

# HIGHER EDUCATION FOR SUSTAINABLE *Food Production*

## **Seventh Joint Meeting of Agriculture-oriented PhD Programs**



**Udine**  
**29 September - 03 October 2025**



Università  
di Catania



UNIVERSITÀ  
DI FOGGIA



UNIVERSITÀ  
DEGLI STUDI  
DI UDINE









Time	Monday 29.09			Chairperson
08:30 - 9:00	WELCOMING			
9:00 - 9:45	KEYNOTE	Tulli	The potential of insects rearing in sustainable agri-food systems	
9:45 - 10:00	Plant & Crop Science I	Bonforte	The use of flower strips and the richness and abundance of pollinator bees in a Mediterranean agroecosystem	G. Rosiello & A. Del Pino
10:00 - 10:15		Parlascino	Innovative approaches for plant health monitoring	
10:15 - 10:30		Ashraf	Differential expressed genes in kiwifruit ( <i>Actinidia</i> spp.) graftings	
10:30 - 10:45		Angione	From the Green Revolution to Green Resilience: Rht genes between productivity and adaptation	
10:45 - 11:00		Canciani	Uncovering Critical Reflectance Wavelengths for Iron-Deficiency Detection Using Machine Learning: A Comparative Study	
11:00 - 11:30	Coffee Break			
11:30 - 11:45	Animal Science & One Health I	Corte Pause	Impact of a carbon-based treatment on plasma biochemical parameters related to energy metabolism, inflammation, oxidative stress, and mineral balance in dairy cows	R. Opere & M. Carentini
11:45 - 12:00		Fabbri	Double trouble: co-infection of <i>Angyostrongylus vasorum</i> and <i>Dirofilaria immitis</i> in golden jackal ( <i>Canis aureus</i> ) in Friuli Venezia Giulia, Italy.	
12:00 - 12:15		Petruzzi	Detection of <i>Salmonella</i> spp. in carcasses and rectal swabs of wild boars ( <i>Sus scrofa</i> ) from the 2024–2025 hunting season in Apulia and Basilicata regions	
12:15 - 12:30		Bertino	Dietary almond skin to lambs: effect on meat oxidative stability	
12:30 - 14:00	Buffet			
14:00 - 14:15	Sustainability, Circular Bioeconomy & Emissions	Bruno	Drivers for double cropping adoption in Friuli Venezia Giulia	E. B. Hmad & G. Marinaro
14:15 - 14:30		Carnevale	Biorefinery Approach for Valorizing Anaerobic Digestate into Agricultural Bioproducts	
14:30 - 14:45		Midolo	Valorization of Low-Quality Sheep Wool for Sustainable Composite Applications: A Preliminary Investigation	
14:45 - 15:00		Panebianco	Bio-based fertilizers and soil improvers for the sustainability of Mediterranean herbaceous cropping systems	
15:00 - 15:15		Pavanello	A Moo-ving story: methane burps, behaviour, and management in the dairy world	
15:15 - 15:30		Rizzo	Long term performance of Nature-Based Solutions as decentralized wastewater treatment: a case study of a retail store in southern Italy	
15:30 - 15:45		Spadanuda	The Italian Durum Wheat Variety Trial Network: A Collaborative Platform for Climate-Resilient Variety Evaluation.	
15:45 - 16:00		Barresi	Effect of novel substrates on the efficiency of dairy wastewater in constructed wetlands.	

Time	Tuesday 30.09			Chairperson
08:30 - 9:00	WELCOMING			
9:00 - 9:45	KEYNOTE	Gusella	Cultivating Growth: A Research Journey from PhD to Agritech	
9:45 - 10:00	Other	Anaclerio	An Experimental Investigation of the Effects of Hydrogen Peroxide on the Autoignition of NH <sub>3</sub> /H <sub>2</sub> /air Mixtures in an HCCI Engine	N. Achkar & S. Boga
10:00 - 10:15	Plant & Crop Science II	La Quatra	Preliminary Screening of Essential Oil-Based Nanoemulsions for the Control of Major Fungal Pathogens of Citrus	
10:15 - 10:30		Furnitto	Fresh biomass estimation from high-resolution multispectral vegetation indices obtained from UAV in Mediterranean "Sulla" pastures	
10:30 - 10:45		Boscarol	Ecophysiological response of maize ( <i>Zea mays</i> L.) to water stress: remote sensing and upscaling techniques for a more efficient management of water resources in agriculture	
10:45 - 11:00		Antoniciello	Implementing the accuracy of canopy transpiration assessment to improve the estimation of tree crop water use in the era of climate change	
11:00 - 11:30	Coffee Break			
11:30 - 11:45	Food Processing I & Technology	Montebello	Influence of thermal processing on the physicochemical and technological properties of mycelium-based meat substitute	P. Marino & F. Petrucci
11:45 - 12:00		Accettulli	White onion PGI valorization through fermentation processes	
12:00 - 12:15		D'Amelio	Application of Artichoke powder for microbial biomass production: a case-study	
12:15 - 12:30		De Stefano	Intestinal inflammation and ketogenic diet: the combined role of $\beta$ -hydroxybutyrate and MCT oil on Caco-2 cells	
12:30 - 14:00	Buffet			
14:00 - 14:15	Plant & Crop Science III	De Santis	Can <i>Pseudomonas</i> spp. isolated from polluted soils be used as possible bioremediation agents for heavy metal removal?	M. Lucai & T. Conte
14:15 - 14:30		Guerrieri	Microbial Diversity of Salt-Tolerant Plant Growth Promoting Bacteria Associated with <i>Cakile maritima</i>	
14:30 - 14:45		Formica	Sustainable Weed Management: Evaluating the Herbicidal Potential of Three Essential Oils on <i>Amaranthus retroflexus</i> L.	
14:45 - 15:00		Al Achkar	Exploitation of rootstock scion combination and microbial consortia on the yield and rhizosphere Microbiome in Tomato under greenhouse	
15:00 - 15:15	Consumer & Market Studies	Conte	Metabolomic analysis of a high performative <i>Streptomyces</i> strain against soilborne fungal pathogens and weed of wheat and tomato crops	
15:15 - 15:30		Reitano	Athletes' preferences and willingness to pay for an innovative high-protein bread: evidence from an experimental study	
15:30 - 15:45		Cofano	Understanding Willingness to Adopt an Open-Loop Innovation: Evidence from Italian Artichoke Farms	
15:45 - 16:00		Carbone	Understanding consumer preferences for herbal teas: A conjoint analysis approach	

Orari	Thursday 02.10				Chairperson	
08:30 - 9:00		WELCOMING				
9:00 - 9:45	KEYNOTE	Colelli	Last Minute Surprise Topic!			
9:45 - 10:00	Plant & Crop Science IV	Chana	Genotyping, Cultivar Screening, and Breeding of Common Buckwheat ( <i>Fagopyrum esculentum</i> Moench) for Enhancing Cropping System in Northern Italy			A. Cabbia & L. Cattani
10:00 - 10:15		Liva	Building better genomes: long-read pipelines for assembly, annotation and functional profiling			
10:15 - 10:30		Sumon	Innate Immune Response and Gene Expression Profiling of <i>Lactococcus petauri</i> - and <i>L. garvieae</i> -Challenged Selected Lines of Rainbow Trout ( <i>Oncorhynchus mykiss</i> )			
10:30 - 10:45		Opere	Preliminary study on the effect of raw chitin from <i>Hermetia illucens exuviae</i> and lauric acid on gut morphology and functionality in rainbow trout ( <i>Oncorhynchus mykiss</i> ) fed semi-purified diets			
10:45 - 11:00		Mangano	Fatty acid metabolism in lambs supplemented with different level of almond skin by-product			
11:00 - 11:30		Coffee Break				
11:30 - 11:45	Plant & Crop Science V	Lucci	Strains of <i>Aureobasidium pullulans</i> from extreme environments: new potential biocontrol agents?			F. M. Sanna & M. G. Morea
11:45 - 12:00		Bonfante	Biostimulant effects of <i>Chlorella vulgaris</i> extract on zucchini ( <i>Cucurbita pepo</i> L.) under tunnel cultivation			
12:00 - 12:15		Giuffrida	Characterization of novel Citrus rootstocks subjected to deficit irrigation techniques			
12:15 - 12:30		Vecchio	<i>Colletotrichum perseae</i> and <i>C. gloeosporioides</i> sensu stricto Causing Stem lesion, Dieback and Fruit Rot on Avocado in Italy and sustainable control			
12:30 - 14:00		Buffet				
14:00 - 14:15	Food Technology & Processing II	Omri	Mediterranean diet and intake of microorganisms and biomolecules of microbial origin: case studies for the design of innovative trends			G. Vinci & R. Parlascino
14:15 - 14:30		Presutto	Lactic acid bacteria from Apulian honeys as functional candidates for fungal biocontrol in food systems			
14:30 - 14:45		Di Palma	Automated and digitally designed food processing operations			
14:45 - 15:00		Khan	Evaluation of Artichoke Powder as a Functional Additive to Design a Synbiotic Beverage			
15:00 - 15:15		Marinaro	Food reformulation and emerging technologies as strategies in supporting healthy nutrition			
15:15 - 15:30		Proetto	Valorisation of Brewer's Spent Grain as a Functional Ingredient to Improve Nutritional and Health Quality in Dry Pasta Formulation			

Orari	Friday 03.10			Chairperson
08:30 - 9:00	WELCOMING			
9:00 - 9:45	KEYNOTE	Franchini	Wildlife and agro-pastoral activities in shared landscapes: which steps towards coexistence?	
9:45 - 10:00	Viticulture & Enology	Bunello	Transcriptome dynamics during budbreak in early and late grapevine cultivars	
10:00 - 10:15		Ereddia	Sustainable viticulture: different strategies to enhance the resistance to the main fungal diseases on Sicilian cultivars	
10:15 - 10:30		Matloob	Deep Learning for Precision Viticulture: Automated Grape Harvesting, Pruning, and Pesticide Detection	
10:30 - 10:45		Trombetta	Assessment of Crop Evapotranspiration, Vine Ecophysiological Performance, and Fruit Quality in a Table Grape Vineyard Protected by Anti-Rain Plastic Sheets and Anti-Hail Nets	
10:45 - 11:00		Vinci	Characterization of grapevine responses to water and nitrogen availability	
11:00 - 11:30	Coffee Break			
11:30 - 11:45	Food Tech & Innovation	Rosiello	Ozone-Enriched MAP: A new prototype for minimally processed fruit and vegetables preservation and packaging compatibility	
11:45 - 12:00		Vit	Towards Selective Detection of Artificial Sweeteners: Electrochemical and Docking Studies for MIP Sensor Development	
12:00 - 12:15	Animal Science & One Health III	Pividori	A one-health approach: from feed to functionalized food	
12:15 - 12:30		Urli	Microplastic Contamination in Meat and Internal Tissues of Cattle and Pork	
12:30 - 14:00	Buffet			



## Posters:

Primo giorno (Lunedì)	Amodio	Deep Learning in Agriculture: Empowering the Transition to Agriculture 4.0
	Barbagallo	Drip Irrigation Performance with Reclaimed Wastewater
	Barrasso	Effects of Extreme Low-Oxygen Conditions on Volatile Compound Production in 'Pink Lady' Apples
	Ben Hmad	Lethal and behavioural side effects of insecticides on the whitefly parasitoid <i>Eretmocerus eremicus</i>
	Boga	MetaBench: assessment of performance of metagenomic approaches
	Bosco	Formulation of a symbiotic blend based on germinated brown rice and probiotics with promising health features
	Bua	Towards predictive and biological control tools for fungal pathogens of Mediterranean crops
	Cabbia	Evaluation of feeding behaviour in growing bulls with an ear-tag accelerometer
	Carchiolo	Influence of Spectral Characteristics of LED Lighting on Nutritional Quality of Vegetable Products: A Systematic Review
	Carlentini	Exploring the Genetic Diversity and Population Structure in three Sicilian horse breeds
	Cattani	Attitude and perceptions of dairy cattle herders towards the compensation system for wildlife damages in North-Eastern Italy.
	Chiarenza	Recovery of bioactive compounds and in particular eriocitrin from citrus processing wastes
	Ciavarella	Regenerative Agriculture in Mediterranean cropping systems: a long-term multi-source assessment
	Del Pino	Physiological responses of plants to abiotic stress in alpine environments
	Faulisi	Pomegranate ( <i>Punica granatum</i> L.) by-products extracts with high antioxidant and antimicrobial action as food additives in a circular economy and environmental sustainability perspective – "From waste to wealth" objective
	Formenti	Arbuscular mycorrhizal fungi inoculation as sustainable tool to improve yield and phytochemical value of <i>Cynara cardunculus</i> L.
	Ganci	Assessing the Value of Sustainability in the Citrus Supply Chain
	Passerelli	Enhancing red raspberry ( <i>Rubus idaeus</i> ) breeding with molecular tools: QTLs for fruit quality and powdery mildew resistance
Secondo giorno (Martedì)	Grasso	Social Networks and Sustainable Innovation in the Etna DOC
	La Iacona	The Exploitation of Nanotechnology in Herbicides and Bioherbicides: A Novel Approach for Sustainable Weed Management
	Lupica	Toward modern pesticide use reduction strategies in advancing precision agriculture: A bibliometric review
	Manerchia Maserà	Integrative Assessment of Citrus Genotypes under Deficit Irrigation: Physiological and Biochemical Perspectives
	Marino	Integrated management of <i>Albugo occidentalis</i> in spinach: climate-driven emergence, varietal resistance, and sustainable control.
	Morea	Sustainable control of fungal soil-borne pathogens on tomato crops by phytochemicals
	Paramasivam	Global pattern and thematic evolution in robotic applications for the transformation of the food manufacturing sector
	Giannoccaro	Greenhouse Gas Emissions from Soil and Strategies for Their Mitigation in the agroecosystems in Mediterranean environment
	Rapisarda	Study of the ecophysiological and productive response of Nerello Mascalese subjected to four different canopy management techniques
	Ricatti	Dissection of genetic bases of plant salt response through the study of halophyte plants as model species and application to Mediterranean crops
	Rutigliano	Innovative strategies for oxidation protection in pistachio products
	Saccone	Bio-products to mitigate the adverse effect of climatic changes on crop performance
	Sanna	Are Volatile Organic Compounds Biomarkers of Grape Trunk Diseases Infection?
	Scavera	Evaluation of removal efficiency in full-scale constructed wetlands for wastewater treatment and reuse: two Sicilian case studies for small settlements.
	Stango	Agri-Food By-Products for Climate-Resilient Sheep Farming: Nutritional Strategies to Enhance Welfare and Milk Quality Under Heat Stress
	Vitaliano	AI-BIM Integration for Cost and Time Optimization in Construction Project Management: A Review



Orari	Mon 29.09		Tue 30.09		Excursion mountain or sea	Thu 2.10		Fri 3.10	
08:00 - 8:15									
8:30 - 9:00	Welcoming		Welcoming			Welcoming		Welcoming	
9:00 - 9:45	Tulli		Gusella			Colelli		Franchini	
9:45 - 10:00	G. Rosiello & A. Del Pino	Bonforte	N. Achkar & S. Boga	Anaclerio		A. Cabba & L. Cattani	Chana	M. Liva & L. Rapisarda	Bunello
10:00 - 10:15		Parlascino		La Quatra			Liva		Ereddia
10:15 - 10:30		Ashraf		Furnitto			Sumon		Matloob
10:30 - 10:45		Angione		Boscarol			Opere		Trombetta
10:45 - 11:00		Canciani		Antoniciello			Mangano		Vinci
11:00 - 11:30		Coffee Break		Coffee Break			Coffee Break		Coffee Break
11:30 - 11:45	R. Opere & M. Carlentini	Corte Pause	P. Martino & F. Petruzzi	Montebello		F. M. Sanna & M. G. Morea	Lucci	A. Grasso & E. Presutto	Rosiello
11:45 - 12:00		Fabbri		Accettulli			Bonfante		Vit
12:00 - 12:15		Petruzzi		D'Amelio			Giuffrida		Pividori
12:15 - 12:30		Bertino		De Stefano			Vecchio		Urli
12:30 - 14:00	Buffet		Buffet			Buffet		Buffet	
14:00 - 14:15	E.B. Hmad & G. Marinaro	Bruno	M. Lucci & T. Conte	De Santis		G. Vinci & R. Parlascino	Omri	Conclusion	
14:15 - 14:30		Carnevale		Guerrieri			Presutto		
14:30 - 14:45		Midolo		Formica			Di Palma		
14:45 - 15:00		Panebianco		Al Achkar			Khan		
15:00 - 15:15		Pavanello		Conte			Marinaro		
15:15 - 15:30		Rizzo		Reitano			Proetto		
15:30 - 15:45		Spadanuda		Cofano		Visit at Vie di Romans winery, Mariano Del Friuli Staff: Michele Canciani		Excursion at Udine historical Center	
15:45 - 16:00		Barresi		Carbone					
17:30 - 19:30	Aperiposter Casa della Contadinanza (UD) Staff: Cristina Pavanello, Daniele Fabbri					Cena sociale Ristorante Eliot, Via Orsari 50, Manzano Staff: Filippo Sanna			
19:30 - 23:00	Free evening		Free evening		Free evening				

### Monday and Tuesday

*Aperiposter* at Casa della Contadinanza, Salita al Castello, Udine. Meeting point at 18:00.

If you are presenting a poster, please hand it to the staff during the morning of the presentation day.

### Wednesday

Meeting point: in front of the library at 8:00, departure by bus at 8:30.

- Sauris group: Arrival at 10:00. Visit to *Prosciuttificio Wolf*. Lunch at 13:00 at *Seike Restaurant*. In the afternoon, ethnographic guided tour, visit to the Ethnographic Museum and *Zahre Brewery*. Departure from Sauris at 17:30.  
(Staff: Francesca Tulli, Filippo Sanna)
- Aquileia/Grado group: Arrival at 10:00. Visit to the *Basilica of Aquileia*. Transfer by bus to Grado. Lunch at 13:00 at *La Dinette Restaurant*. Visit of Grado's historical city center.  
(Staff: Stefano Bovolenta, Michele Canciani)

### Thursday

Departure by bus from the library at 16:00. Arrival at 16:30 at *Vie di Romans Winery* for a guided visit and wine tasting. At 19:00, transfer to *Elliot Restaurant* for the social dinner.

Please note: appropriate dress code required (*smart casual*).

### Friday

Treasure hunt in the historical center of Udine. Meeting point in *Piazza 1° Maggio* at 15:30.

Join us to discover the hidden gems of Udine's historical center!



## **Organizing and scientific Committee:**

### **PhD Students:**

- **Daniele Fabbri**, University of Udine
- **Michele Canciani**, University of Udine
- **Filippo Sanna**, University of Udine
- **Cristina Pavanello**, University of Udine
- **Roberta Montebello**, University of Foggia
- **Alessandro De Santis**, University of Foggia
- **Valentina Formica**, University of Catania
- **Roberto Carbone**, University of Catania

### **Coordinators of PhD courses:**

- **Antonio Biondi**, University of Catania
- **Maria Luisa Amodio**, University of Foggia
- **Stefano Bovolenta**, University of Udine

### **Participating Professors:**

- **Colelli Giancarlo**, University of Foggia
- **Zanin Laura**, University of Udine
- **Di Francesco Alessandra**, University of Udine
- **Tulli Francesca**, University of Udine



## **Oral presentation section**

## The use of flower strips and the richness and abundance of pollinator bees in a Mediterranean agroecosystem

Marta Bonforte<sup>1,3</sup>, Roberto Catania<sup>1,3</sup>, Maria Augusta Pereira Lima<sup>2</sup>, Gaetana Mazzeo<sup>1</sup>

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**Introduction:** Bees, solitary and social, play an essential role in the pollination of cultivated and wild plants, contributing significantly to the stability and productivity of agroecosystems. However, agricultural intensification and habitat fragmentation are critical factors in preserving their diversity. The planting of flower strips within agricultural settings is a proven agroecological measure that can increase environmental heterogeneity and provide food resources as well as refuge sites for pollinators. However, the use of flower strips is widespread in European countries and even outside, while it's not very extensive in Mediterranean crops. Flower strips are a mix of flower seeds, sometimes combined with legume species or grasses, are planted in fields. Thus, improving environmental quality within agro-ecosystems, especially in intensive agricultural areas. This study assessed the impact of flower strips on the composition and abundance of bees in a Citrus orchard in Carlentini (SR), southern Sicily.

