

# AGRICULTURAL RESEARCH INSTITUTE Centre of Excellence in Agriculture and Environment

# REVIEW FOR 2014-2015



MINISTRY OF AGRICULTURE, NATURAL RESOURCES AND ENVIRONMENT LEFKOSIA

October 2016



# FOR 2014-2015



# MINISTRY OF AGRICULTURE, NATURAL RESOURCES AND ENVIRONMENT LEFKOSIA

October 2016

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**Editorial Committee:** Dr M.C. Kyriacou

Dr L.C. Papayiannis Dr G. Hadjipavlou Mr. A. Kyratzis

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Postal Address Agricultural Research Institute

Ministry of Agriculture, Rural Development and Environment

P.O. Box 22016

1516 Lefkosia, CYPRUS

**Telephone** Lefkosia +357 22 403100

**Cables** ARI

Telex4660 MINAGRI CYTelefaxLefkosia +357 22 316770Internet sitehttp://www.ari.gov.cy

#### 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, Rural Development 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, Vegetable Crops and Animal Production and b) the Scientific Support Division, which comprises the Sections of Plant Protection, Natural Resources and Environment, 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 5,000 volumes.

Human resources at ARI consist of 40 scientists, specialised 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 programmes, 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 organisations and research networks, including the Food and Agriculture Organisation 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 optimisation 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 varieties of bread wheat, common vetch, ground nut and barley.

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 vegetable cultural practices and at evaluating new scion and rootstock varieties. Research activity aimed at improving vegetable crop management practices and at preserving postharvest quality through optimised handling methods. Particular attention has been given to rootstock-scion interaction in grafted watermelon and melon with respect to crop performance, fruit quality and storability. Nutritional safety characteristics of salad crops have been evaluated in relation to the impact of nitrogen fertilisation strategies. Postharvest work further examined the ripening physiology, quality and storability of watermelon, melon, and cactus pear, while emphasis has been laid on fruit physicochemical characterisation of local pomegranate clones established in an *ex situ* clonal collection. 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 characterisation

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, while clonal characterisation of olive fruit ripening and its implications on oil attributes is pursued. Genebank and herbarium activities focus on the collection, *ex situ* conservation, characterisation, evaluation and utilisation of plant genetic resources including native plants of the flora of Cyprus and local landraces.

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 characterised 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. Emphasis has also been given to the utilisation of endemic plants for floricultural purposes. 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 characterising 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 utilised 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 both the tireless efforts of 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 aims at improving cultural practices of forage plants, grain cereals, legumes and aromatic plants.

Barley is the cereal most adapted to the often harsh Cyprus agroclimatic conditions. Spring barley is grown in Cyprus during winter as the main rain-fed crop for livestock feed as 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 hybridisation and selection for agronomic performance and quality. Environmental and genetic factors affecting grain yield, water use efficiency and quality of durum wheat are examined and new genetic lines of increased drought tolerance under field conditions have been identified. Cultural practices are also examined for improved production. The cereal technology programme, which is complementary to the improvement programme, aims at identifying varieties of superior quality. 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. Two new varieties that originated from the Triticale improvement programme were examined to be prospectively registered to the National Catalogue.

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.

Research on aromatic and medicinal plants focuses on cultural techniques required for the main aromatic and medicinal plants grown in Cyprus. New species of medicinal and aromatic plants, such as stevia (*Stevia rebaudiana* Bertoni), with multiple prospective benefits to consumers' health, are studied as alternative crops for Cypriot farmers.

#### **CROP IMPROVEMENT**

#### Breeding barley for grain and hay

The specific aims of the barley breeding programme include the development of cultivars with improved adaptation to climate change, improved drought resistance, and improved quality traits, such as threshability, hectolitre weight, grain uniformity and protein content. The development of cultivars

suitable for dual grain and hay consumption, with improved performance stability and reduced interaction with the environment has been a priority. Trials were performed in five 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 trend for healthy and well-balanced human diet, the barley breeding programme has also focused on the development of naked barley cultivars appropriate for human nutrition and on cultivars adaptable to organic cultivation. A new, two-row barley cultivar, named Politiko, has been subjected to the official national tests and is intended for registration to the National Catalogue, while a new, six-row cultivar has entered the national trials. Innovative breeding approaches are being developed, based on the unique properties of the honeycomb selection designs and the prognostic breeding methodology. In collaboration with the John Innes Institute, UK, student training on aspects of the project has been provided.

An important component of the barley and cowpea breeding programmes is bridging the gap between genotype and phenotype, particularly during the crucial procedure of field phenotyping. In this respect, a novel barley mutant, identified through our specific trials, was found to be associated with improved performance, and is being further characterised. (**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. 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". Grown under organic farming conditions, these two varieties exhibited promising performance. New lines with improved quality characteristics are expected to replace these varieties. These new lines will exhibit better adaptability to climatic change and the harsh climatic conditions of Cyprus than the older durum 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. Bread wheat lines having tall, leafy and strong stem are listed in the National Catalogue under the names "Akhelia" and "Yiolou". These varieties proved to be tolerant to dry conditions and yield much better than durum wheat. A sideline to forage improvement

gaining preference among farmers of small ruminant animals, are the awnless bread wheat types created in the last few years. Finally, the most promising triticale lines, in terms of quality and yield, from the ARI development programme were promoted for advanced trials and have been registered in the National Catalogue. (A. Pallides)

#### **Grain and forage legumes**

In recent years, rain-fed 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 Plant Improvement Section's most crucial duties is the maintenance of breeder's seed for all the varieties produced at 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. (A. Pallides). Alfalfa (Medicago sativa) is a high protein feeding stuff of great importance. Four populations have been stabilised and are currently evaluated for their yield and quality potential. One variety "Ntopia" has been accepted for registration in the National Catalogue. (D.A. Fasoula, A. Pallides)

#### Cowpea

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. The project aims at the development of improved cowpea varieties, well adapted to the changing climate conditions of southeast Mediterranean. The project also involves the study of the microbial environment of local cowpea landraces and the relevant plant-microbe interactions. Nitrogen-fixing symbiotic bacteria have been isolated from Cyprus cowpea landraces and are being studied at the molecular level. A participatory breeding component of the project has been very successfully executed, with the involvement of local farmers and extension officers across Cyprus. (**D.A. Fasoula**)



#### AROMATIC AND MEDICINAL PLANTS

#### Stevia field trials

Four stevia varieties (*Stevia rebaudiana*), "CRIOLE", "SRB128", "CANDY" and a candidate variety for registration to the Greek National Catalogue, were studied in field trials conducted at Zygi Experimental Station. The study aimed to investigate: a) variety adaptation under Cyprus conditions, b) the ratio between leaf dry weight to plant fresh weight, and c) sugar content. Plants in the experimental plots were produced from cuttings of mother plants established in 2011. Planting distances were 75cm between rows and 40cm between plants. Drip irrigation was applied. Plants received 3510m³/ha in 2013, 3320m³/ha in 2014, and



3300m³/ha in 2015. Fertilisation was applied though irrigation at the following rates: 50 kg/ha N, 40 kg/ha P and 150 kg/ha K. Sampling was performed 30 days after revegetation (end of April), 60 days after revegetation (end of May), and just before the beginning of flowering (mid-end of July). Harvested plants were dried to constant weight and samples were analysed at the General State Laboratory for determination of the two main sugars of stevia, Stevioside and Rebaudioside A. According to our observations we proposed that stevia can be cultivated as a perennial crop in the coastal areas, since no serious damage was observed by the low winter temperatures. This is a very important advantage because no additional cost is needed for plant re-establishment. The main findings of this study are shown in Table 1. An economic feasibility analysis will be conducted for the cultivation of this crop in Cyprus. (C. Stavridou)

**Table 1.** Plant height, dry leaf weight per plant, and leaf sugar content (mg.g<sup>-1</sup> dw) at harvest just before the beginning of flowering (mid-end of July). Different letters within columns represent significant difference between means (P<0.05).

Variety	Heigh	t (cm)	Dry lea	f weight	Leaf s	ugar conte	nt (mg.g-1	dw)
			per pl	ant (g)	Rebaud	ioside A	Stevi	oside
	2014	2015	2014	2015	2014	2015	2014	2015
Criole	52a	48ab	97a	77a	21a	35a	76a	78a
SRB 128	49a	57b	89a	86a	18a	22a	71a	69a
Candidate variety	53a	48ab	104a	58a	22a	29a	63a	73a
Candy	52a	46a	90a	86a	52b	64b	52a	63a

#### FRUIT TREES

Research activity in the Fruit Trees 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. Current research activity in citriculture focuses on rootstock-scion relations on calcareous soils and the evaluation of citrus varieties as scions grafted onto different rootstocks with respect to productivity and quality. The conservation, management and evaluation of local olive genetic resources remain a key priority. Evaluation of olive genetic resources entails the elaiotechnical characterisation of olive oil as well as the physicochemical characterisation of olive fruit and olive paste with respect to clonal ripening profiles.

#### **VITICULTURE**

#### **Evaluation of table grape varieties**

Evaluation of new and traditional table grape varieties under different environmental conditions is undertaken 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. At the Acheleia Station, 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. The collection of other table grape varieties continues. (**S. Savvides**)

# 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,

#### Fruit Trees

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

Acquisition of primary and secondary descriptor data continues, according to the International Organisation of Wine and Vine (OIV) standards, for the traditional table grape varieties Veriko and Sideritis and the wine grape varieties Mavro, Xynisteri, Ofthalmo, Spourtiko, Maratheftiko, Morokanella, Omio, Promara, Skouro Mavro, and Kanella. (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 old and neglected varieties and to promote their future utilisation. Wild vine plants have been recorded at 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 characterisation of all individual plants. An *ex-situ* collection has been established at Saittas Experimental Station with more than 120 wild vine accessions collected from the above locations. (**S. Savvides**)

#### **OLIVE CULTURE - OLIVE OIL TECHNOLOGY**

#### Conservation, evaluation and management of olive genetic resources

Local olive genetic material is under conservation in the *ex situ* Collection of Olive Genetic Material (OCARICY) situated at the Zygi Experimental Station, in the context of the research project: "Management of the ARI *ex situ* Collection of Olive Genetic Material". The main objectives of the project are: a) proper *ex situ* conservation of the genetic resources, b) characterisation-identification and evaluation of genetic resources, c) implementation of actions for further prospection, collection and installation of local genetic material in the Collection, d) enrichment of the Collection with genetic material from other countries. For identification purposes the evaluation is carried out based on the following elaiographic characteristics of endocarps: Shape in position B, length, width in position B, length/width ratio, weight, symmetry in position A, symmetry in position B, number of grooves on basal end, distribution of grooves on basal end, shape of apex in position A, mucron, shape of base in position A, rugosity of surface, position of the maximum transversal diameter in position B as described in the relevant UPOV protocol for olives. (M.G. Emmanouilidou)



Olive oil characterisation in relation to olive fruit maturity profile and olive fruit postharvest management

Research work

The olive oil constitutes the main product of olive culture. For the Mediterranean people, olive oil is the main source of oils and fats intake; furthermore olive oil production and consumption is concentrated almost exclusively in the Mediterranean basin. It is derived from olive fruits (Olea europaea L.) of various varieties and its extraction from olive fruit requires a series of physicomechanical processes. The various quality standards for olive oil determine the different quality categories of olive oil, with virgin olive oils (extra virgin, virgin, ordinary) comprising the superior categories. The virgin olive oil categories reflect a wide range of variation in physicochemical and organoleptic characteristics. A repository of these characteristics with respect to varietal and edafoclimatic origins can be an effective tool for product differentiation and for product quality control according to specific qualitative and sensorial consumer requirements. The lack of data concerning the physicochemical and organoleptic characteristics of olive oils produced in Cyprus provided the springboard for launching the present ARI research programme on olive oil characterisation which is a joint project of the Olive Technology Laboratory and the Postharvest Technology Laboratory. In this context, we examine the variety effect, the maturity effect as well as the effect of postharvest handling of olive fruits on the physicochemical and organoleptic characteristics of olive paste and olive oil. The main objectives of the project are: a) varietal characterisation of olive fruit ripening profiles, b) varietal characterisation of olive fruit post-harvest behaviour, c) evaluation with respect to fruit harvest maturity and postharvest storage of: i) efficiency of olive oil mechanical extraction, ii) rheological-chemical characteristics of the olive paste, iii) physicochemical-organoleptic characteristics of the olive oil, iv) the oxidative stability of olive oil. (M.G. Emmanouilidou, M.C. Kyriacou)

#### **CITRICULTURE**

Citrus rootstock-scion relations on calcareous soils: Effects of citrus rootstocks on yield and quality characteristics of four citrus scion cultivars ('Delta' orange, 'Lane Late' navel orange, 'Nova' mandarin, 'Ortanique' hybrid)

The aim of the citrus rootstock evaluation programme is to identify rootstocks tolerant to the tristeza virus capable of replacing Sour Orange, the rootstock commercially used in Cyprus, which is highly susceptible to the virus. The commercial cultivars 'Delta' orange, 'Lane Late' navel orange, 'Nova' mandarin, 'Ortanique' hybrid were grafted on various rootstocks and cultivated on calcareous soils with the following main objectives: a) evaluation of rootstock effects on scion performance, b) evaluation of rootstock effects on scion fruit maturation profile, c) evaluation of rootstock effects on scion fruit quality characteristics.

Yield of all varieties studied was higher on Volkameriana rootstock (Table 1), rendering this rootstock the most productive for all the varieties studied. Additionally, all varieties entered production earlier when grafted on Volkameriana compared to the other rootstocks. All varieties profiled during fruit maturation presented an increase in the juice soluble solids content (SSC) and pH while titratable acidity (TA) decreased, resulting in an increase in SSC/TA ratio. 'Nova' mandarin incurred a decrease of the SSC and TA when grafted on Volkameriana and GouTou rootstocks. 'Lane Late' navel orange had the lowest SSC and SSC/TA when grafted on Volkameriana rootstock. Volkameriana also decreased the SSC of 'Ortanique' in comparison to the other rootstocks whose SSC values were not significantly different. Overall, Volkameriana induced the lowest TA values while Citrumelo and GouTou rootstocks induced the highest TA and the lowest SSC/TA values. 'Delta' orange fruits presented the highest and lowest SSC on Carrizo and Volkameriana rootstocks, respectively, and the highest and lowest TA on Citrumelo and Volkameriana rootstocks, respectively. Also on 'Delta' orange, rootstocks Sour Orange and Carrizo caused the highest SSC/TA ratio and Citrumelo the lowest (Table 2). (M.G. Emmanouilidou, M.C. Kyriacou)



Table 1. Mean comparisons for yield (kg) of citrus cvs. Delta, Ortanique, Lane Late, Nova, grafted on Carrizo, Cleopatra, Citrumelo, GouTou, Sour Orange and Volkameriana rootstocks.

	DEI	DELTA	ORTANIQUE	JOUE	LANE LATE	LATE	NO	NOVA
Rootstock	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Carrizo	58.65 b	27.163 b	73.02 b	52.89 ab	61.67	39.07 b	13.85 bc	57.36 bc
Citrumelo	48.16 b	11.950 b	60.57 b	7.82 c	I	I	I	I
Cleopatra	45.45 b	11.043 b	62.92 b	61.57 a	58.87	41.64 b	24.43 b	64.85 b
GouTou	39.99 b	13.237 b	95.83 ab	26.70 bc	92.17	57.31 ab	8.13 c	22.66 c
Sour Orange	66.77 ab	28.387 ab	85.45 b	79.30 a	80.64	57.60 ab	13.71 bc	45.87 bc
Volkameriana	104.92 a	49.696 a	130.08 a	72.82 a	91.46	82.11 a	43.19 a	137.11 a

Means within columns followed by different letters denote significant (P<0.05) differences according to Tukey HSD test.

Table 2. Mean comparisons for soluble solids content (SSC), titratable acidity (TA) and SSC/TA for citrus cvs. Delta, Ortanique, Lane Late, and Nova grafted on Carrizo, Cleopatra, Citrumelo, GouTou, Sour Orange and Volkameriana rootstocks.

