



REPUBLIC OF CYPRUS

**AGRICULTURAL RESEARCH INSTITUTE**

Center of Excellence  
in Agriculture and Environment

# REVIEW FOR 2020-2021



MINISTRY OF AGRICULTURE,  
RURAL DEVELOPMENT AND THE ENVIRONMENT  
LEFKOSIA

FEBRUARY 2023



REPUBLIC OF CYPRUS

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## **THE AGRICULTURAL RESEARCH INSTITUTE**

The Agricultural Research Institute (ARI) was established in 1962 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. It was entrusted to the Government of Cyprus in 1967 and 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 Center of Excellence in Agriculture and Environment.

The ARI is organised 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 Center. 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 31 scientists, specialised in various disciplines of plant and animal production, 31 technicians, six administrative and accounting personnel, as well as 61 permanent labourers. 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 implemented 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 databases of the AGRIS and CARIS systems.



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## DIRECTOR'S REPORT "In Search of Excellence"

In view 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 Organization of the United Nations, the International Atomic Energy Agency, the International Centre for Agricultural Research in Dry Areas (ICARDA), the International Potato Centre, the International Centre for Advanced Agronomic Mediterranean Studies (CIHEAM), and numerous academic institutions. Supported by its wide network of cooperation, the ARI has augmented its efforts for providing solutions to problems arising from the management of agricultural systems and of their footprint on the environment, and for providing novel methods and tools toward 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 Research and Innovation 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, utilising 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 on 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 local pomegranate and carob genotypes. Landmark work has been implemented in characterising the genetic and phenotypic variability of indigenous carob genetic resources and in assessing the impact of cultivation zones on the composition and bioactive traits of carob pods. These results have been exploited for the establishment of an ex situ collection of select carob genotypes. 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 have been evaluated, and in citrus, research work on rootstock evaluation continued. 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 has been pursued. Genebank and

herbarium activities focused 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. 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 were utilised for the prediction of market trends for crop and animal products. 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 constitutes a landmark recognition reflecting on the high standard of scientific research conducted, and on ARI's contribution to agricultural development and the protection of the environment. It is a pleasure for me to acknowledge the tireless efforts of both the ARI staff and their commitment to quality research, which remains the foundation for all future achievements.



**Dr Dora S. Chimonidou**  
Director

## PLANT IMPROVEMENT

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

Barley is the cereal most adapted to the often-harsh agroclimatic conditions of Cyprus. 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, originated from the Triticale improvement programme, were sent for testing and are now under registration procedures to the National Catalogue.

The genetic improvement of Cyprus cowpea landraces with respect to climate change is a long-term programme aiming at the enhancement of adaptation of this important crop using innovative field phenotyping methods. The project has further successfully 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 has progressed 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 consumer health, are studied as alternative crops for Cypriot farmers.

## CROP IMPROVEMENT

### Breeding barley for grain and hay under conventional and organic agriculture

A major aim of the barley breeding programme, both for conventional and organic agriculture, is the creation of varieties that are density-neutral or density-independent, in order to make efficient use of the limited resources in Cyprus environments. Other specific aims include the development of cultivars with improved adaptation to climate change, improved drought and heat 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 stability of performance and reduced interaction with the environment has been a priority. An additional priority is the development of varieties appropriate for organic agriculture with increased tillering capacity and other specific traits using an innovative field selection methodology based on the unique properties of the Honeycomb Selection Designs and the prognostic breeding paradigm. Trials were performed in five different locations across the island, employing a series of crosses 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 six-row barley cultivar, named Myrtou, with improved biomass and tillering capacity has been subjected to the official national tests and is going through the final stages of registration to the National Catalogue, while a new naked barley variety is also waiting for the final approval after passing the official registration tests. In



*A view of the novel barley spike mutant phenotype that is associated with improved field performance*

addition, three new barley lines with constantly good performance in Cyprus conditions have entered the national trials as well. Innovative breeding approaches are being developed. An important component of the barley and cowpea breeding programmes is bridging the gap between genotype and phenotype, employing novel methods of precision phenotyping at the single plant level grown under the Honeycomb Selection Designs and distances that eliminate the masking effects of interplant competition and soil heterogeneity. In this respect, a novel barley mutant, identified through our specific trials, was found associated with improved performance and is being further characterised. **(D.A. Fasoula)**

### National Management Committee Member for the COST Action CA16212 INDEPTH: “Impact of Nuclear Domains on Gene Expression and Plant Traits”

This Action recognises the ability of plants to rapidly adapt their genomic expression in response to environmental changes and explores the epigenetic modifications in plant performance to improve yield and mitigate the effects of climate change. ARI actively participated with its innovative field breeding methodology, using the Honeycomb Field Selection Designs and the Prognostic Breeding Paradigm. **(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 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 of them proceed to Value 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 'Giolou'. These varieties proved to be tolerant to dry conditions and yield much better than durum wheat. A new addition to the breeding programme has emerged from the needs of farmers of small ruminant animals and this is the awnless bread wheat lines created in the last few years. These lines show a huge potential for animal feed since they produce high quality and quantity of forage under the tough Cyprus climatic conditions. Finally, the most promising triticale lines, in terms of quality and yield, from the ARI development programme were promoted for advanced trials and are now under registration in the National Catalogue. **(A. Pallides)**

### Grain and forage legumes

In recent years, rainfed legumes have become increasingly popular, because of their efficient use in rotation systems with cereals and their higher feeding value than cereals, either as grain or as dry forage mass. Thus, in current research projects special emphasis has been placed on the evaluation of common vetch, field peas, and alfalfa, among others, for forage production. One of 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)**. Two new groundnut varieties have been registered in the National Catalogue under the names of 'Ntopia' and 'Nikoklia'. These two varieties have been tested from the farmers and were highly evaluated by them for their potential in replacing the current American cultivar that is being produced in Cyprus. **(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 registered in the National Catalogue. **(D.A. Fasoula and 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 the 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 also in conjunction with the national project MAGNET funded by the Cyprus Research and Innovation Foundation. New crosses with external germplasm are also in progress. **(D.A. Fasoula)**

*Crossing cowpea to increase the available genetic variability*



## AROMATIC AND MEDICINAL PLANTS

The investigation and evaluation of the possibility of exploitation of the edible and medicinal flora of Cyprus continues. Four species are studied: *Crithmum maritimum*, *Helichrysum italicum*, *Capparis spinosa* and *Cistus creticus*. The aim of this research project is to study lesser-known aromatic and medicinal plants in order to promote their cultivation and utilisation by companies engaged in the preparation of plant-based products. In the context of the project, the effect of three levels of irrigation and three levels of fertilisation on plant production are examined. Furthermore, the project assesses the antioxidant and antimicrobial activity of plant extracts and the investigation of the inhibitory action of these extracts against the activity of xanthine oxidase. (C. Stavridou).



**3PRO-Troodos** (<https://3pro-troodos.cyi.ac.cy/en/>)

## An ethnobotanical study on the traditional uses of medicinal and aromatic plants in the mountainous area of Troodos in Cyprus

Troodos mountain range can be considered as a cradle of biodiversity for the island of Cyprus, and local people as the guards of an important portion of the island's cultural heritage. Medicinal and aromatic plants (MAPs) are a distinctive component of the Mediterranean vegetation and their traditional uses constitute a valuable cultural element that should be safeguarded and promoted. The aim of this ethnobotanical study was to collect and document information about MAPs traditionally used in Troodos and to identify the most popular and important taxa.

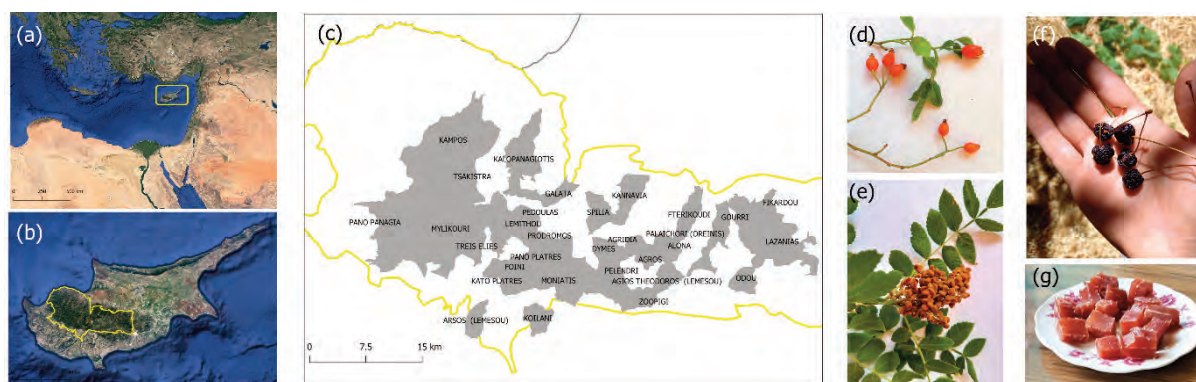
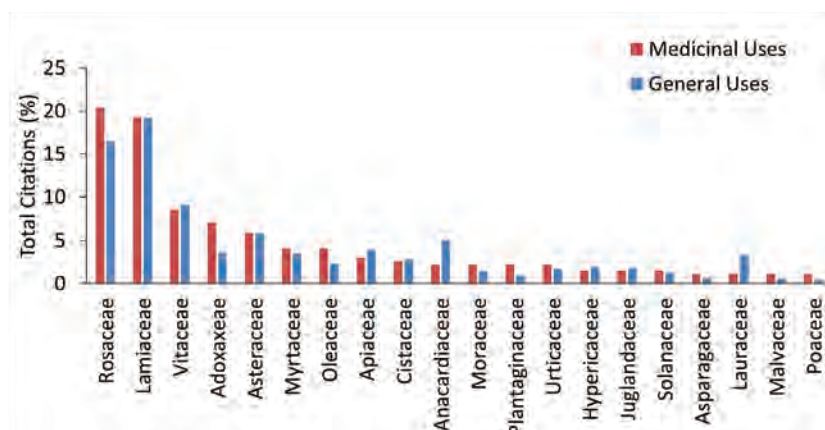
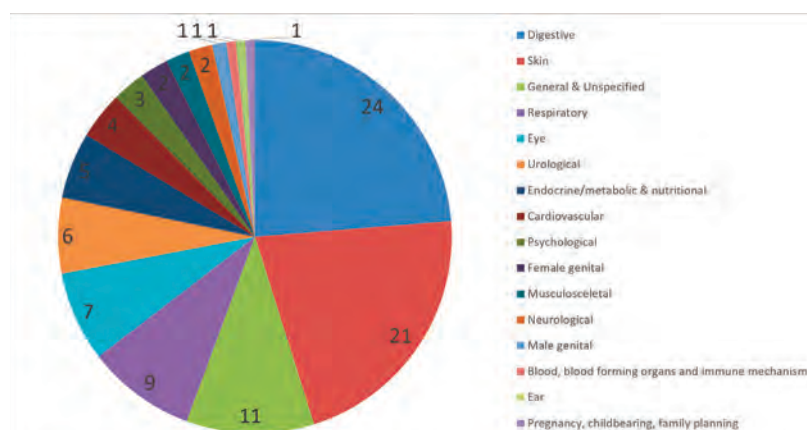


Figure 1: (a) The island of Cyprus (yellow frame) in Eastern Mediterranean, (b) the 2<sup>nd</sup> phytogeographical area (Troodos range) in Cyprus, (c) the communities included in the study within the 2<sup>nd</sup> phyt. area, (d) specimens from *Rosa canina* L., (e) *Rhus coriaria* L., (f) dried fruits of *Prunus avium* L., and (g) a sweet made from fruits of *Cydonia oblonga* Mill. (kydonopasto)

The study site was located within the 2<sup>nd</sup> phytogeographical area of Cyprus (Troodos range; Fig. 1b). Forty semi-structured interviews were conducted in 32 mountainous communities (Fig. 1c). Exponential discriminative snowball sampling was employed for candidate respondents' detection and selection. Semi-structured interviews were conducted in 2019–2020. The knowledge on the traditional plant uses obtained through interviews was classified in general and medicinal use categories, whilst the most popular and important taxa will be identified using well-known ethnobotanical indices.



Total citations (%) per plant family for general (e.g., culinary, medicinal, cosmetics, dyes & colourants) and medicinal uses of MAPs (families achieved >1% for medicinal uses were included)



Total citations (%) per medicinal uses category (based on ICPC-2)

Rosaceae and Lamiaceae families were the two top-cited plant families regarding general and medicinal uses. Medicinal uses regarding “digestive” and “skin” categories accumulated more than 40% of the total citations for medicinal uses. Medicinal uses of plants have been cited for almost all the medicinal categories. Through the study, it was realised and documented that MAPs had a vital role in the daily lives of local people in the past. It was also realised that the transmission and adoption of the knowledge regarding traditional MAPs uses between successive generations (intergenerational continuity) diminishes in time. However, society’s shift towards a healthier lifestyle implies the importance of documenting traditional uses in combination with the current scientific knowledge on MAPs appropriate utilisation.

### Testing of two new indigenous medicinal and aromatic plants

Cultivation of medicinal and aromatic plants (MAPs) may be the source of high added value products. In the framework of 3-Pro Troodos project, two MAPs species namely *Origanum dubium* and *O. majorana* var. *tenuifolium* were tested for cultivation at ARI’s experimental station in Saittas aiming at enhanced yield and product quality, reduced resource (i.e., water) exploitation and introduction of tools for more precise agronomic practices. The above-mentioned were realised by: (1) incorporating a phenological state-dependent (i.e., pre-flowering) deficit irrigation that will potentially allow optimal dry biomass and essential oil content leading to higher essential oil yield per area; (2) initiating a more controlled irrigation methodology for *Origanum* in Cyprus thereby reducing water over-use during cultivation; and (3) providing a thermal time-based model (also considering day length [seasonal] effects) able to be used in forecasting the different developmental stages and harvesting time for *Origanum* in Cyprus. The experimental phase of the study took place between 2020-2022. The expected results are considered to shed light on the optimal incorporation of smart techniques in MAPs cultivation in Cyprus towards reduced resource use and enhanced product quality. (C. Stavridou, A. Savvides, A. Stylianou, S. Ioannidou, G. Adamides, D. Fasoula).



## 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 has continued. In Deciduous Fruit Crops modern farming systems concerning improved agricultural management practices and IoT technologies of pedestrian cherry orchards in Troodos mountainous areas were evaluated along with their impact on phenological, morphological and productive tree characteristics to address climate change impacts mitigation. 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 have remained 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

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

### Evaluation of table grape varieties grafted on American rootstocks

The imported table grape varieties 'Crimson Seedless', 'Autumn Royal', 'Fantasy Seedless', 'Black Emerald' and the autochthonous varieties 'Veriko' and 'Sideritis' grafted on the American rootstocks 110 Richter, 3309 Couderc, 41B, 99 Richter, 420A, 1103P and 140 Ruggeri were evaluated in terms of yield, fruit quality and earliness. The table grape varieties 'Fantasy Seedless', 'Black Emerald' and the autochthonous varieties 'Veriko' and 'Sideritis' were evaluated at the Acheleia Experimental Station and 'Autumn Royal' at the Saittas Experimental Station. 'Crimson Seedless' and 'Veriko' were 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 64 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' were evaluated in terms of yield and quality in comparison to the imported varieties 'Mataro', 'Cabernet Sauvignon', 'Merlot', 'Chardonnay' and 'Sauvignon Blanc'. The above varieties were also tested for distinctness, uniformity and stability in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. **((S. Savvides))**

### **Collection and ampelographic description of traditional table and wine grape varieties**

The acquisition of primary and secondary descriptor data, according to the International Organisation of Wine and Vine (OIV) standards, of the traditional table grape varieties 'Veriko' and 'Sideritis' and the wine grape varieties 'Mavro', 'Xynisteri', 'Ofthalmo', 'Spourtiko', 'Maratheftiko', 'Morokanella', 'Omio', 'Promara', 'Skouro Mavr'o', and 'Kanella' and other autochthonous varieties has continued. **(S. Savvides)**

### **Evaluation of traditional wine grape varieties 'Maratheftiko' and 'Xynistry' grafted on American rootstocks**

Evaluation of the traditional wine grape variety 'Maratheftiko' and 'Xynisteri', grafted on the American rootstocks 110 Richter, 3309 Couderc, 41B, 99 Richter, 420A and 140 Ruggeri, were performed at Koilani and Kannaviou respectively. Both varieties were evaluated in terms of yield, fruit quality and earliness. **(S. Savvides)**

### **Management and conservation of grapevine genetic resources**

The long-term objective of this project is to preserve and study *in situ* populations of wild vines as well as of old and neglected varieties and to promote their future utilisation. Wild vine plants have been recorded in six locations: Ayia, Stavros tis Psokas, Potamos tou Limniti, Potamos tou Pyrgou, Platys and Saramas. All plants are located along water streams and are found as climbers on the stems of pine and deciduous trees. Until now, more than 240 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 130 wild vine accessions collected from the above locations. **(S. Savvides)**

## **DECIDUOUS FRUIT TREES**

### **3PRO: Proactive producer and processor networks for Troodos mountains agriculture**

#### **Sustainable fruit production through the use of protective nets**

The agri-food sector in Cyprus has high potential in the production of fresh fruits and nuts, which are cultivated, due to climatic requirements, in the mountainous and semi-mountainous areas of Troodos, covering the central part of the island. Adverse climatic conditions that prevail in recent years are a major cause of uncertainty in fruit production. The growing negative effects of climate change on the agricultural sector are undermining significantly both yield and fruit quality and thus require the development and use of techniques and tools capable of mitigating these effects. The use of protective nets is one of the most effective and environmentally friendly methods, which might interact positively with the environmental factors, thus affects yield (i.e., prevents production losses) and fruit quality.

The purpose of this study, in the framework of 3-PRO Troodos project, was to evaluate the effects of protective nets (anti-hail and anti-rain nets) on a sweet cherry orchard in terms of microclimate variations, functionality of tree physiological processes, fruit quality and yield efficiency. To this end, Internet of Things (IoT) technologies (e.g., smart sensors) and hand-held instruments have been deployed in an orchard for sensing aerial environment (e.g., solar radiation, air temperature, relative humidity and wind speed), soil factors (e.g., soil temperature, moisture, electrical conductivity), and canopy traits (e.g., leaf temperature and stomatal conductance). Phenological observations, growth and yield assessment as well as fruit and product quality evaluation have been taking place within the two-year experimental period (2021-2022) in the sweet cherry orchard. Microclimate data and canopy traits from a covered and a non-covered, by protective nets, area of the orchard were collected, stored, and analysed aiming at identifying the impact of protective nets on orchard microclimate and canopy traits.

The first results showed that protective nets significantly shaped orchard microclimate, mainly through the reduction of incident solar radiation and the change in light distribution within orchard canopy. The change in orchard microclimate due to the protective nets resulted in reduced reference evapotranspiration and leaf temperature on the top of the canopy. The latter results indicate that protective nets may additionally yield reduced irrigation needs. The current analysis of the results on phenological observations, growth and yield assessment as well as fruit and product quality will significantly add to the conclusions of the experiment. (S. Ioannidou, A. Savvides, G. Adamides and A. Stylianou)



### National catalogue varieties of fruit genera and species

In collaboration with the Horticulture Division of the Department of Agriculture of Cyprus (DoA), deciduous fruit plant varieties are described in order to be acceded to registration in the National Varieties Catalogue of Cyprus according to the provisions of the national legislation for the production and marketing of propagative material of fruit genera and species.