**Methods:** The flower strips, sown in December 2023 with four species attractive to pollinators selected from Ecoschema 5 of the PAC, were set up so that flowering coincided with that of the lime crop. The strips were replicated twice and the seed bank was left intact. Monitoring was conducted on a weekly basis, during the flowering period (April–June 2024), comparing flower strips with the spontaneous vegetation in a close orange grove. The transect walks with the net were carried out in two rounds (9-11 and 14-16). The pan trap triplets were placed in the field and surrounding areas in the morning and removed in the late afternoon.

**Discussion and Conclusion:** A total of 535 bees were recorded by net, with the highest numbers observed in the lime field where flower strips were established, and fewer in the adjacent areas. The genus *Andrena* was the most represented, mainly associated with *Medicago sativa*, planted as cover crops in previous years. Bees from the genera *Amegilla*, *Sphecodes*, *Tetralonia*, and *Xylocopa* were exclusively detected within the flower strips, which also displayed a broader distribution of individuals compared to surrounding areas. These findings indicated that pollinator presence was strongly influenced by both the diversity and abundance of floral resources. Flower strips clearly promoted bee biodiversity in this agroecosystem, offering diverse and beneficial resources for a wider range of pollinator species. Although Shannon's diversity index ( $H'$ ) was similar between flower strips and other habitats, it reflected distinct patterns: species richness was greater in the strips, but their distribution was lower compared to the other habitats, as shown by Pielou's index ( $J = 0.81$  vs  $0.90$ ). Likewise, Simpson's index ( $1-D = 0.94$  vs  $0.96$ ) confirms this trend, with the surrounding habitats presenting more homogeneous, but less rich communities. These results highlight how flower strips are important biodiversity hotspots, playing a complementary role in enhancing species richness and supporting pollinator conservation with semi-natural areas that are vital for ecological stability.

## Innovative approaches for plant health monitoring

Rossana Parlascino<sup>1</sup>, Alessia Cavallaro<sup>2</sup>, Rossella Santonocito<sup>2</sup>, Mario Riolo<sup>1</sup>, Federico La Spada<sup>1</sup>, Nunzio Tuccitto<sup>2</sup>, Giuseppe Trusso Sfrassetto<sup>2</sup>, Santa Olga Cacciola\*

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**Introduction:** The early detection of pathogenic fungi in agri-food products is essential to ensure quality and safety throughout the production and distribution chain. To address this need, we developed FAST (Fluorescent Array Sensor Technology), a portable optical sensing system based on fluorescent organic molecular probes. Initially, FAST demonstrated the ability to selectively detect spores of phytopathogenic fungi, including *Penicillium italicum*, *Alternaria alternata*, and *Fusarium sacchari*, in washing waters from citrus packinghouses. The technology provides rapid results using a common smartphone for signal acquisition, eliminating the need for complex laboratory instruments. Building on these results, we explored a novel application of the sensor: the real-time, non-destructive monitoring of citrus fruit stored in sealed packaging. By detecting volatile organic compounds (VOCs) emitted from inoculated fruit, the sensor can distinguish between healthy and *Penicillium digitatum*-infected citrus. This marks the first reported use of an optical sensor capable of simultaneously monitoring fruit preservation status and fungal contamination inside closed packaging. The system holds strong potential for integration into postharvest handling to enhance food safety and minimize losses.

**Methods:** The FAST platform was employed to detect VOCs emitted by citrus fruits infected with *Penicillium digitatum*, the causative agent of green mold, a major postharvest disease. An optical array composed of 20 fluorescent molecular probes was designed to interact non-covalently with specific VOCs produced during fungal infection and fruit ripening. The mechanisms of interaction include hydrogen bonding,  $\pi$ - $\pi$  stacking, and ionic interactions. The sensor array was placed inside closed fruit packaging to enable real-time and non-invasive monitoring of VOCs. Fluorescent responses were recorded using a smartphone-based imaging system under UV excitation, and data were processed using multivariate analysis techniques to differentiate between infected and healthy fruits.

**Discussion and Conclusion:** The application of FAST technology to the monitoring of VOCs in citrus packaging demonstrated the sensor's high sensitivity and selectivity for detecting fungal infections. The system successfully differentiated between healthy and *P. digitatum*-infected fruit, not only based on the presence of the pathogen but also according to the dynamic changes in VOC profiles over storage time. This approach offers a rapid, non-destructive, and cost-effective solution for in-package quality control. By avoiding the need to open or visually inspect fruit, it enables early intervention while preserving product integrity. The ability to integrate FAST into existing packaging and logistics workflows supports the development of smart postharvest monitoring systems across the citrus supply chain. Beyond fungal detection, the platform's versatility suggests further applications, such as the assessment of fruit ripeness or adaptation to other food products and pathogens. Future research will focus on optimizing probe selectivity and incorporating the system into automated quality control platforms for broader agri-food use.



## Differential expressed genes in kiwifruit (*Actinidia* spp.) graftings

Iqra Ashraf<sup>1</sup>, Guido Cipriani<sup>1</sup>, Gloria De Mori<sup>1</sup>

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**Introduction:** Kiwifruit (*Actinidia* spp.) cultivation has attracted consumers due to its unique flavor, nutritional value, and economic importance. Initially, kiwifruit was not grafted in commercial orchards, but the recent emergence of the kiwifruit vine decline syndrome (KVDS) has established grafting as a mainstay propagation technique. Future cultivation of kiwifruit will rely on graft compatibility and the use of KVDS-resistant or tolerant rootstocks, particularly in regions with challenging environmental conditions. Evidence shows that two wild species of kiwifruit, *A. macrosperma* and *A. valvata* can overcome the KVDS. Therefore, this study aims to evaluate grafting compatibility among three kiwifruit species: *A. macrosperma*, *A. valvata*, and *A. chinensis*.

**Methods:** Micro-grafting experiments were conducted using *A. macrosperma* and *A. valvata* as rootstocks and two commercially important genotypes of *A. chinensis* as scions: 'Soreli' (yellow-fleshed, *A. chinensis* var. *chinensis*) and 'Hayward' (green-fleshed, *A. chinensis* var. *deliciosa*). The study focused on the potential link between graft success and antioxidant and auxin biosynthesis gene expression. These genes mitigate grafting-induced oxidative stress and promote vascular development, respectively. Gene expression analysis using quantitative PCR was performed on ten different genes: Superoxide dismutase 1, Superoxide dismutase 3, Ascorbate Peroxidase, Catalase isozyme 1, Catalase isozyme 3, Auxin efflux carrier components like, *Actinidia* auxin-responsive protein IAA26-like, Indole-3-acetic acid-amino synthetase, Phenylalanine ammonia-lyase, and Ascorbate Peroxidase 3.

**Discussion and Conclusion:** The analysis of differential gene expression among various kiwifruit rootstock and scion combinations is expected to offer critical insights into the molecular mechanisms underlying graft compatibility. It is anticipated that specific genetic markers associated with successful or failed grafts will be identified, providing potential targets for improving grafting outcomes. These findings will contribute to a deeper understanding of the physiological and genetic interactions at the graft union, enabling more informed selection of compatible rootstock–scion. This research will emphasize the importance of optimizing these combinations not only for improved compatibility but also for enhanced resilience to environmental stresses and diseases, particularly KVDS.

## From the Green Revolution to Green Resilience: Rht genes between productivity and adaptation

Giuseppina Angione<sup>1,3\*</sup>, Concetta Lotti<sup>1</sup>, Salvatore Esposito<sup>2</sup>, Ida Colella<sup>3,4</sup>, Patrizio Spadanuda<sup>1,3</sup>, Angelica Giancaspro<sup>1</sup>, Philippa Borrill<sup>5</sup>, Pasquale De Vita<sup>3</sup>

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**Introduction:** Over the past century, the evolution of wheat ideotypes has been primarily driven by the need to maximize productivity through the efficient use of agricultural inputs. During the Green Revolution, the introduction of gibberellin-insensitive dwarfing genes ( $GA_3$ -insensitive *Rht*), such as *Rht-B1b* and *Rht-D1b*, led to the development of shorter varieties, characterized by increased lodging resistance and improved resource-use efficiency, significantly contributing to increase yields. Climate change imposes new challenges, requiring high-yielding varieties, resilient to adverse conditions and more efficient in natural resource use. In this new scenario,  $GA$ -sensitive *Rht* genes are emerging as valuable tools to reduce plant height without compromising early vigour or coleoptile and root development, traits that are crucial for establishment and stress tolerance.

**Methods:** In the present work, we analyzed a segregating population obtained by crossing Castelporziano (medium height, with a long coleoptile and sensitive to  $GA_3$ ) and Atoudur (semi-dwarf, with a short coleoptile and insensitive to  $GA_3$ ). Two contrasting bulks for coleoptile length, plant height, and  $GA_3$  sensitivity were analyzed through QTL-seq and RNAseq approaches.

**Discussion and Conclusion:** Two major QTLs were identified on chr 4B and 6A, two important chromosomes harbouring  $GA$ -insensitive (e.g., *Rht1*) and  $GA$ -sensitive (e.g., *Rht14*, *Rht16*, *Rht18*, *Rht24*) genes. In detail, on chromosome 6A and in line with previous results, no polymorphisms were identified for *TdGA2oxA9* in its promoter or coding regions, although its expression was higher in Castelporziano compared to Atoudur. By exploring the surrounding region ( $\pm 6$  Mbp) on chromosome 6A, a missense mutation was identified in a gene encoding a *Histone H2A deubiquitinase*, an important player potentially involved in chromatin remodelling and epigenetic control of the gibberellin pathway. Validation through TILLING mutants will clarify its role in growth regulation.

## Uncovering Critical Reflectance Wavelengths for Iron-Deficiency Detection Using Machine Learning: A Comparative Study

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**Introduction:** Iron (Fe) deficiency represents a serious agronomic issue, leading to reduced yield and increased management costs if not addressed in time. Although Fe fertilization is a common solution adopted by farmers, delayed responses often result in substantial production losses. Precision agriculture provides valuable tools for the early detection of nutrient deficiencies, enabling timely interventions that minimize both economic and agronomic impacts. Among the most common approaches are machine/deep learning models and vegetation indices (VIs), which are used to classify plant stresses based on spectral information. However, Fe deficiency has been understudied in hyperspectral research, partly because its main visual symptom—leaf yellowing—is common to various other abiotic and biotic stresses. This overlap complicates the accurate identification of Fe-specific stress. The objective of this study is to identify a reduced set of hyperspectral wavelengths that are uniquely associated with Fe deficiency and are not influenced by nitrogen (N) deficiency, with the aim of supporting more accurate and early stress detection in crops.

**Methods:** Hyperspectral data were collected from five plant species (barley, maize, cucumber, tomato, and lupine) grown in a hydroponic system under three nutrient conditions: complete solution, Fe deficiency, and N deficiency. Two feature selection algorithms—Competitive Adaptive Reweighted Sampling (CARS) and SHapley Additive exPlanations (SHAP)—were applied to identify key wavelengths. These wavelengths were then used to develop classification models using four methods: Support Vector Machine, Linear Discriminant Analysis, Random Forest, and Logistic Regression. Finally, a performance comparison was conducted, followed by a testing phase using two independent datasets: the SPECIM dataset and the Polypen dataset.

**Discussion and Conclusion:** On SPECIM testing phase, RF achieved the highest accuracy (0.94) with CARS-15, while Logistic Regression (LR) reached 0.96 using SHAP-6. LDA also performed well (0.94–0.98) with reduced datasets. Tomato and maize were classified with perfect accuracy (1.00) in several combinations. Barley reached up to 0.98, while cucumber and lupine showed lower accuracy, peaking at 0.87. Overall, RF and LR demonstrated strong and consistent performance across datasets, and the selected wavelengths proved effective for distinguishing Fe and N deficiencies in multiple crop species. On Polypen test phase, barley and lupine datasets were classified with accuracy values above 0.90 across all tested approaches. The best result was achieved by LDA on the CARS-5 dataset (0.99), while RF proved to be the most consistent model, reaching up to 0.97 on CARS-5 and 0.98 in species-specific analyses. RF also correctly classified the highest number of test samples for both species. In contrast, Logistic Regression and SVM showed lower accuracy, with SVM peaking at 0.83. The most informative datasets were CARS-13-LR for barley and SHAP-6-LDA for lupine, highlighting the value of targeted wavelength selection. In conclusion, RF is the best model owing to perform well in several selected wavelengths set. The most related wavelengths to iron deficiency are 449.35 nm in blue region, 557.36 nm in the green region and 717.54 nm in red-NIR region.



## **Impact of a carbon-based treatment on plasma biochemical parameters related to energy metabolism, inflammation, oxidative stress, and mineral balance in dairy cows**

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**Introduction:** Uterine infections are among the most significant disorders in dairy cows, with an incidence ranging from 20 to 40%. They are classified based on the uterine layers involved and the presence of clinical signs, and include metritis and endometritis (both clinical and subclinical). The consequences of UIs include reduced welfare, increased incidence of other diseases, decreased productive and reproductive performance, increased culling, and decreased profitability. Prompt and effective treatment is therefore essential, with antibiotics currently representing the most reliable option. However, due to the globally recognized issue of antibiotic resistance, legislation restricting the use of antibiotics has been introduced, particularly in the livestock sector.

In this scenario, the antimicrobial properties of alternative treatments are worthy of being more deeply investigated. The adsorptive capacity of porous carbon matrices (Carb) may enhance pathogens clearance and represent a promising solution to modulate inflammation, energy status, oxidative stress, and mineral metabolism.

**Methods:** Blood samples were collected at 4 different timepoints of the experimental protocol (T0, T7, T14, and T28), centrifuged, and plasma used for biochemical analyses. Haptoglobin, ceruloplasmin, albumin, globulin, total protein, and paraoxonase were analysed as inflammation markers. Cholesterol, bilirubin,  $\beta$ -hydroxybutyrate, glucose, non-esterified fatty acids, urea, glutamate oxaloacetate transaminase, and  $\gamma$ -glutamyl transferase were assessed for energy status and liver functionality. Oxidative stress was investigated through reactive oxygen metabolites, myeloperoxidase, total antioxidant capacity, advanced oxidation protein products, and thiol groups. Calcium and zinc were measured to assess mineral metabolism. Data were analyzed with repeated measures mixed models using the GLIMMIX procedure of SAS.

**Discussion and Conclusion:** Among all evaluated parameters, haptoglobin, albumins, globulins, cholesterol, GOT, and Zn showed statistically significant results between Ctrl and Carb in at least one time point of the experimental protocol. Regardless of the treatment group, time had a significant effect on the variation of all parameters ( $p < 0.0001$ ), except for Zn. Additionally, baseline values (T0) significantly influence the concentrations measured throughout the protocol for cholesterol, globulins, and GOT ( $p < 0.01$  for all of them). Haptoglobin was significantly higher in the Carb group compared to Ctrl at T7 ( $p = 0.04$ ). At T14, globulins were higher in Carb ( $p = 0.04$ ), while albumins were higher in Ctrl ( $p = 0.03$ ). Cholesterol was higher in Ctrl at T28 ( $p = 0.05$ ), glutamate oxaloacetate transaminase (GOT) in Carb at T14 ( $p = 0.04$ ), and zinc in Ctrl at T7 ( $p = 0.05$ ). No statistically significant differences between the Carb and Ctrl groups were observed for total protein and ceruloplasmin, as well as among the evaluated oxidative stress biomarkers. Overall, the results suggest that the treatment with the carbon-based adsorbent matrix tends to induce an acute inflammatory response. However, the return to values comparable to the Ctrl groups before the end of the protocol suggests a transient nature of the response.

## **Double trouble: co-infection of *Angiostrongylus vasorum* and *Dirofilaria immitis* in golden jackal (*Canis aureus*) in Friuli Venezia Giulia, Italy.**

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**Introduction:** The golden jackal (*Canis aureus*) has expanded rapidly across Europe, including into Italy's Friuli Venezia Giulia (FVG) region. This expansion increases the species' potential role in the transmission of parasitic pathogens. *Angiostrongylus vasorum* and *Dirofilaria immitis* are cardiopulmonary nematodes of veterinary and zoonotic relevance, previously reported in golden jackals elsewhere in Europe, but not in co-infection in Italy. Both parasites share similar host ranges and induce serious pulmonary and vascular pathology. The golden jackal's adaptable ecology and increasing contact with domestic and synanthropic animals raise concern for its role in parasite dissemination. This study presents the first documented co-infections of *A. vasorum* and *D. immitis* in golden jackals in Italy, detailing pathological and parasitological findings and emphasising the importance of wildlife disease surveillance.

**Methods:** From 2020 to 2023, 60 golden jackals recovered in FVG were necropsied. The heart, lungs, and pulmonary arteries were examined macroscopically and microscopically for adult nematodes. Morphological identification was complemented by molecular diagnostics using PCR amplification of the 18S rRNA gene. DNA sequencing confirmed parasite identity via BLAST alignment. Histopathological analyses were also performed on lung and heart tissues to assess lesions associated with infection.

**Discussion and Conclusion:** This study confirms the golden jackal as a host of both *A. vasorum* and *D. immitis*, with the first report of *D. immitis* infection in this species in Italy and three confirmed co-infections. Typical pathological features were observed, including granulomatous pneumonia, vascular lesions, and adult nematodes in cardiopulmonary tissues. These findings align with previous reports in wild canids, reinforcing the pathological significance of both parasites. Co-infected jackals showed multifocal haemorrhagic and infarcted lung areas and meningeal haemorrhages, likely due to *A. vasorum*-related coagulopathies. The golden jackal's broad diet, scavenging behaviour, and adaptability to human-dominated environments enhance its contact with domestic and wild species, facilitating parasite transmission. As *D. immitis* is zoonotic and climate change is expanding the range of vector mosquitoes, monitoring wild canids such as the golden jackal is essential within a One Health framework. The species' continued range expansion may contribute to the emergence of new endemic areas for both parasites. Sustained surveillance efforts are necessary to better understand their prevalence and mitigate potential health risks to domestic animals and humans.

## **Detection of *Salmonella* spp. in carcasses and rectal swabs of wild boars (*Sus scrofa*) from the 2024–2025 hunting season in Apulia and Basilicata regions**

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**Introduction:** Wild boar (*Sus scrofa*) is a worldwide distributed species and is increasingly implicated as a reservoir of various pathogens. It has been suggested as a potential sentinel species for antimicrobial resistance (AMR) in wildlife due to its large home ranges, omnivorous diet, and high tolerance for human proximity. So, as part of the 2024–2025 hunting season, a sampling activity aimed to obtain a preliminary estimate of the circulation of *Salmonella* spp. in wild boars hunted in Apulia and Basilicata was conducted, with the objective of collecting data useful for better defining the risk of human infection associated with the handling and consumption of wild boar meat. Our data underscore the potential role of wildlife, in a multidisciplinary *One-Health* approach, as a carrier of zoonotic and antibiotic-resistant pathogens in southern Italy and a food safety risk for game meat consumers.

