



AGRICULTURAL RESEARCH INSTITUTE
Centre of Excellence
in Agriculture and Environment

REVIEW FOR 2012-2013



**MINISTRY OF AGRICULTURE, NATURAL
RESOURCES AND ENVIRONMENT
LEFKOSIA**

June 2014



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THE AGRICULTURAL RESEARCH INSTITUTE

The Agricultural Research Institute (ARI) was established in 1962, shortly after Cyprus gained its independence, as a cooperative project between the Government of Cyprus and the United Nations Development Programme Fund, with the Food and Agriculture Organization (FAO) of the United Nations acting as the executive agency. By the time it was entrusted, to the Government of Cyprus in 1967, it had been firmly established as a research institution staffed predominantly by local scientists. ARI constitutes a Department of the Ministry of Agriculture, Natural Resources and the Environment and is headquartered at Athalassa, on the outskirts of Lefkosia.

The Institute undertakes research within the wider domain of plant and animal production. Its mission is to provide high quality scientific research with the objective of achieving a secure supply of safe, good quality food produced by methods financially, environmentally and socially sustainable. It develops or adapts and evaluates under local conditions scientific findings and technology available from international and regional research institutions. ARI's contribution to the solution of actual problems and to the introduction of new technological methods and approaches in agricultural production is highly valued, both locally and abroad, as reflected preeminently in its selection by the European Commission in 2000 as a Centre of Excellence in Agriculture and Environment.

The ARI is organized in two divisions and eight sections: a) the Production Division which comprises the Sections of Plant Improvement, Fruit Trees and Viticulture, Vegetable and Ornamental Crops and Animal Production and b) the Scientific Support Division, which comprises the Sections of Plant Protection, Soil Science, Rural Development and Agrobiotechnology. The latter Division also includes the Variety Examination Centre. The Institute is further equipped with State-of-the-art laboratories, a gene bank, an herbarium and a library carrying leading international agricultural journals and over 5000 volumes.

Human resources at ARI consist of 40 scientists, specialized in various disciplines of plant and animal production, 64 technicians, 14 administrative and accounting personnel, as well as permanent and seasonal labour force. Most of the scientists are PhD or MSc holders and all technicians have been trained either locally or abroad in their respective fields of work.

ARI's work is outlined in the Institute's Biennial Review. Research work of international interest is regularly published in international scientific journals. Transfer of research results and of new technology to the farming community is effected through radio and television programs, popular articles in local agricultural magazines and the daily press and through the extension services of the Department of Agriculture. Innovative knowledge is also transferred through regular seminars, training sessions, demonstration trials and field days.

The Institute is the national AGRIS Centre collecting, cataloguing and indexing the agricultural literature published in Cyprus and is also the national CARIS Centre collating information on on-going research. All this information is supplied to FAO for inclusion in the global data bases of the AGRIS and CARIS systems.

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DIRECTOR'S REPORT

“In Search of Excellence”

Under the spectre of the national and international economic crisis, the fundamental role of research and development in spearheading the economy has been widely acknowledged. In this current context, the Agricultural Research Institute (ARI) has reaffirmed its role as a driver of innovation and economic growth for the agricultural sector of Cyprus. Since its establishment, the Institute has been actively cooperating with national, regional and international organizations and research networks, including the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, the International Centre for Agricultural Research in Dry Areas (ICARDA), the International Potato Centre, the International Centre for Advanced Agronomic Mediterranean Studies (CIHEAM), and numerous academic institutions. Supported by its wide network of cooperation, the ARI has augmented its efforts for providing solutions to problems arising from the management of agricultural systems and of their footprint on the environment, and to providing novel methods and tools for the optimization of agricultural productivity and sustainability. These efforts have been pursued through a substantial number of research activities, outlined below, many of which are supported by the European Union (EU) and the Cyprus Research Promotion Foundation.

Plant Improvement has focused on the introduction, development and distribution of new varieties carrying the genetic potential for higher production, improved quality characteristics, resistance to pests and diseases and tolerance to abiotic stress, including drought. Particular emphasis has been given to the improvement of cereals, food legumes and fodders, utilizing both field methods and molecular-genetic approaches. New varieties from the ARI's breeding programmes have been registered in the National Catalogue, including two varieties of bread wheat ('Akhelia' and 'Yiolou'), two varieties of common vetch ('Kimon' and 'Zinon'), two varieties of ground nut ('Ntopia' and 'Nikoklia') and a barley variety named 'Pyge'.

Plant Protection methods for the viral-disease-free production of citrus, grapevine and stone fruit stocks have been developed, including clonal and sanitary selection, thermotherapy, tissue culture and virus indexing techniques. The control of insect pests and soil-borne diseases has also been studied using conventional and advanced integrated methods in vegetables, grapevines, citrus and other fruit crops. Within the context of Integrated Crop Management (ICM), sustainable approaches for the control of pests and diseases in agricultural production have been tested, aimed at the production of agricultural commodities with minimal application of pesticides and other chemicals.

Horticultural research programmes have focused on improving cultural practices and at evaluating new scion and rootstock varieties. Research activity on Vegetable and Ornamental Crops aimed at improving crop management practices and at preserving postharvest quality through optimized handling methods. Of particular interest has been the effect of preharvest factors such as varietal selection, grafting and crop management on quality and postharvest performance of fresh produce. Nutritional safety characteristics of salad crops have been evaluated with respect to the impact of nitrogen fertilization strategies. New growing media and methods of Ornamental Crops production have been investigated. Emphasis has been given to the utilization of endemic plants for floricultural purposes. The potential for exploiting salt-tolerant species for hydroponic cut-flower production under saline conditions has been

examined. Work on Viticulture included the evaluation of new table grape varieties on American rootstocks. Local wine grape varieties have been evaluated and their ampelographic description has been in progress along with the collection, evaluation and morphological characterization of *in-situ* populations of wild vines and of old neglected varieties. Local clones of pomegranate and several cherry varieties are evaluated and in citrus, research work on rootstock evaluation continues. The conservation, evaluation and management of local olive genetic resources remain a key priority.

Research activity in Agrobiotechnology encompassed molecular biology, food science, agricultural microbiology and agricultural chemistry projects. Quantitative and qualitative tests for the presence of genetically modified seeds in imported corn and soybean were carried out. The antioxidant and antimicrobial properties of natural products such as essential oils and plant extracts have been investigated. The impact of treated waste water use on the microbial load of fruits and vegetables has been explored. Bacteria able to degrade fungicides and herbicides have been isolated and characterized and the same approach was used for the remediation of other recalcitrant pollutants such as petroleum hydrocarbons. Screening of local isolated microalgae strains was performed in order to isolate efficient and promising strains for biofuel production and added value co-products.

Research activities in Soil Science related to the design and application of closed hydroponic systems adapted to Cyprus conditions. Increasing water use efficiency, preventing pollution and using environmentally friendly local substrates have been among the main objectives. The potential for using biomass for biofuel and energy production in Cyprus and the use of agricultural waste products and by-products have been examined.

In Animal Production research aimed at enhancing productivity through improved breeding methods, management techniques, and feeding practices. The effects of artificial rearing practices on lamb and kid growth and on dam milk performance have been investigated. The genetic improvement of economically important characters, such as milk, meat, and fecundity, has been pursued by methods of selection. A project for combating the scrapie disease in sheep, using molecular-genetic methods, has been successfully implemented. The Chios sheep unit of ARI at Athalassa has been transformed into a nucleus herd of about 500 disease-resistant animals, which provides farmers with scrapie-resistant stock of high genetic value. Additional studies have been initiated to combat the scrapie disease in goats, by characterizing further the various scrapie genotypes in the ARI Damascus goat unit.

Agro-economic studies have been at the core of activity related to Rural Development, as time-series data and market prices are utilized for the prediction of market trends for crop and animal products. Following the membership of Cyprus to the EU, the ARI has been assigned the task of maintaining the Farm Accountancy Data Network in Cyprus. Targeted studies, commissioned by the Ministry of Agriculture, Natural Resources and the Environment, have been conducted on agricultural economics and the trade of agricultural products. Work on robotic technology in agriculture has also been conducted and the newly established Remote Sensing Laboratory (RSL) continued its work on remote sensing and geo-information for agricultural and environmental applications.

The contribution of the ARI to agriculture and the environment has been valuable and substantial; it has contributed through concerted actions to the improvement of agricultural production in Cyprus, by generating solutions to emerging problems and by introducing innovative technology, thus promoting a better future for the Cypriot farmer. Furthermore, the ARI has functioned as a pivotal centre for collection, evaluation and dissemination of information on agricultural matters and technology and has incorporated

in its scope of activities the training of scientists, agricultural extension officers, technicians and farmers. The selection of the ARI by the European Commission in 2000 as a Centre of Excellence in Agriculture and Environment has constituted a landmark recognition reflecting on the high standard of scientific research conducted, and on its contribution to agricultural development and the protection of the environment. It is a pleasure for me to acknowledge the tireless efforts of both the ARI staff and their commitment to quality research, which remains the foundation for all future achievements.



Dr Dora S. Chimonidou
Director

PLANT IMPROVEMENT

The main activities of the Plant Improvement Section concern the improvement through breeding of barley, forage plants, seed and food legumes, durum and bread wheat, triticale, as well as studies of genetic and environmental factors affecting their productivity, quality, and resistance to diseases. Work also involves methods of improving cultural practices of forage plants, grain cereals, legumes, potatoes and aromatic plants. The Section is also responsible for the National Genebank and Herbarium.

Barley is the cereal most adapted to the often harsh Cyprus agroclimatic conditions. Spring barley is grown during winter in Cyprus as the main rainfed crop for livestock feed (hay or grain). The barley breeding programme aims at the development of new, well adapted and drought-tolerant cultivars with improved quality characteristics, and of naked barley cultivars for human consumption and as feed for monogastric animals. Wild species (*Hordeum vulgare* spp. *agriocrithon* Aberg) and landraces are used in crosses to improve biotic and abiotic stress resistance.

The durum wheat improvement programme aims to enhance the productivity and economic value of grain and straw through hybridization and selection for agronomic performance and quality. Environmental and genetic factors affecting grain yield, water use efficiency and quality of durum wheat are within the scope of the current research work. The cereal technology programme is complementary to the improvement programme and aims at identifying varieties with suitable quality for the local farmer. Cultural practices are also examined for improved production. Factors that could enhance the quality of industrially made local bread are also evaluated in collaboration with local mills and bakers.

Forage production has increased both quantitatively and qualitatively through the introduction of new species/varieties, selection of local material and improved management practices. Crops under investigation include barley, oats, bread wheat, vetch, alfalfa, triticale, and peas. Triticale is a fast developing crop introduced mainly by cattle growers in the last few years. In the absence of a locally originated variety, all triticale seeds are currently imported or produced locally by imported varieties. Through the research programme for the development of triticale two new varieties were submitted for examination and registration.

Two bread wheat varieties named Akhelia and Yiolou were registered in the National Catalogue and their distribution will begin in the near future. They are destined both for forage and grain production.

The ARI legume breeding programme has developed and registered in the National Catalogue two new groundnut varieties, called Nikoklia and Ntopia. These were presented to the local farmers who showed a great interest in reproducing and trading them.

A new project for the genetic improvement of Cyprus cowpea landraces with respect to climate change is progressing with good results for this important crop. The project identified exploitable genetic variation in all landraces under study, and isolated symbiotic nitrogen-fixing bacteria from local populations. In addition, work on the on-farm (*in situ*) conservation of Cyprus eggplant landraces through a novel approach is



progressing very satisfactorily. Further work on potato crops has continued with the evaluation of new varieties and clones, and potato material with coloured flesh.

The National Genebank efforts have concentrated on the collection of new genetic material and on the conservation, regeneration, evaluation and documentation of the existing germplasm. Work in the National Herbarium concerned the maintenance and documentation of the existing specimens and further research on the Cyprus flora.

Research on aromatic and medicinal plants focused on cultural techniques required for the main aromatic and medicinal plants grown in Cyprus, with emphasis placed on essential oils and their properties. Work has commenced on examining the impact of deficit irrigation on spearmint (*Mentha spicata* L.) biomass production, oil yield, and oil quality. New species of medicinal and aromatic plants, such as stevia (*Stevia rebaudiana* Bertoni), with multiple benefits to the consumer's health, are studied as potential alternative crops for the Cypriot farmer.

CROP IMPROVEMENT

Breeding barley for grain and hay

Specific aims of the barley breeding programme include the development of cultivars with improved adaptation, particularly in view of climate change, improved drought resistance and improved quality traits, such as threshability, high hectolitre weight, uniform grains and high protein content. In addition, the development of cultivars that would be suitable for both grain and hay consumption has been a priority. Trials were performed in four different locations across the island. A series of crosses was performed between locally adapted material and promising imported germplasm. Trials using internationally available germplasm, in collaboration with CIMMYT and ICARDA, continued. Following the global trends and concerns about a healthy and well-balanced human diet, the barley breeding program has focused on the development of appropriate naked barley cultivars to enhance human nutrition. A new hooded barley cultivar, named Pyge, has been registered in the National Catalogue. Furthermore, as part of the barley breeding programme, exploitation of the capabilities of honeycomb breeding were used to deal with the disturbing effects of genotype by environment (GxE) interactions and incorporate stability and responsiveness to inputs. In addition, a natural barley mutation has been identified and followed through the generations. (D.A. Fasoula)



Whole-plant, crop yield field phenotyping, molecular breeding and studies of epigenetic variation

An important component of the barley and cowpea breeding programmes is the development of novel approaches to address the well-known challenge of bridging the gap between genotype and phenotype. Two novel selection equations have been developed exploiting the unique capabilities of honeycomb breeding for whole-plant, crop yield field phenotyping as well as for the study of individual genomes and epigenetic variation. Part of this work was presented in an invited talk to the COST Action FA0806-Plant Epigenetics Workshop in Switzerland. **(D.A. Fasoula)**

Breeding durum wheat, bread wheat and triticale

The aim of the programme is to develop cultivars of high and stable grain yield under rain-fed and supplementary irrigation conditions. Work is based mainly on two approaches: The first is the crossing of selected cultivars possessing desirable agronomic and quality characteristics, until a desired line is developed and checked in the field. A second and faster approach is the introduction of genetic material under development by CIMMYT and ICARDA, which is checked and selected under the climatic conditions of Cyprus. Sometimes a combination of these approaches is necessary for the achievement of a very good line. The practiced breeding method is a modification of the pedigree method, which retains, for subsequent exploration, F3 lines with a higher frequency of promising genes for high quality and agronomic characteristics positively associated with grain yield early in the programme. Outstanding F5 lines enter the preliminary yield trials and the best proceed to Variety for Cultivation and Use (VCU) tests for registration. The two durum wheat cultivars that are currently cultivated the most are the ARI varieties Ourania and Hekabe. The two varieties were recently grown under organic farming conditions, exhibiting promising performance. Soon, new lines with improved quality characteristics are expected to replace these varieties.

Forage production could be improved both quantitatively and qualitatively through crossing and selection. Thus, the programme aims at identifying new varieties with high forage production and high nutritional value. The most promising lines are tested at several sites, in order to assess dry yield potential, nutritional value, disease and lodging resistance. New bread wheat lines having tall, leafy and strong stem, selected from the bread wheat improvement programme, were further tested for their forage mass production and have been registered in the National Catalogue under the names Akhelia and Yiolou. These varieties proved to be tolerant to dry conditions and have much better yield than durum wheat. In another project, the most promising triticale lines, in terms of quality and yield, from the ARI development programme were promoted for advanced trials and two of them were sent for Variety for Cultivation and Use (VCU) trials and Distinctness, Uniformity and Stability (DUS) tests for the current cultivation season. **(A. Pallides)**

FODDERS AND PASTURES

Grain and forage legumes

In recent years, rainfed legumes have become increasingly popular, because of their efficient use in rotation systems with cereals and their higher feeding value than cereals, either as grain or as dry forage mass. Thus, in current research projects special emphasis has been placed on the evaluation of common vetch, field peas, and alfalfa, among others, for forage production.

One of the Plant Improvement Section's most crucial duties is the maintenance of breeder's seed for all the varieties produced in the ARI. This is also the case for legumes where the Section preserves the purity of many varieties of common vetch, field pea, fava beans, lentils, chickpea, bitter vetch, ground nut and other. Two new varieties of common vetch (*Vicia sativa*), Kimon and Zinon, were registered in the National Catalogue and have become available through seed companies for cultivation. Two groundnut (*Arachis hypogaea*) varieties have passed the variety examination tests and were registered in the National Catalogue under the names of Nikoklia and Ntopia. (A. Pallides)

Alfalfa (*Medicago sativa*) is a high protein feeding stuff of great importance. Four populations have been stabilized and are currently evaluated for their yield and quality potential. One variety is in the final stage for registration in the National Catalogue. (D.A. Fasoula and A. Pallides)

Cowpea improvement

The cowpea is a subtropical legume with substantial tolerance to heat and the drier conditions experienced in Cyprus, and a favourite traditional food among the local population. A relevant project aims at the development of improved cowpea varieties, well adapted to the changing climate conditions of south-east Mediterranean. The project also involves the study of the microbial environment of local cowpea landraces as well as the use of remote sensing technology to facilitate informed irrigation decisions for the farmers. Nitrogen-fixing symbiotic bacteria have been isolated from Cyprus cowpea landraces and studied at the molecular level. Student training associated with the project has been provided. (D.A. Fasoula)



POTATOES

Evaluation of potato clones

Since 2005, the ARI offers its services to breeding stations and companies for the evaluation of potato clones and varieties on a subsidy basis. Potato clones are assessed in cooperation with potato breeding stations in Europe. The objective is to select clones suitable for the local climatic conditions from the early stages of the screening procedure. The clonal material was received from Germicopa SA, France and the Irish Potato Marketing, Ireland. Nineteen clones were tested in 2012 and thirty-two in 2013. (S. Gregoriou)



Multiplication and evaluation of potato genetic material

Thirteen old German varieties with coloured flesh were imported from the Genebank Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) for evaluation and multiplication. In 2013, the plants were planted in a net house at Athalassa and in the open field at Xylotymvou Experimental Station of the Institute. Drippers of 4 l/h capacity on 16 mm diameter polyethylene line were used for irrigation and fertigation. (S. Gregoriou)



CONSERVATION OF PLANT GENETIC RESOURCES

National coordination

Cooperation with Biodiversity International, mainly through participation in the European Cooperative Program for Plant Genetic Resources (ECPGR), as well as with ICARDA and other international and national organizations continued. ARI is the National Focal Point in the European Internet Search Catalogue (EURISCO), the national correspondent for the FAO-World Information and Early Warning System on Plant Genetic Resources and the national representative to the Committee established by the Council Regulation 870/2004. It is also involved in the implementation, at the national level, of the International Treaty on Plant Genetic Resources for Food and Agriculture. (A.Kyratzis)

National Genebank (CYPARI)

Seed collection has focused on native plant genetic resources threatened by genetic erosion or extinction and on useful plants such as rare species, landraces, and crop wild relatives. Around 450 new accessions have been registered during the last two years. More than 485 germination tests have been conducted to assess the germination capacity of conserved seeds. Accessions with low germination



capacity or with few seeds in stock have been regenerated. During the last two years, 394 accessions have been successfully regenerated. In total, around 1,750 accessions of local landraces and wild species, including crop wild relatives and endemic species, are conserved in the Genebank under controlled conditions (0 to 4, and -20 °C). Access to passport data can be gained through the EURISCO database. (A.Kyratzis)

In a collaborative joint mission with ICARDA and the Margot Forde Forage Germplasm Centre (New Zealand), 250 accessions have been collected

representing a wide diversity of native crop wild relatives. Most of the accessions collected belong to the species *Hordeum* sp., *Aegilops* sp., *Medicago* sp., *Trifolium* sp., *Dactylis* sp., *Lolium* sp., *Plantago* sp., and *Brachypodium* sp. Duplications of these accessions have been sent to ICARDA's genebank and to Margot Forde Forage Germplasm Centre for safety duplication. (A.Kyratzis)

The project “Ensuring the survival of endangered plants in the Mediterranean – MAVA project” started in October 2011 and is funded by the MAVA foundation. The consortium comprises six partners from the Mediterranean islands and the Kew Royal Botanic Gardens and serves as the project coordinator. The main goal of the project is to ensure the survival of threatened plant species in the Mediterranean basin through *ex situ* conservation measures. One hundred and fifty accessions from Cyprus have been collected during the last two years. The vast majority of the collected species represent rare, threatened species of the flora of Cyprus as well as endemic species. Extensive germination tests have been conducted and optimum germination conditions have been established for most species. Duplications of these accessions have been sent to Millennium Seed Genebank for safety duplication. (A.Kyratzis)



The main objectives of the project “Linking on-farm and *ex situ* activities to the conservation of vegetable landraces” are to survey and develop an inventory of vegetable landraces still in use, to collect and conserve seeds in the ARI Genebank, to create a seed exchange network and to promote on-farm conservation activities among interested farmers. Efforts have been focused on the collection of landraces without any commercial value and threatened with extinction. Linkages with stakeholders (NGOs) have been established and potential ways of collaboration have been explored. Local eggplant landraces have been chosen for field experimentation as a first case study for the genetic variation existing within local vegetable landraces and the potential for optimized on-farm conservation. (A. Kyratzis and D.A. Fasoula)

National Herbarium

More than 12,000 named specimens are kept in the National Herbarium. Priority has been given to the conservation of the specimens and their protection from pests and diseases. In collaboration with ICARDA, the KEW Gardens and the University of Birmingham, a joint collecting mission of herbarium specimens was organized in May 2012. In total, 333 herbarium vouchers have been collected mostly from native plants of crop wild relatives. In collaboration with the University of Birmingham, the National Strategy for the *in situ* and *ex situ* conservation of native plants that are wild relatives of crops has been

finalized. For the development of the strategy, 178 native wild plant species that are relatives of globally or regionally important cultivated species have been selected. The main areas for *ex situ* and *in situ* conservation and areas which require more survey are summarized in Fig. 1 (A. Kyratzis).



Figure 1. Summary of the Crop Wild Relatives (CWR) conservation strategy for Cyprus. Blue squares are top 10 priority sites for genetic reserves establishment, brown enclosed area requires CWR surveying, green enclosed areas are areas for further *ex situ* collecting, grey enclosed area is the area with the highest CWR richness and existing protected areas are shown as black enclosed areas.

AROMATIC AND MEDICINAL PLANTS

Impact of deficit irrigation on spearmint

In 2011, a spearmint (*Mentha viridis*) plantation was established at the Zygi Experimental Station. The aim of the experiment was to study the impact of deficit irrigation on spearmint biomass production, oil yield, and oil quality. Furthermore, the antioxidant and antimicrobial activity of spearmint essential oil were examined. Three levels of irrigation were applied: 60%, 80%, and 100% of the estimated irrigation requirement. In 2011, all plots were irrigated adequately to establish uniform stands across all plots. During the 2012 and 2013 growing seasons, deficit irrigation management procedures were implemented on these plots. Additionally, three harvests took place per year and the samples were weighed, dried in a laboratory oven and analysed further to characterise the major components of the essential oil. Also, analyses were performed to verify the antimicrobial and antioxidant activity of the essential oil. (C. Stavridou)

In 2013, another research project commenced for the genetic identification of five species *Mentha* sp. with molecular methods. The objectives of this project are the evaluation of cultivated spearmint *Mentha viridis* and its comparison with other mint species that are found in the market. The overall aim is to identify genetic differences in chloroplast DNA with the use of molecular genetic methodology. The identified genetic sequences shall be deposited in the world germplasm bank of NCBI. In 2013, samples from five different species of *Mentha* that exist in Cyprus have been collected and, after appropriate preparation, material has been sent for molecular analyses. (C. Stavridou)

Stevia (*Stevia rebaudiana*) field trial

A field trial of stevia (*Stevia rebaudiana*) was established at the Zygi Experimental Station in 2011. The aim of the experiment is to investigate the adaptation of this plant to the climatic conditions of Cyprus and the possibility for commercial cultivation. The productivity and the steviol glycoside concentration of four varieties are assessed. Additionally, an economic feasibility analysis will be conducted for the cultivation of this species in Cyprus. During 2011/12, we examined the needs of the plant mainly in terms of irrigation, as well as the cultivation methods and disease, pests and other cultivation problems. The harvesting method and the duration of drying were also reviewed. The first year of experimental manipulations was in 2013, during which three harvest dates were tested in order to investigate the influence of this factor on the production of fresh and dry stevia biomass weight and the concentration of sugars in each product. (C. Stavridou)

FRUIT TREES AND VITICULTURE

Research activity in the Fruit Trees and Viticulture Section encompasses methods of managing yield and quality of fruit trees and vines and optimising production cost. In viticulture, research work on evaluation of table grape and wine grape varieties and rootstocks, as well as ampelographic description and conservation of traditional grape varieties continues. Local clones of pomegranate and several cherry varieties are evaluated and in citrus, research work on rootstock evaluation continues. In olive sector, the conservation, evaluation and management of local genetic resources remains a key priority.