### Official description of the almond variety 'Troito'

Specifically, for the purposes of the official description of the almond variety 'Troito', the establishment of the Almonds Reference Collection was completed in the beginning of 2021 at the Saittas Experimental Station (ARI). Besides the variety 'Troito', currently under examination, the Almonds Reference Collection includes genetic material of the varieties 'Marcona', 'Ispaniko' and 'Ferragnes'. All genetic material included in the collection was provided by the DoA. Furthermore, for the purpose of evaluating the almond trees' (*Prunus dulcis*) qualitative and quantitative characteristics, an experimental plantation was created including the following genetic material of local and imported almond varieties: 'Kabourika', 'Afrougia', 'Mavrouthkia', 'Petrathasa', 'Kotsinouthkia', 'Troito', 'Marcona', 'Ispaniko' and 'Ferragnes'. This experimental plantation has been established at Saittas Experimental Station (ARI). (S. Ioannidou)





### Official description of the pear variety “Superfine”

Also, following the work done during the growing seasons 2017-2019, in 2020 the official description of the pear variety (*Pyrus communis* L.) ‘Superfine’ was completed based on phenological observations and qualitative/quantitative measurements. After the detailed examination of 65 tree, leaf, flower and fruit characteristics and according to the results of the report on technical examination the variety ‘Superfine’ is clearly distinguishable from any other variety, is sufficiently homogeneous and stable. (S. Ioannidou)

## 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 Tochni 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 OCARICY with genetic material from other countries. (M.G. Emmanouilidou)



### Elaigraphic description and cataloguing of Cypriot olive cultivars

Following the identification of local Cypriot olive genetic material, based on the unique combination of microsatellite (SSR) genotypes and endocarp morphologies the presence of three local cultivars (‘Kato Drys’, ‘Korakou’ and ‘Ladoelia’) revealed and a full elaigraphic description and cataloguing was held. A total of 24 morphological characters of tree, leaf blade, fruit and stone described based on CPVO-TP/099/1 and UPOV-TG/99/4 Protocols for Distinctness, Uniformity and Stability Tests in Olive (*Olea europaea* L.). In total, 31 accessions were studied with 11 morphological endocarp traits and 14 microsatellite (SSR) markers. The SSR variability allowed segregation of 16 genotypes among accessions, which were clustered into three main groups based on genetic similarity. Similarity indexes among groups ranged from 0.63 to 0.65 and within groups were  $\geq 0.9$ . Lower morphological variability was detected among accessions, which conformed to three morphological profiles; moreover, the three morphological profiles corresponded to the three groups of genetic similarity obtained by SSR markers. The identification, based on the unique combination of SSR genotypes and endocarp

morphologies, revealed the presence of three cultivars ('Ladoelia', 'Kato Drys' and 'Korakou') and 15 molecular variants that presented limited molecular differences but morphological profiles identical to their catalogued cultivars. (M.G. Emmanouilidou, M.C. Kyriacou)



### True Healthy Olive Cultivars (THOC2) Project

The Network of Olive Germplasm Banks of the International Olive Council (IOC), consisting of three international and 22 national banks continues the implementation of THOC2 project. The objectives of this project are the following: a) complete the authentication of 101 most common in national level and most commonly exchanged among IOC network countries commercial cultivars; b) test the absence of the main pathogens that cause epidemics spread by plant interchange; c) establish reference collections of DNA and endocarps from the authenticated cultivars; d) create an online database of the morphological (UPOV) and SSR profiles of the authenticated and pathogen-free cultivars; e) provide members of IOC Network with true-to-type and pathogen-free genetic material to facilitate initial plants for potential olive plant certification programmes in the corresponding countries and f) publish the new world catalogue of olive cultivars. (M.G. Emmanouilidou)

### Evolution of physicochemical constitution and cultivar-differential maturity configuration in olive (*Olea europaea* L.) fruit

Analytical characterisation of the ripening process is prerequisite to establishing cultivar-specific harvest maturity windows and safeguarding the quality of olive products. In this context, the aim of this project was to profile the ripening of select Cypriot olive (*Olea europaea* L.) cultivars 'Ladoelia', 'Kato Drys' and 'Korakou' juxtaposed against introduced 'Koroneiki'. Fruit skin and flesh colour-based maturity index (MI) evolved linearly over time but differentially among local cultivars. Anthocyanin content exhibited better correspondence to MI with progressive maturity. Cultivars varied widely in their sugar content (60.2–180.9 mg g<sup>-1</sup> dw) and their fructose/glucose ratio, which impacted their relative sweetness. Putative connection of declining mannitol levels with oleogenesis during ripening was not supported by 'Korakou', wherein mannitol declined without concomitant oil accumulation. However, fruit firmness correlated negatively with oil content prompting harvest of table olives before oil peak. Succinic and oxalic acid levels may constitute valuable cultivar signature traits. Decline pattern in total phenolic content (TPC) during ripening was cultivar-specific, with oleuropein, rutin and hydroxytyrosol constituting the major constituents in diminishing abundance. No cultivar differences were



observed in rutin content, while hydroxytyrosol and tyrosol levels were independent of maturity. At MI3, 'Ladoelia' had higher total phenolic content (TPC) than 'Korakou' and 'Kato Drys' but inferior to 'Koroneiki' (12.3, 9.9, 2.5 and 20.4 g kg<sup>-1</sup> dw, respectively). Oleuropein relative content in 'Ladoelia' (85.6 %TPC) was higher than 'Kato Drys' (63.6 %TPC) and 'Korakou' (59.2 %TPC). 'Ladoelia' exhibited superior but maturity-dependent phenolic acids content. Our results corroborate a cultivar-specific application of MI supported by additional physicochemical parameters of maturity. The current findings may facilitate cultivar and product specific integrative harvest maturity indices, which are especially critical for dual purpose large-fruited cultivars. **(M.G. Emmanouilidou and M.C. Kyriacou)**

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

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 physicomachanical 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 edaphoclimatic 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 varietal olive oils produced in Cyprus provided the springboard for launching the present ARI research programme on olive oil characterisation, 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 postharvest behaviour, c) evaluation with respect to fruit harvest maturity and postharvest storage: 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.

Oil content, extractability, chemical composition and oxidative stability of three monovarietal ('Ladoelia', 'Kato Drys' and 'Korakou') virgin olive oils (VOOs) were examined at progressive stages of fruit maturity. Oxidative stability was assessed on a Rancimat System, phenols and tocopherols by HPLC-DAD and fatty acid (FA) profiles by GC-FID. Oil extractability was highly cultivar-specific and unaffected by maturity stage. 'Ladoelia' exhibited excellent extractability and satisfactory VOO composition and oxidative stability, with moderate phenolic and diverse tocopherol content. 'Kato Drys' was rich in monounsaturated FA that imparted superior oxidative stability and poor in polyunsaturated and saturated FA. Monovarietal VOOs exhibited higher correlation of oxidative stability with the C18:1/C18:2 ratio ( $r = 0.80$ ,  $p < 0.0001$ ) than with the total phenolic content (TPC;  $r = 0.436$ ,  $p < 0.05$ ), suggesting that phenolic association with oxidative stability might be specific to individual phenolic compounds than to TPC. Late harvest of 'Ladoelia' and 'Kato Drys' was liable to drastic rise in free acidity. Poor extractability, low oil and oleic acid contents undermined the suitability of 'Korakou' for VOO production. The current findings contribute towards the establishment of typicity for Cypriot monovarietal VOOs. **(M.G. Emmanouilidou and 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, and c) evaluation of rootstock effects on scion fruit quality characteristics. **(M.G. Emmanouilidou and M.C. Kyriacou)**

## 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. The Postharvest Technology Laboratory (PTL) constitutes the main analytical facility of the Vegetable Crops Section. It is principally dedicated to the study of pre- and post-harvest ripening physiology of climacteric and non-climacteric fresh horticultural commodities in relation to the configuration of quality and postharvest performance. Work at the PTL concentrates 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 National Genebank constitutes another integral component of the Vegetable Crops Section. It concentrates on the collection, *ex situ* conservation, regeneration, characterisation, evaluation and utilisation of native plants of the flora of Cyprus and local landraces. The ARI Herbarium is an extension of the Genebank dedicated to the collection and documentation of botanical specimens of the Cyprus flora.

The Section's current work focuses on rootstock-scion relations of cucurbits and solanaceous crops with respect to productivity, disease-resistance, stress response, quality and postharvest performance. The biostimulatory action of vegetal-derived protein hydrolysates is also assessed for possible interaction with the ripening of diploid watermelon grafted onto interspecific hybrid rootstock. Work on leafy salad crops examines the effect of planting pattern, nitrogen administration strategies, shading, regulated salinity eustress, seasonal adaptation and time of harvest on yield, quality, shelf-life and consumer safety of leafy vegetables in open and protected cultivation. Male hybrid asparagus cultivars are under trial along with select populations of two wild asparagus species (*A. stipularis* and *A. acutifolius*) tested for adaptability to intensive cultivation. The effect of sequential harvesting on the mobilisation of primary and secondary metabolites of male green asparagus (*Asparagus officinalis* L.) has been examined in order to monitor changes in the sensory and bioactive profile of asparagus throughout the harvest season. The impact of key preharvest factors (genotype, light quality, substrate, nutrition and developmental stage) on the postharvest quality of microgreens has also come under systematic investigation. The Section also coordinates the current ARI project on the evaluation of an *ex situ* collection of indigenous pomegranate clones for fruit physicochemical characteristics, trait stability and phylogenetic structure. Moreover, the PTL and the Genebank have a key role in the project Black Gold, funded by the Research and Innovation Foundation, with the overall objective to help re-mobilise and extend the carob industry in Cyprus into new and innovative food and nutrient supplement products that meet the needs of the growing international market for carob-based products. The Genebank has a central role in the genotyping and phenotyping of indigenous carob genetic resources from the main agroecological zones of Cyprus and the evaluation of the comparative contribution of genotype and environment effects on the variance of agronomic and technological traits. The PTL has focused further on the compositional profile of carobs, with respect to sensory and bioactive properties, and how this profile is modulated by harvest maturity and postharvest storage.

## VEGETABLE SCIENCE

### **Adaptability and performance of two wild asparagus species (*A. stipularis* and *A. acutifolius*) under intensive cultivation: Comparisons between *in situ* and *ex situ* populations**

The adaptability and performance of two wild asparagus species (*A. stipularis* and *A. acutifolius*) under intensive cultivation is 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), *A. stipularis* (five populations) and *A. acutifolius* (two populations). (M.C. Kyriacou, A. Kyratzis and G.A. Soteriou).

### **Changes in the primary and secondary metabolome of male green asparagus (*Asparagus officinalis* L.) as modulated by sequential harvesting**

Rising temperature and solar radiation drive the mobilisation and depletion of crown-deposited metabolites harnessed for asparagus spear regeneration during the spring harvest season. We presently examined how successive same-season harvests impact the sensory, nutritive and bioactive composition of select green asparagus genotypes. Soluble carbohydrates were analysed by HPLC-RI, organic acids and polyphenols by HPLC-DAD and metals by ion chromatography. Higher sugars and lower acids accentuated sweetness and lower polyphenols contributed to reduced astringency at the beginning of the harvest season. This trend was reversed as the season advanced and spear sensory quality was compromised by declining sugars and rising acids; however, functional quality improved as antioxidant capacity increased along with the concentrations of quercetin-3-O-rutinoside (rutin) and ascorbic acid. The compositional changes presently described were uniform across all genotypes examined and thus contribute toward our understanding of seasonal variation in the sensory and functional quality of this acclaimed health-promoting product. (G.A. Soteriou, A. Kyratzis and M.C. Kyriacou).

### **Biostimulatory action of vegetal protein hydrolysate and the configuration of fruit physicochemical characteristics in grafted watermelon**

Foliar application of a vegetal-derived protein hydrolysate as a biostimulant was assessed for possible interaction with the ripening of diploid watermelon grafted onto interspecific hybrid rootstock. Assessment encompassed crop performance; fruit morphometric and sensory quality traits; soluble carbohydrates; macrominerals; and bioactive composition at 10, 20, 30, 40, and 50 days post-anthesis (dpa). The biostimulant effect on yield components was confounded by the vigorous rootstock effect. Pulp firmness declined precipitously with cell enlargement from 10 to 30 dpa, and the biostimulant phyto-hormonal potential on firmness and rind thickness was masked by grafting. Pulp colorimetry was determined solely by ripening and peaked at 40 dpa. The biostimulant effect reduced lycopene content by 8% compared to the control. Total sugars coevolved with soluble solids content, peaked at 30 dpa, and then stabilised. Fructose and glucose prevailed during rapid fruit growth from 10 to 30 dpa and sucrose prevailed at advanced ripeness between 40–50 dpa, whereas acidity

peaked at 20 dpa and then decreased. Potassium, which was the most abundant micromineral, peaked before full ripeness at 30 dpa. The biostimulant effect on the watermelon fruit ripening process is not granted, at least regarding the conditions this study was carried out under. The absence of biostimulant effect might relate to rootstock vigorousness, the grafted watermelon physiology, or the type of biostimulant used. (G.A. Soteriou, M.G. Emmanouilidou, A.C. Kyratzis and M.C. Kyriacou)

### **Preharvest and postharvest evaluation of green asparagus male hybrid cultivars 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 postharvest performance of the five promising male asparagus hybrids, and b) evaluation of the hybrids as to their suitability for cultivation in arid climate. Preliminary results showed no differentiation among cultivars, concerning extra yield (tn ha<sup>-1</sup>), total yield (classes extra & I), mean spear weight or extra spear number (Table 3). 'Giovè' produced the lowest I (class I) yield and I spear number among the cultivars. No differences were recorded among the cultivars concerning classes extra and I spear weight. (G.A. Soteriou, M.C. Kyriacou).

### **Configuration by osmotic eustress agents of the morphometric characteristics and the polyphenolic content of differently pigmented baby lettuce varieties in two successive harvests**

Salinity eustress is one of the pre-harvest factors that can be used to improve the phytochemical profile and the quality attributes of horticultural species, and most of the studies are carried out using NaCl. In this work, we compared the effect of three mildly saline iso-osmotic nutrient solutions (NS) differing in the cation employed (either K, Na, or Ca) in baby lettuce cultivated in a floating system. Specifically, we analysed the impact on key morphological traits and polyphenol composition in leaves in a completely randomised design experiment with the following experimental factors and levels: Two differently pigmented lettuce varieties (green and full red), three NSs (20 mM KCl, 20 mM NaCl, 13.3 mM CaCl<sub>2</sub>, each with a final ionic concentration of 40 mM), and two successive harvests. The lettuce response to mild salinity was multifaceted and with a marked role for the cultivar factor and its interactions, as also indicated by multivariate analysis. The morphological response of baby lettuce to the saline solutions was predominantly affected by the osmolarity, and ion-specific alleviating or detrimental effects were not observed. The phytochemical analysis revealed significant interactions among all tested factors, with ion-specific differences over some of the detected phenolics. This is consistent with the high sensitivity of this class of bioactive compounds to environmental factors. This work indicated that certain combinations of the experimental factors tested can be exploited to improve the biochemical profile and possibly the nutraceutical quality of baby lettuce in a floating system. (G.A. Soteriou and M.C. Kyriacou)

### **Physiological and nutraceutical quality of green and red pigmented lettuce in response to NaCl concentration in two successive harvests**

Nutritional eustress such as salinity or nutrient stress applied in soilless systems, is a convenient pre-harvest factor efficient in modulating the phytochemical components of horticultural crops, by triggering defensive mechanisms and accumulating plant secondary metabolites in plants tissues. Nevertheless, genetic material (cultivars with different pigmentation) dictates lettuce metabolites and physiological response to extrinsic



eustress, with red leaf cultivars being highly nutrient packed notwithstanding the stress. Product quality can be meliorated equally by applying several cuts, a practice proven to increase bioactive compounds accumulation. In this study, we analysed the effects of four salinity levels (1, 10, 20 and 30 mM NaCl) on green and red pigmented Salad Bowl lettuce (*Lactuca sativa* L. var. *acephala*) in two successive harvests cultivated in a floating raft system. The morphological parameters, mineral composition, leaf gas exchanges, bioactive compounds, and antioxidant activity of both cultivars were assessed. The green cultivar exhibited superior crop productivity but was more prone to salinity effect than the red cultivar. Irrespective of cultivar and cut order, the net photosynthesis decreased with increasing salinity in the nutrient solution. The second cut incurred higher dry biomass, greater accumulation of most minerals and higher photosynthetic activity. In red lettuce, 20 mM NaCl proved adequate eustress to increase phytonutrients and beneficial minerals (K, Ca, and Mg) with minimal loss of yield. Mild salinity and sequential harvest have proven effective pre-harvest tools in positively modulating the quality of lettuce. Eustress interaction with genotype was demonstrated as a promising field for future breeding programmes targeting select genotypes for agronomic application of eustress to improve the nutraceutical value of vegetable crops. **(M.C. Kyriacou)**

### **Regulated salinity eustress in a floating hydroponic module of sequentially harvested lettuce modulates phytochemical constitution, plant resilience, and postharvest nutraceutical quality**

A mild salinity stress (eustress) may modulate the induction of the plant defense system in horticultural crops and the synthesis of phytochemical components able to enhance plant resilience, post-harvest performance, and the nutraceutical quality of produce. However, the choice of the correct eustress type and dose to induce the synthesis of these protective phytochemicals is pivotal to avoid potential interference with plant growth and productivity. In order to study how green and red lettuce (*Lactuca sativa* L.) plants equilibrate the nutritional and nutraceutical components of quality with yield components, we applied iso-osmotic concentrations of three different salts (20 mM NaCl, 20 mM KCl, and 13.3 mM CaCl<sub>2</sub>, with a final total ionic concentration of 40 mM) in combination with two successive harvests in a floating raft system. The biometric parameters, mineral composition, bioactive compounds, and antioxidant activity of both cultivars were analysed. The green cultivar had a superior response concerning biometric traits and productivity compared to the red one during the first cut but lower phytochemical content (e.g., ascorbic acid). The effect of cut order, independently of cultivar and salinity treatments, demonstrated that at the first harvest plants could redirect metabolism by increasing the lipophilic antioxidant content (LAA) at the expense of plant yield, therefore increasing plant resilience and post-harvest nutraceutical quality; whereas, at the second harvest, plants reverted principally to tissue expansion. The treatments with iso-osmotic salt concentrations did not affect K and Mg ion contents but further increased LAA and resulted only in a moderate decrease of fresh yield. The lettuce nitrate content was reduced during the second cut only when lettuce plants were treated with NaCl and especially CaCl<sub>2</sub>. **(G.A. Soteriou and M.C. Kyriacou)**

### **Selenium biofortification impacts the nutritive value, polyphenolic content, and bioactive constitution of variable microgreens genotypes**