**Methods:** A total of 148 wild boar specimens were sampled during the 2024–2025 hunting season, including 106 carcass samples collected by sponge sampling and 42 rectal swabs. Isolation of *Salmonella* spp. was performed according to ISO 6579-1:2017. One isolate per positive sample was confirmed by MALDI-TOF MS. Confirmed *Salmonella* strains underwent serotyping according to ISO/TR 6579-3:2014 and MIC testing using Sensititre™ EUVSEC3 plates. MIC results were interpreted based on EUCAST 2025 (v.15) breakpoints.

**Discussion and Conclusion:** *Salmonella* spp. was detected in 5/148 samples (3.37%). In detail, in 3/78 (3.84%) carcasses (Basilicata), 2 strains of *S. Kasenyi* (38:e,h:1.5) and 1 strain of *S. Kottbus* (6.8:e,h:1.5) were isolated. In 2/42 (4.76%) rectal swabs were isolated *S. ser. II* (41:z:1.5) (Apulia) and *S. Kasenyi* (Basilicata). All isolates were sensitive to the antimicrobials tested. The results of this preliminary investigation revealed the presence of *Salmonella* in tested samples, confirming that wild boar could spread it in the environment and, potentially, transfer it to consumers. The isolation of *S. Kasenyi* and *S. Kottbus* represents a relevant finding. While *S. Kasenyi*—previously isolated from wild boars—has not been associated with human infections, *S. Kottbus* has been implicated in multiple human outbreaks. Notably, *S. ser. II* (41:z:1.5) has not previously been reported in wild boars, and no cases have been documented in the European scientific literature to date. In conclusion, the identification of *S. Kasenyi* and *S. Kottbus* in wild boar is relevant to the ecological surveillance of salmonellosis and underscores the potential role of this species as a reservoir of minor *Salmonella* serotypes, with epidemiological implications yet to be clarified.

## **Dietary almond skin to lambs: effect on meat oxidative stability**

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**Introduction:** Incorporating agro-industrial by-products into animal feeding is a strategy to enhance the sustainability of the livestock sector. Since feed production contributes to pollutant emissions in livestock, substituting conventional crops with agro-industrial by-products can reduce the environmental footprint of animal feeding, aligning with a circular economy model. In this context, almond skin could be an alternative feed that can be used for ruminant feeding. In light of the above, this study aimed to investigate the effects of feeding lambs diets containing two levels of almond skin on meat oxidative stability. Thirty Pinzirita × Valle del Belice male lambs (2 months old, body weight  $12.7 \pm 2.07$  kg) were randomly assigned to 3 groups and fed ad libitum for 56 days with: a conventional diet (CON) or diets containing 14% (A14) or 28% (A28) dried almond skin as replacement of corn.

**Methods:** Fatty acid profile and hydrophilic antioxidant capacity were assessed in fresh meat, while color and lipid oxidation were evaluated over 7 days of refrigerated storage. Data were statistically analyzed using a mixed model. No differences in the content of saturated and monounsaturated fatty acids were observed among the treatments ( $P > 0.05$ ). The dietary treatment did not influence the stability of meat colour and metmyoglobin development ( $P > 0.05$ ). Whereas, colour parameters and metmyoglobin development were affected during storage time ( $P < 0.001$ ), showing a typical browning pattern. Regarding the hydrophilic antioxidant capacity, ferrous ion chelating activity (FICA) and DPPH assays showed no differences ( $P > 0.05$ ); while Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and Folin-Ciocalteu assays were significantly affected ( $P < 0.05$ ). In both Folin and FRAP assays – which measure the reducing ability of the extract – smaller values were observed in almond treatments compared to the control. Conversely, the TEAC assay, which assesses radical scavenging ability, showed increased values in the almond groups compared to the control treatment. Dietary treatment and the interaction between dietary treatment and time of storage did not affect the content of secondary lipid oxidation products, analysed with TBARS assay ( $P > 0.05$ ). Whereas, TBARS values increased along 7 days of refrigerated storage ( $P < 0.001$ ).

**Discussion and Conclusion:** The inclusion of both levels of almond skin as a substitute of corn in the lamb diet did not worsen the fatty acid profile nor the colour parameters of the meat. The meat antioxidant capacity assays revealed an increase in radical scavenging capacity, while the reducing ability decreased. No differences in lipid oxidation values were observed between the treatments. In the light of the above, the almond skin could substitute corn in lambs' diet without detrimental effects on FA profile, color stability and lipid oxidation of meat. Further analyses will be conducted to better understand the differences in antioxidant capacity and to evaluate the content of lipophilic antioxidants.

## Drivers for double cropping adoption in Friuli Venezia Giulia

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**Introduction:** Supporting cropping system changes, partly due to climate change, requires knowledge of farmland dynamics to design effective, resilient, and sustainable policies. Diversification practices have been proposed as adaptation, like extension of crop rotations, intercropping, cover crops or double cropping (DC). DC, which requires abundant water and long growing seasons, is constrained in Mediterranean areas by water scarcity. Nonetheless, DC offers supplemental income, reduces fixed cost incidence, improves pest and weed control, and prevents soil erosion. In Friuli Venezia Giulia region (FVG), N-E Italy, DC is commonly practiced thanks to summer rainfall (June-August 613 mm). But very little is known about its spatial distribution and adoption drivers. In this context, our goal is to assess DC patterns via the Land Parcel Identification System (LPIS) database and investigate the factors affecting DC in farms.

**Methods:** To assess DC patterns, we used 2023 LPIS data, the first year with mandatory second crop declarations for FVG. We formulated three hypotheses based on literature and interviews in a sample of DC farms:

1. DC\_ratio (DC\_UAA/farm\_UAA) is higher in smaller farms to mitigate fixed costs and yield instability risks. We correlate farm UAA and DC\_ratio.
2. Access to irrigation (IrrA) supports DC adoption; we compared DC and single cropping hectares by IrrA.
3. Resurgence line (rl) affects water availability and consequentially DC adoption. We compared DC adoption in areas north and south of rl

Analyses were performed on RStudio-2024.12.1-563.

**Discussion and Conclusion:** DC covered barely 10% of farm surface (21341 ha) in 2023. The most abundant spring crops were maize, soybean and sorghum, whereas the winter ones were wheat and barley. The unique DC first/second crop combinations were 171. Soybean, maize and sorghum covered 98% of DC\_UAA as second crop. DCratio has a small positive relation with farm size. IrrA affects DC less than reported in literature at regional level probably due to the rainfall patterns in late spring. Water demand for DC adoption is thus supplied by rainfall patterns. Rainfall patterns changes will require adjustments in DC. Soybean and sorghum are preferred over maize in non-irrigated areas due to their lower water demand. DC ratio is not affected by location; this was unexpected considering the differences in water availability between north and south. Farmer interviews suggest that maize is still perceived as a cash crop but, the low water availability (irrigation/soil) combined to changing rainfall patterns may make preferable DC to maize as main crop. This perception seems to be supported by the higher percentage of SC than DC with IrrA. This study provides a preliminary regional-scale analysis of DC adoption drivers.



## **Biorefinery Approach for Valorizing Anaerobic Digestate into Agricultural Bioproducts**

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**Introduction:** Anaerobic digestion (AD) is a biochemical process that converts organic waste into renewable biofuel (biogas) and nutrient-rich fertilizer (digestate). Widely promoted within European sustainability frameworks, AD is gaining traction as a key technology in the circular economy. Among suitable feedstocks, olive pomace – a by-product of olive oil production – is particularly promising due to its high organic load and regional availability. Despite growing research on digestate applications, insufficient data addresses digestate quality derived from combining olive pomace with complementary agricultural waste products. So, this study explores the chemical composition of such digestate, produced in an industrial-scale AD plant primarily fed with olive pomace. The goal is to develop a biorefinery approach to transform this digestate into high-value bio-based products for agricultural use. This strategy aims to go beyond its conventional role as an N-P-K fertilizer, positioning digestate as a key resource in sustainable agricultural systems.

**Methods:** Raw digestate samples were collected to assess their physicochemical composition through elemental analysis, thermogravimetric analysis (TGA), ionic profile, structural polysaccharides and lignin, VFAs, lipids (total, unsaponifiable and FAMES), macro- and microelements (ICP-OES), total phenolics and antioxidant capacity. Solvent extraction was performed, selecting ethyl acetate for its green properties. Extracts were quantified gravimetrically and analyzed by GC-MS for untargeted compound identification. Additionally, antioxidant activity and total phenolic content were evaluated using colorimetric assays and LC-MS. This multi-analytical approach aimed to characterize bioactive compounds, supporting the sustainable valorization of digestate through a biorefinery perspective.

**Discussion and Conclusion:** Green extraction from whole digestate revealed the presence of unsaponifiable lipids, including terpenoids and sterols. Colorimetric assays confirmed antioxidant activity, mainly attributed to the phenolic fraction. To improve selectivity, membrane filtration with different molecular weight cut-offs is planned for phenolic compound isolation. Preliminary findings suggest that ethyl acetate enables the recovery of bioactive molecules with potential applications in the biopesticide sector. A biorefinery approach applied to digestate from anaerobic digestion of olive pomace could diversify AD plant outputs, enhancing natural bioproducts for agriculture. This strategy aligns with European energy transition and circular economy goals, offering an integrated waste valorization pathway. Moreover, it contributes to scientific progress by enhancing understanding of digestate's chemical profile and extractive potential.

By converting digestate into valuable agricultural products, this biorefinery model supports sustainable farming practices and fully exploits the environmental and economic benefits of anaerobic digestion.

## **Valorization of Low-Quality Sheep Wool for Sustainable Composite Applications: A Preliminary Investigation**

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**Introduction:** The management of low-quality sheep wool remains a significant and ongoing challenge in the livestock sector. Despite its renewable origin and growing recognition in the scientific literature, this resource is often treated as waste. In the absence of dedicated valorization chains, it is frequently disposed of illegally, generating environmental impacts and missing opportunities for circular economy development and local growth. This study presents preliminary tests exploring the use of low-quality sheep wool, both in the form of yarn and as a carding by-product, for the production of bio-composite materials for applications in the construction and industrial sectors.

**Methods:** Three experimental approaches were developed to assess the tensile properties of wool-based composites, using wool as a reinforcing fiber. In the first approach, wool yarn was used to produce a reinforcement grid, which was impregnated with a vegetable-based resin and embedded in a cementitious mortar to form a cement-reinforced composite (CRM). The resulting composite (70×10 cm, 1.5 cm spacing) was tested according to ASTM D2256. The carded wool by-product was explored in two additional ways: (i) as powder,, obtained through cryogenic grinding using liquid nitrogen, then blended with PLA pellets to produce 3D printing filaments with varying wool contents (0%, 5%, 10%, 20%). Samples were tested according to ISO 527-2; (ii) as loose fiber, layered between PLA sheets and processed via hot pressing with wool contents of 10%, 20%, and 30%, alongside a pure PLA control. These samples were tested according to ISO 178.

**Discussion and Conclusion:** Although preliminary and limited to tensile tests, the results provided valuable insights into the challenges associated with using low-quality wool in composite materials. In the CRM composite, strong adhesion was observed between the yarn, resin, and mortar. However, the premature failure of the yarn relative to the matrix limited the overall mechanical enhancement. This suggests that increasing the yarn's cross-sectional area could potentially improve structural performance. In PLA-based composites, developed through both 3D printing and hot pressing, wool demonstrated good compatibility with the matrix, enabling the creation of homogeneous and workable samples. For 3D-printed filaments, further optimization is required to improve fiber distribution along the filament axis. In both processing methods, the best mechanical performance was achieved with a wool content of 20%, indicating this may represent an optimal threshold for reinforcement. Overall, this study serves as an initial step toward future research, highlighting how the valorization of livestock waste, such as wool, can contribute to waste reduction and support more circular and sustainable production models.

## **Bio-based fertilizers and soil improvers for the sustainability of Mediterranean herbaceous cropping systems**

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**Introduction:** Nowadays the agricultural systems are based on two priorities, firstly to maintain or increase crop yield and to reduce the impact on the environment as a consequence of the use of fertilizers. So it is very important to develop new ecofriendly technologies. Currently, it is estimated that 50% of the total population is fed thanks to mineral fertilizers. The increasing demand for food supplies will significantly boost the region's demand for high-quality fertilizers, ultimately having a favorable influence on the agricultural sector. The utilization of bio-waste will convert waste management challenges into economic opportunities by establishing a viable internal market for secondary raw materials of biological origin in the EU.

**Methods:** To evaluate the suitability of local organic residues for bio-based fertilizer production, fish sludge, orange peels, sewage sludge, and solid digestate were collected and characterized through chemical analyses. Based on the results, a pot trial was established to test three fertilizer formulations:

- i. 65% solid digestate + 35% WWTP sludge,
- ii. 50% solid digestate + 50% fish sludge,
- iii. 50% solid digestate + 50% fresh orange peels,

with a mineral fertilizer and an unfertilized control. Treatments ( $80 \text{ kg N ha}^{-1}$ ) were tested on hemp, sorghum, and safflower in randomized block experimental design with five replications.

**Discussion and Conclusion:** The adoption of innovative technologies, such as bio-based fertilizers and controlled-release nutrient systems, offers a promising approach to enhancing nutrient use efficiency and minimizing the environmental footprint of fertilization practices, thereby supporting the transition towards more sustainable and effective bio-based solutions. The project aims to promote a paradigm shift in fertilizer production, valorising residual organic matrices of local origin, as agro-industrial by-products, in order to improve the fertility of Mediterranean soils, reduce  $\text{CO}_2$  emissions and decrease agriculture's dependence on conventional mineral fertilizers. The implementation of these solutions could lead to significant environmental and economic benefits, promoting the circular recycling of organic waste and contributing to a more sustainable management of resources. Moreover, such approaches are in line with European strategies for the ecological transition and can be a valuable support to ensure food security on a global scale, especially in a context of climate change and increasing pressure on natural resources.

## **A Moo-ving story: methane burps, behaviour, and management in the dairy world**

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**Introduction:** The European dairy sector has undergone rapid intensification in recent decades, raising multiple concerns related to environmental, economic, and social sustainability. To address these challenges, an agroecological transition has become essential, and the evaluation, monitoring, and improvement of sustainability are now central goals for dairy production. Among the various indicators developed, the environmental dimension has received the most attention, particularly the contribution of dairy farms to global warming potential (GWP). Enteric methane emissions from cows are a major focus, given their relevance and impact on climate change. In this context, three trials were carried out on a regional dairy farm to investigate enteric methane emissions and their variability across different categories of dairy cattle. Two trials focused on emissions during the three main lactation stages, considering the cows' primary behaviours: feeding, ruminating, and resting. A third trial was conducted to evaluate whether early-life methane emissions in female calves can be modulated by daily supplementation of *Saccharomyces cerevisiae* yeast in milk during the pre- and weaning periods.

**Methods:** In the first two trials, animals were balanced for body weight, days in milk, and body condition score. Feed intake, behavior, milk yield and composition were recorded. Trial one involved 48 animals; feces and rumen fluid were analyzed for VOCs and microbiota. Trial two involved 42 animals during the three lactation stages and behavioral parameter were registered. Trial three included 40 calves supplemented with 20g/day of *Saccharomyces cerevisiae*. Calves were weighed, behavior monitored, fecal microbiota analyzed. For all the trials methane emissions were measured following Sorg et al. (2018).

**Discussion and Conclusion:** In the first trial, the 12 cows with the highest and the 12 with the lowest methane (CH<sub>4</sub>) emissions were selected and assigned to high (HME) and low (LME) methane-emitting groups, respectively. CH<sub>4</sub> emissions were significantly higher in HME than in LME cows (23.9 vs. 12.7 g/kg DMI;  $P < 0.01$ ). LME cows also showed greater neutral detergent fibre (NDF) digestibility (50.4% vs. 46.7%;  $P < 0.05$ ). Volatile organic compound (VOC) and microbiota profiles differed markedly: LME cows had higher levels of propionic acid and a greater abundance of microbes, whereas HME cows showed increased valeric acid and higher prevalence of methanogens such as *Methanobrevibacter* and *Methanobacteriales* ( $P < 0.05$ ).

Milk protein content was lower in early lactation ( $P < 0.01$ ). No significant differences were observed in behavioural parameters across lactation stages, but a significant interaction between behaviour and lactation stage affected methane emissions ( $P < 0.01$ ).

In the third trial, no significant differences in methane emissions or growth performance were found between the control group and the group receiving powdered yeast.

These findings suggest that microbiome modulation and stage-specific interventions may represent effective strategies for reducing methane emissions in dairy cattle. Further research on young animals is warranted.

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## **Long term performance of Nature-Based Solutions as decentralized wastewater treatment: a case study of a retail store in southern Italy**

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**Introduction:** Safeguarding water and wetlands is crucial for sustainable societal and economic progress, assisting climate change adaptation and promotion stability. Treatment Wetlands (TWs) are vital Nature-Based Solutions, providing ecosystem services and proving effective for decentralized wastewater treatment. TWs offer resilience, reduced costs, and environmental benefits. The Mediterranean region, highly vulnerable to climate change and water degradation, can greatly benefit from TW implementation. Despite advantages, some problems such as vulnerability to the influence of climate and temperature, clogging, non-standard design and construction, large area occupation, inadequate management practices, continuous production and emission of greenhouse gases, single ecological service function, etc., continued to appear with time. This study evaluates the long-term efficiency of a hybrid-TW system in Catania, Italy, focusing on clogging in its horizontal subsurface flow (HF) unit, assessing its impact on treatment efficiency and hydraulic behaviour, and determining if the treated water is suitable for irrigation.

**Methods:** Wastewater quality monitoring was conducted roughly one per month, from 2016 to 2023, to quantify chemical, physical and microbiological parameters. Ks values in the HF unit were determined using the falling head method. Four falling head infiltration tests were performed around each of the nine piezometers situated within the HF unit. The hydrodynamic behaviour of the HF unit was analyzed using an impulse-response tracer study. Each test involved the instantaneous injection of a highly concentrated NaCl solution at the HF unit inlet, followed by continuous monitoring of electrical conductivity at the outlet.

**Discussion and Conclusion:** The system has consistently produced high-quality effluent over its 10-year operation, meeting both Italian and EU standards for effluent discharge and water reuse. This makes the treated wastewater suitable for irrigating adjacent green spaces in the parking lot. While the system maintains excellent overall treatment efficiency due to the HF unit's strong buffering, clogging is evident in the HF unit, progressing towards its outlet. This phenomenon is evidenced by ponding, decreased hydraulic conductivity (Ks values), and increased mean residence time. Although partial clogging hasn't yet impacted treatment effectiveness, it necessitates future restoration measures to prevent potential sanitation risks. Effective management should account for varying conditions like seasonal temperatures and visitor frequency. Effluent recirculation at the inlet and saturation of the last VF unit are practical solutions for Mediterranean semi-arid climates. The study's findings will help managers of long-term TW systems in the Mediterranean plan hydraulic, restorative, and vegetation management, ensuring continued system efficacy and operator safety.



## **The Italian Durum Wheat Variety Trial Network: A Collaborative Platform for Climate-Resilient Variety Evaluation.**

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**Introduction:** Durum wheat (*Triticum durum* Desf.) is a staple crop of strategic relevance for the Italian agri-food system, particularly under growing climatic variability and the urgent need for resilient genetic resources. In this context, varietal innovation and improved decision-making at the farm level are essential. The Italian Durum Wheat Varietal Comparison Network, coordinated by CREA Foggia, addresses this need through a large-scale, multi-actor initiative that integrates both public and private stakeholders. Established to evaluate varietal performance under diverse environmental conditions, the Network contributes to the development of climate-resilient agriculture by providing farmers, researchers, and policymakers with robust, location-specific data. It operates at the national scale and plays a vital role in identifying genotypes best suited to Italy's heterogeneous agro-climatic conditions. Since 2024, the Network has expanded its reach to the southern Mediterranean, establishing international collaborations to enhance the global relevance and adaptability of durum wheat germplasm in challenging environments.