VITICULTURE

Evaluation of table grape varieties

For the evaluation of new and traditional table grape varieties under different environmental conditions, two experimental plots were planted at the Saittas and Acheleia Experimental Stations. In Saittas, the newly introduced table grape varieties Red Globe, Superior, Fantasy Seedless, Crimson Seedless, Autumn Royal, Sublima and the traditional varieties Veriko and Sideritis are under evaluation in terms of yield, quality and earliness. In Acheleia, in addition to the above, the varieties Prima, Black Emerald, Early Superior, Summer Muscat, Ora, Victoria, Italia, Calmeria and Flame Seedless were also introduced for evaluation. (S. Savvides)

Evaluation of table grape varieties grafted on American rootstocks

The imported table grape varieties Crimson Seedless, Autumn Royal, Fantasy Seedless, Black Emerald and the autochthonous varieties Veriko and Sideritis grafted on the American rootstocks 110 Richter, 3309 Couderc, 41B, 99 Richter, 420A, 1103P and 140 Ruggeri are evaluated in terms of yield, fruit quality and earliness. The table grape varieties Fantasy Seedless, Black Emerald and the autochthonous varieties Veriko and Sideritis are evaluated at the Acheleia Experimental Station and Autumn Royal at the Saittas Experimental Station. Crimson Seedless is evaluated at both locations. (S. Savvides)



Collection and Conservation of table grape varieties

The aim of this project is to collect and preserve all the table grape varieties imported in Cyprus over the years. At the moment a collection of 53 varieties has been established at the Acheleia Experimental Station (Table 1). The collection of other table grape varieties continues. (S. Savvides)

Table 1: Table grape varieties in the collection at Acheleia Experimental Station

1	Aetonichi aspro	21	Eftakilo	41	Red Globe
2	Aetonichi kokkino	22	Emerald Seedless	42	Rozaki
3	Aetonichi mavro	23	Emperor	43	Ruby Seedless
4	Alphonse lavalle	24	Exotic	44	Seneca
5	Attiki	25	Fantasy Seedless	45	Sideritis
6	Avgoulato aspro	26	Flame Seedless	46	Sideritis ellinikos
7	Avgoulato mavro	27	Flame Tokay	47	Sultanina
8	Autumn Royal	28	Fraoula	48	Summer Muscat
9	Barlinka	29	Gold	49	Superior
10	Beauty Seedless	30	Italia	50	Suplima
11	Black Emerald	31	July Muscat	51	Veriko
12	Blackrose	32	Katomylitiko	52	Victoria
13	Bronx Seedless	33	Loose Perlette	53	Chimoniatiko
14	Calmeria	34	Monukka		
15	Canner	35	Muscat Flame		
16	Cardinal	36	Ora		
17	Crimson Seedless	37	Pearl of csaba		
18	Delight	38	Prima		
19	Early Muscat	39	Princess		
20	Early Superior	40	Queen		

Evaluation and tests for Distinctness, Uniformity and Stability (DUS) of traditional wine grape varieties

The traditional varieties Mavro, Xynisteri, Ofthalmo, Spourtiko, Maratheftiko, Morokanella, Malaga and Lefkada are evaluated in terms of yield and quality in comparison to the imported varieties Mataro, Cabernet Sauvignon, Merlot, Chardonnay and Sauvignon Blanc. The above varieties are also tested for distinctness, uniformity and stability in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. (S. Savvides)



Collection and ampelographic description of traditional table and wine grape varieties

The acquisition of primary and secondary descriptor data, according to the International Organisation

of Wine and Vine (OIV) standards, of the traditional table grape varieties Veriko and Sideritis and the wine grape varieties Mavro, Xynisteri, Ofthalgo, Spourtiko, Maratheftiko, Morokanella, Omio, Promara, Skouro Mavro, and Kanella continues. **(S. Savvides)**

Evaluation of traditional wine grape variety Maratheftiko grafted on American rootstocks

Evaluation of the traditional wine grape variety Maratheftiko, grafted on the American rootstocks 110 Richter, 3309 Couderc, 41B, 99 Richter, 420A and 140 Ruggeri, has been performed in the Kilani area. The variety is evaluated in terms of yield, fruit quality and earliness. **(S. Savvides)**



Management and conservation of grapevine genetic resources

The long-term objective of this project is to preserve and study *in-situ* populations of wild vines as well as of old and neglected varieties and to promote their future utilisation. Wild vine plants have been recorded in six locations: Ayia, Stavros tis Psokas, Potamos tou Limniti, Potamos tou Pyrgou, Platys and Saramas. All plants are located along water streams and are found as climbers on the stems of pine and deciduous trees. Until now, more than 200 individual plants have been recorded. Work is still in progress for the *in situ* evaluation, and morphological characterization of all individual plants. **(S. Savvides)**

OLIVE CULTURE

Conservation, evaluation and management of olive genetic resources

Local olive genetic material is under conservation in the *ex situ* Collection of Olive Genetic Material situated at the Zygi Experimental Station. The aim of this collection is the conservation and complete characterization of the material and its effective management. Special interest has been given to the completion of the varietal characterization and identification of the material. **(M. Emmanouilidou)**

CITRICULTURE

Rootstock evaluation

The aim of citrus rootstock evaluation is to identify rootstocks tolerant to the tristeza virus capable of replacing Sour Orange, the rootstock commercially used in Cyprus, and which is highly susceptible to the virus. The commercial cultivars ‘Delta’, ‘Nova’, ‘Ortanique’ and ‘Lane Late’ are evaluated on various rootstocks. **(M. Emmanouilidou)**

DECIDUOUS FRUIT TREES

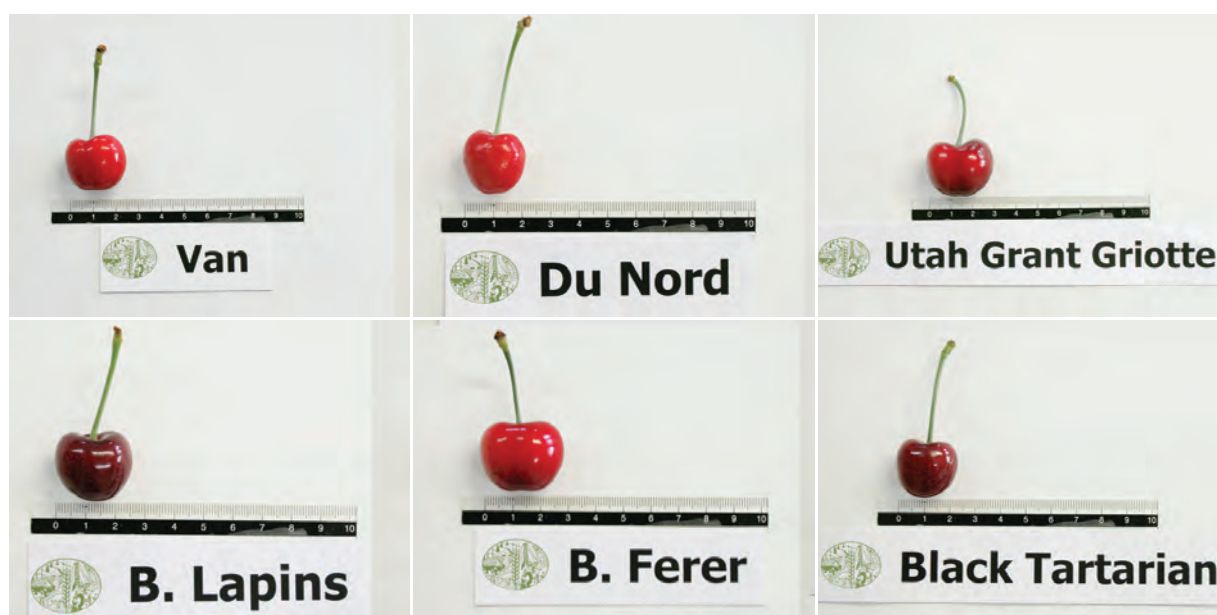
Characterization and evaluation of local pomegranate clones (*Punica granatum* L.)

The evaluation of selected local pomegranate clones, planted at the Zygi Experimental Station, continued. The purpose of this experiment is the characterization and evaluation of local pomegranate clones concerning phenological, pomological-morphological and production characteristics. In particular, phenological development stages, growth and productivity of clones, morphological and qualitative characteristics of fruits, are examined in terms of yield and quality. (S. Ioannidou)



Evaluation of Cherry varieties

Ten cherry varieties (*Prunus avium* L.) are examined in terms of production, fruit quality, sensitivity to tearing and maturation period in an experimental plantation at the Saittas Experimental Station. The varieties currently being evaluated are: Bigarreau Burlat, Black Tartarian, Utah Giant, Bigarreau Ferbolous (Verdel), Bigarreau Summit, Bigarreau Fercer (Arcina), Bigarreau Reverchon (Souche sandar), Griotte Du Nord, Bigarreau Lapins and Van. The aim of this work is to evaluate Cherry varieties under the agro-climatic conditions of Cyprus, in order to select the varieties that have excellent fruit quality and mature at different times within the season, in order to satisfy market needs for longer periods. (S. Ioannidou)



VEGETABLE AND ORNAMENTAL CROPS

The Vegetable and Ornamental Crops Section undertakes research on intensive vegetable production systems, on floral and landscape ornamental crop production, and on the postharvest technology of horticultural commodities. Current research work on vegetable crops focuses on rootstock-scion relations with respect to productivity, disease-resistance, stress response, quality and postharvest performance. Work on leafy salad crops examines the effect of planting pattern, nitrogen administration strategies, shading, seasonal adaptation and harvest age on yield, quality, shelf-life, and consumer safety. Work on ornamental crops examines the effect of salinity, with respect to substrate, developmental stage, and seasonality, on the hydroponic production of cut flowers. The evaluation of endemic species of Cyprus flora for potential use in commercial floriculture constitutes a perennial theme in floricultural research. Work on postharvest technology maps the farm-to-fork etiology of fresh produce quality, by linking preharvest factors to produce quality and postharvest performance. The implications of minimal processing on quality and shelf-life of fresh fruits and vegetables, and the effectiveness of non-chemical postharvest treatments for controlling pathological and physiological loss of quality are within the current scope of postharvest research. The Postharvest Technology Laboratory (PTL) manages national participation in EU Cost Action 1204 - Vegetable Grafting to Improve Yield and Fruit Quality under Biotic and Abiotic Stress Conditions and participates actively in the Action's working group on rootstock-mediated effects on vegetable fruit quality. Finally, the PTL coordinates the current ARI project on the evaluation of an ex situ collection of indigenous pomegranate clones, and spearheads a new ARI project on commercial cultivation of indigenous asparagus species.

VEGETABLE CROPS

Impact of three hybrid rootstocks on yield, quality and nutritive value of five watermelon [*Citrullus lanatus* (Thunb) Matsum & Nakai] cultivars

Most information on effects of rootstocks on watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] quality is derived from studies on disease resistance. The current study was undertaken to determine effects on yield and fruit morphometric and physicochemical characteristics of large-fruited, diploid, and small-fruited, triploid, watermelon cultivars grafted to inter-specific hybrid rootstocks. Two yearly field experiments were conducted between April-July at the Zygi Experimental Station in Cyprus. Grafting increased marketable yield by 37% on average over non-grafted controls. No cultivar-rootstock combination resulted in commercially unacceptable (<10%) soluble solids content (SSC); only triploid cv. Extazy exhibited a rootstock-dependent decrease in SSC. Flesh firmness was increased by grafting on all rootstocks, by an average of 37% over the non-grafted control. Variability in rind thickness was derived mainly from the scion. Rootstocks did not affect lycopene content and their effect on flesh color was minimal. While grafting enhanced yield and fruit quality, choice of rootstock may adversely affect SSC of certain cultivars. (G.A. Soteriou and M.C. Kyriacou)

Table 1. Mean comparison for yield and quality components of watermelon cultivars Celebration, Gallery, Pegasus, Extazy, and Torpila grown self-rooted or grafted on rootstocks TZ148, Bombo, and N101

Cultivar	Marketable yield (Mt·ha ⁻¹)		Fruit Number (ha)		Mean Fruit weight (kg)		Firmness (kg)		Lycopene (µg·g ⁻¹ FW)		TSS (%)	
	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2
Celebration	54.8		5630		9.75		3 b ^a		75.3		11	
Gallery	55.5	95.3	6500	10816	8.62	8.84	3.2 ab	3.6 b	60.1	65.1 ab	12	11.1
Pegasus	53.6	101.8	6080	10592	8.82	9.66	3.5 a	3.9 ab	63.5	60.9 b	12	11.4
Torpilla	57.1	106.2	6370	10336	8.92	10.38	3.6 a	4.1 a	71.8 71	a	12	11.2
<i>Rootstock</i>												
TZ148	62.2 a	108.5 a	6560	11221 a	9.51 a	9.7	3.5 a	4.1 a	67.32	68.3	12	11.2
Bombo	55.2 a	109.8 a	6080	11904 a	9.17 a	9.23	3.6 a	4.1 a	66.75	66.5	12	11
N101	60.3 a	106.8 a	6330	11093 a	9.53 a	9.65	3.6 a	4.2 a	69.33	66	12	11
Non-grafted	43.3 b	79.2 b	5600	8107 b	7.9 b	9.93	2.6 b	3	67.41	61.9	12	11.7
<i>Extazy^b</i>												
TZ148	67.3 a	114.9 a	20610 a	30720 a	3.28	3.78	9.9 a	12.5 a	98.5	113	11 ab	10.3 ab
Bombo	56.5 ab	108.5 ab	17410 a	31872 a	3.2	3.43	11 a	12.8 a	88.9	117	10 b	10.6 ab
N101	59.5 a	119.3 a	17410 a	32000 a	3.4	3.73	11 a	13 a	90.9	116	11 ab	10.1 b
Non-grafted	41.2 b	76.8 b	11710 b	18816 b	3.5	4.11	6.3 b	9.3 b	90	108	12 a	11.6 a

^a Values in columns followed by the same letter are not significantly different, Tukey-Kramer HSD Test, $P \leq 0.05$; mean comparisons are distinct for cultivars and rootstocks. Values not followed by a letter denote absence of a significant effect.

^b Rootstock mean separation performed independently for outlier cv. Extazy.

Yield and quality of four mini watermelon [*Citrullus lanatus* (Thunb) Matsum & Nakai] cultivars grafted on *C. maxima* x *C. moschata* and *Lagenaria siceraria* rootstocks

Over the last years, the market share commanded by mini (3-5kg) watermelon cultivars has been growing across watermelon producing countries. In Cyprus this type of watermelon has not been adopted yet by local producers, mainly because of the unfamiliarity of the local market with the product. The objectives of this study have been to evaluate the field performance of four mini watermelon cultivars as well as their response to grafting on two rootstocks. Yield in all cultivars was above the regional average (52 t ha⁻¹) except from cultivar Vivlos in year 1 (Table 2). Triploids ‘Extazy’ and ‘Petite’ produced greater yield than both diploids only in year 2. ‘Extazy’ had the smallest fruit weight and the highest number of fruit among all cultivars examined. Cultivar differentiation in soluble solids content (SSC) was observed only in year 2 wherein diploid ‘Vivlos’ had higher SSC content than both triploids. Triploid ‘Extazy’ had highest flesh firmness and lycopene content than all cultivars. Cultivars on ‘TZ148’ benefited more than those grafted on ‘Festival’, as proven by the differences observed regarding their tradable yield (Table 2). The TSS content was the same on both rootstocks but fruit produced on TZ-148 had firmer flesh than fruit produced on Festival. (G.A. Soteriou and M.C. Kyriacou)

Table 2. Mean comparison for yield and quality components of watermelon cvs. Petite, Vivlos, Extazy and Esmeralda grafted on two rootstocks types.

Cultivar	Marketable yield (Mt·ha ⁻¹)		Fruit Number (ha)		Mean Fruit weight (kg)		Firmness (kg)		Lycopene (µg·g ⁻¹ FW)		TSS (%)	
	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2	Year1	Year2
Petite	73.52 a ^a	93.76 a	16704 b	17920 b	4.44 a	6.19 a	5.6 b	5.7 b	61.41 b	62.70	11.2	10.7 bc
Vivlos	45.21 c	55.05 d	10752 c	12096 c	4.18 a	5.09 b	4.1 bc	4.3 c	54.41 b	69.05	11.4	11.6 a
Extazy	69.24 ab	82.45 b	21632 a	22059 a	3.18 b	4.15 c	10.3 a	11.4 a	110.45 a	98.97	10.8	10.2 c
Esmeralda	59.66 b	72.60 c	13504 bc	15793 b	4.42 a	5.68 ab	3.7 c	3.5 d	50.71 b	62.88	11.0	10.9 b
<i>Rootstock</i>												
TZ148	70.96 a	81.57 a	16512	17916 a	4.43 a	5.34	6.5 a	7.0 a	72.03	75.01	11.2	10.8
Festival	52.85 b	70.36 b	14784	16018 b	3.68 b	5.22	5.3 b	5.4 b	66.46	71.79	11.1	10.9

^a Values in columns followed by the same letter are not significantly different, Tukey-Kramer HSD Test, P≤0.05; mean comparisons are distinct for cultivars and rootstocks.

Values not followed by a letter denote absence of a significant effect.

Impact of nitrogen fertilization strategies on quality, safety and postharvest behaviour of salad crops

A number of individual experiments for each of four salad crops (lettuce, spinach, rocket and coriander) have been running in order to examine the effects of nitrogen (N) application methods and crop season on pre- and post-harvest residual nitrate/nitrite concentrations and other important biochemical and physical indices of quality. Sampling and sample preparation for nitrate and nitrite analysis have been performed according to EU Commission Regulation 1882/2006 directives. Analysis has been performed by high performance liquid chromatography (HPLC) using a mobile phase of 0.01M methanolic solution of octyl-ammonium phosphate (pH 6.5), a C18 4.6 x 250 mm 5 μ m column for separation, and UV detection at $\lambda=220$ nm. Quantification was performed by use of external standards of sodium nitrate/nitrite with a linearity of calibration ≥ 0.9999 . Retention times were 7.8 and 10.0 min for nitrite and nitrate ions, respectively. The peak-to-peak noise ratio was 0.1213 mAU and the LOD values were 0.09 and 0.15 mg kg⁻¹ f.w. for nitrites and nitrates, respectively.

The objectives of our latest experiments were to evaluate the effects of basal and top dressing N applications on rocket yield, quality, and safety, with respect to nitrate/nitrite residual content, during the summer and winter seasons. A multi-factorial CRD design was deployed, with combined basal (0, 100, 150, 200 kg N ha⁻¹) and top dressing (0, 50, 100, 150 kg N ha⁻¹) N applications in four replications. In both seasons, preliminary results showed that bunch weight and diameter were influenced by basal N application only, whereas nitrate content was also influenced by top dressing. CIELAB colour components were not affected by either of the two application methods examined. Overall higher nitrate content was obtained during the winter season. Postharvest behavior of rocket summer crop was also assessed in a multi-factorial CRD design with two factors: a) nitrogen application rate (0, 100, 200, 250, 300 kg N ha⁻¹) in combined base and top dressing, and b) storage period (0, 5, 10, 15 days) at 5 °C. Further to the above field and postharvest experiments, an extensive survey of the local market has been carried out to determine nitrate and nitrite concentrations in leafy vegetables. A wide range salad crops (cabbage, celery leaves/stalks, coriander leaves, beet leaves, lettuce, parsley, purslane, rucola, spinach and dill) were sampled at retail stores in winter (358 samples) and summer (330 samples). Besides the notable variation between crops and seasons, results also support that cultural practice may be implicated in cases of outlying nitrate and nitrite concentrations. (G.A. Soteriou and M.C. Kyriacou)



POSTHARVEST TECHNOLOGY

Evolution of watermelon fruit physicochemical and phytochemical composition during ripening as affected by grafting

The practice of grafting watermelon [*Citrullus lanatus* (Thunb) Matsum & Nakai] cultivars onto rootstocks resistant to soilborne pathogens has gained ground worldwide owing to the restrictions imposed on the use of chlorofluorocarbon-based soil fumigants. Beyond disease resistance, selected rootstocks are customarily evaluated for their effects on yield and fruit quality. However, the evaluation of quality has relied by convention on a simultaneous harvest of grafted and self-rooted plants, assuming a synchronized maturation. An inherent problem in that approach is that it overlooks the effect of grafting on fruit ripening behavior and may lead to inconsistent results. The magnitude and significance of differences obtained in physicochemical components of fruit quality between grafted and self-rooted plants is thus limited to a snap-shot of quality at harvest while in fact it may partly or solely reflect differences in harvest maturity. Sound prediction of harvest maturity is particularly critical for non-climacteric fruit, such as watermelon, whose physiological maturation terminates at harvest and their quality steadily declines thereafter. The overall objective of our work has been to characterize the evolution of physicochemical and phytochemical components of quality during watermelon ripening in response to grafting. Physicochemical components of quality examined, included flesh reflectance colorimetry, mechanical texture analysis, pH, titratable acidity, and soluble solid and soluble carbohydrate (glucose, fructose, sucrose) content. Phytochemical components of quality included flesh lycopene and citrulline content. For the purposes of this study a diploid watermelon cultivar (cv. Pegasus) was cultivated self-rooted or grafted onto an inter-specific *C. maxima* x *C. moschata* hybrid rootstock TZ148. Fruits were harvested in 5-day intervals from 30 to 50 days post-anthesis (dpa). Grafting increased firmness, titratable acidity and lycopene content though it delayed its peak. Lycopene content was mostly ripening-dependant, highly correlated and synchronous with changes in pulp chroma (C*) and color component a*. The sweetness was affected only by ripening. However, total sugars and soluble solids content peaked later in fruit of grafted plants than in non-grafted ones, and significant interaction of ripening with grafting was observed. Citrulline content increased with ripening in fruit of grafted plants, reaching a peak at 45 dpa; whereas in non-grafted ones it was unchanged between 30-45 dpa and declined at 50 dpa. As ripening overall was retarded by grafting, fruit quality of grafted watermelon may benefit from belated harvest. (M.C. Kyriacou, G.A. Soteriou, A.S. Siomos and D. Gerasopoulos)

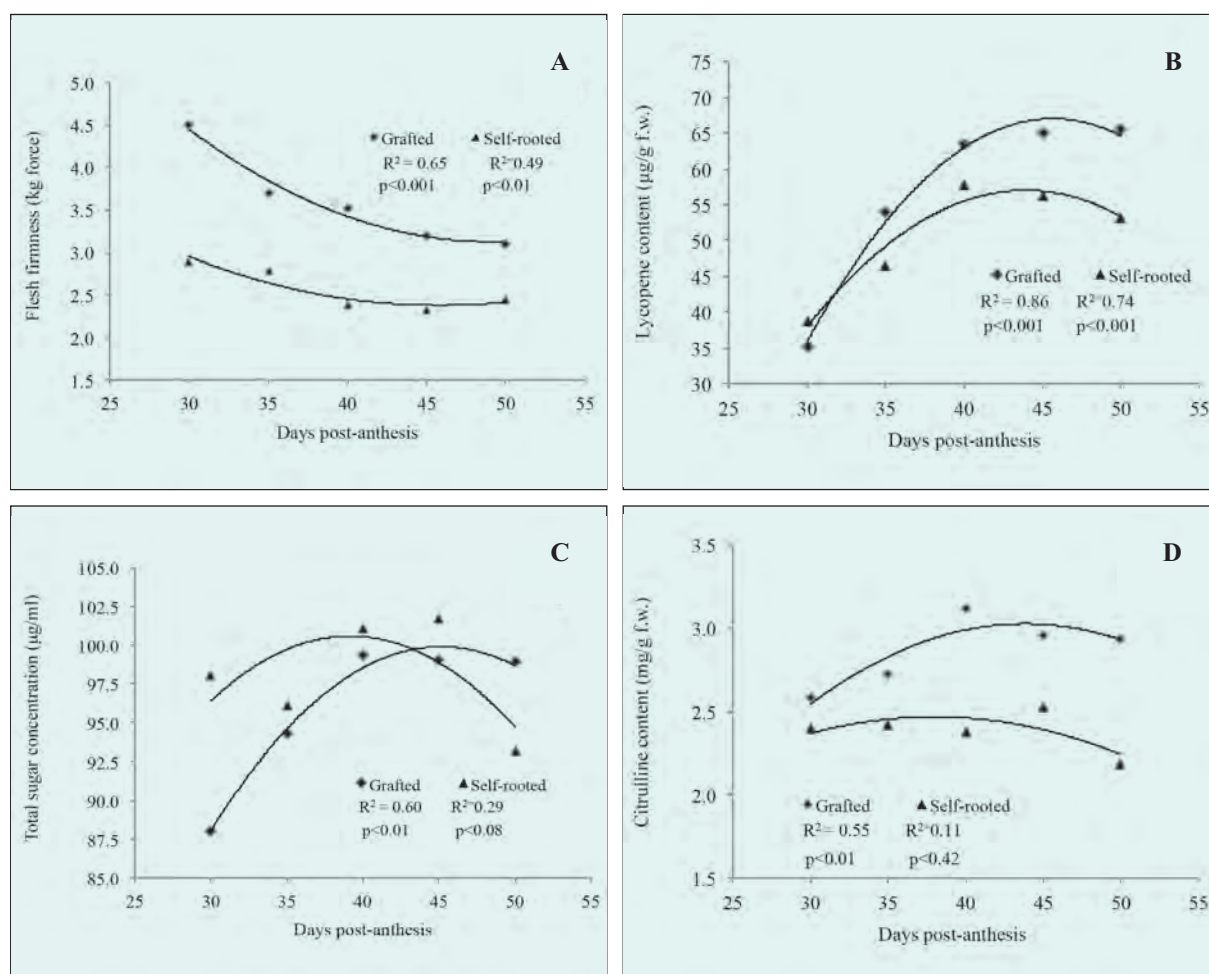


Figure 1. Regression of fruit weight (A), flesh firmness (B), total sugar concentration (C), and citrulline content (D) with maturity for watermelon fruit harvested at 30, 35, 40, 45 and 50 days post-anthesis from plants grown self-rooted (non-grafted) or grafted on *Cucurbita maxima* x *C. moschata* cv. TZ148 rootstock. Data points represent means of four replicates consisting of six sample fruits each.

