

# biodiversity

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# Contents

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2	CREDITS
3	CONTENTS
4	EDITORIAL
6	GREEN HYDROLYSIS CONVERSION OF WOOL WASTES INTO ORGANIC NITROGEN FERTILISERS
7	DONKEY MILK IN PRETERM INFANT NUTRITION
8	YEASTS BIOFACTORIES APPLIED TO THE RESTORATION OF CULTURAL HERITAGE
9	FOOD SECURITY: CONCEIVE PLANT PATHOGEN DETECTION TOOLS TO IMPROVE THE USE OF AGRICULTURAL BIODIVERSITY
10	BIOREMEDIATION OF POLLUTED WATER BY ACINETOBACTER VENETIANUS
11	FOREST GENETIC RESOURCES: A MODEL FOR STUDYING CLIMATIC CHANGE
12	NETTLE, A MULTIPURPOSE PLANT: FIBERS, EXTRACTS AND INSECT REPELLENTS
13	NEW STRATEGIES TO PRODUCE RECOMBINANT PHARMACEUTICAL PROTEINS
14	TOMATOES PRODUCED BY MYCORRHIZAL PLANTS SHOW ENHANCED NUTRACEUTICAL PROPERTIES
15	THE “SOLAR” ENERGY WHICH COMES FROM FRUIT
16	THE PRESERVATION OF CROP GERMPLASM IN APULIA REGION
17	POSIDONIA BEACHED RESIDUES CAN BE USED AS SOIL AMENDMENT AND SOILLESS GROWING MEDIA COMPONENT AFTER COMPOSTING
18	FOCUS ON: SWEETEN OUR CROPS. SUSTAIN A HIGH DIVERSITY OF BENEFICIALS THROUGH “SWEET FOOD” FROM PLANTS

## Biodiversity: a gross value for a *great beauty*

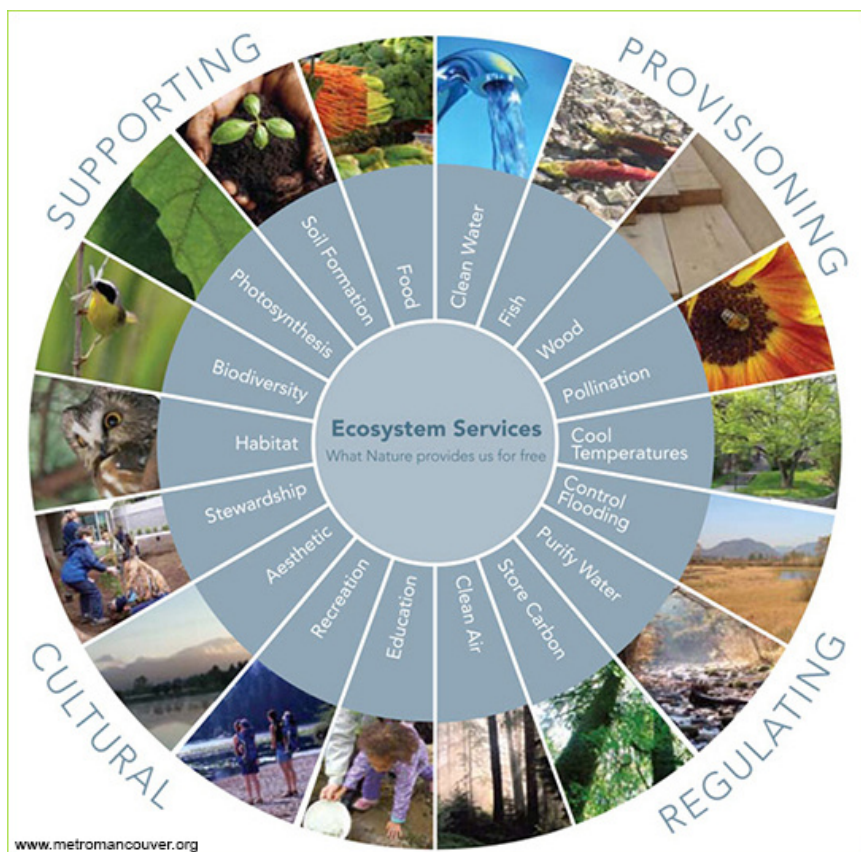
In recent years a rising common concern is looking at biodiversity concept with a new sight, attempting to evaluate its economical value, as ground step for supporting measures proposed by national governments and international committees. Although this utilitarian view applied to a complex concept could cause an underestimation of the true potential of biological resources, nowadays a wide spectrum of direct and indirect quantifiable values has been recognized as tightly correlated to biodiversity.

These economic criteria have been simplistically split into two distinct categories, each of them attempting to reconstitute a concrete outline of biodiversity preservation as a profitable entity. Within this distinction benefits straightly arising from natural resources exploitation are defined as direct-use values, and include consumptive and productive use of biological species. Many examples come easy to mind. Harvesting and direct consumption of plants is a source of food, as well as of active principles extracted for traditional medicine and pharmaceutical purposes. Fish is a relevant source of proteins, widely exploited through fishery and aquaculture.

Fuel is a hugely-exploited product of biodiversity, in form of forestry firewood, natural biogas, or fossils sources like coal and petroleum. In addition, productive values are derivatives of the consumptive values, as they come from primary sources and pass through industrial process, thus increasing their economical value and marketing potential. Examples are given by wool, silk, timber, paper, gum, likewise products banned from trading or commonly perceived as "prohibited" like furs, ivory trunks or coral jewels.

The economic "taste" of direct-use values is easily perceived and continuously growing thanks to the relevant contribution of biotechnologies, that are re-modeling and increasing the mere value of genetic resources. Conversely, it seems often more difficult to quantify and conceive the true incidence of the commonly named indirect or non-use values, apparently lacking in a direct and countable benefit. Nevertheless there is a recognized value provided by the so-called ecosystem services, that are the active mechanisms through which human activities are sustained by natural ecosystems and all the living things they host. The assumption that these natural processes can be thought as a concrete service is consistent with the "industrial" profile held by, for instance, conversion of sunlight to energy through photosynthetic carbon dioxide fixation; retention of nutrients ratio carried





out by carbon, oxygen, nitrogen, sulphur and phosphorus cycles; climate regulation, moisture control and recharge of ground stream through water cycle; recycling of organic waste through biodegradation or moderation of flood damage; control of erosion through soil formation and its interaction with plant root system.

Undoubtedly the better functioning of these ecoservices is strictly related to the preservation of high biodiversity rates. Exactly in the same way as a company employing several and distinct competences will have an higher impact on the market. Nonetheless it results difficult to re-think the value of a natural complex like Amazon forest, given that our mind is instinctively used to consider these entity as a priceless

heritage. A way to estimate this value exists, and is related to the cost we should effort to replace ecosystem action with human-made surrogates. This forecast has been attempted and led to enormous numbers, in the measure of approximately twice the world gross national product. Facing this awareness we have the true perception of how big damage could represent for global economy the past, present and future loss of biodiversity. Every time we create a tear in the ecosystem fabric, we need to pay for it, more and more, to provide again the lost service. In some way, indirect values acquire a fundamental importance, that is even more incident on planet balance than the more easily perceived value of direct exploitation.