**Methods:** Each year, the Network tests 30 recent commercial durum wheat varieties across more than 30 experimental sites in 16 Italian regions, organized into six agro-climatic macrozones. All trials follow a harmonized agronomic protocol to ensure consistent evaluation of key traits such as phenology, yield, and grain quality. The initiative brings together universities, breeding companies, research institutes, and regional authorities. Since 2024, parallel trials are also conducted in Moroccan environments via collaboration with ICARDA, enabling direct comparison of varietal performance under Mediterranean and semi-arid conditions.

**Discussion and Conclusion:** The Italian Durum Wheat Variety Trial Network provides a high-quality, standardized dataset that serves multiple scientific and practical applications. The results support informed varietal selection tailored to specific environments, while also functioning as a reference for advanced predictive models. These include simulations of genotype × environment × management (G×E×M) interactions, yield forecasting based on meteorological and remote sensing inputs, and modelling of disease and pest risks. Moreover, the data contribute to assessments of climate change impacts on crop phenology and productivity, making the Network a valuable tool in climate-smart agriculture strategies.

The inclusion of Moroccan sites since 2024 enhances the Network's scientific reach, offering new insights into varietal adaptability in heat- and drought-prone environments. This Mediterranean linkage strengthens breeding pipelines targeting resilience traits and extends the utility of Italian-bred germplasm to other regions facing similar climatic challenges. Through its collaborative framework, the Network also fosters knowledge exchange and capacity building across institutions and borders.

This research highlights the structure and scientific potential of the Network, emphasising its role in fostering innovation and sustainability in durum wheat production. By promoting broad partnerships and methodological rigour, the initiative serves as a model for integrated, multi-scale crop research platforms. It advocates for increased international cooperation to enhance food security and climate adaptation in the face of mounting global challenges.

## **Effect of novel substrates on the efficiency of dairy wastewater in constructed wetlands.**

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**Introduction:** The dairy sector produces considerable volumes of wastewaters (WW), which represent a serious risk to the environment if not treated and disposed of properly. In general, small and medium-sized farms use conventional systems (pre-treatment and primary treatment) to treat this effluent, but these are often insufficient. A sustainable alternative for the treatment of these WWs could be constructed wetlands (CWs). Nevertheless, the considerable surface area required by the full-scale CW could be a disadvantage. The main goal of this research is based on the optimisation of a CW system for the treatment and reuse of dairy WW by minimising the surface area required. In this regard, several innovative substrates were tested in a lab-scale CW fed with synthetic dairy influent (SDI). Preliminary assessments of both the hydraulic behaviour and the performance of treatment were performed.

**Methods:** The lab-scale hybrid system includes four identical CWs (CW1, CW2, CW3 and CW4), each consisting of two treatment units functioning in series: column (VF) followed by a tank (HF). The columns are filled with from top to bottom with dolomite, perlite, zeolite, expanded clay and bio-balls. The HFs were filled with gravel and expanded clay (inlet of the system).

The SDI was prepared based on the average concentrations of the mother influent reported in the literature. The SDI was prepared every week in the laboratory using skim milk powder, NaCl, Urea 46%, PK 52-34, NH<sub>4</sub>Cl. The porosity of different substrates was determined by drainage method. The hydraulic conductivity ( $K_s$ , cm/s) of VFs and HFs was measured using the constant load method and was determined by using the Darcy's law.

**Discussion and Conclusion:** Preliminary results from physical-chemical analyses show high average removal efficiencies for TSS (61%), COD (95%), TN (73%), N-NH<sub>3</sub> (88%) and TP (42%) already in the VF. The performance of the entire CW system shows a further increase, recording the following average values for TSS (99%), COD (99%), BOD<sub>5</sub> (99%), TN (91%) and TP (95%). Porosity tests shows the following values for gravel, bio-balls, expanded clay, zeolite, perlite and dolomite: 43%, 80%, 41%, 36%, 51% and 41%, respectively. Hydraulic conductivity tests carried out during the start-up phase (time "0"), confirmed the absence of clogging and the general cleaning conditions of the system. The tests conducted on the VFs showed the following  $K_s$  values: 0.479 (cm/s), 0.453 (cm/s), 0.550 (cm/s) and 0.725 (cm/s) for CW1, CW2, CW3 and CW4, respectively. The  $K_s$  measurements performed in the HFs revealed hydraulic conductivity values of 0.200 cm/s (CW1), 0.353 cm/s (CW2), 0.349 cm/s (CW3) and 0.230 cm/s (CW4). These tests will be repeated periodically, in the presence of vegetation and at full system operating capacity, to assess the spatial-temporal evolution of clogging and study the hydraulic behaviour of the system.

## **An Experimental Investigation of the Effects of Hydrogen Peroxide on the Autoignition of $\text{NH}_3/\text{H}_2$ /air Mixtures in an HCCI Engine**

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**Introduction:**  $\text{NH}_3$  and  $\text{H}_2$  are considered among the most promising carbon-free fuels. However, when used in internal combustion engines, they exhibit several limitations. In Homogeneous Charge Compression Ignition (HCCI) engines, autoignition of the fuel/air mixture is the dominant combustion mode. Both  $\text{NH}_3$  and  $\text{H}_2$  show high resistance to autoignition, with ignition temperatures of 923 K and 793 K, respectively, thus requiring very high compression ratios. In HCCI configurations, the use of combustion promoters such as ozone or hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) can be employed to trigger ignition.  $\text{H}_2\text{O}_2$  readily decomposes into  $\text{H}_2\text{O}$  and  $\frac{1}{2}\text{O}_2$  at atmospheric conditions due to its high reactivity. Under engine-relevant conditions, however, its decomposition is further promoted by the third-body reaction  $\text{H}_2\text{O}_2 + \text{M} \leftrightarrow 2\text{OH} + \text{M}$ , which increases the OH radical pool and enhances the autoignition process.

**Methods:** A four-stroke single-cylinder PSA-DW10 engine with a CR of 16.4:1 was used to investigate the influence of  $\text{H}_2\text{O}_2$  on  $\text{NH}_3/\text{H}_2$ /air mixtures with experimental approach. The engine speed was set at 1000 rpm by an electric motor. The  $\text{H}_2\text{O}_2$  was supplied using a direct injection inside the combustion chamber using a conventional gasoline injector. The injection pressure was set at 100.0 bar. For each experimental test 100 instantaneous work cycles were acquired with a Crank Angle Degree (CAD) resolution of 0.1 CAD.

**Discussion and Conclusion:** To evaluate the effect of  $\text{H}_2\text{O}_2$  on  $\text{NH}_3/\text{H}_2$ /air mixtures with 12% of  $\text{H}_2$  content, a baseline case without  $\text{H}_2\text{O}_2$  injection was acquired. The results highlight that the presence of 12% of  $\text{H}_2$  is sufficient to ignite the mixture. Afterwards,  $\text{H}_2\text{O}_2$  was injected into the combustion chamber with a Duration on Injection (DOI) of 300 $\mu\text{s}$  at -30 and -50 CAD bTDC. The results highlight that the injection of  $\text{H}_2\text{O}_2$  can trigger the autoignition of the  $\text{NH}_3/\text{H}_2$ /air mixtures advancing combustion. Indeed, the presence of  $\text{H}_2\text{O}_2$  results in higher in-cylinder pressure exhibiting a shift of the pressure peak towards the Top Dead Centre (TDC). Furthermore, the presence of  $\text{H}_2\text{O}_2$  results in faster Heat Release Rate (HRR). The  $\text{H}_2$  content in the fresh charge was gradually reduced to achieve unstable operating conditions in the presence of  $\text{H}_2\text{O}_2$ . The results show that increasing the degree of injection (DOI) leads to higher in-cylinder pressures, indicating that a larger amount of the combustion promoter enhances ignition strength and reduces the coefficient of variance.

## Preliminary Screening of Essential Oil-Based Nanoemulsions for the Control of Major Fungal Pathogens of Citrus

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**Introduction:** Citrus is a key crop in Sicily, playing a vital role in the region's agriculture and economy. Due to growing concerns related to the environmental and health risks associated with using fungicides, this study explores sustainable alternatives for managing the major citrus fungal pathogens. In this context, essential oil-based nanoemulsions (N-EOs) have emerged as promising bioactive formulations for their antifungal properties. This study evaluates the *in vitro* efficacy of seven N-EOs - clove, fennel, garlic, laurel, lavender, lemongrass and mint - against three fungal pathogens of citrus: *Colletotrichum gloeosporioides*, *C. karstii*, and *Neofusicoccum parvum*. The aim is to identify the most effective N-EOs as potential candidates for integrated disease management strategies in citrus orchards.

**Methods:** Seven essential oil-based nanoemulsions were tested *in vitro* using mycelial growth and conidial germination inhibition assays against three fungal isolates for each fungal species. IC<sub>50</sub> and IC<sub>90</sub> values were calculated for each N-EO-isolate combination by using non-linear regression models and implemented to categorize the treatment effectiveness as high (IC<sub>50</sub> ≤ 0.7% w/v; IC<sub>90</sub> ≤ 1.95% w/v), moderate (0.8 < IC<sub>50</sub> < 1.5% w/v; 1.95 < IC<sub>90</sub> < 2.5% w/v) or low (IC<sub>50</sub> ≥ 1.6% w/v; IC<sub>90</sub> ≥ 2.6% w/v).

**Discussion and Conclusion:** The antifungal efficacy of the N-EOs varied across fungal species and assays. Garlic and clove nano-emulsions consistently demonstrated the highest inhibitory effects in both mycelial growth and conidial germination assays, regardless of the fungal species. Lavender exhibited moderate to high activity in both assays, whereas mint and lemongrass were effective in mycelial growth but showed low efficacy in conidial germination inhibition. Laurel exhibited moderate to high efficacy against *N. parvum* in the mycelial growth trial but was less effective against *Colletotrichum* spp. and in conidial germination assay. Fennel emerged as the least effective N-EO, with the highest IC<sub>50</sub> and IC<sub>90</sub> values in both trials. These findings highlight the potential of N-EOs, particularly garlic and clove, as promising alternatives for sustainable plant disease management in citrus production. While the *in vitro* results are encouraging, they must be validated through *in vivo* studies to assess field efficacy. Furthermore, the contrasting responses observed across assays underscore the complexity of pathogen behaviour and further emphasise the necessity of comprehensive *in vivo* evaluations before practical implementation.

## **Fresh biomass estimation from high-resolution multispectral vegetation indices obtained from UAV in Mediterranean “Sulla” pastures**

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**Introduction:** Pasture biomass estimation is essential for optimizing grazing management, particularly in semi-arid Mediterranean environments where climatic variability strongly influences vegetation productivity. Traditional destructive methods are accurate but impractical for frequent and large-scale monitoring. Recent advances in drone-based remote sensing offer high-resolution, flexible, and non-invasive alternatives. This study focuses on evaluating the performance of UAV-derived vegetation indices for estimating fresh aboveground biomass (AGB) in a rotationally grazed *Hedysarum coronarium* L. (“Sulla”) pasture. The aim is to assess which indices are more effective in reflecting spatial variability and biomass changes during key phenological stages, offering insight into operational monitoring applications. A further objective is to find a correlation between the biomass measured in the field before and after grazing, and the vegetation indices applied to images from UAVs.

**Methods:** Four field campaigns were conducted in a 0.7 ha pasture in Villarosa territory (Sicily, Italy) in 2024 and 2025 years. UAV flights were performed with DJI Mavic 3 Multispectral and DJI Matrice 300 + Altum PT. Fresh biomass was collected in 28 georeferenced plots of 0,50 x 0,50 cm before and after grazing. NDVI, SAVI, NDRE, and EVI were calculated from orthomosaics. Sample data was correlated with the VIs values in an area of 1 m around sampling points.

**Discussion and Conclusion:** Preliminary findings indicate that UAV-derived indices—especially NDVI and NDRE—are closely related with fresh biomass ( $R^2$  from 0,74 to 0,78 for all evaluated VI), being able to capture relevant variations in biomass across space and time. Fresh biomass showed consistent spatial patterns with spectral indices, particularly under different grazing conditions and rainfall regimes. The very high spatial resolution of UAV imagery allows detection of fine-scale vegetation changes, providing detailed information not observable via satellite data and this will be demonstrated by further processing and studies. Although the statistical analysis is ongoing, early insights confirm the potential of UAVs for supporting sustainable grazing through rapid and accurate biomass monitoring. Future processing of data will validate the regression models and explore their applicability across seasons and pasture types and will expand the analysis to dry biomass terms



## **Ecophysiological response of maize (*Zea mays* L.) to water stress: remote sensing and upscaling techniques for a more efficient management of water resources in agriculture**

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**Introduction:** Climate change is reshaping hydrological patterns, with profound implications for agriculture. Maize (*Zea mays* L.), one of the world's most important crops, is highly responsive to such environmental fluctuations. Understanding its physiological and phenological responses is key to enhancing climate resilience. In this context, remote sensing technologies — satellite and drone-based imagery — combined with physiological stress indicators, offer powerful, non-invasive tools to monitor crop responses under dynamic environmental conditions. Moreover, maize kernel safety is increasingly threatened by mycotoxins produced by pathogens such as *Aspergillus flavus* and *Fusarium verticillioides*, whose proliferation and toxin biosynthesis are strongly modulated by temperature and precipitation. Understanding these complex interactions is critical to safeguard food security in a changing climate.

**Methods:** In 2022 and 2023 we monitored 40 maize fields along a pedoclimatic gradient in the Friuli Venezia Giulia Region, including both irrigated and rainfed sites, at four phenological stages. For each field we collected climatic data, morphophysiological and yield traits, kernel mycotoxin concentrations, and conducted multispectral drone flights. The goal was to develop early-prediction models for mycotoxin contamination based on environmental and physiological variables. In parallel, we performed *in vitro* assays to test the effect of selected plant-based bioextracts – obtained from waste materials – on the growth of the maize fungal pathogens *A. flavus* and *F. verticillioides*.

**Discussion and Conclusion:** Our study identified predictive models linking mycotoxin accumulation in maize to climatic conditions and key morphophysiological traits of the plant, highlighting the potential for early risk assessment under variable environmental scenarios. In addition, preliminary analysis of multispectral drone imagery allowed us to extract vegetation indices, which we aim to correlate with plant traits and mycotoxin levels for each phenological stage investigated. This integration could lead to the development of a non-invasive, remote sensing-based tool for real-time monitoring of crop health and contamination risk.

In parallel, *in vitro* assays showed that the *Ulva lactuca* extract significantly inhibited the growth and spore germination of *A. flavus*, while *F. verticillioides* seemed to be unaffected by the extracts tested. Given the promising antifungal activity of the *U. lactuca* extract, it was tested in a small-scale field trial. Although data analysis is ongoing, this approach opens the door to sustainable biocontrol strategies based on waste-derived natural compounds.

Together, these findings contribute to the development of integrated methods to monitor and mitigate mycotoxin risk in maize under changing climatic conditions.

## **Implementing the accuracy of canopy transpiration assessment to improve the estimation of tree crop water use in the era of climate change**

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**Introduction.** In the current era of climate change, where rainfall has drastically reduced and temperatures have soared, it is essential to more accurately estimate crop water use to avoid water waste. Estimating canopy transpiration using appropriately calibrated sap flow sensors is a non-destructive method for continuously assessing plant water use in real time, which must be integrated with soil evaporation. The present study aims to compare different radial sap flow distribution models to improve the estimation of canopy transpiration, which accounts for the majority of tree crop water use.

**Methods.** The study was conducted in a Concha y Toro vineyard in Penciahue (Maule Region, central Chile), a semi-arid area with prolonged drought, where a previous multi-year trial estimated soil evaporation at 30-33% of evapotranspiration (ETa). ETa was obtained from Eddy-Covariance and Surface-Renewal techniques (reference methods) and LI-710 sensors. HRM sap-flow sensors were installed on six *vines*. Transpiration (T) was assessed using four radial flow models from outside to inside the stem cross-section: (a) linear-constant; (b) linear-decreasing; (c) decreasing-quadratic, simulating a parabolic profile; (d) asymmetric-unimodal distribution, with a peak near the cambial region. T was expected to be 70-75% of ETc.

**Discussion and Conclusion.** All models resulted in high values of correlation coefficients (r) between T and ETa (from 0.9631 to 0.9975). However, T was almost always lower than 70-75% of ETa. In detail, applying the linear-constant model (a), which implies a constant flow along the radial section, T was found to be 13.0%, 12.5%, and 19.7% of the ETa assessed with EC, SR, and LI710, respectively; with the linear-decreasing model (b) T was found to be 27.4%, 26.3%, and 41.5% of the ETa; with the quadratic-decreasing model (c) T was only 10.0%, 9.6%, and 15.2% of the ETa; applying the asymmetric-unimodal flow distribution model (d), which is a new one, T was found equal to 69.6%, 67.0%, and 105.6% of the ETa estimated with the three methods. Based on these results, this new model of radial variation of xylem sap-flow within the sapwood seems capable of significantly improving the accuracy of the "sap-flow" estimation method for assessing grapevine transpiration, from 1,5 times that of the linear-decreasing model to 6 times that of the decreasing-quadratic model. Further experiments are planned to verify the reliability of these results, aiming to increase the precision of the estimation of vineyard water use.

## **Influence of thermal processing on the physicochemical and technological properties of mycelium-based meat substitute**

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**Introduction:** Mycoproteins represent a promising alternative protein source due to their high nutritional value, including complete essential amino acids, low fat content (mainly mono- and polyunsaturated fats), and high dietary fiber levels. These are produced through fermentation, which is driven by filamentous fungi belonging to *Ascomycota* and *Zygomycota* phyla. The fermentation process typically occurs via two methods: solid-state fermentation (SSF) or submerged fermentation (SmF), which differ mainly in the moisture content within the medium. A limitation of mycoproteins is their relatively high nucleic acid content (6–11%), particularly purines, which have been associated with elevated uric acid levels and an increased risk of gout or kidney stones. While thermal treatment is a well-established method to reduce RNA content, the lack of standardized and validated protocols remain a significant challenge. In SmF, purines can be more efficiently removed through the liquid phase, in contrast to SSF, wherein no separation occurs. This PhD project is centered on evaluating the effects of different thermal treatments on the physicochemical and sensory properties, purine content, and microbiological risk profile of a mycelium-based meat substitute (MMS) produced via SSF.

**Methods:** The MMS, supplied by Millow AB (Sweden), was produced using SSF with *R. oligosporus* and oat okara as substrate. The product, packaged in 400 g batches, underwent multiple steam pasteurization treatments, which differed for temperatures (from 60 to 90 °C) and times (3–21 min). Microbiological analyses were conducted to detect the presence of pathogenic and non-pathogenic bacteria, in addition to the total bacterial count. Texture profile analysis (TPA) was performed on 10 g samples for each condition. Treated products were also evaluated for their moisture content, water activity, pH and color ( $L^*$ ,  $a^*$ ,  $b^*$ ). Purine content was quantified by high-performance liquid chromatography (HPLC).

**Discussion and Conclusion:** The TPA revealed distinct responses to heat treatments. At 60 °C, hardness progressively decreased, likely due to incomplete protein denaturation and limited cross-linking with  $\beta$ -glucans, resulting in a softer structure. In contrast, at 75–90 °C, an initial increase in hardness was observed, followed by a decrease upon extended heating, suggesting thermal breakdown. Adhesiveness and chewiness were found to be consistent with the trend of hardness. Color changes occurred at 60 °C and at 75 °C (7 min), mainly due to decreased  $L^*$  values, while at 90 °C no substantial color variations were observed. Moisture content, water activity, and pH exhibited slightly variations across the treatments.

The results of the microbiological analysis confirmed the safety of the products under all conditions, including the untreated sample, with no pathogens detected.

The purine analysis focused on adenine and guanine. No significant differences were observed between treated and untreated samples, indicating that heat treatment alone does not effectively reduce the purine content.