Evaluation of an *ex situ* collection of local pomegranate (*Punica granatum* L.) accessions as per their qualitative and technological traits, their antioxidant value and storage performance

An *ex situ* collection of local pomegranate accessions collected over a wide range of localities on the island was established at the Zygi Experimental Station in 2010 for purposes of characterization. Most accessions reached productive age in 2013. Initial evaluation aimed at grouping the clonal material with respect to earliness, mostly on the basis of sugar/acid ratio. Sequential sampling was performed between August and November. Other fruit quality traits examined included individual fruit weight, skin and juice colour. Preliminary results indicated wide variation in harvest maturity among accessions, spanning the period from end August to early November. Promising, very early and very late clones have been identified. Characterization of earliness will be performed for two more years before comprehensive evaluation of the accessions is performed. This will encompass seed, fruit and juice characterization as

well as assessment of fruit storage performance. Fruit quality and composition traits to be examined will include soluble carbohydrates, organic acids, and anthocyanin compounds in the pomegranate juice, characterization of free radical scavenging potential of pomegranate juice and assessment of juice antioxidant value, seed hardness, texture and fibre content, and fruit storage performance. (M.C. Kyriacou, S. Ioannidou, N. Seraphides, L.C. Papayiannis)

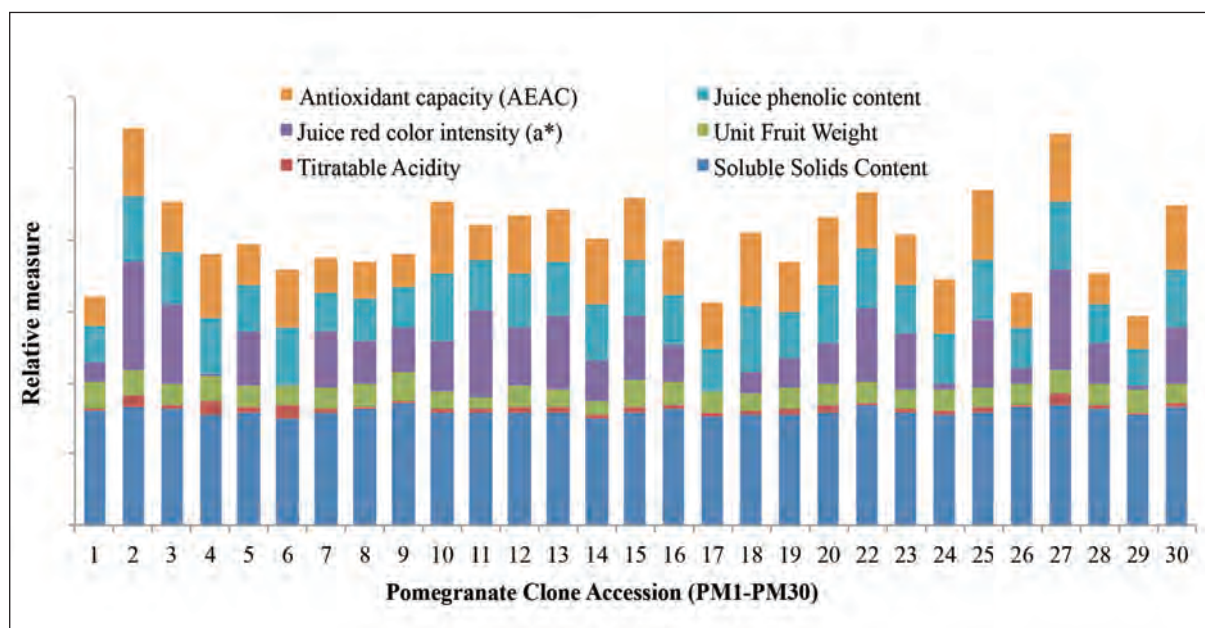


Figure 2. Relative measure of soluble solids content, titratable acidity, fruit weight, antioxidant capacity, phenolic content and juice colour intensity of indigenous pomegranate clones. Harvest performed on 16/9/2013 from *ex situ* collection established at Zygi Experimental Station. N=15.

FLORICULTURE

Cultivation of lisianthus under saline conditions (*Eustoma grandiflorum*)

The effect of salinity was examined on hydroponic production of lisianthus (*Eustoma grandiflorum*). The main objectives of the project are: (a) to evaluate the effect of salinity on productive, morphological, qualitative, and physiological characteristics of lisianthus flowers grown on 2 different substrates, and (b) to evaluate the effects of salinity and substrate type on the postharvest vase-life and physiology of lisianthus cut flowers.

Lisianthus cv. Echo Blue was grown on coco soil and rock-wool substrates in a controlled greenhouse environment at the Zygi Experimental Station using an open hydroponic system. Nutrient solution of four different levels of salinity (expressed as electrical conductivity, EC) was used: 2 (control), 4, 6, and 8 dS m⁻¹. The different levels of salinity were attained by adding sodium chloride in the nutrient solution (control). Irrigation was performed using self-compensating drippers of 4 l/h discharge while drainage was kept constant 20-30% throughout the experiment.

Response to salinity, induced by sodium and chloride ions in the nutrient solution, was evaluated in terms of productive, morphological, qualitative and vase-life characteristics of *lisianthus*. The experimental work both at the greenhouse as regards the preharvest handlings and at the laboratories for the postharvest handlings was concluded and data were collected. The statistical analysis and the results from the project will be disseminated during the year. (L. Vassiliou)



Valorisation of endemic species in commercial floriculture

Research on ornamental crops further entailed work on the valorisation of endemic species in commercial floriculture. Endemic ornamental species, propagated and cultivated in the context of a recently concluded research project, have been promoted for commercial nursery potted-plant production and for use in landscaping. The preliminary experimental work for the new project concerning the development of propagation and cultivation protocols for indigenous tulip species *Tulipa agenensis* for commercial production has been initiated. The preliminary work concerns the collection of propagated material such as bulbs and seeds from the region where the species occur in order to create the propagation protocol. In addition artificial pollination was performed in tulip flowers for further evaluation. (L. Vassiliou)

ANIMAL PRODUCTION

The research activities of the Animal Production Section involve work on nutrition, management, animal breeding and physiology of reproduction. Research is generally directed towards increasing milk and meat yields under semi-intensive or intensive systems of management in sheep, goats and dairy cattle. In addition, genetic methods and animal husbandry practices are employed, aiming at controlling and preventing animal diseases.

In animal breeding, the research work aims at improving the genetic stock with respect to important economic traits in livestock using within-breed selection methods. Genetic evaluations are based on selection indices that combine individual capacity of young animals for growth and milk production of female ancestors. This method is routinely used at all government breeding units for the evaluation and selection of superior breeding stock in sheep and goats. Further research in genetic improvement at ARI is directed toward dissecting the genetic aspects underlying milk production, and further characterising scrapie genotypes in goats.

Research programmes in the area of reproductive physiology of farm animals aim at improving reproductive performance. In this regard, genetic and environmental factors that influence seasonal reproduction, reproductive development and puberty in sheep and goat breeds under local conditions are examined. In farm management, artificial rearing systems are evaluated, with automated feeders and milk substitute being used for lambs and kids from birth to weaning.

A research project in which genetic methods are employed to combat the scrapie disease in Chios sheep is being successfully implemented by ARI in cooperation with the Veterinary Services. The Chios sheep unit of ARI at Athalassa has been transformed into a nucleus herd of scrapie-resistant genotypes. The number of productive animals at the nucleus is maintained around 420 breeding females.

In mid-2008, a programmes had commenced to transform the Damascus goat herd at ARI into a nucleus of scrapie-resistant genotypes. The programme continued at ARI in 2012 and 2013, aiming at creating a nucleus of 300 breeding goats that would be resistant to the disease. This has enabled ARI to issue scrapie-resistant animals to the farmers, in order to significantly contribute in eradicating scrapie from the Cyprus goat population.

The ARI Dairy Cattle Unit

The Friesian/Holstein dairy cattle unit of ARI is situated at Athalassa. The performance of cows during the years 2012 and 2013 is shown in Table 1. The overall performance of the herd has been satisfactory. Milk yield and milk quality were very good. Milk produced was 417,682 l in 2012 and 423,251 l in 2013. Total milk production has increased in 2012-2013 by about 25,000 l, as compared to the production in 2010-2011, roughly corresponding to an increase of about 2 annual cows in 2012-2013. Even so, in 2012 and 2013, the milk yield per annual cow was higher than that of 2010-2011, indicating increased productivity per animal. Finally, cows that suffered from mastitis were removed from the flock. **(G. Hadjipavlou and D. Sparaggis)**

Genetic evaluation and selection to further improve the performance of Chios sheep and Damascus goat nucleus herds at ARI

The ARI small ruminant herds consist of 420 Chios sheep and 280 Damascus goats. Daily milk yield



is recorded automatically during milking in recent years, and this led to improved phenotype collection for genetic evaluations. Male and female replacement stock is selected on the basis of a continuously updated index combining 60-day milk yield of dam and grand dam and individual body weight at 98 days of age for lambs and at 105 days for kids. The breeding scheme is updated yearly and directed toward minimal inbreeding for both sheep and goats. Matings

take place twice each year for each species. Production and reproduction characteristics for the two herds during the periods 2012/13 are shown in Tables 2 and 3. (G. Hadjipavlou)

The effect of artificial rearing on kid growth and milk production of Damascus goats

Research on zero suckling systems in Chios ewes and Damascus goats continued in 2012 and 2013. Lambs and kids on zero suckling were reared artificially on milk substitute using four automatic milk feeding machines. Yearling Damascus goats were allocated as they kidded to either Natural Suckling (NS) or were separated from their kids immediately after birth. NS goats suckled up to two kids, while separated kids were Artificially Reared (AR) on milk replacer. Colostrum was given to AR kids by bottle feeding. All kids were weaned at 49 ± 3 days of age. Following weaning, 15 male kids from each NS and AR groups were fattened for 70 days. AR goats were milked twice daily. NS goats were milked once daily before and twice daily after weaning. Birth weight of kids in both groups was similar. NS kids had a faster ($P < 0.01$) preweaning growth rate than AR kids and were heavier ($P < 0.05$) at weaning than AR kids. After weaning, males of both groups had similar growth rates. Final weight at 120 days of age of AR and NS kids was similar. Milk yield of AR goats during the preweaning period was higher ($P < 0.05$) than that of NS goats. Fat and protein content of milk was similar for AR and NS goats. Post weaning milk yield for both groups was also similar. Total milk yield (205 days) was significantly different for AR and NS goats. These results further supported preliminary results from previous years that artificially reared kids had satisfactory preweaning growth, although slightly lower than that of suckling kids; however, both groups reached the same final weight at 120 days of age. Goats on zero suckling produced more marketable milk over their whole lactation period than suckling goats. Marketable milk of mature goats was 85 kg more than goats that suckled their kids. Therefore, in particular to dual purpose breeds such as the Damascus one, artificial rearing may increase the farmer's income, with no adverse effects on kid growth. (G. Hadjipavlou)

Use of triticale hay and silage in the diet of ruminants

The project commenced in 2011 and shall be completed in 2015. The main aim of the research is to

evaluate the effects of feeding triticale hay and silage to Holstein-Friesian cows, Chios sheep and Damascus goats. Triticale is grown in plots at the Athalassa Experimental Farm and its nutritional, qualitative and quantitative characteristics are evaluated. Participating animals are allocated in two groups and the effect of triticale hay or silage on milk production and composition, and on the animals' weight at the onset of lactation, is being determined, as compared to control groups that receive barley hay or silage. Preliminary data regarding the use of triticale hay show that it can replace barley hay in ruminants' nutrition with no adverse effects on milk production and on milk fat and protein content. Research findings could potentially be used for the production of higher quality and improved triticale hay and silage that will be used as barley hay replacement for ruminants. **(D. Sparaggis and A. Pallides)**

Genetic and molecular techniques for controlling the scrapie disease in sheep and improving the genetic stock provided to farmers

The project for combating scrapie disease in Chios sheep with the use of genetic and molecular methods is being implemented in close cooperation between the ARI Animal Production and



Agrobiotechnology Sections. The present population of the ARI unit consists of breeding ewes and rams of the ARR/ARR genotype, which is resistant to scrapie. For breeding purposes, the number of resistant rams and ram lambs issued to farmers in the period of 2012-2013 was 48 and 339, respectively, and that of surplus female lambs was 328. In addition, 32 ewes were issued to farmers. It is anticipated that by reducing

the incidence of the disease and increasing the frequency of the desirable allele, and consequently of the resistant genotypes, the disease has been controlled and will eventually, be eradicated. It should be emphasized that the project for controlling scrapie in Chios sheep runs in parallel with the project aiming at the genetic improvement of economically important traits (milk yield, growth, prolificacy, etc). Additionally, since 2008, genetic management of the sheep nucleus unit has been significantly upgraded, in an effort to substantially minimize inbreeding that arose based on necessity during the effort to eradicate the scrapie-susceptible genotype (AQQ/AQQ) from the flock. **(G. Hadjipavlou and I.M. Ioannides)**

Genetic and molecular techniques for controlling the scrapie disease in goats and improving the genetic stock to be provided to farmers

The programme for controlling the scrapie disease in goats runs in the framework of an ARI-funded joint research project between the Animal Production and Agrobiotechnology Sections, and in cooperation between ARI and the Veterinary Services. During 2012-2013, the collection of genetic and production

information for Damascus goats continued, with the aim of further studying the different *PrP* genotypes in goats and compile enough phenotypic information to examine potential associations between genotype and production characteristics of the animals. Targeted matings were designed and performed in both breeding seasons of each year, in order to increase the frequency of the D and S alleles at codon 146 of the *PrP* gene, and to simultaneously decrease the N allele at the same locus. Particular emphasis was given on reducing the numbers of homozygous NN animals in the flock. In 2012 and 2013, molecular genotyping of the selected *PrP* alleles was conducted by the Agrobiotechnology laboratory for more than 810 animals. In this period, the Animal Production section provided the farmers with 49 bucks, 13 goats, 140 male kids and 78 female kids. All the animals had proposed scrapie-resistant genotypes, in order to decrease the frequency of the assumed scrapie-susceptible N allele in the Cyprus Damascus goat population. For all genotype classes, records on body weight at birth, at weaning and at 120 days, dam prolificacy and milk production have been collected to examine potential associations between genotype and animal production traits. All information is recorded in an extended, continuously updated database, according to standard procedures of the Animal Production section. (G. Hadjipavlou and I.M. Ioannides)

Use of molecular and quantitative genetics for the improvement of the Chios sheep

This project commenced in 2009 and was completed in May 2012. It was funded by the Research Promotion Foundation and the Cyprus University of Technology, with the latter serving as the project coordinator. Apart from ARI, the Alexander Technological Educational Institute of Thessaloniki and the Scottish Agricultural College also take part in the aforementioned study. The main aim of the research was to identify and characterize genetic regions associated with milk production traits in Chios sheep, utilizing genetic and phenotypic information from the ARI nucleus flock. Research findings could potentially be used for genetic improvement in the Chios and in other sheep breeds. Additionally, the rate of inbreeding in the ARI flock was determined using classical and molecular genetic techniques, and was found to be very low, indicating the success of the long-term breeding scheme followed at ARI.

With respect to the genetic study conducted, ARI Chios ewes were genotyped for various candidate genes that might affect milk production and quality. The genetic diversity of the Chios breed was investigated regarding *β -lactoglobulin* genotypes and the existence of single nucleotide polymorphisms (SNPs) at other loci, already known to affect bovine milk traits. During 2012, a novel genetic polymorphism, identified for the first time in this project, and located in the 3'UTR of the acetyl-CoA acyltransferase-2 (*ACAA2*) gene (HM537015:g.2982T>C), was further examined, with respect to its potential association with ovine milk traits. Mixed model association analysis, using SNP data from 318 animals from 104 paternal half-sib families and first lactation phenotype and pedigree information on 2,405 ewes revealed that this SNP was significantly associated with milk yield. The significance of the SNP effect persisted when milk yield information from up to the third lactation was analyzed. The T allele was associated with increased milk yield, and exhibited partial dominant action. Animals with the g.2982TT or g.2982CT genotype had significantly higher milk yield than those with the g.2982CC genotype, with the g.2982T allele having an additive effect of 13.4(\pm 4.7) kg and a dominance effect of 7.9 (\pm 6.1) kg. Based on estimated allelic effects and sample allele frequencies, the g.2982T>C SNP explained 10% of the additive genetic variance for milk yield. This is the first report regarding ovine *ACAA2* gene variants and, after further validation, the polymorphism may provide a useful marker in future sheep breeding programmes for more efficient selection of ewes with increased milk production. (G. Hadjipavlou)

Research project on “Mediterranean biodiversity as a tool for the sustainable development of the small ruminant sector: from traditional knowledge to innovation” (ARIMNET-DoMEsTic)

The Animal Production Section participates in a European research project, in which Mediterranean countries collaborate on topics of agricultural research. The DoMEsTic project, funded under EU FP7 ARIMNet, began on the 1st May 2012, and aims to enhance our knowledge on pastoral and rangeland sheep and goats production systems. Case studies from Greece, Cyprus, France and Morocco are analysed through field surveys, focusing on livestock farming systems, the genetic management of the breeds, and economical aspects. Surveys were conducted in 2012 and 2013, with personal interviews of the farmers, on the basis of a detailed questionnaire. The comparative analysis of the data collected will assess the associations between the structure of the farming systems, farmer practices and the characteristics of the sheep and goat breeds with the sector’s resilience, competitiveness and overall sustainability.

In terms of the economic aspects of the sheep and goat farming systems, within 2013, information related with the distribution of the products through the value chain and the role of the different stakeholders was collected by semi-structured personal interviews with representatives from local actors. The analysis of the value chains aims to identify the different actors, assess the governance mechanisms in the value chain, analyze the opportunities for upgrades within the chain and gain distribution along the chain. Analysis of all the information collected shall provide an insight on the nature and determinants of smallholder competitiveness in the market chain and to propose alternative policies and optimal interventions in the value chain of local sheep and goat breeds. **(G. Hadjipavlou, G. Adamides and A. Stylianou)**

Table 1. Performance of dairy cattle at the ARI unit, Athalassa

Variable	Year	
	2012	2013
Cows calved	28	36
Heifers calved	19	18
Abortions	0	1
Calves born alive	43	50
Calves born dead	5	6
Calves died	1 (2.0%)	2 (4.0%)
Calving interval (days)	405	404
Days open	129.8	127.1
Duration of pregnancy (days)	277	276
Services/conception	1.9	1.9
Annual cows	47.8	48.1
Milk produced (l)	417 682	423 251
Milk/annual cow (l)	8 765	8 799
Milk fat %	3.31	3.41
Milk protein %	3.32	3.28

Table 2. Production characteristics of Chios ewes at ARI (2012/13)

Trait	Yearlings	Adults
No. of ewes lambing	204	507
Lambs born/ewe	1.80	2.08
Lambs born live/ewe	1.67	1.86
Litter weight at birth (kg/ewe)	6.54	7.37
Lambs weaned/ewe	1.55	1.78
Litter weight at weaning (kg/ewe)	19.2	23.2
60-day milk (kg/ewe)	121	149
Total milk (kg/ewe)	297	439
Days in milk	199	251
Milk fat (%)	4.65	5.46
Milk protein (%)	5.00	5.37

Table 3. Production characteristics of Damascus goats at ARI (2012/13)

Trait	Yearlings	Adults
No. of goats kidding	189	190
Kids born/goat	1.66	2.03
Kids born live/goat	1.61	1.92
Litter weight at birth (kg/goat)	6.39	8.23
Kids weaned/goat	1.45	1.69
Litter weight at weaning (kg/goat)	19.26	24.7
60-day milk (kg/goat)	141	161
Total milk (kg/goat)	350	551
Days in milk	205	247
Milk fat (%)	3.08	3.60
Milk protein (%)	3.79	3.98

PLANT PROTECTION

Research activities in Plant Protection concern the disciplines of plant pathology, entomology and pesticide toxicology. Under each of these disciplines, specialized studies are conducted to address major crop protection problems associated with particular pests (insects, mites) or diseases (viral, prokaryotic and fungal). In addition, joint multi-disciplinary research projects targeting important pests and diseases of particular crops are undertaken. All studies are governed by the considerations of sustainability in crop protection and production, food quality and safety, environmental protection and the utilization of new technologies for the development of more effective crop protection practices. Following the wide scale deployment of modern nucleic acid detection assays for plant pathogens, effort has focused on research and development of new diagnostic tools for assessing the phytosanitary status of plants, for certification and/or quarantine purposes. Fluorescent-based amplification methods using labeled probes have been used for the identification of RNA or DNA targets, SNP's genotyping and gene quantification.

In line with the above principles, one of the main activities of the Section concerns the development of integrated pest management (IPM) strategies, based on various combinations of ecosystem-oriented methods, i.e. biological control, cultural practices, natural products, resistant varieties, resistant rootstocks and minimal use of selected, mild pesticides. This approach ensures sustainability and minimizes pesticide risk to human health and the environment. It can be applied alone or in combination with other ecosystem-oriented production practices, in the context of an advanced system of sustainable agriculture, known as integrated crop management (ICM). Studies to develop IPM/ICM systems were carried out on citrus, grapevines and on vegetable and ornamental greenhouse crops, in cooperation with other Sections of the Institute.

Plant Pathology and Entomology laboratories provided support for the scientific identification of plant pathogens and insect pests, in various samples submitted by the Department of Agriculture, the Medical and Public Health Services, the Department of Forestry, agriculturists from the private sector and farmers.

ENTOMOLOGY

Fruit Fly Pest Prevention and Management in the Balkans and the Eastern Mediterranean

In parts of the Balkans and the Eastern Mediterranean, the Mediterranean fruit fly (*Ceratitis capitata*) causes major damage to fruit production. Medfly reduces fruit production and increases insecticide use,



and therefore exerts a direct impact on the production cost of agricultural commodities. Moreover, the recent introduction of exotic fruit fly species, such as the peach fruit fly, *Bactrocera zonata*, and the species of the oriental fruit fly complex *Bactrocera dorsalis*, in North Africa and the Middle East, poses a high threat to fruit and vegetable production of the Balkan and Eastern Mediterranean countries. To cope with the high risk of fruit fly infestation, the International Atomic Energy Agency (IAEA) initiated a project having as main objectives the knowledge sharing among countries of the region and the development and support of selected fruit fly suppression programmes. ARI is one of the institutions from 12 countries that participate in the project. (N.A. Seraphides)

Development of a warning system for monitoring the California red scale, *Aonidiella aurantii* (Homoptera: Diaspididae)

The California red scale, *Aonidiella aurantii* (Maskell), constitutes a major citrus pest. In Cyprus, four generations per year have been reported, while control of the scale has been a problem to citrus growers because of its continuous reproduction and the overlap of generations. Decision on when treatment is needed is mainly dependent on monitoring male scale populations with the use of pheromone traps. The ARI, in cooperation with the Cyprus University of Technology, CNE Technology LTD and CYPROFRESH, initiated a research programme funded by the Cyprus Research Promotion Foundation concerning the development of a novel automated and energetically autonomous trap for monitoring male red scales. The system, which also records major environmental parameters that affect *Aonidiella aurantii* populations, includes wireless networks, a GIS platform and automatic user notification services via email and SMS. (N.A. Seraphides)



Management of insect pests in pomegranate orchards

In Cyprus, the insect pests of pomegranates have not received much research attention, although numerous insect pests have been reported to attack the crop. Among them, the Mediterranean fruit fly *Ceratitis capitata* Wied, the carob moth *Ectomyelois ceratoniae*, Zeller aphids and scales are the most important. The programme aims to identify, monitor and develop the best strategy to manage insect pests in pomegranate orchards. (N.A. Seraphides)



Rearing of beneficial insect populations

Wide application of chemicals to control insect pests generates many drawbacks for agriculture, the environment and human health. By employing natural enemies to control insect pests, the above



drawbacks could be greatly reduced. For this purpose, populations of natural enemies of greenhouse pests, such as the parasitic wasp *Diglyphus isaea* against leafminers (*Liriomyza* sp.), the predatory bug *Macrolophus pygmaeus* against whiteflies, thrips etc., the lacewing *Chrysoperla carnea* against aphids, whiteflies etc, and the predatory bug *Nesidiocoris tenuis* against lepidopteran eggs, aphids etc, are reared at the main insectary of the ARI. (N.A. Seraphides)

Lethal effects of pesticides on the insect predator *Macrolophus pygmaeus* (Hemiptera: Miridae)

In cooperation with the Cyprus University of Technology, the lethal effects of six insecticides and a fungicide on *Macrolophus pygmaeus* nymphs, a common generalist predator in Mediterranean agroecosystems, were evaluated. Chlorantraniliprole and emamectin-benzoate caused less than 25% mortality to *M. pygmaeus* and were classified as harmless according to the IOBC rating scheme. In contrast, thiacloprid and metaflumizone caused 100% and 80% mortality, respectively, and were classified as harmful. Indoxacarb and spinosad resulted in close to 30% mortality to the predator and were classified as slightly harmful, while the fungicide copper hydroxide caused 58% mortality and was rated as moderately harmful. (N.A. Seraphides)



Acaricide resistance in two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) populations from Cyprus

Five field and greenhouse populations of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), were collected from five different districts across the island of Cyprus, both in field and greenhouse crops, and tested to determine levels of resistance. Standard leaf-disk spray application bioassay procedures were used to determine the LC_{50} s for five chemicals: abamectin, acrinathrin, fenazaquin, pirimiphos methyl, and bifenazate. Selection of these compounds was based on the widespread use by farmers as well as on the frequent control failures against *T. urticae* reported in the past. Resistance of *T. urticae* was detected to abamectin, acrinathrin, fenazaquin and pirimiphos methyl. The resistance ratios were calculated relative to the German susceptible reference strain. The highest resistance ratios at LC_{50} value were recorded for abamectin in a greenhouse rose population

(RR = 3822), followed by a field bean (RR = 1356) and field tomato population (RR = 1320). Significantly high resistance levels were also found for acrinathrin where the highest resistance ratios at LC₅₀ were recorded in a field bean *T. urticae* population (RR = 903). For fenazaquin, the highest resistance levels were recorded in a field tomato population (RR = 310). Lower resistance levels were found for pirimiphos methyl ($13.3 < RR < 77.4$) in all populations. Low susceptibility of *T. urticae* was observed for bifentazate ($2.7 < RR < 24.4$) in all populations. These results suggest that at least the use of abamectin and acrinathrin should be avoided or minimized for the control of *T. urticae* populations in indoor and outdoor environments. (V.A. Vassiliou and P. Kitsis).