		DELTA		)	<b>NETANIQUE</b>	3	$\Gamma$	LANE LATE	(F)		NOVA	
	SSC	TA SSC/TA	SSC/TA	SSC	$\mathbf{T}\mathbf{A}$	SSC/TA	SSC	$\mathbf{T}\mathbf{A}$	SSC/TA	SSC	$\mathbf{T}\mathbf{A}$	SSC/TA
Rootstock	(%)	(%) (g citrate/		(%)	(g citrate/		(%)	(%) (g citrate/		(%)	(g citrate/	
		100ml)			100mI)			100ml)			100mI)	
Carrizo	Carrizo 11.17 a 1.086 b 10.35 a	1.086 b	10.35 a	14.75 a	1.386 bc	10.77 a	9.52 ab	0.705	13.64 a	11.64 a	1.075 a	11.19 ab
Citrumelo	10.43 bc 1.312 a 7.97 c	1.312 a	7.97 c	14.31 a	1.781 a	8.21 b	I	I	I	I	I	I
Cleopatra	Cleopatra 10.28 c	1.082 b	1.082 b 9.53 b	14.01 a	1.266 c	11.13 a	9.94 a	0.717	13.98 a	11.90 a	1.084 a	11.75 a
GouTou	GouTou 9.86 cd 0.991 c	0.991 c	10.01 ab	13.77 a	1.522 b	9.16 b	9.44 b	9/9.0	14.08 a	10.75 b	1.001 b	10.66 b
Sour Orange 10.89 ab 1.051 bc 10.38 a	10.89 ab	1.051 bc	10.38 a	14.12 a	1.360 bc	10.49 a	9.67 ab	0.70	13.91 a	11.91 a	1.086 a	11.82 a
Volkameriana 9.64 d 0.989 c 9.79 ab	9.64 d	0.989 c	9.79 ab	12.25 b	1.084 d	11.37 a	8.80 c	0.70	12.54 b	10.80 b	1.006 b	11.22 ab

Means within columns followed by different letters denote significant (P<0.05) differences according to Tukey HSD test.

#### **DECIDUOUS FRUIT TREES**

#### Characterisation and evaluation of local pomegranate clones

The evaluation of selected local pomegranate (*Punica granatum* L.) clones, planted at the Zygi Experimental Station, continued. The purpose of this experiment is the characterisation 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 with respect to yield and quality. (**S. Ioannidou**)

#### **Evaluation of Cherry varieties**

Ten cherry varieties (*Prunus avium L.*) are examined in terms of production, fruit quality and maturation period. An experimental plantation is established at 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 and investigate the behaviour of Cherry varieties under the local agro-climatic conditions of Cyprus, in order to identify varieties of excellent fruit quality. Also the maturity time within the season is evaluated, in order to satisfy market needs for longer periods. (**S. Ioannidou**)

#### **VEGETABLE CROPS**

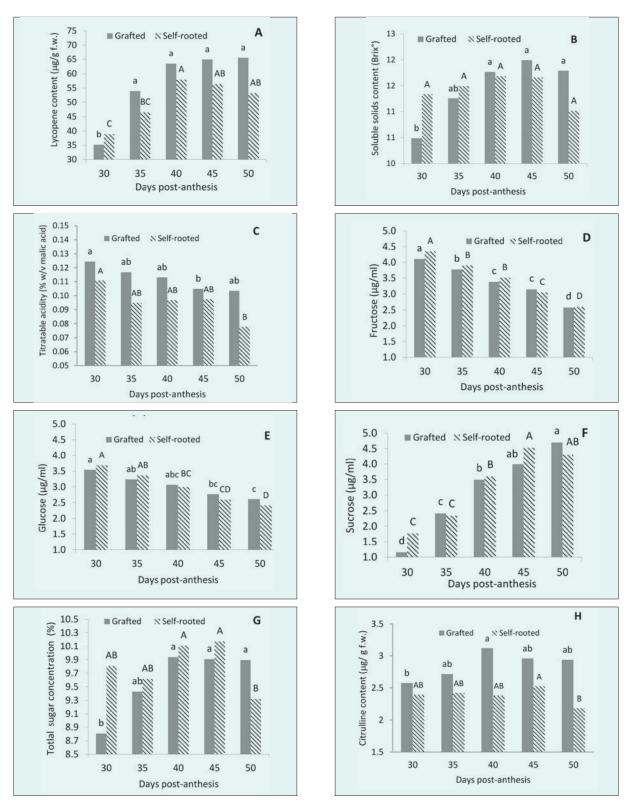
The Vegetable Crops Section undertakes research on intensive vegetable production systems, on the postharvest physiology and technology of horticultural commodities, and on the *ex situ* conservation and utilisation of local plant genetic resources. Current work in Vegetable Crop Science focuses on watermelon and melon rootstock-scion relations with respect to productivity, disease-resistance, stress response, quality and postharvest performance. The Vegetable Crops Section 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. Work on leafy salad crops examines the effect of planting pattern, nitrogen administration strategies, shading, seasonal adaptation and time of harvest on yield, quality, shelf-life and consumer safety. Performance of five male hybrid asparagus cultivars is under trial along with select populations of two wild asparagus species (*A. stipularis* and *A. acutifolius*) tested for adaptability to intensive cultivation.

The Postharvest Technology Laboratory (PTL) is the main analytical facility of the Vegetable Crops Section, dedicated to the study of pre- and postharvest ripening physiology of climacteric and non-climacteric fresh horticultural commodities in relation to the configuration of quality and postharvest performance. The PTL maps the farm-to-fork etiology of fresh produce quality, by studying the impact of pre- and postharvest applications on quality and shelf-life. Analytical emphasis is placed on assessing the implications of harvest maturity for product physiology, on deducing objective indices of physiological and commercial maturity, and on describing physicochemical attributes of quality. The effects of minimal processing on product quality and shelf-life, and the effectiveness of non-chemical postharvest treatments for controlling pathological and physiological loss of quality are also within the scope of PTL current activity. The PTL coordinates the current ARI project on the evaluation of an *ex situ* collection of indigenous pomegranate clones. The National Genebank constitutes another integral component of the Vegetable Crops Section concentrated on the collection, *ex situ* conservation, regeneration, characterisation, evaluation and utilisation of native plants of the flora of Cyprus and local landarces. Finally, the ARI Herbarium, which is an extension of the Genebank, is dedicated to the collection and documentation of botanical specimens of the Cyprus flora.

#### **VEGETABLE CROPS**

# Rootstock-mediated effects on watermelon ripening behaviour and fruit physicochemical and phytochemical composition

Flesh reflectance colorimetry, mechanical texture analysis, pH, titratable acidity (TA), and soluble solid (SS), soluble carbohydrate, lycopene and citrulline content of watermelon fruit were evaluated throughout ripening (30-50 days post-anthesis; dpa) and with respect to grafting. Grafting increased firmness, TA, and lycopene content though it delayed its peak. Lycopene content was mostly ripening-dependant, quadratic in regression, highly correlated and synchronous with changes in pulp chroma (C\*) and color a\* (Fig. 1). The sweetness was affected only by ripening. However, total sugars and SS peaked later in fruit of grafted plants than in non-grafted ones, and significant interaction of ripening with grafting was observed. Citrulline content increased quadratically 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)



**Fig. 1.** Mean comparisons for lycopene (A), soluble solids (B), titratable acidity (C), fructose (D), glucose (E), sucrose (F), total sugar (G) and citrulline content (H) of 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. Mean comparisons were performed according to Tukey-Kramer HSD test. Means followed by different letters denote significant (P < 0.05) differences.

# 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 (3-5 kg) 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. Large fruited diploid cv. Pegasus was used as control. In both years tested, fructose content of the two diploid mini cvs. Vivlos and Esmeralda was higher than that of the two triploid mini cvs. Petite and Extazy, and that of control cv. Pegasus (Table 1). On the contrary, sucrose content of the triploid cvs. Petite and Extazy and diploid cv. Pegasus was higher than that of the two mini diploid cvs. Vivlos and Esmeralda. The glucose content of triploid cultivars was lower than that of diploid cultivars across years. Cultivars differentiated for total sugars content only in year 2, wherein triploid cultivars had invariably lower total sugar content than diploid cvs. Vivlos and Pegasus; however, diploid cv. Esmeralda had higher total sugar content compared only to cv. Extazy but not cv. Petite. No differences were observed among diploid cultivars. Regarding sweetness index, only triploid cv. Extazy differentiated from the diploid cultivars in both years. No significant differences were observed among the triploid or the diploid cultivars in both years with respect to sweetness index. The content of the juice in fructose, glucose and total sugars was not affected by rootstock type. Cultivars grafted on rootstock 'TZ148' had lower sucrose content and sweetness index over rootstock 'Festival' only in year 2. (G.A. Soteriou, M.C. Kyriacou)

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Table 1. Mean comparisons for fructose, glucose, sucrose, total sugars and sweetness index at harvest of watermelon fruit from cvs. Petite, Vivlos, Pegasus, Extazy and Esmeralda grafted on C. maxima × C. moschata (TZ148) and Lagenaria (Festival) rootstocks.

r. Year 1 4.44 b <sup>x</sup> 4.93 a 3.84 c 3.51 c 3.51 c		(mg/m)	(mg/ml)	(11)	(mg/mľ)	(lm)		
4.44 b* 4.93 a 4.93 a 3.84 c 3.51 c 4.98 a		Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
4.93 a 3.84 c 3.51 c 4.98 a	0 7.00 0	2.30 с	2.40 b	3.00 a	9.4	8.82 bc	1,115 ab	1,010.1 bc
3.84 c 3.51 c 4.98 a	a 3.06 a	3.86 a	1.50 c	1.26 b	9.5	9.72 a	1,133 a	1,110.8 a
3.51 c 4.98 a	b 2.73 a	3.36 b	2.79 ab	2.78 a	9.4	9.57 a	1,069 ab	1,046.0 ab
4.98 a	b 2.07 c	2.28 c	3.43 a	2.42 a	9.0	8.20 c	1,037 b	946.3 c
	a 3.12 a	3.44 b	1.28 c	1.28 b	9.4	9.21 ab	1,123 a	1,066.9 ab
TZ148 4.36 3.88	2.66	3.11	2.38	1.98 B <sup>y</sup>	9.4	86.8	1,105	1,019.6 B
Festival 4.32 3.93	2.77	2.98	2.18	2.32 A	9.3	9.23	1,086	1,052.5 A

\*Means within columns followed by different letters denote significant (P<0.05) differences according to Tukey HSD test.

#### Indexing melon physiological decline to fruit quality and vine morphometric parameters

While grafting cucurbits has become essential in the management of soil borne diseases and for improving performance under conditions of abiotic stress, commercial melon grafting has been curbed by the incidence of non-pathological decline, usually expressed right before harvest and attributed to physiological scion-rootstock incompatibility. Prognostication of physiological incompatibility in grafted melon has been hampered by insufficient understanding of the factors that govern scion × rootstock interaction under dynamic agronomic conditions. Moreover, the effect of factors (e.g. high air temperature) suspected of triggering physiological incompatibility is random, causing decline only to a variable fraction of the presumably incompatible scion-rootstock population. Limited evidence exists linking the effect of grafting on plant morphological characteristics to the incidence of plant decline. Hence, extensive field trials are still essential before recommending specific scion × rootstock combinations. Indexing physiological incompatibility to fruit quality and vegetative morphometric parameters in the surviving plant population may provide useful tools for diagnosing latent incompatibility and identifying rootstock-scion combinations prone to decline. It may also enhance our understanding of the physiological mechanisms behind grafted melon plant decline. Accordingly, the objective of the current work has been to investigate the possible expression of physiological incompatibility in plant performance and morphometric parameters, and in fruit physicochemical characteristics. For the purposes of this study scion-rootstock combinations sensitive to physiological incompatibility were selected on the basis of preliminary experimental results and extension service reports on grafted melon decline incidence. Scion selection in particular was based on the relative frequency of physiological incompatibility incidence prior to harvest as follows: (a) infrequent plant mortality – cv. Elario; (b) frequent plant mortality – cv. Raymond; (c) ubiquitous plant mortality – cv. Polynica on inter-specific rootstock 'N101'. Results indicate that plant collapse shortly before harvest is a onetime event that does not necessarily reflect on the performance of the asymptomatic, surviving plants. However, the negative rootstock effect on scion dry weight (7.9 - 42.5%) may constitute a plausible index of incompatibility. The attenuation of the 1st internode's diameter relative to the hypocotyl's ('Elario' 29.1%; 'Raymond' 41.5%; 'Polynica' 44.0%) and the loss of mesocarp firmness reflected the categorical sensitivity of the scions to physiological decline. No systematic pattern was identified connecting fruit soluble carbohydrate (fructose, glucose and sucrose) content to physiological incompatibility and plant decline. However, earliness of harvest maturity, pronounced in sensitive climacteric scions 'Polynica' and 'Raymond', may relate to ethylene-mediated comprehensive acceleration of ripening stressing rootstock-scion synergy to collapse. (G.A. Soteriou, L.C. Papayiannis, M.C. Kyriacou)



# Impact of nitrogen fertilisation 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  $\mu$ 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  $\geq$  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<sup>-1</sup> 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 spinach yield, quality and safety, with respect to nitrate/nitrite residual content, during

the winter season. A multi-factorial CRD design was deployed, with combined basal (0, 100, 150, 200 kg N ha<sup>-1</sup>) and top dressing (0, 50, 100, 150 kg N ha<sup>-1</sup>) N applications in four replications. Winter season preliminary results showed that bunch weight, diameter and nitrate content were influenced by both basal N application and top dressing. CIELAB colour components and texture characteristics were not affected by either of the two application methods examined. Postharvest behaviour of spinach winter 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 15 °C. 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, M.C. Kyriacou)



### Evaluation of green asparagus hybrids and wild asparagus species for intensive culture under local conditions

Green asparagus *Asparagus officinalis* cultivation presents unique features which render it potentially suitable as an alternative crop for Cyprus. It is a crop species considered highly tolerant to drought, however its irrigation requirements during the growth period of the fern (i.e. the above ground vegetative part) are linked to yield and quality. To examine the prospects of green asparagus cultivation in Cyprus,

the Vegetable Crops Section of the ARI, in collaboration with the Horticulture Laboratory, Aristotle University of Thessaloniki, has set up an experimental trial on five promising male hybrids of green asparagus. Expected outputs of the experiment are: a) The determination of earliness, yield and quality of the physicochemical characteristics and post-harvest performance of the five promising male asparagus hybrids, and b) evaluation of the hybrids as to their suitability for cultivation in arid climate.

Moreover, the adaptability and performance of two wild asparagus species (*A. stipularis* and *A. acutifolius*) under intensive cultivation are examined. Both species are seasonally collected *in situ* and marketed in the Cyprus market as wild products. There has been no attempt to characterise the populations of wild species of the genus Asparagus, nor any extensive collection, *ex situ* conservation and study of seed germination physiology. Furthermore, there is no information on crop physiology, crop management, post-harvest physiology, composition and physicochemical quality characteristics of these species. In order to generate this information, the Vegetable Section of the ARI has collected genetic material (seeds) of the two wild species from compact indigenous populations originated at five typical edaphoclimatic-botanical territories of Cyprus. Sample populations consisted of genetic material from 40 individual plants per territory. Subsequently seeds were treated under standard procedures as stated in ENSCONET (2009) protocol. Following seed collection a germination protocol was established (percentage of success >90%) and seedlings were developed for the experimental setup at the Zygi Station. Presently, experimental plots have been established for *A. Officinalis* (five hybrids) and *A. stipularis* (five populations) and another experimental plot will be set up for *A. acutifolius*. (M.C. Kyriacou, A. Kyratzis, G.A. Soteriou)



#### POSTHARVEST TECHNOLOGY

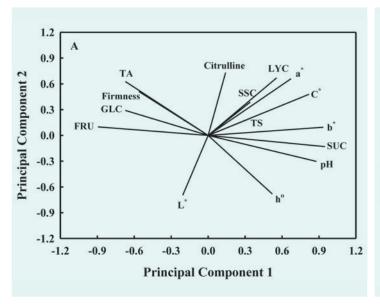
# Quality and postharvest performance of watermelon fruit in response to grafting on interspecific cucurbit rootstocks

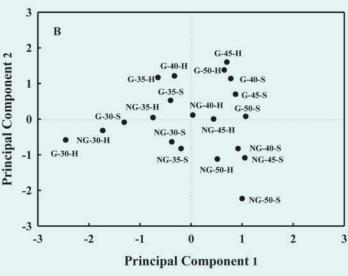
Grafting on disease-resistant rootstocks is a growing practice in watermelon cultivation worldwide. The current work examined the postharvest behaviour at 25 °C of four hybrid diploid cultivars grown self-rooted or grafted onto three *Cucurbita maxima* × *C. moschata* hybrid rootstocks. Despite sucrose accumulation throughout storage at the expense of fructose and glucose, the total soluble carbohydrates and the soluble solids content declined. Lycopene content peaked 7 days postharvest and the intensity of flesh colour increased concomitantly; yellowing of the flesh was detected at 14 days. Rootstocks reduced the soluble solids content only in the second year of experiments by 0.8-1.0 °Brix. Rootstocks improved postharvest flesh firmness and lycopene content and enhanced the colour intensity of the flesh. Rind was thickened minimally by rootstocks and declined with storage. Despite being a non-climacteric fruit, watermelon exhibited significant postharvest anabolic activity in terms of lycopene and sucrose synthesis. Grafting diploid cultivars on inter-specific rootstocks invariably improved fruit quality and storability.

