulation and of a correlation between tran-

scripts and terpene levels (PhD work of Jury Battilana, University of Padova). Further evidence that demonstrates the role of the candidate genes in the expression of aroma is being sought by testing allelic variation for the gene-trait associations in a grapevine germplasm collection provided by INRA-Montpellier, France. We expect that increased knowledge about the molecular mechanisms regulating flavour will lead to the identification of the key gene variants that will permit optimal aroma determination and lead to the adoption of practices which promote their expression, finally permitting consistently high quality grapes for wine production.

The occurrence of unexpected intermixing of feed, in particular with genetically modified (GM) soybean, is emerging as a major problem in animal husbandry. Besides maize (*Zea mays* L.), soybean (*Glycine max* L.) is also an essential ingredient of feed, being an elite energy and protein source for animal nutrition. At least 95% of soybean used in Europe is imported – mostly from the USA, Argentina, Canada and Brazil – where much of the soybean under cultivation is genetically modified, and where regulation on traceability is less restrictive with respect to Europe.

Intermixing may be the result of careless handling in various steps of product importation, occurring during transportation, stocking and distribution. As a consequence, traces of GM soybean may be present in a stock of supposedly GM-free raw materials. Our laboratory analysis confirmed that soybean which is not specifically labelled as having GM content in fact often contains high percentages of transgenic material, whether in feed components or in mixed formulations of fodder.

Such unexpected intermixing is emerging as a problem for the feed and food quality control. Even EU regulations on GM food and feed (1829/2003/CE and 1830/2003/CE), while establishing precise traceability and labelling rules at present do not consider this kind of mixing, even though the rules also contemplate accidental GMO contamination among materials of the same kind. The increasing relevance of stakeholder concern on this subject, however, is pushing authorities and laboratories toward formal GMO control to detect unexpected mixing in food and feed.

In this framework, we developed an analysis that resulted as useful for determining the unexpected occurrence of GM soybean in a single-component meal containing different species (Dalla Costa and Martinelli, 2007, *J Agric. Food Chem.* 55:1264-1273).

Our study was part of a research project supported by the Autonomous Province of Trento that aimed to

set up innovative methodologies for improving the traceability and detection of GMO in food and feed (Project *Detection of Genetically Modified Organisms in the Agrofood Chain*, OSSERVA3).

Real-time PCR in GMO detection

The analytical method is based on real-time PCR. The analysis was set up on a model system where we simulated a soybean intermixing with a sample of maize powder at various mixture levels (0.1%, 0.5% and 1%). The preparation of the soybean-maize mix test samples simulating the intermixing of soybean with maize meal was done carefully, homogeneity of the mixture being a crucial preliminary step of our analysis. DNA for subsequent analysis was extracted from these mix levels.

The aim of our assay was to quantify the soybean intermix and the consequent possible GM soybean component in the total sample. This strategy differs from conventional GMO analysis because it calculates the ratio of soybean (total and GM) in maize. First, we set up two main aspects involved in real time PCR analysis, i.e. the calibrator for building the standard curve and choice of the proper reference



Issues of GMO detection and unexpected intermixing in feed

Lucia Martinelli
Lorenza Dalla Costa



gene. This latter is a species-specific endogenous gene that is proportional to the DNA quantity of the species analysed. It had to fit some basic requirements, such as species-specificity, no intraspecific variability, and presence in single or low-copy number in the genome. In our analysis, the calibrator system needed to be maize DNA at known percentage of soybean intermixing that allows quantification of soybean presence in unknown samples by comparison.

Such a calibrator was not commercially available, thus we built *ad hoc* plasmids. Those contained the sequences of both soybean and maize endogenes in tandem orientation and in a 1:1 ratio. The soybean *lectin* gene is an already accredited gene in literature (Vodkin *et al.*, 1983, *Cell*, 34:1023-1031), whilst for maize the suitability of four different genes (*alcohol dehydrogenase 1*, *high mobility group protein a*, *invertase 1*, and *zein*) has been variously reported (Hernandez *et al.*, 2004, *J. Agric. Food Chem.* 52:4632-4637).

For this reason, we tested four kinds of duplo-plasmids, each one containing the soybean lectin gene alternatively combined with one of the four maize endogenes.

The aim of our assay was to identify the plasmid (or plasmids) that provides the best accuracy and precision performance in quantification. For this reason we performed the analysis on three mixture levels at known percentages of soybean intermixing with maize meal (0.1%, 0.5% and 1%). These samples were prepared by mixing GM soybean (certified Roundup Ready standard Fluka at known percentage) with maize meal. According to our results (Dalla Costa and Martinelli, 2007, *J Agric. Food Chem.* 55:1264-1273), duplo-plasmids containing *adh1-lect1* and *zein-lect1* genes resulted as the most reliable calibration systems for this analysis, providing precise and accurate quantification results. On the contrary, *hmga-lect1* and *ivr1-lect1* systems respectively over- and under-estimated the expected percentage values.

A reliable method for quantifying intermix levels

The method we developed proved to be a reliable analytical assay for determining the unexpected occurrence of GM soybean in different simulated intermix levels with maize meal. The use of duplo-target plasmids as calibrator standards forms the crucial part of the overall analysis, and proved to be a powerful tool for real-time PCR analysis.

Our system offers a strategy that would also be useful for detecting and precisely quantifying the presence of various feed ingredients in a certain feed composition. In fact, once they were built up, specific plasmids with the species-specific endogenous genes of the target plant species, presence and reciprocal proportions could be detected and quantified. This would be particularly useful for ascertaining the source of the protein component in a feed when less expensive alternative protein sources might be employed as substitutes for soybean. As a consequence, the actual value of the product can be determined.

A further application of our method concerns the detection of unexpected intermixing as a consequence of the coexistence between GM and non-GM species in a territory. In Trentino province, this could be the case of some local maize varieties renewed for their specific quality marks that integrity programs need to preserve.





Genome-wide transcriptional analysis of grapevine berry development

Claudio Moser

What's behind a glass of premium wine? "High quality berries and a good job in the cellar", most enologists would reply. Our research unit started to investigate berry quality three years ago because of its economical relevance to Trentino province. Considerable international research efforts are being devoted to shedding light on the molecular mechanisms behind the phenological and biochemical changes that occur during berry development and on their relation to wine quality.

Our functional genomics approach to answering these questions was encouraged by recent dramatic improvements in grapevine genomic resources, such as an extensive EST collection, the availability of commercial *Vitis* microarrays, and a physical map of the cultivar Pinot noir. The project aims on the one hand to identify transcriptional pathways correlated with berry quality and to look at how they are influenced by viticultural practices, and on the other hand to support IASMA grapevine breeding programs with the aim of developing better quality cultivars.

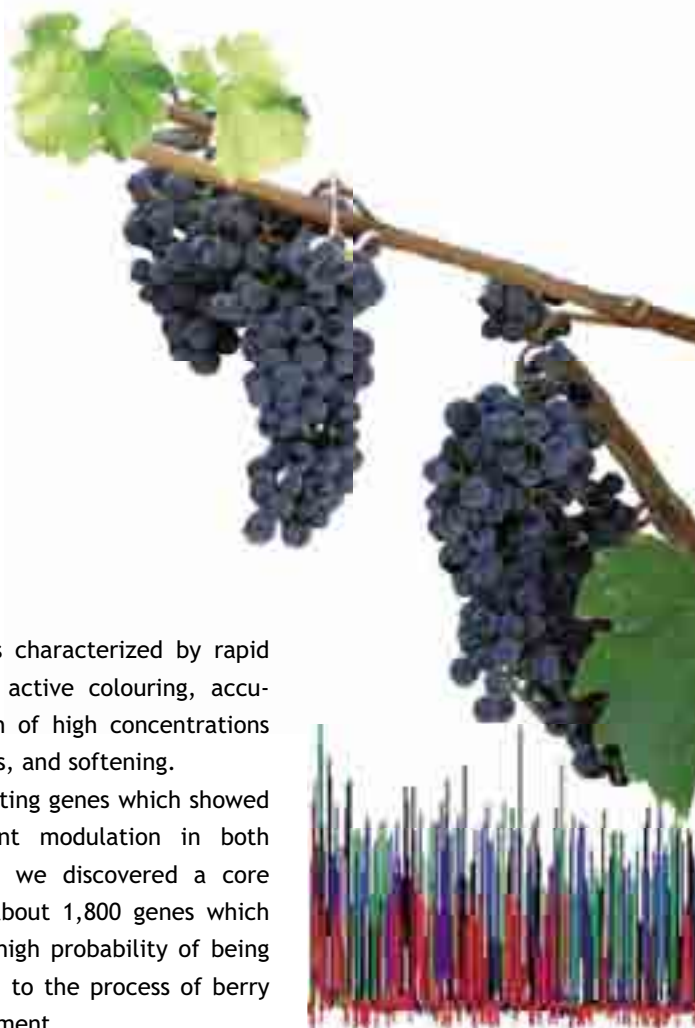
DNA microarrays

We investigated fruit development in the *Vitis vinifera* cultivar Pinot noir at the transcriptional level through the use of dedicated DNA microarrays which contain approximately 14,500 unique genes.

The technology of microarrays is rather new and it has opened up the possibility to study in a single experiment the relative abundance of all the transcripts present in a sample at a given moment. In this way, we obtained gene expression data from berries sampled in three distinct developmental stages in two different growing years. The first stage corresponded to small green berries still accumulating organic acids, the second to berries undergoing colour change from green to red (*véraison*), and the third stage was represented by berries in the ripening phase,

which is characterized by rapid growth, active colouring, accumulation of high concentrations of sugars, and softening.

By selecting genes which showed significant modulation in both seasons, we discovered a core set of about 1,800 genes which have a high probability of being related to the process of berry development.



The genes involved

An early conclusion was thus that the metabolic and phenotypic changes that occur in the berry during its development are strictly associated to broad transcriptional changes. A further line of evidence came by the assignment of the modulated genes to functional categories, which underlined three of them as over-represented. Genes involved in cell wall organisation, secondary metabolism and stress response were specifically induced during the ripening phase. Moreover, the analysis of the times from pre-véraison to véraison showed

a general bias towards induction of the categories involved in regulatory mechanisms, mainly transcription factors, hormone metabolism and signal transduction. This observation suggests a strong cell reprogramming taking place in berry cells up to véraison. From véraison to ripening, instead, a marked negative regulation was evident for categories involved in energy production and cell division, in agreement with the slowing down of cell replication and the loss of photosynthetic capacity and, on the other hand, the prevalence of metabolic processes such as cell wall loosening, sugar accumulation and transport of metabolites responsible for grape colour and flavour.

We also saw at the véraison stage a previously undetected oxidative burst as already reported in tomato and strawberry: glutathione-S-transferases, peroxidases and peroxiredoxins which are key players of the cellular antioxidant system.

Over 17% of the modulated gene

set was characterised by genes involved in regulatory processes, such as transcription factors and transcripts related to hormonal metabolism and signal transduction. We identified 120 modulated transcription factors, among which members of the myb, MADS-box, NAC and WRKY families were the most represented. Amongst the genes related to hormone metabolism, those related to auxin and ethylene were the most abundant, followed by those related to abscisic acid and brassinosteroids. These observations are consistent with our knowledge of the relative importance of these signalling pathways during berry development.

Finally, we were able to separate ripening-specific isoforms within gene families and to identify ripening related genes which, besides the berry growth stage, also appeared strongly regulated by seasonal weather conditions. These genes showed quite different expression profiles in the two seasons under study.



A powerful resource

Time-course gene expression analysis of grapevine berry development identified two very distinct phases during the development process. The pre-véraison phase represents a reprogramming stage of the cellular metabolism, characterised by the expression of numerous genes involved in hormonal signalling and transcriptional regulation. The post-véraison phase is characterised by the onset of a ripening-specialised metabolism responsible for the phenotypic traits of the ripe berry. The large number of regulatory genes we have identified represents a powerful new resource for dissecting the mechanisms of fruit ripening control in non-climacteric plants.



Due to its cultural and economic importance, wine grape was an obvious candidate for the first woody plant to have its genome deciphered. Highlights on grapevine genome have been of great interest since the International Grapevine Genome Program was funded with the aim of stimulating innovation in grape breeding and biology. Our research focused on the elite cultivar Pinot Noir with the multiple goals of genome assembly, gene identification and annotation, and identification of a maximum number of polymorphisms. Of special interest to biologists and breeders are polymorphisms in and around the coding regions. Pinot Noir is highly polymorphic, with two clearly distinguishable haplotypes that reveal several million single nucleotide polymorphisms SNPs and small insertion/deletion (In/Dels). These polymorphisms, along with trait and QTL marker association, represent a substantial resource for molecular breeding programs. A total coverage of seven genome equivalents of libraries of ascending size sequenced by the Sanger method, coupled with systematic highly parallel automated primer walking and 4.2 genome equivalents of 454 Life Science™ sequences, allowed us to create an effective genome sequence. Assembly was then reached by adding sequences of two BAC libraries and a fosmid library which were end-sequenced to assemble large meta-contigs. Contigs were oriented and ordered on appropriate chromosomes by high throughput marker development and genotyping in an F₁ cross of Syrah x Pinot Noir (<http://genomics.research.iasma.it>).

The grapevine genome sequencing project

Riccardo Velasco
Michela Troggio

Crossing population Syrah x Pinot Noir

The mapping population consisted of 94 progeny plants from a cross between *V. vinifera* cvs. Syrah and Pinot Noir (clone 115) obtained and grown at IASMA. Genomic DNA was extracted from young leaves following both the protocol described by Doyle and Doyle (1990) with slight modification (Grando *et al.*, 2003, *Theor. Appl. Genet.* 106: 1213-1224) and the DNeasy Plant Mini Kit (Qiagen, The Netherlands).

SNP-based marker development

A total of 454 EST clusters (Moser *et al.*, 2005, *Funct. Integr. Genomics* 5: 208-217) were selected based on homology with transcription factors or coding sequences putatively involved in sugar, flavonoids, and defense-related metabolic pathways. Also selected were 905 primer pairs defining *in silico* unique BAC-end sequences (BESs). Two different approaches were explored for the identification of SNPs: single-strand conformation polymorphism (SSCP) on both non-denaturing gel and fluorescence-based capillary electrophoresis and resequencing. The largest part of the SNP-based marker polymorphisms was then assessed by the multiplex minisequencing technique on the ABI PRISM® 3100 Genetic Analyz-

er (Applied Biosystems) (Troggio *et al.*, 2007, *Vitis* in press). Polymorphisms discovered by assembling the two haplotypes of the Pinot Noir genome were used to develop additional SNP-based markers using the SNPlex™ Genotyping System. The genomic DNA was prepared following the SNPlex™ Genotyping system 48-plex user guide and the samples were run on an ABI PRISM® 3730xl Genetic Analyzer (Applied Biosystems). Data obtained were analyzed using the software Gene Mapper v. 4.0.