Abundance of the *Bemisia tabaci* B-biotype overexpressing CYP6CM1 in field populations from Cyprus

The tobacco whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is one of the most destructive pests worldwide causing severe damage to various crops via feeding activity, quality reduction (excreta) and virus transmission. In Cyprus, the presence of *B. tabaci* B and Q biotypes has been reported by several researchers in past years, however, in recent studies it was found that the prevailing *B. tabaci* biotype in Cyprus is the B-biotype. *B. tabaci* populations (biotype B) were collected in a one month-old broccoli crop from the area of Klirou (35°01'15''N; 33°10'45''E) of the Lefkosia district, Cyprus. Dead and alive adults from leaf-disk dip bioassays (LC₉₀) conducted with three different neonicotinoid insecticides, i.e. Confidor® (imidacloprid 20.6%, Bayer AG, Leverkusen, Germany, 200 g/l SL), Mospilan® (acetamiprid 20%, Nippon Soda Co., Tokyo, Japan, 200 g/L SP), Actara® (thiamethoxam 25%, Syngenta, Inofyta, Viotia, Greece, 250 g/L WG) (imidacloprid, thiamethoxam and acetamiprid) and the pyrethroid Talstar® (bifenthrin 2.5%, FMC, Philadelphia, PA, USA, 25 g/L) as well as from their combinations with the synergists DEF (inhibitor of esterases) or PBO (piperonyl butoxide, monooxygenase inhibitor) were counted at 24h and preserved in RNAlater® (Ambion, TX) for transcriptional analysis. Controls (susceptible whitefly strain SUD-S) were treated only with acetone. Following analyses such as mRNA isolation, cDNA synthesis, PCR and cloning CYP6CM1, as well as the quantitative real-time PCR (qPCR) were conducted in the Insect Toxicology Laboratory, Department of Entomology at Texas A&M University. Primers sequences were:

BTCYP6CMF: 5' ATG GAA CTG TTG GAA ATA GTT AAG TCA GCC 3'

BTCYP6CMR: 5' TCA TGG CGT CGA CGC CCT TCT CCG GAA GCG 3'

Cytochrome P450 enzyme CYP6CM1 transcriptional expression was analysed by qPCR in order to identify potential insecticide detoxification mechanisms associated with resistance. The whitefly population in Klirou showed enhanced esterase and oxidase metabolism as revealed by DEF and PBO, and possibly target site insensitivity as suggested by results of the acetamiprid bioassays with synergists. CYP6CM1 was over-expressed in the Klirou population from Cyprus, with about four-fold the level of a susceptible strain. In this population, over-expression appears to be associated only with resistance to imidacloprid. (V.A. Vassiliou, D. Kim and P. Pietrantonio)

Study on the biology of palm borer *Paysandisia archon* (Lepidoptera: Castniidae)

Palm borer, *Paysandisia archon* Burmeister (Lepidoptera: Castniidae) and red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), comprise the two key pests of palms in Cyprus and the Mediterranean. In Cyprus, *P. archon* is not well established, while it has been recorded

only in the Larnaka and Pafos districts. It was found on palms (mainly on offshoots) of the *Phoenix dactylifera* and *Chamaerops humilis* species. Both the absence of commercialised pheromone (recent research showed that this insect has no pheromone) and its cryptic life cycle make early detection, population monitoring and management of this pest extremely difficult. For this purpose, biological parameters such as longevity and reproductive potential of *P. archon* were studied under laboratory conditions. Adults are found in nature only during their flight activity, while egg detection is possible after detailed observations. The rest of life stages (larvae, pupae) are found inside different parts of the host (offshoots, trunk, leaves). For this study, adults were captured in nature close to damaged date palms (*P. dactylifera*) during their flight activity (end of May - end of July), using a scoop-net. Captured adults were transferred to the laboratory and under controlled conditions ($t = 25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, $\text{RH} = 65\%$) were placed in separate cages and let to mate. As food source adults were given syrup 10%. Results showed that, under laboratory conditions, this species reached its full development within one year. The average duration from egg to adult males was 348.0 days, while for adult females this period required an average of 362.6 days. Duration of mating was recorded close to 1h, while the beginning of oviposition was recorded at 1.2 days, after mating. The mean number of eggs laid was 21.8 eggs/female ($n = 6$; simple range 10-50 eggs/female). Viability and hatching of eggs was very high and reached 96.2%, starting at the 13th until the 15th day after oviposition. The great majority of eggs hatched during the 13th day after oviposition, while 3.8% and 0.8% of eggs were hatched during the 14th and 15th day, respectively. No hatching was observed after the 15th day. Although no statistical significant differences were observed ($P=0.06$) results showed that the average life span for females was 11.8 days ($n = 6$; sample range: 8-18), while for males life span was recorded at 16.8 days ($n = 5$; sample range: 10-21). In all cases, no feeding activity of adults during their life span was observed. (V.A. Vassiliou and P. Kitsis)

Study on the reproductive potential and longevity of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

In Cyprus, the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) was first recorded in 2006, on palms of the *Phoenix canariensis* species. Today, this insect pest is found all over Cyprus and has destroyed hundreds of palms of the *P. canariensis* and *P. dactylifera* species, while the great majority of damage is recorded on Canary palms. More recently, damages have been recorded on palms of the *Washingtonia filifera* species. Reproductive potential and longevity of adults of red palm weevil were studied under laboratory controlled conditions ($t = 25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and $\text{RH} = 65\%$), in specific insect cages. Male and female adults were collected from pheromone traps installed all over Cyprus (including mountainous areas) as well as directly from damaged palms. One pair of healthy and active male and female were transferred to a separate cage and allowed to mate and lay eggs. Freshly cut apple halves as food source and for oviposition purposes were given to adults every two days. Newly hatched larvae were transferred to a separate cage and kept there for further development. Results showed that female adults exhibited longer lifespan compared to males. Concretely, the mean number of females' lifespan was found to be 137.4 days, while the mean number for males was 127.8 days. The mean number of eggs was found to be 97.3 eggs/female. Regarding the pre-oviposition period of females (from the day transferred to cages until the beginning of egg laying) differences between the adults collected from the coastal areas and those from the mountainous areas (720 m above the sea level) were observed. Adults from the mountainous areas exhibited lower pre-oviposition period (mean 3.8 days), compared to the pre-oviposition period of adults collected from the coastal areas (mean 30.9 days). This may be accounted for by the fact that females from the mountainous areas had already mated. (V.A. Vassiliou and P. Kitsis)

PLANT PATHOLOGY

Sanitation of local citrus varieties and/or clones

The main objectives of the project were the elimination of viruses and viroids from local citrus clones and/or varieties. Micrografting *in vitro* has been used to free from virus and other pathogens valuable local citrus varieties and/or clones. A survey in commercial groves has been conducted for selection of elite mother plants of citrus, including Polyphori and Lapithou lemon (*Citrus limon* Burm f.), Jaffa, Siekeriko and Aematousiki orange (*C. sinensis* L.), Arakapas mandarin (*C. reticulata* Blanco), Frappa (*C. grandis* (L.) Osbeck), Bergamot (*C. bergamia* Risso & Poit.), Coumantantas (*C. comandatore*) and Pummelo (*C. maxima*). All mother plants were tested for viruses and viroids by biological indexing, by ELISA for *Citrus tristeza virus* (CTV), and by RT-PCR for viroids. All mother trees were found free of CTV, *Citrus infectious variegation virus* (CIVV) concave gum and impietratura, but were infected by *Citrus exocortis viroid* (CEVd) and/or other viroids, including *Citrus cachexia viroid* (CcaVd) and *Citrus bent leaf viroid* (CBLVd). Some mother trees were infected by *Citrus psorosis virus* (CPsV). The standard procedure of shoot-tip grafting technique was used for elimination of viroids and CPsV in the selected citrus isolates. Micrografted plants, re-grafted on sour orange seedlings *in vivo*, and successfully established and transferred to the glasshouse, were tested six to nine months later for the viruses and viroids present in meristem donor mother plants. Polyphori and Lapithou lemon, Arakapas mandarin, Jaffa and Siekeriko orange, Bergamot, Frappa and Coumantantas produced by micrografting from different source trees, were found free of all viroids and CPsV and were used as primary source material for the basic citrus plantation of the island and for further propagation to government mother stock plantations, private nurseries and growers. Shoot-tip grafting *in vitro* is being continued for sanitation of all collected mother plants. (T. Kapari-Isaia, L.C. Papayiannis and I.M. Ioannides)

Sanitation of Greek elite citrus varieties.

The main objectives of the project were the elimination of viruses and viroids from Greek elite citrus varieties using the micrografting *in vitro* technique. The initial mother plant material were: Grapefruit (Shambar, Star Ruby), lemon (Adamopoulou, Vakalou, Zambetaki, Interdonato, Nouvel Athos, Verna), mandarin (Clementine of Poros, Chiotiko, Clasuelina, Encore, Marisol, Page, Tardivo di Ciaculli, Clementine SRA-63, Nova), orange (Valencia of Poros, Late Navel of Argos, Moro, Navelate, Salustiana, Valencia Olinda, Navelina, Newhall, Washington navel). All mother trees were found free of *Citrus tristeza virus* (CTV), *Citrus psorosis virus* (CPsV), *Citrus infection variegation virus* (CIVV), concave gum and impietratura, but were infected by *Citrus exocortis viroid* (CEVd) and/or other viroids. Scions derived from these trees were grafted onto healthy sour orange rootstocks in the greenhouse so that new apical meristems were obtained for micrografting, or apical meristems were directly micrografted on young seedlings of trifoliate *in vitro*. Adamopoulou and Verna lemon produced by micrografting was found free of all viroids. Shoot-tip grafting *in vitro* has been continued for sanitation of all collected mother plants. (T. Kapari-Isaia, A.E. Voloudakis, Ev.Mar. Koutsiumari and L.C. Papayiannis)

Chemotherapy *in vitro* for elimination of *Citrus tristeza virus*

The antimetabolite chemical ribavirin was added to the tissue culture medium to prevent virus replication or movement of virus from infected to healthy tissues. The use of this chemical combined with micrografting *in vitro* succeeded eradication of *Citrus tristeza virus*. The chemical was directly

added in the nutrient medium in five concentrations (10, 20, 30, 40 and 50 mg/l). After chemotherapy, meristems were removed from stems cultured *in vitro* and were micrografted onto new virus-free rootstocks. **(T. Kapari-Isaia)**

Thermotherapy *in vitro* for elimination of *Citrus tristeza virus*

High temperatures have been found to reduce virus concentration or inactivate several viruses. Micro-cuttings from infected plants were cultured *in vitro* and they were incubated in a controlled temperature cabinet at 35-40 °C for a period of six to twelve weeks. Eradication of *Citrus tristeza virus* from the whole cutting was impossible without causing severe damage or even death to the new plantlet. Virus was inactivated in new meristem shoots but not at the base of the stem. For the production of new virus-free plants, after thermotherapy, meristems were removed from the heat-treated plantlet and were micrografted *in vitro* onto new virus-free rootstocks. **(T. Kapari-Isaia)**

Production, maintenance and distribution of healthy citrus material - Pre-basic citrus plantations

All virus-free material, which was either selected or imported by the ARI or produced locally by micrografting, has been maintained in a pre-basic plantation under double insect-proof screen at the Acheleia Experimental Station. An alternative pre-basic plantation has been established at the Zygi Experimental Station. Presently, 60 citrus accessions are included in either plantation and are kept in sanitary condition by application of strict measures and regular indexing



in conjunction with optical observation for either fungal problems or genetical aberrations. The plantations provide with citrus material the basic or mother plantations of the Department of Agriculture and the experimental glasshouses of the ARI. **(T. Kapari-Isaia and L.C. Papayiannis)**

Programme for the control of *Citrus tristeza virus*

The main objective of the programme for the control of CTV, which was initiated in 1992, has been the systematic survey of all citrus and the removal of infected trees and/or groves wherever that is feasible. The survey has been conducted by indexing 10-20% of the trees of each grove and samples were tested in the Plant Virology Laboratory by enzyme-linked immunosorbent assay (ELISA). **(T. Kapari-Isaia and L.C. Papayiannis)**

Evaluation of citrus rootstocks for tolerance to *Citrus tristeza virus*

The following citrus rootstocks are evaluated for their tolerance to CTV at Xylotymvou Experimental Station: Carrizo citrange, Citrumelo, Volkameriana lemon, Gou Tou, Cleopatra mandarin and Sour Orange. All trees were grafted with the variety Washington navel sweet orange, whereas half of them

were inoculated by blind bark inoculation with the local citrus tristeza virus isolate 89-197. All trees have been tested by ELISA twice per year for the detection of CTV transmitted by aphids to virus free trees. Two virus-free trees tested positive to CTV by ELISA. **(T. Kapari-Isaia and L.C. Papayiannis)**

Evaluation of five new sour orange hybrid rootstocks for tolerance to citrus viroids

Five citrus hybrid rootstocks are evaluated at Zygi Experimental Station for their tolerance to citrus viroids. The five citrus hybrids are the following: (1) Sour orange × Orange (*Citrus aurantium* L. × *Citrus sinensis*, (L.) Osbeck), (2) Sour orange × Satsouma (*Citrus aurantium* L. × *Citrus reticulata* blanco), (3) Sour orange × Citrumelo 1452 × Lemon (*Citrus aurantium* L. × *P. trifoliata* (L.) Raf × *Citrus paradisi* Macf. × *Citrus limon*), (4) Sour orange × Volkameriana (*Citrus aurantium* L. × *Citrus volkameriana*) and (5) Sour orange × Carrizo (*Citrus aurantium* L. × *Citrus sinensis* (L.) × *Poncirus trifoliata* (L.) Raf). **(T. Kapari-Isaia and L.C. Papayiannis)**

Production, maintenance and distribution of healthy stone fruit material. Pre-basic stone fruit plantations

All virus-free material is maintained in a pre-basic plantation under double insect-proof screen at the ARI Zygi Experimental Station. A second, alternative prebasic plantation has been established at the Experimental Station at Saittas. To the present, 50 stone fruit accessions are included in either plantation, kept in sanitary condition by application of strict measures and regular indexing in conjunction with optical observation for either fungal problems or genetical aberrations. The plantations provide with propagating material for the basic or mother plantations of the Department of Agriculture and for the experimental glasshouses of the ARI. **(T. Kapari-Isaia and L.C. Papayiannis)**

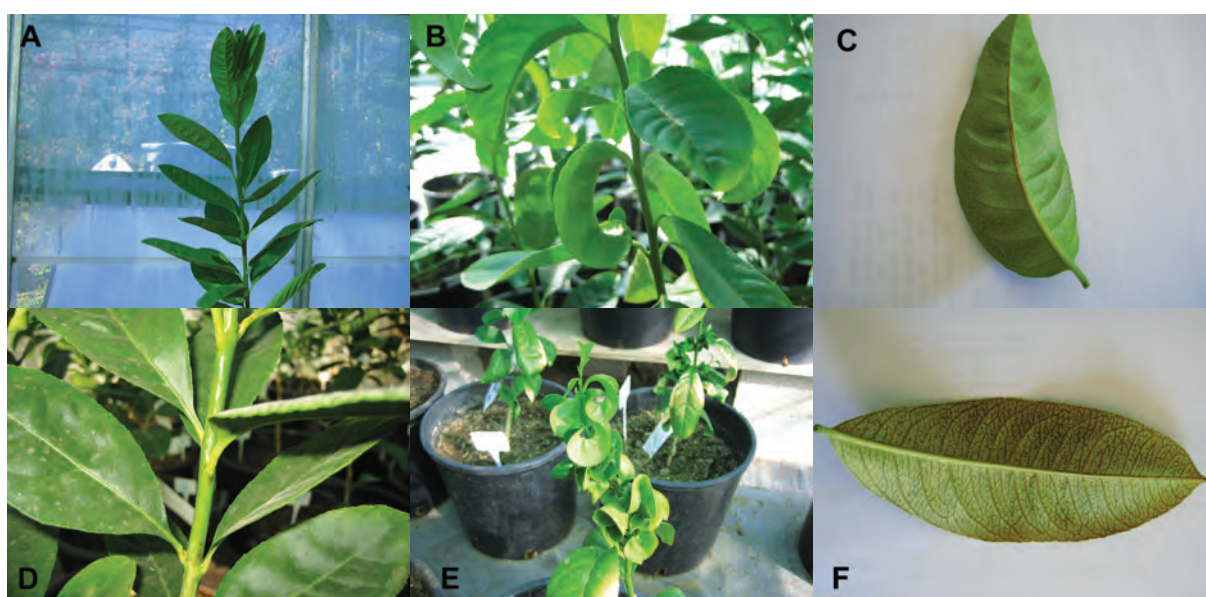
Diagnostic assay for the simultaneous detection of *Citrus exocortis* and *Hop stunt viroids*

Viroids are the smallest plant disease agents and consist of a naked, covalently-closed, circular ribonucleic acid molecule. Until now 29 viroid species have been identified and classified into two major groups (families), namely *Pospiviroidae* and *Avsunviroidae*. *Citrus exocortis* viroid (CEVd) (genus *Pospiviroid*) and *Hop stunt viroid* (HSVd) (genus *Hostuviroid*) are two common viroids that have been reported to infect several plant species. In nature, their host range includes fruit trees, such as citrus, stone fruit and grapevines, vegetable crops and ornamentals, where they can be found in single or mixed infections. Although these pathogens do not always cause notable symptoms, they are considered of great phytosanitary importance, especially for the production of healthy propagating material of fruit tree crops. To support effective disease detection and management, an assay was developed and optimized for the simultaneous detection of CEVd and HSVd. The developed assay, allows for simple, accurate and cost-effective testing of a large number of plant samples, and can be applied in surveys and certification schemes. **(L.C. Papayiannis)**

Co-inoculation of *Citrus* viroids affects symptom expression on Etrog citron

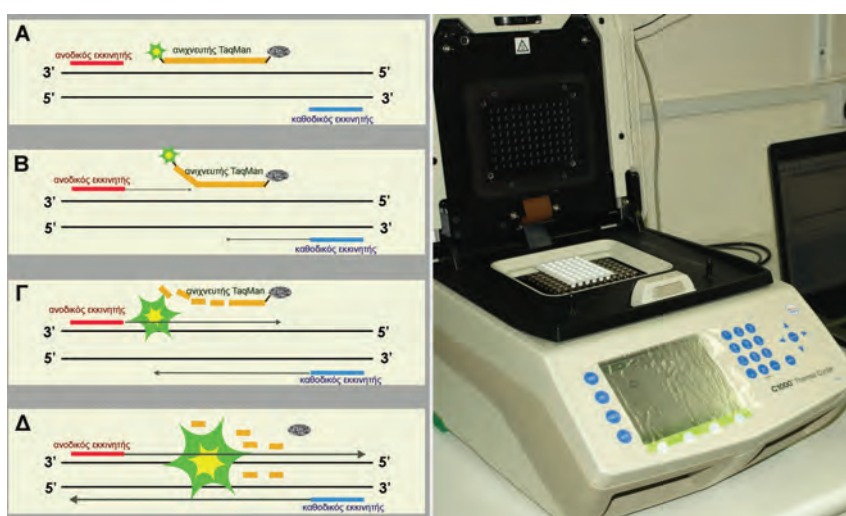
Citrus trees are naturally infected with various combinations of viroids that often lead to different symptom expressions. Etrog citron (*Citrus medica* L. Arizona 861-1S) is an excellent indicator plant often used by researchers for biological indexing of citrus viroids. In this work, the symptom expression

severity was studied on Etrog citron, after inoculation of single or mixed isolates of five different viroids in a controlled environment. For this purpose *Citrus exocortis viroid* (CEVd), *Hop stunt viroid* (HSVd), *Citrus bent leaf viroid* (CBLVd), *Citrus dwarfing viroid* (CDVd) and *Citrus bark cracking viroid* (CBCVd) were inoculated at different combinations on Etrog plants which were produced both as seedlings and grafted on sour orange. Results showed that single infection using CEVd caused the most severe symptoms on all tested trees. Mixed infections caused by CBLVd, HSVd, CDVd and CBCVd also resulted in severe symptoms. On the contrary, symptoms produced by other combinations of these pathogens produced only mild symptoms. Furthermore, viroids reduced the height of citron plants in general, but there was a significant difference in height reduction between citrons grafted on sour orange and citrons ungrafted. Different viroids and viroid complexes reduced the height of citrons grafted on sour orange by 2.4% to 29.0%, whereas the height of infected citron seedlings was reduced by 5.7% to 60.3%. (T. Kapari and L.C. Papayiannis)



Detection and characterization of *Citrus tristeza virus* (CTV) in Cyprus using molecular techniques

Citrus tristeza virus (CTV) was first reported in Cyprus in 1968 and until recently, virus detection has been mainly based on Mexican lime (*Citrus aurantifolia*) indexing and ELISA tests. In view of a national project aiming at the management and characterization of the disease, a new diagnostic protocol was developed and optimized. The protocol is suitable for the generic



detection of all virus isolates associated with severe or mild symptoms in the Mediterranean basin. In addition, for discrimination of virus strains identified in Cyprus, six primer pairs were designed suitable for application in conventional or real-time PCR assays. Primer specificity is based on their small length which varies between 12-15 nucleotides, and on the incorporation of modified bases known as locked nucleic acids (LNAs), which increase hybridization range and melting temperature. Evaluation of these primers in isolates from Cyprus, Greece, and other geographical regions showed capacity to discriminate different CTV strains efficiently and rapidly. **(L.C. Papayiannis and T. Kapari-Isaia)**

Grapevine pre-basic plantations

Efficient control of plant virus, virus-like and prokaryotic diseases starts with the use of healthy propagating material, which is free not only from disease symptoms but also from latent infections. The EU has recently imposed strict directives on the distribution of grapevine material that involve phytosanitary testing of plants for several plant pathogens. Utilization of healthy plant material seems nowadays the most promising tactic that can ensure good results to control these diseases.



During the past decades, the ARI has established long term maintenance of healthy propagative grapevine material under “pre-basic” status. Approximately 60 local or imported varieties are maintained under insect proof net houses at the Zygi Experimental Station. These plants are annually tested for a number of plant viruses including *Grapevine fan leaf virus*, *Grapevine fleck virus*, *Arabis mosaic virus* and *Grapevine leaf roll* associated viruses 1 and 3, viroids and several other prokaryotic pathogens. Tests include adoption of pathogen specific serological and molecular based laboratory techniques. Virus-free grapevine plant cuttings are issued to the Department of Agriculture for further multiplication and distribution to Cypriot farmers. **(L.C. Papayiannis and T. Kapari-Isaia)**

Tomato yellow leaf curl disease in the Eastern Mediterranean Basin: Virus species, incidence, hosts and transmission properties

In view of a bilateral project between the ARI and the Aristotle University of Thessaloniki, an extensive survey was conducted during 2006-2013 in Cyprus, Crete, the Dodecanese and Ionian islands as well as in the mainland of Greece, in order to identify the virus species and *B. tabaci* biotypes involved in Tomato yellow leaf curl disease (TYLCD) epidemics. Approximately 8,000 symptomatic tomato samples, 4,500 weeds and 3,000 whitefly samples were collected and analyzed. The host range of TYLCV and TYLCSV isolates was studied using whitefly transmission tests in several plant species. Transmission efficiency of TYLCV and TYLCSV was evaluated using different *B. tabaci* biotypes colonies which harbored different bacterial endosymbionts. Results showed that in Greece, TYLCV was the most prevalent *Begomovirus* species (94.5%), whereas TYLCSV was found in 4.5% of the total samples tested.

In Cyprus, TYLCV was the only species found to be associated with TYLCD. Molecular identification of *B. tabaci* biotypes showed that Q was the only biotype found in the mainland of Greece, Pelloponese and the island of Crete. Both biotypes (B and Q) are involved in TYLCD spread in Cyprus and Dodecanese islands. Forty nine different weed species belonging to 15 botanical families were tested positive to TYLCV under field conditions, suggesting that the host range of the virus is far more extensive



that previously documented. Transmission studies showed that TYLCV isolates had a broader host range as well as higher transmission efficiency than TYLCSV. Finally, TYLCV transmission was somehow correlated with the presence of *Hamiltonella* sp., within the body of *B. tabaci* insect, as colonies that harbored this bacterium, transmitted the virus more efficiently from and to tomato plants. (L.C. Papayiannis, N.A. Seraphides and N.I. Katis)

Molecular typing of cyst-forming nematodes *Globodera pallida* and *G. rostochiensis*, using novel molecular methods

Globodera pallida and *G. rostochiensis* are two cyst-forming nematodes known to infest potato crops, causing severe economic losses worldwide. Besides direct feeding damages, which often lead to potato plant death, these nematodes are considered of great phytosanitary importance. Once they infest an agricultural field, they are practically impossible to eradicate as their eggs can survive inside cysts in the soil for more than 20 years. Both organisms are listed as quarantine pests in Europe and each member state is enforced to perform annual surveys to identify the PCN incidence and distribution. In Cyprus, both PCN species have been recorded and associated with major yield losses in potato crops. Their differentiation is extremely difficult, since both species have similar phenotypic characters and produce morphologically indistinguishable cysts. In this study, a real-time TaqMan PCR assay was designed and optimized for the simultaneous detection of *G. pallida* and *G. rostochiensis*. The assay shows an excellent analytical and diagnostic sensitivity, and holds much promise as a routine tool for diagnosis of the two pathogens in samples across different countries. The assay can be accomplished either by using total DNA or crude extracts prepared directly from cysts, offering users a reliable, wide range diagnostic tool. (L.C. Papayiannis, M. Christoforou and D. Tsaltas)

NEW PESTS AND DISEASE RECORDS

First record of the Goji gall mite, *Aceria kuko* (Acari: Eriophyidae)

On October 2013 leaves of Goji berry plants, *Lysium barbarum*, exhibiting leaf galling were transferred to the ARI's Entomology lab. It was found that the galls had been formed by the non-indigenous eriophyoid mite, *Aceria kuko*. Adult mites are extremely small with a wormlike body and bear only two anterior pairs of legs. In addition to the host *L. barbarum*, the mite has been recorded to form foliar galls on *Capsicum annuum* (pepper) and *Solanum nigrum* (Black nightshade). (N.A. Seraphides)



New records of phytoseiid mites (Acari: Phytoseiidae) from Cyprus

Predatory mites of the Phytoseiidae family are important biological control agents of phytophagous arthropods including mites. A survey on phytoseiid mites associated with different crops was carried out in many localities in Cyprus during 2009 and 2010. Five species are recorded for the first time from various plants in Cyprus: *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese), *Phytoseiulus persimilis* Athias-Henriot and *Phytoseius plumifer* (Canestrini & Fanzago). In all cases, these phytoseiid mite species were found in great abundance. (V.A. Vassiliou, P. Kitsis and G.Th. Papadoulis)

Presence of Lettuce big vein disease in Cyprus

Lettuce big-vein disease is a viral disease of lettuce (*Lactuca sativa* L.) which is transmitted by the soil borne fungus *Olpidium brassicae*. Symptoms of big vein include chlorosis surrounding the vascular



bundles in the leaf and increased stiffness of the leaves. The causal agent is *Mirafiori lettuce big-vein virus* (MLBVV), an ophiovirus transmitted by the soil-borne fungus *Olpidium brassicae* (Lot *et al.*, 2002). Another virus, *Lettuce big vein virus* (LBVV) that has historically been associated with this disease, causes only latent infection. During 2012 and 2013 symptoms of interveinal chlorosis and stiffness were observed in lettuce crops

cultivated in Lefkosia district (Akaki, Analiontas). Samples from symptomatic plants were collected, transferred to the Laboratory of Virology at ARI and analyzed using serological and molecular methods. Results showed that all symptomatic lettuces were infected both with MLBVV and LBVV. To our knowledge, this is the first record of these plant pathogens in Cyprus. **(L.C. Papayiannis)**

Detection of *Prunus necrotic ringspot virus* (PNRSV) infecting ornamental rose plants

During spring of 2012, yellow mosaic and chlorotic line patterns were observed on leaves of rose plants cultured in gardens of Larnaka and Lemesos Districts. Diseased plants were collected and tested for viruses known to infect members of the *Rosaceae* family at ARI's Laboratory of Virology. Serological and molecular tests identified the presence of *Prunus necrotic ringspot virus* (PNRSV), a plant virus that is widespread in *Prunus* sp. across Cyprus. PNRSV is transmitted by grafting and by infected pollen, and is reported for the first time to cause symptoms on rose plants in Cyprus. Phylogenetic analysis studies of virus isolates revealed high affinity with PNRSV isolates from abroad, indicating that these infected roses were most likely imported. **(L.C. Papayiannis and Y. Markou)**



SOIL SCIENCE

The activities of the Soil Science Section concern soil fertility improvement, plant nutrition, soilless culture, wastewater reuse, integrated water resources management and agricultural engineering. Research on soils mainly aimed at improving soil quality and nitrogen fertilization through organic amendments or implementation of legume rotations. Field and laboratory experiments have been undertaken to investigate the mechanisms by which the incorporation of manure, compost or plant residues influence soil N dynamics and crop yields.