Without forgetting that a rational use of the priceless treasure represented by biological resources, should consider within non-use values, also the social and aesthetic impact of nature on people. Natural landscape, national parks or zoological and botanical gardens, in fact providing several opportunities for recreational activities (e.g. bird watching, diving, eco-tourism, hiking, game fishing, photography, etc), constitute a great attractive for millions of people and therefore represent a significant and growing income for state economy. People are concerned with biodiversity more than governments expect, and there is even an ethical issue, well perceived in many cultures, that is our responsibility to future generations to protect from extinction what Nature gave to us. This final consideration seems irrational to conclude an economical dissertation, but it is also true that the "religious" sense for biodiversity constitutes a surplus value to not underestimate in many contexts. Not only in India where plants and animals like peepal tree, lotus, cow, monkey, peacock or snake are still worshipped as sacred, but even in Western countries, where the belief that nature is God's creation is still cited as a fundamental reason to protect biodiversity.

Given these considerations, we should not forget that when, in 1992 the Convention on Biological Diversity established in Rio de Janeiro recognized biodiversity as a "common concern of humankind", was indirectly affirming that it has an economical value. Unfortunately, more than twenty years later, this value is still culpably unknown and underestimated.

Massimiliano Morelli  
*Redactor*

# Green hydrolysis conversion of wool wastes into organic nitrogen fertilisers

Dr. Claudio Tonin, Institute for Macromolecular Studies, CNR-ISMAC

## The Problem

EU-27 has the second world sheep population, numbered about 100 million heads in December 2011, the majority of which were based in the UK (25 %), Spain (20%), Romania (10%), Greece (10%), Italy (9%), France (9 %) and Ireland (4%) (Source EU-Eurostat 2012). The EU flock is made of crossbred sheep not graded for fine wool production; the primary role is meat, whilst the milk market is relatively small, being confined to Mediterranean regions. Sheep population is declining (4% in 2011), with the highest rate being registered in Italy. Nevertheless, slaughter is forecast rising in 2013, thus increasing dependency of imported sheep meat from the Southern Hemisphere, which amounts to 20% about.

Sheep husbandry plays an important role in the subsistence mixed farming systems and is vital in poorer and rural parts of the EU. The annual wool clip amounts to more than 200 000 t (18-20 are produced in Italy) and its management is a specific problem for the EU livestock sector. Indeed, wool from sheep farming and butchery industry is very coarse and contains a lot of kemps (dead fibres), making it practically unserviceable for the textile industry. The use for filling mattresses has been outclassed by synthetic materials, so that there is no market for crossbred wool, excluding handicraft niches. Unserviceable wool is mostly disposed in landfills or illegally thrown over, with serious ambient threats since it can affect the pastures and bring illness if leave out on soil, where it does not readily degrade. Thus, shearing, storage, transportation and disposal of waste wool in accordance with current EU Regulation, heavily weigh on the profit of sheep farming.



Waste wool



Organic nitrogen fertiliser from green hydrolysis conversion of waste wool

## The Solution

The Life+GreenWoolF project, supported by the LIFE 2012 EU financial instrument, aims at demonstrating that waste wools can be recycled into organic nitrogen fertiliser for grassland management and other cultivation. Controlled hydrolysis with superheated water converts wool keratin (the wool protein) into simpler compounds, tailoring the release speed of nutrients to plants. Wool contains elements such as carbon, nitrogen and other nutrients, which play an essential role in plant nutrition. Wool, when added to the soil, increases the yield of grass grown, absorbs and retains moisture very effectively, reduces run off of contaminants such as pesticides, and can aid in water conservation. The environmental soundness of recycling waste wool into amendment-fertilisers relies on the following items:

- the closed-loop cycle grass-wool-grass is an efficient form of recycling, because the wool-grass step is solar powered and reduces the use of synthetic fertilisers;
- recycling unserviceable wool into amendment-fertilisers is a way of exploiting natural renewable resources, reducing organic wastes disposed in landfills (thus promoting waste prevention);
- amendment-fertilisation of grassland contributes to the reduction of soil degradation, including erosion and decline in organic matter;
- grazing sheep increases soil carbon sequestration on grasslands and fertilisation, if not over-used, can enhance the carbon sequestration rate;
- increasing management yield and extension of the pasture lands may contribute to employment and profit of sheep farming, increase EU sheep population, and reduce dependency of imported meat which is forecast to rise in the next years.

# Donkey milk in preterm infant nutrition

Dr. Laura Cavallarin, Institute of Sciences of Food Production, CNR-ISPA

## The Problem

Breastfeeding and human milk are the normative standards for feeding and nutrition of both term and pre-term infants. Fresh mother's own milk is recognized as the optimal choice for feeding all newborns, including preterm and very low birth weight infants. However, human milk does not fully meet all the nutritional needs of premature infants and needs to be supplemented (fortified) with extra protein. Human milk fortifiers were introduced around 20 years ago to overcome the protein and energy deficiency in many preterm infants. Currently marketed fortifiers are bovine milk based products, which strongly differs from human milk as far as the biochemical, and, in particular, protein profile is concerned.



A donkey dairy farm in Piedmont, Italy

For this reason, there is the need to make a new fortifier of human milk available, able to meet the quality protein requirement of preterm infants, in order to assist the ongoing efforts of neonatologists to improve infants' growth and clinical outcomes.

## The Solution

Donkey milk has been successfully used for feeding infants since the nineteenth century and it has recently aroused scientific and clinical interest among pediatric allergologists. A comparative study among the protein and lipid fractions of donkey's, human and cow's milk that we recently conducted, showed evidences on the existing structural similarities between human and donkey's milk protein and lipid fractions.

This suggested that preterm infants fed with human milk fortified with a donkey's milk based fortifier could have better both short and long term auxological and clinical outcomes in comparison to infants fed human milk fortified with the commercially available cow's milk derived products.

A novel human milk fortifier based on donkey's milk was designed and produced in collaboration with the Neonatal Intensive Care Unit of the University of Torino and three SME of the agro-food sector. For the fortifier production process and the final product composition a patent was filed in Italy (n°. MI2013R004585 - 2013). A blind randomized clinical trial, that will last 18 months and enroll around 120 infants, is now in progress, to evaluate the effect of feeding the novel donkey's milk based human milk fortifier to preterm infant on tolerance, growth and general clinical outcomes, when compared to a bovine milk based human milk fortifier.



Lab-scale production of the donkey milk based human milk fortifier



Donkey milk bottles

# Yeasts biofactories applied to the restoration of cultural heritage

Dr. Patrizia Filetici, Institute of Biology and Molecular Pathology, CNR-IBPM

## The Problem

The challenge in the restoration of cultural heritage is to identify novel approaches and technology for the recovery of degraded artistic stoneworks, frescoes and ancient prints. Biocleaning and the production of engineered microorganisms may provide novel tools in a yet exploited field of biotech research. Rare books and ancient prints belonging to world-famous collections are often damaged by poor storage conditions. Ancient paper and pergamena are frequently deteriorated by spores, fungi and microorganisms and the repair and restoration procedure is often expensive and time consuming. Since the seventies enzymes have been used in book recovery as catalytic scalpels to detach the stiff damaged paper block. Papers sealed by old glues or starch paste could be safely separated by proteolytic or carbohydrate splitting enzymes. After detachment the printed sheet is applied to a new clean support, this approach may be similarly applied to frescoes and paintings. Enzymes with different catalytic activities can be used to solve other



Our pilot study on old book restoration: application of alpha-amylase gel produced in secreting yeast strain and detachment of the paper sheets after treatment

problems like surface cleaning from dust or candles grease and color refreshing. Unfortunately, enzymes are expensive and their catalytic activity must be preserved for optimal results. For these reasons restorers may find not convenient their use.