Mapping

Recombinational values among markers were established by considering the F₁ population of the cross Syrah x Pinot Noir as a cross-pollinator population. Linkage group assignment and ordering of loci was established based on newly developed software that finds the maximum likelihood map using an error-compensating model (Cartwright *et al.*, 2007, *Genetics* in press). Linkage groups were initially determined using the 'grouping' application with a minimum LOD of 8.0 and a maximum distance of 35 cM. An initial order was built using the 'builder' application, which starts with a linkage group in which the mark-

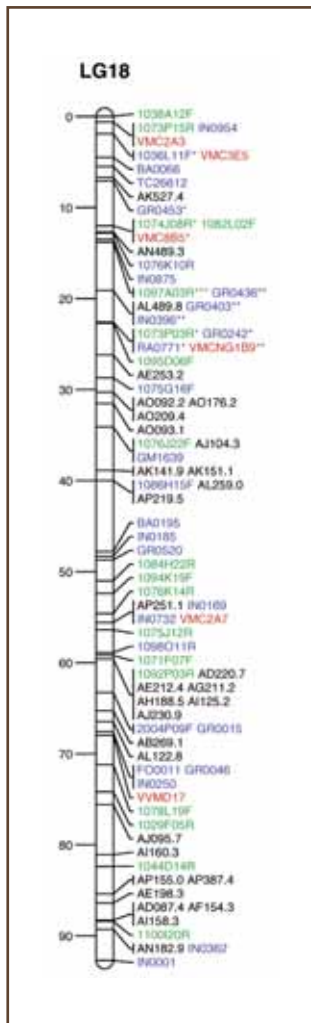
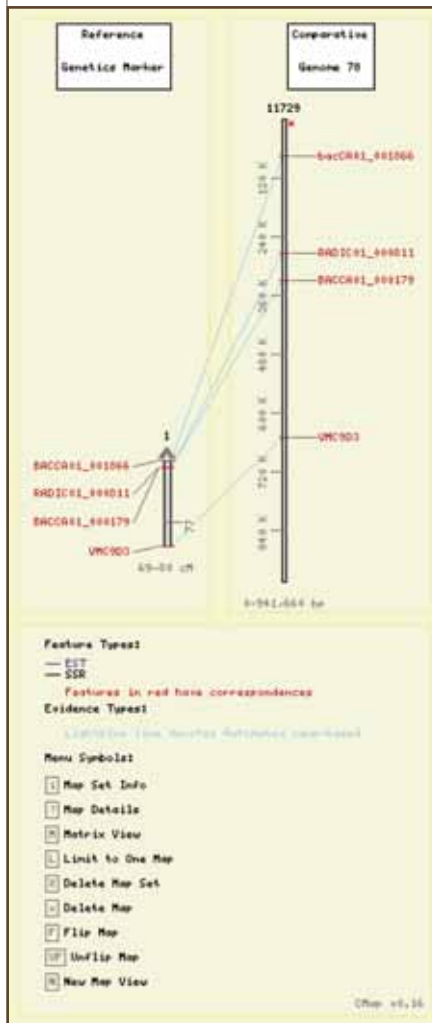


Fig. 1 - Consensus linkage group 18 of *Vitis vinifera* from the Syrah x Pinot Noir cross. Markers from EST and from coding BES regions are shown in blue, non-coding BESs in green, SSRs in red and AFLP markers in black. Distances of markers from the top are indicated on the left in cM

Fig. 2 - Region-dependent estimations of physical and recombination distance. Image displayed by the comparative map tool CMap of linkage group 1 (69-80 cM interval), and metacontig 11729 from the assembled genome (right side). Red colored markers in the CMap display indicate that there are correspondences between marker loci in the genetic map and markers in the assembled meta-contig



Sequencing and genome assembly

Libraries of 2-5 kb, 8-10 kb, and 15 kb in size were produced for clone-end sequencing. Fosmid and BAC clones were then added to the final assembled smaller clone sequences to assemble large meta-contigs. A total of 3.5 billion of nucleotides were sequenced using Sanger from libraries of different sizes. In order to increase the quality of the resulting meta-contigs, a fruitful collaboration with 454 Life Science produced an additional 4.2 genome equivalents (2.2 billions of nucleotides). These were added to the 7 genome equivalents produced by IASMA and Myriad using Sanger sequencing, covering over 25,000 gaps and elongating a similar number of contigs. Finally, merging of genetic and genome data was obtained by identifying the position of the markers on the genome, comparing the two sets of data and correcting reciprocal errors. Currently, 66,164 contigs merged into 2,177 meta-contigs covering 503,1 Mb have been submitted to the Genbank and EBI databases, and are available on the IASMA web site, organised in 19 chromosomes. Gene prediction and annotation was performed by the IASMA bioinformatics team in collaboration with Softberry and gene function was defined following Gene Ontology terms.

In the near future, gene identification and function validation will be the basis for molecular breeding applications and biotechnologically novel approaches that benefit human health and the environment. In particular, our major interest on molecular breeding will regard allele characterisation by single nucleotide polymorphisms (SNPs) of genes involved in metabolic pathways of terpenes, flavonoids, organic acids, sugars, or resistance gene analogues (RGA) putatively involved in plant-pathogen interaction.

ers have not yet been ordered. Markers were interactively removed to see which ones distort the distances greatly. The retained markers for each linkage groups were ordered with the 'improve' command. The Kosambi mapping function was used to convert the recombination frequencies into map distances (cM). MapChart v2.1 software was used for the graphical visualisation of the linkage groups.

For the genus *Vitis*, we developed a dense genetic map and demonstrated its physical anchor to the sequenced genome of Pinot Noir. The map was generated from the segregation of 1110 SNP-based genetic markers (180 from ESTs, 317 from BESs, 613 from genomic contigs), 132 simple sequence repeats (SSRs) and 379 amplified fragment length polymorphisms (AFLPs) in a mapping population of 94 F_1 individuals derived from a *Vitis vinifera* cross of the cultivars Syrah x Pinot Noir (Fig. 1). SNP-based genetic markers were developed from EST and BAC-end sequences and from unique regions of the assembled genome of Pinot Noir (Troggio *et al.*, 2007, *Genetics* in press). Polymorphisms discovered by assembling the two haplotypes of the Pinot Noir genome were used to develop markers for each meta-contig and to correlate them with linkage groups. SSCP, minisequencing, and high-throughput techniques were used to map the SNP-based markers on the F_1 population. All sequenced markers that were well ordered on the genetic map were used to order and orient meta-contigs on the appropriate linkage groups. An example of region-dependent estimations of physical and recombination distance is shown in Fig. 2.

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 a tissue liophylizer, an automatic DNA extractor, a Perkin Elmer Multiprobe IIX expanded automatic liquid handler, an automatic TECAN platform, six ABI 9700 thermocyclers of 96 and 384 wells, four Applied Biosystem DNA Sequencers (one ABI 310 with a sin-

gle capillary, two ABI 3100 with 16 capillaries, and one ABI 3730xl with 96 capillaries).

These instruments are commonly used for DNA sequencing projects, molecular marker and genetic map development, and marker screening and genotyping.

This 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 454 Life Science or an ABI SOLID system.

Facilities and Equipment



Functional genomics platform

The functional genomic platform is composed of four sterile benches for cell and tissue culture where grape, tobacco and Arabidopsis are used for gene function-transgenic assays, three real-time thermocyclers (one ABI 7000, one iCycler iQ Thermocycler (Bio-rad) for gene expression assays, one Amersham Pharmacia Typhoon 9210 plus phosphoimager for DNA analysis, and one radioisotope laboratory.

The functional genomics unit is specialised in automatic RNA extraction from grape and apple tissues for gene expression analysis of single genes, as well as the entire transcriptome (Affimetrix and Combimatrix assays). An additional eight thermocyclers are distributed across the 800 square meters of recently equipped molecular biology laboratories. Recently, a fluorescent stereomicroscope (Leica MZ16FA) was set up for noninvasive GFP reporter gene detection, adding the department's abilities with GUS and NPTII transient and stable transformation analysis. A growth chamber area completes the setup of the functional genomics unit. In 2007, 250 square meters of green houses and three additional growth chambers will increase the amount of stable transformation assays in grape, apple or model systems.

Bioinformatics platform

The prerequisite core of any structural and functional genomics assay is a strong bioinformatics platform. The bioinformatics unit runs a terabyte sized server and large client PC network. This unit manages the grapevine apple genome databank which provides data through a Web interface, as well as through a dedicated private virtual network. The databank provides an overview of the genomes and their functional fractions by integrating genetic maps, physical maps, genomes, and transcriptome assays. Bioinformatics expertise includes semi-automatic genome annotation using novel algorithms based on gene ontology.



Gianni Zorzi

NATURAL RESOURCES DEPARTMENT

The department is organised into four research units: Forest Ecology and Physiology, Limnology and Fish, Biomass and Renewable Energy, and Agrometeorology, Climatology and Aerobiology.

Forest Ecology and Physiology unit

The principal objective of the Forest Ecology and Physiology unit is to study climatic changes and their impact on forest ecosystems, forest physiology and genetics, biodiversity conservation, as well as ecological assessment of forest ecosystems through integrated monitoring. Principal works carried out in recent years include studies on the incidence, damage and biological control of root-rot fungi, investigations on the bioethology, ecology and biological control of phytophagous species, and the taxonomic identification and biodiversity monitoring of Orthoptera communities with the goal of identifying bioindicators of environmental change. Further activities have examined in detail the genetic variability of Norway spruce in Trentino in relation to high quality timber production, and the ecological evaluation of cypress in the Trentino landscape, studying sustainable cypress introduction, its potential diffusion to vocational areas and its exploitation in landscape amenity projects.



Limnology and Fish Research unit

The mission of the Limnology and Fish Research unit is to carry out research on the ecology of lakes and rivers, and to conduct long-term research on selected sites and genetic studies on freshwater species.

In this context, important studies have recently been carried out on the trophic evolution of lake ecosystems in Trentino, including long-term studies on the physical, chemical and biological characteristics of Lake Garda (which has been included recently in the Long Term Ecological Research (LTER) network), and limnological investigations for the restoration and rehabilitation of some Trentino reservoirs. Further studies have been 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 functionality indices in freshwater ecosystems based on the study of epilithic and epiphytic diatoms. Beyond the monitoring of fish in freshwater ecosystems for the fisheries management plan of Trentino, ichthyological research has addressed genetic diversity in and aquaculture potential of natural salmonid populations, as well as the distribution, characterisation, and rearing protocols of salmonids in southern alpine lakes. In particular, specific studies have examined the sustainable management of marble trout (*Salmo trutta marmoratus*) with the goal of its conservation in the Adige basin through both genetic and ecological characterisations.



Biomass and Renewable Energy unit

The Biomass and Renewable Energy unit carries out investigations on energy production from renewable sources, recovery and recycling of organic waste, and mechanical-biological treatment of solid waste before landfill disposal.

Recent studies on composting have investigated an aerobic treatment process aimed at organic matter recovery from various kinds of waste biomass and at the maintenance of soil fertility. Many related research

topics include: optimisation of the technological and process aspects; application of modern parameters to assess product stability (static and dynamic respiration index); identification and measurement of the environmental impact utilising new techniques such as olfactometry and electronic noses; definition of the best ways to use compost in agriculture and landscape-environmental projects.

In the field of renewable energy, researchers have studied the development and application of new technologies for biomass

treatment aimed at promoting its use as a renewable energy source. In this area, integrated management-systems for animal waste (husbandry) in mountain areas with high tourist vocation (odour reduction, soil and water protection, energy recovery) were specifically defined. The biological process under study is anaerobic digestion aimed at recovery of energy (power and thermal energy) and at reducing the environmental impact from odour emission. Finally, the unit looks at final disposal methods that protect soil and water.

Agrometeorology, Climatology and Aerobiology unit

The mission of the Agrometeorology, Climatology and Aerobiology unit is to carry out studies of agrometeorology, microscale climatology, and aerobiology. The unit's agrometeorology researchers were recently involved in a project aimed at the study of frost dynamics and climate in alpine areas (establishment of the most suitable forecasting, alarm and protection techniques). Further investigations examined the possibility of using purified wastewater in Trentino for irrigation purposes. A dissemination project promoted diffusion of global climate change knowledge in the agricultural world.

The unit is also involved in service and monitoring activities, including maintenance of a station network for agrometeorology and alpine ecosystem monitoring; testing of innovative irrigation technologies aimed at optimising use of water resources; pre-processing of meteorological data for infection modelling; application of geomatic techniques for the territory analysis of meteorological data, and, more generally, of data concerning agrometeorological issues (water balances, meteorological downscaling, high-resolution climatology, etc.); local climatology investigations and consequences of climatic change on the environment and on the local agricultural system; improvement of weather forecast techniques for agricultural use and phenological surveys and modelling of crop and forest species. Recent activities in the field of aerobiology include the collection of information about atmospheric pollen content for diagnosis and prevention of respiratory allergopathy; the monitoring of air pollutant effects, using different bio-monitors such as lichens and pollen, and the testing of innovative methods for bioaerosol identification.



Ecological evaluation in alpine forest ecosystems by integrated monitoring

Cristina Salvadori

In a typical mountain province such as Trentino, where woods cover more than 55% of the surface area, the forest constitutes a priceless asset of primary importance. This is why the Autonomous Province of Trento (APT) participates in integrated monitoring programmes (ICP IM) for assessing forest health promoted by the United Nations Economic Commission for Europe and the EU. These programmes aim at gathering a full and comparable set of data on the cause-effect interactions between pollutants and forest damage at three survey levels. Thus, in the early 1990s, two intensive monitoring areas were added to the European network. The areas were set up for surveying (level II plots) and checked weekly to study the stability of the forest in relation both to natural and anthropic disturbances. A variety of research had already been carried out over the previous few years for other studies, but most was limited to individual parameters or based on short-term surveys (see table 1).

The EFOMI project

The EFOMI project (Ecological Valuation in Alpine Forest Ecosystems by Integrated Monitoring) offered the opportunity to obtain wider and more in-depth knowledge through an interdisciplinary study. EFOMI considered ecological aspects, economic sustainability and improvement of air quality through the balance of carbon sink, with the aim of obtaining a complete evaluation of the ecosystems examined. One area is situated at Lavazè Pass (Val di Fiemme, 1780m a.s.l.) and is made up of sub-alpine spruce woods. The stand is of natural origin and has a composite, multi-level structure. A second area is a mixed, thermophilous oak wood situated above Pomarolo (about 700m a.s.l.) in Val Lagarina. This wood is made up of mature trees, mostly *Quercus pubescens* growing on superficial soil.

The EFOMI project, co-ordinated by IASMA and involving national and European research institu-

Tab. 1 - The Integrated Monitoring subprogrammes carried out to date, partly with the relative frequency of acquisition, in the two plots (sampling intervals: c=continuous, d=daily, w=weekly, m=monthly, y=yearly)

IM SUBPROGRAMMES	SAMPLING FREQUENCY	BIOINDICATION		
		Arthropods	Micromammals	Trap control
AM: Meteorology	c/d			
AC: Air chemistry	c/w			
PC: Precipitation chemistry	w	<i>Araneae</i>	Insectivora	<i>Ips typographus</i>
TF: Throughfall	w	<i>Isopoda</i>	Rodentia	<i>Xyloterus lineatus</i>
SC: Soil chemistry	5 y	<i>Diplopoda</i>	Macromycetes	<i>Pityogenes chalcographus</i>
SW: Soil water chemistry	m	<i>Chilopoda</i>		<i>Tomicus minor</i>
RW: Runoff water chemistry	w	<i>Collembola</i>	Determination Radioactivity	<i>Tomicus piniperda</i>
FC: Foliage chemistry	2y	<i>Ins. Orthoptera</i>		<i>Rhyacionia buoliana</i>
LF: Litterfall chemistry	y	<i>Ins. Heteroptera</i>	Soil	<i>Tortrix viridana</i>
VG: Vegetation	1-5 y	<i>Ins. Auchenorrhyncha</i>		<i>Orgyia antiqua</i>
EP: Trunk epiphytes	1-5 y	<i>Ins. Coleoptera</i>	Ectomycorrhiza and Fine-Root System	<i>Lymantria dispar</i>
SF: Stemflow	w	<i>Ins. Lepidoptera</i>		<i>Lymantria monacha</i>
FD: Forest damage	y	<i>Ins. Diptera (Sirphidae, Cecidomyiidae)</i>	Soil Microbiology	<i>Thaumtopoea pityocampa</i>
AL: Aerial green algae	y	<i>Ins. Hymenoptera</i>		
MB: Microbial decomposition	5 y			
PH: Phenology	w			

tions, was organized in seven work packages:

- Vegetation description
- Procedures and models to estimate the biomass of forest stands
- Climatology and meteorology
- Chemical characterisation of environment
- Characterisation of zoocoenosis
- Ecological and physiological analysis
- Forest ecosystems sustainability.