In conclusion, this study highlights how different thermal treatment conditions significantly affect the physicochemical and sensory characteristics of the product. However, since the purine content remained unchanged, it would be advisable to explore alternative processing technologies that could more effectively reduce their levels.

## White onion PGI valorization through fermentation processes

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Introduction: Fermentation represents a traditional biotechnological approach that is gaining renewed interest for improving vegetable shelf life, safety, and nutritional properties. Lactic acid bacteria (LAB), especially species belonging to *Lactobacillus*, *Leuconostoc*, and *Lactococcus*, play a key role by promoting acidification, inhibiting pathogens, and improving sensory attributes. Onion (*Allium cepa* L.), among the most widely consumed vegetables globally, is recently emerging as a functional food due to its health-promoting properties. In particular, the "Cipolla Bianca di Margherita PGI" (CBM-PGI), a sweet onion from Apulia (Italy), is a local variety with distinctive agronomic and sensory features.

This study aimed to develop a standardized fermentation process for CBM-PGI using selected autochthonous LAB strains. These were first characterized for relevant technological traits and then tested in fermentation trials alongside commercial starters. A factorial design was used to optimize salt concentration, brine/onion ratio, and temperature, while monitoring acidification, microbial growth, and volatile compound profiles (VOCs).

Methods: Twenty-five autochthonous LAB strains were isolated from fresh CBM-PGI onions and screened for salt, temperature, and pH tolerance, as well as acidification ability. The strain with the best starter performance was used in fermentation trials conducted to optimize the process by using a 2<sup>k</sup> factorial design and varying brine/onion ratio (1:3–1:6), salt (0–5%), and temperature (25–35 °C). Fermentation time to reach the critical pH threshold of 4.4, ensuring hygienic and microbiological safety, was calculated using the modified Weibull equation. VOCs were also analyzed to generate odour profiles with an electronic nose.

Discussion and Conclusion: Optimization trials were performed comparing a commercial *Lactiplantibacillus plantarum* strain (DSM 1055), an autochthonous isolate L1, and spontaneous fermentation. The autochthonous strain L1, taxonomically identified as *Lactococcus* spp., exhibited excellent acidification properties and stress tolerance, showing performance levels equivalent to commercial starter cultures. All inoculated samples reached the safety pH threshold (<4.4) within 24 hours, with L1 showing slightly faster kinetics. Fermentation time (FT) was significantly influenced by temperature and salt concentration: higher temperatures (35 °C) and lower salt levels (0–2.5%) favoured faster acidification. Optimal fermentation conditions (1:3 onion/brine ratio, 5% salt, 35 °C) resulted in an FT of 0.17 days for strain L1. VOCs analysis showed that spontaneous fermentation produced higher levels of aroma compounds, but inoculated samples ensured better safety and reproducibility. In conclusion, the use of the autochthonous strain L1 offers a promising approach to develop high-value fermented products starting from CBM-PGI onions, combining tradition, safety, and innovation.

## **Application of Artichoke powder for microbial biomass production: a case-study**

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Introduction: Globe artichoke (*Cynara cardunculus* var. *scolymus*) is a crop originates from the Mediterranean region. Italy is one of the world's leading producers of this crop. Although it is known for its nutritional, functional, and health properties, a major issue in industrial artichoke processing is the large amount of waste produced, consisting mainly of external bracts and stems, unsuitable for human consumption. These by-products are a valuable source of biomass and bioactive compounds that can be used to produce innovative bioproducts for agricultural, food industry and environment applications, thus contributing to reducing waste and promoting a sustainable circular economy model.

The aim of this research was therefore to develop an innovative model for the integrated valorisation of artichoke powder, produced from by-products and waste, by using them as substrates for microbial biomass production.

Methods: Several methods of incorporating artichoke powder (infusion and maceration) were tested, then optimized using a Design of Experiments (DoE) in which different operational and compositional variables, such as pH (5 – 6.5), temperature (25°- 35°C), agitation (0 – 150 rpm), dehydrated artichoke powder concentration (2 – 4 %) and basic medium composition, were used for optimization on process variables. This activity was conducted on two target strains belonging to the DAFNE Predictive Microbiology Laboratory collection, *Pseudomonas migulae* (23P), a promising Plant Growth-Promoting Bacterium, and *Lactiplantibacillus plantarum* (C19), a probiotic strain.

Discussion and Conclusion: Results indicated that the artichoke powder supported microbial growth, in a strain-specific and condition-dependent manner, with notable interaction with pH and temperatures. Namely, artichoke powder influenced the lag phase, while not the maximum amount of biomass, which was on the other hand affected by other variables, for example agitation.

Independently by concentration and methods of incorporation, artichoke powder could support microbial growth with biomass level similar to conventional media, thus suggesting its potentiality as a low-cost ingredient to produce microbial biomass.



## **Intestinal inflammation and ketogenic diet: the combined role of $\beta$ -hydroxybutyrate and MCT oil on Caco-2 cells**

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**Introduction:** Chronic intestinal inflammation is a major risk factor for colorectal cancer, particularly in inflammatory bowel diseases (IBD) like Ulcerative Colitis and Crohn's disease. Persistent inflammation creates a pro-tumorigenic environment through activation of molecular pathways like NF- $\kappa$ B, STAT3, and IL-6/IL-10, promoting cell proliferation, immune evasion, and metastasis. Ketogenic diets (KD), characterized by low carbohydrate, high fat, and adequate protein intake, are gaining interest for their potential anti-inflammatory effects on the gut. KD stimulates hepatic ketogenesis, producing  $\beta$ -hydroxybutyrate (BHB) and acetoacetate (AcAc), which support mucosal integrity, fuel enterocytes, and reduce inflammation. Medium-chain triglycerides (MCTs), such as C6:0, C8:0, C10:0, and C12:0, further enhance ketogenesis via rapid portal absorption and liver oxidation. These mechanisms suggest a protective role for BHB and MCTs on intestinal health. This study evaluates their synergistic anti-inflammatory effects using CaCo-2 cells, a human colorectal adenocarcinoma cell line that serves as a reliable in vitro model of the intestinal epithelial barrier.

**Methods:** CaCo-2 cells were treated with 5 mM BHB and 0.5 mM MCT, alone or combined, with or without 1  $\mu$ g/ml LPS for 24 and 48 h. Cell viability, wound healing, and ELISA assays were performed to assess proliferation, migration, and cytokine production. Western blot analysis evaluated the expression of adhesion proteins E-cadherin, Cx43 and ZO-1 to determine barrier integrity. The study aimed to assess the anti-inflammatory and barrier-protective effects of BHB/MCT under inflammatory conditions, mimicking intestinal damage.

**Discussion and Conclusion:** Both BHB and MCT independently improved cell viability, migration, and the production of anti-inflammatory cytokines in CaCo-2 cells. Notably, their combined administration resulted in a significantly greater effect, suggesting a synergistic interaction in promoting intestinal epithelial function and inflammation resolution. Western blot analysis is currently underway to evaluate whether treatment with BHB and MCT can mitigate the LPS-induced alteration in the expression of junctional proteins, including key adhesion components such as E-cadherin and ZO-1, and the gap junction protein Cx43. This analysis aims to determine whether the combination treatment contributes to the preservation of epithelial barrier integrity under inflammatory conditions. If confirmed, these findings would support the hypothesis that BHB and MCT act synergistically to enhance epithelial restoration and suppress inflammation. The study, therefore, has the potential to highlight the therapeutic relevance of ketogenic strategies, particularly exogenous ketone supplementation, as supportive interventions in chronic intestinal inflammation. However, additional in vivo and clinical investigations will be necessary to validate the safety and effectiveness of this approach in patients with IBD or related inflammatory disorders. Ultimately, this line of research could inform the development of novel dietary-based or adjunctive therapies aimed at protecting intestinal epithelial health.

## **Can *Pseudomonas* spp. isolated from polluted soils be used as possible bioremediation agents for heavy metal removal?**

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**Introduction:** The illegal dumping of urban waste severely compromises soil health and biodiversity through the accumulation of persistent heavy metals such as Pb, Cd, Cr, Cu and Zn. These elements alter soil microbial communities and reduce agricultural sustainability. Among bioremediation approaches, microbial strategies offer an eco-compatible solution by exploiting native metal-resistant bacteria. In this context, the genus *Pseudomonas* is widely recognized for its resilience and capacity to mitigate environmental pollutants through mechanisms such as biosorption and bioaccumulation. This study aims to isolate and characterize *Pseudomonas* spp. from soils affected by uncontrolled dumping, evaluating their phenotypic traits under stress and their potential for heavy metal removal.

**Methods:** One hundred *Pseudomonas* isolates were recovered from contaminated soils in Southern Italy. Strains were screened morphologically and biochemically and their adaptability to temperature, pH, and osmotic stress was evaluated using growth index assays. Resistance and removal capacity for Zn, Pb, Cr, Cu, and As were tested in liquid cultures at increasing concentrations (100–300 mg/L). Selected isolates were genotyped via 16S rRNA sequencing and phylogenetic analysis. Removal efficiencies were quantified by ICP-OES after incubation in metal-enriched broths.

**Discussion and Conclusion:** The functional screening of *Pseudomonas* isolates revealed notable inter-strain variability in their ability to withstand environmental stressors and to grow in the presence of toxic metals. PCA analysis highlighted a subset of strains highly tolerant to extreme pH and temperature, suggesting robust physiological plasticity. Similarly, osmotic stress assays identified some isolates as highly resilient, maintaining stable growth even at 20% PEG. Resistance to heavy metals was strain-specific and concentration-dependent: several isolates retained growth above 75% GI at 200–300 mg/L, particularly in arsenic and copper modified broths. Among the most promising candidates, two isolates demonstrated broad-spectrum tolerance and efficient metal removal. ICP-OES quantification confirmed their capacity to eliminate over 75% of multiple metals, including Pb, Cu, and As, with some strains exceeding 90% removal. These results indicate that polluted environments exert selective pressure, fostering microbial communities with enhanced bioremediation traits. The phenotypic robustness, together with high removal rates, suggests a realistic potential for testing these strains in in-situ bioremediation strategies. Such applications not only support the recovery of soil health but also contribute to environmental sustainability goals by mitigating contaminant dispersal and restoring ecosystem services in degraded agroecosystems. Future studies should focus on genomic profiling and pilot-scale validation of these isolates *in vivo* scenarios.

## **Microbial diversity of salt-tolerant plant growth-promoting bacteria associate with *cakile maritima***

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**Introduction:** Microorganisms inhabiting saline environments possess unique genetic and physiological traits that allow them to thrive under extreme conditions. Halophytic plants, which naturally grow in saline areas, host halotolerant Plant Growth Promoting Bacteria (PGPB) in their rhizosphere and endosphere. These beneficial bacteria can enhance plant growth, increase yields, and help plants resist both pathogens and environmental stresses, without causing pathogenicity. For their properties PGPBs are valuable for sustainable agriculture, particularly given the increasing threat of global soil salinization to crop productivity. The use of these microorganisms could offer an eco-friendly solution to improve salt tolerance in conventional crops.

This study focuses on isolating and characterizing potential PGPB from *Cakile maritima* (sea rocket), a salt-tolerant plant native to coastal regions ranging from the Arctic to the Mediterranean. Samples were taken in Margherita di Savoia (north-eastern Apulia, Italy), aiming to identify bacterial strains with promising PGPB traits for future application in saline agriculture.

**Methods:** Microbiological sampling of *C. maritima* was performed at three phenological stages (seedling, vegetative growth, flowering) of the plant life cycle. A total of 180 (150 rhizobacteria and 30 endophytes) bacteria were isolated and morphologically and biochemically characterized through Gram staining, catalase, oxidase, urease test, microscopic observation, spore production, and motility. To assess the presence of PGPB traits, the isolates were tested for their capacity to solubilize phosphate and silicon, produce indole acetic acid and siderophores, generate ammonia, resist drought, and high salinity. The soil samples were subjected to metagenomic sequencing. The most representative isolates were identified by 16S rRNA gene.

**Discussion and Conclusion:** The isolation of 180 bacterial strains from *C. maritima* demonstrates the rich microbial diversity associated with this halophytic plant. The predominance of Gram-positive spore-forming bacteria and Gram-negative Pseudomonadaceae aligns with previous findings in saline environments, where these bacterial groups are known to thrive due to their robust stress tolerance mechanisms. The finding that 36% of isolates demonstrated phosphate solubilization capability is particularly significant, as phosphorus availability is often limited in saline soils due to precipitation with calcium and magnesium ions.

These results demonstrate that halophytic plants like *C. maritima* may serve as valuable reservoirs of halotolerant PGPB with multiple beneficial traits. Three promising strains (42C, 134C, and 178C) with complementary capabilities were selected and identified as belonging to the genera *Pantoea* and *Bacillus*, two genera well known for their PGPB activities and environmental adaptability.

In conclusion, the combination of salt tolerance with multiple PGPB traits in the selected isolates suggests their potential application in salt-affected agricultural lands. Future research should focus on field validation of the selected strains under various crop systems and salinity levels, and on interaction studies to evaluate compatibility with existing soil microbiomes and potential synergistic effects.

## **Sustainable Weed Management: Evaluating the Herbicidal Potential of Three Essential Oils on *Amaranthus retroflexus* L.**

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**Introduction:** *Amaranthus retroflexus* L., commonly known as redroot pigweed, is one of the most troublesome weeds in various agroecosystems worldwide due to its competitive growth and high resilience. Its aggressiveness is due to prolific seed production and high adaptability to various climates, leading to dense infestations across temperate and tropical regions. The increasing resistance of *A. retroflexus* to herbicides reduces the effectiveness of conventional chemical control, highlighting the need for integrated and sustainable weed management strategies. Natural compounds are gaining attention for their biodegradability and low environmental impact. Essential oils rich in isoprenoids from medicinal and aromatic plants (MAPs) inhibit weeds' seed germination and hypocotyl growth. Specifically, thyme, rosemary, and sage oils show promising herbicidal activity. This study aimed to evaluate the effects of these Mediterranean essential oils, applied at three concentrations, in an in vivo experiment conducted under controlled greenhouse conditions.

**Methods:** A post-emergence trial was conducted under greenhouse conditions using *A. retroflexus* at the three-leaf stage. Essential oils from thyme (THY), rosemary (RO), and sage (SA) were applied at three different concentrations (8, 12, 18 µL for THY, 12, 24, 48 µL for RO, 16, 32, 64 µL for SA) mixed in FITOIL (0.02 µL) and water (5 mL) and finally applied for each pot. Damage score and efficacy were evaluated at 1, 3, 7, 10, 15, and 30 days post-treatment through visual analysis. At 30 days, fresh and dry weights (shoot, root, total) were collected. The lengths of the aerial part and root system were measured using Digimizer 6.6.2 software. ANOVA analysis was performed with STATA SE 19.5.

**Results and Conclusion:** Essential oil treatments had a statistically significant effect on all measured growth features ( $p < 0.001$ ), including total fresh and dry biomass, length variables, efficacy, and damage score. Among the oils tested, rosemary and sage exhibited strong herbicidal effects, significantly reducing total length and biomass (fresh and dry), while thyme showed the highest damage score for all doses applied during the experiment. Dose-dependent effects were observed for both rosemary and sage: the highest concentrations (RO-48 and SA-32/64) caused up to 100% inhibition of growth and biomass accumulation. Statistical analysis confirmed significant group separations, especially between rosemary and thyme in terms of damage scores and weed growth inhibition. Specifically, *A. retroflexus* treated with the RO-48 dose exhibited minimal growth (2.36 cm total length; 0.01 g dry biomass), compared to sage, which showed similar effects at lower doses. These findings highlight the potential of essential oils as an effective and sustainable alternative for weed management. Their ability to significantly suppress *A. retroflexus* growth supports their application in integrated weed management. By replacing or reducing synthetic herbicides, these natural compounds could contribute to more environmentally friendly farming systems, especially in Mediterranean agroecosystems where pressure from herbicide-resistant weeds is increasing. Further research is needed to validate these effects under field conditions and explore formulation strategies for practical use.

## **Exploitation of rootstock scion combination and microbial consortia on the yield and rhizosphere Microbiome in Tomato under greenhouse**

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**Introduction:** Grafting in horticultural crops is an agricultural practice used to increase productivity by mitigating biotic and abiotic stresses at the soil level. In tomato, the development of resistant varieties maintaining the high qualitative traits in fruit is still until today a complicated process and highly time consuming due to the genetic complexity of the tomato plant thus, grafting emerged as an agronomic strategy, particularly effective in safeguarding the productivity and the quality within the Solanaceae family. In parallel, the application of microbial consortia, (PGPR), has become a sustainable agronomic strategy for enhancing plant resilience and soil biological function. In this context, the aims of this study is to evaluate how grafting onto interspecific rootstocks and microbial amendments, interact to influence rhizosphere microbiome composition and diversity in tomato, and their combined effects on plant performance in a monoculture greenhouse during the long growing season under mediterranean conditions.

**Methods:** A mix of commercial microorganism consortia was evaluated on two commercial hybrid rootstocks for tomato with two scions a mid-plum hybrid "101MVS" and a Sicilian landrace "Pizzutello" during the winter season under a traditional greenhouse in a Split plot design. The rhizosphere microbiome of each grafting combination, and the two controls: the auto-grafted and un-grafted scions, was collected 120 days after transplanting. Microbiome DNA extracted was then sequenced by amplifying two specific regions ITS1-1F for Fungus and 16SV34 for bacteria. Once microbiome was profiled for each combination, species were then correlated to the yield roots components registered along the season

**Discussion and Conclusion:** The metagenome sequencing of the microbiome in the rhizosphere highlighted the changes in the fungal colonies depending on the grafting combinations. Bacterial abundance tended to change more in relation to the microorganisms' treatments rather than grafting conditions. The rootstock showed to be the main manipulator of bacterial and fungal abundance although the effect of the scion was also noticed for some microbial species. Interestingly, none of the microbial component of the products applied, was noted to increase in the treated plots compared to the non-treated ones. On the other side, the addition of such of the such consortia had a remarkable effect on other species from different genera of fungi. It ameliorates the abundance of some taxa, playing a role of stimulant for beneficial microbial species

This study highlighted the effect of two agricultural practices on the rhizosphere microbiome and their potential microbial communities modification. Future work should aim to resolve the functional roles of unclassified species present and to clarify the role of the environmental conditions on the exogenous rootstocks, particularly under biotic stress conditions, to inform the rational design of microbial consortia for enhanced plant performance and resilience.

## Metabolomic analysis of a high performative *Streptomyces* strain against soilborne fungal pathogens and weed of wheat and tomato crops

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**Introduction:** In the last decade, the European Community strictly limited the use of chemical pesticides due to their negative effects for human health and environment, promoting at the same time the research of natural means, such as agricultural wastes (AWs) and biocontrol agents (BCAs), for the management of the biotic adversities. Among the BCAs, *Streptomyces* spp. are considered the largest natural biofactory of metabolites, of which some are known to be fungicide, herbicide or larvicide. Based on previously experiments performed in laboratory and greenhouse, a strain of *Streptomyces* spp. *Strep\_22* resulted the best strain among all those tested. The same strain was used for study the metabolomics profile that resulted to be more interesting. The aim of the study was to perform an extraction of the strain metabolites and test them singularly to find all the active compounds.

**Methods:** The strain *Strep\_22* was incubated in GYM (Glucose 4 g/L; Yeast extract 4 g/L; Malt extract 10 g/L) for 21 days at 28°C at 100 rpm in the dark. Then, the mycelium was filtered and the cultural broth used to perform the extraction with ethyl acetate. The organic phase of the strain *Strep\_22* was divided in nine phases through a direct column in chloroform:isopropanol (9:1), and then purified by separation on column, TLC or HPLC, allowing to collect about thirty compounds. For each compound, proton nuclear magnetic resonance and mass spectrometry were performed to detect its structure.