Selenium (Se) is considered essential for human nutrition as it is involved in the metabolic pathway of selenoproteins and relevant biological functions. Microgreens, defined as tender immature greens, constitute an emerging functional food characterised by overall higher levels of phytonutrients than their mature counterparts. The nutraceutical value of microgreens can be further improved through Se biofortification, delivering Se-enriched foods and potentially an enhanced content of bioactive compounds. The current study defined the effect of sodium selenate applications at three concentrations (0, 8, and 16 µM Se) on the bioactive compounds and mineral content of coriander, green basil, purple basil, and tatsoi microgreens grown in soilless

cultivation. Analytical emphasis was dedicated to the identification and quantification of polyphenols by UHPLC-Q-Orbitrap-HRMS, major carotenoids by HPLC-DAD, and macro micro-minerals by ICP-OES. Twenty-seven phenolic compounds were quantified, of which the most abundant were: Chlorogenic acid and rutin in coriander, caffeic acid hexoside and kaempferol-3-O(caffeoyl) sophoroside-7-O-glucoside in tatsoi, and cichoric acid and rosmarinic acid in both green and purple basil. In coriander and tatsoi microgreens, the application of 16  $\mu\text{M}$  Se increased the total phenols content by 21% and 95%, respectively; moreover, it improved the yield by 44% and 18%, respectively. At the same Se dose, the bioactive value of coriander and tatsoi was enhanced by a significant increase in rutin (33%) and kaempferol-3-O(feruloyl)sophoroside-7-O-glucoside (157%), respectively, compared to the control. In green and purple basil microgreens, the 8  $\mu\text{M}$  Se application enhanced the lutein concentration by 7% and 19%, respectively. The same application rate also increased the overall macroelements content by 35% and total polyphenols concentration by 32% but only in the green cultivar. The latter actually had a tripled chicoric acid content compared to the untreated control. All microgreen genotypes exhibited an increase in the Se content in response to the biofortification treatments, thereby satisfying the recommended daily allowance for Se (RDA-Se) from 20% to 133%. The optimal Se dose that guarantees the effectiveness of Se biofortification and improves the content of bioactive compounds was 16  $\mu\text{M}$  in coriander and tatsoi, and 8  $\mu\text{M}$  in green and purple basil. (M.C. Kyriacou and G.A. Soteriou)

## POSTHARVEST TECHNOLOGY

### Physicochemical characterisation and trait stability in a genetically diverse *ex situ* collection of pomegranate (*Punica granatum* L.) germplasm from Cyprus

Proximity to the center of origin and geographical isolation shaped a unique genetic diversity of pomegranate in Cyprus that constitutes a valuable resource for the crop. Physicochemical characters and trait stability were studied for three years in an *ex situ* collection of 29 pomegranate accessions from Cyprus. Accession signature traits with exceptional yearly stability were titratable acidity and the citrate/malate ratio. Overall, the Cypriot germplasm was characterised by juice of low anthocyanin content ( $\bar{x}$  = 20.6 mg/L) and moderate phenolic content ( $\bar{x}$  = 613.0 mg/L) with glucose ( $\bar{x}$  = 69.1 g/L) and fructose ( $\bar{x}$  = 74.2 g/L) as predominant sugars. Juice antioxidant capacity was associated primarily with total phenolics and less so with juice colour and anthocyanin content. Total sugar content was higher in large-fruited accessions with darker juice. In most accessions the titratable acidity was low (< 0.50% w/v), with citric ( $\bar{x}$  = 44.7%), malic ( $\bar{x}$  = 39.1%) and succinic ( $\bar{x}$  = 15.8%) being the main organic acid fractions detected. Based on the maturity index (SSC/TA), six accessions were sweet-sour or borderline sweet-sour and 23 accessions were sweet. Cluster analysis of phenotypic characteristics and genetic data revealed a core group of thirteen genetically and phenotypically close accessions constituting the Cypriot pomegranate landrace, characterised by moderate fruit weight, high juiciness, thin rind, moderately hard seeds and light-coloured juice low in acidity, anthocyanins, phenolics and antioxidant capacity. The present work advances the understanding of genetic and environmental contribution to the configuration of pomegranate physicochemical fruit composition. (M.C. Kyriacou, S. Ioannidou, N. Seraphides, L.C. Papayiannis and A.C. Kyratzis)

### Effects of deficit irrigation on 'Koroneiki' olive tree growth, physiology and olive oil quality at different harvest dates

Agricultural water demand in the Mediterranean region is expected to increase due to climate change. The application of deficit irrigation practices may improve the efficiency of water use in orchards, by accounting for changes in environmental conditions and in the tree growth. The main goal of this study was to improve our understanding on the effect of deficit irrigation on physiology, morphology, yield, and oil quality of 'Koroneiki'

olive trees. The effect of two deficit irrigation treatments and three harvest dates on olive yield and oil quality were investigated in a 17-year-old, low-density 'Koroneiki' orchard in Cyprus, during a high-crop load year. For the sustained deficit irrigation (SDI) treatment 70% ETC was applied over the irrigated period, while the regulated deficit irrigation (RDI) consisted of 70% ETC during water-stress sensitive growth stages (shoot growth, flowering, bud initiation) and 35% ETC during water-stress tolerant growth stages (pit hardening, oil accumulation). Environmental conditions and volumetric soil water content of the 70-cm rootzone were monitored. A Kc of 0.37 was derived for the irrigated period from daily water balance computations, excluding rain days. No significant effects of the two irrigation treatments were found on morphology, physiology, fruit yield and oil quality. Midday stem water potential reached  $-4$  MPa in September as a result of increasing fruit load. Leaf conductance ranged between 65 and 228 mmol m<sup>-2</sup> s<sup>-1</sup>, with highest values obtained under high soil water conditions and a vapour pressure deficit of 3.6 kPa. Yields were approximately 9 tons ha<sup>-1</sup> in both irrigation treatments while water productivity was 1.4 and 1.0 kg oil m<sup>-3</sup> irrigation water in SDI and RDI, respectively. Maximum phenolic content was found in early December, reaching on average 202 mg kg<sup>-1</sup> oil in both irrigation treatments. Overall, irrigation water use in RDI was 32% less than in SDI, whereas oil quality was only affected by the harvest date. **(M.C. Kyriacou and M.G. Emmanouilidou)**

### **Grafting as a sustainable means for securing yield stability and quality in vegetable crops**

Grafting is among the most ancient agricultural techniques, having been practiced since 2000 BC. Nowadays, this old technique holds a significant margin for improvement by adding contemporary advances in plant science and technology. Vegetable grafting is widely used in Cucurbitaceous (cucumber, melon and watermelon) and Solanaceous crops (eggplant, pepper and tomato). Grafting provides opportunities to exploit natural genetic variation for specific root traits to influence the phenotype of the shoot. By selecting a suitable rootstock, grafting can manipulate scion morphology and physiology and can manage biotic stresses including foliar and soil borne pathogens, arthropods, viral diseases, weeds and nematodes, as well as abiotic stresses such as thermal stress, drought, salinity, nutrient deficiency and imbalances in soil, adverse soil pH (alkalinity and acidity), heavy metals contamination and organic pollutants. The current Agronomy-MDPI research topic "Grafting as a sustainable means for securing yield stability and quality in vegetable crops" compiles 12 research papers and two review articles that examine the implications of vegetable grafting for crop growth and productivity, resource use efficiency (water and fertiliser), nutritional and functional quality of the produce as well as tolerance to biotic and abiotic stress. The present research topic contains scientific articles of high standard coming from several prestigious research groups. As such, it is geared to increase knowledge among scientists, breeding companies and farming communities on the benefits of grafting vegetables towards securing productivity and stability of the agricultural sector, thus improving food security. **(M.C. Kyriacou)**

### **Grown to be blue—Antioxidant properties and health effects of coloured vegetables. Part I: Root vegetables**

During the last few decades, the food and beverage industry faced increasing demand for the design of new functional food products free of synthetic compounds and artificial additives. Anthocyanins are widely used as natural colourants in various food products to replenish blue colour losses during processing and to add blue colour to colourless products, while other compounds such as carotenoids and betalains are considered as good sources of other shades. Root vegetables are well known for their broad palette of colours, and some species, such as black carrot and beetroot, are already widely used as sources of natural colourants in the food and drug industry. Ongoing research aims at identifying alternative vegetable sources with diverse functional and structural features imparting beneficial effects onto human health. The current review provides a systematic description of coloured root vegetables based on their belowground edible parts, and it highlights species



and/or cultivars that present atypical colours, especially those containing pigment compounds responsible for hues of blue colour. Finally, the main health effects and antioxidant properties associated with the presence of colouring compounds are presented, as well as the effects that processing treatments may have on chemical composition and colouring compounds in particular. **(M.C. Kyriacou)**

### **Grown to be blue—Antioxidant properties and health effects of coloured vegetables. Part II: Leafy, fruit, and other vegetables**

The current trend for substituting synthetic compounds with natural ones in the design and production of functional and healthy foods has increased the research interest about natural colourants. Although colouring agents from plant origin are already used in the food and beverage industry, the market and consumer demands for novel and diverse food products are increasing and new plant sources are explored. Fresh vegetables are considered a good source of such compounds, especially when considering the great colour diversity that exists among the various species or even the cultivars within the same species. In the present review we aim to present the most common species of coloured vegetables, focusing on leafy and fruit vegetables, as well as on vegetables where other plant parts are commercially used, with special attention to blue colour. The compounds that are responsible for the uncommon colours will be also presented and their beneficial health effects and antioxidant properties will be unraveled. **(M.C. Kyriacou)**

### **Ontogenetic variation in the mineral, phytochemical and yield attributes of brassicaceous microgreens**

Microgreens constitute novel gastronomic ingredients that combine visual, kinesthetic and bioactive qualities. The definition of the optimal developmental stage for harvesting microgreens remains fluid. Their superior phytochemical content against mature leaves underpins the current hypothesis of significant changes in compositional profile during the brief interval of ontogeny from the appearance of the first (S1) to the second true leaf (S2). Microgreens of four brassicaceous genotypes (Komatsuna, Mibuna, Mizuna and Pak Choi) grown under controlled conditions and harvested at S1 and S2 were appraised for fresh and dry yield traits. They were further analysed for macro- and micro-mineral content using inductively coupled plasma optical emission spectrometry (ICP-OES), carotenoid content using high-performance liquid chromatography with a diode-array detector (HPLC-DAD), volatile organic compounds using solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME-GC/MS), anthocyanins and polyphenols using liquid chromatography-high resolution-tandem mass spectrometry (LC-MS/MS) with Orbitrap technology and for chlorophyll and ascorbate concentrations, well as antioxidant capacity by spectrophotometry. Analysis of compositional profiles revealed genotype as the principal source of variation for all constituents. The response of mineral and phytochemical composition and of antioxidant capacity to the growth stage was limited and largely genotype-dependent. It is, therefore, questionable whether delaying harvest from S1 to S2 would significantly improve the bioactive value of microgreens while the cost-benefit analysis for this decision must be genotype-specific. Finally, the lower-yielding genotypes (Mizuna and Pak Choi) registered higher relative increase in fresh yield between S1 and S2, compared to the faster-growing and higher-yielding genotypes. Although the optimal harvest stage for specific genotypes must be determined considering the increase in yield against reduction in crop turnover, harvesting at S2 seems advisable for the lower-yielding genotypes. **(M.C. Kyriacou, G.A. Soteriou and A.C. Kyratzis)**

### **Phenolic constitution, phytochemical and macronutrient content in three species of microgreens as modulated by natural fiber and synthetic substrates**

The present study examined the modulatory effects of natural fiber substrates (agave fiber, coconut fiber and peat moss) and synthetic alternatives (capillary mat and cellulose sponge) on the nutritive and phytochemical

composition of select microgreens species (coriander, kohlrabi and pak choi) grown in a controlled environment. Polyphenols were analysed by UHPLC-Q-Orbitrap-HRMS, major carotenoids by HPLC-DAD, and macro-minerals by ion chromatography. Microgreens grown on peat moss had outstanding fresh and dry yield but low dry matter content. Natural fiber substrates increased nitrate and overall macro-mineral concentrations in microgreens compared to synthetic substrates. The concentrations of chlorophylls, carotenoids and ascorbate were influenced primarily by species. On the contrary, variability in polyphenols content was wider between substrates than species. Out of twenty phenolic compounds identified, chlorogenic acid and quercetin-3-O-rutinoside were most abundant. Hydroxycinnamic acids and their derivatives accounted for 49.8% of mean phenolic content across species, flavonol glycosides for 48.4% and flavone glycosides for 1.8%. Peat moss provided optimal physicochemical conditions that enhanced microgreens growth rate and biomass production at the expense of phenolic content. In this respect, the application of controlled stress (eustress) on microgreens growing on peat moss warrants investigation as a means of enhancing phytochemical composition without substantial compromise in crop performance and production turnover. Finally, nitrate deprivation practices should be considered for microgreens grown on natural fiber substrates in order to minimise consumer exposure to nitrate. **(M.C. Kyriacou and G.A. Soteriou)**

### **Preharvest nutrient deprivation reconfigures nitrate, mineral, and phytochemical content of microgreens**

While imparting gastronomic novelty and sensory delight, microgreens also constitute rudimentary leafy greens packed with nutrients and phytochemicals. As such, they comprise an upcoming class of functional foods. However, apart from bioactive secondary metabolites, microgreens also accumulate antinutritive agents such as nitrate, especially under conducive protected cultivation conditions. The current work examined nutrient deprivation before harvest (DBH), applied by replacing nutrient solution with osmotic water for six and twelve days, as a strategy for reducing microgreen nitrate levels in different species (lettuce, mustard, and rocket). The three species were sown on a peat-based substrate, cultivated in a controlled climate chamber, and harvested 18 days after sowing, when the first two true leaves emerged. DBH impact on major constituents of the secondary metabolome, mineral content, colorimetric, and yield traits was appraised. Nitrate and mineral content were determined through ion chromatography, phenolic composition through UHPLC-Q-Orbitrap HRMS, and carotenoid composition through HPLC-DAD. Nutrient deprivation was effective in reducing nitrate content; however, effective treatment duration differed between species and decline was more precipitous in nitrate hyperaccumulating species such as rocket. Quercetin and kaempferol glycosides were the flavonol glycosides most abundant in brassicaceous microgreens, whereas lettuce microgreens were steeped in caffeoyl quinic acid. DBH interacted with species as it increased the total phenolic content of lettuce, decreased that of rocket, but did not affect mustard. Further research to link changes in phenolic composition to the sensory and *in vivo* bioactive profile of microgreens is warranted. Notably, brief ( $\leq 6$  days) DBH can be applied across species with moderate or no impact on the phenolic, carotenoid, and mineral composition of microgreens. Brief DBH applications also have limited impact on microgreens' yield and colorimetric traits hence on the commercial value of the product. They can therefore be applied for reducing microgreen nitrate levels without significantly impacting key secondary metabolic constituents and their potential bioactive role. **(M.C. Kyriacou, G.A. Soteriou and A.C. Kyratzis)**

### **Sensory attributes and consumer acceptability of 12 microgreens species**

Microgreens are gaining increasing recognition among consumers, acclaimed for their freshness and health promoting properties associated with densely fortified secondary metabolites. These immature greens enhance human diet and enrich it with sharp colours and flavors. While numerous species are being tested for agronomic and nutritional suitability, consumer acceptance of appearance, texture, and flavor is critical for the microgreens'

marketplace success. This study investigates whether sensory attributes and visual appearance affect consumer preference for microgreens and their willingness to consume them. By means of a consumer test, the sensory attributes of 12 microgreens species were evaluated, wherein a partial least squares structural equation model was developed to link sensorial attributes to willingness to eat the product. The results showed that although visual appearance of the microgreens was largely appreciated, consumer acceptance overall was mainly determined by flavour and texture. In particular, the lower the astringency, sourness, and bitterness, the higher the consumer acceptability of microgreens. Among the 12 examined species, mibuna and cress scored the lowest acceptance by consumers, while Swiss chard and coriander were the most appreciated, being therefore good candidates to be introduced in Western country markets. In addition, both Swiss chard and coriander have been identified by previous literature as good dietary source of phenolic antioxidants. **(M.C. Kyriacou)**

### **Variation in macronutrient content, phytochemical constitution and *in vitro* antioxidant capacity of green and red butterhead lettuce dictated by different developmental stages of harvest maturity**

Rising life expectancy and the demanding modern lifestyle drive the growing appeal of healthy and balanced diets centered on vegetable and fruit consumption. Functional, phytonutrient-packed and principally raw food is in high demand. Microgreens constitute such a novel functional food that combines a high sensory and bioactive value, which invites comparison to their mature-leaf counterparts. For this purpose, a controlled environment chamber experiment was carried out to compare the mineral, phytochemical and antioxidant capacity attributes of two-pigmented *Lactuca sativa* L. var. capitata cultivars (green and red Salanova®) harvested at the microgreens and the mature-leaf stage. Macronutrients were assessed through ion chromatography, while carotenoids and polyphenols were assessed and quantified through HPLC-DAD and UHPLC-Q-Orbitrap HRMS, respectively. Calcium and magnesium were higher in microgreens irrespective of the cultivar; conversely, phosphorous, potassium and nitrate were higher in mature leaves. All pigments including chlorophyll, lutein and  $\beta$ -carotene augmented at advanced maturity stage and were more concentrated in the red pigmented cultivar at both stages. Total polyphenols accumulated more densely in red Salanova, particularly in the microgreens stage; whereas, in green Salanova, the accumulation was significant but less pronounced in the microgreens stage. Chlorogenic acid, quercetin malonyl glucoside, rutin and coumaroyl quinic acid were the most concentrated phenolic acids in microgreens, while feruloyl tartaric acid was predominant in mature leaves. Finally, when a high carotenoids content is sought, mature lettuce leaves should be the prime culinary choice, whereas high polyphenolic content is dictated by both the cultivar and the harvest stage, with red Salanova microgreens being the most nutrient-packed choice. **(M.C. Kyriacou)**

## **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 13 new accessions have been collected during the last two years. Germination tests have been routinely conducted to assess germination capacity of the CYPARI conserved accessions.

### A common approach to the conservation of threatened island vascular plants: First results in the Mediterranean basin

The Mediterranean islands represent a centre of vascular plant diversity featuring a high rate of endemic richness. Such richness is highly threatened, however, with many plants facing the risk of extinction and in need of urgent protection measures. The CARE-MEDIFLORA project promoted the use of *ex situ* collections to experiment with *in situ* active actions for threatened plants. Based on common criteria, a priority list of target plant species was elaborated, and germplasm conservation, curation and storage in seed banks was carried out. Accessions were duplicated in the seed banks of the partners or other institutions. Germination experiments were carried out on a selected group of threatened species. A total of 740 accessions from 429 vascular plants were stored in seed banks, and 410 seed germination experiments for 283 plants species were completed; a total of 63 *in situ* conservation actions were implemented, adopting different methodological protocols. For each conservation programme, a specific monitoring protocol was implemented in collaboration with local and regional authorities. This project represents the first attempt to develop common strategies and an opportunity to join methods and methodologies focused on the conservation of threatened plants in unique natural laboratories such as the Mediterranean islands (A. Kyratzis and M.C. Kyriacou).