Climates and bioindicators
Methodology and results of EFOMI are exhaustively described in the specially issued publica-

tion dedicated to the project (Salvadori & Ambrosi, 2005, *St. Trent. Sci. Nat. Acta Biol.* 81, (Suppl. 1): 1-276). IASMA's main activities concentrated on the ecology and physiology of forest trees, biodiversity of zoocoenosis, bioindication, climatology, soil and precipitation chemistry. Concerning the main climatic features, Pomarolo appeared as a site with "temperate, middle latitudes climate, with no dry season" and a moderate oceanicity. The temperature has significantly increased in the two last decades, whereas no evident trend for yearly precipitation was detected. The elevated summer temperature predisposes the area to deficit periods (Eccel *et al.*, 2005, *Int. For. Review* 7 [5]:99). The Lavazè Pass site presents a "microthermal climate, humid all year round", with continentality features. Winter is a cold

and relatively dry season. Rainfall amounts are lower than mean precipitation in the central-eastern Alps. Nevertheless, high humidity makes the area generally not drought-prone. Concerning the chemical characterisation of environment, the two forest soils are an iron-humic podzol (Lavazè) and a brown lessived soil (Pomarolo), with no evident anomalies or pollution by heavy metals and trace elements. As regards the chemical composition of atmospheric depositions, annual loads of acidity, nitrogen and sulphur were calculated for each site and seem good in alignment with their impact on forest health. The mean values (2001-2004) of the three variables are 21.8 eq ha⁻¹, 6.7 and 10.4 kg ha⁻¹ at Lavazè and 27.4 eq ha⁻¹, 9.8 and 16.1 kg ha⁻¹ at Pomarolo. The arthropodofauna at the two areas is dominated by a char-

acteristic set of species, part of which is strongly specialised. The suitability of three groups of insects as bioindicators (Auchenorrhyncha, Coleoptera Staphylinidae, Lepidoptera) was tested at both sites and critically evaluated. The research pointed out their value in monitoring status and change in alpine woodlands and in assessing a range of environmental problems such as pollution effects, land use changes,

long-term degradation and recovery of ecosystems. The results demonstrate the excellent suitability of leafhopper fauna for bioindication of changes in several factors such as rainfall, temperature and air pollution. Numerous stenotopic species of lepidoptera were observed both at Lavazè and Pomarolo. Many of these are related to very specific habitats and are often strictly monophagous.



The study area of Passo Lavazè (1780 m a.s.l., Trentino, Italy)

Current status and future priorities

This feature is key to successful host-plant oriented bioindication. On this basis, we were able to indicate good choices for bioindicators for use in future surveys at these monitoring sites. The study of small-mammal communities and the assessment of epiphytic lichen biodiversity supplied additional indications on the state and naturality of the ecosystems investigated. The studies carried out in the project framework were an interdisciplinary approach to the issue of the ecological stability of Trentino forests in a context of global change. Bearing in mind the knowledge acquired to date, EFOMI contributed to increasing the range of data gathered since 1992. From the data, we can draw the following conclusions:

- The condition of the forests we monitored, although affected negatively by past anthropic activities, did not show evident stresses from local or transboundary air pollution.
- Climate is the main conditioning factor on the two ecosystems studied, acting directly or in combination with particular site conditions. Water stress and winter dissection, in particular, are recurrent phenomena with a decisive role in the status of forest stands: the first accentuates the characteristics of aridity of some stations, slowing down the biogeochemical cycles, while the second interferes with the physiology of trees growing at the timberline.
- The elevated biodiversity of flora and fauna found to date and good crown conditions indicate a high degree of homeostatic capacity necessary to support and overcome adverse situations.
- The sustainability of the ecosystems studied calls for forest management increasingly directed towards maintaining high ecological stability, above all in protected forests or in those with difficult edaphic conditions.

In conclusion, the ecological evaluation of forests should be considered a priority research activity. Many more aspects have to be investigated in greater depth, such as the effects of ozone and fine dust, critical loads of pollutants, and climatic changes.



Improving minimum temperature prediction for frost control

Emanuele Eccel

Despite an increase in average global temperatures, frost is still a recurrent problem with reference to the consequent early spring vegetation recovery. The GEPRI (GELate PRImaverili or spring frosts) project studied the various items involved in spring frost research: frost climatology (including phenological modelling for apple), frost micrometeorology, protection technology testing, and temperature prediction improvement. This article sums up our findings and the operational applications of temperature prediction. Prediction of minimum temperature is a high priority for farmers. To assist them, agricultural tech-

nical services need to find and adopt a suitable means of comparing frost episodes. Since the 1920s, descriptive models, calibrated by empirical coefficients, were developed to determine the nocturnal minimum based on variables measured at sunset. However, these empirical methods were developed for plains and open areas.

Applying them in a morphologically irregular area is problematic. More recently, machine learning techniques have enabled modellers to include into prediction algorithms variables other than just temperature and wind velocity, and at the same time they exploit the potential

of nonlinear relations among predictors. Furthermore, their suitability in dealing with many predictors, makes them particularly fit in downscaling raw numerical weather prediction (NWP) output. Indeed, the latter is determined on a coarse grid, whose nodes are scattered unevenly over the geographical domain. Typical grid spacing ranges from a few dozen kilometres for general circulation models (GCM) down to a few kilometres for limited area models (LAM). The temperature at 2m above the surface is closely tied to the topographic position that the model assigns to any grid point in the digital terrain model.

Finally, our research looked at two approaches: nowcasting, starting from measured values at sunset, and forecast downscaling carried out on the NWP outputs from the previous morning. The meteorological network under study covers a fruit-growing area in Trentino situated in the Adige valley, from Piana Rotaliana to the north and Vallagarina to the south.

Nowcasting models

A temperature decrease can be modelled by a parabolic function, with a constant heat-loss rate given by the Stefan-Boltzmann law, which can be corrected by other quantities, such as sky cover, wind velocity, and air and soil moisture.

Fig. 1 - Error distribution in classes for nowcasting models (from Ghielmi and Eccel, 2006 *Comp. Electr. Agr.*, 54(2):101-114)

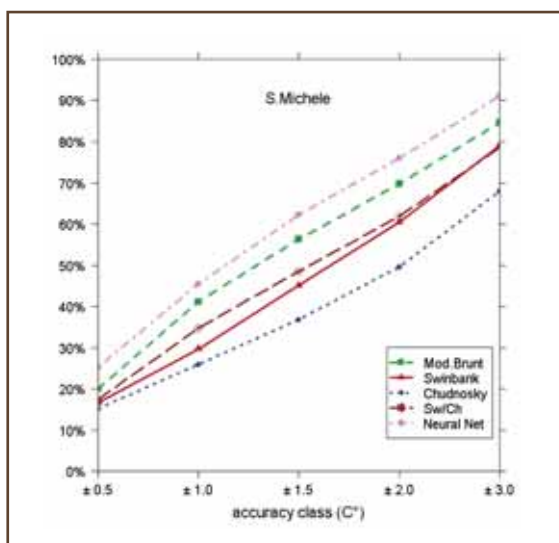
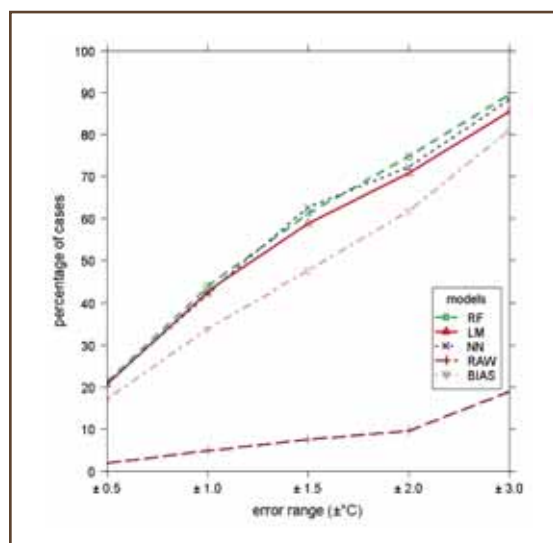


Fig. 2 - Error distribution in classes for downscaling models. RF: Random Forest; LM: multilinear model; NN: neural networks; RAW: direct model output; BIAS: simple bias correction. From Eccel *et al.*, *Nonlinear Processes in Geophysics*, 14



All tested models envisage calculation of long-wave radiation balance, where the outgoing term is lost by the soil-air interface towards the atmosphere ($RL\uparrow$), as the agent responsible for cooling the atmospheric layer in contact with soil. Yet, each model varies in the way $RL\uparrow$ is calculated. The methodologies we applied were Brunt, Swinbank, and Luerstein-Chudnosky.

For neural network models, we employed the Multilayer Percep-

trons algorithm. The main variables inputs to the neural networks were:

- Air temperature at 2m, soil temperature, relative humidity, and wind velocity (at sunset).
- Maximum and minimum temperature in the previous 24h.
- Night duration.

The comparison among models was performed on the basis of several indices: contingency tables (namely hit rate (HIR),

false alarm rate (FAR), and others), calculation of the root mean square errors (RMSE), and distribution of absolute error in classes. In general, among “traditional” equations, the choice of the best-performing one is non-ivocal and strongly site-dependent. Notably, differences arise among sites on frost-favourable nights, according to their liability to cold-air accumulation. But, in general, neural networks outperformed other models (Fig. 1).

Downscaling models

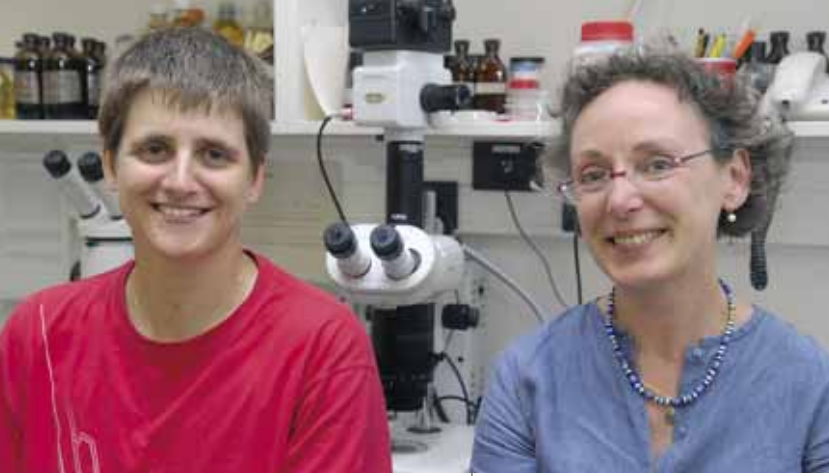
A model output statistics (MOS) approach was followed (Wilks, 1995: Statistical methods in the atmospheric sciences. Academic Press). Functional relationships (explicit or not) are built between numerical forecasts and observations which are obtained by using the model’s outputs as predictors (e.g. for a model run at 00 Coordinated Universal Time (UTC), temperature forecast at different grid points at a lead time of 30 hours after run-time), and measured quantities as predictands. Temperature at 06 UTC averaged over three stations inside the middle reach of Adige valley was selected as the MOS target. Two NWP were tested: one GCM (the routine ECMWF T511 - L60 from Reading), both for the 00 UTC and 12 UTC runs, and one LAM (Limited Area Model Italy, LAMI), for the 00 UTC run. The MOS techniques employed were the following:

- **Mean bias correction.** In both models, we selected the grid points that best predicted temperature for the target area, calculated the mean bias and corrected the prediction.
- **Multilinear Regression.** We applied a backward stepwise multilinear regression.
- **Machine learning techniques.** We applied both artificial neural networks and a “random forest” algorithm (RF), implemented in an R package (Liaw and Wiener: <http://stat-www.berkeley.edu/users/breiman/RandomForests>). An RF is formed by growing several regression trees, resulting in the average of the single tree’s outcomes. One of the most important features of the RF method is that it limits overfitting, even

when the ensemble contains thousands of trees. Site-related calibration improves temperature forecasts obtained from single equations. Even the optimal equation changes from one site to another. Yet, compared to ‘traditional’ models, improvement was evident when using neural network techniques. The approach can capture specific features of the climatic conditions of any site even if the neural network does not explicitly represent functional relationships with nocturnal cooling. Also, use of neural networks offers the unquestionable advantage of including explicitly within the pool of the input variables quantities that are absent in traditional equations or are only qualitatively taken into account. In the downscaling model, the residual error (expressed as MAE) is probably as low as possible after post-processing, yet it is still 1.2°C in the best case.

The very good agreement among multiparameter algorithms (especially the non-linear ones, Fig. 2) shows that there is a technical limit to the improvement that MOS methods can obtain. Postprocessing algorithms can calibrate and correct systematic errors, provided that relationships exist between these errors and output variables. It is not, however, effective in reducing errors of different origin. This would apply, for example, to errors in the prediction of night-sky cloud cover or wind speed by NWP. Further improvements in forecasting have to be sought in NWP itself, rather than in complex statistical downscaling. For more information, go to <http://217.222.71.209/meteo/agricoltura/gelate.php>.





The reddening of Lake Tovel - solving the mystery of “Glenodinium”

Giovanna Flain
Ulrike Obertegger

Lake Tovel, a cold oligotrophic lake in the Brenta Dolomites, is one of the most famous Italian lakes. Aside from its natural beauty, its fame is due to the bright red blooms that once graced the lake’s Red Bay. Scientifically these blooms were unique, since ‘red tides’ caused by dinoflagellates are usually restricted to marine environments. That explains why ecological studies of Lake Tovel and its surroundings have been an ongoing affair for the last 150 years. These investigations culminated with the in-depth work of the famous Italian limnologist (Baldi 1941, Mem. Stor. Nat. Ven. Tri, 6:1-297); Baldi placed particular emphasis on the life cycle of the dinoflagellate *Glenodinium sanguineum*, considered the causal agent of the red blooms. Since then, researchers have used Baldi’s studies as their starting point.

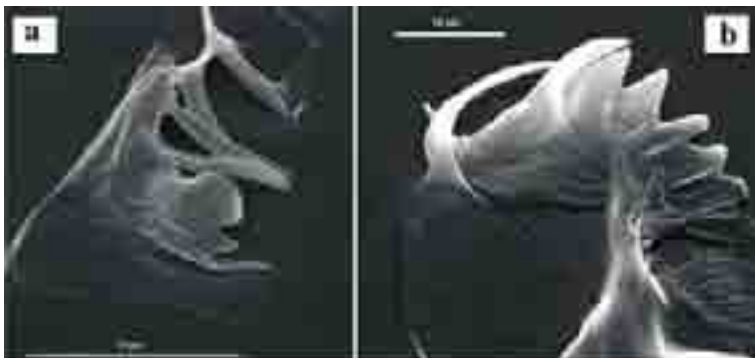
The abrupt end of these summer blooms in 1964 has been the source of argument, prompting the Autonomous Province of Trento to finance a research project aimed at investigating the causes of bloom cessation. The SALTO project, headed by the former director of IASMA, Basilio Borghi, and involving local, national and European research institutions, provided in-depth knowledge of Lake Tovel and its valley. Many aspects were studied, including local history, landscape ecology, paleoecology, hydrology, and limnology, all focusing on their possible relationship with the red bloom.

Perhaps the most interesting aspects to emerge from this project were the importance of taxonomy and hydrology—the former for a correct understanding of biological phenomena, and the latter as an important driving force in determining the composition of lake plankton.

Fig. 1 - Cysts of *Tovellia sanguina* (a) *Borghiella dodgei* (b) and *Baldinia anauniensis* (c)



Fig. 2 - Trophus of *Synchaeta kitina* (a) e *Synchaeta lakowitziana* (b)



A species complex revealed

The methodology and results of SALTO are detailed in the special volume dedicated to the project (Borghi *et al.*, 2006, *St. Trent. Sci. Nat. Acta Biol.* 81:1-476). IASMA’s main activities concentrated on limnology and plankton ecology with expertise on dinoflagellate taxonomy provided by Øjvind Moestrup’s Phycology group of the University of Copenhagen.

One of the problems that emerged during SALTO was the handling of plankton samples. Key planktonic organisms did not tolerate transportation from the lake to the laboratory.