**Discussion and Conclusion:** The proton nuclear magnetic resonance and mass spectrometry confirmed that three of the main metabolites produced by the strain *Strep\_22* are Heptyl  $\alpha$ -D glucopyranoside, Pimprinine and Pimprinethine. Of the latter, pimprinine and pimprinethine are already known as produced by *Streptomyces* spp. and have bioactivity against fungal pathogens, weeds and insects. Indeed, these two metabolites showed inhibitory activities against fungal and bacterial pathogens of plants and human. Pimprinine and pimprinethine show inhibitory effects also of tobacco mosaic virus (TMV), without displaying phytotoxic activity on the tested tobaccos even at high concentration. Moreover, their derivatives show herbicidal, insecticidal and nematocidal activities. In conclusion, the *Streptomyces* spp. are very promising tools in the control and management of the fungal pathogens, weeds and insects. Most of the *Streptomyces* metabolites are still unknown, as well as a few information is available also for those having the structure. To confirm the results obtained until now, the bioactivity of *Streptomyces* strains should be tested in open field conditions. Moreover, another important topic of the project consists of evaluating the efficacy of metabolites as bioactive compounds against fungal pathogens, and continuing to characterize the remaining metabolites produced by *Strep\_22* and from other strains.

## **Athletes' preferences and willingness to pay for an innovative high-protein bread: evidence from an experimental study**

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**Introduction:** The rise of diet-related diseases has pushed consumers toward healthier choices—from nutritional attention and exercise to greater control over food intake—motivating industries to design strategies aligned with this growing demand for wellness. Within this trend, the demand for functional foods has expanded significantly, highlighting the need to understand their preferences and willingness to pay for innovative products like high-protein bread. This research investigates how sensory attributes, product information, and food values influence purchasing decisions of athletes', a key consumer segment for protein-enriched foods. This research follows two complementary lines of investigation, the first line examines how order of information and sensory evaluation impact willingness to pay, challenging assumptions that athletes neglect taste for functionality. The second line studies segments of athletes based on their "food values" to identify heterogeneity in preferences and willingness to pay drivers. Both underscore athletes' dual focus on product information and sensory appeal.

**Methods:** A sample of 189 athletes participated to a non-hypothetical experimental auction. The first line of research was based on two-treatment design: Treatment 1 provided product information before sensory evaluation; Treatment 2 reversed this order. Willingness to pay was elicited through a second-price auction alongside 9-point hedonic scales for sensory attributes (colour, odour, taste, texture, liking degree). The second line employed a Best-Worst Scaling to rank nine food values (price, convenience, naturalness, appearance, safety, taste, healthiness, sport performance, claim), followed by a cluster analysis to segment athletes. The mean willingness to pay for each cluster were compared to identify differences between them.

**Discussion and Conclusion:** Both studies reveal that athletes balance sensory attributes and product information, but information dominates willingness to pay. In the first line of research, information provision increased willingness to pay significantly more than sensory evaluation, particularly when presented first (+0.19€ vs. +0.07€). Among sensory drivers, "taste" and "odour" emerged as most influential factors of willingness to pay. The second line of research identified five clusters: "Budget-Focused Athletes", the cluster with the highest willingness to pay (1.93€), valued most price and sport performance. However surprisingly, although the target group selected for the study was athletes, "sport performance" ranked low overall, contradicting assumptions that athletes associate the sporting effectiveness of a food as an important value in their food choice. Instead, safety, taste and healthiness were primary values. Conclusions that can be drawn from the results of these two lines of research emphasize that product information is important to athletes, particularly information relating to safety and health, even though taste remains important for acceptance. Company product development should therefore incorporate clear product information without compromising taste. Future research should extend these insights to other functional foods and broader contexts.

## **Understanding Willingness to Adopt an Open-Loop Innovation: Evidence from Italian Artichoke Farms**

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**Introduction:** Economic activities, particularly food production, significantly impact the environment due to the prevailing linear economic model. In contrast, the Circular Economy (CE) promotes sustainability through reuse, recycling, and waste valorization. In the agri-food sector, utilizing production residues is crucial. Artichoke cultivation, in Italy -the world's leading producer- generates substantial waste, with 80-85% of the plant discarded. These by-products, rich in bioactive compounds like inulin and polyphenols, offer valuable opportunities for open-loop innovations, including the development of a biofertilizer. This product, derived through a multi-step process, can improve soil health and waste management. However, transitioning to CE models remains challenging. This study investigates factors influencing farmers' willingness to adopt such innovation. Guided by the Theory of Planned Behavior (TPB), the study examines attitude, subjective norm, and perceived behavioral control, alongside environmental commitment, green economic incentives, sustainable supply chain design, and relationship management. Understanding these factors can support the transition to CE.

**Methods:** A pilot study was carried out in order to conduct a national survey with a convenience sample of approximately 200 Italian artichoke farms. Data were collected using a questionnaire with two sections: firm characteristics and variables related to adoption of a biofertilizer innovation. Constructs like Attitude (A), Subjective Norm (SN), Perceived Behavioral Control (PBC), Environmental Commitment (EC), Green Economic Incentives (GEI), Sustainable Supply Chain Design (SSCD), and Supply Chain Relationship Management (SCRM) were measured using validated scales. Willingness to adopt (WTA) biofertilizer was assessed as a binary variable. A logistic regression will be performed to identify key determinants of WTA.

**Discussion and Conclusion:** The growing interest in CE and the agri-food sector's need for sustainable practices, such as waste management, make it essential to understand the factors influencing the adoption of innovative solutions. This study explored the factors influencing farms' willingness to adopt an open-loop innovation, specifically an inulin-based biofertilizer, in the context of artichoke production. Preliminary results revealed a strong positive attitude towards CE, with farms recognizing its efficiency in terms of energy and resource use. Subjective norms, such as technological advancements and institutional expectations, as well as internal factors like human and infrastructural capabilities, play a key role in supporting the transition to CE. While farms showed environmental commitment and engagement in sustainable supply chain practices, green economic incentives had a more limited impact. All surveyed farms expressed a positive WTA towards the proposed biofertilizer, and future research will proceed with the implementation of the binary regression model. In terms of implications, the study highlights the need for farms to promote supply chain collaboration to improve circular practices and to eliminate non-value-added activities. For policymakers, it underscores the importance of implementing targeted economic support measures and communication strategies to foster the transition towards CE.



## **Understanding consumer preferences for herbal teas: A conjoint analysis approach**

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**Introduction:** The global herbal tea market is rapidly expanding, driven by growing consumer interest in health, sustainability, and product personalization. However, knowledge regarding consumer preferences for specific product attributes remains limited. This study investigates the factors influencing purchasing decisions in the functional herbal tea segment, focusing on five key attributes: perceived function, certification, price, mode of use, and packaging colour. By also examining the role of socio-demographic variables and attitudes toward health and sustainability, the research aims to provide actionable insights for product innovation and strategic market positioning.

**Methods:** An online survey was conducted in Italy and Denmark (N=1,000) between March and July 2025. The questionnaire comprised: (i) questions on consumption habits, (ii) a conjoint task involving 12 product profiles designed using an orthogonal design, and (iii) validated scales assessing health consciousness, environmental concern, trust in certification systems, and preferences for natural products. The data were analysed to estimate the relative importance of the attributes and their interactions with individual-level variables.

**Discussion and Conclusion:** The results highlight a strong influence of perceived function and certifications on consumer preferences, followed by price, mode of use, and packaging colour. Italian consumers exhibit greater sensitivity to organic certifications, whereas Danish consumers place higher value on convenience and both social and environmental sustainability. Preferences are significantly shaped by psychographic factors: individuals with high environmental consciousness and a strong health orientation assign greater importance to certifications and functional benefits. Interaction analysis suggests that packaging colour can enhance or diminish the perceived functionality and sustainability of a product, particularly among consumers who are visually oriented. These findings offer strategic insights for industry stakeholders: developing functionally oriented products, clearly communicating health benefits, and investing in recognizable design and certifications can enhance product appeal. Moreover, understanding distinct consumer segments may increase the effectiveness of promotional campaigns. From a scientific perspective, the study contributes to filling a gap in the herbal tea literature by proposing an integrated approach that combines consumer psychology with sustainable marketing.

## **Genotyping, Cultivar Screening, and Breeding of Common Buckwheat (*Fagopyrum esculentum* Moench) for Enhancing Cropping System in Northern Italy**

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**Introduction:** Common buckwheat (*Fagopyrum esculentum* Moench) is a pseudo-cereal gaining renewed interest for its high-quality protein, low input requirements, and short growth cycle. However, the genetic feature of the genotypes is poorly understood and the productivity of cultivated varieties are constrained by environmental stresses, mainly by late spring frosts and summer heat waves. To enhance the diversification of cropping systems in the Friuli-Venezia Giulia (FVG) region, where crop rotations are short and dominated by summer crops, identifying and development of resilient and high-performing buckwheat cultivars for spring and summer sowing are imperative. Therefore, we adopted a multi-faceted strategy combining the collection and multiplication of accessions, genotyping, variety screening and pollinator assisted crossing to overcome the limitation of manual pollination. This integrated approach aims to identify buckwheat cultivars with stable agronomic performance and improved resilience to abiotic stresses, ultimately enabling their integration as main crops into diversified cropping systems in the region.

**Methods:** 96 buckwheat accessions from Europe and Asia were genotyped using double-digest restriction-site associated DNA sequencing (ddRAD-seq). Multivariate analysis to assess genetic diversity and sources of major variation to be exploited for breeding were executed. Accessions with potential breeding value were propagated in isolation on farmers' plot in both mountainous and lowland areas to ensure a supply of quality seed. Cultivar screening under spring and summer sowing conditions in a randomized complete design with 10 commercial cultivars is ongoing. In parallel, we established tunnel structures to test inter varietal crossing approach that combines unsupervised bumble bee mediated pollination with hyperspectral imaging.

**Discussion and Conclusion:** A Preliminary analysis of genotypic data from nearly 100 accessions provided by the first contacted germplasm bank (IPK, Germany) revealed relatively scarce nucleotide diversity in the overall panel and evidence for rich but underrepresented sources of diversity from the less abundant accessions of Asian origin, which compel further characterization. This information is currently guiding the selection of accessions of major interest for seed multiplication at farmers' fields. Preliminary results from the 2024 field trials revealed promising variation among the tested commercial cultivars. Grain yield ranged from 0.52 to 1.1 t ha<sup>-1</sup>, with 'Esquire' and 'Billy' representing the lowest and highest performers, respectively. Notably, 'Billy' also exhibited the largest seed size, with a thousand seed weight (TSW) of 32 g, while 'Hajnalka' had the lowest TSW (24 g). Further sowing of commercial cultivars is expected in 2026 to see the performance across environments. The experimental setup for the pollinator-assisted crossing test has been completed and is going to provide preliminary results at the end of the summer-fall sowing season. Ultimately, integrating highly performing cultivars from screening with our ongoing work on diversity study, and advanced selection of common buckwheat will contribute sustainable food systems and enhance food security in northern Italy.

## **Building better genomes: long-read pipelines for assembly, annotation and functional profiling**

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**Introduction:** Third generation sequencing technologies have made the assembly process easier, thanks to the good quality of the obtained reads and bigger length. Moreover, long reads can be used to span over longer repetitive regions, enabling the reconstruction of telomere-to-telomere assemblies, and the study repetitive regions, such as centromeric regions and long structural variants (SVs). Information of the epigenetic status such as DNA methylation of cytosine can be also inferred during the same sequencing experiment, making possible the detection of both genetic and epigenetic profile at the same time. Here we present a comprehensive pipeline that uses long read sequences to build highly accurate, haplotype specific reference genomes of several grapevine varieties, and annotate them structurally and functionally. The reconstruction and comparison of centromeres has permitted the structural and functional characterization of these elusive regions. Gene prediction, together with long read transcriptomic data, was also used to study allele-specific expression (ASE).

**Methods:** Assemblies of 3 cultivated grapevine accessions, one wild accession of the same species and one interspecific hybrid were obtained using PacBio HiFi and ONT long-reads and the Hifiasm software. Genomic ONT reads were also aligned to the assembled references using minimap2 and filtered based on haplotype concordance to get haplotype specific alignments. Methylation levels of cytosines in all contexts were calculated using Modkit and structural variants were detected using a custom pipeline (sniffles2 and cuteSV). Gene prediction was performed using BRAKER while ONT reads of gene expression data were aligned to the diploid reference and assigned to the belonging haplotype.

**Discussion and Conclusion:** A large fraction of chromosomes was reconstructed from telomere to telomere. Haplotype specific methylation levels showed expected methylation patterns in expressed genes, as well as in regions populated by TEs. Moreover, the use of cDNA long reads has facilitate the attribution of the reads to the haplotype of origin, allowing the estimation of the expression rate between correspondent genes in the two haplotypes. The completeness of the assembly has allowed, in some cases, the comparison of up to 8 different haplotype-specific centromeric regions. These regions were populated by highly ordered and symmetrical structures composed of tandem repeats of different modules, intermixed with retrotransposons mainly belonging to the Athila and chromovirus families of Gypsy elements. Extreme variability was observed in terms of structure and repeat composition both among different chromosomes as well as among haplotypes of the same chromosome and haplotypes of different accessions, attesting a high rate of evolution of these regions. Analysis of methylation state and ChIP-seq data of centromeric-specific histone variants showed a hypermethylation pattern at a broad level, with a hypomethylation at a fine scale, overlapping with arrays of a 59-mer repeat and peaks of ChIP-seq data, giving hints about the functional repeat.

## **Innate Immune Response and Gene Expression Profiling of *Lactococcus petauri*- and *L. garvieae*-Challenged Selected Lines of Rainbow Trout (*Oncorhynchus mykiss*)**

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**Introduction:** Lactococcosis, primarily caused by *Lactococcus garvieae* and more recently by *Lactococcus petauri*, poses a significant threat to rainbow trout (*Oncorhynchus mykiss*) aquaculture. This study investigates the immunological differences between resistant and susceptible rainbow trout strains experimentally challenged with *L. garvieae* and *L. petauri*. Disease progression and clinical signs were monitored, and targeted sampling was performed at multiple timepoints post-infection. *Lactococcus petauri* has recently emerged as a pathogenic threat to aquaculture, with increasing reports of its association with Lactococcosis outbreaks in rainbow trout (*Oncorhynchus mykiss*). While its close phylogenetic relationship with *Lactococcus garvieae* has prompted concern, the immune response it elicits in teleost remains poorly understood. This study aimed to evaluate the non-specific immune markers and gene expression changes in rainbow trout following experimental infection with both of those bacteria separately, in order to gain insights into host-pathogen interactions and immune defense mechanisms.

**Methods:** Two selected lines, i.e. Resistant (R) and Susceptible (S) rainbow trout, were experimentally infected with a virulent strain of *L. petauri*/*L. garvieae* via intraperitoneal injection, while a control group received sterile PBS. Samples were collected at different post-infection time points. Non-specific immune parameters—including lysozyme activity, antiprotease activity, total protein and immunoglobulin, and serum bactericidal activity—were measured spectrophotometrically. In parallel, PCR was performed to assess the expression of key immune-related genes, including *cytokines*, *acute phase proteins*, *antimicrobial peptides*, *complement factors* and *caspases* of pre- and post-infection R and S samples.

**Discussion and Conclusion:** Results demonstrated significant modulation in innate immune markers and gene expression profiles suggesting triggering of a robust and temporally coordinated innate immune response in selectively bred rainbow trout. This study provides new evidence on the early immune dynamics of rainbow trout in response to *L. petauri* and *L. garvieae*, underlining the importance of innate defenses during the initial stages of infection. The insights gained here could contribute to improved disease management strategies, including the development of diagnostic markers or targeted immunostimulants for aquaculture systems.

## **Preliminary study on the effect of raw chitin from *Hermetia illucens* exuviae and lauric acid on gut morphology and functionality in rainbow trout (*Oncorhynchus mykiss*) fed semi-purified diets**

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**Introduction:** Natural functional feed additives can enhance both fish growth and health through the interplay between bioactive compounds and intestinal mucosa. Insects and their derivatives are considered a promising feed ingredient and additive to promote animal growth and health enhancement. This study aimed to describe the effects of two compounds derived from black soldier fly (*Hermetia illucens*, BSF): a polysaccharide (chitin from *exuviae*, C-CHI) and a fatty acid (lauric acid, LAU). A feeding trial was performed with juvenile rainbow trout (*Oncorhynchus mykiss*) to assess if and to what extent raw chitin from BSF used alone or in combination with increasing levels of lauric acid can affect liver and intestinal morphology by focusing on goblet cells mucus composition, intestinal cell proliferation and differentiation as bio-markers.

**Methods:** Four semi-purified diets were formulated for juvenile rainbow trout, including a control (CTRL), chitin-only (C-CHI), and two diets with chitin (1.5%) plus lauric acid at 0.1% (C-CHI-LAU1) or 0.2% (C-CHI-LAU2). Growth performance was assessed, and liver and intestinal samples were collected for histological and immunohistochemical analyses. Hematoxylin-eosin-stained sections were used to evaluate the general morphology, PAS and Alcian Blue staining to analyze the overall complex carbohydrates, and immunostaining for proliferating cell nuclear antigen (PCNA) and peptide transporter 1 to assess cell proliferation and peptides absorption respectively. The histochemical detection of alkaline phosphatase (ALP) was used to detect fully differentiated enterocytes.

**Discussion and Conclusion:** This study showed that including BSF *exuviae* and LAU into trout diets resulted in no differences in growth performances and biometric indices, similarly to what was observed in the same species with an inclusion of 1.6%, but unlike what was reported in sea bream fed diet including 0.02% LAU.

Raw chitin alone caused a reduction in the fold's length, and it affected goblet cell mucin production in rainbow trout fed diet with BSF meal. Furthermore, *exuviae* decreased PepT1 signal intensity, indicating reduced intestinal peptide absorption, similar to what was observed when trout were fed a plant-protein based diet. None of the diets affected PCNA and ALP expression, indicating that both raw chitin or LAU, did not induce proliferative state of intestinal tissue and cellular damage in the rainbow trout intestine. Cell proliferation is a hallmark of cellular damage, repair, and carcinogenesis. In this case, neither diet affected PCNA nor ALP expression, indicating that neither *exuviae* nor lauric acid induced intestinal cellular damage in rainbow trout. Overall, these findings indicate that 1.5% *exuviae* supplementation can negatively impact intestinal morphology, mucin production, and PepT1 expression, whereas a combination with 0.1% LAU in the diet seems to ameliorate the adverse effects of chitin.

## **Fatty acid metabolism in lambs supplemented with different level of almond skin by-product**

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**Introduction:** Feed production significantly contributes to greenhouse gas emissions from the livestock sector (Gerber et al., 2013; FAO, 2023). To reduce environmental impact and feed-food competition, using agro-industrial by-products as feed has been proposed. These by-products, such as hazelnut skin and tomato pomace, can also improve meat fatty acid (FA) profiles (Priolo et al., 2021). Almond skin (AS), a by-product of almond blanching, is rich in dietary fiber, fats, proteins, and phenolic compounds, with oleic and linoleic acids as its main FAs (Mandalari et al., 2010; Ingegneri et al., 2023). Phenolics in AS may modulate rumen biohydrogenation, increasing beneficial FA accumulation in meat. Despite its potential, research on AS in ruminant diets is limited. This study assessed the partial replacement of maize with AS (14% and 28%) in lamb diets, evaluating effects on growth performance and FA composition in the rumen, abomasum, and intramuscular fat, aiming to improve meat quality and sustainability.