## The Solution

Molecular genetic of yeast makes it feasible to produce cells expressing selected proteins, in our case freshly produced enzymes like amylase, lipases, proteases. Bacteria producing selected enzymes have been previously reported however the application of bacteria on antiques surface may not be safe. In our laboratory at IBPM we have applied a new protocol to produce *S.cerevisiae* yeast strains expressing and secreting selected enzymes in the culture medium. In other words the yeast cell becomes a biofactory able to produce and secrete the enzyme of

interest outside of the cell. As a result the growth medium contains the desired enzyme. This approach not only provide fresh enzymatic activity in the medium but, in addition, avoids the application of living cells directly on the substrate. This is extremely important since it prevents any contamination of the surfaces with living microorganisms.

In our laboratory we inserted the gene coding for Alfa-amylase from barley in a yeast vector and expressed in a yeast strain. Alfa-amylase was successfully secreted in the culture medium and was further assayed in a pilot study in collaboration with a center for paper restoration in Spoleto. The amylase produced was applied on different substrates: old and new paper, pergamena etc. A ready and clean detachment of the paper sheets was obtained. The amylase was also incorporated in a gel which may turn to be very useful for restoration of substrates in semidried condition. The easy and low cost of biotech yeast technology for production of active molecules makes our strategy innovative in the field of cultural heritage restoration. Biotech enzymes may easily be adopted by non expert restorers. Single yeast strain or combination of different enzyme producing strains could also be used for different kind of interventions.

This pilot study may therefore open a new biotech way for the production of useful enzymes applied to the restoration of cultural heritage, it represents a novel translational effort linking biotechnology to antiques restoration.



# Food security: conceive plant pathogen detection tools to improve the use of agricultural biodiversity

Dr. Lilia Formica, AGRITEST S.r.l.

## The Problem

Re-thinking the plant diagnostics business is the coming up challenge of our company. How could the plant health sector be linked to the whole ecosystem health? How can we assess the risk of plant pathogens along the agri-food chain to ensure the well being of humans, animal and environment?

Agritest was set up 20 years ago with the objective of sustaining the nursery industry to certify the phytosanitary status of its products.

In the light of assisting the agriculture sector to compete in the global free market, plant diagnostics were conceived to add value to the marketable products according to the nursery 'quality control' frameworks (regulatory or not).

Specifically, we worked hard to meet the grapevine nursery industry needs, focused on implementing its quality control process in a sustainable and efficient way. Our experience in assisting the nursery industry and its linked partners was plenty of exiting experiences and constructive feedback. Moreover, we gained the prospect of looking at the plant health sector trough a wide angle, exploring the valuable environment living around the farm place.

What we see indeed is a network of interests aimed at achieving 'plant health' for diverse purposes, all converging into the need of using plant biodiversity. Such scenario makes us consider whether our expertise in developing diagnostics for phytosanitary certification would be suitable for ensuring the long-term conservation of genetic resources, improving the availability of agricultural biodiversity, and preventing plant disease out-breaks.

## The Solution

Differently from the diagnostic systems designed for known and predictable targets (i.e. the regulatory defined list of pathogens), safeguarding the ecological health requires its own diagnostic strategies.

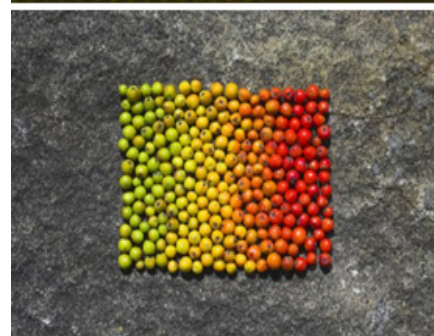
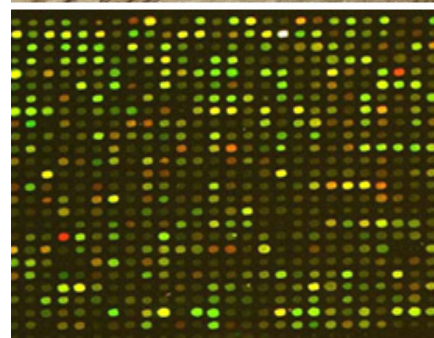
As a diagnostic manufacturer enterprise the key question we face is: how to conceive a detection tools system for unpredictable pathogens on a wide range of plant hosts? Having in mind such challenge, we have identified our needs to design our offer in the short -medium term. We would like to gain three basic conditions: a rapid access to a reliable network of knowledge on plant pathology; easy, yet safe movement of the samples to be analyzed; and feasible diagnostic technologies, fast applicable and robust.

Scientific support on plant pathology is crucial since any detection technology itself might mislead to under/over estimate the health risks. On that respect Agritest, along 20 years of collaboration with CNR-IVV and University of Bari, has established a solid scientific network for technology assessment evaluation against the plant pathology state-of-art.

Agritest is confident that its in-house developed platforms are suitable for designing new diagnostics to meet the eco-health market demand: multiple target detection, multiple samples analysis, large-scale detection for monitoring the environment

Our basic expertise on designing poly probe reagents (on the way for being patented), screening for recombinant antibodies, and handling pathogen reference material and recombinant pathogen proteins is to be re-organized for a new diagnostic offer.

On that respect, we welcome any feedback from scientific or biotechnology business across the human, animal and plant sectors with the objective of implementing the efficient use of the biological resources.



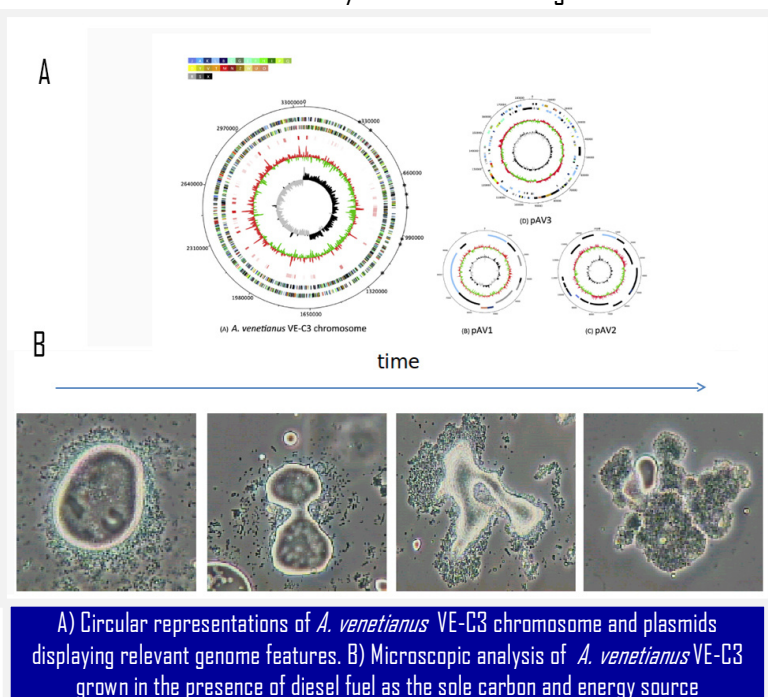
# Bioremediation of polluted water by *Acinetobacter venetianus*

Dr. Ermanno Rizzi, Institute of Biomedical Technologies, CNR-ITB  
Dr. Marco Fondi, Department of Biology, Università di Firenze

## The Problem

The marine environment is subjected to the contamination by organic pollutants from a variety of sources, with crude oil being one of the most important substances. Alkanes are the major components of crude oils and are commonly found in oil-contaminated environments. Aerobic n-alkane degradation is a widespread phenomenon in nature, and several microbial species/strains and enzymes involved in n-alkane degradation have been identified, isolated and studied in detail. Some key enzymes for alkanes degradation are the cytochrome P450 and alkane hydroxylases (pAHs). Several bacterial strains, such as *Acinetobacter* sp. EB104, mycobacteria, rhodococci and proteobacteria are able to degrade C<sub>5</sub> and C<sub>10</sub> alkanes and contain these key enzymes.