On the other hand, they underwent morphological distortion when preserved with fixatives. This proved to be responsible for the misidentification and the under-identification of important components of the plankton in previous studies. Progressing from Baldi’s observations that the dinoflagellate responsible for the red blooms exhibited substantial polymorphism, we were able to clarify that ‘*Glenodinium sanguineum*’ as intended by Baldi and later on by Dodge *et al.* (1987, *Algol. Stud.* 47:125-138) is actually a species complex composed of three different dinoflagellates (Flain *et al.*, 2004, *Phycologia* 43:737-743).

The species are morphologically very similar in the vegetative stage

and especially difficult to distinguish in fixed samples, but have distinct cysts (Fig. 1). The presence of these cysts in the original Baldi samples (courtesy of the Plankton Museum at Pallanza) confirmed that '*Glenodinium sanguineum*' was indeed more than one species. While all three dinoflagellates are found in Red Bay, they occupy different ecological niches (Flaim *et al.*, 2006, *St. Trent. Sci. Nat. Acta Biol.* 81:447-457) and are genetically distinct (D'Andrea *et al.*, 2005, *Verh. Int. Ver. Limnol.* 29:478-481).

Mystery solved

Only one of these species is responsible for the red blooms and is now classified as *Tovellia sanguinea* (Moestrup *et al.*, 2006, *Eur. J. Phycol.* 41:47-65). Its intense red colour is due to the production of unusually high amounts of astaxanthin esters (Frassanito *et al.*, 2006, *Biochem. Sys. Ecol.* 34:843-853). In addition, the other two dinoflagellates also belong to newly erected genera: *Baldinia anauniensis* (Hansen *et al.*, 2006, *Phycologia* 46:86-108) and *Borghiella dodgei* (Moestrup *et al.*, *gen inedit, spec inedit*).

SALTO also helped clarify some taxonomical features in the rotifer genus *Synchaeta*. This ubiquitous soft-bodied zooplankton is rarely identified to species level because of morphological distortion in fixed samples compounded by confusion in taxonomic keys.

Our study (Obertegger *et al.*, 2006, *Zool. Anz.* 245:109-120) helped distinguish several *Synchaeta* species in fixed samples on the basis of trophus morphology (Fig. 2), a feature which does not undergo distortion upon fixation.

Furthermore, SALTO showed how lake hydrology, using water residence time as a proxy and ultimately reflecting precipitation events, can determine plankton dominance. The occurrence of documented bloom events was tied to summer precipitation, with dry summers favouring the formation of *Tovellia* blooms in the Red Bay (Flaim *et al.*, 2006, *Verh. Int. Ver. Limnol.* 29:1327-1330). Water residence time was shown to be an important factor for the structuring of the zooplankton community. Low water residence time tends to favour rotifer dominance, while high water residence time tends to favour crustacean dominance among the zooplankton (Obertegger *et al.*, 2007, *Aquatic Sci.* in press).

Besides clarifying the identity of the dinoflagellate responsible for the red blooms, SALTO also proposed a plausible explanation to the central question posed at its onset, i.e. why the red blooms ceased. Results from various studies indicate that change in land use in the lake's catchment were responsible for less nutrients reaching the lake, nutrients most probably needed to sustain an algal bloom. Both the start of the blooms in the 1860s and their termination in the 1960s coincide with changes in animal husbandry practices in pastures overlooking the lake (Borghi *et al.*, *ibid*).



The interplay of lake physics and biology

While taxonomy is in a state of constant flux, creating havoc among ecologists, it reflects the current state of the art in terms of what we know about a species. Proper identification of organisms is essential to their study and conservation. This is especially true for small organisms such as plankton which are easily overlooked and distorted upon preservation. Only if we know what we have can we understand ecological processes in nature.

However, ecological research is not just the result of its biological components, but should also consider physical aspects. Our work has contributed to understanding how hydrology acts as an important but often overlooked factor in structuring the plankton community. Lake physics will become an increasingly important aspect as climatic change increases the number of extreme hydrological events. Biodiversity studies of plankton can also provide unexpected results: small organisms are often a source of exciting biochemical compounds giving them an anthropocentric value in addition to their intrinsic biological one.



Integrated systems for sustainable management of animal manure in alpine regions

*Silvia Silvestri
Luciano Sicher*

A cow on a green pasture is one of the most familiar images of Europe's alpine landscape. In our mind, it is connected to nature, to walks in the summer season and, finally, to a healthy environment.

In some areas of Trentino, as in many other alpine regions, a process of intensification and specialisation of animal husbandry has taken place in recent decades. A new reality has emerged that doesn't fit that earlier image: milking cows spend all their lifetime in the stable, eating forage and concentrates mainly bought outside of the territory. Permanent meadows and pastures are often underutilized. At the same time, insufficient storage capacity, inadequate application of animal waste slurry using obsolete equipment, increased ammonia emission, and release of odours have a strong impact on alpine ecology.

This puts pressure on the relationship between residents and tourists. Agriculture and tourism have

to coexist in the same areas, but with different needs in terms of landscape use and requirements. Thus, both farmers and local governments are urging new approaches in the treatment of animal manure based on the sustainability principles. New European policies and actions aimed at promoting and stimulating the use of biomass as renewable energy source are helping to identify the best available techniques for the livestock manure stabilisation.

The solution adopted depends on the overall evaluation of the environmental aspects, the agronomical requirements and the economical sustainability. An integrated system approach starts out by considering farm characteristics and the local environmental impact factors related to animal husbandry. Innovative proposals have been explored regarding the possibility of converting a negative impact factor of manure into a renewable energy resource.

Evaluating composting and anaerobic digestion

Specific feasibility studies on manure as renewable energy source addressed four different areas. A deep cognitive survey was carried out on the livestock production (effective animal stock, husbandry systems, stable, manure collection, manure storage, and manure application), plant production (usable agricultural area, crop systems and productivity, intensity, yield, nutrient uptake, and mineral fertilizer) and nutrient balance (nitrogen and phosphorus) at both the farm and district level.

The evaluation of potential risks of soil pollution and worsening of air and water quality completed the local survey. The second step of the study analyzed the technological solutions which could be effective in reducing the environmental impact of the manure. Both composting and anaerobic digestion (AD) were considered, highlighting advantages and disadvantages of each one. A third aspect considered the end-use of digested manure with a proposal of introducing innovative machinery and equipment to minimize odour release and to enlarge the application windows during the growing season.

Finally, the energy balance of the proposed technical solutions was considered, i.e. the expected efficiency in terms of biogas production and the possible allocation of thermal and electrical energy. Results and possible solutions differed considerably from one area to the other with respect to the starting situation. The amount per year and dry matter (DM) content of manure, i.e. solid manure (20% DM) or liquid manure (10% DM) suggests in most cases the following technology: composting in the first case, and AD in the second one.



Table 1 - Results of the cognitive survey on the *Giudicarie Esteriori* study area

ITEM	AUGUST 2006	NOTE
Farms n°	68	Dairy/beef cattle, pigs, rabbits, broiler
Livestock Units (LU)	3.847	3.586, 142, 50, 69
Slurry + liquid manure (m ³ y ⁻¹)	70.000	
Solid manure (tons y ⁻¹)	12.000	
Maize silage 70 ha (tons y ⁻¹)	4.550	To improve energy production
Grass silage + apple + potatoes (tons y ⁻¹)	1.200	To improve energy production
Arable crops + permanent meadows (ha)	2.132	Usable agricultural area of the 4 municipalities excluded biotops and the steepest permanent meadows (417 ha)
Animal density (LU ha ⁻¹)	1,80	Rural Development Plan 2000-06: Dairy cattle max 2,5 LU ha Beef cattle max 2,0 LU ha

Table 2 - Expected outputs in the option of one centralised plant (with the technical support of specialised companies)

ITEM	YIELDS	INPUT BIOMASS
Total input biomass (tons y ⁻¹)	~ 87.750	First hypothesis
Biogas (m ³ y ⁻¹)	~ 3.222.000	Animal manure + maize silage + grass silage + apple + potato
Electric energy (kWh y ⁻¹)	6.260.000	Excluded the self consumption (8%)
Thermic energy (kWh y ⁻¹)	3.800.000	Excluded the self consumption (50%); available heat for the local cheese factory and other users
Installed electric power of cogenerator (kW)	997	

Future trends

Deep knowledge of the local situation helped us to work out an integrated proposal for sustainable manure management in alpine areas with a strong livestock farming vocation. In detail, these are the components: adjustment to storage capacity, replacement of obsolete slurry tankers with innovative machinery and equipment for slurry application, definition of local schedules for fertilisation with the digested slurry (times and amounts for each crop), anaerobic digestion for renewable energy production, identification of local users of heat.

As concerns the research aspects of biomass connected to AD, future trends include a study on optimisation of biogas production using selected microorganisms, an improvement of biogas quality before feeding fuel cells to produce electric energy, and an increase of hydrogen production instead of methane.

Studies on the laboratory and pilot plant scales are in the planning phase with the technical support of research institutes and private companies.

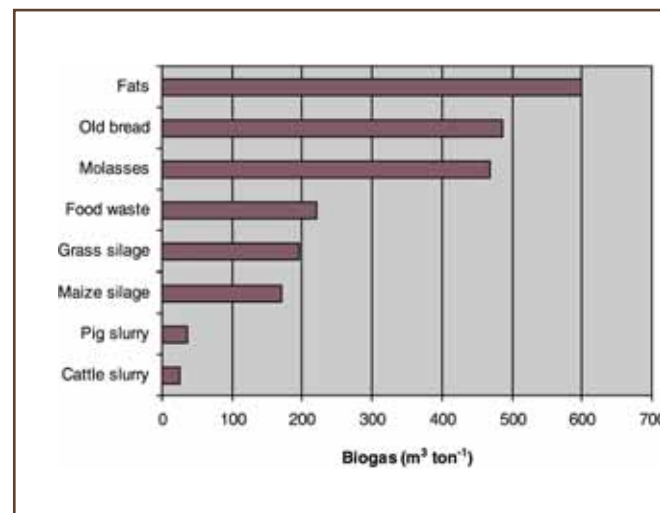
Renewable energy source

Anaerobic treatment of manure makes it possible to convert the current problem into a resource by producing biogas and renewable energy, in alignment with European trends. Other advantages of AD are the abatement of bacteria and viruses, inactivation of plant seeds, reduction of odour impact, and greenhouse gas emission. Regarding the results of the feasibility studies, Tables 1 and 2 summarize the most significant data of the proposal for one specific study area among the four investigated.

The general proposal to realize one centralized AD plant seems to be the best hypothesis when considering the total number of local farms and the whole usable agricultural area. It also takes into account the necessity of preserving protected areas such as biotopes and the steepest permanent meadows (Table 1). The co-digestion of manure and maize or grass silage is suggested because these are energy-crops which can considerably increase biogas yield (Figure). At the same time, the AD of organic residues from agriculture simplifies the authorisation procedure for the digested slurry application in the field.

Concerning renewable energy production, two main streams need to be used the right way: electrical power obtained by cogeneration is put in the network; heat (thermal energy), which is substantial in terms of quantity (Table 2) is partly re-used within the plant (digester thermostating), the rest is available for external users, here the local cheese factory.

Figure - Biogas yields of different biomass (modified from: Mitterleitner, H. 2000 - Landesanstalt für Landwirtschaft, Landtechnik Weihenstephan, Landtechnik bericht n.34)



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, Clinometer, Blitterlich Relascope Optical distancemeter, telemeters, binoculars, Pressler's increment borers, 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 lab facilities include a Gradient thermocycler, Gradient multiple robotisable thermocycler, microcentrifuges, fluorimeter, horizontal and vertical electrophoretic chambers, a Phytotrone for tissue and plant growth, as well as freezers and refrigerators.

Limnology and fish

The unit relies on several laboratories. These include the hydrobiology and microscopy laboratory, equipped for the analyses of basic limnological variables (photosynthetic pigments, dry weight, dissolved oxygen, water turbidity, macrophytes) and analysis of phytoplankton, picoplankton and periphyton samples (microscopes, invertoscopes, fluorescent microscopy, image analysis). The analytical and environmental chemistry laboratory is equipped for the analysis of the major ions and nutrients (phosphorus, nitrogen and silica) carried out by spectrophotometry and ion chromatography. The genetics laboratory includes the principal analytical tools, i.e. PCR, horizontal and vertical electrophoresis, sequencing (capillar electrophoresis), software for data analysis. Finally, the research unit may rely on a large pisciculture equipped for the breeding and reproduction of several fish-stocks.

Biomass and renewable energy

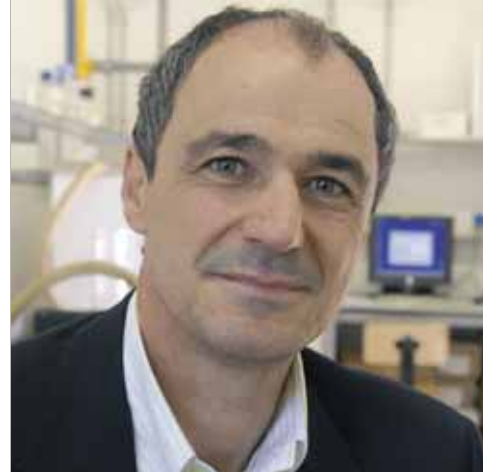
The laboratory is equipped with three dynamic respirometers for assessing biological stability of samples collected during composting and after the mechanical biological pretreatment of residual solid waste. An olfactometer TO7 and an electronic nose are used to monitor odour release and environmen-

tal impact of 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 biotunnel phase with air treatment using a biofilter, a curing section, and a compost sieving area.

Agrometeorology, climatology and aerobiology

The meteorological center manages a network of 80 real-time meteorological stations spread over the main agricultural and forest areas of Trentino province. Data are collected by a three-tiered information system which consists 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, TDR reflectometer, a data logger for temperature and humidity, multichannel dataloggers, and other sensors. The aerobiology sector has a set of five automatic pollen samplers, a geomatics sector with four workstations, an A0 plotter, and precision GPS devices.



Claudio Ioriatti

PLANT PROTECTION DEPARTMENT

The mission of the department 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 Trento province. The department handles a range of activities from plant-disease diagnostic services to pesticide registration support, and from research on sustainable crop protection strategies to transferring 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). The prompt identification of the etiological agent contributes to improving efficacy of the control measures that the extension service suggests, as well as helping to identify introduced diseases and pests.

The department provides research and technical services that assist the agrochemical industry in registering pesticide compounds for agricultural applications. 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), as well as in conformance with European Plant Protection Organization (EPPO) guidelines. This activity helps to maintain a critical link between researchers and extension service consultants.

The department promotes research beneficial to the region by enhancing the sustainability of fruit production. We are dedicated to discovering and accessing knowledge that contributes to safe food production, maintaining environmental quality, and preserving 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 pesticide effects 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 an important task 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 into a crop for extended periods of time so as to suppress pest reproduction by interfering with mate finding. This pest management tactic holds considerable promise for management of moths and other pests that rely on long-distance chemical communication.

Trentino is recognised as a pioneer region in Italy in applying behavioural modification tactics for pest control both in apple and grape. Improvement to this pest control tool are possible 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 probably in sympatric speciation. Knowledge of the chemical signal which attracts gravid females is essential for 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 programs 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 program is now threatened by the development of insecticide resistance



in codling moth *Cydia pomonella*. Early detection of insecticide-resistant populations is of paramount importance to implementing effective integrated resistance management programs. 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 programs for field use.

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

Apple proliferation disease, a serious disorder in apple, was first reported in Trentino in the early 1950s. However, a serious epidemic only developed 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. The noncultured, phloem-restricted phytoplasmas are transmitted by man through grafting, and latently infects 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 applicable, control of the insect vectors is the most promising short-term way to prevent the 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 program to develop resistant plant material.

Apple proliferation (AP) disease occurs in all central and southern European countries, but its highest rates of incidence are in the apple growing regions of the Trentino province and southwestern Germany. The disease causes small-sized fruits with poor taste and results in significant economic loss. All currently grown cultivars and rootstocks are susceptible to the disease and no cure is available. *Candidatus Phytoplasma mali*, a phytoplasma, is associated with the disease. AP was reported in Trentino starting from the early 1950s, but serious epidemics began to break out only from the late 1990s. Start-

ing in 2001, IASMA and a team of German research institutes, AlPlanta - Institute for Plant Research (Neustadt/Weinstrasse) and Biologische Bundesanstalt, Institut für Pflanzenkrankheiten im Obstbau (Dossenheim), began an interdisciplinary research project called SMAP which continues today.