Reduction in soluble solids content in response to grafting may be observed but it is limited and not detrimental to fruit quality. (M.C. Kyriacou, G.A. Soteriou)

# Configuration of watermelon fruit quality in response to rootstock-mediated harvest maturity and postharvest storage

The configuration of watermelon fruit quality was analysed in a multi-factorial approach accounting for the effects of grafting, harvest maturity and postharvest storage. Diploid, seeded, hybrid cv. Pegasus, cultivated as scion on interspecific hybrid squash rootstock TZ148 and as non-grafted control, was stored at 25°C following sequential harvests from the onset of ripening to over-maturity. Delayed rootstock-mediated climax in pulp lycopene and chroma was observed, while both were heightened by postharvest storage when harvest preceded full maturity (Fig. 2). Pulp firmness was increased by 46.5% on TZ148, while postharvest decrease in firmness was non-significant. Non-grafted fruits attained their peak in pulp carbohydrate content earlier during ripening. Monosaccharide content declined and sucrose content increased both preharvest and postharvest; overall sugar content declined by 4.3% during storage. Pulp acidity decreased steadily with ripening but was moderately increased by grafting. Citrulline content increased by 12.5% on TZ148; moreover, it climaxed with ripening and declined with storage only in grafted fruit. Grafting enhances pulp texture and bioactive composition. Potential suppression of sugar content as a result of grafting is minimized at full commercial maturity. Brief postharvest ambient storage enhances pulp lycopene and chroma, especially in early-picked fruit, notwithstanding the depletion of monosaccharides and citrulline and a limited deterioration of texture. (M.C. Kyriacou, G.A. Soteriou)





**Fig. 2**. (A) Principal component loading plot and (B) scores of principal component analysis of physicochemical traits of watermelon fruit as a function of grafting (G, grafted; NG, non-grafted), harvest maturity (30, 35, 40, 45, 50) and postharvest storage (H, at harvest; S, storage at 25°C for 10 d). FRU, fructose; GLC, glucose; TA, titratable acidity; SSC, soluble solids content; LYC, lycopene; C\*, chroma; TS, total sugars; b\*, yellowness; SUC, sucrose; ho, hue angle; L\*, lightness.

# 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 characterisation. 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, fruit weight, skin and juice colour. Sequential sampling was performed between August and November. 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. Comprehensive evaluation of the accessions for physicochemical quality attributes is performed, including soluble carbohydrates, organic acids, anthocyanins, phenolic compounds in the pomegranate juice, characterisation of free radical scavenging potential of pomegranate juice and assessment of juice antioxidant value, seed hardness, texture and fibre content, and fruit storage performance. Also, genetic diversity among the 30 pomegranate genotypes was assessed using 10 microsatellites representing 10 loci. All the microsatellites were polymorphic. The number of alleles per locus ranged from 2 to 5, with an average of 3.3. Our results demonstrate the existence of significant genetic diversity within the collection, which renders the clonal evaluation highly valuable. (M.C. Kyriacou, A. Kyratzis)

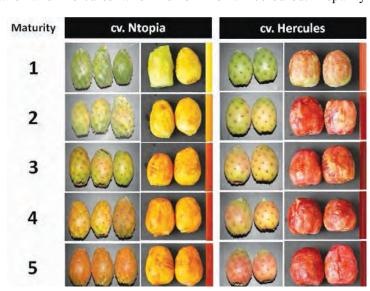
# Asynchronous ripening behaviour of cactus pear (*Opuntia ficus-indica*) cultivars with respect to physicochemical and physiological attributes

Resilience to conditions of prolonged drought and high temperature, owing mainly to crassulacean acid metabolism, make the Cactaceae family subject of renewed agronomic interest for cultivation under arid and semi-arid climates, expedited by the ensuing climate change. The growing exploitation of cactus fruits and cladodes, in particular of Opuntia species, in the cosmetics and neutraceutical industries, and the heightened interest in their fresh fruits, widely referred to as cactus pears, stem from the unique functional properties and composition of this fruit. While expansive genetic diversity within Opuntia is found in the countries of South America, prominently in Mexico, introduction of these fruiting species into the Old World, possibly in the 16th century, has long provided a naturalised genetic basis for the resurgence and valorisation of this crop in southern Europe over the last two decades. Cultivation of mostly Opuntia ficus-indica L. genotypes is now widespread in southern Europe, particularly on the islands of Malta, Sicely, Crete and Cyprus. Characterisation of cultivated cactus pear genotypes has displayed wide variation in important compositional traits such as their phenolic, carbohydrate, pigment and soluble/insoluble fibre contents. Previous workers have reported on significant genotypic, environmental and cultural effects that contribute to compositional variation in cactus pear fruits. On the contrary, work on the on-tree ripening behaviour of cactus pears and the effect of harvest maturity on fruit quality and physicochemical composition remains scarce, owing perhaps to the termination of physiological maturation of cactus pear fruit at harvest and the absence of acute climacteric rise in respiration and ethylene production during postharvest ripening. Physicochemical assessment of fruit quality is thus commonly limited to a snap-shot of fruit composition at harvest, and what may be construed as genotypic differences in quality attributes may in fact reflect differential ripening behaviour affecting harvest maturity. Accordingly, the overall objective of our work has been to characterise the ripening behaviour of two prominent Opuntia ficus-indica cultivars widely cultivated in Cyprus. Physicochemical and physiological changes with maturity were profiled in relation to skin colour

#### Vegetable Crops

development, which was referenced as a non-destructive index of harvest maturity. Fruits of orange-fleshed cultivar 'Ntopia' and red-fleshed cultivar 'Hercules' were harvested at five stages of maturity based on skin colouration ranging from mature green to fully coloured, overripe fruit. Physical components of quality examined included seed and pulp relative weights, pulp reflectance colorimetry and fruit mechanical texture analysis. Phytochemical components of quality examined entailed pulp pH, titratable acidity, soluble solids and carbohydrates (glucose, fructose, sucrose), phenolic compounds, ascorbate, betalains (betacyanins and betaxanthins), and the pectins and neutral sugars of the fractionated alcohol-insoluble cell wall material. Fruit physiological attributes examined included respiratory activity and ethylene production rate. Analysis of the above components highlighted cultivar-maturity interaction and revealed cultivar profiles of asychronous ripening behaviour that are critical for determining optimum harvest maturity and postharvest handling protocols. The two cultivars presented differential sugar accumulation behaviour during ripening with significant implications for their harvest maturity: 'Ntopia' should be harvested near full skin colouration and 'Hercules' when fruit skin is half-coloured. Disparity

between pulp betacyanin accumulation and delayed skin colouration renders skin color a dubious harvest maturity index for red-fleshed cultivar 'Hercules'. The current work highlights the unique behaviour of cactus pear with respect to cell wall metabolic events and textural changes during ripening: No pectin solubilisation but weakening of glycan bonding and extensive loss of neutral sugars; while further complication in interpreting fruit compressibility is the high correlation of seed weight and seedto-pulp ratio to uniaxial fruit compression analysis. (M. Kyriacou, M. Emmanouilidou, G. Soteriou)



#### CONSERVATION OF PLANT GENETIC RESOURCES

#### **National coordination**

Cooperation with Biodiversity International, mainly through participation in the European Cooperative Programme for Plant Genetic Resources (ECPGR) and other international and national organisations 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 and on useful plants such as landraces and crop wild relatives. Around 1,200 new accessions have been collected

during the last two years. In collaboration with the Dutch Seed Company "Rijk Zwaan", two joint collecting missions have been organised targeting the collection of vegetable wild relatives. In total, 690 new accessions have been collected. In collaboration with ICARDA, 472 new accessions of cereal, legumes and vegetable wild relatives have been collected during the two joint collecting missions. The accessions have been safety duplicated at Rijk Zwann, ICARDA and Millenium Seed Bank.

Germination tests have been routinely conducted to assess germination capacity of the CYPARI conserved accessions. During the last two years, 184 accessions have been successfully regenerated. In total, around 2010 accessions of local landraces and wild species, including crop wild relatives and endemic species, are registerd in the Genebank and conserved under controlled conditions (0 to 4, and -20 °C). Access to passport date can be gained through the EURISCO database. (A. Kyratzis)

# Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives – Trust Project

The "Trust Project" was founded by the Government of Norway and coordinated by the Global Crop Diversity Trust and the Millennium Seed Bank, Royal Botanical Gardens KEW. The consortium comprises partners from all crop diversity hotspots of the world as well as partners with research experience on genetic resources and plant breeding of the most important crops for food and agriculture. The main goals of the project are the collection, characterisation, evaluation and utilisation of crop wild relatives. Crop wild relatives can support efforts for adaptation to climate change through plant breeding as they may possess traits that can impart crops improved resilience and adaptability under the abiotic stress conditions projected under climate change. The ARI has successfully collected, regenerated and conserved 256 accessions of crop wild relatives. Duplications of these accessions have been sent to ICARDA and to Millennium Seed Bank, Royal Botanical Garden, KEW. (A. Kyratzis)

#### **Global Tree Seed Bank Project**

The "Global Tree Seed Bank Project" is a global initiative which will secure the future of more than 2,000 of the world's rarest, most threatened and most useful trees. It is funded by the Garfield Weston Foundation and it is coordinated by the Millenium Seed Bank, Royal Botanical Gardens KEW. In Europe, the project will collect and conserve in seed banks at least 200 native European tree species. The national coordinator for Cyprus is the "Nature Conservation Unit" of the Frederic University. Seed collecting has been done by the Department of Forests and the Nature Conservation Unit while seed cleaning and conservation will be done by ARI genebank. Until now, 40 accessions of native trees and shrubs of Cyprus have been collected. Duplications of these accessions will be sent to Millennium Seed Bank, Royal Botanical Garden, KEW. (A. Kyratzis)

#### Linking on-farm and ex situ activities to the conservation of vegetable landraces

The main objectives of this project 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 devoid of commercial value and threatened with extinction. Linkages with stakeholders (NGOs) have been established and potential ways of collaboration have been explored.

#### Vegetable Crops

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 optimised on-farm conservation. The project is currently dealing with local landraces of garlic. (A. Kyratzis, 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 the context of the project "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives – Trust Project" around 80 new botanical specimens have been collected. The specimens concerned native taxa of the flora of Cyprus that are wild relatives of vegetable, cereal, legume and fodder crops. (A. Kyratzis)

### ANIMAL PRODUCTION

Research in the Animal Production Section focuses on the topics of nutrition, management, animal breeding and physiology of reproduction. All projects are ultimately directed towards increasing milk and meat yields under semi-intensive or intensive management systems in sheep, goats and dairy cattle. In addition, genetic methods and animal husbandry practices are employed, aiming at preventing and controlling animal diseases. Research work on animal breeding aims at improving the genetic stock with respect to important economic traits 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 for the evaluation and selection of superior breeding stock in sheep and goats. Research in genetic improvement is also directed toward dissecting the genetic aspects underlying production traits, including further characterisation of scrapie genotypes and productive output in goats. Research programmes in the area of reproductive physiology of farm animals examine genetic and environmental factors that influence seasonal reproduction, reproductive development and puberty in sheep and goat breeds, under local conditions. 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 employing genetic methods to combat the scrapie disease in Chios sheep has been successfully implemented by ARI in cooperation with the Veterinary Services. The Cyprus Chios sheep unit of ARI at Athalassa Experimental Farm has been transformed into a nucleus herd of scrapie-resistant genotypes. The number of productive animals at the nucleus is maintained around 350 breeding females. Further research in genetic improvement at ARI is directed toward dissecting the genetic aspects underlying production traits, and further characterising scrapie genotypes in goats. A programme is currently in progress to transform the Cyprus Damascus goat herd at ARI into a nucleus of scrapie-resistant genotypes. The project continued in 2014 and 2015, 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, which contribute significantly 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 2014 and 2015 is shown in Table 1. The overall performance of the herd was satisfactory, since milk yield per annual cow was on average 8,400 l in 2014 and 7,600 l in 2015. The total milk produced on farm was 401,279 l in 2014 and 374,227 l in 2015. Calving interval and return rate (services/conception), and mastitis incidence, all improved significantly during 2014-15. Finally, cows that suffered from mastitis were removed from the flock. (**G. Hadjipavlou, D. Sparaggis**)

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

The ARI small ruminant herds consist of 351 Cyprus Chios sheep and 330 Cyprus Damascus goats. Daily milk yield is recorded automatically during milking, and this led to improved phenotyping 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 two times per year for each species. Production and reproduction characteristics during the period 2014/15 are shown in Tables 2 and 3. (G. Hadjipavlou)



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

Research on zero suckling systems in Cyprus Chios ewes and Cyprus Damascus goats continued in 2014 and 2015. 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. Results in 2014 and 2015 further supported findings from previous years that artificially reared kids had satisfactory pre-weaning 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. 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 Dried Distillers Grain byproducts in the diet of Cyprus Chios sheep

The project was conducted in 2014 as a joint effort between the ARI and the Cyprus University of Technology. The objective was to examine the effect of Dried Distillers Grain with Solubles (DDGS), an alternative protein feed, on milk production and milk quality of Chios sheep. The DDGS is a dried cereal byproduct of the distillation process. A total of 45 ewes of the Chios breed were allocated to three groups of 15 animals each and fed three iso-nitrogenous and iso-energetic mixtures of concentrates with different inclusion rates of DDGS, replacing part of the soya bean, as follows: G0 no inclusion, G10 with 10% and G20 with 20% inclusion of DDGS on DM basis. The experiment ran for 9 weeks and milk samples, collected once weekly, were analysed for fatty acid (FA) profile. Results showed no significant differences between the three groups in milk yield, protein, lactose or solid non-fat content of milk. Fat content, though, was affected by the inclusion of DDGS, with lower values observed in milk from the G20 group (total means of 6.0, 5.9 and 5.0 for the D0, D10 and D20 group, respectively, P<0.001). FA composition of fat was also affected. Expressed in g/100g of fat (for the D0, D10 and D20 groups, respectively), milk from ewes fed DDG had less saturated FA (means of 67.5, 65.7 and 60.2, P<0.001) more mono-unsaturated (23.5, 25.2 and 28.2, P<0.001), and more poly-unsaturated FA (6.0, 6.6, and 8.2, P<0.001) compared with the control milk. Health related FA, such as the conjugated linoleic (18:2 e9,

t11) and linolenic (C18: 2 cis-9 cis-12 cis-15) acids were also increased (P<0.01) throughout the experiment in G10 and G20 groups. Overall, the inclusion of DDGS as an alternative protein feed reduced the total fat content of milk, improved the FA profile of milk fat, and had no effect on other milk constituents or milk yield. (**D. Sparaggis**)

#### Use of triticale hay and silage in the diet of ruminants

The main aim of the project is to evaluate the effects of triticale hay and silage feeding on Holstein-Friesian cows, Chios sheep and Damascus goats. Triticale is grown 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 determined and compared to control groups that receive

barley hay or silage. Preliminary data regarding the use of triticale hay showed 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, A. Pallides**)



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

Combating scrapie disease in Chios sheep with the use of genetic and molecular methods is a joint project of the ARI Animal Production and Agrobiotechnology Sections. The present population of the ARI unit consists of breeding ewes and rams of the scrapie-resistant ARR/ARR genotype. For breeding purposes, the number of resistant rams and ram lambs issued to farmers in the period of 2014-2015 was 24 and 275, respectively, and that of surplus female lambs was 198. In addition, 14 ewes were issued to farmers. By increasing the frequency of the desirable allele in Cyprus Chios sheep, and consequently of the resistant genotypes in the population, the disease has been controlled and will be eventually eradicated. It should be emphasised 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 minimise inbreeding that arose out of necessity during the effort to eradicate the scrapie-susceptible genotype (AQQ/AQQ) from the flock. (G. Hadjipavlou, 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 ARI-funded joint research between the Animal Production and Agrobiotechnology Sections, and in cooperation

between the ARI and the Veterinary Services. During 2014-2015, the collection of genetic and production information for Damascus goats continued, with the aim of further studying the different *PrP* genotypes in goats and compiling 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 simultaneously decrease the N allele at the same locus. In 2014 and 2015, molecular genotyping of the selected *PrP* alleles was conducted by the Agrobiotechnology laboratory on more than 789 animals. In this period, the Animal Production section provided the farmers with 36 bucks, 12 goats, 220 male kids and 138 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 from birth, 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, I.M. Ioannides**)

# 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 participated in a European research project, in which Mediterranean countries collaborated on topics of agricultural research. The DoMEsTIc project, funded under EU FP7 ARIMNet, ran from May 2012 to April 2015, and aimed to enhance our knowledge on pastoral and rangeland sheep and goats production systems. Case studies from Greece, Cyprus, France and Morocco were analysed through field surveys, focusing on livestock farming systems, the genetic management of the breeds, and economic aspects. The comparative analysis of the data collected assessed 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.