**Methods:** Thirty male Pinzirità × Valle del Belice lambs were selected from a local dairy farm. Animals were housed individually, and randomly assigned to three equal groups, balanced for body weight, for a 56-day trial. The control group (CON) received a typical ovine diet, while the other two groups, ALM14 and ALM28, received the same diet of the CON with the inclusion of 14% and 28% of almond skin, respectively. After the trials in a commercial abattoir, all animals were sacrificed where rumen and abomasum digesta, and *longissimus thoracis et lumborum* (LTL) muscle were collected to perform fatty acids analysis.

**Discussion and Conclusion:** The diet did not affect final body weight, carcass weight, or dry matter intake. However, NDF, ADF, and ADL intake was higher in the A14 and A28 groups ( $P < 0.001$ ). The intake of  $\alpha$ - and  $\gamma$ -tocopherol, phenols, tannins, total fatty acids, and specific fatty acids such as oleic, linoleic, and  $\alpha$ -linolenic acids increased with almond skin inclusion. In the rumen, A14 and A28 diets increased C18 content, reduced saturated fatty acids (SFA), and altered the composition of OBCFA and DMA ( $P < 0.001$ ). A28 showed the highest levels of biohydrogenation intermediates and trans fatty acids. In the abomasum, MUFA, trans-MUFA, and C18 increased, while SFA decreased with A28. In the meat, the A28 diet increased rumenic acid, vaccenic acid, and C18:0 isomers, but reduced n-3 fatty acids, oleic acid, and ALA. In the polar and neutral fractions, A28 increased PUFA, n-6, and CLA while reducing the atherogenic index. The effect was dose-dependent, with progressive nutritional improvements as almond skin inclusion increased. In light of the findings, almond skins appear to be an excellent by-product in the lamb diet, increasing the quality of the meat without worsening the animals' performance.

## **Strains of *Aureobasidium pullulans* from extreme environments: new potential biocontrol agents?**

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**Introduction:** Climate change and environmental degradation, largely caused by human activities, so sustainable solutions are required. Microorganisms, such as yeasts, thrive in extreme environments due to unique genetic adaptations, making them valuable for eco-friendly applications. *Aureobasidium pullulans*, a poly-extremotolerant black yeast, is widespread and known for its strong stress resistance and potential as a biocontrol agent. However, the mechanisms behind its resilience and antagonistic activity are still not fully understood. This study focuses on 67 *A. pullulans* strains from extreme environments with the aim to i) evaluate their growth under various media, temperatures, and pH levels; ii) assess the production of cell wall degrading enzymes (CWDEs) and siderophores, and iii) test by *in vitro* and *in vivo* assays their biocontrol potential against *Monilinia fructicola*, causal agent of brown rot of stone fruits.

**Methods:** Between winter 2023 and spring 2024, 117 microorganisms were isolated from extreme environments. Sixty-seven *A. pullulans* strains were characterized by their growth under different temperatures (from 0°C to 45°C), pH (from 2 to 12), and media (Czapeck, NYDA) and tested for biocontrol activity against *M. fructicola* by *in vitro* and *in vivo* on 'Redhaven' peaches. The DNA was extracted by using CTAB (2.5%) and sequenced for phylogenetic analysis. Twelve strains were analysed for the production of CWDEs and siderophore (CAS). Data were analyzed using ANOVA, NMDS, and Random Forest to evaluate phenotypic traits and strains clustering, identifying fundamental key factors.

**Discussion and Conclusion:** Diverse extreme environments were considered such as deserts, alpine regions, coastal zones, and urban centres. Molecular characterization and phylogenetic analysis confirmed species identity and revealed strains grouping by geographical origin. The strains demonstrated remarkable adaptability, thriving at temperatures from 0 °C to 45 °C and pH levels from 2 to 12. Strains from the Algerian Desert and France showed strong growth capability in extreme conditions, while strains from Alto Adige and Fusine's Lake were more limited. *In vitro* and *in vivo* assays against *M. fructicola* revealed significant biocontrol potential in specific strains (e.g., RB\_7, ACB\_10) achieving up to 100% of inhibition *in vivo*. All tested strains produced cellulase and xylanase, but no siderophores. Multivariate analysis identified temperature, pH, and antagonistic activity as key traits driving strains differentiation, linked closely to their environmental origins. Overall, this work highlights the ecological plasticity and biocontrol efficacy of *A. pullulans* strains from extreme habitats, supporting their potential for use in sustainable postharvest disease management, particularly under variable or challenging environmental conditions.

## **Biostimulant effects of *Chlorella vulgaris* extract on zucchini (*Cucurbita pepo* L.) under tunnel cultivation**

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**Introduction:** Increasing demand for sustainable agricultural practices has led to the growing interest in natural biostimulant. Among these, microalgae offer an environmentally friendly and renewable source of bioactive compounds capable of enhancing plant growth, nutrient uptake, and stress tolerance. *Chlorella vulgaris* is rich in proteins, amino acids, phytohormones, and antioxidants that can positively influence plant metabolism. Although its potential has been widely demonstrated in laboratory conditions, field-scale validation under commercial horticultural settings is still limited. This study investigates the biostimulant effects of an extract obtained from *Chlorella vulgaris* CCAP 211/19 through ultrasound-assisted extraction, applied to zucchini (*Cucurbita pepo* L.) cultivated under tunnel. The extract was tested for its ability to stimulate vegetative growth, modulate enzymatic activity related to nitrogen metabolism, and ultimately enhance production. The overarching goal is to promote a natural and sustainable alternative to synthetic plant growth regulators, aiming to improve crop performance without relying on exogenous phytohormones such as IAA.

**Methods:** The trial was carried out in 12 tunnels of 215 m<sup>2</sup> each one located in Siracusa (Italy) during autumn 2024, using a randomized block design with three replicates. Four treatments were tested: (1) standard control following the farm's conventional practice, including the use of synthetic plant growth regulators (IAA); (2) root application of the microalgal extract (0,5 L/ha); (3) foliar application at 1:10000 dilution; and (4) foliar application at 1:1000 dilution. All treatments were applied every 15 days from transplanting and did not include exogenous phytohormones. Agronomic traits, pigment content, protein concentration, and enzyme activities (NR, GOGAT, MDH, PAL) were monitored through field measurements and laboratory analyses.

**Discussion and Conclusion:** The application of *Chlorella vulgaris* extract significantly influenced the physiological and biochemical performance of zucchini plants. Foliar treatments, especially at a 1:1000 dilution, enhanced vegetative growth and increased levels of chlorophyll a, chlorophyll b, and carotenoids, indicating improved photosynthetic efficiency and stimulation of primary metabolism. Treated plants also showed elevated total protein content, aligning with increased activity of nitrogen-related enzymes such as GOGAT and MDH. This suggests that the extract may improve nitrogen use efficiency and promote coordination between carbon and nitrogen metabolism.

Root application (0.5 L/ha) also had beneficial effects, though less pronounced, possibly due to reduced bioavailability or delayed uptake, with effects evident during the first two sampling points but not the third. Despite this, root treatment produced a total yield comparable to the control.

The quality of the harvested fruits is still under evaluation. However, these preliminary findings underscore the potential of *C. vulgaris* extract as a biostimulant under protected cultivation conditions. The study supports the use of microalgae-based products as sustainable alternatives to synthetic growth enhancers in horticulture. Future research will focus on unraveling the molecular mechanisms behind the observed effects and evaluating the long-term agronomic impacts across different crop systems and environmental conditions.



## Characterization of novel *Citrus* rootstocks subjected to deficit irrigation techniques

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**Introduction:** In recent years, due to climate change and global warming, *citrus* cultivation is facing several environmental problems. Among these, the reduction of water availability is one of the most challenging, especially in the Mediterranean area. Modern citriculture relies on the use of rootstocks to guarantee adaptability to environmental and biotic constraints, so it is of pivotal importance the characterization of novel citrus rootstocks. In addition, to obtain and establish a potential unlimited number of homogeneous plants of a specific clone, the use of rootstock plays a fundamental role to obtain uniformity in the production of high-quality fruit. This study is focused on the characterization of novel citrus rootstocks, also subjected to deficit irrigation techniques, in terms of production, quality of the fruits and their physiological capacity to withstand medium levels of Deficit Irrigation (DI).

**Methods:** The comparative trial was established in June 2021 and includes plants of TDV Tarocco blood orange grafted onto 9 rootstocks, and Meli Tarocco grafted onto 42 rootstocks. A total of 20 rootstock/scion combinations were subjected to DI and only a small sub-dataset was subjected to physiological analysis because of their performance obtained in the morphological analysis. These include stem water potential SWP, photosynthetic gas exchange, max quantum efficiency of PSII and chlorophyll content. According to their harvesting data, production has also been evaluated as well as their pomological aspects regarding Brix grade, pH, acidity, anthocyanins and polyphenols.

**Discussion and Conclusion:** According to the preliminary observation related to plant growth, productivity and response to DI, the best scion/rootstock combinations were TDV grafted onto FAO30951 and FAO30584 (Sunki mandarin × *Poncirus trifoliata* and Cleopatra mandarin × *Poncirus trifoliata*, respectively) and Meli grafted onto M5761 and M5723 (Cleopatra Mandarin × Swingle citrumelo) with values of SWP that did not exceed -2 Mpa which is considered a threshold for the Mediterranean environment, the rate of photosynthesis which was stable during the whole summer season, indicating that the plants did not suffer from the DI imposed and they were able to efficiently use the limited water available for their growth and development. The Fv/Fm ratio varied between 0.5 and 0.8, which indicates that the photosystem was not damaged from the imposed DI. The level of chlorophyll was principally influenced by the rootstocks and the day of the experiment (DOY) and was unaffected by the water regime (WR).

This preliminary data shows the good adaptability of some of the genotypes to the pedoclimatic conditions of the Mediterranean environment as well as their ability to withstand mild level of deficit irrigation in a period when water is an essential resource for sustainable agriculture.

## ***Colletotrichum perseae* and *C. gloeosporioides sensu stricto* Causing Stem lesion, Dieback and Fruit Rot on Avocado in Italy and sustainable control**

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**Introduction:** Tropical and subtropical crops, like mango (*Mangifera indica* L.) and avocado (*Persea americana* Mill.), are becoming more popular and are rapidly expanding in Southern Italy. In particular, the cultivated area of avocado in Sicily has increased in recent years. However, the occurrence of fungal diseases has also significantly expanded, leading to significant production losses. From various investigations conducted in 2024, new symptoms such as stem lesion and dieback often starting at the graft point were observed in nursery and open field on young plants. Given the trend to replace chemicals with more environmentally friendly products, it is crucial to find new biological control methods that can control fungal diseases.

**Methods:** To determine the causal agent of the disease, isolations were carried out, followed by fungal morphological and molecular characterisation. Thus, to corroborate the pathogenicity of the pathogens recovered, pathogenicity tests were conducted on stem of young two-year-old avocado plants and on fruit. In addition, a disease control trial was conducted. The objective of this trial was to evaluate the effectiveness of five commercial biological products in reducing infections caused by *C. perseae*. The treatments were performed 96 hours before and at the same time of the pathogen inoculation. Five avocado fruits per treatment were used with two points of inoculation for each fruit. The mean lesion was measured after 5 days.

**Discussion and Conclusion:** *Colletotrichum*-like colonies were frequently isolated from symptomatic tissues and identified as *Colletotrichum perseae* and *C. gloeosporioides sensu stricto*. In the pathogenicity tests, *C. gloeosporioides* and *C. perseae* induced necrotic lesions on stem after two months and fruit rot after 5 days. In the biological control trial, all treatments significantly reduced lesion size caused by *C. perseae* compared to the positive control, with Amylo-X® showing the highest efficacy. Serenade®, Prevatect®, T-34 Biocontrol®, and Remedier® exhibited intermediate effects, with no statistically significant differences among them. The results obtained are of considerable interest. The use of biological control has become increasingly relevant, since fungicide application is limited in the EU by restrictive regulations. This trend highlights the growing need for sustainable and ecologically sound strategies, positioning biological control as important component in the development of integrated disease management programs.

## **Mediterranean diet and intake of microorganisms and biomolecules of microbial origin: case studies for the design of innovative trends**

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**Introduction:** Dietary microbes, which are live microorganisms naturally present in foods, are gaining importance for their potential roles in supporting gut health, immune modulation, and metabolic function. The Mediterranean diet, characterised by a high intake of plant-based and fermented foods, offers a unique framework for exploring the nutritional and functional contributions of these microbes. This study aims to characterise the microbial content of Mediterranean food matrices, assess the effects of processing on microbial loads, with a special focus on fermented table olives as a model Mediterranean fermented matrix, characterised also by means of a cross-over fermentation strategy.

**Methods:** Foods commonly associated with the Italian Mediterranean diet were classified by microbial load using culture-dependent techniques and information from the scientific literature, as follows. For 'Bella della Daunia' Italian table olives and their brines, together with the standard microbiological characterisation, these matrices were also used as starters to ferment diverse substrates, namely wheat flour, grape juice, and milk, following a fermentation cross-over approach as a novel approach to explore microbial diversity associated with table olive microbial communities. Although microbial dynamics were not monitored over time, quantitative data were obtained at defined stages. In addition, lactic acid bacteria (LAB) isolated from various food matrices were identified by 16S rRNA gene sequencing and screened for antimicrobial activity against selected foodborne pathogens.

**Discussion and Conclusion:** We classified Mediterranean foods based on their microbial profiles in low ( $<10^4$  CFU/g), medium ( $10^4$ – $10^7$  CFU/g), and high ( $>10^7$  CFU/g) categories; in addition, for several products, we also report information about the exact counts for microbes of specific interest (e.g. lactic acid bacteria and yeasts). In general, the objective was to support the design of microbe-depleted and microbe-rich diets. The findings contribute to create a clearer categorisation of microbial loads across Mediterranean food types and support the strategic design of microbiota-enriching diets. Table olives, in particular, contain dense and diverse microbial communities, demonstrated strong potential for initiating fermentation in other food matrices (i.e. wheat flour, grape juice, and milk). Isolation and characterization, through culture-dependent strategies, starting from these fermenting matrices, has been explored as an indirect method to study the microbial diversity associated with fermented table olives. This work lays the foundation for ongoing studies integrating culture-independent methods to optimise dietary microbial intake and promote a health approach through microbiome-informed nutrition. Finally, several LAB strains isolated during this PhD work exhibited notable antimicrobial effects, highlighting their role in food safety and preservation and expanding the knowledge on the desired properties of dietary microbes.

## **Lactic acid bacteria from Apulian honeys as functional candidates for fungal biocontrol in food systems**

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**Introduction:** Lactic acid bacteria (LAB) are increasingly recognised as effective natural agents for microbial biocontrol, due to their ability to produce a wide range of antagonistic metabolites, including organic acids, bacteriocins, bioactive peptides, and volatile organic compounds (VOCs). In this context, apicultural matrices represent unconventional microbial sources of interest, as they may harbour bacterial species with largely unexplored functional traits. This study evaluated the antifungal potential of selected LAB strains isolated from 11 varieties of artisanal Apulian honey. Strains were chosen from a broader collection based on biochemical traits associated with antibacterial activity. Antifungal assay was assessed *in vitro* against five representative fungal targets (*Aspergillus niger*, *Penicillium crustosum*, *Penicillium roqueforti*, *Aureobasidium pullulans* and *Geotrichum* spp.), with the aim of identifying novel microbial candidates for bioprotective use. The findings support the application of LAB from non-conventional matrices as natural protective agents in food systems, promoting “green” solutions aligned with sustainable innovation.

**Methods:** A total of 32 presumptive LAB isolates were obtained from 11 varieties of artisanal Apulian honeys and phenotypically characterised through visual inspection of colony morphology and pigmentation, Gram staining and catalase test. A selection of strains, identified based on their phenotypic profiles, was subjected to antifungal activity testing through a sequential *in vitro* assay combining the agar overlay method with the “Plate-on-Plate” technique, using common foodborne fungal contaminants as microbial targets. The most effective isolates in terms of mycelial growth inhibition were subsequently identified at the species level using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS).

**Discussion and Conclusion:** This study confirmed the antifungal potential of selected LAB strains isolated from artisanal Apulian honeys, previously characterised for antibacterial properties, highlighting the strain- and target-specific nature of the antagonistic effects. Several isolates exhibited marked inhibitory activity against *Aureobasidium pullulans* and *Penicillium* (*P.*) *roqueforti*, whereas *P. crustosum* and *Geotrichum* spp. proved generally more tolerant, indicating differential fungal susceptibility. The most active strains were identified by MALDI-TOF MS as *Lactiplantibacillus plantarum* and *Leuconostoc* (*Leuc.*) *mesenteroides*, isolated from honeydew, rosemary, coriander, *Eucalyptus* and wildflower honeys. Notably, the strain *Leuc. mesenteroides* MME-4 achieved complete inhibition of *Aspergillus niger* mycelial growth in the ‘Plate-on-Plate’ assay, pointing to the potential role of VOCs released in the headspace. These findings reinforce the value of apicultural matrices as reservoirs of functional microbial biodiversity. In particular, VOC-mediated inhibition may represent a promising strategy to limit airborne fungal spread in food environments and in specific packaging solutions. Further investigation into the underlying metabolic pathways and validation in model food systems will be crucial to support their application as sustainable bioprotective tools.

## **Automated and digitally designed food processing operations**

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**Introduction:** While robotic applications are well established in secondary food processing operations – such as packaging or sorting – their integration into primary operations, including mixing and shaping, remains limited. This thesis aims to bridge that gap by demonstrating how digitally designed and controlled movement as well as automation and robotics can be successfully applied in key food processes operations to enhance precision and product quality. Specifically, it explores the integration of digital technologies and automation in food processing, focusing on morphing food and mixing operations. The research investigates the potential of digitally designed superficial grooves to induce dynamic shape changes in food during cooking and baking. It also explores the use of a Delta Robot for the mixing of a oil-in-water emulsions with unconventional mixing paths.

**Methods:** Two main research activities were developed. Firstly, 3D-printed molds were used for precise groove patterning on pasta and snack samples under various experimental conditions. The dehydration/rehydration kinetics and the morphing behavior were studied by empirical (e.g. Peleg, Weibull, 4P-Logistic model) and finite element models, developed with COMSOL Multiphysics. For the second research activities, a Delta-Robot followed digitally designed paths to mix O/W emulsions. A Central Composite Design and random forest model helped assess the impact of speed, time, and path on the particle size distribution and viscosity of emulsions.

**Discussion and Conclusion:** Results on morphing food of pasta showed that thinner samples with deeper grooves rehydrated faster and bent more rapidly, with the remaining dough layers below the grooves playing a key role. Also, by increasing the applied pressure, a progressive increase of the rate of bending was observed even though such differences were significant ( $p < 0.05$ ) in samples 1.5 and 1.0 mm thick. A semi-automated system was then developed to precisely generate grooves on cereal-based snacks before baking. The computer-controlled system was capable to create grooves with defined depths, thereby altering the dehydration rate and bending angles. Thinner samples of 1.89 and 1.24 mm achieved the desired shape changes with an increase of the groove depths. The experimental data obtained from the evaluation of mixing performances using a Delta Robot demonstrated a significant effect of time and an interactive effect of time and speed on the particle size distribution of oil droplets. Furthermore, different trajectories of mixing movements influenced all the investigated parameters. Overall, this work demonstrates how digital design and automation can improve a controlled dynamic shape change of food during cooking and baking and optimize emulsion mixing, offering new perspectives for future food manufacturing systems.

## Evaluation of Artichoke Powder as a Functional Additive to Design a Synbiotic Beverage

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**Introduction:** Artichoke (*Cynara scolymus* L.) is known for its prebiotic potential due to its inulin content and polyphenolic compounds. This study aimed to evaluate the effects of artichoke powder extract on the growth, stress tolerance, and functional properties of selected functional microorganisms under various environmental conditions and in a dairy matrix.