In plant nutrition and soilless culture, activity focused on both education and research. Work at the “hydroponics training centre” continued with the training of agronomists on advanced management of hydroponic systems. Experiments focused on precision growing of plants for sustainability and profitability. Plant nutrition aspects have been examined when nutrients in irrigation water exceed recommended levels or when salty water was used. Different types of hydroponic systems were tested taking into consideration production, water use efficiency and fertilizer expenditure. Ongoing research relates to closed hydroponic systems and nutrient uptake concentrations. Further, the Section participates in a study using remote sensing and geographic information systems to record the changes in irrigation needs of some water consuming crops.

Research on integrated water resources management is currently devoted mainly to wastewater reuse in agriculture. Field experiments are performed for appraising the effects of wastewater reuse for irrigation purposes on the environment and on public health. Also, a field survey regarding these effects has been carried out in all districts of Cyprus. In addition, a field and a hydroponic experiment have been carried out to evaluate xenobiotic compounds (pharmaceuticals) uptake by plants (tomato, lettuce and alfalfa).

Research in agricultural engineering examines the potential use of biomass for biofuel (biodiesel, bioethanol, biogas and solid fuel) and energy production under Cyprus conditions. Moreover, agricultural engineering is actively involved in a large number of EU and RPF Research Projects: «Adaptation of agricultural production to climate change and the limited water resources-Adapt2change», «Designing an environmental friendly management scheme for agrochemicals plastic packaging waste-Agrochepack», «Testing of new technology greenhouse plastic covers-GreenFilm», and «Study of the growth of micro-algae for the production of biodiesel-Med-Algae».

PLANT NUTRITION AND SOILLESS CULTURE

Research activity focused on plant nutrition aspects of greenhouse vegetables, particularly strawberry, melon, and cucumber, in order to generate up-to-date, practical and location-specific information. A parallel activity concerns the irrigation management in hydroponics under Mediterranean climatic conditions. Precision agriculture offers the promise of increasing productivity while decreasing production cost and minimizing environmental impact. Therefore, the collection and reuse of the fertigation effluents produced in greenhouses was employed. In addition, (i) nitrogen requirements and (ii) leaf nutrient concentration levels of selected crops were studied. Plant nutrition when nutrients in raw water exceed recommended levels was also examined. Finally, the Soil Science Section participated in three EU projects with the following main objectives: to adapt farming to climate change and limited water resources, to develop and implement halophytes utilization, and to facilitate smart specialization in promoting high value berry crops.

Impact of different nutrient macrocation ratios on macronutrient uptake by melon (*Cucumis melo* L.) grown in NFT

The aim of the present study was to determine standard uptake ratios between macronutrients and water for melon (*Cucumis melo* L. cv. Dikti) grown in closed soilless cropping systems. The obtained data are needed to establish standard nutrient solution compositions for melon grown in closed hydroponic systems under Mediterranean climatic conditions. Nutrient uptake by plants in the closed system was compensated for by supplying a Replenishment Nutrient Solution (RNS) with different K^+ : Ca^{2+} : Mg^{2+} ratios to test their impact on uptake ratios between macronutrients and water. The RNS used as control treatment had an Electrical Conductivity (EC) of 1.74 dS m^{-1} and contained $6.5 \text{ mM } K^+$, $2.8 \text{ mM } Ca^{2+}$, and $1.0 \text{ mM } Mg^{2+}$ ($K^+ : Ca^{2+} : Mg^{2+} = 0.63 : 0.27 : 0.10$). Control RNS was compared with two other RNS, both with a high Ca^{2+} ion level (4.2 mM), while their K^+ and Mg^{2+} ion levels were (i) either not altered (corresponding to a ratio of $K^+ : Ca^{2+} : Mg^{2+} = 0.55 : 0.36 : 0.09$; $EC = 2.0 \text{ dS m}^{-1}$); (ii) or increased to maintain the same $K^+ : Ca^{2+} : Mg^{2+}$ ratio as in control RNS ($EC = 2.45 \text{ dS m}^{-1}$). Nutrient to water uptake concentration (UC) was assessed by two alternative methods, i.e. (i) nutrient and water removal from the system and (ii) nutrient recovery from plant biomass and water consumption. Over the two methods, mean UC were: N, 15.4; P, 1.31; K, 5.47; Ca, 3.78; and Mg, 1.02 mmol l^{-1} and measured tissue analysis resulted in a total plant composition molar ratio of $K : Ca : Mg = 0.55 : 0.34 : 0.11$. In addition, UC tended to decrease as the crop aged although in absolute values, the mass of nutrients absorbed increased following dry weight accumulation. Further, the results revealed that the damage caused by the increase of the EC when attempting to maintain a target K:Ca:Mg ratio in the RNS, is higher than the benefits from the optimal cation ratio. Increasing K^+ and Mg^{2+} ions concentration in addition to that of Ca^{2+} to maintain a standard $K^+ : Ca^{2+} : Mg^{2+}$ ratio raises the EC in the root zone (4.62 dS m^{-1}), associated with increased accumulation of nutrients outside roots and impairment of fruit yield (20% decrease) due to reduction of mean fruit weight. Leaf gas exchange, chlorophyll parameters and fruit taste quality did not reveal any differences according to the treatment, whereas phenolics and antioxidant capacity in melon fruits increased under high EC. (D. Neocleous, D. Savvas)



The effect of grafting and silicon on physiological parameters and nitrate disappearance from the nutrient solution of melon (*Cucumis melo* L.) plants

Grafting of *Cucurbitaceae* to some rootstocks seems to be especially beneficial for the nitrogen nutrition of these plants. Moreover, melon (*Cucumis melo* L.) has been considered as a crop that could benefit from the addition of Silicon (Si) in the Nutrient Solution (NS). Thus, two successive experiments were carried with the following objectives: (i) to investigate how grafting affects nitrate uptake concentrations (following the disappearance of nitrates from NS), leaf water relations, leaf gas exchange, chlorophyll parameters and antioxidant activity and (ii) to assess which of the previous physiological factors could be associated with Si supply in melon plants grown in a floating system. Results revealed that either grafting or Si application enhances nitrate uptake in melon plants. However, grafting did not have a consistent effect on measured physiological parameters. (D. Neocleous)

Irrigation management techniques in soilless culture under Mediterranean climatic conditions

In order to evaluate the irrigation frequency based on solar radiation on growth and reproductive development of cucumber plants (*Cucumis sativus* L.) an experiment was conducted under greenhouse conditions. Plants were cultivated in rockwool slabs and irrigation was performed whenever accumulative solar radiation outside greenhouse reached 1307 kJ/m² for high irrigation frequency, 1960 kJ/m² for medium irrigation frequency and 3049 kJ/m² for low irrigation frequency. The amount of water



applied on every irrigation event was 120 ml/plant, 180 ml/plant and 280 ml/plant at high, medium and low irrigation frequencies, respectively. The concept of precision irrigation, especially in high investment, cultivations should include some measurement of actual plant physiological responses to the dynamics of substrate moisture content. In respect to the above objective this experiment aims at correlating irrigation frequencies with solar radiation and real time plant water status by using different kinds of sensors. For that purpose, a phytomonitoring system was set up monitoring leaf temperature, steam micro variation, substrate electrical conductivity, temperature and volumetric water content. Results are expected to have a potential for application for commercial implementation. (D. Neocleous, G. Nikolaou)

Assessment of different strategies to balance high Mg levels in the irrigation water when preparing nutrient solution for soilless strawberry crops

In some cases, the levels of dissolved Mg in the irrigation water for strawberry (*Fragaria × ananassa* Duch.) may exceed recommended concentrations for hydroponic Nutrient Solutions (NS). Using a NS with a standard Mg level (1.4 mM) as control, three different strategies were compared for managing

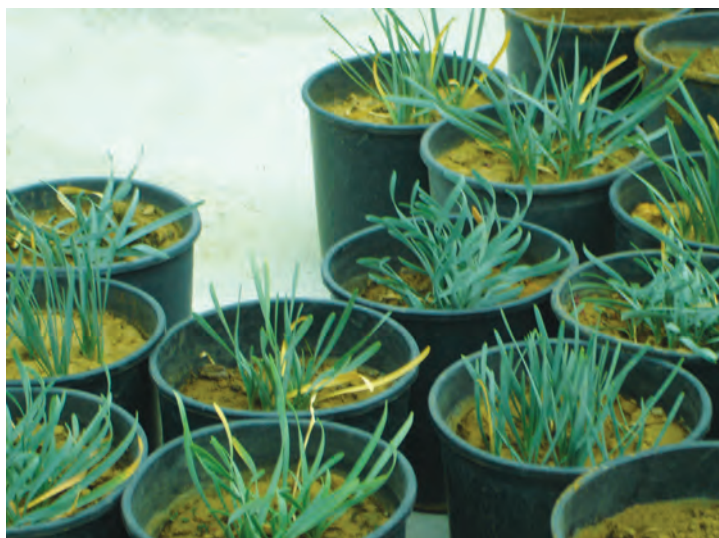
excessive Mg concentration (2.1 mM) in irrigation water when preparing NS for hydroponically-grown strawberry, so as to minimize crop damage by excess Mg. Management of excessive Mg level was performed according to the following strategies: (i) electrochemically balanced by equally increasing the sulphates, while not altering the levels of the other macrocations (Strategy I), (ii) by commensurate increases in the K, Ca and $\text{SO}_4\text{-S}$ levels, thereby maintaining the same K:Ca:Mg ratio as in the control treatment (Strategy II), (iii) compensated for by proportional decreases in the K and Ca levels, so as to maintain the same total salt concentration as in the control treatment (Strategy III). The Electrical Conductivity (EC) in the control NS was 1.8 dS m^{-1} but increased to 1.9 and 2.6 dS m^{-1} by Strategies I and II, respectively. When Strategy II was applied, the fresh shoot and fruit weights decreased to 78% and 84%, respectively, compared to the control treatment, despite the application of an optimal K:Ca:Mg ratio. These results indicate that, for strawberry performance, the EC of the supplied NS is more important than the K:Mg or Ca:Mg ratios. The reduction of plant growth and fruit yield by Strategy II was associated with an increase of EC to 3.4 dS m^{-1} in the drainage solution and significant reductions from -0.57 to -0.85 MPa in leaf water potential and from -1.1 to -1.4 MPa in leaf osmotic potential, while leaf turgor pressure and relative water content were similar in all treatments. Gas exchange parameters and chlorophyll levels were not affected by the increased Mg level, regardless of the strategy used to compensate for the excess Mg in the irrigation water. (D. Neocleous, D. Savvas)



Antioxidant responses of native to Cyprus halophyte *Pancratium maritimum* L. under salinity conditions

The purpose of this study was to look into antioxidant responses of the near threatened *Pancratium maritimum* L. (sea daffodil), a native bulbiferous halophyte, naturally thriving along coastal sand dunes of Cyprus. Besides its ecological and floral value, this halophytic species may present interest as an

alternative natural source of antioxidants. The plants were grown under protected cultivation in pots containing 5 l of quartz sand and were supplied for 30 days with Hoagland Nutrient Solution (NS) containing 0, 50 or 200 mM NaCl. Leaves and bulbs were harvested after salt treatment period mainly to determine antioxidant molecules such as: ascorbic acid, phenolics, flavonoids and antioxidant activity (FRAP and DPPH assays). The response of salt-treated plants revealed that low salinity (50 mM NaCl) enhances the secondary metabolism enabling leaves and bulbs to respond to external signals (NaCl stress) as indicated by higher contents of antioxidants. However, antioxidant molecules in plant tissues were significantly restricted at elevated salinity level (200 mM NaCl). Leaves showed higher values of antioxidants compared with bulbs. The results indicate that native to Cyprus halophyte *P. maritimum* may be interesting for production of antioxidant compounds in some instances.



(D. Neocleous)

INTEGRATED WATER RESOURCES MANAGEMENT AND IRRIGATION

Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity

In recent years, wastewater reuse for agricultural crop irrigation has become a common practice in Cyprus. More than 70% of treated wastewater, representing 14 to 15 × 10⁶ m³ per annum is currently used for crop irrigation. Appraisal of treated wastewater irrigation effects on soil physicochemical properties and on public health has been carried out since 2011. Tomatoes have been irrigated with treated wastewater supplied from Limassol and Anthoupoli Wastewater Treatment Plants (WWTP), and with control water derived from a well located in the Athalassa Research Station. Irrigation with treated wastewater from both WWTPs sustained soil electrical conductivity at lower levels in comparison to well water irrigation, whereas no statistically significant differences were reported regarding other soil physicochemical properties, such as pH, organic matter content, total C, total N and heavy metal content. Furthermore, no microbial contamination was detected in tomato fruits in all treatments, whereas crop productivity was the same among all three treatments. Results revealed that treated wastewater constitutes a valuable and safe water resource that can be integrated into Cyprus water resource balance for mitigating water shortage. (A. Christou. G. Maratheutis)

Assessment of long-term wastewater irrigation impacts on the soil geochemical properties and the bioaccumulation of heavy metals to the agricultural products

An extensive field survey was performed for assessing the impact of long-term wastewater irrigation of forage crops and orange orchards in three suburban agricultural areas in Cyprus, as compared to rainfed agriculture, on the soil geochemical properties and the bioaccumulation of heavy metals (Zn, Ni, Mn,

Cu, Co) in the agricultural products. Both ryegrass fields and orange orchards in areas I and II were continuously wastewater irrigated for ten years, whereas clover fields in area III for 0.5, four and eight years. The results revealed that wastewater reuse for irrigation caused a slight increase in soil salinity and Cl^- content in areas I and II, and a remarkable increase, with strong correlation with the irrigation period of the



wastewater, in area III. Soil salinization in area III was due to the high Electrical Conductivity (EC) of the wastewater applied for irrigation, attributed to the influx of seawater into the sewage collection network in area III. In addition, the wastewater irrigation practice resulted in a slight decrease of the pH values in area III, while a subtle impact was identified regarding the CaCO_3 , Fe and heavy metal content in the three areas surveyed. The heavy metal content quantified in the above ground parts of the forage plants was below the critical levels for phytotoxicity and the maximum acceptable concentration in dairy feed, whereas heavy metals quantified in orange fruit pulp were below the Maximum Permissible Levels (MPLs). Heavy metal phytoavailability was confined due to soil properties (high pH and high clay content), as evidenced by the estimated low Transfer Factor (TF). (A. Christou)

Uptake and translocation of four pharmaceuticals (diclofenac, trimethoprim, sulfamethoxazole and 17 α -ethynylestradiol) to different organs of hydroponically grown tomato, lettuce and alfalfa plants

Technologically advanced quantification techniques enable scientists to accurately measure Compounds of Emerging Concern (CECs) in the water matrix and in the environment in general. A group of CECs that raise concern among water specialists are pharmaceuticals, which render Waste Water Treatment Plants (WWTPs) as potential hotspots for the introduction of these micropollutants in the environment. However, the fate of pharmaceuticals in the environment, particularly their potential uptake by plants and entry into the food chain, remains largely unexplored. The aim of this research work, undertaken in collaboration with the Department of Civil and Environmental Engineering of the University of Cyprus, is to evaluate the potential of uptake of four pharmaceuticals by plant species, such as tomato, lettuce and alfalfa. These four pharmaceuticals are frequently found in treated wastewater in Cyprus. A hydroponic experiment was set up for evaluating this hypothesis. The results are expected to shed light into this unexplored topic. (A. Christou)



SOIL FERTILITY IMPROVEMENT

Grain legumes in rotation systems

Rainfed grain legumes are cultivated in rotation systems in Cyprus on a much smaller scale than it would be expected based on their apparent economic and environmental benefits. A more thorough understanding of the mechanisms through which they influence subsequent crops would further support their cultivation. The objective of this work was to provide evidence on the effects of faba bean and chickpea on the dynamics of soil N availability and yield parameters of wheat in a two-year rotation. Benefits were evaluated in comparison with the more extensively used vetch-wheat and wheat-wheat systems. Two- year legume-wheat rotations were established in small plots. Soil samples were taken from these plots just before wheat sowing, analyzed for residual N and incubated under optimum conditions in the laboratory to assess N mineralization potential, soil respiration and N immobilization after incorporation of plant residues. Soil that had grown vetch showed the highest residual N six months after its harvesting and mineralized at the end of the incubation almost 120 mg N kg⁻¹ soil; an amount largely capable of supporting even the highest wheat production without fertilization. This soil showed also the greatest CO₂ release and the smallest N immobilization after residue incorporation. Smaller values of mineral N release (80 kg N kg⁻¹ soil) were shown by faba bean soil, but this mineralization potential would still be capable of meeting the requirements of a medium-size yield. Results on mineralization and immobilization were contrasted to with grain, biomass and N yield during the second year of field rotation. The unfertilized wheat removed from the soil greater amounts of N as straw and grain and showed significant yield surplus when grown after vetch and faba bean. Yield surplus was also found in wheat plots cultivated previously with chickpea although, apart from soil respiration, this legume and wheat manifested no differences in residual N and N mineralization or immobilization potential. It was concluded that the removal of seed N at harvesting does not cancel the N credit of grain legumes in rotations.

(P. Dalias)



Compost stability

Stability is an important characteristic that determines the effects of compost when used for soil improvement or for replacing fertilizer. For example, the spreading of unstable compost on soil can highly stimulate microbial activity and provoke nitrogen immobilization or soil oxygen depletion. This work aimed at investigating the possibility of controlling the stability of compost by manipulating the composition of feedstock. Three mixtures of organic materials were composted in rotating metal drums. The proportions of the different materials were such as to obtain the same carbon-to-nitrogen ratio. Shredded prunings of *Ficus microcarpa* were mixed with legume residues, or animal manure, or grass. When composts reached maturity, samples were analyzed for microbiological oxygen consumption, chemical properties and phytotoxicity. Results indicated small but statistically significant differences in stability, with the *Ficus*-manure compost showing the smallest oxygen consumption in laboratory incubation tests.

(P. Dalias)

Agricultural Plastic Packaging Waste - AgroChePack Project

Agricultural Plastic Packaging Waste (APPW) management practices constitute a major environmental problem in many countries, by polluting soil, air and water resources and compromising product safety and public health. The AgroChePack Project established the basic design principles of an environmental friendly and economically viable APPW management scheme for Europe. The adoption of such a scheme would protect the environment and public health, would enable the sustainable development of agriculture and would allow the exploitation of APPW material. Within the framework of the project, a pilot application of the management system in Cyprus was also designed and operated for more than one year. Local collection stations in the region of Xylofagou and Sotira and a central consolidation station in the Experimental Station of ARI in Xylotymvou were designed, constructed and functioned according to operational guidelines. Farmers participated on a voluntary basis. Based on data gathered from this pilot trial an economic analysis was carried out on the capital and operating cost of the system and different scenarios were examined depending on the final use of the collected plastic containers. (P. Polycarpou and P. Dalias)



AGRICULTURAL AND ENVIRONMENTAL ENGINEERING

Application of renewable energy sources in agriculture

Greenhouse horticulture and floriculture is burdened with demand for heating during winter, which is absolutely necessary for maintaining production. In fact, heating contributes considerable increase in production costs. Therefore, alternative energy sources, such as solar, wind, shallow geothermal, biomass etc., should be considered. The work carried out deals with the possibility of using biomass from agriculture and forestry as a renewable energy source. Alternative energy plants are investigated as potential energy sources for solid fuel production, without disturbing the food or feed chain. Biomass can be processed in briquettes, pellets or loose chips. Examples of such plants are *Acacia saligna* and *Leucaena leucocephala*. Furthermore, biomass could be used for the production of liquid bio-fuels, such as bio-diesel and bio-ethanol for transportation and biogas for energy production. Different plant species are being tested in order to select the most



suitable ones for the production of bio-fuels under Cyprus conditions, by exploiting also the possibility of utilizing treated wastewater that cannot be otherwise exploited. Some of the species tested in this programmes are: *Sinapis alba*, *Ricinus communis*, *Onopordum cyprium*, *Ferula communis*, *Cynara cardunculus* and *Asphodelus aestivus*. (P. Polycarpou)

Adaptation of agricultural production to climate change and limited water resources

The European project ADAPT2CHANGE: “Adaptation of agricultural production to climate change and limited water resources”, “Adapt agricultural production to climate change and limited water supply LIFE 09 ENV/GR/000296” started in September 2010. Lead Partner is TEI Larissa’s in Greece,. The project is 50% funded by the EU and involves the University of Athens, the Agricultural Research Institute of Cyprus, TEI Piraeus, and Europliroforisi S.A. The overall objective is to enhance the adjustment of agricultural production to climate change and limited water supply. In particular, it aims to reduce the water volume used in agricultural production by introducing a method of recycling water through a closed, fully automated hydroponic greenhouse system. The project aims to develop and demonstrate in rural areas of Greece and Cyprus the possibility of increasing agricultural production and the income of farmers in a sustainable and environmentally friendly manner, while reducing consumption of energy and natural resources such as water. (P. Polycarpou and D. Neocleous)



New technology in greenhouse plastic covers

The Project, funded by the RPF “GreenFilm”, started also in 2011 and concerns the testing of latest technology greenhouse plastic covers that incorporate nano-particles as pigments imparting special properties to the cover. (P. Polycarpou)

Study of the growth of micro-algae for the production of biodiesel

Efforts to replace petroleum products and reduce greenhouse gas emissions have currently intensified. One of the main problems facing biodiesel production is the availability of sufficient raw material for production. A solution to this problem seems to be the cultivation of microalgae found in seawater or wastewater, which have a higher yield for biodiesel production compared to conventional energy crops. The European ENPI Project “MED-ALGAE” was signed in December 2011 and involves the study of the growth of micro-algae for the production of biodiesel. The consortium consists of research organizations, academic institutions, energy agencies and private organizations from six countries: Cyprus, Greece, Italy, Malta, Lebanon and Egypt. ARI is the Lead Partner coordinating the Project. This novel technology project can contribute towards the goals targeted by the EU strategy on “Climate change and energy.” The methodology includes all stages in the production of biodiesel from microalgae.

Furthermore, the project aims to collect all available data on microalgae in the participating countries, to conduct a study on the available state-of-the-art technologies and to provide feasibility studies for the implementation of research findings. (P. Polycarpou, I. Ioannidis, M. Omirou and P. Dalias)



Study of the use of macroalgae for wastewater treatment and energy production

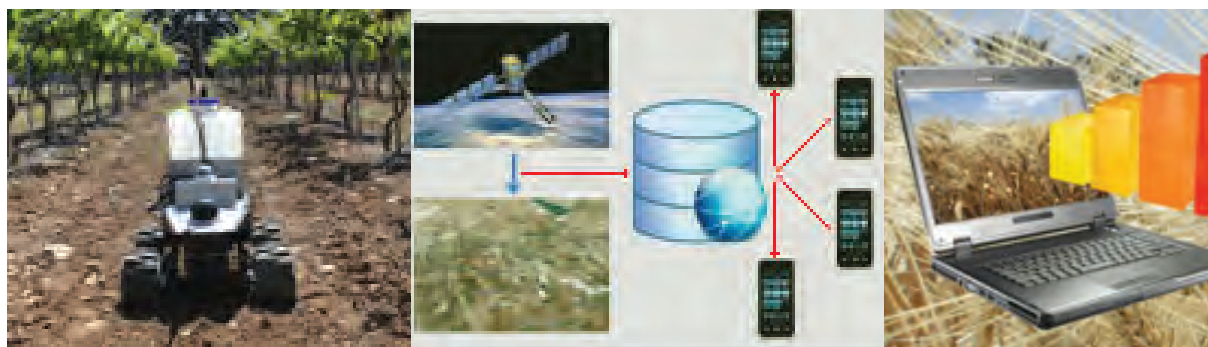
The Programme “PENEK/0311/21”, funded by the Cyprus Research Promotion Foundation, commenced in 2013 aiming at co-treatment of wastewater and production of renewable energy from waste using macroalgae. The proposed project, in which the ARI is a partner, will rely on the following steps to be taken: 1. Design and develop a suitable methodology for swine farm wastewater treatment using the most favorable species of macroalgae at an experimental plant based on real conditions. 2. Examine the quality of the treated water based on conventional quality techniques and ensure harmonization with EU legislation, and R.A.A 737/2003. 3. Use the excess Macroalgal biomass to produce biofuel, such as: a) quality biodiesel in compliance with the EN14214 standard, and b) bioethanol in compliance with EN15376 standard.

Study on the use of olive pomace for energy production in Cyprus

In 2012 the project “KEDELEA” funded by the European Territorial Cooperation Programme between Greece and Cyprus 2007-2013 was launched. The general objectives of KEDELEA are the construction and operation of a management centre for the collection, processing and disposal of olive husk for energy recovery purposes, as well as the development of a comprehensive framework for the operation of olive husk management centres in Greece and Cyprus.

RURAL DEVELOPMENT

The Rural Development Section of the Agricultural Research Institute conducts research aiming overall at the enhancement of the agricultural sector in Cyprus and at the improvement of the quality of life of Cypriot farmers. The Section manages the research programme titled “Rural Development and Trade of Agricultural Products”. Within the framework of this programme two studies have been carried out during 2010-2013, regarding agricultural information sharing among researchers, extension officers and farmers. The first study was on the usage of PC and the Internet and the other on the use of mobile phones. Work on robotic technology in agriculture has also been conducted within the framework of a project funded by the Research Promotion Foundation. The newly established Remote Sensing Laboratory (RSL) continued its work on remote sensing and geo-information for agricultural and environmental applications. Finally, the Section provided support to the Ministry of Agriculture, Natural Resources and the Environment by conducting targeted studies on agricultural economics and trade of agricultural products.