The objectives of the project are: to understand the spread of the disease in Trentino so as to enable risk assessment; to establish short-term solutions by controlling the transmitting insect vectors; and to find a durable solution by developing AP-resistant plant material.

Identifying apple proliferation vectors

AP is spread naturally by psyllid vectors. IASMA was the first to identify *Cacopsylla picta* as a vector of *Ca. Phytoplasma mali*, a finding confirmed in Germany. In Aosta-Valley, however, *Cacopsylla melanoneura* was also reported as a vector. SMAP carried out extensive research on both these species in Trentino and Germany. The biological cycle of both species was elucidated, the natural infection rate determined, and the vectoring capacity studied in transmission trials. In this work, *C. picta* was identified as main vector of *Ca. Phytoplasma mali* in Trentino and Germany. The role of *C. melanoneura* in the spread of the disease remained unclear because naturally infected individuals were found, but a successful transmission of the phytoplasma was obtained only once out of 278 transmission trials. Therefore, quantitative real-time PCR was applied to monitor the multiplication capacity of the phytoplasma in the different vector species.

The results showed that *Ca. Phytoplasma mali* can be acquired from infected test plants by both species, but it multiplies up to 30 times higher concentrations in *C. picta*. Because phytoplasmas are transmitted in a persistent manner and need to colonise and multiply in the salivary glands of the insect vector, these data strongly indicate that *C. melanoneura* is not an efficient vector and, thus, does not represent an important risk for disease spread. The same approach was also used to exclude aphids as potential vectors, although they can acquire the phytoplasma. SMAP research also discovered another important way for the disease to spread, namely transmission via naturally formed root bridges between adjacent trees. For the first time, this was experimentally demonstrated and a transmission rate of 12% over two years was reached. Since the identification of psyllids as an AP-vector, more than 300 insecticides were evaluated for their efficiency against both *Cacopsylla* species which are usually not considered as pests. Pyrethroid ethofenprox was found to be very efficient in controlling the psyllids before blossom.



Research activities on apple proliferation disease at IASMA

Wolfgang Jarausch





Prevention methods identified

The development of new generations on apple was preventable through the use of organophosphates and neonicotinoids. The control strategies are widely applied now in Trentino and the psyllid populations drastically decreased in 2005 and 2006. Because the indicated insecticides have side effects on beneficials, less harmful products were evaluated in standardised insecticide trials. The high efficiency of the standard treatments was confirmed, but some alternative products were found for potential use in the future.

Naturally resistant root stocks

Natural resistance to AP was discovered at BBA Dosenheim in wild, apomictic *Malus* species, namely in *Malus sieboldii*. Crossings of these wild *Malus* species with *M. domestica* were carried out in the 1950s and 1970s in order to obtain apomictic rootstocks for apple. Although the obtained progeny turned out to be too vigorous for modern apple culture, a certain number of genotypes remained resistant to AP. A durable, long-term solution to AP is, therefore, expected from the development of AP-resistant rootstocks. Phytoplasma-infections in the susceptible cultivar are eliminated each year during the renewal of the phloem in early spring. This phenomenon was confirmed under Trentino conditions by studying seasonal colonisation behaviour of *Ca. Phytoplasma mali* in infected trees. Therefore, it is possible to base a resistance strategy solely on resistant rootstocks.

Malus sieboldii and its apomictic rootstock derivatives are being used in new breeding programs in combination with dwarfing rootstock genotypes such as M9. The objective is to develop AP-resistant rootstocks

for use in modern apple orchard management. More than 3,000 seedlings have been produced in 19 cross combinations. In 2006, a first backcross with M9 was done with the resistant breeding progeny from 2001. All seedlings were examined by microsatellite analysis in order to distinguish recombinant from non-recombinant, apomictic progeny. All recombinant seedlings were experimentally graft-inoculated with *Ca. Phytoplasma mali* to test for resistance. The preliminary results indicate that resistance could be successfully inherited by a part of the progeny. Efficient multiplication systems based on micropropagation or green cuttings were developed and validated in 2006 to multiply the resistant genotypes for further evaluation.

Because resistance screening in the field takes a long time, an *in vitro* screening system was established. Parental and promising progeny genotypes were introduced into an *in vitro* culture and each genotype was graft-inoculated *in vitro* in repetitions under standardised conditions. Resistance was evaluated by recording transmission rates and by determining the phytoplasma titre in the inoculated plant by quantitative PCR.

We confirmed resistance of the parentals and some of the breeding progeny. To facilitate resistance screening, the project is currently developing molecular markers. Susceptible and resistant recombinant progeny of one cross (H0909 x M9) was individually fingerprinted with SSR markers. The association of the markers to the resistance trait using bulk segregant analysis is under way. Furthermore, cDNA-AFLP analysis is being employed to find genes differentially expressed in healthy resistant vs. susceptible genotypes, or in infected vs. healthy genotypes. cDNA-AFLP fragments corresponding to over expressed genes were cloned and sequenced, and the differential gene expression of these genes is being evaluated by a newly developed gene expression analysis system based on RT-qPCR.

Implementation status

The agronomic evaluation of AP-resistant rootstocks under Trentino conditions began in 2005. Two field trials were planted in Val di Non and Val d'Adige using available *M. sieboldii* hybrids from 1970s breeding. The first results obtained in 2006 indicate that the reduced agronomic value due to high vigour of these genotypes could be improved if cultural practices were adapted.

The importance of *Phytoseiids* for biological control of phytophagous mites in apple orchards and vineyards has been widely demonstrated over the last 30 years. The biological and ecological characteristics of this generalist predator make phytoseiids one of the most dominant beneficial mites in European orchards and vineyards.

Its beneficial traits include high reproductive potential on various mite species and pollen, as well as the possibility to discriminate

between hetero- and conspecific phytoseiids.

Over the last 20 years, IASMA has carried out a large number of field and laboratory investigations on the toxicity of the pesticides most used in orchards and vineyards to phytoseiids, in particular *Amblyseius andersoni*, *Typhlodromus pyri* and *Kampidromus aberrans*. Organophosphate (OP) and dithiocarbamate (EBDC) agrochemicals were, for a long time, claimed to be detrimental to phytoseiids. Strains

of *A. andersoni* resistant to OPs were found in Trentino orchards from 1988 onwards (Forti *et al.*, 1992, *Inf. Fitop.*, 5: 57-59; Angeli *et al.*, 1996, in: Haskell P.T. and McEwen P.K. (eds.), *New Studies in Ecotoxicology*, 1-4). Field resistance to EBDC fungicides by an *A. andersoni* strain has been monitored since 1991 and confirmed by laboratory investigations (Angeli and Ioriatti, 1994, *Exp. Appl. Acarology*, 16: 669-679; Angeli *et al.*, 2001, *IOBC/WPRS Bulletin* 24 (4): 53-60).

Standard monitoring protocols

It is known that the response to pesticides of different phytoseiid populations evolves continuously. The changing situation requires field monitoring, as well as laboratory and semifield tests. Field tests are fundamental, but expensive, and the interpretation of results is sometimes difficult since mortality effects cannot be distinguished from those linked to reduction of fecundity and fertility. Since 1988, field tests on apples and grapes have been carried out at IASMA, using standardized protocols (Dal Rì and Angeli 1989, *Boll. ISMA* I, 2:18-21; Ioriatti *et al.*, 1992, *Exp. and Appl. Acarol.* 15: 109-116; Angeli and Ioriatti 1994, *Exp. and Appl. Acarol.* 16:669-679; Angeli and Maines, 1997, *Inf. Fitop.* 11:52-56; Angeli *et al.*, 1997, *Inf. Agr.* 53:74-77; Angeli *et al.*, 2000, *Inf. Agr.* 56(17): 71-73; Angeli *et al.*, 2001, *IOBC/WPRS Bulletin* 24 (4):53-60; Angeli and Baldessari 2005, *IOBC/WPRS Bulletin*, 27(8):193-196; Duso *et al.*, 2006, *Acarology* XI, 113-126).

The evaluation of the side effects of pesticides on phytoseiids requires not just reliable identification, but also inexpensive methods. IASMA has proposed laboratory and semifield testing methods to solve some of these problems (Castagnoli *et al.*, 2002, *J. Pest Science* 75 (5): 122-127). Since 1996, the results of field tests performed on *A. andersoni* have been included in comparative studies performed by the IOBC Working Group "Pesticides and Beneficial Organisms" (Angeli *et*



A synopsis of phytoseiid mite and pesticide research at IASMA

Gino Angeli

al., 2001, *IOBC/WPRS Bulletin* 24 (4): 53-60). To compare the results of tests on species that occur in different regions of Europe, IASMA participated in work groups to propose the standardization of testing methods (Candolfi *et al.*, 2000, *J. Pest Science* 73(6):141-147). However, climatic conditions, agricultural practices and species composition differ greatly among European geographic regions.

Nutritional interactions

The dietary effect of airborne pollens in improving the survival and reproduction of Phytoseiids under the effects of pesticides was investigated in the field by means of a study on functional response. A relevant difference in toxicity levels of each insecticide, acaricide and fungicide tested towards two Phytoseiids species was demonstrated. It showed that toxicity depends on the amount of pollens in the plot. In the mowed-lawn plot, a more pronounced negative effect of the agrochemicals was present when compared to the unmowed plot. The effect appeared shortly after the treatment and lasted for a long time. The increased negative effects of the agrochemicals in the mowed-lawn plot was due to the lack of pollen provided by the flowers. This meant that the Phytoseiids were not as well nourished as in the unmowed plot which produced a higher quantity of pollen. Hence, careful grass-flower management is required (Baldessari *et al.*, 2005, *IOBC/WPRS Bulletin*, 28 (7) 133-137).



Successful importation of tolerant mites

In the late 1990s, a *K. aberrans* strain collected in the Veneto region was successfully released in some farms located in Trentino. The phytoseiid spread to apple orchards despite the fact that the latter were colonised by natural populations of the endemic beneficial mites (strains with poor resistance to pesticides), and became the dominant species. After 7 years, the imported mite species is still persistent in these

orchards as the dominant species and has occupied new orchards. The adaptation of *K. aberrans* to Trentino environmental conditions, its tolerance to pesticides, and its competitiveness towards other phytoseiid species suggest a potential role of this species in the biological control of phytophagous mites in Trentino apple orchards (Duso *et al.*, 2007, *Bio-Control*, in press). In conclusion, pesticides can affect mite communities, reducing biodiversity at different levels

or altering relationships among different components. Therefore, studies on the side-effects of pesticides on beneficial mites is a fundamental aspect of integrated pest management. However, data on this topic can rapidly become obsolete; new outcomes may, in fact, arise with any change in agricultural practice (including pesticide use and variety choices) and, in some ways, they are strongly locality-dependent and affected by the past phytoiatric management.



The grapevine moths *Lobesia botrana* (Dennis *et* Schiffermüller) and *Eupoecilia ambiguella* (Hübner) (Lepidoptera Tortricidae) are major grape pests in most European wine-growing areas. The larvae feed on grape and various fungi (in particular *Botrytis cinerea*), developing very rapidly and causing an entire grape cluster to rot.

Currently, insecticides represent the most widespread control method for this pest, both for economic and practical reasons. However, some of the most effective compounds are being withdrawn from the market due to adverse toxicity.

Increased public concern about the environmental impact of vineyard pest management has

also led to development of biotechnical methods that reduce impact and that are also applicable in organic agriculture.

These methods include pheromone mating disruption, false-trail following, autoconfusion, and attract and kill (Ioriatti *et al.*, 2005, *Redia* 87:117-128; Angeli *et al.*, 2007, *JAE* 1172).

Among them, the most used is pheromone mating disruption. This consists in field application of a relatively low number of dispensers (500 dispensers per hectare) loaded with 170-350mg of synthetic sex pheromone. In Trentino-South Tyrol (Italy), mating disruption has been successfully used to control grapevine moths in 9,400 hectares, i.e. 65% of the vineyard area.

Enhancements to pheromone-based methods

Even so, this method has been adopted only on approximately 1% of European vineyards, due to economic, agronomic and social constraints. Among the reasons for the low adoption are control method failure when pest population density is high and when the size of the treated area is not large enough, for example in the case of isolated vineyards. Further studies are being conducted in order to make this safe technique more effective and economical (Anfora *et al.*, 2005, *EEA* 117:201-207; Witzgall *et al.*, 2005, *JCE* 31(12):2923-2932; Angeli *et al.*, 2007, *JAE* 1172). To enhance pheromone-based methods, plant volatiles can be used to manipulate

the behaviour of egg-laying females (Tasin *et al.*, 2006, *NatWis* 93(3):141-144; Schmidt *et al.*, 2006, *IF* 5: 17-24). Knowledge about the volatile compounds involved in host finding and egg laying will significantly improve the efficacy of pheromone-based methods by providing complementary strategies for environmentally safe pest management. Grape berries and leaves release a more than 100 volatiles (Tasin *et al.*, 2005, *JCE* 31 (1):77-87). Grape odour attracts grapevine moth females to lay eggs on flower buds and berries in different phenological stages. Antennae of grapevine moth females specifically respond to grape volatiles. Processing of plant odour signals in the antennal lobe, the olfactory centre of the insect brain, is under study. It has recently

Grapevine chemoecology traits involved in moth attack

Gianfranco Anfora,
Marco Tasin,
SafeCrop Centre

Claudio Ioriatti,
Plant Protection Department

been shown that a blend of 10 synthetic grape volatiles that elicit a response in female antennae can attract as many grapevine moth females by upwind orientation flights as a bunch of green grapes, or headspace collections made from the same grapes (Tasin *et al.*, 2006, *Chem* 16 (2):87-92). This blend contained redundant compounds, since it could be

reduced to three terpenoids, (*E*)-beta-caryophyllene, (*E*)-beta-farnesene and (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), without significant loss of behavioural activity (Tasin *et al.*, 2006, *NatWis* 93 (3):141-144). Blending the three terpenoids had a strong synergistic effect on female attraction.

This three-component blend acted as a unit, and the female response dropped significantly when any one compound was subtracted. It is unclear if this blend conveys specificity in natural habitats, since the same compounds are released by many other plants. The presence of ubiquitous terpenoid compounds in the background odour released from other plants and grapevine will affect attraction to artificial blends. The plasticity in the response of grapevine moth females to different volatile blends observed in the flight tunnel probably reflects the natural variation in grape odour, signalling suitable oviposition sites.



Identifying volatile signals from background

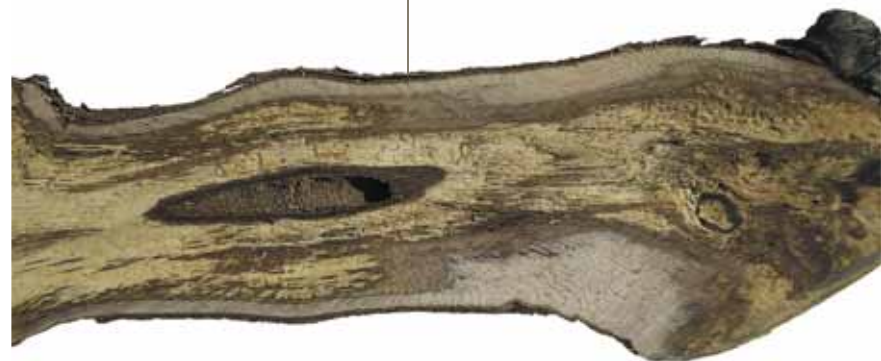
Grapevine moth feeds on all cultivars of *Vitis vinifera* used for wine production, but not all cultivars are equally susceptible. Grape volatiles may account for such differences.

Deterrent compounds or lack of attractants may lead to differentiation of grape cultivars by egg-laying females. With sex pheromone blends, flight tunnel attraction usually matches field trap captures.