The main outcomes of DoMEsTIc were the development of methodologies and frameworks to support local breeds and enhance their positive impact on rural economies. More specifically, (1) a regulatory framework was developed to establish and reinforce coordination among local actors; (2) value chain analysis and analysis of the factors affecting marketing and channel choice decisions were pursued at the farmer's level; (3) a framework supported by a "checklist" of levels was developed, describing how the breed can play a mediating role between product valorisation and genetic management; (4) indicators for assessing sustainability of whole production systems were chosen, based on the collected data. Value chain analysis for Cyprus revealed that the majority of sheep and goat milk is sold to large dairies, even though the farm gate price received does not differ significantly among market channels. Mean animal milk production is significantly higher in farms selling their milk to large dairies, and this is even more pronounced for goat milk producers. The majority of contracts for milk sales are oral and all written contracts made were with large dairies. Price satisfaction is very low for all market channels and, in most cases, farmers receive payment in the form of cheque or credit, not cash. Additionally, the farmer profile is well correlated to his/her choice of milk market channel. Full time farmers, who keep records on farm and animal management, and can afford technical supervision and successors, in most cases sell their milk to the large dairies. (G. Hadjipavlou, G. Adamides, A. Stylianou)

### Country report on the state of animal genetic resources (AnGR)

The preparation of the report is a conventional obligation of Cyprus to the FAO. The ARI is the focal point for AnGR and, in 2014, was responsible for preparing the latest country report, which was then submitted to the FAO. The report provided an overview of Animal Production in Cyprus, the state of AnGR concerning various species and breeds, livestock sector trends, the impact of changing climatic conditions and other factors on AnGR management and the efforts to utilise, conserve and develop AnGR. Country reports on AnGR were subsequently used by FAO to prepare a universal document entitled "The second report on the state of the world's animal genetic resources for food and agriculture", which was published in 2015 (ISBN 978-92-5-108820-3). (**G. Hadjipavlou**)

**Table 1.** Performance of dairy cattle at the ARI, Athalassa Experimental Farm

	Year	
Variable	2014	2015
Cows calved	39	34
Heifers calved	14	11
Abortions	0	0
Calves born alive	48	42
Calves born dead	5	3
Calves died	1 (2.1%)	2 (4.8%)
Calving interval (days)	396	399
Days open	118.4	120.9
Duration of pregnancy (days)	277	277
Services/conception	1.7	1.6
Annual cows	47.8	49.2
Milk produced (l)	401,279	374,227
Milk/annual cow (l)	8,400	7,612
Milk fat %	3.27	3.51
Milk protein %	3.34	3.33

**Table 2.** Production characteristics of Chios ewes at ARI (2014/15)

Trait	Yearlings	Adults
No. of ewes lambing	202	318
Lambs born/ewe	1.78	2.06
Lambs born live/ewe	1.68	1.91
Litter weight at birth (kg/ewe)	6.30	7.16
Lambs weaned/ewe	1.54	1.70
Litter weight at weaning (kg/ewe)	18.2	20.5
60-day milk (kg/ewe)	86	100
Total milk (kg/ewe)	226	329
Days in milk	254	279
Milk fat (%)	4.97	5.47
Milk protein (%)	4.97	5.35

Table 3. Production characteristics of Damascus goats at ARI (2014/15)

Trait	Yearlings	Adults
No. of goats kidding	176	270
Kids born/goat	1.62	2.09
Kids born live/goat	1.60	2.04
Litter weight at birth (kg/goat)	6.10	8.33
Kids weaned/goat	1.45	1.68
Litter weight at weaning (kg/goat)	18.2	21.3
60-day milk (kg/goat)	80	109
Total milk (kg/goat)	217	391
Days in milk	212	233
Milk fat (%)	3.43	3.70
Milk protein (%)	3.73	3.72

### PLANT PROTECTION

Plant Protection research covers subjects in the disciplines of plant pathology and entomology. Specialised studies are conducted to address important crop protection problems associated with particular plant diseases (viral, prokaryotic and fungal) and pests (insects, mites). Studies are governed by the considerations of sustainability in crop protection and production, food quality and safety, environmental protection and the utilisation of new technologies for the development of more effective crop protection practices. Plant Pathology research includes the implementation of modern nucleic acid diagnostic assays for plant pathogens. Research effort has been focused on the development of new effective detection tools in order to identify the phytosanitary status of plants, for certification and quarantine purposes. 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 environmentally-friendly methods, including cultural practices, biological control, resistant varieties and rootstocks, natural products, and minimal use of mild pesticides. This approach ensures sustainability and minimises pesticide risk to human health and the environment. It can be applied alone or in combination with other 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 fruit tree plantations, vegetable and ornamental greenhouse crops, in cooperation with other Sections of the Institute. The Plant Pathology and Entomology laboratories provided support for the scientific identification of plant diseases and insect pests, on various samples provided by the Department of Agriculture, the Medical and Public Health Services, the Department of Forestry, agriculturists from the private sector and farmers.

#### PLANT PATHOLOGY

#### **Programme for the control of Citrus tristeza virus (CTV)**

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, L.C. Papayiannis)



#### Molecular characterisation of Citrus tristeza virus (CTV) isolates from Cyprus

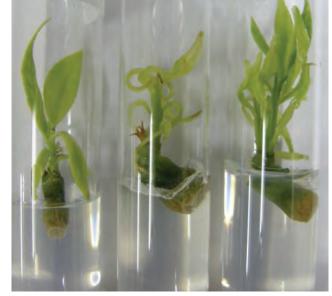
A nested reverse transcription (RT) polymerase chain reaction (PCR) was developed to allow a rapid and sensitive amplification of the viral coat protein gene (CPG). Thirty one isolates collected from different areas of Cyprus causing a wide diversity of symptoms were tested and characterised. Symptoms on field trees ranged from inconspicuous to twig die back, decline and death of sweet orange or grapefruit trees on sour orange rootstock. Similarly, on Mexican lime, symptoms ranged from barely noticeable leaf vein clearing to vein corking, stem pitting and plant stunting. CPG amplicons were digested by a

selection of restriction enzymes and characterised with single strand conformational polymorphism (SSCP). The nucleotide sequence of the CPG was determined and phylogenetic analysis was performed. Results showed that 22 symptomless isolates from Cyprus clustered among the mild strains reported from Spain, Portugal and Africa. In addition, five isolates that were responsible for decline of sweet orange, grapefruit and mandarin trees showed high similarity with strains reported in Africa (B249) whereas four other isolates that caused stem pitting symptoms clustered with T36, an American severe strain from Florida. The SSCP technique and the subsequent nucleotide analysis of the Cypriot CTV isolates enabled their clear distinction in mild and severe, their comparison to universal isolates/strains. The molecular techniques used in the present work enabled, in addition to the differentiation of mild and severe isolates, the establishment of relationships of Cypriot isolates to universal isolates, including the severe isolates T36 and T3 from Florida, B246 from South Africa, B-CTV from India and the mild isolate 28C from Portugal. These results substantiate our belief that CTV was introduced in Cyprus with imported budwood from South Africa in the 1930's when there was not much knowledge about viruses and more recently from other countries, as travelling became easier during the last three decades. (L.C. Papayiannis, T. Kapari).

## Micrografting, chemotherapy and coldtherapy in vitro for elimination of Citrus tristeza virus, and/or viroids from infected citrus plants

The coldtherapy, chemotherapy and micrografting *in vitro* techniques have been used since 2010 for sanitation of citrus species and varieties in Cyprus and Greece infected by *Citrus tristeza virus* (CTV) and/or citrus viroids. Fourteen citrus trees including the lemon varieties Adamopoulou, Verna, Polyphori and Lapithou, the orange varieties Navelina, Washington navel, Jaffa, Siekeriko and Aematousiki, the mandarin varieties Clasuelina, Page and Arakapas, Frappa and Bergamot were selected. Four Mexican lime (ML) trees infected by severe or mild CTV isolates from Cyprus were used. All trees were tested for viruses and viroids by biological indexing, by ELISA for CTV and CPsV, and by RT-PCR for viroids. They were all found free of CPsV, CVV, concave gum and impietratura. Thirteen trees were infected by CEVd and/or other viroids and one was infected by both CTV and viroids. The micrografting technique *in vitro* was used for elimination of viroids and CTV in the selected citrus isolates. Chemotherapy *in vitro* was tested in ML trees infected by CTV. Coldtherapy *in vitro* was tested in Lapithou lemon tree

infected by CEVd and HSVd. Micrografted plants were re-grafted on sour orange seedlings in vivo, or were potted and those which were successfully established were transferred to the glasshouse. They were tested 6-9 months later for CTV and viroids present in meristem donor mother plants. One or more plants produced by micrografting from all mother plants were found free of CTV and/or viroids. Elimination of CTV on apical meristems was achieved by chemotherapy in vitro using 30, 40 and 50 mg/lt ribavirin or 30, 40 and 50 mg/lt methotrexate. Elimination of viroids by coldtherapy at 10°C for 5 weeks was not possible, but was successful by coldtherapy at 4°C for 5 weeks. (T. Kapari-Isaia, L.C. Papayiannis)



#### Evaluation of six citrus rootstocks for tolerance to local isolate of Citrus tristeza virus

The main aim of this project was to find tolerant rootstock for replacement of the CTV-sensitive sour orange, which is commercially used in Cyprus. Twenty four plants of the following citrus rootstocks were grafted with Washington Navel sweet orange and are being evaluated for their tolerance to CTV in a replicated complete block design, at Xylotymbou experimental Station of the Institute: Carrizo citrange, Swingle citrumelo, Volkameriana lemon, Gou Tou, Cleopatra mandarin and Sour Orange. Of these trees 50% were graft-inoculated with CTV and the rest remained healthy. Infected and healthy trees are being evaluated and compared with regard to their performance in the field, fruit production and quality. Most of the trees grafted on citrumelo rootstock showed severe stunting and chlorosis. (**T. Kapari-Isaia**, **L.C. Papayiannis**)

### Citrus certification programme

A mandatory certification programme has been established and implemented since the mid 1990's. The responsibility for the implementation of the relevant legislation rests with a seven-member Board under the Minister of Agriculture. The foundation or pre-basic block is kept and maintained under insect-proof screen by the Agricultural Research Institute, whereas the multiplication and mother blocks, protected also under insect-proof screen, are maintained by the Department of Agriculture. In addition, the private nurseries are obliged to keep their mother plants and the production of seedlings and budded treelets also under screen. The foundation block provides with virus-tested material the mother blocks of the Department of Agriculture which in turn provide with budwood the private nurseries or directly the growers. Citrus budwood, which is introduced from overseas sources, is kept in a post-entry quarantine station and undergoes thorough indexing for the known virus and virus-like diseases before entering the foundation block. Local varieties are being cleaned from the known virus problems by micrografting.

All virus-free material, which was either selected or imported by the Horticulture Section of the Institute or produced locally by micrografting, is maintained in a pre-basic plantation under double insect-proof screen at the Agricultural Research Station of Zygi. To the present 60 citrus accessions are included in the plantation and are kept in clean state by application of strict sanitary measures and regular indexing in conjunction with optical observation for either fungal problems or genetic aberrations. The plantation provides with citrus material the basic or mother plantations of the Department of Agriculture and the experimental glasshouses of the Institute. (**T. Kapari-Isaia, L.C. Papayiannis**)

### Production of healthy local citrus propagating material

For several commercial varieties, it is possible to reintroduce currently healthy material from overseas Citrus Production Centres. However, this includes the risk of the introduction of new unknown diseases, which is not possible in the case of local varieties. For these reasons it was decided to employ contemporary techniques, as micrografting *in vitro* to free from virus and other diseases valuable local citrus varieties and/or clones, including "Lapithou" and "Polyphori" lemon, "Arakapa" mandarin, local Jaffa orange and others. The micrografting *in vitro* technique was used in Cyprus since 2000 to free from virus and other pathogens valuable local citrus varieties and/or clones. A survey in commercial groves was conducted for selection of elite mother plants of citrus, including "Polyphori" and "Lapithou" lemon (*Citrus limon* Burm f.), Jaffa, Siekeriko and Aematoysiki 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 CTV, and by RT-PCR for viroids. 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 6-9 months later for the viruses and viroids present in meristem donor mother plants. (**T. Kapari-Isaia, L.C. Papayiannis**)

#### 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 migrografted on young seedlings of trifoliates *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, L.C. Papayiannis**)

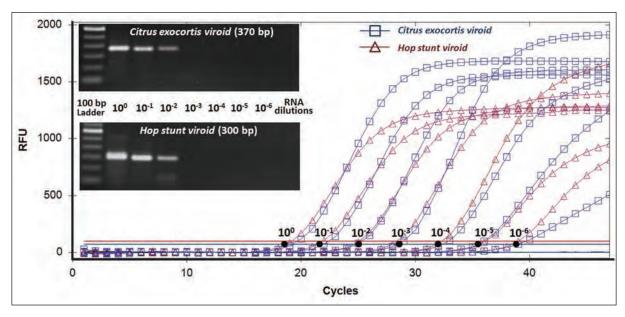
#### Evaluation of five new sour orange hybrid rootstocks for tolerance to citrus viroids

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, L.C. Papayiannis)

#### **Incidence of Citrus viroids in Cyprus**

Viroids are currently the smallest recognised disease agents of plant species. They are transmitted by grafting or other mechanical means and can cause infectious diseases to several vegetable, ornamental and fruit tree crops. Citrus viroids are listed among the most important pathogens that negatively affect the citrus industry worldwide. In view of a research project aiming at the identification and management of citrus diseases in the island, an extensive survey was conducted in order to define the presence and incidence of citrus viroids using molecular based techniques and in selected samples biological indexing. Results showed that *Citrus exocortis viroid* (CEVd) was the most widespread viroid with an incidence of 80.5%, followed by *Hop stunt viroid* (HSVd) which was detected in 53.3% of the 500 samples tested. *Citrus bent leaf viroid* (CBLVd), *Citrus dwarfing viroid* (CDVd) and *Citrus bark cracking viroid* (CBCVd) were also identified at lower rates of 12.2, 9.6 and 7.7 %, respectively. CEVd+HSVd was the most common viroid combination (69.3%), while CEVd+CBLVd, CEVd+CDVd and CEVd+CBCVd were detected at 9.7, 9.5 and 9.4% of the double viroid mixtures (25.4%). Triple viroid combinations were also recorded at 9.8% of the tested samples with CEVd and HSVd identified in all mixtures. *Citrus viroid* V was not detected in any of the samples. Sequencing analysis showed that all HSVd shared 100% nucleotide identity and were closely related to other variants from the Mediterranean basin, while CEVd

isolates shared 97-100% homology to exocortis isolates from neighbouring countries, in Africa and Asia, suggesting multiple intoductions through contaminated budwood. Only 7.5% of the samples tested were negative to viroid infection, indicating the need for dissemination of virus/viroid-free propagating material. (**L.C. Papayiannis, T. Kapari-Isaia**)



## 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 pre-basic 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 propagating material for the basic or mother plantations of the Department of Agriculture and for the experimental glasshouses of the ARI. (T. Kapari-Isaia, L.C. Papayiannis)

#### Production and maintenance of grapevine healthy plant material

EU has recently imposed strict directives and legislation on the distribution and the marketing of material for the vegetative propagation of grapevines material. 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. Utilisation of healthy plant material seems nowadays the most promising tactic that can ensure good results to control these diseases. During the past years, ARI has established a long term project on the maintenance of healthy propagative grapevine material under "pre-basic" status. Approximately 70 local or imported varieties are maintained under insect proof net houses at 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 are performed using pathogen specific serological and molecular based laboratory techniques. Virus-free grapevine plant cuttings are then given to the Department of Agriculture for further multiplication and distribution to Cypriot farmers. (L.C. Papayiannis, T. Kapari-Isaia)