COMPUTER SCIENCE

Computers in agriculture - information society in rural areas

The research project “Information Society in Rural Areas: Knowledge Sharing Using Information and Communication Technology” (<http://ruraldev.ari.gov.cy>) was initiated to study current practices of agricultural research information sharing and new opportunities that emerge by exploiting Information and Communication Technology (ICT). In order to examine the usage of Personal Computers (PC) and the Internet by Cypriot farmers, a stratified random sample of 949 producers was developed, covering all areas under the effective control of the Republic of Cyprus. The sample was based on applicant-beneficiaries of the Rural Development Plan 2007-2013, and specifically on beneficiaries of the Measures 1.5.1 “Modernization of agricultural holdings” and 1.2 “Setting up of young farmers”. The majority of the beneficiaries cultivate fruits and vegetables, potatoes and cereals, while of beneficiaries engaged in animal production most were sheep and goat farmers, cattle farmers and swine farmers. Between October 2010 and March 2011, 219 rural communities were visited for personal interviews, wherefrom 526 questionnaires were completed. Using a structured questionnaire, the participants were asked to answer several questions related to the usage of PC and the Internet for business purposes. The results of the study showed that 60.6% of the participants make direct use of the PC and 54.2% of the Internet. Furthermore, logit models were used to examine the socioeconomic characteristics of the Cypriot farmers that may affect the usage or not, of the PC and the Internet.

From the results of the logit models (Table 1) it appears that gender, age and education level of the principle farm owner, the annual income, the farm type (crop or livestock farming), the employment type (full-time or part-time), the participation in a Producers’ Organization and the district, are factors that

significantly influence the usage of PCs by farmers. About the usage of the Internet, it appears that it is significantly affected by the age and the education level of the farmer, the income level, the farm type (livestock farming) and the farm location, as well as from the employment type. In general, younger and more educated farmers with high income, mainly livestock farmers, who are employed part-time in agriculture, have a greater probability of using ICTs (PC and Internet). (**G. Adamides and A. Stylianou**)

Table 1. Logit models for factors affecting PC and Internet usage by farmers

Variables	Logit model for PC usage Coefficient (Standard Error)	Logit model for Internet usage Coefficient (Standard Error)
Gender	0.472* (0.272)	0.048 (0.313)
Age		
18 to 28 years	1.073** (0.473)	0.785* (0.473)
29 to 39 years	1.103** (0.375)	0.756** (0.348)
40 to 50 years	0.958*** (0.360)	0.720** (0.326)
51 to 61 years	0.910** (0.365)	-0.720** (0.319)
Education level		
Completed secondary education	1.477*** (0.248)	0.429*** (0.248)
Completed tertiary education	1.626** (0.313)	1.475*** (0.314)
Income		
€5 000 to €20 000	-0.239* (0.276)	-0.085 (0.290)
€20 000 and above	0.351** (0.303)	0.641** (0.325)
Agricultural activity		
Crop farming	-0.222* (0.564)	-0.269 (0.598)
Livestock farming	0.958*** (0.363)	0.836* (0.315)
Employment type (full-time/part-time)	-0.469** (0.222)	-0.554** (0.246)
Producers' Organizations	0.703*** (0.301)	0.471 (0.266)
District		
Famagusta	-0.524* (0.384)	-0.386 (0.392)
Larnaka	0.599* (0.395)	0.602 (0.399)
Pafos	-0.360* (0.306)	-0.501 (0.350)
Limassol	0.499 (0.413)	0.670 (0.441)
Likelihood Ratio	-3.015.003	-3.888.099
R-Squared	0.20	0.21
Observations	526	526

* Indicates significance, at the 10% level; ** indicates significance at the 5% level; *** indicates significance at the 1% level.

Use of mobile phones in agriculture

Mobile phones are used by farmers not only in person-to-person voice communication but also for providing access to information via Short Messages Service (SMS), Multimedia Messages Service (MMS) and access to the Internet. Mobile technology now includes mobile phones, smartphones, tablets and affiliated technologies. A survey was carried out in Cyprus in 2012, about the use of ICT and mobile phones by Cypriot farmers, specifically as a mean for access to agricultural information. The results (Table 2) showed that nearly 98% of the farmers in Cyprus use the mobile phone as a source of agricultural information. The second most favorable source of information is actually other farmers (89%), followed by Extension Service visits to the field (85%), private sector Extension consultants (81%) and input suppliers (74%). No statistically significant differences were found between educational groups and between crop farmers and their livestock counterparts, concerning mobile phone usage. The results of this research provide strong evidence that Extension Service may benefit from the development of applications targeted to farmers and their information needs. This study concluded that mobile phone is a very common medium for farmers obtaining agricultural information and it could therefore be used for the efficient dissemination of agricultural information to the farmers. (G. Adamides)

Table 2. Sources of agricultural information (N=142)

Information source	%*
Mobile phone	98
Through other farmers	89
Extension Service	85
Extension consultants	81
Input suppliers	74
Newspapers/Magazines	69
TV (agricultural related programmes)	67
Cooperatives	51
Radio (agricultural related programmes)	42
Internet	40
Experts	15

*Note: Multiple answers were allowed.

Robotics in agriculture

The research project titled “The supportive role of robotics technology for sustainable agriculture” (AgriRobot - <http://agrirobot.ouc.ac.cy>) is funded by the Research Promotion Foundation and deals with the aspect of human-robot interaction (HRI) and examines how humans can interact efficiently and effectively with robots to carry out agricultural tasks. The supportive role that robots can play in the field or inside a greenhouse may contribute towards a sustainable agriculture. This can be accomplished by the introduction of existing robotic technology that can augment farmers’ capabilities in carrying out repetitive and tedious agricultural tasks. In addition the introduction of robots can help tackle the high costs of production deriving from increased labor costs, and overcome the observed shortage of labor.

The research methodology focuses on user interface design, implementation and evaluation, specifically for a human-robot interaction system. It involves the study of volatile literature in the areas of human-computer interaction, human-robot interaction, and agricultural robotics.

During 2013 a field study was conducted to demonstrate the feasibility of the Agricultural Robot Sprayer. In addition, the study investigated two interaction modes to determine which would be more suitable to efficiently and effectively teleoperate the robot. In this context, participants were asked to use two different interfaces in order to navigate the robot, avoiding obstacles, while spraying (target identification). The experiments took place during August-September 2013 at the Zygi, Acheleia and Saittas Experimental



Research Stations. Thirty participants were involved in the study (7 females, 23 males), aged 28 to 65 ($M=39.8$, $SD=9.3$). Seventeen participants were farmers, and 13 were agriculturalists. Analysis of the collected data showed that users in the additional views condition sprayed significantly more grapes and teleoperated the robot with significantly less collisions with obstacles [$t(29) = 9.06$, $p < 0.001$, $r = 0.86$], compared to users who did not have these aids [$z = 3.86$, $p < 0.001$, $r = 0.50$ respectively]. However, participants also required significantly more time to achieve this performance; $t(29) = 2.78$, $p < 0.01$, $r = 0.46$. In addition, it was found that the number of views did not have any effect on the participants' perceived usability of the system, as measured by the System Usability Scale; $t(29) = 0.32$, $p = 0.751$. (**G. Adamides**)

AGRICULTURAL ECONOMICS

Commissioned by the Ministry of Agriculture, Natural Resources and the Environment, two studies were performed towards the Midterm Financial Framework 2014-2020: 1) Economic impact of the remote geographical position of Cyprus on the Cypriot agricultural sector; 2) Effect of climate variability and climate change on crop production and water resources in Cyprus. (**M. Markou and A. Stylianou**)

Moreover, ongoing research activity is under way in collaboration with the Department of Agriculture regarding the variable and fixed cost of all agricultural products. The project aims to support the new Rural Development Plan (RDP) 2014-2020 regarding sustainability studies for specific RDP measures. (**G. Papadavid**)

The Agricultural Research Institute in collaboration with the “Promotion of Agricultural Products” Section of the Ministry of Energy, Commerce, Industry and Tourism, and the Department of Agriculture, conducted a survey about the perspectives of production and disposal of fresh vegetables. The research revealed that there is a large production margin for legumes, cereals and fodder for domestic consumption, as for okra and selected aromatic plants for export. However, for the appropriate planning of the production, for the reduction of the production costs, for the conclusion of production contracts with the

traders, and for the promotion of the short supply chains, the full activation of Fruit and Vegetables Producers' Organizations is needed. (**M. Markou, A. Stylianou, S. Ioannou, M. Giannakopoulou, A. Iordanou, A. Mazeris, E. Mili, C. Zoumides**)

In 2012 the innovative research project "Investigating the sustainability of the Agricultural Productive System in Cyprus: case studies on the wider rural areas of Larnaka and Ammochostos" was initiated, aiming to examine the economic sustainability of the current Agricultural Productive System in Cyprus and its component systems, using advanced multivariate statistics and econometric models. The project is based on a highly representative sample and is using a well-structured questionnaire for collecting the primary data. Moreover, the project uses both quantitative and qualitative analysis. (**A. Stylianou**)

EU/ EXTERNALLY FUNDED PROJECTS

The Section participates in the ICT-PSP funded project "Virtual Open Access Agriculture & Aquaculture Repository: Sharing Scientific and Scholarly Research related to Agriculture, Food, and Environment" (VOA3R – <http://voa3r.eu>). The VOA3R platform (<http://voa3r.cc.uah.es/>) aims at re-using existing and mature metadata and semantics technology to deploy an advanced, community-focused integrated service for the retrieval of relevant open content and data that includes explicit models of the scholarly methods and procedures used and of the practical tasks targeted by applied research. The service will enable researchers to formulate their information needs in terms of elements of the scientific methods established in their field (variables, techniques, assessment methods, kinds of objects of interest, etc.) combined with topical descriptions as expressed in metadata. The community approach will facilitate the enhancement of information seeking, with extended evaluation elements that complement and go beyond the traditional, anonymous peer review process whose results are not made available openly. (**G. Adamides**)

The ARI is a member of the consortium of the ERA-NET cooperation project titled "Facing sustainability: new relationships between rural areas and agriculture in Europe" (RURAGRI - <http://www.ruragri-era.net/>). The aim of the project is to bring together the cooperated research parties from EU member states, record their current research activities and conduct joint research. (**M. Markou, G. Adamides and V. Vassiliou**)

The Section also participates in the project "Options for sustainable agricultural production and water use in Cyprus under global change" (AGWATER Project) which is funded by the Research Promotion Foundation. The overall goals of the project are to provide recommendations for the adaptation to climate change of the agricultural sector in Cyprus and the wider Mediterranean region, and to establish a consortium of excellence in research on natural resource management in Cyprus for tackling the challenges imposed by climate change. The project will document and analyze available knowledge and data, fill in critical knowledge gaps and develop the Cyprus green-blue water model to compute sustainable irrigation water supplies, agricultural water use, crop yields and economic indicators for 2020-2050. The databases, maps and models developed in this project will provide a simulation of potential short and long term costs and benefits for agricultural water use and crop production in Cyprus. (**M. Markou, S. Gregoriou, D. Fasoula, A. Stylianou**)

As of October 2013 the Rural Development Section coordinates the Lifelong Learning Programme project "Improving skills for Smartfarming as an innovative tool for rural development and economic growth" which aims to create and develop a training programme, a long-term superfood electronic platform and a guide book. The purpose of these outputs is to enhance the dissemination and transfer of

knowledge and innovation in the specialized agricultural areas of the selected superfoods (e.g. goji berries, blueberries etc.). To enable the attainment of the objectives set forward by the project, the conceptual as well as empirical principles of Smart Specialisation are adopted. The operationalisation of the conceptual principles and Smart Specialisation will lead to the exchange of knowledge and expertise in the selected regions that are represented by the consortium partners. The project will be implemented by a consortium of seven partners from five EU countries spanning from North to South of Europe (Cyprus, Spain, Latvia, Greece and Portugal). The consortium includes education and training organizations and authorities responsible for rural development, thus allowing combining different views and experiences. **(G. Adamides, A. Stylianou, G. Papadavid, D. Neocleous)**

The Section represents ARI in the “Coordination of the Agricultural Research in the Mediterranean Area” project (ARIMNet <http://www.arimnet.net>). ARIMNet is an ERA-NET action supported and funded under the 7th Framework Program (FP7) of the European Commission. The project aims are the following: to coordinate national research activities, to identify common research programmes among the countries of the Mediterranean region, to fight fragmentation and to exploit synergies. **(M. Markou, G. Adamides and V. Vassiliou)**

In 2012, the first call of ARIMNet project proposals was initiated. The Rural Development Section cooperates with the Animal Production Section, which serves as coordinator for Cyprus in the ARIMNET-selected project DoMEsTic (http://www.arimnet.net/index.php?p=fp_domestic). The project aims to investigate the factors that influence the sustainability of pastoral and rangeland production systems in the four participating countries (Cyprus, Greece, France and Morocco), focusing on sheep and goat production, and to examine the adaptation strategies to the current socio-economic and environmental challenges. **(G. Adamides, A. Stylianou, G. Hadjipavlou)**

REMOTE SENSING LABORATORY

The Remote Sensing Laboratory (RSL) was established at the Acheleia Experimental Station in order to provide scientific data for applications in agriculture and environmental research. The Laboratory has been involved in two research projects regarding crop evapotranspiration and its contribution is expected to prove valuable for all the ARI Sections through collaborative research.

The RSL was involved in the research project for the Genetic Improvement of cowpeas and in the research project ΥΔΩΡ (water, in Greek) which was successfully completed towards the end of 2013. Results have shown that the collaboration of research institutions along with SME's could yield innovative methods or products of direct impact on agriculture and the environment. The 'ΥΔΩΡ' application is a mobile phone widget that was released in a pilot stage and has shown that farmers can indeed have a more orthological irrigation management using the specific application. The application is running on Android mode and can be installed in almost any smartphone. The user-friendly application simply indicates the appropriate crop water needs of specific crops in the area of interest taking into account the phenological stages of each crop at the time being. Using satellite images which are transformed into crop evapotranspiration maps, and by modelling crop canopy factors, the user estimates the crop water requirements of each plot and sends the data through an internet database directly to farmers' mobile phones in order to inform them on the current irrigation needs of their plants. **(G. Papadavid)**

The main purpose of the new research proposal submitted under the title: “Effects of Climate Change on irrigation cost of water-intensive cultivations in Cyprus, using remote sensing”, is to find out the impact of climate change on crop irrigation needs and how these change affects producers’ income. Another research objective is to investigate whether these crops have managed to adapt to the new conditions or if their lifecycle has differentiated according to their irrigation needs and climatic conditions. The research activity regarding the project is already launched with *in situ* measurements on the different crops and of the meteorological conditions. These parameters will become inputs in the form of empirical models for crop evapotranspiration algorithms for estimating Crop Coefficients (Kc) and Crop Evapotranspiration (ETc). (G. Papadavid, D. Neocleous, A. Stylianou)

DISSEMINATION ACTIVITIES

The Section is responsible for maintaining the ARI website (<http://www.ari.gov.cy>) and various social media network services. It also maintains the digital version of the ARI library catalogue at <http://e-library.ari.gov.cy/dspace>. The electronic library of ARI is an open access system containing more than 1,000 digitized versions of all of ARI publications since 1965. Recently, in December 2013 the digitization of articles published in the “Agrotis” magazine by ARI research officers since 1965 was completed and they are now available on our website.

With the emergence of the social networking media, the Rural Development Section makes use of several tools such as Facebook,

(<https://www.facebook.com/ARICyprus> Twitter (@ari_rd),

YouTube (<http://www.youtube.com/user/aricyprus>),

Issuu (<http://issuu.com/ari-rd>), SlideShare (<http://www.slideshare.net/ARIWebinars>), and finally a web blog at (<http://blog.ari.gov.cy>).

These services are used to share information about several topics related to ARI activities, while the blog website is used for sharing articles, news and updates from the ARI research activities. (G. Adamides)



AGROBIOTECHNOLOGY

The Agrobiotechnology Section was established in 2008. Its purpose is to provide solutions to arising agricultural problems with the application of advanced, modern techniques using state-of-the art laboratory equipment. The research activity of the Section focuses on the following scientific areas: Molecular Biology, Agricultural Chemistry, Food Technology and Agricultural Microbiology. The research interests of the Section are fully aligned with the latest scientific needs of the country and the research strategic plan and priorities of the Institute. Along with its research activities, the Agrobiotechnology Section performs chemical analyses in support of research programs pursued by other Sections of the Institute as well as analysis for GMO presence in corn grain imports.

MOLECULAR PLANT-MICROBE INTERACTIONS AND ECOLOGY OF AGRICULTURAL ECOSYSTEMS

Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress only

Horticultural crops in the Mediterranean basin have to cope with severe drought conditions. The effect of inoculating watermelon plants grown under limited water availability conditions with arbuscular mycorrhizal (AM) fungi on the fruit yield, water use efficiency (WUE), root-N and -P content was examined. We focused on the impact of watering level and inoculation with allochthonous AM fungi on the diversity and presence of AM fungi in the watermelon roots using molecular techniques. An open field experiment was conducted and plants were grown with (M) and without AM fungal inoculum (NM), subjected to water stress (NW) and no stress conditions (W). Suboptimal water application (NW) resulted in significant reduction of fruit yield, root-N and -P content. Inoculation of plants grown under water stress resulted in a significant increase of WUE (19%), fruit yield (19%), root-N (27%) and -P (40%). However, only root-P responded to AM inoculation under non water stress conditions demonstrating 23% increase in M plants. DNA extracted from root samples was subjected to PCR– denaturing gradient gel electrophoresis (DGGE) analysis (Fig.1). The native mycorrhizal population colonized watermelon roots, as indicated by DGGE bands in NM treatments. Some members of this colonizer community appeared sensitive to the introduction of allochthonous inocula and to water stress conditions. Cloning and sequencing of AM fungi revealed that watermelon roots were colonized by *Glomus* and *Paraglomus* species. A TaqMan real-time PCR assay was also carried out targeting the 18S rRNA gene for the quantification of AM nucleic acids. The 18S rRNA copy numbers of AM fungi were significantly increased in M plants compared to NM plants under water stress. On the contrary, under non- stress conditions M and NM plants did not show significant differences, indicating that inoculation with AM fungi was related to the response of plants to water stress conditions. Principal coordinate analysis of the DGGE banding patterns showed that the diversity of AM fungal colonizers was strongly affected (i) by inoculation and (ii) by water stress in the inoculated plants (Fig. 2). Inoculation affected fungal presence under water limitation conditions only. The latter was in line with the significant beneficial effect of inoculation on both WUE and yield only under water limitation. (**M. Omirou, I.M. Ioannides**)

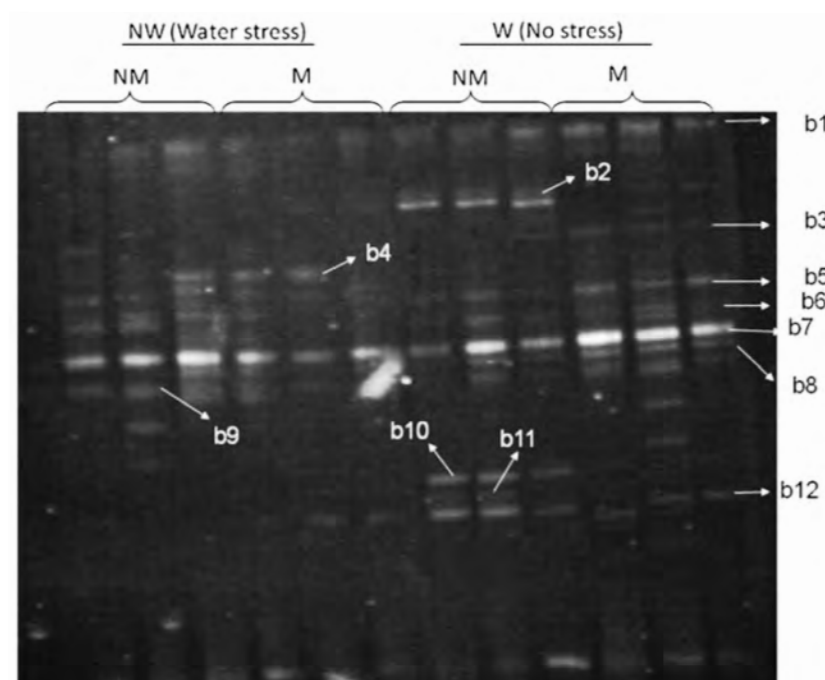


Figure 1. DGGE analysis of partial 18S rRNA gene sequences of the AM fungal assemblage in watermelon roots. Arrows indicate bands corresponding to sequenced clones.

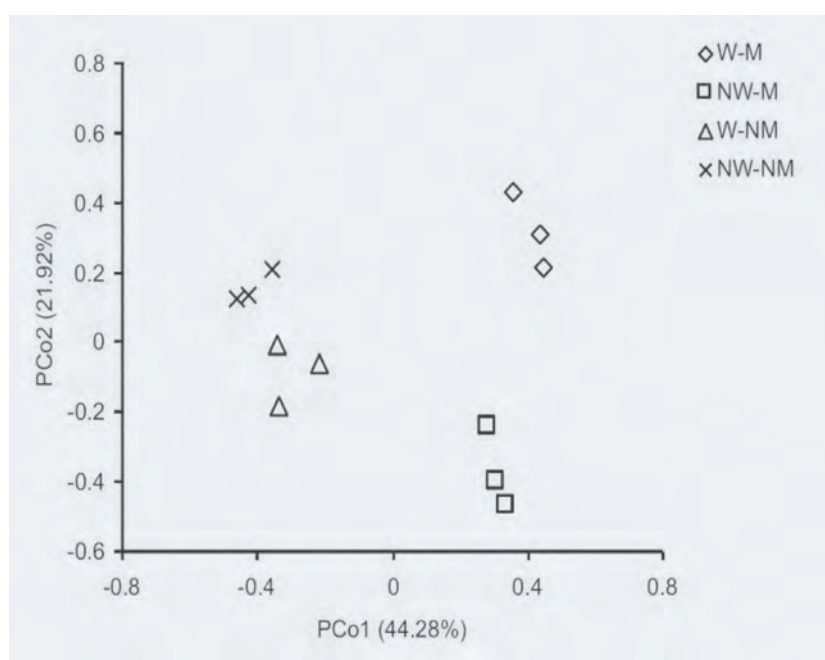


Figure 2. Principal coordinate analysis (Jaccard similarity index) of the DGGE banding pattern for the AM fungal community of watermelon roots grown under different inoculation and water availability conditions (M: AM fungal inoculum, NM: without AM fungal inoculum, NW: water stress and W no water stress).

Tripartite symbiosis influences cowpea performance and mycorrhizal community structure under limited nitrogen conditions

Legumes could be a major crop in sustainable agricultural systems since they can effectively associate with symbiotic microorganisms, such as nitrogen fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) leading to efficient resource management. The response and the efficiency of tripartite symbiosis rely on biotic and abiotic factors, with the associated symbiotic microbial communities being crucial for the sustainability and the efficiency of the system. In the current study, we investigated the influence of a local *Bradyrhizobium* isolate and a commercial mycorrhizal inoculum on cowpea performance and on the mycorrhizal community structure under limited nitrogen conditions using molecular approaches. A factorial randomized design was established in sterile and non-sterile soils, including control plants (without inoculation), inoculated plants with a *Bradyrhizobium* (NFB) strain, a commercial mycorrhizal inoculum (AMF) and their combination. Mycorrhizal colonization and nodule formation were also evaluated whereas AMF assemblage diversity was examined using PCR-DGGE approach (Fig. 3). Inoculation resulted in a significant increase ($p < 0.05$) of shoot and root biomass compared with the control plant under both soil conditions. Soil sterility resulted in a significantly lower shoot biomass of control plants stressing the importance of local microbial community on cowpea performance. A synergistic effect between NFB and AMF was also observed in the nutrient content of cowpea plants, except for magnesium (Mg) where no response was noticed. Mycorrhizal colonization and nodule formation on control plants suggest an active local mycorrhizal and rhizobial community in the soil used which was also demonstrated by the molecular fingerprints of the samples. Cloning of PCR-amplified sequences revealed that cowpea plants were colonized by *Rhizophagus*, *Funneliformis*, *Claroideoglomus*, and *Paraglomus* related species (Fig. 4). Overall, the local AMF community was positively influenced by NFB, AMF and dual inoculation since increased diversity was noticed compared to control plants. In addition, the AMF colonization in dual inoculated plants was significantly higher compared to AMF and NFB plants indicating a synergism between AMF and the *Bradyrhizobium* isolate. It could be claimed that under N-limiting conditions, inoculation with NFB and AMF interacts with and stimulates functional local AMF strains, resulting in increased cowpea performance through a more balanced nutritional status of the plants. (M. Omirou, D.A. Fasoula, I.M. Ioannides)

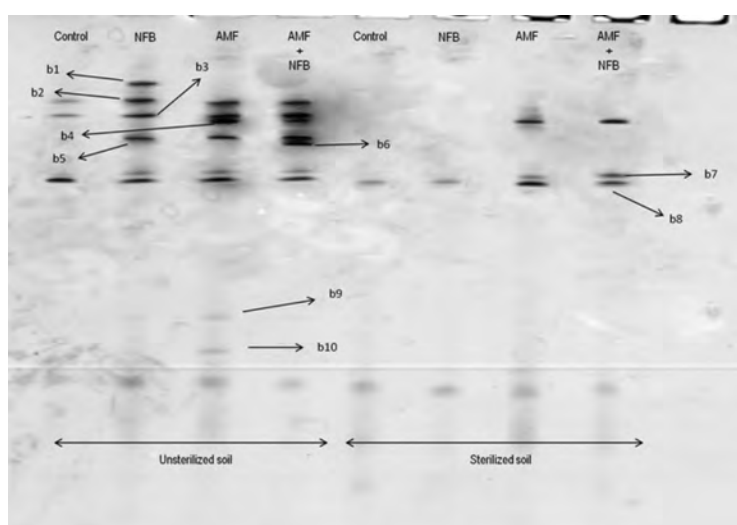


Figure 3. DGGE analysis of partial 18S rRNA gene sequences of the AMF fungal assemblage in cowpea roots. Arrows indicate bands corresponding to sequenced clones.