Genomes of oil-degrading bacteria have been obtained in recent years, including those of *Acinetobacter* sp. DRI, *Alcanivorax borkumensis* and *Marinobacter aquaeolei* VT8. Some light has been shed on the different strategies for diesel fuel degradation adopted by different *Acinetobacter* strains, suggesting a good efficiency in this process by *A. venetianus*. One *A. venetianus* strain, VE-C3, able to grow on C<sub>1</sub> and C<sub>14</sub>, was isolated in 1993 from the superficial waters of the former industrialized Marghera Port in the Venice lagoon. This area has been polluted due to oil refineries for decades up to late nineties although, today, it is a dismissed and remediated area.



## The Solution

The overall genetic and functional understanding of VE-C3 strain biologically and biotechnologically relevant phenotype, including its adhesion to hydrocarbon molecules, its subsequent metabolism and its adaption to its peculiar ecological niche is far from complete. To address these points whole genome sequencing of the marine, hydrocarbon-degrading bacterium *A. venetianus* VE-C3 was performed by the use of a

comprehensive approach that combined the next-generation sequencing (NGS) platforms Roche/454 and Illumina with the classical Sanger sequencing of PCR products. Genome analysis yielded interesting insights into the biology of this strain, allowing the identification of putative niche-adaptation and bioremediation-related specific gene sets. The sequencing data of VE-C3 strain showed a chromosome of 3.56 million bases large plus 2 small plasmids, of 10,820 and 15,135 bases and a newly identified large plasmid of about 186,446 bases. Beside the identification of alkanes degradation pathway genes, such as alk-like sequences and P450-cytochrome sequences, it was identified the *wee* gene cluster, responsible for the biosynthesis of the polymeric bioemulsifier that could provide the ability to adhere to oil fuel droplets. Important genetic features, related to the environmental adaptation were also identified such as the gene clusters potentially involved in heavy metals resistance (for Arsenic, Chrome, Copper, Cobalt and Zinc). The genome annotation analysis provided information about a massive presence of recombination related genes, showing a high level of horizontal gene transfer (HGT) and recombination events in the *Acinetobacter* genus. Furthermore, using other *Acinetobacter* genus sequences available in databases, it was created an *Acinetobacter* pangenome to evaluate the VE-C3 strain position in the phylogenetic tree. This analysis showed a high phylogenetic distance between the VE-C3 strain from the human pathogen *A. baumannii*.

The findings reported in this work provide a valuable background for future biotechnological applications in the bioremediation field as well as for deeper in silico analyses of *A. venetianus* VE-C3 metabolism.

Results described in this article are available at this link: <http://www.ncbi.nlm.nih.gov/pubmed/23528645>

# Forest genetic resources: a model for studying climatic change

Dr. Andrea Piotti and Dr. Giovanni Giuseppe Vendramin, Institute of Biosciences and Bioresources, CNR-IBBR

## The Problem

The effects of the global climate change (GCC) are easily recognizable in the Mediterranean region. GCC has clearly accelerated since 1970, with an average warming of nearly 2 °C and a rainfall decrease by up to 20% in some areas of the Mediterranean region. The entire region is projected to experience more frequent, prolonged and severe drought as a consequence of GCC, which is expected to generate a reduction of the precipitation and soil water availability, and an higher frequency and intensity of extreme climatic events, e.g. summer heat waves. Among GCC consequences, drought stress is likely to be dramatically relevant for the biodiversity of the Mediterranean in general. Mediterranean forests are fragile ecosystems also threatened by grazing, fires and intensive forest cuttings. Interacting with such disturbances, GCC is fuelling an ever-increasing fragmentation process and the loss of ecosystem services.

The Mediterranean region is among the most important hotspots of plant biodiversity worldwide. Furthermore, Southern Europe had a crucial role during several Pleistocene glacial cycles in terms of number and extension of refugial areas for European flora, nowadays it represents the area where most of plant genetic diversity can be found, and this pattern is particularly well-documented for forest tree species.



## The Solution

What can trees do to cope with GCC? The main strategies are to migrate, to adapt to new environmental conditions or to rely on their phenotypic plasticity. Contrary to early conclusions about a limited migration potential for forest trees, on the basis of recent evidences it is emerging that forest trees have been indeed able to share genetic variability at long distance, in particular through pollen dispersal, that can be effective at  $10^2$ - $10^3$  km. But to cope

with current CCG a similarly large seed dispersal capability is required, which is not the case for many species. It has been estimated that spread rates that allowed a successful escape from past glaciations are far below what would be necessary for species migration to track future climatic warming. The adaptive potential could nonetheless be high in forest trees despite possible dispersal limitations. In fact, besides tracking their ecological niche spatially through migration (without adapting), tree populations could adapt to the ongoing GCC in the short-term through individual physiological tolerance (phenotypic plasticity), and/or in the long term through evolutionary response to climate-induced selective pressure. Common garden experiments and genetic trials have demonstrated that most forest trees populations indeed harbour large genetic diversity for adaptive traits. In addition, local adaptation may be favoured by high levels of within-population genetic variation, large population sizes and extensive pollen immigration characterizing forest tree populations.

Notwithstanding large genetic variation and potentially fast adaptation, there is only anecdotal evidence that forest trees can genetically adapt to contemporary environmental change over a limited number of generations. When environmental conditions deteriorate at a rate to cause demographic decline (e.g. at the rear edge of current species' distribution), adaptive challenges faced by declining populations are markedly different from those experienced during demographic expansion (e.g. at the leading edge of current species' distribution). Adaptation then consists in a race between demographic decline and evolutionary change: if genetic modifications are too slow, the population would go extinct before it can adapt to the new environmental challenges. Only when environmental changes are not too rapid and severe, when the population is initially large and not too isolated, and when evolutionary potential is high favouring fast adaptation, a population can be rescued.

In the near future, a deep knowledge of evolutionary processes involved in forest tree responses to GCC is required. This must rely on a sound multi-disciplinary approach upon which effective strategies for the conservation of forest genetic resources should be based. Future research should be focused on the comprehension of mechanisms and on the development of appropriate management practices for coping with an extraordinary rapid GCC. Only through a joined multidisciplinary effort by ecologists, ecophysiologicals, geneticists and climatologist, high quality data can be generated to understand the biological processes coming along with ongoing GCC and what is their relative relevance. If this research approach is faster than GCC and atropogenic modifications, effective tools for conserving the biodiversity of Mediterranean forests will be provided.

# Nettle, a multipurpose plant: fibers, extracts and insect repellents

Dr. Francesca Camilli and Dr. Sara Di Lonardo, Institute of Biometeorology, CNR-IBIMET

## The Problem

The sustainable use of environmental and agricultural resources is central to the current scientific debate. Natural resources are often neglected or not correctly exploited, with the consequent pauperization of lands, soils and local biodiversity. In the last years, consumer awareness of safer products for human health and the environment has been rising, urging research and policies to find sustainable solutions for the exploitation of natural and agricultural resources.