## Epidemiology and evolutionary studies of criniviruses associated with tomato yellows disease

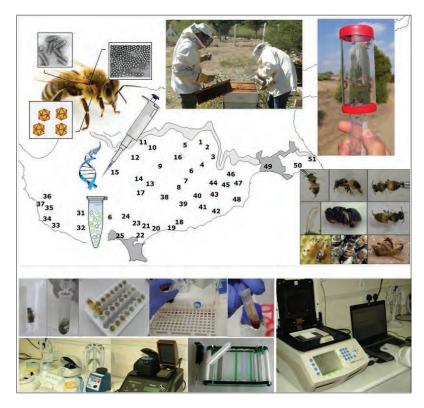
Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TICV) are two whitefly transmitted viruses which are classified in the genus Crinivirus of the family Closteroviridae. Both viruses induce similar yellowing symptoms in tomato and are responsible for severe economic losses. ToCV is transmitted by Bemisia tabaci Gennadious, Trialeurodes vaporariorum Westwood and Trialeurodes abutilonea Haldeman, whereas TICV is transmitted only by T. vaporariorum. An extensive study was conducted in order to identify the viruses involved in tomato yellowing symptoms in Greece mainland and the islands. Results showed that TICV prevailed in tomato crops (62.5%), while ToCV incidence was lower (20.5%) and confined in southern Greece. ToCV was detected in lettuce plants with mild yellowing symptoms for the first time in Greece. Approximately 13% of the weeds tested were found to be infected. TICV was the predominat virus in weeds with an incidence of 10.8%, whereas ToCV was detected only in 2.2% of the analysed samples. These results indicate that the host range of TICV and ToCV in Greece is far more extensive than previously believed. T. vaporariorum was the most widespread whitefly species in Greece (80%), followed by B. tabaci (biotypes B and Q) (20%). Sequence analysis of the CP and CPm genes from Greek tomato and weed isolates of ToCV and TICV showed that even though both viruses have very wide host ranges, their populations show very low evolutionary divergence. (L.C. Papayiannis)

## Geographical spread of viruses involved in tomato yellow leaf curl disease and whitefly vectors in Greece and Cyprus

During an extensive survey which was conducted during the past six years in Cyprus and Greece (including Greek mainland, Peloponnese and the islands) in order to investigate the epidemiology of the virus species and the whitefly vectors involved in Tomato yellow leaf curl disease (TYLCD), more than 8,000 symptomatic tomato samples, weeds and whitefly samples were collected and analysed. Differentiation of Begomoviruses and B. tabaci biotypes was based on various real-time TaqMan PCR assays developed in this study. Results showed that in Greece, TYLCV was the most prevalent Begomovirus species (94,5%), whereas TYLCSV was found only in 4,5% of the total samples tested. In Cyprus, TYLCV was the only species found to be associated with TYLCD. Molecular characterisation of TYLCV showed that TYLCV-IL and TYLCV-MLD strains co-exist in the two countries, with an incidence of 90 and 10%, respectively. Sequencing analysis results showed that Greek and Cypriot TYLCV isolates possess high similarity with corresponding isolates from Israel, Lebanon, Turkey and other Mediterranean countries, whereas TYLCSV sequences were identical to a Begomovirus reported previously in Italy. Molecular identification of B. tabaci biotypes showed that Q was the only biotype found in the mainland of Greece, Peloponnese and the island of Crete, and seems to be involved in TYLCD spread. 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. (L.C. Papayiannis)

#### Research on bee (Apis mellifera) viruses

A new research activity was initiated during 2015 in collaboration with the Centre of Beekeepers and the Department of Agriculture in order to investigate the presence of bee viruses in Cyprus. More than 80 apiaries from the districts of Lefkosia, Lemesos, Larnaka, Pafos and Ammochostos were surveyed and approximately 25 adult bees per apiary were randomly collected and stored at -80°C. A total of 1,088



samples were tested for virus presence using molecular based techniques and results showed that Black queen cell virus (BQCV) was the most widespread virus with an incidence of 88%, followed by Deformed wing virus (DWV) which was detected in 34%. Acute bee paralysis virus (ABPV) and Varroa destructor virus -1 (VaDV-1) were also identified at a very low incidence of 3 and 2% respectively. DWV, ABPV and VaDV-1 were found in mixed infections with BQCV. Kashmir bee virus (KBV), Chronic bee paralysis virus (CBPV) and Sacbrood bee virus (SBV) were not detected. (L.C. Papayiannis, Y. Markou)

#### NEW PESTS AND DISEASE RECORDS

#### First report of potato powdery scab in Cyprus

Powdery scab is a serious disease of potatoes (*Solanum tuberosum* L.) that can cause extensive surface defects on susceptible potato cultivars. During spring of 2013, potato tubers (cv. Spunta) collected from Kokkinochoria area exhibited symptoms of brownish pustule-like swellings that turned into depressions of variable size filled with powdery spore masses. Light microscopic preparations from infected tubers and serological assays revealed the presence of *Spongospora subterranea* f. sp. *subterranean*, widely

known as powdery scab. The disease has since been reported in many potato fields spanning the Kokkinochoria area in Larnaka District. Although powdery scab is a widespread disease in most potato-producing areas of the world, this was the first report of the disease in Cyprus. This work was conducted in collaboration with researchers from the Department of Agricultural Sciences, Biotechnology and Food Science of Cyprus University of Technology and Officers of the Department of Agriculture. (L.C. Papayiannis)



#### **Identification of Pelargonium viruses**

Geranium is the common name for Pelargonium (*Pelargonium* spp.). Pelargonium plants showing symptoms of leaf mottle, mosaic and necrotic lesions were collected during 2014 from private gardens

of Lefkosia and Lemesos Districts. Serological and molecular tests identified the presence of *Pelargonium flower break virus* (PFBV), *Pelargonium line pattern virus* (PLPV) and *Tomato spotted wilt virus* (TSWV). To our knowledge, this is the first report of



PFBV and PLPV in Cyprus and the first record of TSWV in pelargonium plants on the island. Both PFBV and PLPV are among the most common viruses affecting pelargonium, causing detrimental effects on both production and quality of this ornamental plant. PFBV can be transmitted by cutting, grafting, sap and thrips, PLPV is transmitted using mechanical means whereas TSWV with thrips. However, the primary means of transmission is by vegetative propagation, as pelargonium plants are mainly produced from cuttings. (L.C. Papayiannis)

#### **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 comodities. Moreover, the recent introduction of exotic fruit fly species, such as the peach fruit fly, *Bacrocera zonata*, and the species of the oriental fruit fly complex *Bactrocera dorsalis*, in North Africa and the Middle East poses

a high threat to fruits and vegetable production of the Balkan and Eastern Mediterranean. To cope with the high risk of infestation from fruit flies, the International Atomic Energy Agency (IAEA) initiated a project with main objectives the knowledge sharing among the 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)

#### **Management of insect pests in Pomegranate orchards**

In Cyprus, insect pests of pomegranates have not received much attention by researchers, 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 is aiming to identify, monitor and develop the best strategy to manage insect pests in Pomegranate orchards. (N.A. Seraphides)



### Rearing of beneficial insect populations

Wide applications of chemicals to control insect pests generate many drawbacks concerning agriculture, environment and human health. By releasing 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 ARI. (**N.A. Seraphides**)

## Biological control of the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechidae)

Tuta Absoluta is a devastating pest of tomato and other solanaceous crops. Following its introduction into Europe, North America and the Middle East, *T. absoluta* has already caused extensive economic damage to tomato production. In cooperation with the University of Cyprus, the intra and interspecific interactions between two predators, *Nesidiocoris tenuis* and *Macrolophus pygmaeus* feeding on T. absoluta eggs are being evaluated. (N.A. Seraphides)



## Checking the mating behaviour of the palm borer *Paysandisia archon* (Lepidoptera: Castniidae) using cossid's leopard moth *Zeuzera pyrina* pheromone

For the last decade the invasive neotropical castniid moth *Paysandisia archon* became a serious pest of palm trees mostly around the Mediterranean basin. At present it occurs permanently in Spain, Italy, Portugal, France, Switzerland, Slovenia, Greece, Cyprus and Bulgaria, with occasional findings in England and Denmark. In 2012-2013, a collaborative research between Cyprus and Spain was conducted, aimed at observing the mating process of this species, based on the results obtained by Sarto i Monteys *et al.* (2012) where a long range pheromone released by females of *P. archon* to attract males seemed to be lacking. Since cossid and castniid moths are close phylogenetically and no pheromones are known for castniids, we used the leopard moth's (*Zeuzera pyrina*) pheromone to check for possible attraction of *P. archon* males. Indeed, pheromone cross-attraction among closely related groups is well known. Field tests were carried out in Spain in sunny days of July using filter paper and paper dummies (depicting an adult of *P. archon*) impregnated with 1µg of *Z. pyrina*'s pheromone dissolved in hexane. Such gadgets were set on palm trunks (both *Trachycarpus fortuneii* and *Chamaerops humilis*) within commercial gardens heavily infested by *P. archon*. After 2-3 hours of continuous observations, not a single *P. archon* male approached neither the filter papers nor the dummies, suggesting this cossid moth's pheromone does not attract *P. archon* males (**V. Vassiliou**).

### First record of the sisal weevil Scyphophorus acupunctatus, in Cyprus

The sisal or agave weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) (syns: *S. anthraxcinus*, *S. interstitials*, *S. robustior*, *Rhynchophorus asperulus*) (Coleoptera: Curculionidae), is a species native to Nearctic Region (USA, Mexico, Cayman islands, Costa Rica, Cuba, Netherlands Antilles, Dominican Republic, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Nicaragua, Virgin islands, Belize, Brazil, Colombia, Venezuela). It has also been found in Africa (Kenya, South Africa, Tanzania), in Asia (Indonesia, Saudi Arabia) and Oceania. In Europe, it was first reported in 1980 in the Netherlands, on imported ornamental Yucca plants as well as several times on imported ornamental plant species such as the Beaucarnea, Dasylirion and Yucca. It was also recorded in Italy on *Beaucarnea recurvata* Lem., and on *Agave americana* L., in France, in Spain and in Greece on *A. americana* and *Agave spp*.

During 2013 and early 2014, sisal weevil was found in all the districts across Cyprus, in funnel and pitfall pheromone traps placed for the monitoring of red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae). Adults of *S. acupunctatus* are small brown-black or black weevils without dorsal scales, 10-19mm long. They feed on leaves and bore into the bole of plant where females may oviposit from 25 to 30 eggs. Generally, there are 5 larval instars but their number may vary depending of the type of food that larvae were fed. The fully developed larva is about 18mm long, creamy white and legless. Pupation takes place within a cocoon made of plant fibres and debris. The total life cycle requires 50-90 days, with 4 or 5 generations per year. Male adults produce a pheromone that attracts both sexes. Both larvae and adults are found in roots, lower leaves, and inside the heads. Adult damage consists of groups of feeding punctures on young leaves. In addition to feeding damage, the larvae may transfer bacteria that favor the development of secondary fungal or bacterial rots leading to premature death of the host. Most often, the infestation is not apparent until the damage is severe. Application of insecticides is the most commonly used suppression method for *S. acupunctatus* (V. Vassiliou)

## Entomopathogenic fungi for the control of red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

The pathogenicity of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* was evaluated in the laboratory against larvae and adults of the red palm weevil, *Rhynchophorus ferrugineus*. Isolates of the fungi were obtained from soil samples from Greece and Cyprus using *Galleria* bait method. Inoculation was achieved via immersion of individuals into conidia suspensions of different concentrations (106 – 108 conidia/ml in 0,1% Tween 80 solution). All three fungal species proved highly pathogenetic against larvae and adults of the weevil, causing 90–100% mortality. Average survival times differed significantly among treatments and life stages of the weevil: Larvae treated with fungus *Metarhizium anisopliae* survived significantly less days, as well in total, larvae survived significantly less days than adults post infection. Results of the present study indicate the potential of indigenous (from Greece and Cyprus) strains of *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* as biological control agents against the invasive red palm weevil. (V. Vassiliou)

### NATURAL RESOURCES AND ENVIRONMENT

The research work of the Natural Resources and Environment (previously Soil Science) Section over time concerned irrigation and fertilisation of crops, soil fertility, the application of new technologies in greenhouses, the use of treated waste water for irrigation and the use of renewable energy in agriculture. Experimentation involved all major crops of the area, such as tree crops, grapes, vegetables grown in greenhouses and outdoors, forage crops and aromatic plants. An important part of its research work has been carried out within the framework of cooperation programmes of various funding agencies, such as the European Union, the International Atomic Energy Agency and the Research Promotion Foundation of Cyprus. A key feature of recent activities is the extension of research studies to new topics related to climate change, the sustainable use of soil and water, precision agriculture and the use of native and endemic species, in order to assist efforts for sustainable and viable agriculture.

#### PLANT NUTRITION AND SOILLESS CULTURE

Research activity focused on plant nutrition aspects of crops in order to generate up-to-date, practical and location-specific information. A parallel activity concerns irrigation management under the pressing need for climatic change adaptation, particularly in the Mediterranean region. In this context, reassessment of water requirements of major irrigated crops in Cyprus was performed. Precision agriculture offers the promise of increasing productivity while decreasing production costs and minimising environmental impacts. In view of the above, the collection and reuse of greenhouse fertigation effluents was employed. There is also participation in three EU projects whose main objectives are to adapt farming to climate change and limited water resources, to study the comparative advantages of organic versus conventional farming and their products, and to facilitate smart specialisation in promoting high value crops.

## NaCl accumulation and macronutrient uptake by a melon crop in a closed hydroponic system, in relation to water uptake

To optimise nutrient supply in melon (*Cucumis melo* L.), cultivated in closed-loop hydroponic systems under Mediterranean climatic conditions, the process of salinity build-up has to be better understood. To attain this objective, two experiments were conducted in two cropping seasons (winterspring and spring-summer) in order to: (i) establish relationships between Na<sup>+</sup> and Cl<sup>-</sup> concentrations in the root zone and uptake concentrations (UC) of Na<sup>+</sup> and Cl<sup>-</sup>, respectively, i.e. Na<sup>+</sup>/water and Cl<sup>-</sup>/water uptake ratios, and (ii) test whether macronutrient UC in melon grown in closed hydroponic systems are influenced by the gradual salinity build-up. The salt accumulation model developed in this work was of the type  $C_{xu} = \alpha C_{xs}^{\ \ b}$ , (where  $C_{xu}$  = uptake concentrations of Na<sup>+</sup> and Cl<sup>-</sup>;  $C_{xs}$  = concentrations of Na<sup>+</sup> and Cl<sup>-</sup> in the closed system;  $\alpha$  and b calibration constants). The NaCl-salinity up to the tested level had no significant effect on the UC of macronutrients (i.e. N, P, K, Ca and Mg). The mean UC of Ca and N were higher than those reported under northern-European climatic conditions. The obtained results may be used through on-line operating Decision Support Systems to optimise nutrient supply and minimise salinity impacts in melon grown in closed hydroponic systems when the quality of the irrigation water is sub-optimal. (**D. Neocleous**)

## Physiological, nutritional and growth responses of melon (*Cucumis melo* L.) to a gradual salinity built-up in recirculating nutrient solution

Minimising salinity impacts on yield in melon crops cultivated in closed-loop hydroponic systems requires better understanding of the physiological impact of gradual salt accumulation in the recycled

solution. To attain this objective, different NaCl concentrations in the irrigation water, i.e. 0.7, 2.5, and 5 mM, were applied in two cropping seasons (winter-spring; WS and spring-summer; SS). In both seasons plant biomass and yield were negatively affected only in high NaCl-treated plants, due to stomatal limitations, which restricted CO<sub>2</sub> diffusion into the leaf, osmotic and salt-specific effects. However, a progressive NaCl built-up to maximum concentrations



in the root zone solution of 15 (WS) and 20 mM (SS), enabled plants to preserve several physiological mechanisms, thereby adjusting growth and yield without impairing fruit quality. Our results suggest that the use of irrigation water containing up to 2.5 mM NaCl, is feasible in melon crops grown in closed-loop hydroponic systems, without yield and quality losses. (**D. Neocleous**)

## Modeling transpiration of soilless greenhouse cucumber using the simplified Penman – Monteith equation as affected by the greenhouse microclimate

Two experiments (spring and autumn-winter seasons) with soilless cucumber crop (cv. Phenomeno) were conducted in order to: (i) calibrate the simplified Penman - Monteith model equation as affected by the greenhouse microclimate and (ii) validate the prediction efficiency of the model at different climatic conditions. To determine model parameters related to the plants such as transpiration and leaf area index (LAI), several environment variables (i.e. radiation, temperature humidity) were recorded. Results revealed that the determined model parameters were suitable for the whole cucumber cultivation cycle and a wide range of climatic conditions. However, parameterisation of the model using autumn-winter crop data revealed superiority compared to spring data, as indicated by the correlation coefficients.