### Morphological diversity, genetic characterisation, and phytochemical assessment of the Cypriot tomato germplasm

Tomato (*Solanum lycopersicum* L.) is considered one of the most valuable and versatile vegetable crops globally and also serves as a significant model species for fruit developmental biology. Despite its significance, a severe genetic bottleneck and intense selection of genotypes with specific qualitative traits have resulted in the prevalence of a restricted number of (geno)types, also causing a lack of diversity across widespread cultivated types. As a result, the re-emergence of landraces as well as traditional and heirloom varieties is largely acknowledged as a countermeasure to restore phenotypic, phytochemical and genetic diversity while enriching the aroma/taste tomato palette. On those grounds, the Cypriot tomato germplasm was assessed and characterised. Ten landrace accessions were evaluated under greenhouse conditions and data were collected for 24 IPGRI discrete phenotypic traits. Grouping of accessions largely reflected the fruit shape and size; four different fruit types were recorded across accessions (flattened, heart-shaped, rounded and highly rounded). Moreover, a single run panel consisting of ten SSRs was developed and applied in order to genetically characterise 190 Cypriot genotypes and foreign heirloom varieties. Based on genetic indexes it was established that tomato landraces have a rather low level of heterogeneity and genetic variation. Finally, mineral and phytochemical analyses were conducted in order to estimate biochemical attributes (total phenolics, ascorbic acid, lycopene, b-carotene, total soluble content, titratable acidity) across genotypes; thus, ascertaining that the Cypriot panel has a high nutritional value. Due to the thermo-drought adaptation and tolerance of these genotypes, the current study serves as a roadmap for future breeding efforts in order to incorporate desirable traits or develop novel tomato lines combining resilience and alimentary value (A. Kyratzis).

### **Rostraria hadjikyriakou (Poaceae), a new gypsophilous and endemic species from Cyprus**

*Rostraria hadjikyriakou* (Poaceae) is described as new to science. This gypsophilous taxon occurs in three disjunct sites in the eastern half of Cyprus. From a morphological point of view there are similarities to the polymorphic and widespread *R. cristata*. However, it differs by a number of discontinuous characters from this species as well as all other members of the genus. Provisionally, it can be classified as “Vulnerable” (A. Kyratzis).

## **PROJECTS FUNDED BY THE RESEARCH AND INNOVATION FOUNDATION**

### **Carobs, the black gold of Cyprus: Science meets industry – Black gold**

The overall objective of the project is to help re-mobilise and extend the carob industry in Cyprus into new and innovative food and nutrient supplement products that meet the needs of the growing international market for carob-based products. This objective of re-establishing the carob economic sector in Cyprus will be based on the in-depth scientific analysis of key market valued properties possessed by carobs in Cyprus.

The sector is responsible for the characterisation of the genetic, morpho-physiological and compositional Variability of Local Carob Genetic Resources (Work package 3). Such information is crucial for the conservation of the species and the selection of most promising genotypes for cultivation to the appropriate environments. This will be accomplished through: (1) Genotyping and phenotyping of indigenous carob genetic resources from the main agroecological zones of Cyprus where carobs are traditionally cultivated. (2) Evaluation of the comparative contribution of genotype and environment effects on the variance of agronomic and technological traits. (3) Characterisation of morphological, physiological and compositional changes with respect to carob fruit on-tree ripening and postharvest storage.

(M.C. Kyriacou and A.C. Kyratzis)

### **Pod morphology, primary and secondary metabolite profiles in non-grafted and grafted carob germplasm are configured by agro-environmental zone, genotype, and growing season**

Carob is a predominantly rainfed tree crop of high nutritive value and a long history of adaptation to the edaphoclimatic stress conditions of the Mediterranean. However, declining attention to the carob tree in recent decades has aggravated genetic erosion. The extant *in situ* germplasm varies, both in terms of pod morphology and composition, reflecting the genetic and physiological divide chiefly among grafted and non-grafted material, and possibly the impact of variable agro-environments. Accordingly, the present study aimed to establish a systematic categorisation of the genetic and phenotypic diversity encountered across carob germplasm identified *in situ* throughout Cyprus, a historical center of production and genetic diversity for the species. Linking pod morphology, primary and secondary metabolite profiles with genotyped source material originating in different agro-environments and crop seasons would provide a framework for interpreting (a) the interaction of these factors in configuring carob pod physicochemical constitution, and (b) the relative stability of phenotypic traits against environmental and seasonal variation. Microsatellite analysis discriminated 36 genotypes out of the 124 trees located in nine traditional agro-environmental zones and revealed low genetic diversity within the grafted germplasm. Two landraces were identified: “Tillyria,” which is widespread and predominant, and “Kountourka,” which is mainly localised to the northeastern peninsula of Karpasia. Morphological traits, such as seeds-to-pod weight ratio, pod width and thickness were principally under genetic control. Contrarily, compositional traits, particularly total phenolic content-including condensed tannins, *in vitro* antioxidant capacity and to a lesser extent gallic acid, organic acids and minerals were under agro-

environmental control. Agro-environmental zone also modulated principally fructose and glucose; sucrose was modulated equally by genotype and agro-environment, while total sugars were under genetic control. Statistically significant differences between seasons were detected for all traits except for the seeds-to-pod weight ratio, pod length and width. Hierarchical cluster analysis corroborates that Cyprus may be divided into two major agro-environmental zones modulating the compositional properties of the carob pulp. The present study provides a comprehensive insight into the extant carob genetic resources of Cyprus and advances our understanding of how genetic, agro-environmental and seasonal factors interact in shaping carob pod morphology and composition. **(A.C. Kyratzis, L.C. Papayiannis and M.C. Kyriacou)**

### **Heat- and ultrasound-assisted aqueous extraction of soluble carbohydrates and phenolics from carob kibbles of variable size and source material**

Aqueous extraction of carob kibbles is the fundamental step in the production of carob juice and carob molasses. Improving the theoretical yield in sugars during organic solvent-free aqueous extraction is of prime interest to the food industry. Collateral extraction of phenolics, however, must be monitored as it influences the sensory and functional profile of carob juice. We presently examined the impact of source material, kibble size, temperature, and duration on the efficiency of extracting sugars and phenolics aqueously by conventional heat-assisted (HAE) and ultrasound-assisted (UAE) methods. Source material was the most influential factor determining the concentration of phenolics extracted by either method. Source material also influenced the relative proportions of sucrose, glucose, and fructose, which may impact the perceived sweetness of the juice. Kibble size (medium size M = 9–13 mm; powder size P = 1–4 mm) was more influential with UAE than HAE for both sugars and phenolics but was rendered less influential with prolonged UAE duration. Increasing HAE temperature (from 25 °C to 75 °C) favoured the extraction of phenolics over sugars; however, prolonging extraction at 25 °C improved sugar yield without excessive yield in phenolics. Disproportionate extraction of phenolics over sugars limits the use of heat-assisted extraction to improve sugar yield in carob juice production and may shift the product's sensory profile toward astringency. Prolonged extraction at near ambient temperature can, however, improve sugar yield, keeping collateral extraction of phenolics low. Ultrasound agitation constitutes an effective means of extracting sugars from powder-size kibbles. Industrial application of both methodologies depends on the targeted functional and sensory properties of carob juice. **(C. Antoniou, A.C. Kyratzis and M.C. Kyriacou)**

### **Mapping the primary and secondary metabolomes of carob (*Ceratonia siliqua* L.) fruit and its postharvest antioxidant potential at critical stages of ripening**

Six critical stages corresponding to major morphophysiological events in carob fruit ripening were defined, and changes in the primary and secondary metabolome and *in vitro* antioxidant capacity were examined in two genotypes collected at low (15 m) and high (510 m) altitudes from genetically identified and georeferenced trees. Soluble carbohydrates were analysed by HPLC-RI, macro-minerals by ion chromatography coupled to conductivity detection and polyphenols by UHPLC-Q-Orbitrap-HRMS. Spectroscopy facilitated assays for condensed tannins and *in vitro* freeradical scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP). The fruit respiration rate and moisture content declined sharply during the transition from the breaker to green pedicel stage. Sugar accumulation spiked at the onset of fruit colouration and culminated at  $498.7 \pm 8.4$  mg g<sup>-1</sup> dry weight (dw) in the late ripe stage, while the ratio of reducing sugars to sucrose decreased from  $3.45 \pm 0.32$  to  $0.41 \pm 0.02$ . The total phenolic compounds and condensed tannins declined with ripening, particularly during the transition from the breaker to green pedicel stage. Eighteen polyphenols were identified and quantitated, with catechins and hydrolysable tannins being dominant until the onset of fruit colouration. The transition to the green pedicel stage signaled a precipitous decline (90.9%) in catechins, hydrolysable tannins (60.2%) and flavonol glycosides (52.1%) concomitant to the



rise in gallic acid, which was putatively fueled by the enzymatic hydrolysis of gallotannins in immature fruit. Catechins, hydrolysable tannins and flavone glycosides were more abundant at higher altitudes and gallic acid at lower altitudes. An antioxidant capacity was also favoured by higher elevations and declined with ripening, particularly after the breaker stage. Correlations with FRAP and DPPH assays were significant for the total phenolic content, condensed tannins, catechins and hydrolysable tannins. The highest correlation factors were obtained for epigallocatechin-gallate ( $r = 0.920$  and  $r = 0.900$ ;  $p < 0.01$ ). Although the sharp drop in hydrolysable and nonhydrolysable tannins and catechins compromised the *in vitro* antioxidant capacity at physiological maturity, it also reduced the astringency and configured a palatable organoleptic fruit profile. These changes unraveled significant episodes in the ripening-related secondary metabolism of the carob fruit. They further highlighted the value of immature carob as a potent source of gallotannins, with putative *in vivo* anti-inflammatory action, and of catechins beneficial in preventing and protecting against diseases caused by oxidative stress. **(M.C. Kyriacou and A.C. Kyratzis)**

### **Configuration of the volatile aromatic profile of carob powder milled from pods of genetic variants harvested at progressive stages of ripening from high and low altitudes**

Carob powder is increasingly valued as a substitute for cocoa and as a flavour-enhancing component of processed foods. However, little is known about the impact of preharvest factors such as fruit maturity, genotype and altitude on its volatile organic compounds (VOCs) composition. The current study examined the VOCs composition of powder milled from pods of two genotypes cultivated at 15 and 510 m altitude and harvested at six progressive stages of maturity, ranging from fully developed immature green (RS1) to late ripe (RS6). Fifty-six VOCs categorised into acids, esters, aldehydes, ketones, alcohols, furans, and alkanes were identified through HS-SPME GC-MS analysis. Maturity was the most influential factor, followed by altitude and least by genotype. Aldehydes and alcohols correlated positively ( $r = 0.789$ ;  $p < 0.001$ ), both accumulated in immature carobs and decreased with progressive ripening, resulting in the attenuation of green grassy aroma. Conversely, acids increased with ripening and dominated the carob volatilome at full maturity, correlating negatively with aldehydes and alcohols ( $r = -0.835$  and  $r = -0.950$ , respectively;  $p < 0.001$ ). The most abundant VOC throughout ripening (17.3-57.7%) was isobutyric acid, responsible for the characteristic cheesy-acidic-buttery aroma of carob powder. The pleasurable aroma detected at the immature stages (RS2 and RS3) was traced to isobutyrate and methyl isobutyrate esters, rendering unripe green carob powder a potential admixture component for improving the aroma of novel food products. Lower altitude favoured the accumulation of acids linked to less pleasant aroma, whereas isobutyric acid was more abundant at higher altitude. This constitutes a significant indication that higher altitude enhances the characteristic carob-like aroma and sensory quality of carob powder. **(A.C. Kyratzis, G.A. Soteriou and M.C. Kyriacou)**

## 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 2020 and 2021, 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 2020 and 2021 is shown in **Table 4.1**. The overall performance of the herd was satisfactory, since milk yield per annual cow was on average 8 833 in 2020 and 8 944 in 2021. The total milk produced on farm was 420 447 in 2020 and 432 885 in 2021. Good reproductive management led to low abortion numbers in 2020 and 2021. Mastitis incidence was further reduced during 2020-21. (G. Hadjipavlou and 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 350 Cyprus Chios sheep and 300 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 120 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 2020-21 are shown in **Tables 4.2** and **4.3**. (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 2020 and 2021. 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  $49 \pm 3$  days of age. AR goats were milked twice daily. NS goats were milked once daily before and twice daily after weaning. Results in 2020 and 2021 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)



### **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 2020-2021 was 36 and 147, respectively, and that of surplus female lambs was 107. 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 and I.M. Ioannides)**

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

The programme for controlling the scrapie disease in goats runs in the framework of ARI-funded joint research between the Animal Production and Agrobiotechnology Sections, and in cooperation between the ARI and the Veterinary Services. During 2020-2021, 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 2020 and 2021, molecular genotyping of the selected *PrP* alleles was conducted by the Agrobiotechnology laboratory on 428 animals. In this period, the Animal Production section provided the farmers with 35 bucks, 120 male kids and 50 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 and I.M. Ioannides)**

### **Research project on the establishment of a “CYprus AGRicultural Genomics cENTre” (AGRICYGEN)**

In 2021, the nationally funded AGRICYGEN project has commenced following a Council of Ministers Decision, published on the 27<sup>th</sup> of November 2019 (Decision Number 88.605). The AGRICYGEN project aims at the establishment of a Centre of Excellence in Cyprus, which will take advantage of cutting-edge technologies and expertise in order to facilitate:

- a) Increased milk production from local sheep and goat breeds
- b) Increased production of local ruminant feed
- c) The ecological enrichment of Cyprus soils

The project outcomes will have direct impact on the Cyprus economy and society, and will significantly contribute to sustainable development of agriculture and animal production on the island. The initial focus of the project will be on employing research approaches for fulfilling the halloumi cheese PDO requirements in



small ruminant milk and feed production, within the harsh and fluctuating environmental and climatic conditions of Cyprus. AGRICYGEN will also aim at further advancing research and innovation activities in various domains connected with the primary production sector.

Within 2021, relevant infrastructure upgrades were made at the ARI sheep and goat experimental farm in Athalassa area, and the genomic analysis laboratory of the Animal Production section was established and properly equipped, with cutting-edge high-throughput genotyping equipment. In addition, DNA material from 1 061 sheep, 1 060 goats and 738 barley samples were analysed using mid-range SNP beadchip microarray technology, in order to facilitate genomic evaluations and selection, along with further agrigenomic research activities in subsequent years. **(G. Hadjipavlou, D. Fasoula, M. Omirou and I.M. Ioannides)**

### **Recovery and Resilience Plan (RRP)-funded project on the genetic improvement of the Cyprus sheep and goat populations**

In conjunction with the AGRICYGEN research project, and within the context of the preparation of the Cyprus Government RRP, a reform project aiming at the genetic improvement of the Cyprus sheep and goat populations was designed, proposed and negotiated with the European Commission (EC). The reform was approved for funding within the Cyprus RRP, both by the Cyprus Council of Ministers Decision, published on the 14th of May 2021 (Decision Number 91.256) and by the Council of the European Union Implementing Decision number 10686/21 on the approval of the assessment of the recovery and resilience plan for Cyprus.

The central objective of this reform is to pursue the implementation of the national plan on genetic improvement of the Cyprus sheep and goat populations, through the following: support of the sheep and goat farmers to upgrade their record keeping on farm, their production processes, product quality evaluation efforts and participation in the nationally funded AGRICYGEN project that will provide them with advanced knowledge and guidance on the genetic merit of their animals. Knowledge on the genetic value of their animals will allow them to make informed decisions on animal reproduction to achieve significant improvement of their farm productivity, primarily in terms of milk production, in an accelerated and accurate manner.

This RRP-funded project will provide incentives and subsidies and will enable the establishment of long-term collaboration between ARI and the sheep and goat breeders. In order to receive these incentives, eligible breeders will have to go through an application and evaluation process, and, if selected, will need to upgrade their farm equipment, digitalise on-farm data recording, conduct analyses to determine the quality of the milk produced and sold to dairy units and use cutting-edge methods for reproduction and genetic selection of animals. The ARI, through the nationally funded research project AGRICYGEN, will provide the latter service, in order to improve animal and farm productivity. **(G. Hadjipavlou)**



**Funded by the  
European Union**  
NextGenerationEU

**Table 4.1.** Performance of dairy cattle at the ARI, Athalassa Experimental Farm

Variable	Year	
	2020	2021
Cows calved	32	31
Heifers calved	18	25
Abortions	1	4
Calves born alive	49	56
Calves born dead	4	7
Calves died	1 (2.04%)	3 (5.36%)
Calving interval (days)	398.9	400.7
Days open	124	128.8
Duration of pregnancy (days)	273.2	271.5
Services/conception	1.88	1.67
Annual cows	47.6	48.4
Milk produced (l)	420 447	432 885
Milk/annual cow (l)	8 833	8 944
Milk fat %	3.23	3.29
Milk protein %	3.46	3.39

**Table 4.2.** Production characteristics of Chios ewes at ARI (2020/21)

Trait	Yearlings	Adults
No. of ewes lambing	133	294
Lambs born/ewe	1.70	2.07
Lambs born live/ewe	1.50	1.76
Litter weight at birth (kg/ewe)	5.54	6.49
Lambs weaned/ewe	1.29	1.66
Litter weight at weaning (kg/ewe)	17.4	20.6
60-day milk (kg/ewe)	112	136
Total milk (kg/ewe)	408	461
Days in milk	212	218
Milk fat (%)	5.68	6.20
Milk protein (%)	4.89	5.20

**Table 4.3.** Production characteristics of Damascus goats at ARI (2020/21)

Trait	Yearlings	Adults
No. of goats kidding	71	320
Kids born/goat	1.61	1.86
Kids born live/goat	1.54	1.78
Litter weight at birth (kg/goat)	5.49	6.86
Kids weaned/goat	1.22	1.49
Litter weight at weaning (kg/goat)	15.8	20.0
60-day milk (kg/goat)	78	110
Total milk (kg/goat)	361	487
Days in milk	204	210
Milk fat (%)	4.03	4.24
Milk protein (%)	3.61	3.73

## PLANT PROTECTION

The research work of the Plant Protection Section encompasses Plant Pathology and Entomology. Specialised studies are conducted to address major crop protection problems associated with particular virus and virus-like diseases, prokaryotic microorganisms and insect pests. Joint multi-disciplinary research projects are undertaken, targeting various important pests and diseases of cultivated vegetable and fruit tree crops. All research activities 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 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 ecosystem-oriented methods, i.e., biological control, cultural practices, natural products, resistant varieties, resistant rootstocks and minimal use of selected, mild pesticides. This approach ensures sustainability and minimises pesticide risk to human health and the environment. It can be applied alone or in combination with other ecosystem-oriented production practices, in the context of an advanced system of sustainable agriculture, known as integrated crop management (ICM). Studies to develop IPM/ICM systems were carried out on citrus, grapevines and on vegetable and ornamental greenhouse crops, in cooperation with other Sections of the Institute.