This is probably not the case with plant volatiles, and a more complete identification of the grape volatiles mediating upwind flight

attraction of grapevine moth females will require coordinated field and laboratory assays. The principle of insect attraction to their plant hosts by volatile compounds has been well established. The challenge is now to identify those volatile signals which allow insects to discriminate suitable larval hosts from the background chemical environment, and which trigger upwind orientation flights towards plants for oviposition. Knowledge of these chemicals and the behavioural mechanisms involved in host recognition is fundamental for the study of plant-insect interactions, including host-race formation and

sympatric speciation. It is also a crucial input for development of novel insect monitoring and control techniques of the grapevine moth (e.g. mass trapping and killing of females and push-pull strategies).



The department shares its new laboratories with SafeCrop Centre. Equipment includes current versions of instruments necessary for:

- Genetic analysis of plants and micro-organisms.
- Olfactory reaction analysis of living insects and antennae.
- Efficacy trials of plant protection agents from laboratory to small and large field plots.
- Climatic data collection at the plot level.

- Pest and disease diagnostics.
- Micropropagation of plants.

TECAN Freedom EVO® workstation

A TECAN Freedom EVO® workstation (automated multichannel pipetting robot) supplemented by a versatile vacuum system for automate filtration processes allows for 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 electrophoretic apparatus are also available. High throughput mechanical extraction equipment and appropriate centrifuges complete the set up.

Facilities and Equipment

Wind tunnel with flight section

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 recording of the reaction of single antennae to specific volatile metabolites with the real-time identification of the particular substance. The equipment is complemented by an instrument for single sensillum recording, as well as single-cell recording with similar characteristics.

Field equipment

Field equipment includes:

- Motorized sprayers, from single knapsack to multitank airblast sprayers, ranging in size from self driving to tractor pulled, allow application of experimental products on plots of various sizes on IASMA farms. A tractor carried tunnel spray system is employed specifically to avoid drift in row grown grapes. A laboratory and green houses with necessary infection towers, sprayers and safety equipment are available.
- A large number microclimate sensor 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 is supplemented by the necessary equipment for ELISA DAPI and co-uses the equipment 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 sanitized lines used in meristem culture of progeny genotypes of breeding programs for Apple Proliferation resistance and for new rootstock genotypes.



Heinz Saedler

CENTRE FOR THE STUDY OF BIODIVERSITY IN TRENTINO

The Centre for the Study of Biodiversity of Trentino (CSBT) is a joint project between the Istituto Agrario of San Michele all'Adige (IASMA) and the Max Planck Institute for Plant Breeding Research of Cologne (MPIZ, Germany). The aim of the project is perform a long-term study of various levels of biological diversity in plants to understand the mechanisms which generate and maintain diversity in natural environments. To this aim, the natural environment of Trentino has been selected as an “outdoor laboratory”, providing a wealth of wild plant species, habitats and ecosystems. Despite the steady increase of anthropical impact witnessed by European natural environments during the last two centuries, alpine regions have up to now undergone more limited changes than those seen in densely populated areas in the rest of Europe. For this reason as well as for its inherent richness of species, the Alps represent a hotspot of biodiversity in Europe and a unique opportunity to study it.

Modern descriptors of biodiversity

Traditionally, biodiversity has been described through surrogates, the most common of them being species richness. Despite its usefulness, the description of biological diversity resulting from this approach

alone remains superficial. We are, therefore, trying to integrate the traditional descriptors into the more modern genetic and molecular approaches. In doing so, our analyses range from populations to species and ecosystems, using approaches that include phylogeny, ecology, population genetics, and molecular biology.

At the population level, CSBT is focusing on species that for their life history traits (annuality, limited seed dispersal, large populations, variable selfing/outcrossing rates, etc.) represent good indicators for intraspecific patterns of biodiversity.

Moreover, the choice of close congeneric species within selected genera of Lamiaceae and Brassicaceae has the added benefit of permitting a comparison of the intraspecific with the interspecific patterns of genetic diversity as a function of different ecological determinants such as the reproductive system and the altitudinal preference of the species.

This baseline data can be used for modeling purposes to correlate environmental stresses such as human impact or climate change with genetic variability in natural plant populations. We are also working on identifying mutations in reproductive traits (corolla pigmentation) in natural populations, their characterisation at both the ecological and genetic level and the identification of the mutated genes. These studies will help us understand how phenotypic variability in traits relevant for reproduction is generated and maintained in natural populations. Because corolla pigmentation is necessary for plants to attract pollinators, we have also used it as a case study for uncovering the relationship between phenotypic and genetic variability in the mutant populations.

New technologies and methods

At the interspecific level, CSBT activity focuses on both the development of technologies and on their application for comparative purposes. We have developed two kinds of tools. The first group are





universal molecular markers. Because they are universal, the same markers can be used on a wide array of plant species to either determine the true phylogeny of the species used, or they function as reference neutral markers when comparing genes with an adaptive value. The second kind of tool are methods for amplifying orthologous sequences from many species, i.e. genomic sequences having the same functions and which originated in the ancestral species before the evolution of the derived species. Concerning the molecular markers, we have been able to show that Conserved Ortholog Sets of genes (COS genes) are a valid support for phylogenetic reconstruction of closely related genes. We are currently evaluating their potential for phylogeographic and population genetic studies (see accompanying divulgative article). Concerning the isolation of orthologous sequences, we have successfully exploited microsyntenic relationships to improve the speed and efficiency of orthologs from species belonging to the same family.

Once we define the phylogenetic relationships among closely related species, the comparative analysis of selected adaptive traits can be carried out. To this

aim, we carried out a comparative approach based on closely related species (congeneric) that differ in adaptive traits. We isolated genes involved in cold adaptation, viral resistance and photomorphogenesis from various species and deduced evolutive constraints and differential adaptations by comparing patterns of nucleotide variability. Going further, we used complementation in model species to complete the dissection of the molecular basis of plant adaptation to different habitats such as lowlands versus high-altitude alpine regions or open field versus nemoral habitats.

Applications

Ultimately, the aim of the work carried out is to understand the molecular determinants of interspecific biologic diversity and, in light of this information, the interpretation how such adaptations are generated at the intraspecific level within the populations that constitute a single species.

This knowledge will be applied to both the informed management and conservation of biodiversity, progressively recognized as a relevant asset and a moral responsibility of a nation.

Instruments and technological platforms

We perform 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 precompiled lists. In this way data acquisition is carried out directly in digital format without need of transcription. The computers are equipped with GIS software for direct acquisition of georeferenced 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. Highly standardized, quantitative and semi-automatic scoring of AFLP fingerprinting is carried out by means of the AFLP-quantar software. Additional software realised in-house allows for automation of repetitive tasks associated with sequence validation, reformatting and screening for homology in comparison with public databases.



Development and validation of COS genes as universal markers for phylogenetic reconstruction of species

Claudio Varotto

Phylogenetic reconstruction contributes substantially to solving a number of biological problems, including classification, biogeography, polyploidy, comparative genomics, trait evolution, and speciation. Molecular markers, called sequential markers, are based on DNA sequencing data.

The sequential markers most commonly used for these aims in plants are plastid genes (cpDNA like *rbcl*, *atpB*, and *matK*) and nuclear ribosomal genes (nrDNA, mostly represented by internal transcribed spacers [ITS]).

While cpDNA and nrDNA generally provide good resolution at the taxonomic level of families and above, the inference of phylogenetic relationships among closely related plant species by means of these markers is often poorly resolved due to insufficient variation (Hughes *et al.*, 2006, *Philos Trans R Soc*

Lond B Biol Sci. 361:211-25).

Using a genome-wide comparative approach (Fulton *et al.*, 2002 *Plant Cell* 14:1457-67), sets of conserved orthologous genes have been identified in plants.

These conserved ortholog set (COS) markers, were demonstrated to be single or low copy in various genomes and to have remained relatively stable in sequence since the early radiation of dicotyledonous plants.

In this study, we describe the selection, development and testing of nine candidate phylogeny COS (pCOS) markers across various eudicotyledonous orders.

We further describe the development and testing of an additional 18 markers whose amplification range spans from monocotyledon to dicotyledon species and we suggest their possible uses in the field of molecular ecology.

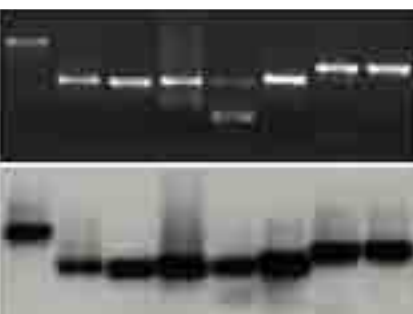
Nine COS markers tested

A first set of nine COS markers was tested for PCR amplification from a total of 87 species which represent 67 families mostly distributed across dicotyledonous angiosperms, with a few representatives from monocotyledonous angiosperms, gymnosperms, bryophytes and pteridophytes. Primer pairs were designed manually on the basis of alignments obtained between the *Arabidopsis thaliana* COS genes and homologous sequences retrieved from public sequence databases. The amplification results were compared to that obtained for *rbcl*, one of the most commonly used universal phyloge-

netic markers. The COS markers amplified from the different families tested with an average efficiency of 69% as compared to *rbcl* 100%.

The nine COS markers selected were further characterised and compared to the traditional markers ITS, *matK*, *rbcl* and *ndhF* by amplification and sequencing from a set of 25 species sampled from selected orders and families throughout the angiosperms ("phylogeny set"). For the selected COS markers, the percentage of confirmation by sequencing ranged from 76% to 100%. On the average, about 50% of the markers developed were useful for phylogenetic reconstruction. The remaining markers





Amplification products and southern blot hybridization (lower)

were present in multiple copies and would, therefore, require development of additional primer pairs to avoid incomplete sampling of paralogous sequences from different species. Using a total of four different COS markers and ITS, we conducted phylogenetic reconstruction of seven congeneric species plus three outgroups, each represented by individuals from three different populations. Our results indicate that:

- 1) COS markers have a degree of polymorphism that ranges between the values for traditional cpDNA and nrDNA markers, depending on the marker selected.
- 2) When used in association with nrDNA, the selected COS markers greatly improve the accuracy of phylogenetic reconstruction for closely related species.
- 3) The level of polymorphism for some of the markers is sufficiently high as to be useful for distinguishing individuals and populations within species.

We used a second set of 18 COS genes to semi-automatically develop primer pairs using GeMprospector software (Fredslund *et al.*, 2006, *Nucleic Acids Res.* 34:W670-5) and compared all predicted *Arabidopsis thaliana* COS with a database of EST as well as genomic sequences from selected Poaceae.

The resulting markers, tested on a set of 32 plant species ranging from monocotyledons to dicotyledons, turned out to have an average amplification success rate of about 50%. Three of the markers developed were particularly useful, yielding strong amplification products from about 70% of the species in the test set. The figure shows an example of the amplification products obtained and of the corresponding southern blot hybridization probed with the *Arabidopsis thaliana* amplification product. On the average, the testing of all 18 COS markers on any randomly chosen species provides nine markers for further characterisation.

Promising markers identified

The availability of easily amplifiable molecular markers is of paramount importance for several applications connected to the study of biodiversity at both the intra and interspecific levels. On the one hand, for model species and closely related taxa, the number of such markers is large. As soon as the focus moves to the plethora of non-model species (by far the majority of both plant and animal species), the development of suitable molecular markers becomes a work-intensive and time-consuming task.

With the aim of developing widely applicable plant molecular markers, COS genes from *Arabidopsis* were used to develop primer pairs able to produce robust amplification products from both monocotyledons and dicotyledons. We demonstrated that the markers obtained are useful for phylogenetic reconstruction of closely related species, one of the most interesting and difficult areas of research connected to interspecific comparative studies of biodiversity. The same markers also hold promise for use in other applications in molecular ecology at the intraspecific level. Such applications could include determination of selfing or migration rates, genetic differentiation among populations, individuation of genes under adaptive selection and phylogeography. More importantly, screening of the primer pairs already developed ensures that, independently from the plant species considered, one can easily obtain between five and nine molecular markers for either interspecific or intraspecific downstream applications. This drastically reduces the investment necessary for the development of species-specific markers.

The refinement of the methods for automatic primer design and the increase in the number of markers available to plant biologists are the two main goals for the further development of this research area.



Facilities and Equipment

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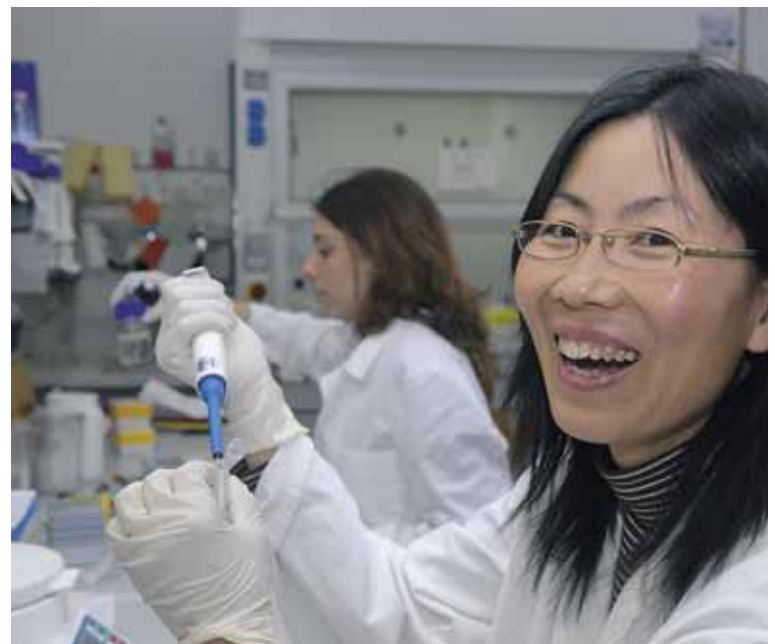
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Cesare Gessler

The SafeCrop Centre originates from a project funded by the Autonomous Province of Trento to implement innovative research on plant protection with the goal of developing sustainable agricultural methods with low environmental impact and reduced use of chemicals. It is hosted by the Istituto Agrario S. Michele all'Adige (IASMA), which is also a partner of the network together with leading European institutions (see partner list, *next page*).

The SafeCrop Centre creates synergism in knowledge building and focuses on the constraints that hamper the large-scale application of low-impact technologies. It is a bridge between scientific research and the public, translating research results into products, processes and services. SafeCrop backs innovative projects from concept to successful application on farms. Its research fills knowledge gaps and fosters innovative ideas. Research activities at SafeCrop are based on joint goals and the integration of several sciences: biology, biotechnology, information technology, sociology, economics, psychology, engineering and agronomy.

Its researchers take advantage of all partner facilities

and knowledge by working for the time needed at the qualified partner institution.

The centre promotes high-level instruction and training of young researchers in an international, collaborative mode of research. SafeCrop hosts and organises meetings and congresses, promotes scientific workshops and technical meetings with technicians, farmers, researchers and private companies to create networking of all the players in the agricultural production chain. The centre also provides contract research services for customers whether private companies or public institutions.

Research

All scientific activities are project oriented and well defined in content and timeline. SafeCrop is divided into three research units: plant pathogen control, insect control and risk assessment, and molecular tools and side effects of nonchemical control methods.

The centre concentrates mainly on grapevine, apple, soft fruits and horticultural crops.