Model validation showed a good fit between simulated and measured data, allowing implementation in commercial soilless practice. With respect to the greenhouse microclimate, cooling affected the daily mean air temperature and vapour pressure deficit as model coefficients. These results may be of value in Mediterranean greenhouses, enabling a more efficient water resource management without significant losses in agricultural productivity. (**D. Neocleous**)

#### **European Programme Leonardo (LLP) - SmartFarmer**

A simple and concise Cultivation Guide aiming to provide some useful tips/guidelines to the Learner for the cultivation of key superfoods (blueberries, raspberries/blackberries, goji berries, aronia and strawberries) in the frame of the SmartFarmer project was prepared. (**D. Neocleous, A. Stylianou, G. Adamides**) http://www.smartfarmerproject.eu/SmartFarmerPlatform/module.php?module\_id=3

### **European Programme LIFE - Adapt2change**

A closed greenhouse system was constructed. Water and nutrients were recycled and re-used in order to minimise the environmental impact. Fertigation Strategies: Most of the nutrient solution controlling systems are currently based on electrical conductivity (EC) control. Typically, in commercial closed-loop substrate systems with drip irrigation, the fertigation water is automatically prepared by mixing drainage nutrient solution with raw water and subsequently adding stock solutions of fertilisers in this mixture, in order to achieve pre-set values of EC and pH. In this experimental system, the control of nutrition in the crop was based on the target composition of two nutrient solutions: a) The standard nutrient solution supplied to the crop, and b) the nutrient solution in the root zone. In the open system, the irrigation solution was simply prepared by adding standard amounts of fertilisers per litre of irrigation



water. In the closed system, the recycling of nutrient solution was managed by mixing the drainage solution with raw water at a ratio resulting in a target EC, followed by the injection of fertilisers on the basis of imposed EC values for the mixture and the outgoing solution to the controlling system. The target EC values were selected according to the concept of "drainage solution plus raw water" based on literature recommendations. (D. Neocleous, P. Polycarpou) https://www.adapt2change.eu/en/home

### **European Programme LIFE - ORGANIKO**

The ORGANIKO LIFE + project (http://ec.europa.eu/environment/life/projects/index.cfm? fuseaction=search.dspPage&n\_proj\_id=5354) started in September 2015 and its duration is 4 years. The aim of the project is to demonstrate the comparative advantages of organic versus conventional farming and their products, using indicators of mitigation efficiency to climate change, agronomic and environmental quality and decreased children exposure to diet-based pesticides. (M. Omirou, D. Neocleous, P. Dalias, A. Stylianou, S. Ioannidou, D. Fasoula, I. Ioannides)

### INTEGRATED WATER RESOURCE MANAGEMENT AND IRRIGATION

Research on integrated water resource 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 provinces of Cyprus. In addition, field and hydroponic experiments are carried out in order to evaluate xenobiotic compounds (pharmaceuticals) uptake by plants (tomato, lettuce and alfalfa).

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

Wastewater irrigation of forage crops and orange orchards is of pivotal importance for the Cyprus agricultural sector, since water shortages restrict any other water resource from being used for this purpose. Wastewater irrigation of ryegrass fields and orange orchards in Lefkosia and Lemesos districts caused no significant modulations in soil geochemical properties and heavy metal content, except for an increase of soil EC by an average equal to or lower than 0.98 mS cm<sup>-1</sup>, which could not affect soil sustainability and ryegrass plants and orange trees productivity in the long term. On the other hand, wastewater irrigation of clover fields modified some of the soils' physicochemical properties evaluated in the Larnaka district, as compared to the adjacent neighbouring rainfed fields. The most pronounced

impact of wastewater irrigation was the salinisation of the irrigated sites, since soil EC and Cl<sup>-</sup> content increased considerably. Soil salinisation in the Larnaka district may pose serious threats to clover crop production and economic sustainability, once clover is considered as a moderately sensitive forage crop. Therefore, the Larnaka district should be considered as a special case that needs to be handled properly, since the sealing of the sewage collection network could moderate the treated wastewater's salinity and allow the long-term wastewater irrigation of forage crops without considerable economic losses. In addition, the low heavy metal phytoavailability, due to the soil properties of the surveyed sites, appears to ensure the agricultural products' safety. Overall, proper management and monitoring are required in order to ensure the long-term environmental sustainability and public health safety of wastewater irrigation in Cyprus. (A. Christou, G. Maratheftis)

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

Advanced tertiary treatment and disinfection technologies have enabled the production of wastewater (WW) with quality complying with the established criteria for reuse in agriculture. We assessed the impact of tomato crop irrigation with two qualitatively distinct treated WW effluents, as compared to control tubewell water (TW) irrigation, on soil geochemical properties, tomato fruit safety and crop productivity. The treated effluents reused for irrigation were produced in two Municipal Wastewater Treatment Plants (MWTPs) utilising two discrete tertiary treatment and disinfection technologies, i.e. Slow Sand Filtration and chlorination (Lemesos-Moni WWTP), and Membrane Bioreactor and UV radiation (Anthoupoli WWTP), respectively. The impacts on soil pH, electrical conductivity, total organic C, Cl<sup>-</sup>, NO<sup>3-</sup>and heavy metal (Zn, Mn, Ni, Cu, Co) content were evaluated. In addition, the heavy metal content in tomato fruits and leaves, as well as the microbial load in fruit flesh and peel was determined. Crop productivity was measured by the mean fruit weight and maximum diameter, and by the number of fruits per harvest. Irrigation with either WW did not significantly affect the soil pH, organic C and heavy metal content, as well as crop productivity, in comparison to control TW irrigation. Furthermore, the heavy metal content of tomato fruits and leaves in all irrigation treatments was found to be below the maximum permissible levels set for fruit safety and the critical tissue concentration for phytotoxicity, respectively. Moreover, no microbiological contamination (total coliform, fecal coliform, Escherichia coli, Salmonella spp., Listeria spp.) of tomato fruits was found from any irrigation treatment. Overall, results obtained with regard to the parameters examined, strongly suggest that advanced tertiary treated effluent of good quality might be safely reused, in terms of both environmental sustainability and public health safety, for vegetable irrigation, concurrently promoting water use efficiency in dry areas. (A. Christou)

## Stress-related phenomena and detoxification mechanisms induced by common pharmaceuticals in alfalfa (*Medicago sativa* L.) plants

Pharmaceutically active compounds (PhACs) have been recently shown to exert phytotoxic effects. In a study we explored the uptake, systemic translocation and abiotic stress responses and detoxification mechanisms induced by the exposure of alfalfa plants to four common, individually applied PhACs (10  $\mu$ g L<sup>-1</sup>) (diclofenac, sulfamethoxazole, trimethoprim, 17a-ethinylestradiol) and their mixture. The plants were grown in sand under greenhouse conditions. Stress physiology markers and gene expression levels of key plant detoxification components were evaluated. PhACs were detected in significantly higher concentrations in roots compared with leaves. Stress related effects, manifested via membrane lipid peroxidation and oxidative burst, were local (roots) rather than systemic (leaves), and exacerbated when the tested PhACs were applied in mixture. Systemic accumulation of  $H_2O_2$  in leaves suggests its involvement in signal transduction and detoxification responses. Increased antioxidant enzymatic

activities, as well as upregulated transcript levels of GST7, GST17, H<sup>+</sup>-ATPase and CytcOx, propose their role in the detoxification of the selected PhACs from plants. The current findings provide novel biochemical and molecular evidence highlighting the studied PhACs as an emerging abiotic stress factor, and point the need for further research on wastewater flows under natural agricultural environments. (A. Christou)

#### SOIL FERTILITY IMPROVEMENT

Research on nitrogen fertilisation through organic amendments has been focused on the use of animal manure. Laboratory incubation studies are being carried out to estimate nitrogen mineralisation potential of manure in order to better schedule its application in soil assuring maximum crop uptake and protecting water quality. During the last year a new research activity has been initiated on the production of biochar from waste biomass, the characterisation of the produced material and the study of its use as a growing medium. (**P. Dalias**)

## Biochar production and preliminary trials to characterise its chemical, physical and biological properties

Biochar, a carbonaceous solid product of thermal decomposition of organic materials in absence of oxygen (pyrolysis) has received great interest due to its wide applications in agriculture and industry. Biochar is highly stable and can, therefore, be used as a carbon sequestration tool. As a soil amendment, it has significant beneficial effects on nutrient cycling, water balance, soil microbial community and activity, and on crop productivity. A pilot pyrolysis unit for the production of biochar was constructed at the premises of the Agricultural Research Institute. The unit has the capacity to produce about 80 L of biochar per load. It consists of a pyrolytic stove which is formed by two metal cylinders, one inside the other, and a system to burn the exhausted gases and collect the bio-oil produced. Biomass destined to become biochar is put inside the inner cylinder, where very little oxygen is available during the process, transforming organic materials into charcoal. The unit has been tested for the production of biochar coming from wood but also from manure or leaves and branches of greenhouse crops (tomatoes, cucumber and pepper plants). These materials as well as biochar and hydrochar produced elsewhere were chemically and biologically characterised. More precisely, they were tested for their stability, microbial respiration after incorporation in soil and seed germination. (**P. Dalias**)





## Mineralisation of nitrogen after soil incorporation of the main types of animal manure produced in Cyprus

The study aims at estimating the rate of mineralisation of nitrogen (N) after incorporating cow, sheep/goat, poultry or pig manure in soil and to establish a relationship between the N mineralisation rate and the degree of decomposition/composting of the manure storage pile prior to soil addition. The rate of mineralisation is estimated in soil-manure mixtures after incubating samples at optimal temperature and moisture conditions. The incubations of these mixtures are carried out in small plastic containers which are removed from the incubation chamber at successive intervals up to about three months. Soil inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N), that gradually accumulates in samples, is extracted at each time point with 2N KCl and determined by colorimetric methods. Nitrogen mineralisation data are then fitted in a single compartment exponential model of the type  $N_t = N_{\text{max}}(1 - e^{-kt})$  to determine the potentially mineralisable N  $(N_{\text{max}})$  and the mineralisation rate constant (k). The asymptote of N mineralisation curves  $(N_{\text{max}})$  obtained during the three month incubation at optimum conditions corresponds to the N release potential in the field during the growing season. Preliminary analysis of results showed that apart from poultry, samples from the other three manure types, when taken from fresh storage piles, showed significant net immobilisation of N, which lasted almost till the end of the incubation. This immobilisation phase was progressively shortened when piles remained for decomposition/composting for 3 or 6 months respectively. On the contrary, poultry manure always showed a net mineralisation phase and an increasingly greater proportion of its initial organic N content to be released as mineral N after 0, 3 or 6 months of manure composting. It is anticipated that data of mineralisation of N for the main types of animal manure produced in Cyprus will enable more precise calculation of the necessary quantities of manure that should be incorporated in the field depending on the needs of the crop, and consequently will contribute to a more rational use of the resource as a fertiliser and to less environmental concerns of nitrate leaching. (P. Dalias)

#### AGRICULTURAL AND ENVIRONMENTAL ENGINEERING

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, a large number of relevant EU and RPF Research Projects are described in detail below.

#### Application of renewable energy sources in agriculture

Greenhouse horticulture and floriculture heavily depends on some form of energy source, since heating during winter is absolutely necessary to maintain production. Heating, however, contributes to a considerable increase in production costs. Therefore, alternative energy sources, such as solar, wind, shallow geothermal and biomass energy, should be considered. The work carried out examined the possibility of using biomass from agriculture and forestry as a renewable energy source. Alternatively, energy plants are investigated so as to select plants that could be used as potential energy sources for solid fuel production, without interfering with the food or feed chain. Biomass can be processed in briquettes, pellets or loose chips. (**P. Polycarpou**)

#### Adaptation of agricultural production to climate change and limited water resources

The European Life+ project ADAPT2CHANGE: "Adapt agricultural production to climate change and limited water supply LIFE 09 ENV/GR/000296" started in September 2010 and is active until 2016. The TEI Larissa in Greece is the lead partner and the ARI is the Partner for Cyprus. The project is funded by 50% by the EU and additionally involves the University of Athens, TEI Piraeus, and Europliroforisi S.A. The overall project objective is to show the adjustment of agricultural production to climate change and limited water supply. In particular, it aims to reduce the water 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 the possibility of increasing agricultural production in a sustainable and environmentally friendly manner in rural areas in Greece and Cyprus, while reducing consumption of energy and natural resources such as water and increasing the income of farmers (**P. Polycarpou, D. Neocleous**).

### New technology in greenhouse plastic covers

The Project funded by the RPF "GreenFilm" was completed in 2014 and concerned the testing of new technology greenhouse plastic covers that involved nano-particles as pigments that offer special properties to the cover. (**P. Polycarpou**)



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

The European ENPI Project "MED-ALGAE" involves the study of the growth of micro-algae for the production of biodiesel. ARI is the Lead Partner coordinating the Project. Efforts have been currently intensified in order to find alternative fuels to replace petroleum products and reduce greenhouse gas emissions. One of the main problems with biodiesel is to have sufficient raw material available for production. A solution to this problem seems to be the cultivation of microalgae found in seawater or



wastewater. The microalgae have higher yield for biodiesel production than energy crops. The consortium consists of research organisations, academic institutions, energy agencies and private organisations from 6 countries: Cyprus, Greece, Italy, Malta, Lebanon, and Egypt. This is a new technology project which can contribute to the goals of 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 also to provide feasibility studies for the implementation of research findings. (P. Polycarpou, I. Ioannides, M. Omirou, P. Dalias)

#### FLORICULTURE/NATIVE PLANTS

Research activity focused on the evaluation of crop production systems and practices of ornamentals in the open field and/or greenhouses, covering all stages of flower production. More recently, the research activities have been focused on the evaluation of species from the Cyprus flora for potential use in commercial floriculture and landscape. Moreover, there is a close cooperation with the European Botanic Group Consortium, since the ARI represents Cyprus to the meetings of the consortium. During 2014-15, there was also a participation in a Leonardo Da Vinci project for transferring innovation in urban greening systems for the Mediterranean Region and also cooperation with the Estonian University of Life Sciences to study the genetic diversity and reproduction ability of a terrestrial orchid.

## Evaluation of endemic and native species of the Cyprus flora for potential use in commercial floriculture and landscaping

This research project was initiated in September 2015 in collaboration with the Department of Environment and the Forestry Department of the Ministry of Agriculture, Rural Development and

Environment, and the Frederick University, under the coordination of the Floriculture lab of ARI. The Mediterranean basin is characterised by a reach flora where unique species occur. Similarly, Cyprus due to the special climatic conditions, has developed a rich and unique flora with high percentage of endemism that accounts to 8.6%. These species are ideal for use in commercial floriculture as pot plants and in landscaping due to the fact that they are well adapted under the climatic conditions of Cyprus and possibly require a decreased amount of inputs when under cultivation. The aim of the project is the creation of a collection of different species from the Cyprus flora that have a potential use in commercial floriculture and the in-depth study and evaluation of these species. Specifically, research activities concern the collection of the material from the wild where these species naturally occur and the development of propagation protocols both for sexual (from seeds) and asexual (from cuttings) propagation. Furthermore, after the propagation of these plants, the growth and development of these plants will be studied and evaluated so as to create cultivation protocols. (L. Vassiliou)

### Joint research activity with the Department of Botany, Estonian University of Life Sciences for the terrestrial orchid *Anacamptis Pyramidalis*

The aim of this research project, which was initiated in January 2014 and concluded in December 2015, was to evaluate the genetic diversity and reproduction ability of deceptive terrestrial orchid Anacamptis pyramidalis in its distribution range, in Estonia, which serves as the northern border of the distribution area of this species, and in the populations located at the southern edge of the range, on the island of Cyprus. In addition, populations from Slovenia and Spain were evaluated. Fresh leaf samples were collected from four populations at the southern margin (Cyprus) in the spring and summer of 2014. A total of 17 populations throughout the distribution range of A. pyramidalis were collected from the other countries named above. Fresh leaf samples were dried and stored in silica-gel bags until DNA extraction. DNA was extracted from dried leaf material following the CTAB extraction protocol. The quality of the obtained DNA was evaluated and AFLP procedures were performed. The tests for the DNA were performed in the labs of the



Department of Botany of the Estonian University of Life Sciences. (L. Vassiliou)

## Lifelong Learning Programme, Leonardo Da Vinci, transfer of innovation. Project Title: "Urban Greening Systems for the Mediterranean Region" "UGreenS"

UGreenS (http://www.ugreens.eu/), a Leonardo Da Vinci Project was initiated in October 2013 and concluded in September 2015. It was a partnership of European Universities and Research Institutes engaged in research in the field of green roofs and living walls, with Small-Medium Enterprises engaged in the development and the implementation of such technologies in Europe. The aim of UGreenS was the introduction of the practical know-how for the implementation of Urban Greening Technologies, Green Roofs and Living Walls in the context of Sustainable Construction and Development, from countries with expertise in these domains (Spain, Greece and Malta) to Cyprus. The benefits of pertinent Urban Greening (UG) technologies can be categorised as environmental, aesthetic, social and economic. Environmental benefits include the mitigation of the heat island effect, mitigation of urban storm-water runoff, enhancement of urban biodiversity and thermal insulation of buildings. The economic benefits are related to energy savings produced by enhancing the energy efficiency of buildings. As a result of the aforementioned benefits, the sustainability of urban environments is significantly enhanced, contributing to the sustainable development of the country. The acquired knowledge was introduced to professionals (engineers, architects, agronomists and other scientists) in Cyprus through trainings and workshops that were delivered during the Project. (L. Vassiliou)

### RURAL DEVELOPMENT

The Rural Development Section of the Agricultural Research Institute conducts research aiming towards the sustainable development of the agricultural sector in Cyprus and the improvement of the quality of life of Cypriot farmers. The activities of the Section encompass topics related to agricultural economics, marketing and trade, as well as to the usage of Information and Communication Technology (ICT) for agricultural information sharing. Furthermore, work on precision agriculture includes topics on applications of ICT and robotic technology in agriculture and remote sensing technologies. The Section is also actively involved in several EU funded projects. Finally, the Section collaborates with the Ministry of Agriculture, Rural Development and Environment and undertakes targeted studies and technical reports on agriculture economics, trade and policy, supporting decision making.