## The Solution

Stinging nettle (*Urtica dioica* L.) is a very common weed. This plant is undervalued as it could be cultivated as a multi-purpose crop for different production sectors by exploiting all parts of the plant: stem, leaves, roots and seeds. Moreover, nettle is a perennial crop (about 10 years) that could alleviate current problems in agriculture such as over-fertilized soils, soil erosion and resources depletion. For all these reasons, a trial was performed in Tuscany to evaluate the agronomic feasibility of this multi-purpose crop. A selected clone was used and protocols were set up for *in vivo* and *in vitro* propagation. Leaf and stalk tissues were analyzed to characterize the phenolic composition in different periods of the growing season and fiber was extracted from the stems with different methods. Nettle stalks



Plant of *Urtica dioica* L.

attained the quality standards required for fiber processing only in the 2nd and 3rd year of cultivation when the fiber content reached 16% of stalk dry matter at the end of July. In order to take this result into account for managing the crop as a multi-purpose production, the first cutting (at end of April) could be used for fodder, medical or industrial purposes, such as chlorophyll extraction; a second cutting (at end of July) for fiber production and a third (in September) to collect leaves. In addition, water from the retting process gave good results when tested as an aphid repellent. This research showed promising uses for nettles, which could be extended in the future and also generate interesting opportunities also for the green textile markets if technical problems of fiber extraction can be solved.

This work is a just a glimpse of a wider research conceived and designed by our colleague Dr. Laura Bacci, whose memory we would like to honor. Her scientific expertise, her enthusiasm for investigation and simple but efficacious approach to finding solutions set a perfect example for future research. *Grazie Laura.*

Year of cultivation	Stalk density (n·m <sup>-2</sup> )	Height (cm)
2 <sup>nd</sup>	131.7±16.8	170.8±25.7
3 <sup>rd</sup>	97.7±20.2	160.4±10.5

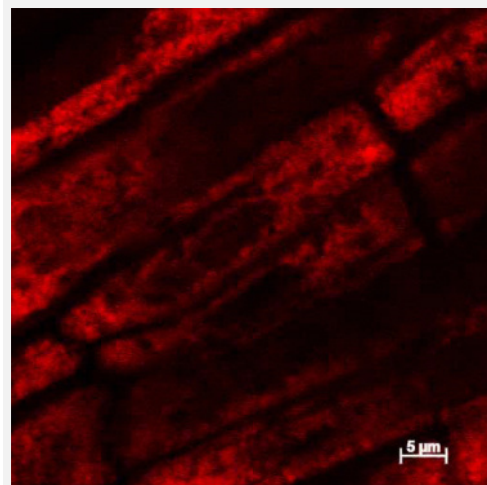
Stalk density and height of stalks of 2<sup>nd</sup> and 3<sup>rd</sup> cultivation year nettle.

# New strategies to produce recombinant pharmaceutical proteins

Dr. Alessandro Vitale and Dr. Emanuela Pedrazzini, Institute of Agricultural Biology and Biotechnology, CNR-IBBA

## The Problem

Most pharmaceutical proteins (such as antibodies, vaccines, therapeutic enzymes, hormones, growth factors) are now produced as recombinant molecules via genetic engineering in bacteria, yeast or mammalian cell cultures, grown in industrial bioreactors. This has reduced production costs and increased safety for patients, two features that can be further greatly improved by exploiting plant cells as bioreactors, either as cell cultures or whole organisms. Unlike industrial bioreactors, growth of plants in controlled greenhouses is a low technology system that can be scaled up or down according to the specific requirements and, most importantly, can be easily adopted by developing countries. Recombinant protein stability is a major bottleneck for high production in any biological system and is dependent both on the protein of interest and the subcellular compartment where it is addressed. The endoplasmic reticulum (ER) is the port of entry of the secretory pathway, which delivers proteins to the cell surface or inner hydrolytic compartments, mainly using the Golgi complex as a transit station. In plant cells, the lumen of ER can tolerate elevated concentration of protein, a feature that has allowed high accumulation of several recombinant proteins targeted and retained in the lumen of this compartment by protein engineering, but the strategy is not always successful.

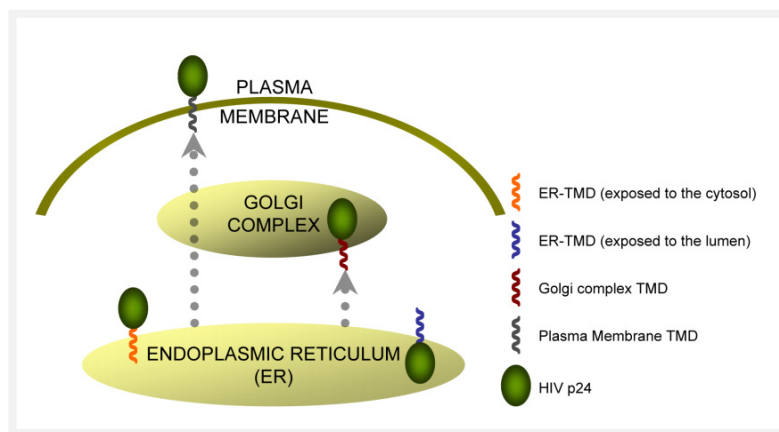


Recombinant HIV p24 fused to a TMD that retains it into the ER membrane and a fluorescent reporter protein (RFP) for detection by microscopy. RFP-p24-TMD highlights the reticular ER pattern in the leaf epidermal cells shown in the image.

## The Solution

Many proteins are naturally anchored to intracellular membranes via hydrophobic amino acid stretches present in their sequence. We have exploited these TransMembrane Domains (TMD) to test whether membrane anchorage can stabilize p24, a soluble protein of the human immunodeficiency virus (HIV) and a potential component for an anti-HIV vaccine. p24 has been previously produced in plants but accumulation either in its native cytosolic location or as a secreted form was not satisfactory. We have tested TMDs that allow

anchoring and retention in the ER membrane, exposing the anchored p24 either into the ER lumen or towards the cytosol, or that allow traffic of the recombinant protein from the ER to the membranes of the Golgi complex or the external membrane of the cell (plasma membrane). Targeting to the plasma or Golgi membranes did not stabilize p24, but anchorage to the ER in both topologies markedly increased the accumulation of the HIV protein. This indicates that not only the ER lumen but also its membrane can be exploited for recombinant protein production in plants, thus electing the plant ER as a compartment of choice for high accumulation. In particular, exposing the ER-anchored protein to the cytosol can be especially convenient to allow correct folding of cytosolic proteins



Schematic representation of strategies to anchor HIV p24 to intracellular membranes via specific transmembrane domains (TMD)

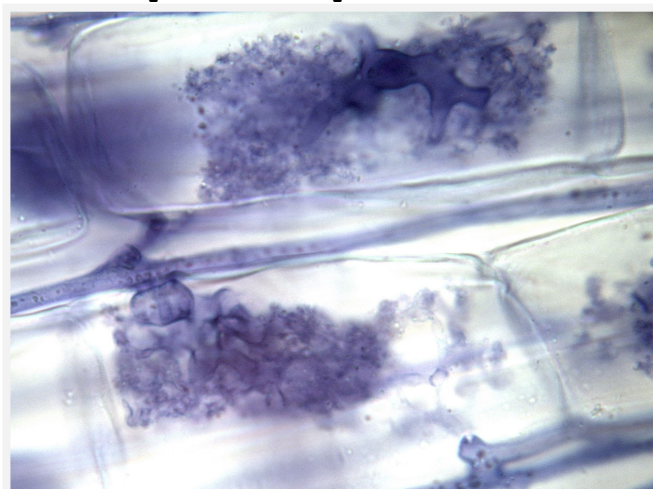
such as p24 in their native environment and at the same time avoid cytosolic degradation pathways. Membrane anchoring can also facilitate downstream purification, by first separating the membrane from the other cellular structures.