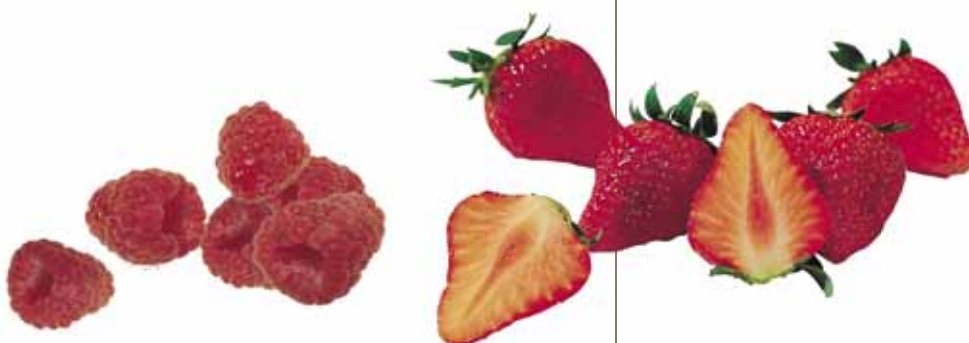
SAFECROP
Centre for Research
and Development of
Crop Protection with
low environment and
consumer health impact

Pathogen Control

Yigal Elad

The long-term objective of the unit is to reduce chemical fungicide use on crops by implementing the use of microorganisms, their metabolites or analogs, and their integration. Specific methods include:

- Selection and identification of field-condition-effective biocontrol agents (BCAs).
- Characterisation of the BCA activity spectrum and modes of action.
- Production, scale-up and formulation of potential BCAs.
- Evaluation of activity, consistency, survival, diffusion and constraints of BCAs applied under commercial production conditions.
- Integration of BCAs with other environmentally friendly methods.



Insect Control

Sylvie Derridj

The long-term objectives of this unit are to develop effective, environmentally friendly control and monitoring techniques to replace traditional insecticides by exploiting natural insect population regulatory control factors. This includes:

- Identification of the biological activity of insect semi-chemicals, plant volatiles and metabolites involved in host-plant selection and oviposition site selection by insects.
- Study of the detection mechanisms used by the insects to recognise pheromones and gustatory signals involved in feeding and reproductive behaviour.
- Identification of parasitoids and development of mass-rearing and release techniques.
- Development of biological products to combat insect pests.
- Investigation of the impact of abiotic environmental factors on survival and performance of bio-insecticides.
- Integration of chemical and biological control measures commonly used in integrated pest management.

Risk assessment, molecular tools and side effects of nonchemical control methods

Ilaria Pertot

The unit's objectives are to supply molecular tools and furnish the know-how for evaluating long-term ecological and economical impacts of BCAs. In particular, the unit seeks to describe the fate of the BCAs in agricultural ecosystems and to ascertain the lack of environmental and health risk in the use of BCAs. The work includes:

- Development of markers to trace BCAs.
- Monitoring spread and colonisation of BCA to nontarget sites.
- Understanding plant-pathogen-BCA interactions in the field and study of environmental effects on BCAs.
- Chemical characterisation and identification at the molecular level of the modes of action of bioactive metabolites.
- Risk assessment of nonchemical fungicides and insecticides.
- Side-effect evaluation of BCAs, insect behaviour regulators and bioactive metabolites on nontarget organisms and beneficials.
- Evaluation of the effects of BCAs on processed agricultural products.
- Risk evaluation of forced pathogen evolution towards avoiding BCA-effects.

Partners

- Swedish University of Agricultural Sciences, Department of Crop Science Alnarp, Sweden, www.slu.se
- Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control Darmstadt, Germany, www.bba.de
- Institut National de Recherche Agronomique, Unité 1272 UPMC, INRA, INA-PG Versailles Cedex, France, www.versailles.inra.fr
- Swiss Federal Institute of Technology Zürich, Institute of Integrative Biology: ecology, evolution and infectious diseases Zürich, Switzerland, www.pa.ipw.agrl.ethz.ch
- Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, www.agri.gov.il



Integrated Studies

The activity of the centre's three units is integrated with socio-economic evaluations, programs for communication and dissemination to the farming community, market analyses, information technologies and computer systems applied to agriculture, statistics and ecotoxicology.



Pests, weeds, and pathogens have a profound impact on agriculture. As a result, enthusiasm for pesticides is high because they are reliable, simple to apply and fast acting. Thanks to their use, crop yields and quality have increased in a way that was unthinkable 50 years ago. All this has led to a strong dependency on pesticides.

Countering this enthusiasm, pesticide pollution has become a highly controversial issue that has resulted in legislation which limits and regulates pesticide use. Recently, consumers have been confronted with food-safety incidents, and occurrence of pesticide contaminants has gradually eroded confidence in food safety.

Although the European food supply is generally regarded as safe by regulatory standards, a number of Europeans are stricken by food illnesses each year (Krchard, 2001, *The Lancet* 357(9264):1276).

Increased desire for food safety

Most studies of public health indicate that pesticides seldom lead to striking or widespread problems. However, while legislation on pesticide safety evaluation typically considers active molecules individually, the large set of data may give a false sense of security, because the long-term effects of chemical combinations are not considered and are largely unknown. It is worth noting, though, that contamination by pesticides above legal levels is reported in the range of 1-5% of food samples taken.

Not surprisingly, consumers have increasingly opted for organically produced food. However, production and retailer costs are higher, and a broad switch in production from conventional/integrated pest management to organic is unrealistic.

Pesticide residues come from four sources: on-farm and post-harvest pesticide use, non registered pesticide use on imported food, and cancelled pesticides that persist in the environment.

Regardless of its origin, consumers and policy makers are demanding lower pesticide input in order

to increase food quality and safety along the whole food chain, while keeping farmer costs and consumer prices in check.

Therefore, reduction or replacement of pesticides is the only feasible future approach.

Soft control methods

Food consumed raw is the area of highest concern. Thus, the aim of research is to substitute pesticide input in fruit and vegetable crops with a variety of soft control methods, including:

- Biocontrol agents that out-compete pathogens.
- Mycoherbicides.
- Chemicals not applied directly to products that influence behaviour of pests.
- Nontoxic compounds that induce resistance in the plant.
- Natural compounds that are only toxic to pests and pathogens.

Implementation issues

The expertise of research institutions, farmers, extension service advisors, businesses, and other professionals must be integrated throughout the production chain. This joint effort is needed to develop the tools, assemble the strategies and, finally, test and implement the tools and strategies at the regional and the European levels.

Among the most significant causes of poor quality and reduced yield are fungal diseases. At present, multiple chemical fungicide treatments are necessary to control plant pathogens. Considerable experience has been gained in the biological control of soil-borne and foliar diseases of agricultural crops

Improving quality and safety in low input agricultural production systems

*Ilaria Pertot
Yigal Elad
Cesare Gessler*



during cultivation or at post-harvest. Pathogen infection can be reduced under field conditions by pre-inoculation of plant surfaces with biocontrol agents (BCAs). Biocontrol offers attractive alternatives and supplements to the use of conventional methods for disease control. Experts believe that microbial BCAs damage the environment less. Furthermore, the generally complex mode of action of these agents makes it unlikely that a target pathogen will develop resistance to them, unlike to modern chemical pesticides.

Interest in biocontrol is reflected in the number of scientific publications on this subject. But enthusiasm for biocontrol needs to be balanced by the reality that to date few microbial agents have been registered as commercial products. Even if in recent years, some new nonchemical alternatives and microbial BCAs have been successfully developed, only a few of them are currently fully commercialised (Paulitz & Belanger, 2001, *Annu. Rev. Phytopathol.* 39:103-133).

Consequently, biopesticides still represents less than 1% of the total crop protection market, and most of this is accounted for by products based on *Bacillus thuringiensis*. Many experimental BCAs await commercial development and implementation. Unfortunately, research on BCAs is fragmented.

The major constraints resulting from this fragmented approach are difficulties in scaling up production, inappropriate formulation and unsuitable applica-

tion technologies. Several BCAs require improvements to be effective under field conditions and they need to be better integrated into local agricultural systems. In many cases, BCAs are developed for a narrow spectrum of plant diseases or crops of importance and are awaiting development for wider uses in different patho-systems.

Microbial biocontrol preparations have a number of drawbacks compared to chemicals. This can include: higher price, lower effectiveness, complicated use, narrow activity spectrum and dependence on environmental conditions. The plant protection market for some microbial BCAs might be better if production costs were lowered, shelf-life was longer, efficacy was better and application simpler (Pertot and Gessler, 2007, *IOBC-WPRS Bulletin* 30, in press).

In other words, the market is ready for microbials, but only if microbials are developed in ways that make them profitable alternatives. To improve the implementation prospects for biological control, intensive research is needed to minimize inconsistency and maximise the efficacy of microbial BCAs. This includes the study of integrated multi-BCAs use, the effect of environmental factors on the activity of BCAs, and integration of BCAs with other control strategies. In the future, it is likely that we will face microbial products with longer shelf-life, better survival in the plant environment and higher efficacy.

Current issues and future research

New control technologies, even if shown to be safe and as targeted as possible, can present various problems, including:

- Unwanted effects on nontarget organisms.
- Low acceptance by farmers and consumers due to lack of clear data on contamination and its effect (perceived risk).
- Inappropriate studies due to legal requirements based on phantom risks (Gessler, 2004, *IOBC-WPRS Bulletin* 27(8):417-419).

Objectives for future research in plant protection should include:

- Reduce pesticide inputs by using BCAs that are applicable and acceptable both in IPM and organic agriculture.
- Overcome the problems that limit BCAs-based methods to control plant pathogens.
- Increase efficacy of BCAs in plant disease biocontrol under field conditions.
- Broaden the spectrum of disease problems that are controlled by individual BCAs.
- Identify common platforms for the development of BCAs in Europe.
- Harmonise experimentation and resolve fragmentation among research groups.



Our laboratories carry out basic and highly specialised analysis services, allowing IASMA to play an important role regionally, nationally, and internationally. These services support, safeguard, and develop products and processes using staff involved in research.

IASMA laboratories work to fully contextualise data, offering a deeper vision, not just a purely analytical one.

Service activities provide a means of staying in contact with fundamental issues, leading to ideas for new research and to choices in research direction. Services covering a range of agrifood fields are available to institutions and private companies.

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 matrixes 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 of specific territories for botanical and zoological characterisation of alpine dairy farms, economic analysis of alpine farming, and data collection 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 agro-pharmaceuticals in the laboratory, greenhouse or field. The SafeCrop Center has greenhouses facilities and equipment for evaluating the efficacy of biocontrol agents and low environmental impact compounds.

Accreditation

Since 1998, the research centre has had SINAL accreditation, currently with 38 analyses in three departments (AgriFood Quality Department, 36; Genetics and Molecular Biology Department, 1; Agricultural Resources Department, 1). Of these tests, 24 are norm-based and 14 were developed internally. The applicable norms are UNI CEI EN ISO/IEC 17025 and the General Requirements for the Competence of Testing and Calibration Laboratories.

The accreditation scheme was set up by the European Union to permit the free movement of goods and products between member states, following mutually recognized checks. Italy's accreditation authority, SINAL, guarantees laboratory impartiality and competence through periodic technical verification. An updated list of tests is available at www.sinal.it.

The coordinator of IASMA quality systems is Anita Dalla Serra, +39.0461.615346, anita.dallaserra@iasma.it

Services and Support to Stakeholders







Research Projects

Core projects

The Autonomous Province of Trento (PAT) provides core fundings for the following projects

PROJECT	DEPARTMENT
Agrometeorology, climatology and aerobiology	Natural Resources
Apple breeding	Agricultural Resources
Apple genome sequencing	Genetics and Molecular Biology
Biomass and renewable energy	Natural Resources
Diagnostic and testing facility	Natural Resources
Food processing and technology	Agrifood Quality
Food safety and traceability	Agrifood Quality
Forest ecology and physiology	Natural Resources
Genomics and bioinformatics of grape berry development	Genetics and Molecular Biology
Grape crop physiology and management	Agricultural Resources
Grape pest management	Plant Protection
Innovative tree husbandry and fruit production methods	Agricultural Resources
Integrated plant protection and biocontrol approaches	Plant Protection
Limnology and fish research	Natural Resources
Management of grape germplasm banks, genetic improvement and selection	Agricultural Resources
Molecular breeding of grape, apple, and soft fruits	Genetics and Molecular Biology
Mountain farming systems	Agricultural Resources
New technologies for fruit and vegetable storage and processing	Agricultural Resources
Oenology	Agrifood Quality
Plant gene expression	Genetics and Molecular Biology
Quality, health and nutrition	Agrifood Quality
Soft fruits protection strategies	Plant Protection
Translational plant genomics	Genetics and Molecular Biology

Other projects

The following projects are supported by various sources

PROJECT	FUNDING	COORDINATOR
ABTM Database of balsamic vinegars	Italy	Fulvio Mattivi
ALPINET GHEEP Alpine network for sheep and goat promotion for a sustainable territory development	European Union	Giorgio De Ros
AMICA New high automation methods for isolating genes in vine, and for mapping genes through association, and identifying candidate genes	PAT	Riccardo Velasco
ASSAGRARIA RIVA Effect of terroir on the quality of Merlot wines	Italy	Giorgio Nicolini
BACCO Analysis of the grape genomic structure towards isolation of relevant genes to improve grape quality	PAT	Riccardo Velasco



PROJECT	FUNDING	COORDINATOR
BIOINNOVA Use of kairomones in the control of <i>Cydia pomonella</i> (L.): development of innovative biotechnologies for sustainable agriculture	PAT	Claudio Ioriatti
BIOMARKER New markers for tracing organic fruits	European Union	Federica Camin
BIOSTABILIZATION OF SOLID WASTE Monitoring of the MBT of solid waste before landfill disposal	Italy	Silvia Silvestri
CARPESCI Harvesting, genetic characterisation, farming, and distribution of native salmonid fish of the alpine lakes of Italy	Italy	Francesca Ciutti
CARPOL Development of innovative methods for identifying and quantifying allergenic pollens	Italy	Elena Gottardini
COLLI di PARMA Improvement of the quality of Malvasia & Sauvignon Blanc DOC Wines	Italy	Giorgio Nicolini
CONCAST Food-chain quality of Grana Trentino cheese	PAT	Giorgio De Ros
COST 858 Viticulture: biotic and abiotic stress, grapevine defence mechanism and grape development	European Union	Stella Grando
COST 864 Pome fruit health	European Union	Ilaria Pertot
DEMARCATÉ Development of molecular taxonomic markers for the psyllids of agronomic interest	PAT	Valeria Malagnini
DOW Laboratory and field studies on the efficacy of autoconfusion technique against <i>Cydia pomonella</i>	Italy	Gino Angeli
EcoGenEtic.Com "Eco-friendly" genes: from scientific research to risk management, ethical issues and communication	PAT	Lucia Martinelli
ENO-CAVIT Chemistry, technology and microbiology in winemaking	Italy	Giorgio Nicolini
EU-INTAS YSF Plant Bioreactor System	European Union	Nicola La Porta
FhG Convention with Fraunhofer Gesellschaft Aachen	European Union	Riccardo Velasco
FINGRAPPA Food Quality	Italy	Giuseppe Versini
Food Quality The role of red wine and its minor components in the prevention of chronic degenerative diseases	Italy	Fulvio Mattivi
GAME Sustainable management of marble trout (<i>S. t. marmoratus</i>) in the Adige basin: genetic, phenotypical and ecological characterisation for conservation purposes	Italy	Andrea Gandolfi
GARDA Long term development of the physical, chemical and biological characteristics of Lake Garda	Italy	Nico Salmaso



PROJECT	FUNDING	COORDINATOR
GeReCa Subtle characterisation of the Vitis genetic resources in the IASMA collection	PAT	Marco Stefanini
GRAPEGEN06 Management and conservation of genetic resources	European Union	Stella Grando
HiDRAS High-quality disease resistant apples for sustainable agriculture	European Union	Matteo Komjanc
ISAGRO Disorientation of vine moths	Italy	Gino Angeli
IMALP Implementation of sustainable agriculture and rural development in alpine mountains	European Union	Giorgio De Ros
Interberry Interdisciplinary integrated study for the improvement of the quality of berries and for the design of new products with high added value	PAT	Lara Giongo
MESVIT Basic and applied research for the control of grapevine esca disease	Italy	Ilaria Pertot
MILACT Exploitation and preservation of the autochthonous microflora associated to Italian cheeses	Italy	Agostino Cavazza
MIPAAF Research and investigations to upgrade the national isotopic data bank of food and beverages	Italy	Federica Camin
PARMA Edible, aromatic and medicinal plants of the Alps: a resource to value	PAT	Matteo Komjanc
PGM Evaluation of expressed characteristics of lines of genetically modified poplars and their potential environmental impact	Italy	Fulvio Mattivi
POPSAL Genetic diversity and aquaculture potential of natural salmon populations in Trentino	PAT	Stella Grando
PROALPE Investigation of alpine terroirs for the characterisation and protection of mountain dairy products	Italy	Luana Bontempo
Profiles Analysis of genetic expression profiles during Vitis Vinifera berry ripening process by means of DNA matrixes	PAT	Riccardo Velasco
QTL_A QTL characterisation of aroma in grapevine	PAT	Stella Grando
RAME Studies to comply with limits on copper residue with low-dosage formulations or alternatives	Italy	Ilaria Pertot
REPCO Replacement of copper fungicides in organic production of grapevine and apple in Europe	European Union	Ilaria Pertot
SAMPPA Development and application of predictive methods for quality control and origin of agrofood products	PAT	Flavia Gasperi