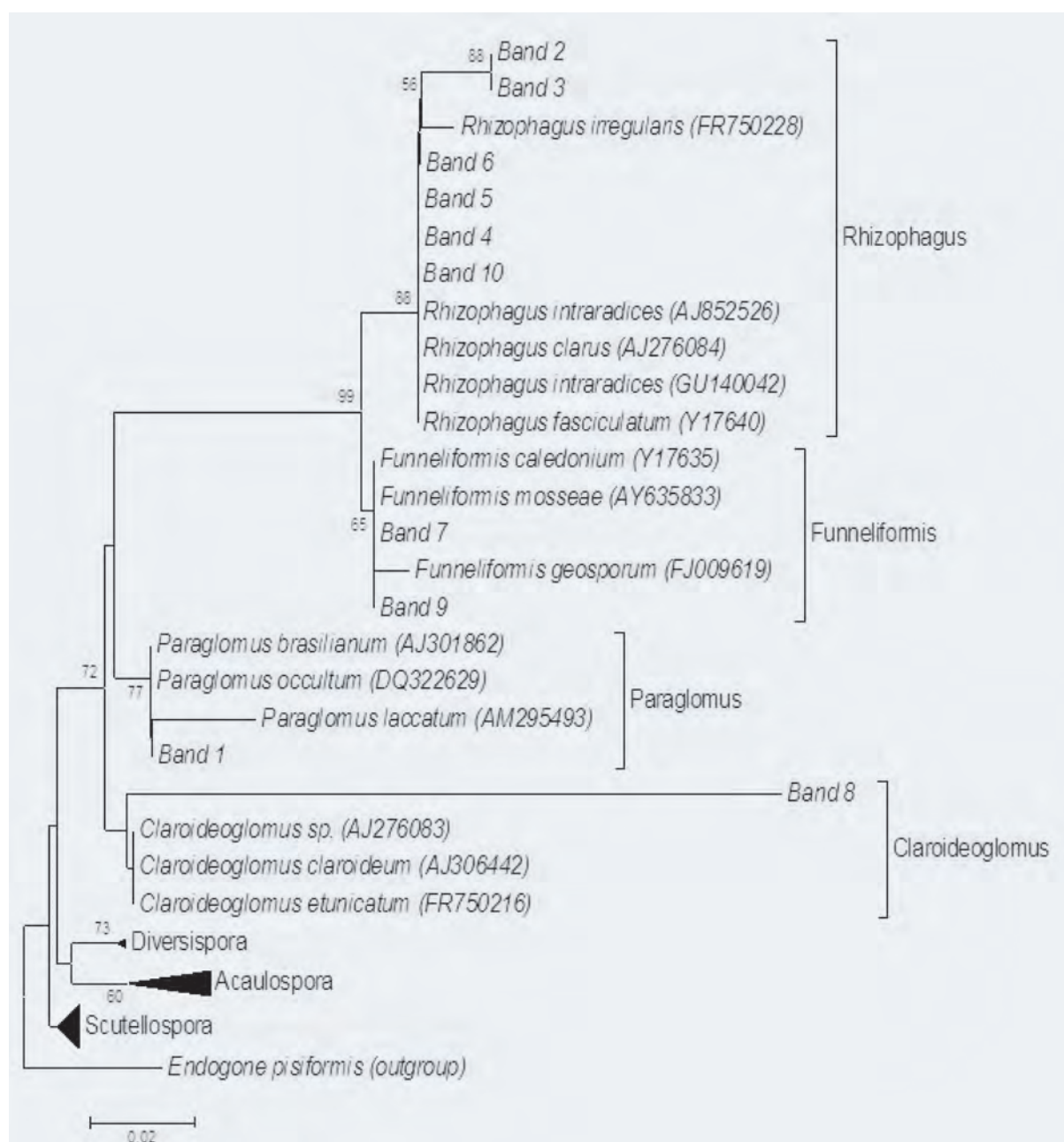


Figure 4. Evolutionary relationships of AMF assemblages in cowpea roots. The evolutionary history was inferred using the Neighbor-Joining method.

Impact of a beneficial and of a pathogenic *Fusarium* strain on the fingerprinting-based structure of microbial communities in tomato (*Lycopersicon esculentum* Mill.) rhizosphere

Fusarium solani strain FsK (FsK), isolated from a plant-pathogen suppressive compost, grows endophytically in tomato roots and controls infestations by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL). The effect of root colonization by the two fungi on the diversity of rhizosphere microbial community was studied. Tomato plants were inoculated with FsK and/or FORL and rhizosphere soil was

collected 8, 15 and 30 days post inoculation (dpi) and analyzed by DGGE of PCR-amplified internal transcribed spacer (ITS) sequences of fungi and 16S rRNA gene sequences of common rhizosphere bacterial guilds like alpha-proteobacteria and pseudomonads (Fig. 5). Cluster analysis of DGGE fingerprints showed that FsK had a transient impact on the fungal and alpha-proteobacterial community only during its endophytic stage (15 dpi), while FORL had a readily distinguished and persistent effect on the fungal community. The changes observed in the rhizosphere fungal and bacterial communities may depict the interactions of the two fungal inocula with the plant. Cloning of selected DGGE bands stimulated by FsK showed that the responsive bacteria were closely related to species known to include biological control agents (BCA). Overall, the inoculation of FsK in the tomato rhizosphere did not appear to have a significant impact on the diversity of non-target microbial groups inhabiting plant rhizosphere. (M. Omirou, D. Karpouzas, K.K. Papadopoulos)

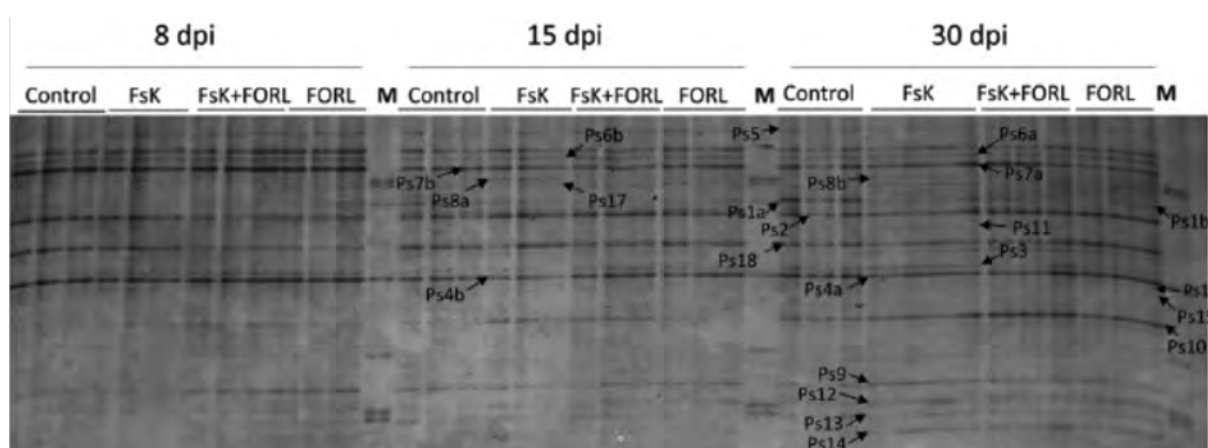


Figure 5. DGGE analysis of partial 16S rRNA gene sequences of the community of pseudomonads in rhizosphere soil.

Dissipation of pure and broccoli-released glucosinolates in the soil under high and low moisture content

Glucosinolates (GSLs) are secondary metabolites found in the *Brassica* species. Upon tissue disruption GSLs are hydrolysed by myrosinase enzymes to isothiocyanates (ITCs) which are highly toxic to microbes. Therefore, the incorporation of fresh Brassicaceae tissues into soil for the control of soil-born plant pathogens is viewed as a biofumigation process. Its efficacy relies on the rate of GSL conversion to ITCs, but also on environmental factors controlling GSL availability in the soil matrix. We studied the dissipation of GSLs, applied either by broccoli leaves or as pure compounds, in a clay loam soil at two soil moisture content levels. Regardless of their mode of application, GSLs were rapidly dissipated in soil with half-life values ranging from 3.2 to 15.5 h. Increasing moisture from 20 to 90% of the soil water holding capacity significantly accelerated their dissipation. Indolyl broccoli-derived GSLs dissipated faster than aliphatic GSLs at high moisture levels, while at low moisture levels a three-fold reduction in their dissipation rates was observed. The dissipation of aliphatic GSLs was less affected by soil moisture levels. Application of pure GSLs resulted in increased soil metabolic quotients (qCO_2), suggesting a decline in microbial metabolic efficiency (Fig.6). Their dissipation was related to myrosinase activity apparently derived from soil microbes. (M. Omirou)

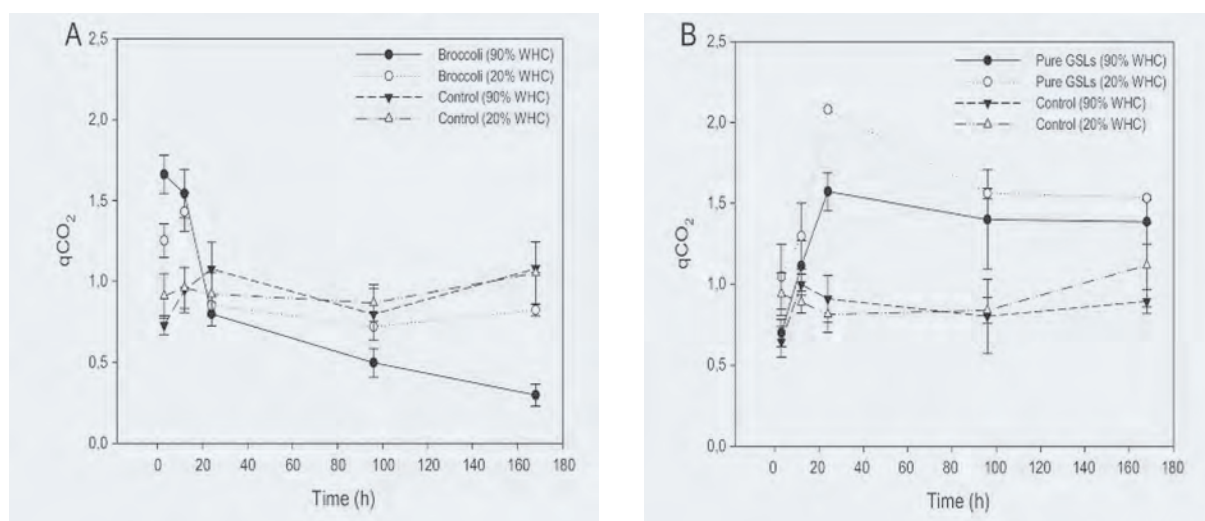


Figure 6. Soil metabolic quotient (qCO_2) in soils amended with broccoli leaves (A) and pure GLS (B). Soil moisture was adjusted to 20% (open symbols) or 90% (closed symbols) of the soil WHC. Error bars show standard errors of means ($n=3$).

FUNCTIONAL FOODS AND FOOD SAFETY

Effect of processing and cooking treatments on certain anti-nutrient compounds in cowpea

Cowpeas can synthesize a variety of undesirable chemical substances termed anti-nutrients that are known to exert a deleterious effect when ingested by humans or animals. These substances include flatulence-producing oligosaccharides (mainly raffinose and stachyose) as well as tannins which can cause adverse physiological responses or diminish the availability of certain nutrients to animals or humans. In this project, we studied the effects of different processing and cooking treatment on the nutritional and anti-nutritional attributes of cowpea. The results showed a considerable decrease of anti-nutrients (oligosaccharites) following certain ways of soaking and cooking while minimal changes on other nutrients like protein and metals were observed. (G. Maratheftis, D.A. Fasoula, M. Omirou)

Antimicrobial and antioxidant activity of plant secondary metabolites

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. The objective of our research was to evaluate different extracts of plant origin to determine their antimicrobial activity against *Bacillus cereus*, *Salmonella enterica*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The evaluation was performed using two different screening methods: agar dilution and minimal concentration. Extracts from rocket leaves (*Eruca sativa*), *Nerium oleander* and essential oil from mint (*Mentha spicata*) have been tested. The lowest minimum inhibitory concentrations were 0.01% (v/v) of *Mentha spicata* oil against all tested microbial pathogens. Higher inhibitory concentrations ranging from 0.01% to 0.05% were observed for *Eruca sativa* methanolic extracts and *Nerium oleander* oil. These results support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives. (G. Maratheftis, M. Omirou)

The impact of wastewater irrigation on crop products safety

The availability of clean and high quality water has been recognized as one of the most crucial factors affecting the agricultural food chain production, especially in Mediterranean countries confronted with severe and frequent seasonal water shortage. In order to overcome water shortage, the European Water Framework Directive encourages and promotes the use of treated urban wastewater in agriculture. The use of poor quality water in agriculture poses potential health risks. The application of wastewater through drip irrigation poses public health concerns, especially regarding the occurrence of human pathogens. The objective of the current study was therefore to assess whether irrigation of tomatoes with tertiary-treated water is associated with increased human health risks. The microbial quality of tomato fruits produced using treated urban wastewater and tube well water were compared at experimental sites in Lefkosia. Faecal contamination was estimated by enumeration of the faecal indicator *Escherichia coli*. Our studies showed no microbial contamination of tomato fruits with this human pathogen. (G. Maratheftis, A. Christou)

Relationships between nitrogen, dry matter accumulation and glucosinolates in *Eruca sativa* Mills - the applicability of the critical $\text{NO}_3\text{-N}$ levels approach

Rocket salad (*Eruca sativa* Mills) is one of the major leafy vegetables produced worldwide and has been characterized as a rich source of chemoprotective glucosinolates (GSL). The relationship between N fertilization and the resulting plant biomass and N status with GSL quantity and quality in rocket leaves was examined. A pot experiment was conducted, applying ten different N-rates and destructive sampling was carried out 15, 30 and 45 days after transplanting (DAT). The Mitscherlich equation was used to establish $\text{NO}_3\text{-N}$ critical levels at each growth stage and as an indicator of N demand to determine relative maximum dry matter accumulation and glucosinolate content and composition. Glucosinolate content was significantly influenced by N rate, growth stage and their interaction. Different GSL types showed dissimilar responses to N fertilization: aliphatic GSLs were significantly reduced under increased N rates whereas indole GSL showed the reverse. Under excess N fertilization (>1.04 g/plant), dry matter accumulation remained constant, $\text{NO}_3\text{-N}$ was significantly increased and total GSL content was significantly reduced, factors that could lead to an anticipated product quality decline. The application of the critical $\text{NO}_3\text{-N}$ level approach used to identify optimal N fertilization rates for plant growth could serve as means to obtain optimized GSL content in the edible plant parts. (M. Omirou)

Application of sodium nitroprusside results in distinct antioxidant gene expression patterns in leaves of mature and senescing *Medicago truncatula* plants

Sodium nitroprusside (SNP) represents one of the most commonly used NO donors in biological sciences, which acts as a signal molecule in plants responsible for the regulation of the expression of many defence-related enzymes. This study attempts to provide novel insight into the effect of application of low (100 μM) and high (2.5 mM) concentrations of SNP on antioxidant gene expression (cAPX, GST, FeSOD, CAT, and AOX) in mature (40 day) and senescing (65 day) *Medicago truncatula* plants. Quantitative real-time RT-PCR suggests that low concentration of SNP applied in mature leaves leads to an overall induction of antioxidant gene expression, while increasing concentration results in suppression of these genes. Conversely, older plants demonstrate a much more variable regulation which appears to be time dependent (Fig. 7). The observed transcriptional regulation pattern in mature *M. truncatula* plants comes in support of the previously documented protective or damaging effect of SNP depending on concentration applied, whereas senescing *M. truncatula* plants demonstrated a general suppression in antioxidant gene expression levels regardless of SNP concentration, indicative of reduced overall plant defence capacity against free radicals. (I.M. Ioannides, D.A. Fasoula, P. Alexios, V. Fotopoulos)

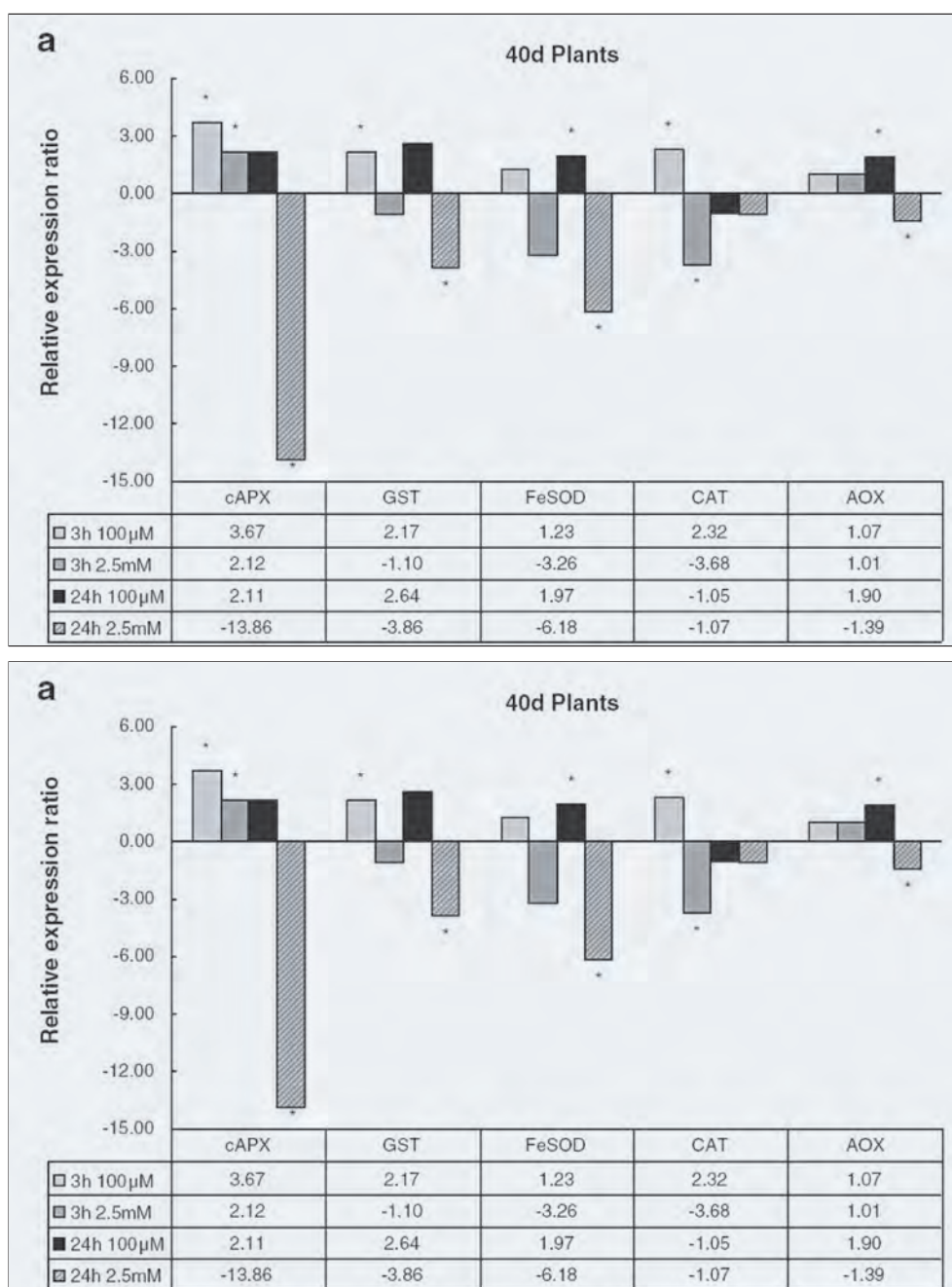


Figure 7. Effect of SNP application on gene expression profiles of antioxidant enzymes (cAPX, GST, FeSOD, CAT, and AOX) determined by qRT-PCR in leaves of mature (a 40 days) and senescing (b 65 days) *M. truncatula* plants vacuum infiltrated with 100 µM and 2.5 mM SNP at 3–24 h.

Developmental stage- and concentration-specific sodium nitroprusside application results in nitrate reductase regulation and the modification of nitrate metabolism in leaves of *Medicago truncatula* plants

Nitric oxide (NO) is a bioactive molecule involved in numerous biological events and has been reported to display both pro-oxidant and anti-oxidant properties in plants. Several reports exist which

demonstrate the protective action of sodium nitroprusside (SNP), a widely used NO donor, which acts as a signal molecule in plants responsible for the expression regulation of many antioxidant enzymes. This study attempts to provide a novel insight into the effect of application of low (100 μ M) and high (2.5 mM) concentrations of SNP on the nitrosative status and nitrate metabolism of mature (40 d) and senescing (65 d) *Medicago truncatula* plants (Fig. 8). Higher concentrations of SNP resulted in increased NO content, cellular damage levels and reactive oxygen species (ROS) concentration, further induced in older tissues. Senescing *M. truncatula* plants demonstrated greater sensitivity to SNP-induced oxidative and nitrosative damage, suggesting a developmental stage-dependent suppression in the plant's capacity to cope with free oxygen and nitrogen radicals. In addition, measurements of the activity of nitrate reductase (NR), a key enzyme involved in the generation of NO in plants, indicated a differential regulation in a dose and time-dependent manner. Furthermore, expression levels of NO-responsive genes (*NR*, *nitrate/nitrite transporters*) involved in nitrogen assimilation and NO production revealed significant induction of *NR* and *nitrate transporter* during long-term 2.5 mM SNP application in mature plants and overall gene suppression in senescing plants, supporting the differential nitrosative response of *M. truncatula* plants treated with different concentrations of SNP. (I.M. Ioannides, D.A. Fasoula, P. Alexios, V. Fotopoulos)

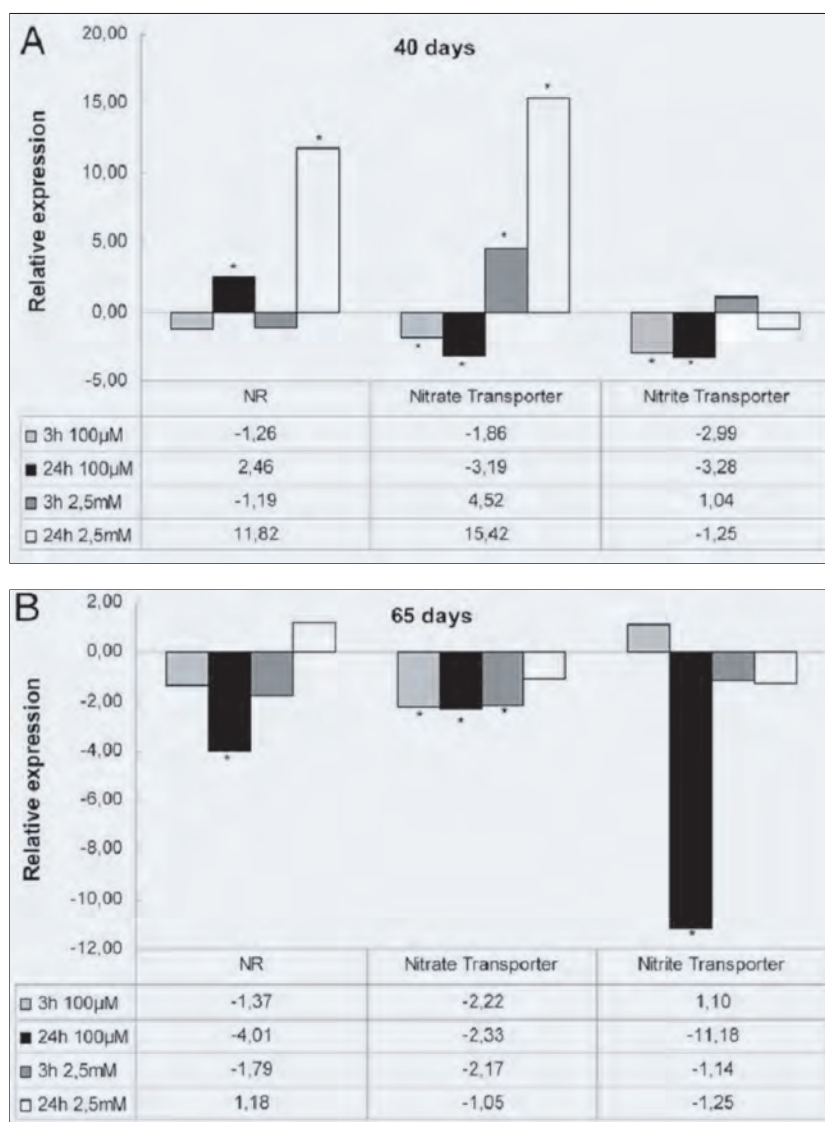


Figure 8. NO effect on gene expression profiles of NO-responsive genes (*NR* and *nitrate/nitrite transporters*). Gene expression analysis was determined by qRT-PCR in leaves of *Medicago truncatula* Jemalong A17 plants vacuum-infiltrated with 100 μ M and 2.5 mM SNP at 3/24 h in mature 40 d (A) and senescing 65 d (B) plants. Asterisks denote statistically different values according to pairwise fixed re-allocation randomization test ($p < 0.05$) ($n = 3$).