#### **COMPUTER SCIENCE**

#### **Computers in agriculture - Information sharing**

The research project "Information Society in Rural Areas: Knowledge Sharing Using Information and Communication Technology" (http://ruraldev.ari.gov.cy) continues the study of current practices related to agricultural research information sharing and new opportunities that emerge by exploiting Information and Communication Technology (ICT). In late 2015, a new study was initiated to investigate the feasibility of developing an online e-learning platform, within the framework of the Rural Development Programme 2014-2020. A structured questionnaire was prepared and a representative sample was selected. Personal interviews with farmers and meetings with focus groups (agronomists) are scheduled for 2016. (G. Adamides, A. Stylianou)

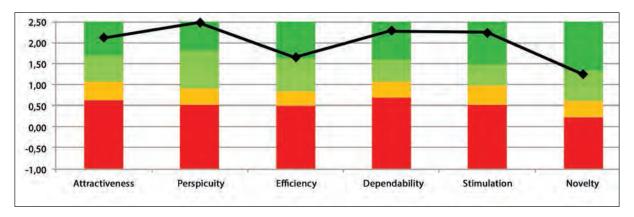
#### **Robotics in agriculture**

The research project titled "Semi-Autonomous Vineyard Agricultural Sprayer" (SAVSAR http://savsar.gr) was implemented during July 2014 - October 2015. This work was financed by the Greek General Secretariat for Research and Technology. Semi-autonomous teleoperation of an agricultural robotic system can help to effectively manage the complexity and performance limitations that current autonomous robots face due to the dynamic and unstructured agricultural environment. Semiautonomous tele-operation implies the existence of a user interface that supports human-robot interaction (HRI). Such a user interface needs to meet specific non-functional requirements, such as reliability, efficiency and usability. A field experiment took place at the Experimental Station of the Agricultural Research Institute at Saittas, Cyprus and involved end-users (farmers). The main goal of this experiment was to evaluate the user experience of the final version of the user interface. Five participants took part in the experiment; 3 male and 2 female with an average age of 38.8. This number of participants is adequate to uncover the most important usability issues, particularly in systems with specialised users or users that are hard to find/reach in specific times, as in our case. Participants were asked to follow a user scenario in order to move the robot along a path and spray identified targets. During the participants' interaction with the system, the following measures were documented: a) time on task, b) number of targets sprayed, and c) number of collisions. After the experiment, participants were asked to complete three questionnaires: a) A questionnaire to collect demographic data, b) the System Usability Scale (SUS), and c) the User Experience Questionnaire (UEQ). Both SUS and UEQ are standardised questionnaires that provide reliable and valid results in terms of the constructs they measure.

In terms of interaction effectiveness, all participants had a task success rate of 100% in both spraying the identified targets and managing to avoid collisions (0 collisions with obstacles for all participants).

Interaction efficiency was measured as the time required (in seconds) to complete the whole task, that is to navigate in the robot pathway, approximately 50 meters, and to spray the four targets. The average time for this was 330 seconds (5.5 minutes).

In terms of perceived usability, the average SUS score for the system was 74.5. According to a dataset of over 3,500 surveys and 273 studies, the evaluated system is characterised as "good to excellent". Regarding overall user experience the system was evaluated positively (>0.8) on the UEQ scales. Comparisons with existing benchmark data for UEQ showed that SAARSv2 was perceived as "excellent" in terms of attractiveness, perspicuity, efficiency, dependability, and stimulation, and "good" in terms of novelty. All in all, SAARSv2 was rated among the 10% best results in all but one (novelty) of the subscales. (G. Adamides)



#### AGRICULTURAL ECONOMICS

#### **Technical Reports and Policy Papers**

During the period 2014/15 the staff of the Rural Development Section was actively involved in the preparation of several technical reports and policy papers, most of them commissioned by the Ministry of Agriculture, Rural Development and Environment.

The creation of a policy paper titled "Contract Farming in Cyprus" was initiated and completed in 2015 aiming to the development of a National Action Plan for the promotion of contract farming in the agricultural sector. The paper included, among others, a detailed description of contract farming, a number of success stories, the possibility of contract farming implementation in the Cypriot agricultural sector and proposed actions for its promotion. (**A. Stylianou**)

The Section is also involved in the development of a policy paper for the promotion of family farming in Cyprus. The paper aims to promote family farming in Cyprus and to raise public awareness of products derived from family farming through a number of different actions. The development of the policy paper started in 2015 and will be completed in early 2016. (**G. Papadavid, A. Stylianou**).

A study titled "Water Management and Crops Restructuring" was conducted by the Agricultural Research Institute in collaboration with the Departments of Agriculture, Environment, Water Development and Meteorology. The main goals of the study were the reassessment of water and irrigation crop needs, the review of the water pricing policy and the estimation of crop water productivity expressed in physical and economic terms. Furthermore, the study provided basic guidelines for better crop water management. (M. Markou, G. Papadavid, D. Neocleous, A. Christou, A. Stylianou)

Within the framework of the Strategic Plan for the development of the Floricultural Sector in Cyprus, a study titled "Consumer Buying Behaviour towards floricultural products in Cyprus" was conducted by the Rural Development Section in collaboration with the Department of Agriculture. The main goal of the study was to examine consumers' attitudes, preferences, intentions and decisions for cut flowers

and pot plants. For that purpose, a sample of 500 consumers was determined. A self-administered questionnaire was used as a main research tool. The final number of questionnaires suitable for statistical analysis was 370 giving a high response rate (74%). The analysis showed that 66% of the consumers buy cut flowers and 85% pot plants, while the average consumer spends €139 per annum on floricultural products. Moreover, the two most important factors influencing consumers' repeat purchase behaviour were the quality and the price of the products (Table 1). Among the different types of cut flowers, the consumers indicated that the most expensive ones are orchids and birds of paradise (*Strelitzia reginae*), whereas the least expensive are carnations and chrysanthema. Regarding consumers' preferences, the analysis showed that older consumers prefer to buy common types of cut flowers such as carnations and roses, while younger consumers prefer uncommon ones, for instance tulips and orchids. Finally, as expected, a positive statistically significant relationship was found between consumers' income and money spent for floricultural products. In other words, consumers with high income are more likely to spend more money for cut flowers and pot plants. In contrast, no statistically significant relationship was found between educational level and money spent on floricultural products. (A. Stylianou)

Factor	0/0	Rank
Quality of cut flowers and pot plants	24.8	1
Price	23.2	2
Distance from home	14.4	3
Wide variety of flowers	10.4	4
Florist's technique in flower arrangement	5.6	5
Florist's attitude	5.6	6
Flower's durability	4.0	7
Flower shop's offers	4.0	8

Table 1. Factors influencing consumers' repeat purchase behaviour

#### Investigating the sustainability of the Agricultural Production System in Cyprus

The goal of this research project is to examine the economic sustainability of the current Agricultural Production System in Cyprus and its sub-systems, using advanced multivariate statistics and econometric models. To achieve this goal, quantitative and qualitative research methods (multi-strategy design) will be integrated in order to increase the reliability of the results. Within the context of quantitative approach, a representative sample of 354 farmers was determined by using proportionate stratified random sampling method. To obtain the necessary primary data, a long and structured questionnaire was developed, including closed-ended and open-ended questions, most of them related to farm economics. After face-to-face meetings with the farmers, 324 fully completed questionnaires were collected. The quantitative research is a work in progress and is expected to be completed in late 2017.

The qualitative analysis was completed in 2015. Within the context of the qualitative approach, semi-structured face-to-face interviews were conducted with 100 farm holders. The basic qualitative research tool was an Interview Guide including a number of open-ended questions. All interviews were also audio-recorded and then transcribed into text format. The method of qualitative content analysis was used for the processing and interpretation of the data. The results showed that some of the most important and traditional sub-systems of the agricultural production system in Cyprus, like the sheep and goat system, may not be sustainable. This may be, mainly, attributed to the high input prices and the relatively low output prices, the lack of organisation of the agricultural holdings and to marketing and trade problems,

in conjunction with the recent financial crisis and its relevant consequences (Table 2). Producer Groups and Organisations may significantly contribute to the sustainability of the agricultural sector in Cyprus, by including qualified staff in their workforce and, at the same time, by following the guidelines of the State. The provision of additional incentives to the farmers is also considered to be an important requirement for the sustainability of the sector. (A. Stylianou)

**Table 2:** Main issues for Agricultural Production Systems in Cyprus, in order of importance (1=the most important)

Issue	Rank
High input prices / high production cost / low output prices	1
Delayed payments / trade and marketing problems	2
Financial crisis / reduced consumption / lack of liquidity	3
Irrigation water shortage / extreme weather events	4
Lack of organisation among farmers	5
Competition with cheaper imported products	6
Ageing of farm population / farm succession problem	7
Low educational level	8

### **Agricultural Education and Training**

The Rural Development Section provides training to the staff of the Department of Agriculture and to farmers in topics related to agricultural economics, farm management and agricultural marketing. In 2015, the staff of the Section was responsible for developing an important part of the training material which will be used in the framework of the Rural Development Programme 2014-2020 for training farmers. The material developed by the Section concerns the thematic units "Agricultural Production Economics" and "Marketing of Agricultural Products". In 2016, the staff of the Section is expected to train the staff of the Department of Agriculture and the farmers on the aforementioned topics.(A. Stylianou, G. Papadavid)

#### **EU FUNDED PROJECTS**

The Rural Development Section coordinated the Lifelong Learning Programme project "Improving skills for Smartfarming as an innovative tool for rural development and economic growth" (http://www.smartfarmerproject.eu). The project was completed in 2015 and was 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 included education and training organisations and authorities responsible for rural development, thus allowing the merge of different views and experiences. The main outcome of this project was the development of an e-learning platform integrating the curriculum and learning material authored by the SmartFarmer project partners. The online training material consists of three (3) modules which give basic information about Strategic Planning and Marketing of "smart" organic agro-foods, Marketing Mix and Organic Farming, Quality Schemes and Eco-agriculture. The SmartFarmer training material can be used for individual, self-directed learning or as supporting material in training courses. The e-learning platform along with an e-book is available at http://e-platform.smartfarmerproject.eu/ (G. Adamides, A. Stylianou, G. Papadavid, D. Neocleous)

Moreover, the staff of the Rural Development Section is actively involved in two LIFE+ projects initiated in late 2015. The first project titled "Adaptation to Climate change Impacts on the Mediterranean islands' Agriculture" (ADAPT2CLIMA) aims to increase knowledge on the vulnerability of EU

Mediterranean agriculture to climate change and to support decision making for adaptation planning. The methodology is based on the deployment of a set of climate, hydrological and crop simulation models for the assessment of climate change impacts on agriculture, as well as on the development of a decision support tool for the elaboration of adaptation strategies for the agricultural sector. The National Observatory of Athens is the coordinator of the project, while the project partners are the Agricultural Research Institute, the Institute of Biometeorology of the National Research Council of Italy, the National Technical University of Athens, the Department of Agriculture, Rural Development and Mediterranean Fisheries of the Region of Sicily (Italy) and the Region of Crete (Greece). (M. Markou, G. Papadavid, A. Stylianou)

The other LIFE+ project titled "Revamping organic farming and its products in the context of climate change mitigation strategies" (ORGANIKO) aims to demonstrate the comparative advantages of organic versus conventional farming and products using indicators of mitigation efficiency to climate change, agronomic and environmental quality, and decreased children exposure to pesticides, promoting healthy food for better children's health. The project is coordinated by the Cyprus University of Technology, whereas the Agricultural Research Institute, the Department of Environment and the Kyoto Club are project partners. (M. Omirou, D. Neocleous, P. Dalias, D. Fasoula, A. Stylianou, S. Ioannidou, I.M. Ioannides)

#### REMOTE SENSING LABORATORY

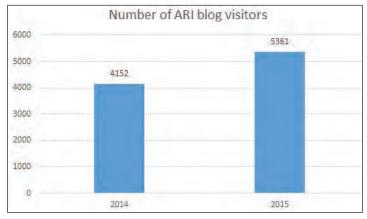
The Remote Sensing Laboratory (RSL) was established at the Acheleia Experimental Station in order to provide scientific data for applications in agriculture and environmental research. The Laboratory has been involved in two research projects regarding crop evapotranspiration and its contribution is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

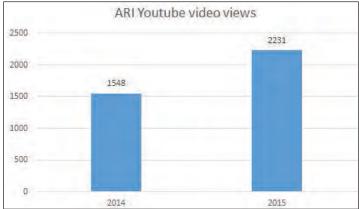
The main purpose of the research project "Effects of Climate Change on irrigation cost of water-intensive cultivations in Cyprus, using remote sensing" is to determine the impact of climate change on crop irrigation needs and how this change affects producers' income. Another research objective is to 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)

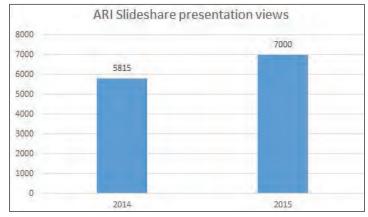
#### **DISSEMINATION ACTIVITIES**

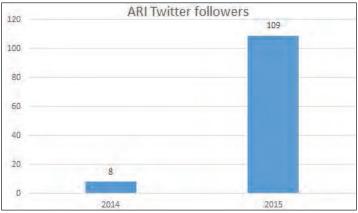
The Section is responsible for maintaining the ARI website and various social media network services. ARI publications since 1965 have been digitised and full-text articles are available from the Institute's website <a href="http://www.ari.gov.cy">http://www.ari.gov.cy</a>. Likewise, articles published in the "Agrotis" magazine by ARI research officers, are available in electronic format. An electronic newsletter (ARI e-Newsletter) is issued quarterly (January, May, August, and October) each year and is sent to subscribers by email; it is also available on our website.

The Rural Development Section makes use of several social networking tools such as Facebook, <a href="http://www.twitter.com/ari\_rd">http://www.twitter.com/ari\_rd</a>, YouTube <a href="http://www.twitter.com/ari\_rd">http://www.twitter.com/ari\_rd</a>, YouTube <a href="http://www.slideshare.net/ARIWebinars">http://www.slideshare.net/ARIWebinars</a>, and maintains also a web blog at <a href="http://blog.ari.gov.cy">http://blog.ari.gov.cy</a>. 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. It's worth mentioning that there is a steady increase of the users-visitors in our social networking tools. (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. 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. Research on traditional products derived from grape juice like sutzioukkos, kiofteri and palouzes has been initiated. The impact of treated wastewater use on the microbial load of fruits and vegetables has been explored. Bacteria able to degrade fungicides and herbicides have been isolated and characterised. Screening of local isolated microalgae strains was performed in order to isolate efficient and promising strains for biofuel production and added value co-products. The section research activities also involve participation in different EU funding projects such as HORIZON2020, LIFE+ and FACCE-SURPLUS.