PROJECT	FUNDING	COORDINATOR
SEDAMA Semiachemicals of <i>Dasineura mali</i>	PAT	Claudio Ioriatti
Selectivity and collateral effects of phytosanitary products on arthropoda	Italy	Gino Angeli
SicilBerry Project for the improvement of the quality of berries and development of their nutritional and antioxidant potential	Italy	Lara Giongo
SICILIA_GEN Quality plant-growing and characterisation system, and gene-based certification and traceability of the whole agro-food production chain	Italy	Stella Grando
Sipcam Decadienoato (DA) for controlling damages from <i>C. pomonella</i> L.	Italy	Gino Angeli
SyrTox Mechanism of action of <i>Pseudomonas</i> spp. metabolites and their potentiality in biocontrol	PAT	Ilaria Pertot
SMAP II Apple Proliferation	PAT	Wolfgang Jarausch
Syngenta Integrated control of the major phytophaga and pathogens of apple and grapevine	Italy	Gino Angeli
TOBLINO-CANZOLINO Limnological studies on Toblino and Canzolino lakes	PAT	Monica Tolotti
TRACE Tracing food commodities in Europe	European Union	Giuseppe Versini
UPaNiVi Uptake and partitioning of nitrogen in <i>Vitis</i>	PAT	Duilio Porro
VITIS Development and application of analytical methods in support of clonal selection	Italy	Fulvio Mattivi
WIPS In-process wine fermentation monitoring system: Oenological fermentation monitoring with biosensors and mathematical models	European Union	Agostino Cavazza



Affiliations

SOCIETY	WEBSITE	PERSON
American Phytopathological Society (APS)	http://www.apsnet.org	Nicola La Porta
COST Action 858, member of management committee	http://www2.univ-poitiers.fr/cost858	Stella Grando
Fifth International Workshop on Grapevine Downy and Powdery Mildew, member of the scientific committee	http://www.safecrop.org	Ilaria Pertot
Groupe Polyphenols, GP board member	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 Riccardo Velasco
International Workshop on Arthropod Pest Problems in Pome Fruit Production, member of scientific committee	http://web.udl.es/700/iobc-pomefruitpests	Claudio Ioriatti
Italian Association for Oceanology and Limnology, member of the board of directors	http://www.aiol.info/home.htm	Nico Salmaso
Italian Biotechnologists Association (ANBI)	http://www.biotechnologi.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 Matteo Komjanc Lucia Martinelli Claudio Moser Riccardo Velasco
Italian Society for Horticulture	http://www.soihs.it	Stella Grando Marco Stefanini Dulio Porro
Italian Society for Sensory Science, founding member and council member	http://www.scienzesensoriali.it	Flavia Gasperi
Italian Society for Vegetal Physiology	http://www.sifv.it	Riccardo Velasco
Journal of Limnology	http://www.iii.to.cnr.it/	Nico Salmaso
Long Term Ecological Research, coordinator of LTER-Italy scientific committee	http://www.ilternet.edu/networks/	Cristina Salvadori
Marie Curie Fellows Association	http://mcfa.eu/	Nicola La Porta
Scientific Council for biotechnologies in agriculture, Lombardy region		Cesare Gessler
Società Italiana di Patologia Vegetale	http://www.agr.unipi.it/sipav/	Giorgio Maresi
Società Italiana di Selvicoltura e Ecologia Forestale (SISEF)	http://www.sisef.it	Nicola La Porta Giorgio Maresi
Society for the study and development of alpine zootechnical systems (SoZooAlp)	http://www.sozooalp.it	Walter Ventura
Subgroup "Pome fruit arthropods", convenors	http://www.iobc-wprs.org/people/index.html	Claudio Ioriatti



Editorial Board Participation

JOURNAL	WEBSITE	PERSON
Mitteilungen Klosterneuburg	http://bundesamt.weinobstklosterneuburg.at/seiten/index.php/view.84/service.true/	Fulvio Mattivi Urška Vrhovsek
Phytopathology Informer	http://www.edagricole.it/r_22_dett.asp	Cesare Gessler
Rivista di Viticoltura ed Enologia	http://www.inea.it/isv/RivistaViticEnol.htm	Fulvio Mattivi
South African Journal of Oenology and Viticulture	http://www.sasev.org/journal	Lucia Martinelli Fulvio Mattivi
Tree Genetics and Genomes	http://www.springer.org	Riccardo Velasco
VITIS - Journal of Grapevine research	http://www.bafz.de	Fulvio Mattivi Lucia Martinelli
VQ - In vite qualitas, in vino excellentia	http://www.tecnichenuove.com/epages/Store.sf?ObjectPath=/Shops/TN/Products/VQ/SubProducts/VQ-0001	Fulvio Mattivi

Recognition

PRIZE	INSTITUTION	PERSON
Achievement in Biosciences Prize	Oxford University Press	Flavia Maia Moreira



Academic Theses at IASMA in 2006

Doctoral theses

PERSON	UNIVERSITY
Gurioli Davide	Bari
Moreira Flavia	Milano
Pedron Luca	Firenze
Pindo Massimo	Modena - Reggio Emilia
Schmidt Silvia	Piacenza
Valgimigli Maria Chiara	Verona
Villa Michela	Piacenza
Zamboni Anita	Verona

Five-year theses

PERSON	UNIVERSITY
Berghi Francesco	Padova
Consonni Michela	Milano
Dal Ri Antonio	Verona
Denis Bassetti	Padova
Di Franco Pierpaolo	Milano
Emanuelli Francesco	Verona
Fedrizzi Bruno	Padova
Martini Alice	Padova
Mazzoli Paola	Milano
Pellegrini Alberto	Padova
Pisetta Michele	Padova
Sordo Maddalena	Verona

Three-year theses

PERSON	UNIVERSITY
Agosti Giacomo	Padova
Beozzo Marco	Udine
Carlotto Michela	Udine
Cesconi Lorenzo	Udine
De Moro Gianluca	Padova
Donolato Daniela	Bolzano
Faettini Luca	Consorzio Trento-Udine-Geisenheim
Fischer Christoph	Consorzio Trento-Udine-Geisenheim
Galimberti Alberto	Udine
Manca Daniele	Udine
Misseroni Andrea	Verona
Murru Gianpiero	Udine
Panizza Claudio	Udine



PERSON	UNIVERSITY
Pasqualini Jonathan	Reggio Emilia
Piotto Martina	Padova
Ress Daniele	Udine
Risatti Stefania	Milano
Rohregger Stephan	Geisenheim, Germany
Ruatti Tiziano	Università di Bolzano
Sandri Nadia	Consorzio Trento-Udine-Geisenheim
Segnana Michela	Padova
Slaghenaufi Davide	Udine
Sora Ramon	Milano
Stenico Loris	Udine
Tamburini Matteo	Padova
Zanon Nicola	Consorzio Trento-Udine-Geisenheim
Zendri Lorenzo	Udine
Zucchelli Massimiliano	Milano



Publications 2006

The results of research activities carried out at 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 most relevant publications reporting the research papers and chapters published in the international press (J) and in proceedings (P). For the international audience of this book, we omitted publications in Italian.

- Alaphilippe A., Derridj S., Elad Y. (2006). Method to study the resistance induced by spraying epiphytic yeast against an insect pest (*Cydia pomonella* L.). *IOBC/WPRS bulletin*, 29, (8): 1-4. J
- Alaphilippe A., Elad Y., Derridj S., Gessler C. (2006). Effect of a potential biocontrol agent of apple diseases on the egg laying of *Cydia pomonella* (L.). In: *Workshop on Arthropod pest problems in pome fruit production: Universitat de Lleida (Spain), 4-6 September 2006*: 18. P
- Amsalem L., Freeman S., Rav David D., Nitzani Y., Sztjenberg A., Pertot I., Elad Y. (2006). Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. *European journal of plant pathology*, 114, (3): 283-292. J
- Anfora G., Isidoro N., Ioriatti C. (2006). Biological interactions between the apple leaf curling midge, *Dasineura mali* (Kieffer), and its inquiline, *Macrolabis mali* Anfora. In: *Workshop on Arthropod pest problems in pome fruit production: Universitat de Lleida (Spain), 4-6 September 2006*: 41. P
- Angeli D., Di Marino E., Maines L., Mescalchin E. (2006). Grapevine powdery mildew and the mycoparasite *Ampelomyces quisqualis* in Trentino vineyards (northern Italy). In: *5th International workshop on grapevine downy and powdery mildew: San Michele all'Adige (TN), 18-23 June 2006* (edited by I. Pertot (et al.)). San Michele all'Adige (TN): Istituto Agrario di San Michele all'Adige: 99-101. ISBN: 88-7843-009-9. P
- Angeli D., Di Marino E., Mescalchin E. (2006). Colonization of grapevine powdery mildew cleistothecia by the mycoparasite *Ampelomyces quisqualis* in Trentino, Italy. *IOBC/WPRS bulletin*, 29, (11): 89-92. J
- Angeli D., Ferrari A., Elad Y., Pertot I. (2006). Evaluation of new control agents against grapevine powdery mildew under greenhouse conditions. *IOBC/WPRS bulletin*, 29, (11): 83-87. J
- Angeli D., Ferrari A., Longa C., Maines L., Elad Y., Simeone V., Assaf H. A., Pertot I. (2006). Efficacy evaluation of new control agents against grapevine powdery mildew under greenhouse conditions. In: *5th International workshop on grapevine downy and powdery mildew: San Michele all'Adige (TN), 18-23 June 2006* (edited by I. Pertot (et al.)). San Michele all'Adige (TN): Istituto Agrario di San Michele all'Adige: 83-84. ISBN: 88-7843-009-9. P
- Angeli D., Maines L., Pertot I. (2006). Efficacy evaluation of integrated strategies for powdery and downy mildew control in organic viticulture. *IOBC/WPRS bulletin*, 29, (11): 51-56. J
- Angeli D., Maines L., Simeone V., Yildiz L., Pertot I. (2006). Efficacy evaluation of integrated strategies for powdery and downy mildew control in organic viticulture. In: *5th International workshop on grapevine downy and powdery mildew: San Michele all'Adige (TN), 18-23 June 2006* (edited by I. Pertot (et al.)). San Michele all'Adige (TN): Istituto Agrario di San Michele all'Adige: 172-173. ISBN: 88-7843-009-9. P
- Angeli G., Rizzi C., Dorignon A., Ioriatti C. (2006). Population injury levels of the apple rust mite *Aculus schlechetndali* (Nalepa) on Golden delicious apple fruits. In: *Workshop on Arthropod pest problems in pome fruit production: Universitat de Lleida (Spain), 4th-6th September 2006*: 30. P
- Angeli G., Simoni S. (2006). Apple cultivars acceptance by *Dysaphis plantaginea* Passerini (Homoptera: Aphididae). *Journal of pest science*, 79, (3): 175-179. J
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- Arroyo Garcia R., Ruiz Garcia L., Bolling L., Ocete R., Lopez M.A., Arnold D., Ergul A., Söylemezoglu G., Uzun H.I., Cabello F., Ibañez J., Aradhya M.K., Atanassov A., Atanassov I., Balint S., Cenis J., Costantini L., Gorislavets S., Grando M.S., Klein B.Y., McGovern P., Merdinoglu D., Pejic I., Pelsy F., Primikiriou N., Risovannaya V., Roubelakis - Angelakis K.A., Snoussi H., Sotiri P., Tamhankar S., This P., Troshin L., malpica M., Lefort F., Martinez-Zapater M. (2006). Multiple origin of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphism. *Molecular ecology*, 15, (12): 3707-3714. J
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- Baudler R., Adam L., Rossman A., Versini G., Engel K.H. (2006). Influence of the distillation step on the ratios of stable isotopes of ethanol in cherry brandies. *Journal of agricultural and food chemistry*, 54, (3): 864-869. J
- Bavaresco L., Bertamini M., Iacono F. (2006). Lime-induced chlorosis and physiological responses in grapevine (*Vitis vinifera* L.cv. Pinot blanc) leaves. *Vitis*, 45, (1): 45-46. J
- Beltrami M. E., Ciutti F., Pierdominici E., Mancini L. (2006). Quality assessment and diatom communities analysis of two Italian streams. In: 20. *Treffen der deutschsprachigen Diatomologen mit internationaler Beteiligung: Trebon (Süd-Böhmen), 23-26 März 2006*. Trebon: Institut of botany ASCR: 6. P
- Bertamini M., Muthuchelian K., Nedunchezian N. (2006). Shade effect alters leaf pigments and photosynthetic responses in Norway spruce (*Picea abies* L.) grown under field conditions. *Photosynthetica*, 44, (2): 227-234. J
- Bertamini M., Muthuchelian K., Rubinigg M., Zorer R., Velasco R., Nedunchezian N. (2006). Low-night temperature increased the photoinhibition of photosynthesis in grapevine (*Vitis vinifera* L. cv. Riesling) leaves. *Environmental and experimental botany*, 57, (1-2): 25-31. J
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- Biasioli F., Gasperi F., Mott D., Aprea E., Marini F., Märk T. D. (2006). Characterization of strawberry genotypes by PTR-MS spectral fingerprinting: a three years study. In: *Proceedings of the fifth international strawberry symposium: Coolool beach, Queensland, Australia, September 5-10, 2004* (editor G. Waite). Leuven: ISHS. (Acta Horticulturae 708): 497-500. ISBN: 9066054999. P
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- Budic-Leto I., Lovric T., Gajdoš Kljusuric J., Pezo I., Vrhovsek U. (2006). Anthocyanin composition of the red wine Babic affected by maceration treatment. *European food and research technology*, 222, (3-4): 397-402. J
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- Cestaro A., Dematté L., Fontana P., Perazzolli M., Segala C., Velasco R., Pilati S., Moser C. (2006). Annotation of the Affymetrix *Vitis vinifera* GeneChip® using the gene ontology vocabulary. In: *9th International congress on grape genetics and breeding: programme and abstracts: Udine Fiera congress center, 2-6 July 2006*: Poster 1.9. P
- Ciccotti A. M., Bianchedi P., Bragagna P., Deromedi M., Filippi M., Forno F., Mattedi L. (2006). Natural and experimental transmission of "Candidatus phytoplasma mali" by root bridges. In: *XXth International symposium on virus and virus-like diseases of temperate fruit crops and XIth International symposium on small fruit virus diseases: Antalya, Turkey, May 22-26, 2006*: 125. P

- Ciutti F., Beltrami M.E., Cappelletti C., Corradini F. (2006). Littoral diatoms and trophic state of three subalpine lakes (Trentino, Italy). In É. Ács, K.T. Kiss, J. Padišák & K. É. Szabó (eds). *6th International Symposium on Use of algae for monitoring rivers*. Hungary, Balatonfured, 12-16 sept 2006: 33-36. ISBN 963 06 0497 3 \n. P
- Ciutti F., Beltrami M.E., Cappelletti C., Della Bella V., Mancini L. (2006). Use of diatoms to evaluate water quality in Italy: towards implementation of the European Water Framework Directive. In É. Ács, K.T. Kiss, J. Padišák & K. É. Szabó (eds). *6th International Symposium on Use of algae for monitoring rivers*. Hungary, Balatonfured, 12-16 sept 2006: 37. ISBN 963 06 0497 3. P
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GETTING TO SAN MICHELE ALL'ADIGE



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