# Tomatoes produced by mycorrhizal plants show enhanced nutraceutical properties

Dr. Cristiana Sbrana, Institute of Agricultural Biology and Biotechnology, CNR-IBBA

## The Problem

Metabolic and degenerative illnesses, including cardiovascular diseases and cancer, are increasing worldwide, and a good relationship between functional degeneration and enhanced oxidative cell damage has been observed. Actually, cellular antioxidant systems are able to repair most, though not all, the estimated 10,000 oxidative damages occurring per human cell per day! In addition to endogenous antioxidant defense systems, intake of dietary antioxidants through the consumption of fruits and vegetables may lower cellular damage and risks of degenerative diseases, without side effects. However, the content in vitamins, mineral nutrients, dietary



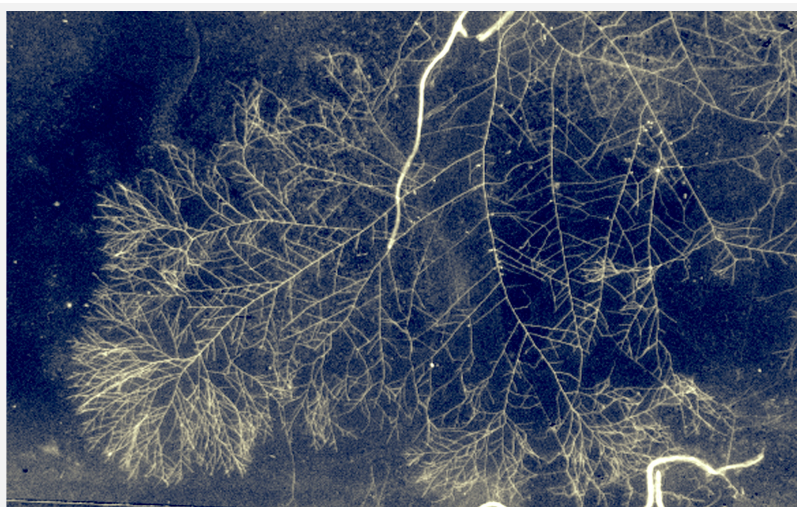
Fungal arbuscules formed in root cells to exchange nutrients with the host

fibers and plant secondary metabolites in fruits and vegetables is not stable, but is affected by plant genotype, crop management, harvest season, soil quality, available nutrients, light and water. The consumer demand for healthy and health-promoting food is growing, and new tomato lines with 10–25 times increased concentration in  $\beta$ -carotene have been developed. Though, scientists have raised concerns on their safety, since high concentrations of secondary metabolites like polyphenols in plant food can be genotoxic.

## The Solution

Soil quality play a key role in the production of plant foods through the action of some beneficial soil microorganisms, the arbuscular mycorrhizal fungi (AMF), living in close association with plant roots.

These mycorrhizal fungi produce an extensive network of extraradical mycelium which absorb and translocate mineral nutrients (P, N, Zn, Cu and others) to the host, enhancing plant nutrient status. The symbiosis also modify plant metabolism, producing higher levels of health-promoting phytochemicals in plant and fruit tissues. Multidisciplinary studies carried out by CNR Institute of Biology and Agrobiotechnology and the Department of Agriculture, Food and Environment, University of Pisa and published in *Plant and Soil* (2010) and *British Journal of Nutrition* (2012), showed that plants used for human nutrition may enhance their nutraceutical value by establishing symbioses with AMF. In a field study, globe artichoke previously inoculated with AMF produced more flower heads with higher content in phenolics and antioxidant activity. Mycorrhizal tomato plants grown in the greenhouse showed a larger production of marketable fruits, whose content in some mineral nutrients such as P, Ca, K and Zn and in lycopene was higher compared to controls. Beside antioxidant content, the increase in mineral nutrients may contribute to biofortification of tomato fruits. Moreover, extracts from tomatoes produced by mycorrhizal plants did not accumulate genotoxic



Fungal mycelium spreading from host roots to absorb nutrients from the environment

compounds and showed larger anti-oestrogenic activity, suggesting their efficacy in antagonizing the activity of estrogen-like molecules possibly contaminating food products and water, and in preventing the development of oestrogen-related cancers. Future agriculture should implement sustainable practices to reduce the environmental impact of intensive management, by using strategies involving the use and maintenance of fundamental ecosystem services provided by beneficial soil microorganisms, such as AMF.

# The "Solar" energy which comes from fruit

Dr. Giuseppe Calogero and Dr. Gaetano Di Marco, Institute for Chemical and Physical Processes, CNR-IPCF

## The Problem

Sun is one of the most promising renewable energy source with minimal adverse environmental impact. Sunlight, in fact, is clean, abundant, environmentally friendly and readily available in most regions on the Earth's surface. In order to preserve cleaned environment, the direct conversion of solar energy into electricity appears as an attractive solution to energy crisis. In this regard, photoelectrochemical solar cells or Dye-sensitized solar cells (DSSCs) are emerging as important low cost third-generation photovoltaic devices. Since their appearance in the late eighties, DSSCs have attracted a lot of attention from the scientific community due to their easy fabrication with respect the traditional crystalline silicon-based ones. In a DSSC the primary charge separation is achieved by means of a photoexcited dye capable of performing the electron injection into the conduction band of a wide band-gap semiconductor, usually  $\text{TiO}_2$  (see Fig. 1). A dye used in DSSC technology must bind strongly to  $\text{TiO}_2$  by means of an anchoring group, typically carboxylic or hydroxyl groups, and ensure efficient electron injection into the titania conduction band. Furthermore it has to strongly absorb a wide range of the solar radiation (visible and near-IR region). Moreover, the dye excited state ( $S^*$ ) must be sufficiently high in energy for efficient charge injection into the  $\text{TiO}_2$ , and its ground state ( $S$ ) be sufficiently low in energy for efficient regeneration of the oxidized dye by the redox couple (iodide/triiodide). The electron

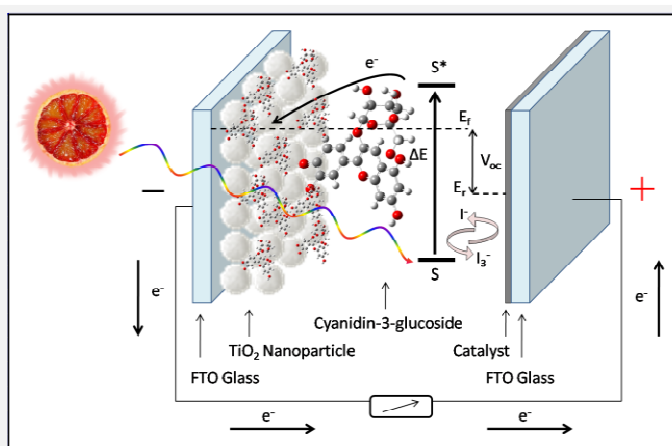


Fig. 1. Cross section view of a DSSC, the dye reported is cyanidin 3-glucoside naturally occurring in red fruits.