Further to the Section's systematic research work, the Plant Pathology and Entomology laboratories provided support for the scientific identification of plant diseases and insect pests, in 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

### The situation of *Citrus tristeza virus* in Cyprus

*Citrus tristeza virus* (CTV) was introduced in Cyprus in 1929 by infected budwood imported from South Africa, and was first identified during the late 1960s. A long-term research project was performed in order to investigate the virus presence on the island, to characterise the existing isolates, to remove the infected trees and to establish a citrus certification programme. Survey results indicated that the recorded mean infection on the island was approximately 4.2%. At the same time, healthy citrus varieties were selected, tested and placed in insect proof screenhouses at the Agricultural Research Institute experimental stations. This plantation is officially certified as the National Prebasic plantation of citrus material. Furthermore, a study was conducted for the molecular characterisation and strain differentiation among CTV isolates, which were selected on the basis of different symptomatology on host plants that ranged from inconspicuous to twig die back, decline and death of sweet orange or grapefruit trees on sour orange rootstock. Seventy-five virus isolates were partially sequenced and phylogenetic analysis results showed that Cypriot isolates were clustered in six major groups which include both severe and mild strains originated from Africa, America, Europe and Asia. These results indicate the multiple introductions of several isolates from different geographical regions. Although the incidence of mild CTV isolates is higher than those responsible for severe symptoms, the identification of severe

strains linked to quick decline is of major importance since the use of sour orange rootstock is widely used on the island. **(L.C. Papayiannis and T. Kapari-Isaia)**

### **Citrus rootstock evaluation for tolerance to Cypriot isolates of *Citrus tristeza virus***

The main goal of this project was to investigate 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. Half of these trees were graft-inoculated with CTV and the rest kept 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 and L.C. Papayiannis)**

### **Citrus certification programme**

A mandatory certification programme has been established and functioning 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 virus-tested material to the mother blocks of the Department of Agriculture, which in turn provide budwood to private nurseries or directly to 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 known viruses via micrografting. All virus-free material, which was either selected, or imported by the Horticulture Section of the Institute or produced locally so far 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 citrus material to the basic or mother plantations of the Department of Agriculture and the experimental glasshouses of the Institute. **(T. Kapari-Isaia and L.C. Papayiannis)**

### **Production of healthy local citrus propagating material**

For several commercial varieties, it is possible to reintroduce presently healthy material from overseas Citrus Production Centres. However, this always includes the risk of introduction of new unknown diseases. In addition, for local varieties there is not such possibility. For these reasons, it was decided to employ contemporary techniques such as micrografting *in vitro* to eliminate viruses and other disease problems from 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 eliminate viruses and other pathogens from 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 and L.C. Papayiannis)**

### Elimination of citrus pathogens from infected citrus plants by micrografting *in vitro*

*In vitro* micrografting technique has been used for sanitation of citrus species and varieties infected by *Citrus tristeza virus* (CTV) and/or citrus viroids. Several citrus tree varieties including the lemon varieties Adamopoulou and Verna, the mandarin varieties Clasuelina, Page were selected. All trees were tested for viruses and viroids by biological indexing, by ELISA for CTV and CPsV, and by RTPCR for viroids. They were all found free of CPsV, CVV, concave gum and impietratura. The micrografting technique *in vitro* was used for elimination of viroids and CTV in the selected citrus isolates. 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 6-12 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. **(T. Kapari-Isaia and L.C. Papayiannis)**

### Molecular typing 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. Until recently, *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd) variants were the only viroid species identified in Cyprus associated to exocortis and cachexia disease, respectively. In view of a long-term project aiming at the identification and management of citrus diseases in the island, an extensive survey was conducted during 2020-2021 in order to identify the presence and incidence of citrus viroids using molecular based techniques and in selected samples biological indexing. Results showed that CEVd was the most widespread viroid with an incidence of 80.5%, followed by HSVd which was detected in 49.3% of the 528 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 (71%), while CEVd+CBLVd, CEVd+CDVd and CEVd+CBCVd were detected at 10.7, 9.2 and 8.6% of the double viroid mixtures. Triple viroid combinations were also recorded at 11% of the tested samples with CEVd and HSVd identified in all mixtures. *Citrus viroid V* was not detected in any of the samples. Only 5% of the samples tested were negative to viroid infection. **(L.C. Papayiannis and T. Kapari-Isaia)**

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

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

### *In vitro* techniques for elimination of pathogens in infected Citrus

*In vitro* micrografting, chemotherapy and cold therapy techniques have been used for sanitation of citrus species and varieties infected by *Citrus tristeza virus* (CTV) and/or citrus viroids. Several citrus tree varieties 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/lit ribavirin or 30, 40 and 50 mg/lit methotrexate. Elimination of viroids through cold therapy at 10 C for 5 weeks was not possible, but was successful via cold therapy at 4 C for 5 weeks. **(T. Kapari-Isaia and L.C. Papayiannis)**

### Citrus rootstock evaluation for tolerance to Cypriot isolates of *Citrus tristeza virus*

The main goal of this project was to investigate 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 ARI's Xylotymbou Experimental Station: Carrizo citrange, Swingle citrumelo, Volkameriana lemon, Gou Tou, Cleopatra mandarin and Sour Orange. Fifty percent of these trees were graft-inoculated with CTV and the rest remained healthy. Infected and healthy trees were 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 and L.C. Papayiannis)**

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

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

### Production, maintenance and distribution of healthy stone fruit material

Virus-free material is maintained in a pre-basic plantation under insect-proof screen at the Zygi and Saittas Experimental Stations. Currently, 52 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 genetic aberrations. The plantations provide with propagating material for the basic or mother plantations of the Department of Agriculture and for the experimental glasshouses of the ARI. **(T. Kapari-Isaia and L.C. Papayiannis)**

### Grapevine viruses and grapevine pre-basic plantation in Cyprus

More than 80 distinct virus species have been reported to infect grapevines worldwide. A survey was conducted to investigate the presence of viruses in grapevine crops in Cyprus and approximately 1 200 samples were randomly collected from the districts of Lemesos, Pafos and Lefkosia. All samples were tested using serological and molecular methods for the presence of 15 viruses involved in leafroll associated and rugose woody disease complex, as well as fleck, infectious degeneration and decline disease complex. Results showed that among the viruses associated to leaf roll disease, *Grapevine leafroll-associated virus 3* (GLRaV-3) was detected in more than

45% of the samples tested, followed by GLRaV-1 (8%) and GLRaV-2 (1%). The incidence of viruses involved in rugose woody complex was 18, 7 and 2% for *Grapevine virus A* (GVA), *Grapevine rupestris stem pitting-associate virus* (GRSPaV) and *Grapevine virus B* (GVB), respectively. *Grapevine fanleaf virus* (GFLV) and *Grapevine fleck virus* (GFkV) were also detected at 8 and 0.5% respectively. Two new viruses, *Grapevine roditis leaf discolouration associated virus* (GRLDaV) and *Grapevine pinot gris virus* (GPGV) were identified in a selection of samples from Greek and other imported varieties. Finally, *Arabidopsis mosaic virus* (ArMV), *Tomato ringspot virus* (ToRSV), and the grapevine leafroll-associated viruses 4 and 7, were not detected. Interestingly, most viruses were detected in mixed infections of various combinations. The presence of these viruses has most likely resulted from the use of infected plant material combined with possible spread by vectors.

Efficient control of plant virus, virus-like and prokaryotic diseases starts with the use of healthy propagating material that is free not only from disease symptoms but also from latent infections. EU has recently imposed strict directives on distribution of grapevine material that involve phytosanitary testing of plants for several plant pathogens. Utilisation of healthy plant material seems nowadays the most promising tactic that can ensure good results to control these diseases. During the past decades, 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*, *Arabidopsis 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 then given to the Department of Agriculture for further multiplication and distribution to Cypriot farmers. **(L.C. Papayiannis and T. Kapari-Isaia)**



### Studies of tomato viruses in Cyprus

A study has been conducted in order to assess the identity and prevalence of viruses infecting crops of Cyprus, using serological and molecular based diagnostic tools. Results showed that TYLCV was the only *Begomovirus* species involved in tomato yellow leaf curl disease epidemics and is widely distributed. TYLCV was also detected in several weed species belonging in different botanical families, which could serve as natural virus reservoirs. ToCV was also identified in plants showing chlorotic symptoms with a low incidence. In nature, all of these viruses are transmitted with *Bemisia tabaci* MEAM1 (also known as biotype B), which is currently the most abundant and important whitefly vector in Cyprus. *Tomato brown rugose fruit virus* (ToBRFV) is a new Tobamovirus that has emerged in several Mediterranean and European countries during the past five years. ToBRFV was detected for the first time in Cyprus infecting greenhouse tomatoes causing symptoms of chlorosis, mosaic with dark brown bulges and necrosis on leaves as well as yellow spots, with occasional rugose symptoms on the fruits. Detection of ToBRFV was performed with conventional and TaqMan reverse transcription polymerase chain reaction assays as well as with enzyme linked immune sorbent assay. In some cases, the virus was identified in mixed infections with PepMV. ToBRFV transmission is mainly mechanical, but it can also occur via contaminated seeds or fruits over long distances, such as for other common tobamoviruses. The mechanical transmission of this new pathogen within crops can occur through direct contact with infected plants or infected sap from different surfaces (operator, clothing, pots, packaging, consumption of tomatoes coming from a different crop, transport equipment, working tools, nutrient solutions), propagation materials (grafts, cuttings) and seeds. **(L.C. Papayiannis)**





## ENTOMOLOGY

### 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)**

### South Eastern Mediterranean Environment Project (SEMEP)

SEMEP is an interdisciplinary environmental education project under the auspices of UNESCO, which focuses primarily on the South Eastern Mediterranean Sea region. The project promotes education for sustainable development through science and intercultural dialogue and the network is coordinated by the Ministry of Education. Public and private schools of secondary education participate in the programme. The Plant Protection Section of the Agricultural Research Institute is responsible for the supervision of the project "Identification of the main pests of greenhouse vegetables and ways to control them" in the Paralimni and Polis Chrysochous Lyceums. **(N.A. Seraphides)**



## NATURAL RESOURCES AND ENVIRONMENT

The main research activities of the Natural Resources and Environment (NRE) Section focused on the rational management of natural resources, precision agriculture, environmental protection, enhancing biodiversity and mitigating the effects of climate change on agriculture. An important part of the research work has been accomplished within the framework of competitive programmes from various funding agencies, such as the European Union, and the Research and Innovation Foundation of Cyprus.

### PLANT NUTRITION AND SOILLESS CULTURE

Soilless culture (SC) is the leading cultivation technology in modern greenhouses, recognised globally for its ability to promote resource-efficiency toward an environmentally friendly agriculture and a promising tool in terms of food security. Sustainable production mainly relies on hydroponic systems with reuse of drainage water since a close control in the supply of water and nutrients is impossible in soil-grown crops. However, in Mediterranean countries, SC systems have been performing below their potential and this needs to be changed.

#### Nitrate supply limitations in tomato crops grown in a chloride-amended recirculating nutrient solution

This study suggests that replacing 1/3 of the standard  $\text{NO}_3^-$  supply by  $\text{Cl}^-$  in closed hydroponic tomato crops (beefsteak and cherry types) enhances N use by two-fold ( $\text{kg produce kg}^{-1}$  N supply) and eliminates  $\text{NO}_3^-$  losses to one-half, with no significant effects on assimilation processes, fruit biomass production and nutritional quality, suggesting that  $\text{Cl}^-$  at appropriate concentrations is not only an essential micronutrient but also a beneficial macronutrient. Tomato plants supplied with  $\text{Cl}^-$ -amended nutrient solution increased leaf  $\text{Cl}^-$  content to macronutrient level ( $35 \text{ mg g}^{-1}$  dry weight), retaining photosynthetic rates and crop yield potential at lower stomatal conductivity and transpiration. Nutrient to water uptake ratios (mass of nutrient per water volume absorbed), which are commonly termed 'uptake concentrations', were defined in different cropping seasons and ranged as follows: 12.1-13.5 (Nitrogen-  $\text{NO}_3^- + \text{NH}_4^+$ ), 1.25-1.35 (Phosphorus-P), 6.1-6.3 (Potassium-K), 3.6-4.0 (Calcium-Ca), 1.0-1.3 (Magnesium-Mg,  $\text{mmol L}^{-1}$ ), 13.0-14.3 (Iron-Fe), 7.6-8.4 (Manganese-Mn), 5.1-5.2 (Zinc-Zn) and 0.7-0.9 (Copper-Cu,  $\mu\text{mol L}^{-1}$ ). The obtained uptake concentrations may be used through on-line operating decision support systems to optimise nutrient supply and save precious water in Mediterranean greenhouses.

(D. Neocleous)





### Impact of chelated or inorganic manganese and zinc applications in closed hydroponic bean crops on growth, yield, photosynthesis, and nutrient uptake

(Mn) and zinc (Zn) chelates on common bean grown in hydroponics on physiological and agronomical responses. Inorganic sulphate forms of Mn and Zn were compared to their synthetic chelate forms in the replenishment nutrient solution (RNS). Nutrient (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) to water uptake ratios (termed uptake concentrations; UCs), growth, pods yield and quality, photosynthetic parameters and tissue nutrient status were evaluated in different cropping seasons (spring-summer and autumn-winter crops). Mean UCs of nutrients ranged as follows: 10.1-12.4 (N), 0.8-1.0 (P), 5.2-5.6 (K), 1.8-2.2 (Ca), 0.9-1.0 (Mg) mmol L<sup>-1</sup>; 12.2-13.4 (Fe), 5.2-5.6 (Mn), 4.4-4.9 (Zn), 0.9-1.0 (Cu)  $\mu$ mol L<sup>-1</sup>. Tissue macronutrient status remained unaffected in both seasons; however, Mn chelates in the RNS affected Fe within plants. Pod yield and quality, growth, photosynthesis and water uptake did not differ among treatments; however, seasonal variations were observed. Results suggest that the chelate forms of Mn and/or Zn in the refill solution for hydroponic beans do not lead to any changes adding superiority in yield, photosynthesis, and nutritional status of the crops compared to their mineral forms. **(D. Neocleous)**



### Composition of nutrient solutions for cherry tomatoes grown in hydroponics

In recent years there has been an increasing trend in the production of small-fruited tomatoes such as cherry tomatoes with better taste, attractive appearance, more lycopene and vitamins, which has a particular export interest. For this purpose, the evolution of hydroponics as a method of cultivation of small-fruited tomatoes in the greenhouse is closely related with the complete control of nutrition via the nutrient solution. The following nutrient solution recipes were successfully tested in cherry tomatoes grown in soilless culture systems.

	EC	K	Ca	Mg	NH <sub>4</sub>	SO <sub>4</sub>	NO <sub>3</sub>	P	Fe	Mn	Zn	Cu	B	Mo
A	2.6	9.50	5.40	2.40	1.20	4.40	15.00	1.50	15.00	10.00	5.00	0.75	30.00	0.50
B	2.6	11.0	4.90	2.15	1.20	4.40	15.00	1.50	15.00	10.00	5.00	0.75	30.00	0.50
C	2.5	9.50	5.40	2.40	0.20	4.40	15.00	0.50	15.00	10.00	5.00	0.75	30.00	0.50

A: Vegetative stage, B: Fruit set, C: End of season. EC, dS m<sup>-1</sup>, NO<sub>3</sub>, NH<sub>4</sub>, SO<sub>4</sub>, P, K, Ca, Mg mmol L<sup>-1</sup>, Fe, Mn, Zn, Cu, B, Mo  $\mu$ mol L<sup>-1</sup>. **(D. Neocleous)**



### Other studies related to climate change, sustainable use of soil and water, precision agriculture and environmental issues, 2020-2021

(i) Smart farming techniques for climate change adaptation in Cyprus. Irrigation of potato crops was fine-tuned using volumetric soil water sensors. Based on the collected data, it is suggested to retain soil water content within the upper 30 cm of the soil close to 40%. Overwatering and large fluctuations in the upper portion of the root zone moisture, can cause tuber cracks and eventually yield losses. **(G. Adamides and D. Neocleous)**;

(ii) Sustainable irrigation in water-scarce regions such as Cyprus under the impact of climate change. Protected cultivation systems such as greenhouses or screenhouses, equipped with artificial intelligence systems present another sustainable option for improving water productivity and may help to alleviate water scarcity in these regions;

(iii) Irrigation groundwater quality evaluation in Cyprus showed possible restrictions in groundwater use for irrigation in relation to its salt content and specific ions toxicity having adverse effects on sensitive and several moderate sensitive crops in some instances;

(iv) In the content of "precision agricultural farming systems", nutrient uptake modelling, phyto-sensing, smart and sustainable technologies must be applied for monitoring and evaluating water and nutrients crops supply. **(D. Neocleous)**;

A holistic approach for evaluating potential risks of lead (Pb) and chromium (Cr) contamination of soils, sediments, water and vegetation suggests management measures. **(A. Christou and D. Neocleous)**

### European and National (RIF) Programmes, 2020-2021

#### Horizon 2020 - Ploutos

Ploutos aims to create opportunities for changes that can rebalance the value chain in the agri-food system towards a more environmentally, socially and economically sustainable system. Ploutos particularly focuses on interoperability architectures that effectively integrate with the growing technological offerings for farm management, precision farming and integrated supply chain data management. **(A. Stylianou and D. Neocleous)** <https://ploutos-h2020.eu/>



### Horizon 2020 - IoT4Potato / CySloP

An innovative, market-ready smart farming solution supports irrigation, pest management and fertilisation. Leveraging a network of telemetric IoT stations combined with satellite data and scientific models tailored to the specificities of the geographic areas, helps small-scale farmers to tackle those challenges. The results show that a significant 20% reduction in ferti(irri)gation and pesticide application could be obtained (**A. Stylianou, G. Adamides and D. Neocleous**) <https://www.iof2020.eu/>

### RIF / RESTART 2016-2020 - Magnet

The aim of the action is to provide the basis of a new approach of soil and water resource management in Cyprus that is related to farming, as a means of adaptation to climate change. The novelty of the proposed approach of crop fertilisation and irrigation is that it allows farmers, through geographic information systems (G.I.S.) that combine different layers of information, to retrieve and manage data in a more useful manner. (**M. Omirou, P. Dalias and D. Neocleous**)

## INTEGRATED WATER RESOURCE MANAGEMENT AND IRRIGATION

Research on integrated water resource management is currently devoted mainly to the challenges related to the release of contaminants of emerging concern (CECs) (i.e., pharmaceuticals, antibiotic resistant bacteria, antibiotic resistance genes) to the agricultural environment being irrigated with treated wastewater (TW) or amended with biosolids, and the mediated potential public health risks. In addition, the effects of the contamination of irrigation water with hexavalent chromium [Cr(VI)] on agricultural crops growth and development, as well as on public health are also evaluated.