Rapid remediation of soil heavily contaminated with hydrocarbons: a comparison of different approaches

To improve our understanding of the dissipation kinetics of total petroleum hydrocarbons (TPH), we tested two bioremediation strategies in soil heavily contaminated with hydrocarbons. In one strategy, different fertilizers and composted winery products were used to stimulate a local microbial community capable of dissipating petroleum hydrocarbons. In the second approach, polluted soil was used for the enrichment and isolation of potential hydrocarbon-degrading bacteria that were subsequently used for implementing a bioaugmentation strategy. The efficacy of both strategies was evaluated using the Hockey-Stick and first-order kinetics models. Two *Pseudomonas* isolates designated as el20 and el15 were able to readily degrade TPH and n-alkanes both *in vitro* and in a microcosm study. Phylogenetic analysis based on 16sRNA gene sequencing revealed that both strains showed a high similarity with *Pseudomonas otitidis* and *P. stutzeri*. Enrichment with both compost and nitrogen fertilizer enhanced the dissipation of TPH. Overall, compost-treated soils exhibited the highest degradation rates, with half-life ($T_{1/2}$) values ranging from 13 to 37 days. Urea-treated soils exhibited the lowest TPH $T_{1/2}$ values among the soils treated with inorganic fertilizers. The addition of glucose to soils treated with inorganic nitrogen fertilizers retarded the degradation rate of TPH, with estimated $T_{1/2}$ values ranging from 70 to 140 days. Different dissipation patterns were noted among the different n-alkane fractions, with short chain molecules (up to 14 carbon atoms) being rapidly reduced within the first 21 days, whereas long chain molecules were more recalcitrant. We demonstrated that *Pseudomonas*-like strains and *in situ* soil incorporation of compost derived from winery by-products can be effectively used for the rapid bioremediation of soil heavily polluted with hydrocarbons. (I.M. Ioannides, M. Omirou)

Exploring the potential of biobeds for the depuration of pesticide-contaminated wastewaters from the citrus production chain: laboratory, column and field studies

High wastewater volumes produced during citrus production at pre- and post-harvest level present a serious pesticide point-source pollution for groundwater bodies. Biobeds are used for preventing such point-source pollution occurring at farm level. We explored the potential of biobeds for the depuration of wastewaters produced through the citrus production chain following a lab-to-field experimentation. The dissipation of pesticides used pre- or post-harvest was studied in compost-based biomixtures, soil, and a straw-soil mixture. A biomixture of composted grape seeds and skins (GSS-1) showed the highest dissipation capacity. In subsequent column studies, GSS-1 restricted pesticides leaching even at the highest water load (462 L m^{-3}). Ortho-phenylphenol was the most mobile compound. Studies in an on-farm biobed filled with GSS-1 showed that pesticides were fully retained and partially or fully dissipated. Overall biobeds could be a valuable solution for the depuration of wastewaters produced at pre- and post-harvest level by the citrus fruit industries. The efficacy of the system is a continuous process and the constructed biobed is currently used for remediation of the pesticides used in the Experimental Research Station at Zygi. Different treatments of the exhausted material are under investigation to enhance the environmental sustainability of the system. (M. Omirou, P. Dalias, I.M. Ioannides)



Isolation and characterization of pesticide degrading microorganisms

Contamination of waters by xenobiotic compounds such as pesticides presents a serious environmental problem affecting European water resources. The aim of this work has been to evaluate the ability of several bacterial species for biodegradation of the pesticides imidacloprid, pendimethalin, imazalil, thiabendazole and ortho-phenyl phenol in batch liquid cultures (Fig. 9). Several bacterial species have been isolated and screened for their ability to metabolize these pesticides via the enrichment process. Despite the relatively high persistence of the tested pesticides, the results obtained so far showed that *Acinetobacter* and *Sphingomonas* like species have a high capability for biodegradation of these compounds. These isolates could prove valuable as active pesticide-degrading microorganisms, increasing the efficiency of pesticide bio-purification systems such as biobeds or specifically designed bio-filters. (M. Omirou, I.M. Ioannides)

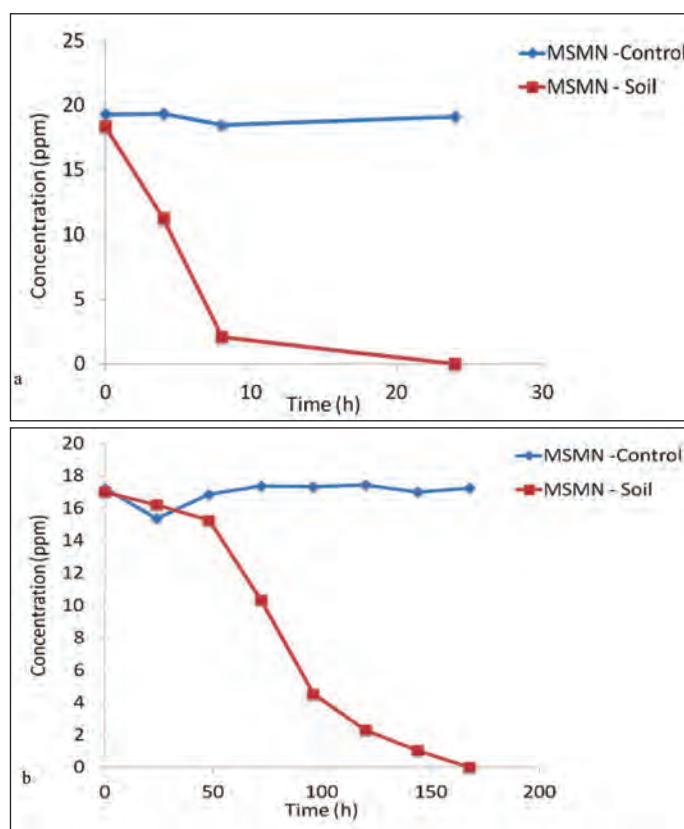


Figure 9. Dissipation of a) ortho-phenyl phenol and b) thiabendazole in batch liquid cultures.

BIOTECHNOLOGICAL MITIGATION TOOLS FOR CLIMATE CHANGE

Screening of marine microalgal species for the production of biodiesel and carbon sequestration

Recently, microalgae have been recognized as a promising platform for biofuel production as well as biorefineries. Biodiesel production from microalgal lipids has been identified as a more feasible and sustainable production approach compared to second generation biodiesel derived from terrestrial, cultivated crops. High growth rates and biomass productivity relate to their ability for efficiently fixing large quantities of CO₂, rendering them suitable biological systems for the absorption of carbon dioxide emissions. The aim of our study has been to isolate and characterize marine microalgal strains and blooms able to produce elevated biomass and intracellular lipid content. We enriched local seawater samples from eutrophic coastal areas and the individual strains have been isolated via streaking approach. Eight of the 33 blooms created exhibited a very interesting biomass productivity and lipid content. In particular, the total lipid percentage of these blooms was higher than 30% while the biomass productivity ranged from 0.8 to 1.2 g/L (Fig. 10). Trans-esterification of the lipids yielded 19 to 37% fatty acid methyl ester (FAME) of high quality biodiesel. (I.M. Ioannides, M. Omirou, P. Polycarpou)

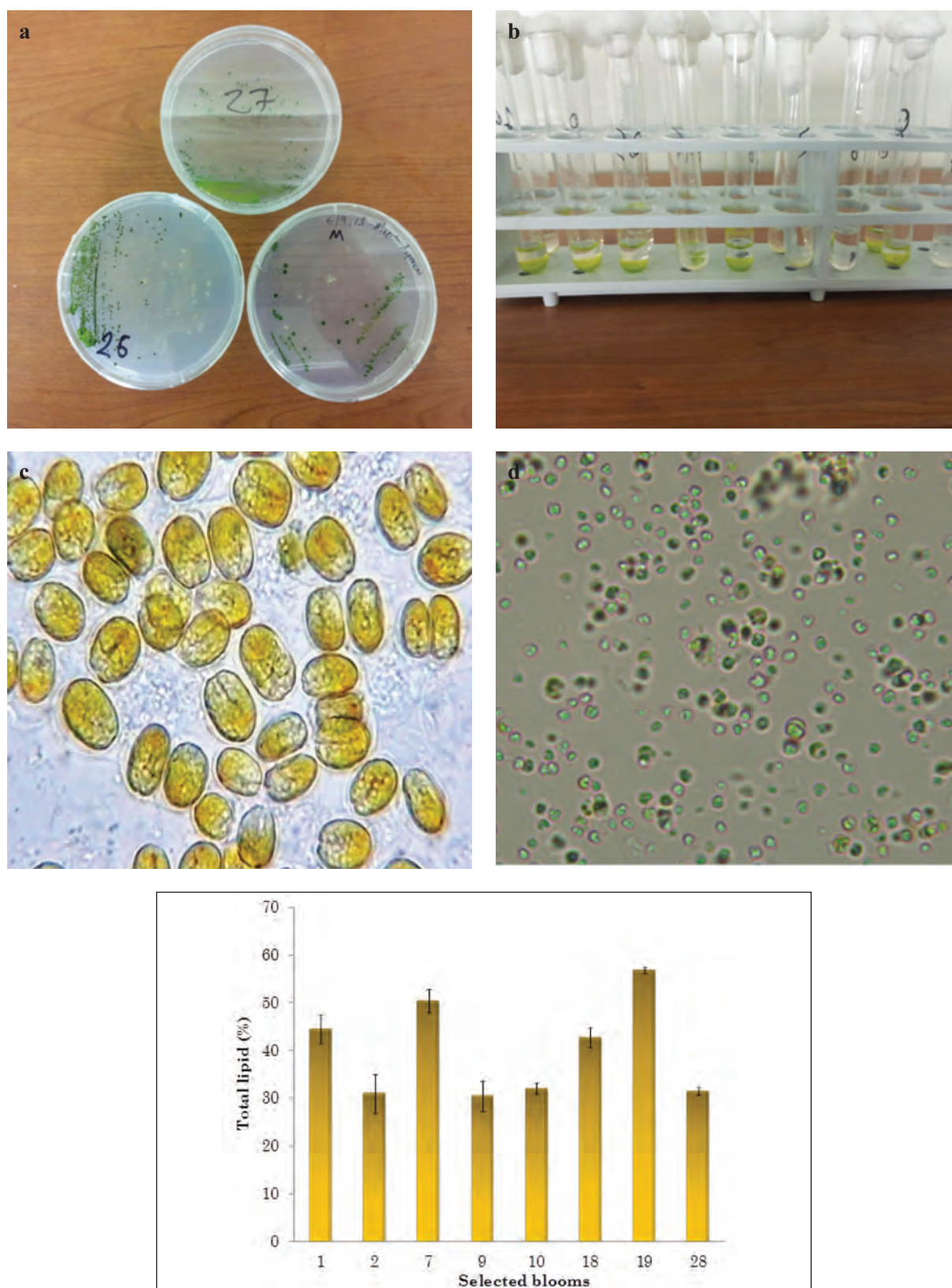


Figure 10. Total lipid percentage content of selected microalgal blooms.

Impact of nitrogen and phosphorus on *Chlorella stigmatophora* performance

Microalgae have a high potential for removing inorganic nutrients from wastewater and for producing biodiesel. Effects of nitrogen and phosphorus concentrations on growth, lipid content, carotenoids and chlorophyll concentration of marine the microalga *Chlorella stigmatophora* were studied. The growth of the microalga was performed in a photo-bioreactor (MC1000) under different temperature simulating summer, spring/autumn and winter (Fig. 11). *Chlorella stigmatophora* could accumulate up to 14% of its biomass in lipids and this was affected by both seasonal variation and nutrient content in the growth medium (Fig. 12). Growth rate and biomass were significantly affected by temperature while pigment accumulation was enhanced under N surplus conditions. On the contrary, high phosphorus levels in the growth medium had no effect on pigment and biomass production. Ongoing research from our group now addresses how to enhance lipid content and lipid productivity of this microorganism in custom-made photo-bioreactors. (I.M. Ioannides, M. Omirou)

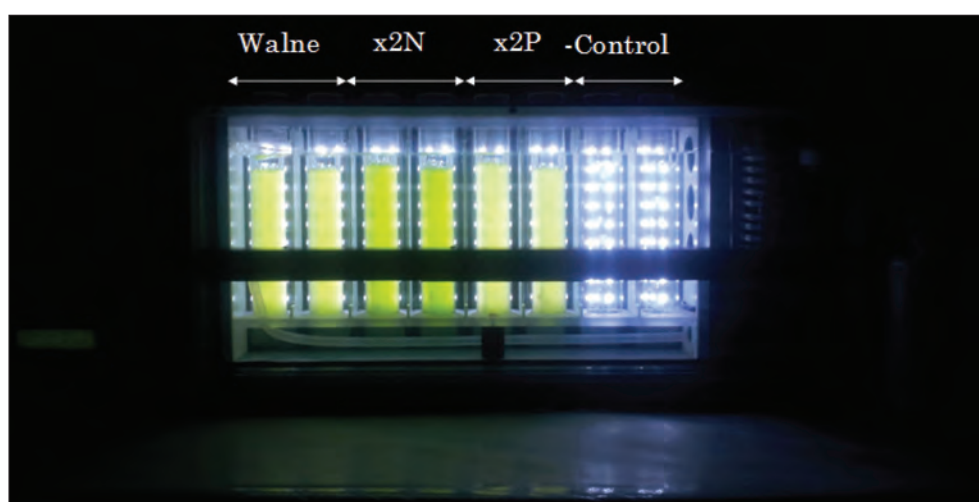


Figure 11. Experimental set up for assessing the impact of different nutrient concentrations on growth, chlorophyll content, dry weight and total lipids.

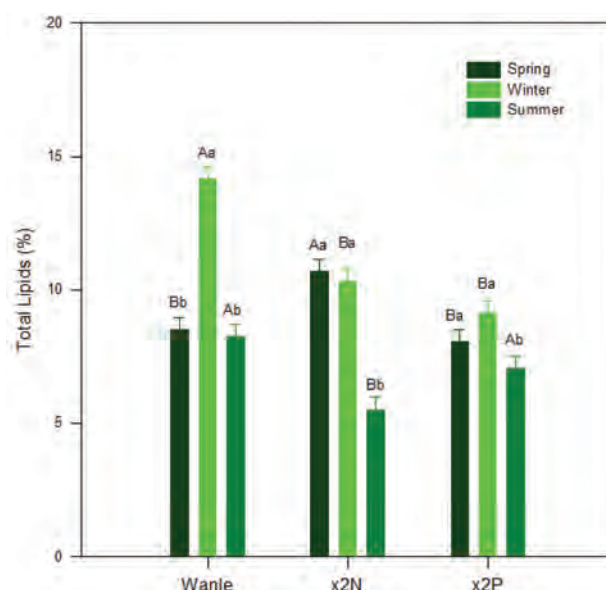


Figure 12. Impact of different nutrient concentrations on total lipids (%) under seasonal variation.

Characterization of PrP genotypes in sheep and goats

Scrapie is a fatal, neurodegenerative disease of sheep and goats that belongs to the family of transmissible spongiform encephalopathies. Sheep susceptibility to scrapie is associated with polymorphisms in the ovine prion protein (PrP) gene at codons 136, 154 and 171. Genomic DNA was isolated and purified from peripheral blood leucocytes using standard procedures. The identification of the allelic variants present in the DNA samples was performed in a simple multiplex PCR reaction and melting curve analysis of the PrP gene (Fig. 13). Genetic analysis of blood samples of the Chios sheep (1,157 samples) continued in 2012/13, in order to identify and select genotypes resistant to the scrapie disease. Presently at the ARI, the Chios sheep nucleus unit counts over 400 breeding animals exclusively of the scrapie resistant genotype ARR/ARR.

In addition, the molecular fingerprinting for goats is in progress. In 2012/13, 814 blood samples were tested for scrapie disease genotypes. The data were submitted to the laboratory molecular data bank for further analysis. Also, based on scrapie genotyping, a nucleus unit with all the different goat genotypes is being established at the Research Farm of the Animal Production Section. **(I.M. Ioannides and G. Hadjipavlou)**

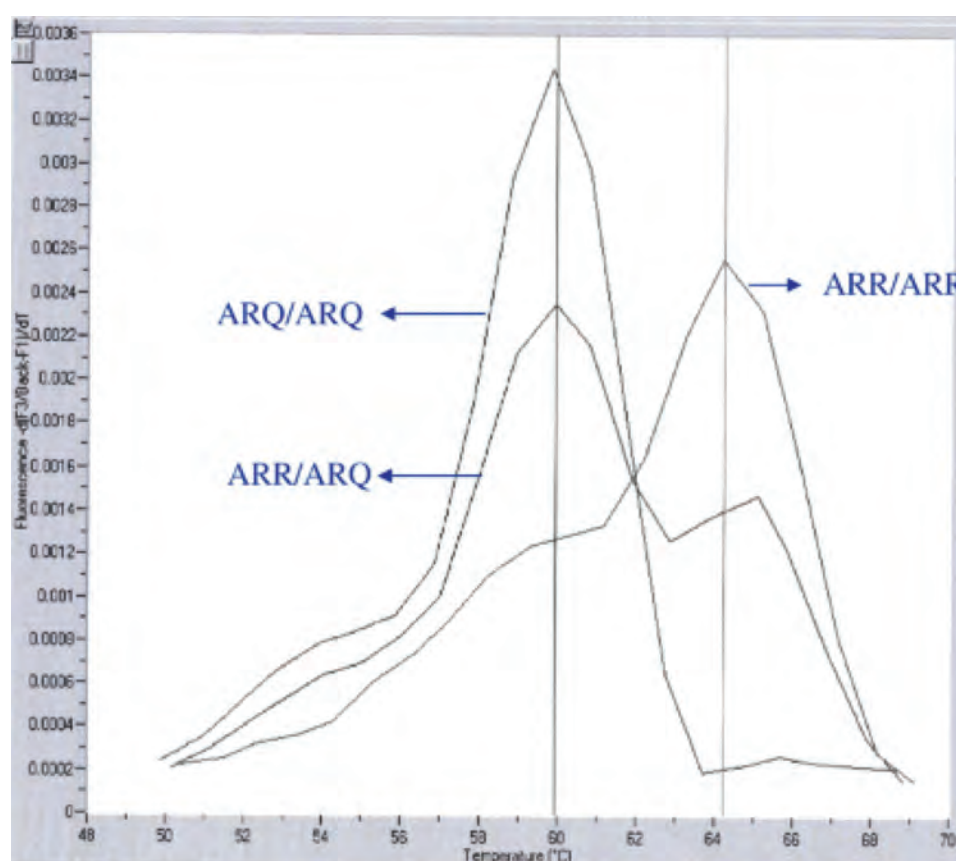


Figure 13. Melting curve analysis of PrP gene performed in a single multiplex PCR reaction in channel F3.

65 °C indicates the presence of the scrapie-resistant variant ARR/ARR, 60 °C indicates the presence of the scrapie-susceptible ARR/ARQ and the two melting points at 60 °C and 65 °C indicate the presence of the ARR/ARQ.

Detection and geographical distribution of the organophosphate resistance-associated 3Q ace mutation in the olive fruit fly, *Bactrocera oleae* (Rossi)

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is the most important pest of olives. Its control is based mostly on organophosphate (OP) insecticides, a practice that has led to resistance development. OP resistance in *B. oleae* has been associated with three mutations in the acetylcholinesterase (AChE), the product of the ace gene. The current work presents new diagnostic tests for the detection of the ace mutations and aims at monitoring the frequency of the 3Q mutation, which appears associated with resistance at higher OP doses in natural olive fly populations. An allele-specific polymerase chain reaction (PCR), a PCR-RFLP (restriction fragment length polymorphism) and a Taq-Man test were developed for the 3Q mutation detection and a new duplex quantitative PCR assay was designed for the G488S and I214V mutations (Fig. 14). Moreover, the frequency of 3Q mutation was examined in ten populations of eight countries in the Mediterranean basin. The highest frequencies (10%) were found in and , whereas a gradual decrease of 3Q frequency towards the western was noted. Robust tests for insecticide resistance mutations at their incipient levels are essential tools to monitor the increase and geographical spread of such mutations. (I.M. Ioannides, M. Omirou, C. Mathiopoulos)

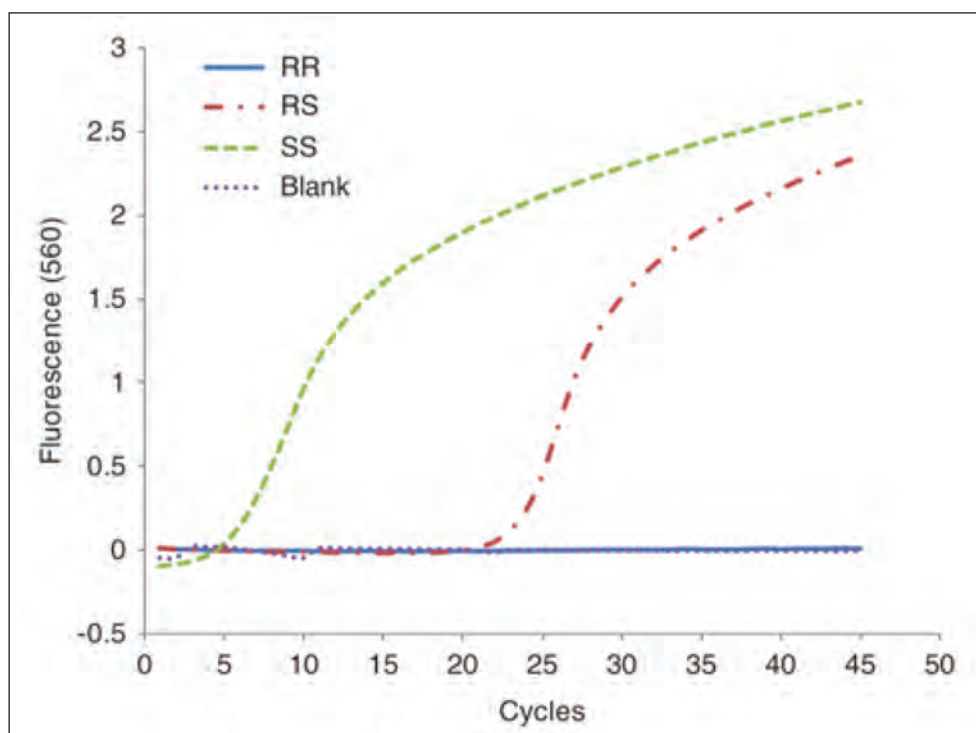


Figure 14. Cycling of FAM-labeled probe specific for the wild-type (SS) and the heterozygous (RS) alleles of 3Q mutation using Taq-Man chemistry. No amplification indicates a homozygous (RR) individual mutant carrying the 3Q deletion. Substantial increase in FAM fluorescence signal indicates a wild type individual and an intermediate increase indicates a heterozygote individual.

VARIETY EXAMINATION CENTRE

Research activity at the Variety Examination Centre focused on the performance of field trials required for the registration of varieties of several agricultural species. In order for varieties to become tradable, they must be registered in the National or Common Catalogue. For the purposes of registration, an application must be submitted to the Designated Authority (Department of Agriculture) by the improver, the legal conservator or their authorized legal representative residing in Cyprus. The procedure followed for examining varieties prior to their registration in the National Catalogue is described below.

Distinctness, Uniformity and Stability trials

The Distinctness, Uniformity and Stability (DUS) trials take at least two years to be completed and include observations on morphological, physiological and agronomic characteristics of varieties according to the protocols of the Community Plant Varieties Office (CPVO) and the International Union for the Protection of New Plant Varieties (UPOV). During the growing season of 2012/13, three groundnut varieties (Ntopia, Kouklia and Nikoklia) and one triticale variety (SIS TC1/Zagor) were subjected to their second year trials. The results were reported to the Designated Authority (Department of Agriculture). The triticale variety (SIS TC1/ Zagor) and two of the three groundnuts varieties (Ntopia and Nicoklia) were registered in the National Catalogue of Cyprus. Additionally, in the 2012/13 growing season, the barley variety Pyge entered its second year of trials and two new barley varieties Alona and Politico were tested for their first year. **(K. Mina).**



Value for Cultivation and Use trials

The Variety for Cultivation and Use (VCU) trials are strictly conducted in the territory of Cyprus by the Variety Examination Centre and have a duration of at least two years for all crops except alfalfa, for which the duration of trials is three years. During the 2011/12 growing season, the aforementioned groundnut varieties Ntopia, Kouklia, and Nikoklia and triticale variety (SIS TC1/ Zagor) were tested for the second year. Alfalfa variety Ntopia was tested for the third year. In the 2012/13 growing season, the barley variety (Pyge) was tested for the second year and the two barley varieties (Politico and Alona) for the first year. The results were reported to the Designated Authority (Department of Agriculture). The final decision for the variety registration in the National Catalogue shall be made by the Technical Committee of Multiplicative Material and Seeds. (**K. Mina**)



Optimization of hay cereal yields to feed sheep and goats in Cyprus

In 2010, a new research programme concerning the cultivation of fodder plants was launched. In 2012/13, the five species were evaluated at four different growth stages with respect to yield and quality. (**K. Mina**)

FARM ACCOUNTANCY DATA NETWORK (FADN)

The ARI has the presidency of the Farm Accountancy Data Network (FADN) National Committee of Cyprus, which monitors the smooth implementation of the FADN. The ARI officers responsible for FADN participate in the FADN Community Committee and the Organization for Economic Cooperation and Development (OECD). For the accounting years 2011 and 2012, returns for 479 and 487 farm units, respectively, were, in turn, submitted to the Community FADN Committee in 2012 and 2013. The data was collected through personal interviews from a relevant sample and processed according to the existing regulations. Processing of the 2012 FADN data and preparation for 2013 have also been initiated. In addition, the 2013 Cypriot selection plan for holdings participating in the FADN sample was submitted to the Community FADN Committee.

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Dados, A., K. Demetriou, C. Papastefanou, M. Omirou, and M.I. Ioannides. 2013. Total petroleum hydrocarbons dissipation efficiency of two *Pseudomonas* strains isolated from diesel contaminated soil in Cyprus. *17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region*, September 28 – October 1st, 2013. Istanbul – Turkey.

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SEMINARS

A total of eight seminars were presented in 2012 and ten in 2013. The seminars were chaired by Messers G.A. Soteriou and A. Kyratzis.

February 16, 2012	E. Maloupa: Protection and utilization of native species of the flora of Greece
March 15, 2012	C. Kadis: Flora of Cyprus: Richness, threats, conservation activities
May 24, 2012	G. Hadjipavlou: Use of molecular and quantitative genetics for the improvement of the Chios sheep breed
June 14, 2012	A. Christou: Appraisal of vegetable wastewater irrigation impact on soil physico-chemical properties, and fruit heavy metal content and microbial contamination
June 21, 2012	A. Stylianou: Dissemination of agricultural research through Information and Communication Technology (ICT)
September 27, 2012	L.C. Papayiannis: Whitefly-transmitted viruses in the Eastern Mediterranean basin.
October 17, 2012	R. Cohen: Grafted vegetables in Israel: Current status and future challenges
December 20, 2012	L.C. Papayiannis and T. Kapari-Isaia: Fifty years of research in Plant Pathology by Agricultural Research Institute: Achievements and future perspectives
February 21, 2013	A. Constantinou: Challenges-experiences-results from the Cyprus EU presidency concerning the agricultural sector
February 22, 2013	A. Degen: Livestock production by the Bedouin in the Negev Desert, Israel
March 14, 2013	G. Hadjipavlou: Use of quantitative genetics for association analysis of candidate genes with milk traits in Chios sheep
March 21, 2013	P. Dalias: The N benefits of grain legumes in crop rotation
June 05, 2013	D. Prophetou-Athanasiadou: Organic agriculture: principles, current situation, problems, prospects of successful implementation in Greece and Cyprus
June 18, 2013.	A. Siomos: The prospect for asparagus production in Cyprus: cultivation, postharvest handling, marketing and related research interest
July 19, 2013	M. Omirou and I. Ioannides: AMF inocula, plant nutrition and water relationships: are we targeting the right selection attributes?
September 12, 2013	N. Lygeros: The value of zeolite in agriculture
October 10, 2013	A. Barnea: Israeli water and environmental technologies: combining innovation with entrepreneurship
December 10, 2013	A. Tebawi and R. Obaid: Olive sector in Palestine