Fig. 2. Digital clock powered by natural dye solar cells

transfer from the dye to the  $\text{TiO}_2$  should be faster than the decay-rate to the ground state of the dye. Finally, the oxidized and the reduced forms of the ground and excited states of the dye must be stable. Up to now, considering the crucial role played by the DSSC sensitizer in an extensive effort has been directed towards the development of new families of dyes. Usually  $\text{Ru(II)}$  polypyridine complexes and more recently Zinc porphyrins are used as efficient sensitizers in DSSC. These dyes, however, require a complex long multistep synthesis involving toxic and environmentally harmful chemicals.

## The Solution

Unlike artificial dyes, natural ones are readily available, easy to prepare, inexpensive, non-toxic, fully biodegradable. In short an example of green chemistry. The main families of naturally occurring dyes are three: anthocyanins, chlorophylls and betalains. Of course, progress in design optimization of natural sensitizer is not possible, because their structure is designed by "nature". For this reason, the current research effort is devoted to select natural pigments. The main goal is to isolate the best sensitizer, and at the same time, finding the optimal operating condition to build a DSSC (concentration, pH, extraction techniques, size of titanium oxide nanoparticles, photoanode film thickness, soaking time, electrolyte composition, etc.).

Researchers of the CNR-IPCF, in Messina, have exploited some fruits or vegetables as sensitizers in DSSC. In particular Calogero and Di Marco have patented red Sicilian orange, purple eggplant, cactus prickly pear fruit, syrah grapes, nero d'Avola red wine, etc... Their best result has been obtained with the Sicilian Prickly Pear fruit juice, achieving the value of ~2% for power conversion efficiency with a current density of  $8.80 \text{ mA/cm}^2$ . Remarkably, these "Fruit Solar batteries" are able to replace two 1.5 V AA batteries in a digital quartz clock having a large display (as shown in Fig. 2).

# The preservation of crop germplasm in Apulia Region

Dr. Luigi Trotta, Policies for Rural Development, Regione Puglia

Dr. Costantino Silvio Pirolo, Dep. of Soil, Plant and Food Sciences, Università di Bari

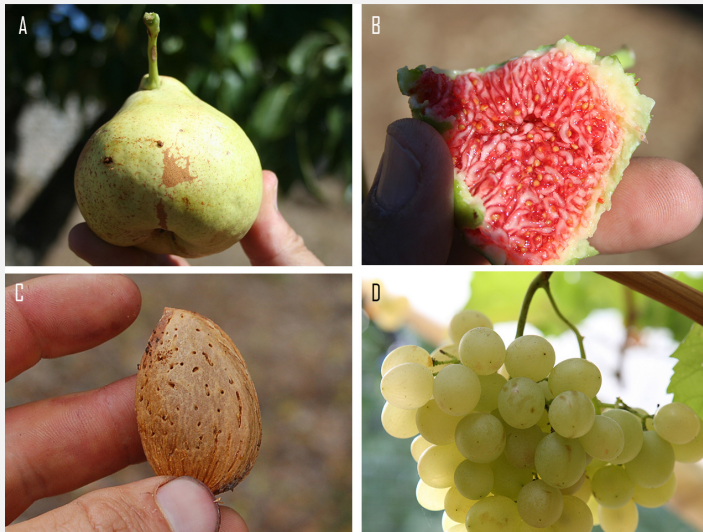
Dr. Grazia Valentino, National Institute of Agricultural Economics, INEA

Dr. Pasquale Venerito, Research, Experimentation and Training Center in Agriculture "Basile Caramia", CRSFA

Dr. Pierfederico La Notte, Institute of Plant Virology, CNR-IVV

## The Problem

Apulia Region, both for its geographical position in the Mediterranean and for historical reasons, is one of the richest Italian regions for agrobiodiversity. In a long period of many centuries the high number of local varieties entered, generation after generation, in the modelling of agricultural landscape, in the life of local communities, in slang terms and dialects, in the typical food recipes, in the history of both aristocratic (an example is the wine grape Primitivo landed in Manduria brought as dowry by the Countess Sabina of Altamura) and popular families (as example the emigrants who carried overseas, in their poor carton suitcase, buds of their varieties). Today, many varieties are officially endangered or missing, replaced by a few other more productive, often imported or obtained through genetic improvement/breeding.



Autoctonous varieties: A) Pear "Pero d'inverno"; B) Fig "Lunghetto"; C) Almond "Mandorla del Gargano"; D) Grape "Baresana"

## The Solution

To prevent the gradual erosion of genetic and cultural heritage, the Apulia Region, in accordance with the FAO Treaty on Plant Genetic Resources, funded for some years several initiatives for the restoration and valorization of ancient cultivated varieties.

Through projects such as "Clonal and sanitary selection of local wine, table grapes and their rootstocks" or "Molecular characterization of cultivars and valuable local sweet cherry germplasm in the province of Bari", etc. the Region started a first important cognitive action concerning the varietal heritage. However, a significant progress in the actions of protection has been achieved through two instruments: the National Plan for Biodiversity of agricultural interest, born out as an instrument of national coordination which finally produced the national guidelines for the in situ, on farm and ex situ conservation of biodiversity, and the Rural Development program of Apulia 2007-2013. In the latter, the Apulia Region wanted to lay solid foundations for a "regional system of biodiversity", relying on specific financing measures: i) Measure 214, Action 3 "Protection of Biodiversity"; ii) Measure 214/4a "Integrated projects for biodiversity"; iii) Measure 214/4b "Creating the network of biodiversity". The measure 214/3 "Protection of biodiversity" acknowledges financial assistance to farms, whose conductors are committed to preserve in situ for 5 years old endangered varieties as indicated in specific lists. The current beneficiaries, qualified as "guardians farmers", are in number about 350, to which are added the same number with a recent new call. The lists were integrated with varietal identification fiches, realized through a preliminary project, for the purpose of verifying the trueness to type of plant varieties eligible for the financial aid. The study produced fiches of 21 vine varieties, 31 olive varieties, 72 different fruit species, 8 varieties of legumes, 14 of vegetables and, in addition successively, 53 new varieties of figs and vegetables. The action 4a is especially important, because it will allow to collect a large amount of plant material at risk of erosion, but also a large amount of information, useful to the implementation of additional forms of protection. The five "integrated projects for biodiversity", one for each species or group of species (vine, olive, fruit plants, legumes/cereals, horticultural crops) have been entrusted in the last days by partnerships between public and private partners include research institutions, trade associations, municipalities, farms, etc. The projects include activities related to 8 different actions: 1) Bibliographical surveys of published and unpublished sources on the indigenous genetic resources (spread, traditional uses, etc.); 2) Search and recovery of reproductive material; 3) Ex situ conservation of local genetic resources; 4) Morphological and genetic characterization; 5) Sanitation of propagation and reproductive materials; 6) Creating inventories, germplasm gene banks and databases; 7) Setting up of varietal descriptive fiches; 8) In situ conservation of local genetic resources at risk of genetic erosion.

The commitment and determination of Apulia Region for the preservation and valorization of crop biodiversity is also demonstrated by the approval of the Regional Law "Protection of indigenous genetic resources of agricultural interest, forestry and livestock" as well as by the will to confirm and even further develop the interventions in the next Rural development Plan 2014/2020, aimed at strengthening the biodiversity regional system.