### Effects of prescription antibiotics on soil- and root-associated microbiomes and resistomes in an agricultural context

In order to evaluate the potential risks associated with the spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in agricultural settings due to TW irrigation, we analysed microbiomes and resistomes of soil and *Lactuca sativa* L. (lettuce) root samples from pots irrigated with tap water spiked with 0, 20, or 100 µg L<sup>-1</sup> of a mixture of three antibiotics (Trimethoprim, Ofloxacin, Sulfamethoxazole). The presence of antibiotics induced changes in bacterial populations, particularly in soil, as revealed by 16S rDNA sequence analysis. Parallel shotgun sequencing identified a total of 56 different ARGs conferring resistance against 14 antibiotic families, also indicating multidrug resistance. These results may act as a warning that TW irrigation may result in increased loads of ARBs and ARGs in edible plant parts, therefore constituting a potential risk for human consumers. (**A. Christou**)

### Occurrence and distribution of hexavalent chromium in ground and surface waters in Cyprus

The origin and distribution of hexavalent chromium [Cr (VI)] over four seasonal cycles was investigated through a conceptual model that included three aquifer systems in Cyprus [Kokkinochoria (A1) and Kiti-Pervolia (A2) aquifers, and the Troodos massif (A3)]. Analytical results give a first insight to the presence of Cr (VI) in Cyprus ground and surface waters. The highest Cr (VI) value of 26 µg L<sup>-1</sup> is observed in the Troodos area (A3) where Cr(VI) is detected in all sampled water systems (surface and ground/spring). Nonetheless, the highest median values of 4.6 and 4.5 µg L<sup>-1</sup> are observed in the Kokkinochoria (A1) and Kiti-Pervolia (A2) study areas respectively, where the local aquifers are under increased pressure from agriculture, urbanisation and seawater intrusion. (**A. Christou**)

### **Uptake of hexavalent chromium by tomato (*Solanum lycopersicum* L.) plants and mediated effects on their physiology and productivity, along with fruit quality and safety**

Tomato (*Solanum lycopersicum* L.) plants were exposed to incremental Cr(VI) concentrations (0.05, 0.5, 1, 5 and 10 mg L<sup>-1</sup> of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in irrigation water), aiming at investigating potential Cr(VI)-mediated effects on (1) yield, (2) stress responses in leaves, (3) the biosafety of fruits and (4) the mediated alterations in tomato fruit quality attributes. Plants in all Cr(VI) treatments displayed similar phenotype. Plants irrigated with water containing 10 mg L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> had lower photosynthetic pigment concentration and suffered from oxidative stress in their leaves. Elevated Cr(VI) treatments (5 or 10 mg L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) resulted in fruits with lower weight and smaller size, along with increased soluble solids, lycopene,  $\beta$ -carotene and soluble carbohydrate (fructose, sucrose, total sugars) content. The concentration of total Cr in all fruit samples was far below the maximum permissible limits, thus indicating safety for human consumption. **(A. Christou, P. Dalias and D. Neocleous)**

## **SOIL FERTILITY IMPROVEMENT**

### **Effects of biochar amendment on the growth, physiology and quality attributes of field-grown lettuce plants**

This study evaluated the effects of soil amendment (5% v/v) with distinct types of biochar (biosolids-derived biochar, BDB; cattle manure-derived biochar, MDB; spent coffee grounds-derived biochar, SCGDB) on the growth, physiology and quality attributes of field-grown lettuce (*Lactuca sativa* L.) plants. Plants grown on MDB-amended soil showed a significant increase of biomass production rate compared with all other treatments. None of the treatments impacted the photosynthetic pigment content, however they did reduce the nitrates content in leaves. Moreover, plants grown in MDB- and BDB-amended soil showed increased total antioxidant capacity. Overall, results showed that BDB and MDB applied as soil amendments may serve as means for enhancing the growth, and partially the nutritional value of lettuce plants. **(A. Christou, P. Dalias and D. Neocleous)**

### **Soil fertility mapping**

The production of soil fertility maps illustrates the geographic variation of soil physical and chemical properties or indices (e.g. pH, EC, organic C available P, available K) in agricultural soils. One of the main intended applications of soil fertility mapping is the improvement of fertilisation schedules. Excessive use of fertilisers increases production costs increases greenhouse gas emissions and pollutes waters. **(P. Dalias and D. Neocleous)**

### **LIFE project AgrOassis**

The primary objective of AgrOassis project is to assist climatic change adaptation of the agricultural sector of Cyprus and Greece, two of the most vulnerable to desertification countries of the Mediterranean. The project will attempt to demonstrate innovative techniques and promote best practices and developed solutions on areas which have been selected as particularly prone to desertification. The suggested measures aim at i) changing attitude towards land management in Cyprus and Greece by introducing minimum tillage and no-tillage farming, ii) establishing drought adapted hedgerows around fields or in the erosion gullies in-between fields to decrease erosion, reduce surface temperatures, increase soil moisture and enhance biological activity of the soil, and iii) producing and incorporating in soils compost coming from municipal green waste or mixtures of chicken manure and green waste. **(P. Dalias)**

## AGRICULTURAL AND ENVIRONMENTAL ENGINEERING

### Lead contamination of soils, sediments, and vegetation in a shooting range and adjacent terrestrial and aquatic ecosystems: A holistic approach for evaluating potential risks

The degree of Pb contamination of soils, sediments and vegetation within the boundaries of a highly visited shooting range, as well as the fluvial transport and dispersal of Pb, and therefore the contamination of adjacent river and water reservoir, were evaluated. Soils in the shooting range were severely contaminated with Pb, with concentrations (ranking from  $791 \text{ mg kg}^{-1}$  to  $7265 \text{ mg kg}^{-1}$ ) being even hundreds of times higher compared with control samples. The degree of Pb contamination was negatively correlated with the distance from the shooting range. To this effect, the degree of contamination of the river and the water reservoir being in the vicinity of the shooting range was negligible. However, cultivated (olives) and wild native plant species grown within the shooting range accumulated high concentrations of Pb in their tissues (>50-folds compared with control). The severe contamination of soils, sediments and vegetation in the studied shooting range can provoke very high ecological risks. **(A. Christou, P. Dalias, D. Neocleous and E. Hadjisterkotis)**

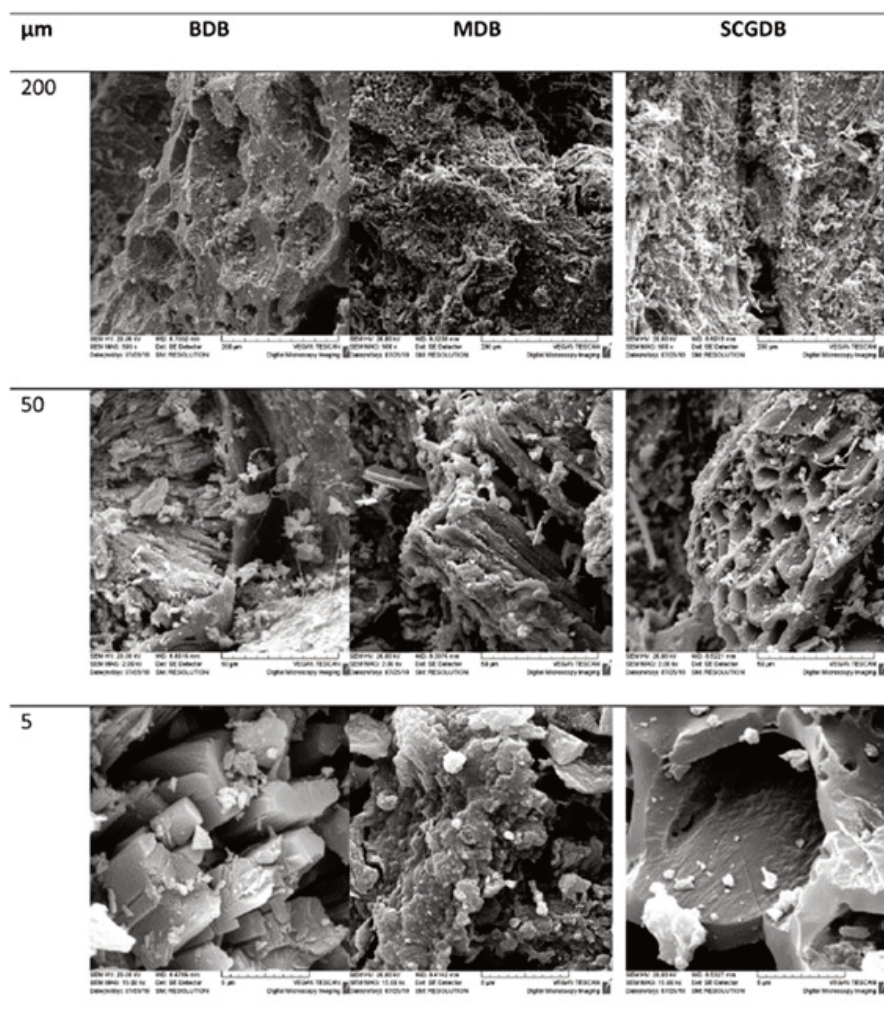


*The study area, including the shooting range and the adjacent terrestrial and aquatic (river and water reservoir) environments*

### Physicochemical and structural characterisation of biochar derived from the pyrolysis of biosolids, cattle manure and spent coffee grounds

Biochars were produced from three distinct biowastes (i.e., manure from cattle waste, MDB; biosolids, BDB; spent coffee grounds, SCGDB) through slow pyrolysis ( $550^\circ\text{C}$  for 1.5h) in a pilot kiln (20-24 kg capacity). The physicochemical characterisation of biochars showed the production of alkaline materials with similarities and variations in their characteristics, depending on feedstock. The biochars' surface area was 14.03, 3.98 and  $1.53 \text{ m}^2/\text{g}$  for MDB, BDB and SCGDB, respectively. The high %C content, the low H/C ratio and the FTIR adsorption peaks revealed high aromaticity, polymerisation and carbonisation of the biochars, suggesting promising properties for environmental applications. **(A. Christou, P. Dalias and P. Polycarpou)**





*SEM images of the BDB, MDB and SCGDB biochars*

## PROJECTS FUNDED BY THE CYPRUS RESEARCH AND INNOVATION FOUNDATION

### **Design and manufacturing of a novel Low-Density Polyethylene (LDPE) Film for the construction industry, using recycled agricultural plastic waste (APW), Recy-Film (ENTERPRISES/0618/0131)**

The overall objective of the project that started in 2019 and ended in 2021 was to give a solution to the accumulated agricultural plastic waste by recycling them to a useful product. Cyprus is one of the main consumers of plastic preparations per hectare of cultivated land, resulting in the accumulation of large volumes of agricultural plastic waste (APW) that should normally be returned for recycling or energy recovery if they are not recyclable. In particular, 700 tons of agricultural plastic waste are produced annually.

Current agricultural plastic waste (APW) management practices in Cyprus include uncontrolled disposal in fields or near water sources or uncontrolled burning of the waste. These practices lead to irreversible degradation of the natural environment through contamination of soil and water resources and cause air pollution and public

health problems. Recy-Film solved all these problems by giving a solution and suggestions on how to collect these materials and manage them until the production of a useful recycled product: An irrigation pipe suitable to irrigate parks, reforested areas, green areas, etc. This product is the first to be produced in Cyprus within the principles of circular economy.

Elysée Irrigation Ltd company is the lead Partner and the ARI and Frederick University are Partners.

**(P. Polycarpou and P. Dalias)**

## FLORICULTURE/ NATIVE PLANTS

Research activity focused on the evaluation of ornamentals and species from the Cyprus flora. New collaborations and participation in European and other external funded projects were reinforced.

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

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. Specifically, research activities concern the collection of the material from the wild and the development of propagation protocols (i.e., seeds). Subsequently, their growth and development are studied and evaluated to create cultivation protocols and define possible uses. **(L. Vassiliou)**

Seed germination			
A/A	Taxon	Germination test conditions	Germination percentage
1	<i>Glaucium flavum</i>	24h dark, 15°C, 1% agar + 2.5ppm GA3, 60% RH	54%
2	<i>Arabis purpurea</i>	8h light/16h dark, 15°C, 1% agar, 60% RH	85%
3	<i>Gladiolus italicus</i>	8h light/16h dark, 15°C, 1% agar, 60% RH	83%
4	<i>Cistus creticus</i>	8h light/16h dark, 15°C, 1% agar, 60% RH	61%
5	<i>Cistus monspeliensis</i>	8h light/16h dark, 15°C, 1% agar, 60% RH	72%
6	<i>Cistus parviflorus</i>	8h light/16h dark, 15°C, 1% agar, 60% RH	65%
7	<i>Limonium meyeri</i>	8h light/16h dark, 20°C, 1% agar, 60% RH	50%
	<i>Limonium mucronulatum</i>	8h light/16h dark, 20°C, 1% agar, 60% RH	58%
8			
9	<i>Limonium avei</i>	8h light/16h dark, 20°C, 1% agar, 60% RH	67%
10	<i>Limonium aucheri</i>	8h light/16h dark, 20°C, 1% agar, 60% RH	63%
11	<i>Achillea cretica</i>	8h light/16h dark, 20°C, 1% agar, 60% RH	85%

### European and National (RIF) Programmes, 2020-2021

**RIF / RESTART 2016-2020 - ICARUS** (<http://icarus.frederick.ac.cy/index.php>)

ICARUS research effort brings together the skills and scientific expertise of different Cyprus' research groups in the fields of Antennas/Microwaves namely Frederick Research Center, University of Cyprus and Agricultural Research Institute with the profound experience of University of Bologna (Italy). The main objective is to implement, and experimentally assess, novel Wireless Power Transfer (WPT) technologies in order to facilitate the wireless charging of flying Unmanned Aerial Vehicles (UAVs) and RFID-enabled sensors. It also investigates fundamental research problems related to the deployment of Wireless Powered Sensor Networks (WPSNs) and especially the implementation of "green" nearly fully recyclable sensors with wireless power scavenging features.

**(L. Vassiliou and G. Papadavid)**

### RIF / RESTART 2016-2020 – SPACES

The general objective of the project is to develop the technology that can monitor soil parameters. That technology is the missing link between the classical approach of providing water and fertilisers to plants at certain amounts without knowing the actual state of the soil, and precision agriculture of providing exactly what is required by monitoring the state of the soil, a technique featuring to be the approach of the future. (**L. Vassiliou**)

### GreenNet (<http://greenet.omegatech.gr/>)

GreeNet aims at improving the inclusion of people with intellectual disabilities through fostering specific actions within the framework of Adult Education and Rehabilitation for People with Intellectual Disability. It aims to create a complete training course for Adult Education in Horticulture (Assistant Gardener – PID) and promote Work Based Learning (WBL) and co-training and also involve the trainees' work in the community. GreenNet will provide people with intellectual disability the opportunity to obtain a certification for the learning units earned or for the entire curriculum. The course will address the latest technological advancements in the supported employment (Assistive Technology solution), in order to bridge this lack of skills, to increase autonomy and maximise social impact. An Assistive Technology toolkit will be prepared to monitor in real time garden conditions, to improve services, to reduce time and resources required, and also to reduce the possibility for human error. The toolkit will include a weather station, soil moisture sensors, sunlight exposure, etc. In the framework of the GreeNet project the “Train the trainers” learning activity was hosted in Cyprus, in which all the consortium partners participated. The trainees that participated in the event and succeeded in the assessment are now certified trainers of the GreenNet project. (**L. Vassiliou and C. Stavridou**).

### COST Action CA18201 “ConservePlants”

(<https://www.cost.eu/actions/CA18201/#tabs|Name:overview>)

The main aim of the COST Action “An integrated approach to conservation of threatened plants for the 21st Century” is to improve plant conservation in Europe through the establishment of a network of scientists and other stakeholders who deal with different aspects of plant conservation. (**L. Vassiliou**)





## SUSTAINABLE ENVIRONMENT AND PROTECTION FOR WILDLIFE

### Mushroom taxonomy

This study presents the results of a survey of the Mycenas of Cyprus, describing the first 19 species collected: *Mycena amicta*, *M. haematopus*, *M. sanguinolenta*, *M. seynii*, *M. pseudocorticola*, *M. epipterygia*, *M. capillaripes*, *M. algeriensis*, *M. aurantiomarginata*, *M. Olevaceomarginata*, *M. polyadelpha*, *M. Mucor*, *M. filopes*, *M. hiemalis*, *M. viridimarginata*, *M. galopus* var. *galopus*, *M. pura* var. *rosea* and *M. pura*. Most of them are recorded for the first time on the island of Cyprus. *M. haematopus* was reported for the first time on olive trees.

(E. Hadjisterkotis)



*Mycena haematopus* inside the stem of an olive tree with two drops of red fluid after piercing with twizers. (Photo: E. Hadjisterkotis, ARI)

### The study of the rodents of Cyprus

The presence of *R. rattus* on Cyprus has long been established, while that of *R. norvegicus* is still debated. In this study, the first body and cranial measurements as well as molecular typing of *R. norvegicus* specimens were provided. The analysis of mtDNA D-loop sequence confirmed that the analysed samples were *R. norvegicus* and pointed out the occurrence on Cyprus of at least two maternal lineages of this species. Rats are the most damaging alien predators to have been introduced on Cyprus, having a negative effect on island biota and causing huge damages to agriculture. A natural enemy of rats is the barn owl (*Tyto alba*). Barn owls are species with a restricted habitat, limited by the presence of suitable natural cavities/holes for nesting. In cooperation with the University of Cyprus, under the project 'Black Gold': Revival of the cultivation of carob tree in Cyprus' a new more efficient design of nesting box for barn owls was installed. (E. Hadjisterkotis)



Barn owl (*Tyto alba*) nesting in an artificial nest installed by the Agricultural Research Institute at Orites carob plantation (Photo: E. Hadjisterkotis, ARI).