## MOLECULAR PLANT-MICROBE INTERACTIONS AND ECOLOGY OF AGRICULTURAL ECOSYSTEMS

#### The efficiency of symbiotic nitrogen fixing bacteria in cowpea

Cowpea (Vigna unguiculata) cultivation is one of the most important legume crops in Cyprus. Its capacity to establish relationships with symbiotic nitrogen fixing bacteria is crucial for the reduction of production costs and the risks of the excess use of nitrogen fertilizers. This study assessed the efficiency of new strains of symbiotic nitrogen fixing bacteria isolated from local cowpea varieties. Totally, thirty strains have been isolated from different cowpea genotypes and evaluated under axenic conditions about their impact on cowpea growth. From these strains, two have been scored as the most promising and selected for further evaluation. A pot experiment using sterile sand as a substrate was employed and a completely randomised design with 10 replicates was implemented to test the efficiency of the inoculation of ARIbrad#7-12-5, ARIbrad#9-17-3 on cowpea performance. The aforementioned strains were compared to a strain ARIbrad#2-15-1 with no effect on cowpea and non-inoculated controls (with and without mineral nitrogen). The highest biomass and N content measured in plants received mineral nitrogen followed by the inoculation treatments. The lowest performance was noticed in non-inoculated plants grown under nitrogen free conditions. Significant correlations between nodule numbers, dry biomass production and N content were calculated demonstrating the beneficial effect of inoculation on cowpea performance. Our group is currently evaluating the ability of these strains to promote cowpea productivity using less nitrogen fertilisers under field conditions. (M. Omirou, D. Fasoula, I.M. Ioannides)

#### Soil microbial community structure in different agricultural systems

It has been debated that agricultural systems influence the structure of soil microbial communities, which are the core stone for soil fertility. In this research our aim is to gain further insight into how organic and conventional agricultural systems influence soil microbial communities. A factorial experiment (2x3) consisted of the 2 systems and 3 crop rotation schemes established at the experimental Station of Acheleia. The experiment is a randomised complete block design in a split-plot arrangement with 3 replicates. First results showed that both factors have a significant effect on soil microbial communities while this is also related to phosphorus levels in soils. The research continues and the results are expected to reveal the differences between the two systems and test the hypothesis that specific

microbial guilds are favored by crop rotation scheme. The study is partially funded by the European Union in the context of the LIFE+ORGANIKO (2015-2019) project. (**M. Omirou, D. Fasoula, I.M. Ioannides, P. Dalias**)

### Diversity of symbiotic nitrogen fixing bacteria and mycorrhiza in different species

The symbiosis of cultivated plants with functional soil microbial guilds is extremely important for agroecosystems productivity especially in low input agricultural systems. In this study we started the description of the abundance and the diversity of symbiotic nitrogen fixing bacteria and mycorrhiza in different underutilised cereals, maize, chickpeas and faba beans. The study is funded by the European Union in the context of the Horizon2020 project with the acronym "DIVERSIFOOD" (2015-2019). (M. Omirou, D. Fasoula, I.M. Ioannides)

#### FUNCTIONAL FOODS AND FOOD SAFETY

#### 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 and antioxidant properties. The objective of our research was to evaluate different extracts of plant origin to determine their antioxidant activity. The evaluation was performed using two different screening methods: DPPH and RANCIMAT. Methanolic extracts from rocket leaves (*Eruca sativa*) and essential oil from mint (*Mentha spicata*) extracted by hydrodistillation have been tested. We tested various concentrations of the methanolic extract and essential oil against the recommended concentration of the synthetic antioxidant TBHQ (0.02%). The results showed that although the methanolic extract and essential oil presented considerable antioxidant activity, this was significantly weaker than that of the synthetic antioxidant. (G. Maratheftis, M. Omirou)

### The impact of wastewater irrigation on crop products safety

The availability of clean and high quality water has been recognised 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 strawberries and lettuce with tertiary-treated water is associated with increased human health risks. The microbial quality of strawberries and lettuce produced using treated urban wastewater and tube well water were compared at experimental sites in Lefkosia. Contamination was estimated by enumeration of Total Aerobic microorganisms, Total Coliform and the absence of human pathogens *E. coli*, *Salmonella* spp. and *Listeria* spp. Our studies showed no microbial contamination of strawberries and lettuce with these human pathogens. (G. Maratheftis, A. Christou)

### Impact of drought stress on Eruca sativa Mills secondary metabolites

Rocket salad (*Eruca sativa* Mills) is one of the major leafy vegetables produced worldwide and has been characterised as a rich source of chemoprotective glucosinolates (GSL). A pot experiment was conducted applying drought stress for 11 days while plants normally irrigated served as control. Drought

stress had a detrimental effect on *E. sativa* performance. Photosynthetic machinery was significantly suppressed while total N and P content in rocket leaves were reduced. Glucosinolate content and their corresponding biosynthetic genes are under evaluation. (**M. Omirou, D. Fasoula, I.M. Ioannides, A. Christou**)

## Chemical composition and microbial guilds of traditional products from grape juice: Sutzioukkos, kiofteri and palouzes

Traditional products obtained from grape juice such as sutzioukkos, kiofteri and palouzes constitute an economically important sector of Cyprus rural areas. The aim of this study was to identify the chemical composition of these products and evaluate their microbial community structure. Our hypothesis was that the use of different local varieties (Xynisteri and Mavro) during the preparation of these products affects their chemical and microbiological characteristics. During this study we also recorded the preparation process that is implemented by the producers of these products. The grape variety used for the preparation of these products has a significant effect on their chemical composition and especially in polyphenols (anthocyanins and stilbenes). Finally an analytical method for the detection of the addition of sucrose was developed and tested in real samples. The study continues and it is expected to be a useful tool for the producers of these traditional products. (G. Maratheutis, I.M. Ioannides, M. Omirou)

## Isolation and characterisation of pesticide degrading microorganisms and their formulation

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. Several bacterial species have been isolated and screened for their ability to metabolise 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. The degradation product of TBZ derived from *Acinetobacter* activity was identified. The active strains are currently evaluated in different formulations in order to scale up their application in biobed systems. (M. Omirou, I.M. Ioannides)

### BIOTECHNOLOGICAL MITIGATION TOOLS FOR CLIMATE CHANGE

#### Screening of marine microalgal species for the production of biodiesel

Biodiesel production gained increased attention during the last decade due to the energy crisis and the increased prices of fossil fuels. In the current study we developed 8 blooms from 2 coastal areas of the Republic of Cyprus. We also isolated the dominant species found in these blooms. All blooms exhibited high amounts of saturated and mono-unsaturated FAME. The sum of these types of FAME was more than 60% while the major type of FAME detected was palmitic, palmitoleic and oleic acid. The most promising bloom was BL1LCA and the dominant species found was *Nannochloropsis* sp. The biomass and lipid productivity measured for this bloom was 0.727 and 0.155 g.L<sup>-1</sup>.day<sup>-1</sup> while the major fatty acid found was palmitoleic acid. However, BL1LCA exhibited the highest amount of eicosapentanoic acid (19 mg.g<sup>-1</sup> dw) and accounted to the 17% of the total FAME detected. (**I.M. Ioannides, M. Omirou, P. Polycarpou**)

#### The use of wastewater for the production of biodiesel

In the current study we examine the growth characteristics of microalgal blooms and the corresponding dominant species on wastewater in order to minimise the cost of the scale up process. A completely randomised experimental design was employed and both B11LCA and *Nannochloropsis* sp. were grown in different ratios of seawater and wastewater. Increased amount of wastewater content in the growth medium resulted in a significant reduction of lipid content and FAME concentration as well as biomass productivity. Our study clearly demonstrated that the general assumption that wastewater can be used as an alternative nutrient source for microalgal growth must be used cautiously since species-specific response seems to take place. (M. Omirou, I.M. Ioannides)

## Bio-prospection and industrial opportunities for biodiesel production in Cyprus using promising microalgal blooms

Detailed studies were performed using a microalgal bloom BL1LCA developed in ARI and its dominant species that was characterised as a *Nannochloropsis* sp. Their biomass productivity, lipid and FAME content were carefully monitored in open ponds and flat-bet PBRs during 3 seasons. Differences were noticed between season and type of culture (PBRs and open ponds). In particular, biomass productivity was higher during the summer while oil content was higher during the winter. Both *Nannochloropsis* sp and BL1LCA exhibited higher biomass productivity in PBRs compared to open ponds in all seasons. Two different biodiesel production scale-up scenarios were implemented. Both scenarios considered the use of 2000 m³ medium/ha for the cultivation of blooms in open ponds. For the flat bed PBRs the first scenario assumed the use of 600 m³ of medium/ha and the second scenario the use of 450 m³ of medium/ha. A 2-way ANOVA was conducted for both scenarios and significant main effects and interactions between culture method and season were noticed (p<0.01). For the first scenario, the annual biodiesel production using PBRs is estimated to be 6.3 tn/ha compared to 4.5 tn/ha/year productivity of BL1LCA bloom grown in open ponds. (M. Omirou, I.M. Ioannides, P. Polycarpou)

#### Algal treated biomass: Added value remaining waste product?

Understanding the chemical composition of microalgal biomass is of great importance since microalgae have the potential to contribute significantly to the biofuels pools. This knowledge will give us the opportunity to evaluate the most potential viable strategy for converting algal biomass into lipid-based and carbohydrate-based biofuels. In addition, the chemical composition of the processed microalgal biomass will also provide useful knowledge about the added value of this biomass. The scope of the current study was to evaluate the chemical composition of the biomass of several microalgal species and the blooms developed throughout MED-ALGAE project as well as the biomass cake produced after biodiesel production. Our results clearly showed a different chemical composition between the developed blooms and their dominant species. Biomass process resulted in a detrimental effect on the chemical composition of microalgal biomass. A 30 to 81% increase of protein content was noticed in microalgal biomass previously treated for biodiesel production depending on the microalgal bloom and the dominant species. Our results indicate that the remaining treated biomass could be a valuable protein resource and more research is needed to reveal possible uses of the waste. (**I.M. Ioannides, M. Omirou**)

## Athrospira platensis: the growth response and chemical characteristics in tertiary treated wastewater

An imperative need for the development of new clean energy resources has emerged in order to sustain natural resources and satisfy our future demands. Renewable energy resources are crucial to avoid

environmental degradation caused by greenhouse gas emissions, climate change as well as fossil fuel exhaustion. Athrospira platensis has been extensively studied in the last decades due to its commercial applications as a source of proteins, vitamins, amino acids and other added value secondary metabolites. The mixotrophic properties of A. platensis classified the organism as one of the most appropriate microalgae for wastewater treatment since its contribution to organic compounds removal from wastewater is significant. In the current study we evaluated growth characteristics of A. platensis as well as FAME, total lipids, and chlorophyll concentration of the cyanobacterium, grown under different tertiary wastewater concentration under two different temperatures simulating seasonal variations. Wastewater addition as well as temperature had a significant effect on biomass productivity, growth rate and chlorophyll content of A. platensis. Two – way ANOVA also revealed significant interactions between the treatments. The major FAME found was C16:0 and ranged from 8.01 to 48.19 mg.g<sup>-1</sup> dw. The unsaturated fatty acids C18:2ω3 and C18:3ω3 were also found to be major components of the total FAME detected and their concentration ranged from 4.40 to 23.59 mg.g<sup>-1</sup> dw and 6.73 to 21.37 mg.g<sup>-1</sup> dw respectively. Our study clearly demonstrated that tertiary treated wastewater could be used as an alternative water source for A. platensis growth. Future research however is needed to explore the public health safety issues as well as the impact of this treatment on secondary metabolites and protein content and quality. (I.M. Ioannides, M. Omirou)

### Characterisation 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. Genetic analysis of blood samples of the Chios sheep (755 samples) continued in 2014/15, 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 2014/15, 789 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, G. Hadjipavlou**)

### **VARIETY EXAMINATION CENTRE**

According to EU legislation, commercialisation of seeds for sowing is only allowed for varieties that are registered to the National Catalogues of the Member States and/ or to the Common European Catalogue. Research activity at the Variety Examination Centre focused on the execution of field trials required for the registration of varieties to the National Catalogue. For the purposes of registration, an application must be submitted to the Designated Authority (Department of Agriculture) by the breeder, the maintainer or their authorised 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 complete 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 2013/14, two triticale varieties (TR4 and TR5) were subjected to trials for the first year and one barley variety (Politico) for the second year. Also one wheat variety (Gavdos) and one durum wheat (Ourania) were tested to renew their registration in the National Catalogue. In the 2014/15 growing season, the triticale varieties TR4 and TR5 were tested for the second year and one barley variety (Kalopsida) and two durum wheat (Josephina and Hekabe) were tested to renew their registration in the National Catalogue. The results were reported to the competent Authority (Department of Agriculture). (**K. Mina**)

#### **Value for Cultivation and Use trials**

The Variety for Cultivation and Use (VCU) trials are conducted at several sites representing the environments where the main rainfed crops are grown. They last for at least two consecutive growing seasons wherein grain yield, yield related characteristics and quality traits are examined. During the 2013/14, the barley variety Politico and triticale varieties TR4 and TR5 were tested for the second and first growing season, respectively. In the 2014/15, the two triticale varieties (TR4 and TR5) were tested for the second growing season. The results were reported to the competent Authority (Department of Agriculture). (**K. Mina**)

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#### **Other Publications**

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Kyriacou, M.C., and G.A. Soteriou. 2015. Rootstock mediated effects on watermelon field performance and fruit quality characteristics. Enimerotiko Deltio 5. Agricultural Research Institute Publications (ISSN 1986-1370). Nicosia, Cyprus (in Greek). 32p.

Orfanidou, C.G., L.C. Papayiannis, L. Lotos, C. Dimitriou, E. Diogenous, V.I. Maliogka, and N.I. Katis. 2015. Etiology of yellows disease in cucurbit crops in Greece. *Hellenic Plant Protection Journal* 8:48-49.

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Papayiannis, L.C., A. Kyriakou, and T. Kapari-Isaia. 2015. Detection and characterization of *Citrus tristeza virus* (CTV) in Cyprus. *Hellenic Plant Protection Journal* 8:49-50.

Papayiannis, L.C., and Theodora Kapari-Isaia 2015. Viroids: The microscopic and dynamic pathogens of plants. Enimerotiko Deltio 6, Agricultural Research Institute Publications (ISSN 1986-1370). Nicosia, Cyprus (in Greek). 28p.

### **SEMINARS**

A total of seven seminars and day conferences were presented in 2014 and sixteen in 2015. The seminars were chaired by Mr. A. Kyratzis and Mrs. M. Emmanouilidou

January 16, 2014	V. Symeou: Innovative approaches to increase phosphorous digestibility in pig farming
February 7, 2014	P. Polycarpou, I. Ioannides, M. Omirou: Preliminary results of the project "MED ALGAE". Biofuel production from microalgae – Day conference
March 13, 2014	M. Emmanouelidou: Olive culture, current trends of a traditional crop
March 20, 2014	L.C. Papayiannis, T. Kapari-Isaia: Viroids, the smallest but extremely infectious plant pathogens
April 11, 2014	J. Eitzinger: Trends and future challenges in agrometeorology
September 9, 2014	C. Zoumides, A. Bruggeman, S. Pashiardis, Z. Zomeni, C. Camera, S. Gregoriou, M. Markou: Options for sustainable agricultural production and water use in Cyprus under global change "AGWATER" project. Day conference
September 17, 2014	A. Kyratzis, C. Nikiforou, T, Papachristoforou: Results of the project "Ensuring the survival of endangered plants in the Mediterranean"
January 29, 2015	V. Symeou: Recent developments in pig nutrition and management
February 4, 2015	E. Maloupa, G. Tsoktouridis, C. Stavridou: Aromatic and Medicinal Plants – Day Conference
February 13, 2015	H.F. Paulus: Sexual Deception – Tricks in the pollination biology of the Mediterranean Orchid Genus <i>Ophrys</i>
March 20, 2015	S. Harbaz: Innovative agricultural research conducted at the Agricultural Research Organization, Israel, and methods for its implementation
April 30, 2015	G. Hadjipavlou: The Cyprus sheep and goat sector: Results from the DoMEsTIc project on economics and social aspects
May 5, 2015	T. Vassilev: Agricultural Academy – organization structure, research activities and priorities. Participation of the Agricultural Academy in the new EU program for education, training, youth and sport "ERASMUS+" – current and future projects
May 5, 2015	I. Tsevetkov: Agrobi institute – current state and development prospects.
May 25, 2015	P. Polycarpou, C. Karapitta, M. Omirou, I, Tzovenis, C. Kordatos, C. Maragos: Biofuel production from microalgae in the Mediterranean, Results of the MED –ALGAE project – Day conference
June 17, 2015	C. Kittas: Intelligent crop-based environmental monitoring and control of sustainable greenhouse eco-systems
June 19, 2015	L.C. Papayiannis: Diagnosis of new diseases caused by viruses and viroids in Cyprus

Seminars	
June 25, 2015	M. Kyriacou: Short training course on postharvest physiology and technology of horticultural products
July 10, 2015	D. Neocleous, M. Douglas: Adapt agricultural production to climate change and limited water supply – Day conference
September 24, 2015	T. Kapari-Isaia: Methods for elimination of viruses and related pathogens from plant propagating material
December 4, 2015	G. Lemesios, M. Markou, A. Karali, M. Papadopoulou, L. Brilli: Launching event "LIFE2014 - Adapt2clima project"
December 9, 2015	V. Symeou: Use of aromatic and medicinal plants from Cyprus in pig nutrition
December 18, 2015	A. Kyratzis, C. Nikiforou: Crop Wild Relatives. Results of the recent research activities