# Posidonia beached residues can be used as soil amendment and soilless growing media component after composting

Dr. Angelo Parente and Dr. Francesco Montesano, Institute of Science of Food Productions, CNR-ISPA

## The Problem

*Posidonia oceanica* (L.) Del. is a marine monocotyledon endemic of the Mediterranean forming extensive underwater meadows, covering about 40,000 km<sup>2</sup> of the seabed. The plant plays a key role in the marine ecosystem, as it contributes to the production of oxygen (about 14 L·m<sup>-2</sup>·day<sup>-1</sup>) and the stabilization of the seabed. *Posidonia* can produce up to 10 t·ha<sup>-1</sup>·year<sup>-1</sup> of biomass, and has



From left to right: banquette of beached posidonia leaves (A), fibers (B) and egagropili (C)

been estimated that about 25% is accumulated along the coast in the form of beached residues, often in characteristic piles of leaves ("banquette"), fibers (consisting of fractions of rhizome and degraded leaves) and egagropili (spherical conformations originating from fibers and residues of leaves intertwined). As an example, in the coastal site of Mola di Bari (Bari, Italy), were measured approximately 8,800 m<sup>3</sup> of posidonia residues in an area of about 7,400 m<sup>2</sup> in August 2011, while in a stretch of coast approximately 600 m long and 4-5 m wide, in Torre Canne (Fasano, Italy), were measured in the period from August to October 2011 about 2,600 m<sup>3</sup> of residues. Although the beached posidonia residues play an important ecological role for the protection of coasts from erosion, the presence of such biomass along some stretches of the coast represents a complex problem for the coastal municipalities when, for example, the accumulation interferes with economic and recreational activities, first of all the touristic use of beaches. The presence of high amounts of residues in urban areas may also represent a hardship because of the odors developing from the accumulation, or even a danger because of the possibility of fires, with consequent problems of public health and safety. In all these cases, the coastal municipalities are forced to remove seagrass residues that, in the absence of alternative solutions, are disposed of in landfills resulting in both environmental charges, due to the significant amount of organic matter removed from the ecosystem, and economic due to disposal costs. The search for alternatives to landfill, providing on the contrary for the re-use of this biomass, is strongly encouraged.



Use of posidonia compost as growing media component in nursery industry

## The Solution

The residues of beached posidonia can be reused in agriculture through the composting process. Italian legislation (Legislative Decree no. 75 of 29 April 2010) allowed fairly recently the use of posidonia residues as raw material for the production of compost, although at a maximum rate of 20% by fresh weight of the composting mixture. High presence of sand mixed to residues after removing from beaches and high salinity levels represent at the moment the main obstacles to the large use of this biomass as raw material for composting. According to Italian regulations, sand has to be separated and left on beaches before composting these biomass (has been found that 1 m<sup>3</sup> of posidonia residues accumulated on a sandy

beach can hold up to 150 kg of sand). Studies have been conducted, in the framework of the Life09 "Posidonia Residues Integrated Management for Eco-sustainability" (P.R.I.M.E.) project financed by the European Commission, with the aim to increase the application of posidonia residues for compost production. The sand has been effectively separated from the organic material through the use of a "rotative sieve" prototype, and the salinity has been easily reduced by storing the collected material and simulating the rain. The compost obtained can be used as a substrate, alone or in mixture, for the vegetable transplants production or in soilless cultivation in replacement of peat or other growing media, as well as a soil amendment. This is also in line with European Union guidelines that since 2001, in order to encourage the recovery of organic matter, gives peat-free growing media the "Eco-label" certification. The compost obtained using the residues of posidonia is of good quality: the physical-chemical characteristics are within the limits imposed by Italian legislation for commercialization and the endowment of nutritional elements for plant growth results interesting. The production of compost, in addition to recovering the organic substance otherwise disposed of in landfills, allows to obtain growing media for the seedlings production and for growing plants in soilless conditions, by adapting the physical characteristics of this material to the cultivation system and crop requirements through proper mixtures.

## Sweeten our crops. Sustain a high diversity of beneficials through “sweet food” from plants

Dr. Roberto A. Pantaleoni, Institute of Ecosystem Study, CNR-ISE

A sweet tooth overhangs the terrestrial ecosystems, it is nursed by plants constantly producing pollen and nectar and indirectly honeydew and honey. Truly the “green” Earth is “a land flowing with milk and honey” for myriads of species: from lizards to bees, from birds to flies, from man to ants, through marsupials, beetles, bats and so on. This sweet plant-provided food is the powerful basis of pollination and of many other multi-trophic interactions.

Plants provide nectar and pollen to attract pollinators, in addition plants provide food as a protection strategy attracting predators that can act as bodyguards. The frequent presence of extrafloral nectaries is a direct evidence of this strategy. A wide range of predaceous insects (and other arthropods), in any developmental stages, requires sweet plant-provided food as a needful part of their diet.

The role of sweet food in plant-herbivore-carnivore interactions is important in biological pest control practices. Biological control workers had the awareness in a long time that in agroecosystems the absence of non-prey food sources could constrain the effectiveness of natural enemies and that adding food sources could enhance the effectiveness of biological control programs. Three approaches have been developed to manage the sweet food sources in modern monocultures: i) the diversification of the landscape (intercropping, uncultivated margins, etc.); ii) food sprays or other artificial food supplements; iii) crop varieties producing extra-floral nectar (e.g. cotton varieties with extra-floral nectaries).



An adult of *Hypochrysa elegans* is eating  
(by Claudio Labriola)

### *A terminological issue*

In scientific English, there are terms to indicate the nectar-feeder (nectarivorous or nectar-feeding), the pollen-feeder (pollenophagous or pollen-feeding), and also the honeydew-feeder (if we are satisfied with the term honeydew-feeding), but there is no general term to indicate a mixed diet of nectar, pollen and honeydew (if not also honey). Unfortunately a strict specialization on nectar or pollen is not a rule.

So e.g., in order to define the adult lacewings belonging to the genera that are not predaceous, we had to write “nectar and pollen-feeding adults” (ignoring the indeed very important honeydew). This is a very inelegant convoluted wording and we miss the simple Italian word “glicifago” of Greek derivation (γλυκός sweet + φαγος feeder) that means feeder on sweet food (similar words exist in the other Romance languages).

Even in English the use of the word *glyciphage* (and its derivatives *glyciphagous*, *glyciphagy*) would be more clear, precise and concise in order to indicate the diet of the several hundreds of thousands of species which feed generically on some or all of the sweet plant-derived foods.

The green lacewings (Neuroptera Chrysopidae) are a paradigmatic group of beneficials that needs sweet food. Whereas the diet of adults is based in many genera on non-prey food, the larvae are considered only predaceous. The fact that the larvae ingest sweet liquids has been well known but this behavior was commonly considered occasional, and overall required in order to survive during periods without prey. Only recently some authors found out that extra-floral nectar provides nutritional benefits enhancing the growth and fasting the development of the lacewing larvae.

Laboratory studies, in which a solution of fructose was been available to newly-hatched larvae, demonstrate as this behavior has a more complex adaptive reason. Actually, fructose solution is never refused by larvae, it has a significant effect on reducing mortality and it is able to ‘cover for’ the absence of a protein source for at least 24 hours. Development times are shorter if fructose is given. The sugar gives a necessary, important energy supply in some particularly delicate moments of the development cycle. Especially at the post-hatch time when the larva has to face great difficulties in searching for, attacking and overpowering prey.

Probably similar needs are shared with other groups of beneficials regarded as exclusively predaceous, so the influence of the sweet tooth in agroecosystems is probably wider than believed.