### **The management and conservation of the hazelnut forests of Pitsilia**

The Agricultural Research Institute in collaboration with the Community Council of Alona and the Sports and Cultural Association of Alona (APOA) undertook a study on the biodiversity of the hazelnut forest of the Pitsilia area, aiming at the understanding of ecology and its proper conservation and maintenance. At the same time, the work included the study of traditional ways of collecting hazelnuts and improving them, for more efficient and less effortful collection of the fruits. (E. Hadjisterkotis)

### **Recording of life and agricultural occupations in the villages of Agios Sozomenos, Vretsia, Statos and Agios Fotios during the previous centuries**

The Agricultural Research Institute, in cooperation with the University of Bournemouth UK undertook a study on the history, architecture, and the evolution of agriculture and animal husbandry through the centuries in the abandoned and destroyed villages of Agios Sozomenos, Vretsia, Statos and Agios Fotios. Innovative pioneering techniques were developed for this work. (E. Hadjisterkotis)

### **The recent taxonomic developments and molecular findings on the Cyprian mouflon (*Ovis gmelini ophion*) and the Sardinian mouflon (*Ovis gmelini musimon*)**

The classification of Mediterranean and Asiatic mouflons, urials and their hybrids are under constant debate, taxonomic confusion and spelling inconsistencies. Based on morphological criteria, karyological and biochemical evidence and geographic distribution, several classifications and taxonomical revisions of these species have been proposed during the last two centuries. In 2003, the International Committee for Zoological Nomenclature (ICZN), ratified the published 1996 Bulletin of Zoological Nomenclature opinion that the Asian mouflon shall be called *O. orientalis* and the European/Mediterranean mouflons were grouped with the domestic sheep, *O. aries*, something which degraded the Cypriot mouflon from endemic subspecies together with



domestic sheep. ARI mtDNA studies in cooperation with the University of Sassari, as well as morphological studies, concluded that the Cyprus mouflon, should not be attributed to the species *O. aries*, but rather be managed as a separate and genetically distinct taxonomic conservation unit, namely *O. gmelini ophion*. In addition, the same team characterised the first complete mtDNA sequence of a Sardinian mouflon with the aim to shed light on the influence of different genetic and environmental parameters on the current mouflon diversity patterns, and on the phylogenetic relationship between Sardinian mouflon and its wild and domestic relatives. The results pointed out that Sardinian mouflons maintained their original genetic features, which became lost in the relatives living in the European mainland countries, and that there is no relationship with domestic sheep. **(E. Hadjisterkotis)**



*Cyprus mouflon (Ovis gmelini ophion) male at Stavros tis Psokas Forest Station (Photo: E. Hadjisterkotis, ARI)*

## RURAL DEVELOPMENT

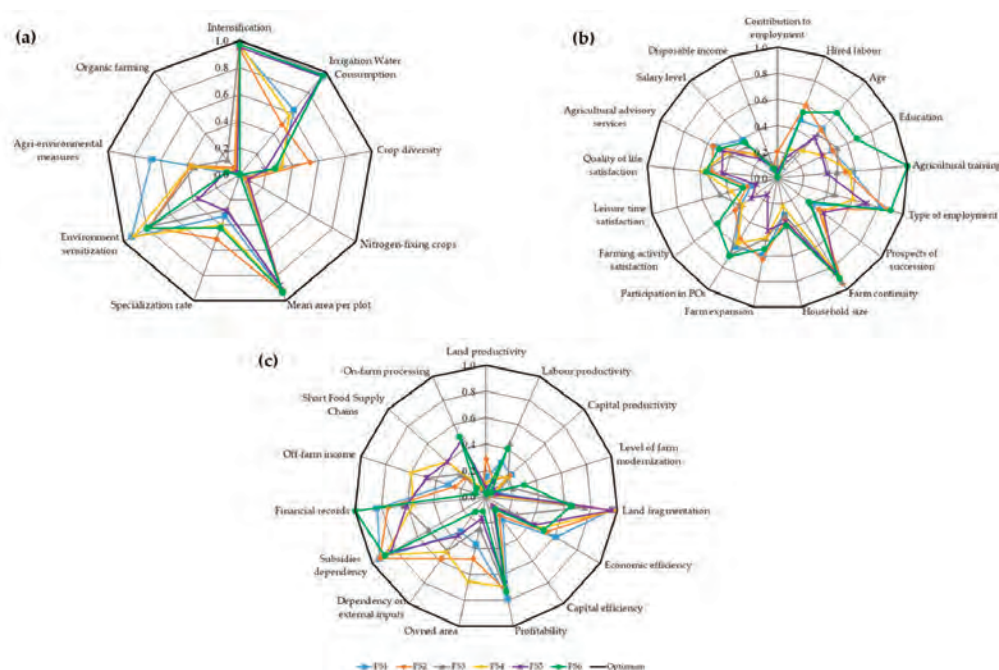
The Rural Development Section of the Agricultural Research Institute conducts research aiming towards the sustainable development of the agricultural sector in Cyprus and the improvement of the quality of life of Cypriot farmers. The activities of the Section encompass topics related to agricultural economics and sustainability assessment of farming systems, marketing and trade, as well as work on smart agriculture, which includes research on applications of Information and Communication Technologies, Remote Sensing, Internet of Things (IoT), and Robotics in agriculture. The Section collaborates with the Ministry of Agriculture, Rural Development and Environment and undertakes targeted studies and technical reports on agricultural economics, trade and policy, supporting decision-making.

## AGRICULTURAL ECONOMICS

### Investigating the sustainability of the agricultural production system in Cyprus

In the agricultural sector, sustainability assessment is a key step in building sustainable farming systems (FSs) viz. that are environmentally friendly, economically viable for farmers and socially acceptable. Although agricultural sustainability is of major concern in Cyprus, no attempt has been made to date to empirically assess the sustainability of the Cypriot agricultural sector. In this context, the goals of this study are, for the first time, to identify and characterise the divergent FSs that exist in southeastern Cyprus and, subsequently, to assess and compare, in a holistic manner, their sustainability performance. For this, Larnaka and Ammochostos districts were chosen as case studies. To achieve these goals, a three-step methodological framework was adopted. First, the sustainability context was set and a list of 9 environmental, 17 social and 15 economic base indicators was created. The calculation of indicators was mainly based on primary data from a representative sample of 324 farms in the study area. Second, multivariate statistical analysis (Principal Component Analysis, Hierarchical Cluster Analysis and k-means clustering) was employed to identify FSs and construct a farm typology. Finally, the sustainability of FSs was assessed by combining numerical and visual integration approaches. Numerical integration involved the construction of four composite sustainability indices (environmental, social, economic, global), while visual integration was concerned with the presentation of base indicators' scores and values with radar graphs and tables. Within the context of numerical integration, the base indicators were normalised using the min-max technique and aggregated with the linear aggregation method, whilst the minimum and maximum observed values in the sample (viz. the lowest-performing and best-performing farms) were used as basic benchmarks. The results of multivariate analysis proposed six significantly different FSs, of which four (FS1–FS4) are crop-oriented and two (FS5–FS6) are livestock-oriented. In terms of environmental sustainability, livestock systems are the least sustainable, while FS5 (medium-sized sheep/goats farms) is also the least sustainable with regard to social and global composite indices. On the other hand, FS6 (large dairy cattle farms) performs best on social sustainability. The values of the economic index are comparatively low for all FSs. FS3 (large farms with market-oriented rainfed cereals and fodder crops) is the least economically viable, mainly due to its high dependency on subsidies. Surprisingly, FS4 (small farms with open field vegetables and permanent crops) is the most economically sustainable. FS2 (small to medium-sized farms with greenhouse and open field vegetables) and FS1 (medium-sized farms with potatoes and open field vegetables) obtain the highest scores for the global composite index. Worryingly, prospects of succession are low for all FSs, while off/non-farm income is sought mainly for farm households' survival and it is not invested on the farm. Moreover, a weak complementarity is observed between the main system components (crop, livestock, off/non-farm income), which might negatively affect the sustainability of FSs. At the same time, results cast doubt on the effectiveness of subsidies in providing the basis for a more sustainable agricultural sector. The importance of membership in producers' organisations for farm sustainability is also highlighted. The results for FS1 indicate that sustainable

intensification of agriculture is feasible in the study area. In contrast, FS5 is the least sustainable with several weaknesses. Therefore, its viability should be the priority of decision makers. The results of this study can be used for sustainability benchmarking, for a more focused agricultural policy, tailored to the different constraints and opportunities of FSs, but also to inform public decision makers who are responsible to design and implement agricultural policy in Cyprus, or in other European Mediterranean regions with similar FSs and agro-ecological conditions. The next step of the study is to conduct internal consistency and sensitivity analyses, in order to verify the robustness of the proposed method, as well as to examine the interaction (trade-offs and synergies) between indicators. **(A. Stylianou)**



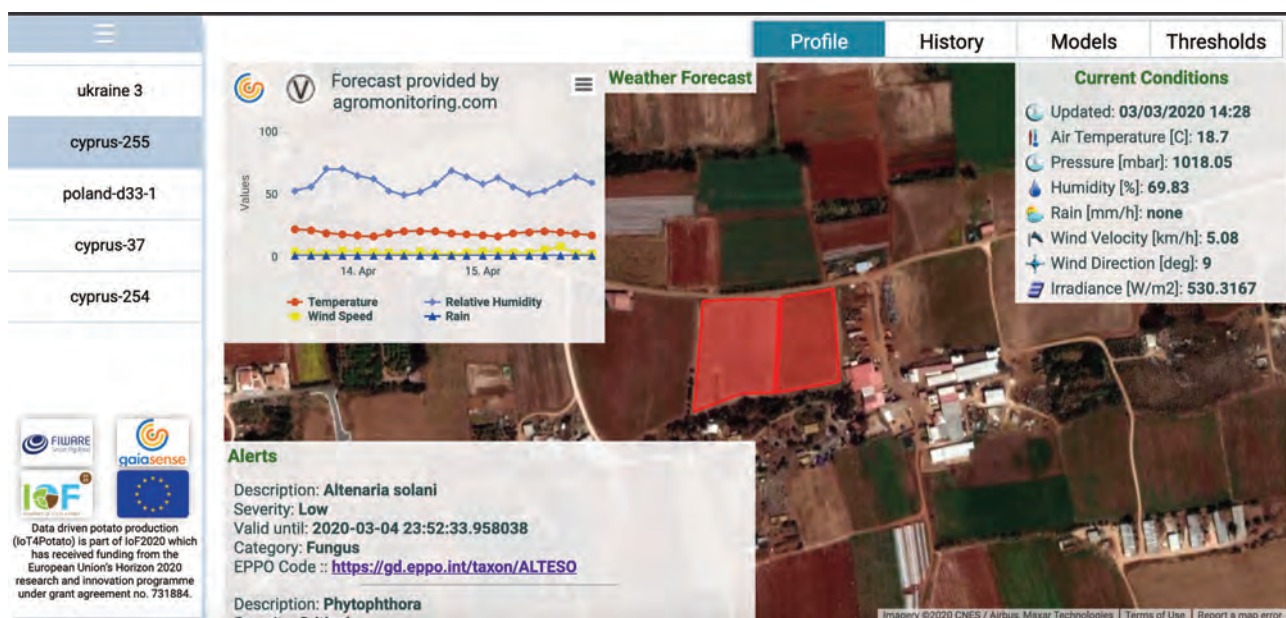
(a) Visual integration of sustainability base indicators; indicators were normalised using as thresholds the worst and best performing farms of the overall sample; (b) indicators of the environmental pillar; indicators of the social pillar; (c) indicators of the economic pillar; POs: Producer Organisations.

## SMART FARMING

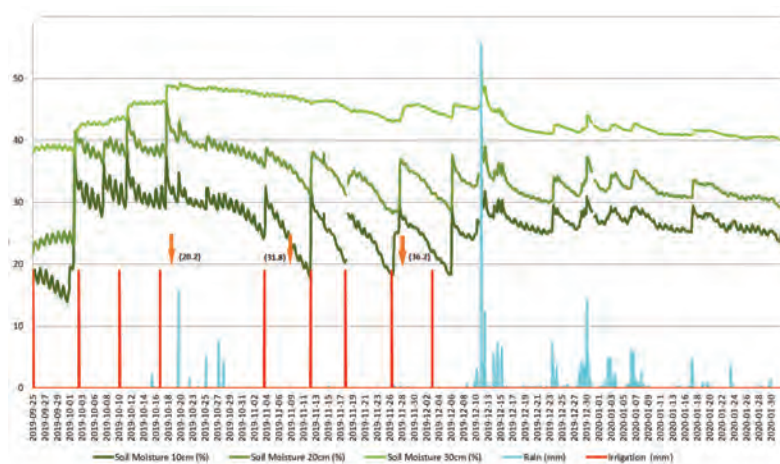
The two-year (2019–2020) project “Data-driven potato production - IoT4Potato” (<https://www.iof2020.eu/trials/arable/data-driven-potato-farming>) was funded by the large scale H2020 project “Internet of Food and Farm - IoF2020” (<https://www.iof2020.eu>). The goal of IoT4Potato was to build on top of giasense, an innovative market-ready IoT-based smart farming solution, which is considered suitable for both small- and large-scale farmers, and extend it to enable the development of services for irrigation, pest management and fertilisation for potato producers in Cyprus, Poland and Ukraine. The services aimed at reducing the production costs of potato per hectare of utilised agricultural area and improving product quality, by employing scientific models tailored to the specificities of the targeted areas, the different potato varieties and the specific requirements of the targeted markets. The models were fed with data from a network of autonomous telemetric IoT stations (gaiatrons) installed in the field, collecting atmospheric and soil measurements, satellite data, as well as information about agricultural practices provided by the farmers



themselves. All the aforementioned data were collected to a central cloud computing repository, where they were stored, processed, combined and converted into facts based on advanced data analytic techniques. The outcomes of the processing were analysed by experts (e.g. agronomists) in order to generate farming advice towards the optimisation procedures of irrigation, pest management, and fertilisation tailored to the context of the targeted fields. The advice, along with selected agri-environmental measurements, were then mediated to the farmer through web-based applications. In the case of Cyprus, two potato fields located in the Kokkinochoria area were used as pilot studies. The overall results of the pilots showed a potential reduction of up to 22% on total irrigation needs and important optimisation opportunities on pesticides use efficiency. This work offers opportunities for innovation targeting and climate change adaptation options (new agricultural technologies), and could help farmers to reduce their ecological footprint. Moreover, engaging more farmers will support the extraction of additional results facilitating the identification of the best practices towards the large-scale realisation of smart farming paradigm in Cyprus. **(A. Stylianou, G. Adamides, D. Neocleous, M. Giannakopoulou and V. Vassiliou)**



Current environmental conditions, active alerts and weather forecast referring to the selected pilot parcels.



Recorded soil moisture in different depths combined with irrigation and rain events. Arrows imbedded in the figure show irrigation events (mm) and amounts according to Food and Agriculture Organization of the United Nations CropWat tool.



## Digital Ecosystem Utilisation

(<https://www.iof2020.eu/trials/vegetables/digital-ecosystem-utilisation> CYSLOP), is a sub-grantee open call project pilot study within the H2020 project “Internet of Food and Farm - IoF2020” (<https://www.iof2020.eu>), that aimed to the delivery of tailored services to farm operators based on IoT data acquired on-field. By utilising data stemming from IoT devices in the field, cloud computing and analytics technologies, this use case timely notified the farmer to proceed with such activities. The result from synergised parameters such as air humidity, temperature and other weather conditions was a service that increased the total farm productivity and contributed to food security for an increasingly populated world. Furthermore, information tailored to farmers’ individual needs lowers their resource costs through early warnings regarding crucial factors in irrigation. By incorporating innovative traceability technology, this use case was among the first to integrate information from the entire food value chain (from farm to shelf) to a marketplace, offering elaborate value propositions to users. Hence, it significantly improved consumers’ perception of food safety while enabling stakeholders in the agri-food sector to participate in an innovative digital ecosystem. The Agricultural Research Institute had arranged the practicalities of FINT’s visit and organised the visits at the three locations. Fruit farms with strawberries, berries, hydroponic tomato and cherry trees were equipped with soil and air sensors. The overall results of the pilots showed a potential reduction of up to 10% on overhead costs, as well as on total irrigation needs and important optimisation opportunities on pesticides use efficiency. (G. Adamides, A. Stylianou, D. Neocleous, M. Giannakopoulou and V. Vassiliou)

**“Data-driven sustainable agri-food value chains - Ploutos”** is an EU research project funded by H2020. Ploutos aims to create opportunities for changes that can rebalance the value chains in the agri-food sector in Europe towards a more environmentally, socially and economically sustainable system. This will be achieved through its Sustainable Innovation Framework (SIF), that applies a systemic approach to the agri-food sector, building on three pillars: Behavioural Innovation, Sustainable Collaborative Business Model Innovation and Data-driven Technology Innovation. The Ploutos SIF will be assessed and validated through eleven Sustainable Innovation Pilots (SIPs) around Europe, covering a large range of agri-food ecosystems, such as arable, horticulture (both open fields and greenhouses), perennials and dairy production. All of the SIPs were launched in the year 2021. Moreover, a Ploutos Innovation Academy will be established as a vehicle for integrating the know-how, best practices and assessments developed across the project and derived from the SIPs. Ploutos consortium consists of partners that represent all the actors of the value chain, from farmers and farmers’ organisations, food industries and traders to NGOs, public authorities and public service providers. GAIA EPICHEIREIN, an innovative Greek organisation, is the coordinator of the project. In total, 33 partners from more than ten European countries, including Greece, the Netherlands, Ireland, Spain, North Macedonia, Serbia, Cyprus, Slovenia, Italy and France are part of the project. The Rural Development Section of ARI leads the SIP in Cyprus (SIP7), which aims at improving the sustainability performance of the wine sector by integrating smart farming technologies with human component (e.g. researchers, agronomists). This integration will allow to minimise the use of inputs, such as pesticides, improve fruit quality and reduce the environmental footprint of the production. In the end, a digital label will be developed to improve market transparency, enhance product traceability and generate high value-added products. During the period October, 2020-December, 2021, a fully operational network of IoT agrometeorological stations was installed in the fields of Oenou Yi winery in Omodos and interconnected with the cloud computing infrastructure of Neuropublic (service provider) for data collection and analysis. In this context, a smart farming web-based app has been developed and made available to SIP7 partners. The parcels geometries, all data recordings and applications on the field for the 1st cultivation period are rendered through the app in a user-friendly way. In addition, smart farming advice on fertilisation, pest management and irrigation is provided to the winery on an ongoing basis. The next important step of SIP7 is

to connect the systems of Oenou Yi and gaiasense smart farming solution with the Ploutos traceability solution to feed the collected data to a digital labelling solution that will promote the related product quality and sustainability information to the consumers. Finally, in 2022 several workshops/meetings between Ploutos experts and wine value chain stakeholders will be organised in Cyprus in order to achieve a deeper understanding of SIP7 and the behavioural challenges faced on the ground. **(A. Stylianou, G. Adamides, D. Neocleous, M. Giannakopoulou, V. Vassiliou and S. Savvides)**



*GaiaSense agri-environmental sensing equipment installed in a vineyard of Oenou Yi winery in Omodos.*

**“Proactive Producer and Processor Networks for Troodos Mountains Agriculture – 3PRO-TROODOS”** (2019–2023) is a project funded by the RIF of Cyprus and coordinated by the Cyprus Institute. The goal of the 3PRO-TROODOS project is to improve agricultural production and food processing in the Troodos Mountains of Cyprus, through social innovation, sustainable natural resource management and climate change adaptation. The project aims (a) to develop a voluntary Troodos quality certification label via a fully participatory process; (b) to improve processing through innovation and linkages between producers and processors; (c) to quantify current and future irrigation water demands based on high-resolution climate projections (2020–2050); (d) to develop guidelines for sustainable irrigation; (e) to test four climate resilient innovations with pilot farmer groups; and (f) to analyse the costs and benefits of tested innovations and the socio-economic impacts of the quality certification label through input-output modelling. Through the integration of people, scientific and practical knowledge and experiences, the project can add value to farming, make agriculture climate resilient and sustain the beautiful cultural landscapes of the Troodos Mountains. The Section is the scientific leader of Work Package 3 (WP3) (Linking Mountain Farming to Policies and Markets), while it is highly involved in WP5 (Adding Value to Farming: Agricultural Product Certification and Improved Food Processing) and WP7 (Socioeconomic Impact Assessment). In addition, the Section actively supports the implementation of Task 6.2. (Sustainable fruit production through the use of protective nets) and Task 6.3. (Ethnobotanic survey and testing of two new indigenous medicinal and aromatic plants). **(A. Stylianou, G. Adamides, S. Ioannidou, C. Stavridou and D. Fasoula)**

## OTHER PROJECTS – COLLABORATIONS

### **Innovative and sustainable measures of keeping water in the agricultural landscape**

AGRIWATER is an ERASMUS+ project of the European Union under key action 2: Cooperation for Innovation and the Exchange of Good Practice that started in October 2020 and scheduled to run until September 2022 (AGRIWATER- <https://agriwater.eu>). The project's main goal is to help farmers maintain their agricultural lands by implementing innovative adaptation measures to droughts, as well as raise awareness amongst landowners about why it is necessary to care for their lands in this way. The project operates across Belgium, the Czech Republic, Cyprus, Germany, Italy and Spain. AGRIWATER project will create an educational platform where farmers and landowners can gain knowledge on the practical implementation of different adaptation strategies and water management systems, which they can then use on their own land to better cope with droughts. The free and open access innovative educational materials will be developed as outputs from the AGRIWATER project, which will be made available on the project website. The material will cover current situations and possibilities in partner countries and best practice examples of innovative adaptation tools to drought from each partner country. The main expected results of the project are to bring the real experience and knowledge of different drought adaptation possibilities and water management systems to people who live, work and/or own agricultural lands, fields and other related areas. Through the developed educational materials, it is expected that the target groups will gain enough knowledge on the practical implementation of different adaptation strategies and water management systems, which they can then realise, settle, start, and maintain on their lands to better cope with drought periods (**M. Markou, A. Christou, A. Stylianou, S. Ioannou and M. Giannakopoulou**).

### **Perceptions of Cypriot consumers towards the traceability of food products**

In the framework of the research programme "Rural Development and Marketing of Agricultural Products", a project titled "Perceptions of Cypriot consumers towards the traceability of food products" is underway. The ultimate goal of this project is to examine the perceptions of Cypriot consumers towards the traceability of food products (specifically fresh agricultural products). Specifically, this research aims to study the degree of importance that consumers attribute to traceability information (i.e., what is indicated on the packaging / label), when purchasing their food products. For the purposes of this study, a self-administered questionnaire is under development. A survey will take place, the survey panel of which will consist of primary household grocery shoppers (i.e., persons primarily responsible for most of the grocery shopping in their household) aged 18 and over, living in Cyprus. (**M. Giannakopoulou, S. Ioannou, G. Adamides, A. Stylianou and M. Markou**)

### **Cypriot consumer behaviour towards online grocery shopping**

In the framework of the research programme "Use of Information Technology", a new project titled "Cypriot consumer behaviour towards online grocery shopping" is underway. The purpose of this survey is to provide a detailed description of the behavioural profile of the Cypriot consumer towards online grocery shopping. Due to the Covid-19 pandemic, it is important and timely to investigate whether consumer behaviour has changed regarding the purchase of food products (shopping with physical presence in retail stores versus online shopping). The survey set to enable the thorough study of the existing behaviours and behavioural intentions of Cypriot consumers towards online grocery shopping and to what extent consumer attitude, perceived social influence, and perceived control of the performance of the behaviour drive the development of behavioural intentions and lead to behaviours of online grocery shopping. The study was based on an online survey in Cyprus using a structured questionnaire, adapted from previous studies. The survey was conducted through a self-administered questionnaire that was posted online using Google Forms and was administered between July and September 2020, during the Covid-19 pandemic and the related 'stay at home' lockdown and other related

measures to prevent infection in Cyprus. The choice of online survey was selected because it is cheap, easy to set up, convenient to the participant (in terms of time and place), and does not require physical contact between the interviewers and the respondents, that was a concern (and restriction) during the Covid-19 outbreak. The survey panel consisted of primary household grocery shoppers (i.e., persons primarily responsible for most of the grocery shopping in their household) aged 18 and over, living in Cyprus. The sampling method that was used was the stratified random sampling of 284 persons (5% margin of error and 95% confidence level). Prior to the survey being administered to the general public, a pretest (pilot study) of 18 respondents was conducted with the goal to increase the validity and reliability of our testimonial survey evidence. **Table 1** displays sample averages for several demographic and household variables. The total number of questionnaires collected was 364, with 302 of the respondents indicating that they were the primary household grocery shopper, while ensuring regional (district) quotas were met. These 302 questionnaires were taken into consideration for further analysis, since when respondents answered 'No' to the first question, whether they were the primary household grocery shopper, the interview was terminated. The respondents' median age was 41, which is in line with that of Cyprus population 37.7. At 67.4%, women are dominating, which seems to be in accordance with various studies, indicating that the grocery shopping is a task performed mainly by women.

**Table 1**

Survey sample demographics		
Survey variable respondents (n=302)	Mean	% of respondents
Age	41.71	
Gender		
Male		32.56
Female		67.44
District		
Nicosia		42.05
Lemesos		29.80
Larnaka		13.91
Ammochostos		6.29
Pafos		7.95
Educational level		
Primary education		0.66
Gymnasium		1.32
Lyceum		8.94
Diploma		8.94
Bachelor		28.15
Master/PhD		51.99
Number of household members	3.50	
Number of household members < 18	1.26	
Marital status		
Single		19.87
Married		73.18
Divorced		4.30
Widower		1.66
Other (cohabitation)		0.99
Occupation		
Private sector employee		36.75
Public sector employee		47.02
Freelancer		5.63
Retired		3.31
Housewife		2.32
Student		2.65
Unemployed		2.32

The logistic regression model was statistically significant  $\chi^2(296) = 40.99, p < .001$ . The model correctly classified 65.9% of cases. Decreasing age and increasing monthly family income were associated with an increased likelihood of online grocery shopping (**Table 2 and Figure 2**).

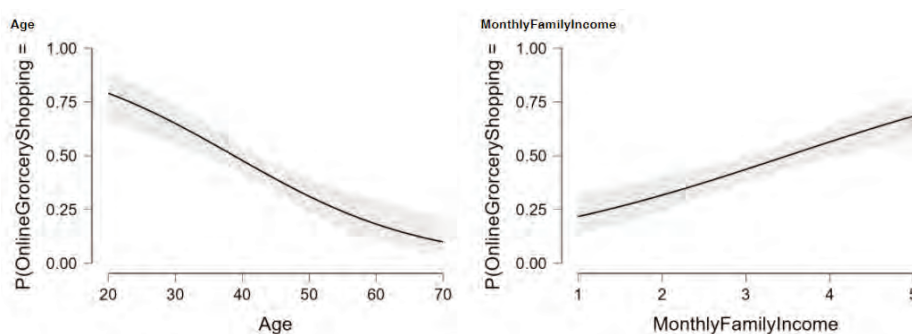
**Table 2**

**Logistic regression model Coefficients**

	Estimate	Standard Error	Odds Ratio	z	Wald Test		
					Wald Statistic	df	p
(Intercept)	1.15	0.59	3.17	1.96	3.84	1	0.05
Age	-0.07	0.02	0.93	-4.60	21.12	1	< .001
Monthly Family Income	0.51	0.12	1.67	4.42	19.54	1	< .001

*Note.* Online Grocery Shopping level '1' coded as class 1.

**Figure 2**



*Estimates plots – Age and monthly family income*

Similar to other studies, no statistically significant difference was found between men (41%) and women (48%). Likewise, the education level, profession, and marital status were not found to have any statistically significant difference, which are also consistent with similar studies. Further analysis should investigate consumers' attitude towards online grocery shopping, the factors that enable online grocery shopping, behavioural intention and related social norms. (**M. Giannakopoulou, G. Adamides and A. Stylianou**)

### **The meat chain in the Republic of Cyprus**

In 2021, the Agricultural Research Institute in collaboration with the Department of Agriculture and Veterinary Services conducted a survey on "Meat Chain in the Republic of Cyprus". The main objectives of the survey were to study the situation of the Cypriot meat market and to determine the values (costs and/or added costs) at each stage of the supply chain, i.e., from the producer to the final consumer. To achieve these objectives, a review of existing national and European legislation governing the movement of meat from the producer (farmer) to the final consumer and a literature review of the food and fresh meat supply chain, price transmission/transfer and empirical studies on price transmission/transfer along the food supply chain were carried out. In addition, primary information was collected using a specifically designed questionnaire from a sample of stakeholders in the Cypriot meat supply chain and additional information was obtained from a sample



of butchers. The primary survey provided useful information on the role of stakeholders, the structure and functioning of the meat distribution chain and recorded live weight and carcass prices, slaughter and transport costs. It also estimated the difference between the carcass price when stakeholders buy live animals and all transport and slaughtering costs from the carcass price when stakeholders buy carcass meat from butchers and calculated the profit margins of meat traders (**M. Markou, G. Adamides, A. Stylianou, M. Giannakopoulou and S. Ioannou**).

## 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, agricultural microbiology and agricultural chemistry projects. The section research activities also involve participation in different EU funding projects such as HORIZON2020, Restart as well as RPF.

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

#### Soil microbial community structure in Cyprus soils

The different types of land-use and soil lithology in urban and peri-urban areas of modern cities compose a complex mosaic of soil ecosystems. It is largely unknown how these differences result in changes in bacterial community composition and structure as well as in functional guilds involved in N cycling. To investigate the bacterial composition and the proportion of denitrifiers in agricultural, forested, schoolyard and industrial areas, 24 samples were collected from urban and peri-urban sites of Lefkosia. Bacterial diversity and the proportion of denitrifiers were assessed by NGS and qPCR, respectively. Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria and Planctomycetes were identified as the most dominant phyla across all sites, while agricultural sites exhibited the highest bacterial diversity. Heavy metals such as Co, Pb, V and Al were identified as key factors shaping bacterial composition in industrial and schoolyard sites, while the bacterial assemblages in agricultural and forested sites were associated with Ca. Variance partitioning analysis showed that 10.2% of the bacterial community variation was explained by land use management, 5.1% by chemical elements due to soil lithology, and 1.4% by sampling location. The proportion of denitrifiers varied with land use management. In industrial and schoolyard sites, the abundance of the nosZII bacterial community increased while nirK abundance declined. Our data showed that land use and lithology have a moderate impact on the bacterial assemblages in urban and peri-urban areas of Lefkosia. As the nosZII bacterial community is important to the N<sub>2</sub>O sink capacity of soils, it would be interesting to elucidate the factors contributing to the proliferation of the nosZII clade in these soils. (M. Omirou and I.M. Ioannides)

#### Impact of N fertilisation on biogenic volatile organic compound emitted from soils

The biogenic volatile organic compounds (BVOCs) emissions from cultivated plants-soil systems and N fertilisation's role on these emissions are mostly unknown. This study aimed to elucidate the BVOCs emissions from a plant-soil system receiving N fertilisation using *Rosmarinus officinalis* as a model case study. We put forward the hypothesis that N fertilisation changes the BVOCs emissions patterns irrespectively the presence of *Rosmarinus officinalis* and that the emissions are related to N availability and bacterial abundance. To test our hypothesis, we established a microcosm (glass static chambers) study in a fully randomised design with four treatments: soil (S), soil and plant (S + P), soil and fertiliser (S + F), soil, plant, and fertiliser (S + P + F); using NH<sub>4</sub>NO<sub>3</sub> as N source. BVOCs were sampled from the microcosms, using the static headspace sampling technique with a solid-phase microextraction (SPME) device and analysed by gas chromatography–mass spectrometry (HS-SPME-GC–MS). Sampling occurred 1, 2, 3, 4, 7, 10, 15, 17, 22, 25, and 31 days after applying the NH<sub>4</sub>NO<sub>3</sub> and besides BVOCs, we measured soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>–</sup> concentration as well as 16S rRNA bacterial abundance. Time drives a significant portion of the variation in the chemical diversity and quantity of the different VOCs found throughout the experiment (PERMANOVA; R<sup>2</sup> = 0.28, p < 0.001); however, this was dependent on the

presence of plant and fertiliser leading to a significant interaction (PERMANOVA;  $R^2 = 0.33$ ,  $p < 0.001$ ). Individual BVOCs emissions were strongly affected by nitrogen fertilisation, plant presence and declined during the time. The most dominant BVOCs detected were benzyl alcohol, acetone, toluene, benzaldehyde, and caprolactam. Interestingly, the BVOCs concentration was negatively associated with soil concentration to  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and total bacterial abundance. **(M. Omirou and I.M. Ioannides)**

### Impact of rhizobial inoculation on mycorrhizal presence in cowpea plants

Cowpea can effectively form tripartite symbiotic associations with nitrogen-fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) although the selection of compatible AMF species and rhizobial strains which are promoting cowpea growth remains a challenge. The aims of the current research were 1) to evaluate the response of cowpea plants to a symbiotic NFB and a multi-AMF inoculum and 2) to explore any interaction between the symbiotic NFB and the different AMF isolates. In a pot gnotobiotic trial, cowpea plants grown under limited N supply, were inoculated with or without a symbiotic nitrogen-fixing bacterium, *Sinorhizobium meliloti*, and combinations of three different AMF species namely *Dominikia disticha*, *Claroideoglomus etunicatum* and *Rhizophagus irregularis*. Experimental evaluation was determined through the measurement of above ground biomass, nutrient content and AMF root colonisation. The presence of AMF species on cowpea roots was also determined with cloning and sequencing. Inoculation with both AMF and *S. meliloti* led to increased cowpea biomass production compared to inoculation with AMF only, but the presence of a positive effect depended on the specific AMF partners used. Inoculation with AMF alone had a highly positive impact on the growth and P uptake of cowpea, but the NFB inoculation was needed to address N deficiency in planta. The presence of both symbionts generally led to increased AMF colonisation of the cowpea roots, however, plant colonisation depended on the AMF species, and became even negative, when all three AMF inocula were used together. The AMF composition in plant roots was also altered in the presence of the *S. meliloti*. Plant nitrogen content of cowpea plants significantly increased under the presence of both symbionts compared to AMF alone, while phosphorus content was hardly affected by dual inoculations. The results show positive synergistic effects of the different AMF species and *S. meliloti*. Inoculation with all AMF isolates and *S. meliloti* led to high above ground biomass production and accumulation of N. The presence of *S. meliloti* increased P content in plants not inoculated with AMF. Finally, the efficiency of synergism depends on the specific AMF partners used and it is not related to their colonisation levels. **(M. Omirou, D. Fasoula and I.M. Ioannides)**

## ENVIRONMENTAL BIOTECHNOLOGY AND MICROBIOLOGY

### 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 (560 samples) continued in 2020/21, in order to identify and select genotypes resistant to the scrapie disease. Presently at the ARI, the Chios sheep nucleus unit counts over 350 breeding animals exclusively of the scrapie resistant genotype ARR/ARR. In addition, the molecular fingerprinting for goats is in progress. In 2020/21, 988 blood samples were tested for scrapie disease genotypes. The data were submitted to the laboratory molecular databank for further analysis. Also, based on scrapie

genotyping, a nucleus unit with all the different goat genotypes has been established at the Research Farm of the Animal Production Section. **(I.M. Ioannides and G. Hadjipavlou)**

### **Depuration of citrus fruit industry fungicides**

The application of the fungicide thiabendazole (TBZ) in fruit packaging plants (FPP) results in the production of effluents that are often disposed in adjacent field sites. These require remediation to prevent further environmental dispersal of TBZ. We assessed the bioaugmentation potential of a newly isolated TBZ-degrading bacterial consortium in a naturally contaminated soil (NCS) exhibiting a natural gradient of TBZ levels (12000, 400, 250 and 12 mg kg<sup>-1</sup>). The effect of aging on bioaugmentation efficacy was comparatively tested in a soil with similar physicochemical properties and soil microbiota, which was artificially contaminated with the same TBZ levels (ACS). The impact of bioaugmentation and TBZ on the bacterial diversity in the NCS was explored via amplicon sequencing. Bioaugmentation effectively removed TBZ from both soils at levels up to 400 mg kg<sup>-1</sup> but failed at the highest contamination level (12000 mg kg<sup>-1</sup>). Dissipation of TBZ in bioaugmented samples showed a concentration-dependent pattern, while aging of TBZ had a slight effect on bioaugmentation efficiency. Bioaugmentation had no impact on the soil bacterial diversity, in contrast to TBZ contamination. Soils from the hotspots of TBZ contamination (12000 mg kg<sup>-1</sup>) showed a drastically lower  $\alpha$ -diversity driven by the dominance of  $\beta$ - and  $\gamma$ -proteobacteria at the expense of all other bacterial phyla, especially Actinobacteria. Overall, bioaugmentation with specialised microbial inocula could be an effective solution for the recovery of disposal sites contaminated with persistent chemicals like TBZ. **(M. Omirou and I.M. Ioannides)**

### **The effects of different soil nutrient management schemes in nitrogen cycling**

Nitrogen has a significant contribution to global warming and its reduction in agriculture is expected to reduce N<sub>2</sub>O emissions having, however, adverse effects on the productivity of agricultural ecosystems. Maintaining systems productivity with alternative N sources i.e., manure and composts could be a strategy also to mitigate N<sub>2</sub>O emissions. In this paper, we present the effect of different N sources (organic and chemical) on field N<sub>2</sub>O emissions and how these emissions are associated with soil available N forms (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in three different rainfed crops namely barley, pea and vetch grown in Cyprus for two growing seasons. The daily emissions ranged from -3.11 to 12.3 g N-N<sub>2</sub>O/ha/day, while cumulative emissions ranged from 119 g N-N<sub>2</sub>O/ha to 660 g N-N<sub>2</sub>O/ha depending on crop and nitrogen source type. The emissions showed a seasonal pattern and WFPS has been identified as a critical soil parameter controlling daily N<sub>2</sub>O emissions. The daily N<sub>2</sub>O fluxes in the current study derives mainly from nitrification irrespectively crop type or nitrogen source type. Specific emission factors for each crop cultivated under different N source type were calculated and ranged from 0.03%  $\pm$  0.02–0.34%  $\pm$  0.09. The application of manure and chemical fertilisers cause similar intensity of N<sub>2</sub>O emissions while compost exhibited the lower emission factors. These findings suggest that composts could be integrated in a nutrient management strategy of rainfed crops with less N<sub>2</sub>O emissions. The high background emissions found suggest also that other factors than external inputs are associated with N<sub>2</sub>O emissions and further studies including the response of microbial community structure and their contribution and association with N<sub>2</sub>O emissions. **(M. Omirou and I.M. Ioannides)**

### **National Soil Genetic Resources Bank and Greenhouse Gas Emissions soil inventory**

Through the RESTART call, the Research Promotion Foundation funded the establishment of the first National Soil Genetic Resources Bank. The aim of this infrastructure is to isolate, characterise and store soil microbes from terrestrial ecosystems of the Republic of Cyprus. Also, a Next Generation Sequencing platform has been established. The objective of the unit is to map and characterise the soil microbiome of Cyprus terrestrial ecosystems. In addition, functional microbial guilds for important crops have been isolated and breeding processes have been linked with rhizosphere related traits. In the same context, we have been acquired substantial funding through RPF scheme to calculate National Emission Factors for the main GHG, to reveal the mechanisms responsible for these emissions and propose specific measures to mitigate the overall GHG emissions from agricultural soils. **(M. Omirou, D. Fasoula, D. Neocleous, P. Dalias and I. M. Ioannides)**

## VARIETY EXAMINATION CENTER

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 Center 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 2020/21, three barley varieties (EV7, EV8, EV9), one peanut variety (Italian), one durum wheat variety (DUR7) and one bread wheat variety (Olivia) was subjected to trials for the first year and in 2021/22 the same varieties for the second year. Also, in the growing season 2020/21, three barley varieties (Kythraia, Lysi and Athinais) and one bread wheat variety (Acheleia) 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. Grain yield, yield related characteristics and quality traits are examined. During the 2020/21 and 2021/22 the peanut variety 'Italian', three barley varieties (EV7, EV8, EV9), one durum wheat variety (DUR7) and one bread wheat variety (Olivia) were tested for the first and second year, respectively. The results were reported to the competent Authority (Department of Agriculture). **(K. Mina)**



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