**Methods:** *Bifidobacterium animalis* subsp. *lactis* DSM 10140, *Bifidobacterium longum* subsp. *infantis* DSM 20088, *Lactiplantibacillus plantarum* DSM 1055, *Lpb. plantarum* c19, and *Lactobacillus acidophilus* La-5 were tested for their growth and survival in saline solutions containing 0.05–0.5% (w/v) artichoke powder. Experiments included evaluation of initial inoculum effect, survival under varied pH (4.5, 6.5, 8.5) and temperatures (15, 37, 45°C), and a full factorial design to examine interactive effects. Additionally, an "active drink" was developed using UHT milk supplemented with and without 0.3% artichoke powder, fermented by *Lpb. plantarum* c19 and *L. acidophilus* La-5. Viability, pH kinetics, and chemical composition (fat, protein, and lactose) were assessed.

**Discussion and Conclusion:** Artichoke powder 0.3% improved the viability of several microorganisms, especially under temperature and pH stress. The factorial design showed the best survival at 37°C for *L. acidophilus* La-5 and 30°C for *L. plantarum* c19, depending on pH. In milk-based drinks, artichoke addition enhanced fermentation performance and maintained probiotic stability during 28 days of cold storage. These results support its use as a functional ingredient in probiotic foods.

In conclusion, Artichoke powder extract supports probiotic viability and can serve as a functional additive in fermented dairy products. This study underscores its applicability in synbiotic food formulations and its potential role in gut-targeted health products.

## **Food reformulation and emerging technologies as strategies in supporting healthy nutrition**

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**Introduction:** In response to global food waste and the rising of malnutrition, this PhD project highlights the potential of plant-based sustainable ingredients, derived from by-products and underutilized species, combined with emerging technologies and reformulation strategies to develop innovative healthy foods.

The study focused on three key areas:

1. Incorporating an ancient wheat variety and glasswort—valued for its salty taste and other nutritional properties—for the development of savoury snacks with the aim to contribute to salt reduction, without compromising sensory quality;
2. valorising okara—a soy manufacturing by-product with 20–30% protein and up to 50% fibre—to create nutrient-enriched buns;
3. integrating okara with 3D food printing, identified by the FAO as a key future food technology, to realize personalized, visually appealing snacks, combining air-frying to support oil absorption modulation and fat reduction, contributing to healthier food alternatives.

**Methods:** 1.Savoury snacks were obtained with an ancient (Autonomia B) and modern (Bolero) wheat flour. The effects of salt (0%; 1%; 2%) and glasswort (0%; 4%; 8%) on physicochemical, textural, functional and sensory properties of the snacks were evaluated.

2.Okara powder was substituted to wheat flour at three concentrations (0%, 5%, 10%) to obtain enriched buns. The leavening performances, protein content and other physical properties were analysed.

3.Okara-based snacks were 3D printed (nozzle 0.84-1.55 mm; infill 40-80%) and air-fried (180°C). Baking kinetic was described with the Weibullian model. Then, oil absorption, peroxide values and textural properties were analysed.

**Discussion and Conclusion:** 1.Bolero snacks exhibited lower porosity, denser structure and higher hardness compared to snacks obtained with the ancient cultivar. Increasing glasswort substitution reduced hardness in both varieties, while in Bolero snack hardness increased from 53.47N to 71.96N with 0% and 2% of salt concentration, respectively. Glasswort improved saltiness and contributed to functional benefits including reduced rapidly digestible starch, higher antioxidant activity, and lower peroxide values.

2.Okara addition increased protein content but reduced leavening performance. Chewiness increased from 4.201 N·mm in control sample to 5.455 N·mm in 5% okara samples. Microstructural analysis revealed a denser crumb structure and smaller pores in okara samples (79.68% vs. 75.29% porosity).

3.Results of experimental activities of 3D-printed snacks revealed a higher oil uptake when using smaller nozzle diameters (0.84) and for denser structures (80% infill density) due to a larger increased surface area available for contact during spraying and frying.

These results suggest that the integration of emerging technologies and reformulation strategies can effectively support the realisation of innovative and healthy food products, aligning with consumer health trends and sustainability goals.

## Valorisation of Brewer's Spent Grain as a Functional Ingredient to Improve Nutritional and Health Quality in Dry Pasta Formulation

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**Introduction:** Brewer's spent grains (BSG) are the main by-product of the brewing industry, constituting approximately 85% of the by-products but potentially capable of representing a resource for the food industry. The aim of this study is to enhance the nutritional and health benefits of pasta. The primary focus was on drying and milling the BSG to produce flour (BSGF). Three semolina pasta formulations with different percentage of substitution (PBSG25, BSG40 and PBSG50) were created and extruded into tagliatelle. These were then compared with control samples of semolina (PS) and whole grain semolina (PW) and analysed for their chemical, physical, nutritional and technological properties. This included in vitro digestion and enzyme inhibition tests to evaluate their potential health benefits.

**Methods:** BSG were supplied by '24 Baroni' brewery in Nicosia (En) and were dried at 40°C for 48 hours to produce BSGF. The five pastes were characterised according to standard AOAC methods for moisture, water activity, protein, ash, lipids, reducing sugars, total dietary fibre, beta-glucans and total carbohydrates. The optimal cooking time (OCT), water absorption (WA), cooking loss (CL), colourimetric parameters and ability to modulate the glycemic index were also evaluated through  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition assays, as well as in vitro starch digestion. The prebiotic properties were evaluated by testing the growth of *L. rhamnosus* and *L. paracasei* in MRS broth supplemented with pasta samples.

**Discussion and Conclusion:** The characterisation of BSGF revealed high protein ( $20.13 \pm 0.37\%$ ) and fibre ( $65.00 \pm 0.06\%$ ) content, indicating its potential as an ingredient. Pasta formulations containing BSGF showed a significant increase in protein and fibre content. In particular, the PBSG50 formulation reached  $20.18 \pm 0.32\%$  protein and  $36.50 \pm 0.22\%$  total dietary fibre, which exceeds the typical values for semolina and whole wheat pasta and defines it as a functional food. While the addition of BSGF impacted technological properties such as cooking loss (which increased in the PBSG40 and PBSG50 formulations to values of 8.31% and 8.56%, respectively; these differences were significant at  $p < 0.05$ ), the cooking time remained acceptable. PBSG50 exhibited a darker colour, resembling whole wheat pasta. Preliminary tests indicated that PBSG50 exhibits approximately 42% inhibitory activity against the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase. This suggests potential benefits in the management of postprandial glycaemia and the prevention of type 2 diabetes. Furthermore, microbiological analyses revealed that the pasta formulations had a positive effect on the growth of probiotic bacteria, such as *L. rhamnosus* and *L. paracasei*. This suggests that the pasta formulations have promising prebiotic properties. Further studies are underway to optimise the methods and confirm these in vitro properties.



## Transcriptome dynamics during budbreak in early and late grapevine cultivars

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**Introduction:** Changes and fluctuations in environmental stimuli such as temperature are among the main factors affecting the annual phenological development of perennial species such as grapevine, especially during the transition from winter dormancy to budbreak and growth resumption in spring. Due to climate change, higher winter temperatures are increasingly recorded, causing an anticipation of dormancy release and budbreak. This in turn anticipates subsequent developmental stages and, more critically, increases the exposure of vulnerable tissues to the damage caused by spring frost events, thus negatively impacting bud survival and productivity. However, a high degree of variability in budbreak timing is observed among grapevine cultivars, resulting in a different susceptibility to frost damage between early and late varieties. Understanding the molecular regulation of dormancy release and budbreak, as well as the determinants shaping cultivar variability, is therefore essential to support the selection of varieties better suited to the changing climate.

**Methods:** An experimental setup based on the use of single-bud cuttings under controlled conditions was implemented to monitor the bud-to-dormancy transition in the early- and late-budbreak cultivars Chardonnay (CH) and Cabernet Sauvignon (CS). Differential Thermal Analysis (DTA) was used as measure of bud cold hardiness and proxy for dormancy progression. Bulk transcriptomes from five timepoints were analysed to compare the temporal gene expression dynamics between the two cultivars and identify cultivar-specific responses and budbreak drivers. In addition, pilot experiments for the application of single-nucleus transcriptome sequencing were carried out to investigate transcriptome signatures over individual cell types within the bud.

**Discussion and Conclusion:** The experimental setup was successfully applied to both cultivars, allowing replication in the lab of the phenotypic differences in budbreak timing observed in the field. The time course used was suitable to capture key stages during dormancy and budbreak showing clear transcriptomic differences between the two cultivars, suggesting the presence of cultivar-specific gene expression programs that regulate dormancy release and budbreak. During dormancy CH showed a stronger upregulation of pathways related to cell-wall remodelling, cell cycle and metabolism, suggesting a priming for growth resumption prior to deacclimation, while CS exhibited a profile typical of a deep dormancy state. Upon budbreak induction, both cultivar-specific up- and downregulation of gene expression and delayed responses of CS to forcing conditions were observed, particularly affecting genes involved in processes such as cell cycle regulation, metabolism, cellular and organ development, hormonal regulation, and responses to abiotic stimuli. Moreover, several known regulators of dormancy and budbreak in perennial species, including *EARLY BUDBREAK 1*, and *FLOWERING LOCUS C* and *T*, showed distinct expression trends between the two cultivars. Analysis of single-cell transcriptomes is ongoing and holds considerable potential to reveal cell-type-specific expression signatures and pathways driving dormancy release and budbreak.

## **Sustainable viticulture: different strategies to enhance the resistance to the main fungal diseases on Sicilian cultivars**

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**Introduction:** Grapevine is one of the most relevant fruit crops in the world for both production and economic impact. However, its cultivation is threatened by widespread diseases like powdery and downy mildew. Obtaining resistant varieties is a key priority for sustainable disease control, however, most breeding programs focus on international genotypes. Resistance can be achieved by introducing genes like *RUN* and *Rpv*, or by silencing susceptibility genes such as *MLO* and *DMR6* using new genomic techniques (NGTs). This PhD project, founded by Istituto Regionale del Vino e dell'Olio (IRVO) of Sicily, aims to develop Sicilian grapevine varieties resistant to both diseases through crossing and genome editing. In the first approach, pollen from resistant hybrids provided by CREA-VE was used to cross-pollinate local varieties. In the second, genome editing was applied to embryogenic masses and protoplasts using a geminivirus-based vector, which enables gene editing without the integration of exogenous DNA into the plant genome.

**Methods:** Six grape sicilian varieties were used for the research: 'Carricante', 'Catarratto', 'Frappato', 'Grillo', 'Nerello mascalese' and 'Nero d'Avola'. Crosses were carried out over three years. The plants obtained were selected using Marker-assisted selection (MAS) by detecting SSR markers linked to resistance traits. Hybrids carrying at least one marker were phenotypically screened by *in vitro* inoculation with *Erysiphe necator* to assess powdery mildew resistance. For genome editing, embryogenic masses were obtained from *in vitro* culture of anthers and pistils on MSII medium. Embryogenic masses and protoplasts, generated via enzymatic digestion, were transformed using a geminivirus-based vector, assembled via GoldenGate, targeting MLO3, MLO7, and MLO13/17. Embryos were transformed using *Agrobacterium tumefaciens*, while protoplasts by PEG-mediated transfection.

**Discussion and conclusion:** For crossing approach, MAS identified at least one resistant marker in 41 hybrids. These were screened *in vitro* for powdery mildew resistance and 13 were confirmed as resistant. For the genome editing approach, the varieties 'Nero d'Avola' and 'Frappato' were used. Embryogenic masses were transformed with *A. tumefaciens* for the editing of MLO3, MLO7 and MLO13/17 in 'Nero d'Avola' and MLO7 in 'Frappato'. All vectors were used for protoplasts transfection in both cultivars. Editing success was evaluated using NESTED PCR and sequencing. Editing was confirmed in 'Nero d'Avola' embryos edited with MLO3 and MLO13/17, and in protoplasts for MLO3 and MLO7; in 'Frappato' editing was confirmed in embryos and protoplasts for MLO7. Plant regeneration protocols are in progress. This study represents a significant advancement toward the development of Sicilian grapevine varieties resistant to fungus diseases, contributing to more sustainable viticulture in Sicily. Future efforts will focus on field evaluation and agronomic characterization of the selected genotypes, with the goal of contributing to a more resilient and environmentally friendly grape production system.

## **Deep Learning for Precision Viticulture: Automated Grape Harvesting, Pruning, and Pesticide Detection**

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**Introduction:** Agriculture has undergone transformational changes due to the rapid growth of artificial intelligence (AI) technologies. The traditional harvesting and pruning methods are time-consuming, costly and labour-intensive; therefore, the use of innovative technologies is needed for the agricultural system. This study presents the development of a deep learning-based vision model tailored for robotic grape harvesting, cane pruning, and grape quality assessment through pesticide residue detection. The primary objectives of the research are:

- to design an object detection model for grape localisation and optimal picking point identification to support robotic harvesting
- to segment grapevines and canes for enabling robotic pruning
- to detect and classify pesticide residue levels on grapes using hyperspectral imaging

**Methods:** RGB images were acquired from vineyards near Foggia, Italy, using an iPhone 13 and a Nikon digital camera under varying lighting conditions and angles to improve model robustness. For grape detection, 500 images were annotated with bounding boxes into four classes: ripe grapes, unripe grapes, rotten grapes, and picking points, using LabelMe software. For vine and cane segmentation, 70 RGB images were polygon-annotated into trunk and cane classes. All datasets were split into training (70%), validation (20%), and test (10%) sets. The YOLOv11 deep learning architecture was employed for object detection and segmentation.

To detect pesticide residues, a mixture of two commonly used pesticides, Flint (Trifloxystrobin) and Switch (Cyprodinil, fludioxonil), was prepared in a 1:5.33 ratio with water. This stock solution was further diluted to five concentrations: 100% (T1), 80% (T2), 60% (T3), 40% (T4), and 20% (T5), then sprayed onto grape samples. Hyperspectral images were captured using a line-scan hyperspectral scanner (Version 1.4, DV srl, Padova, Italy) with two sensors: Vis-NIR (400–1000 nm) and NIR (900–1700 nm). Imaging was conducted at 13 time points post-treatment (from 24 hours up to 35 days) to develop a time series classification and regression model.

**Discussion and Conclusion:** The developed deep learning models demonstrated promising performance across all three target applications: grape harvesting, vine pruning, and pesticide residue detection. The harvesting model, trained over 100 epochs, achieved an overall precision of 70%, with high detection accuracy for ripe (69.6%) and rotten grapes (78.9%). Moderate performance was observed for unripe and spotted grapes (mAP@0.5: 57–60%), while picking point detection remained challenging, likely due to class imbalance and visual complexity. The pruning model attained a precision of 65%, with more accurate identification of trunks than canes, indicating reliable segmentation capability for structured vineyard environments.

For pesticide residue classification, PLS-DA analysis revealed limited class discrimination using Vis-NIR spectra, with an overall accuracy of less than 70%. However, the NIR spectra provided significantly better classification performance. Cross-validation accuracy improved over time, showing a high overall accuracy: T1 (98%), T2 (88%), T3 (75%), T4 (70%), and T5 (94%). In conclusion, the integrated deep learning framework exhibits strong potential for application in autonomous vineyard management. Further enhancements in class balance, data diversity, and spectral analysis could improve detection robustness and enable real-time deployment in precision agriculture systems.

## **Assessment of Crop Evapotranspiration, Vine Ecophysiological Performance, and Fruit Quality in a Table Grape Vineyard Protected by Anti-Rain Plastic Sheets and Anti-Hail Nets**

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**Introduction.** Table grape production is important for the agricultural economy of hot-arid regions, where vineyards are often protected by anti-rain plastic sheets and anti-hail nets to reduce environmental hazards and stresses and adjust harvest timing. Coverings alter the vineyard microclimate: anti-rain plastic sheets increase air temperature and humidity while decreasing wind speed and solar radiation, leading to reduced evapotranspiration and water use. Accurate estimation of crop evapotranspiration (ET<sub>c</sub>) is crucial for efficient irrigation management. The FAO method, which uses reference evapotranspiration (ET<sub>o</sub>) and crop coefficients (K<sub>c</sub>), is widely applied, though K<sub>c</sub> values require local adaptation. Additionally, a specific “coverage coefficient” should be included for covered vineyards to improve ET<sub>c</sub> estimates. This study aims to investigate microclimatic changes under anti-rain plastic sheets and anti-hail nets compared to uncovered vineyards, quantify reduction in evapotranspiration, derive appropriate plastic-coverage coefficients, and evaluate the effects of covers on vine ecophysiology and productivity, and on grape quality.

**Methods.** In a vineyard (cv. Sweet Celebration) partially covered with transparent plastic film, partially with white anti-hail net, and partially uncovered, three weather-stations monitor the microclimate outside and under the covers. Reference evapotranspiration (ET<sub>o</sub>) is calculated using the Penman-Monteith equation. Specific “plastic-coverage coefficients” for both net and plastic sheet are derived following Moratiel and Martínez-Cob (2012). Transpiration is measured with sap flow sensors using the Heat Ratio Method (HRM); soil evaporation is assessed using weighing micro-lysimeters. Vine water status, stomatal conductance, LAI, productivity and grape quality are assessed using well-established methods.

**Discussion and Conclusion.** Comparing the two covering systems, the anti-hail net slightly reduced the average air temperature, while the anti-rain plastic sheet induced a moderate increase. Both coverings increased relative humidity, reduced wind speed, and decreased incoming solar radiation compared to open-field. Consequently, reference evapotranspiration decreased significantly, mainly due to the lowered wind speed and radiation. The reduction in ET<sub>o</sub> was greater under the plastic sheet than under the net, highlighting the need for specific coverage coefficients for different covers. The covers exerted different influences on vine ecophysiology: the plastic sheet improved vine water status and stomatal conductance, especially under high atmospheric demand, while the net promoted vegetative growth, increased pruning weight and leaf area index (LAI). Reproductive performance was enhanced under the plastic sheet, with higher fertility and clusters per shoot. Regarding grape quality, berries under the plastic net were larger, had higher sugar content and deeper color; those under the plastic sheet showed greater firmness. These findings highlight the importance of improving evapotranspiration estimation in protected vineyard by introducing “plastic-coverage coefficients”. A better understanding of these relationships is essential for enabling more accurate irrigation management in covered vineyards.

## Characterization of grapevine responses to water and nitrogen availability

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**Introduction:** The increasing frequency and severity of drought events in several regions can have negative impacts on grapevine photosynthesis, yield, and berry quality. To maintain adequate yields under sub-optimal conditions, farmers often apply large quantities of fertilizers, with negative environmental consequences. While the individual impacts of water deficit and nutrient deficiency in grapevine are well documented, their combined effects remain poorly understood. Moreover, grapevine responses to water deficit depend on several factors, including genotype. This study aims to evaluate the interplay between water and nitrogen (N) availability in two grapevine cultivars, known to exhibit different sensitivities to water stress.

**Methods:** Two-year-old *Vitis Vinifera* cv. Cabernet Sauvignon and Grenache, grafted on S.O.4. rootstock, were grown in soil pots under semi-environmental conditions. Plants were maintained well-watered or subjected to a controlled water deficit irrigation. Moreover, different N:K fertilization regimes were applied. Morphological and physiological parameters were assessed, including plant growth rate, midday stem water potential, and stomatal conductance. In addition, leaf samples were collected at flowering, veraison, and maturity stages for multi-elemental analyses and transcriptomic analyses. The varietal dependency of drought response was further investigated by analyzing the microbiome at the root level and the concentration and distribution of nutrients in roots.

**Discussion and Conclusion:** A significant reduction in plant growth under water deficit conditions was observed, with Grenache displaying a slower growth rate than Cabernet Sauvignon. The response of Cabernet Sauvignon to water and N limitations varied seasonally at both physiological and nutritional levels. At flowering, water treatment was the main factor influencing plant response. Water deficit reduced stomatal conductance ( $g_s$ ), improving water-use efficiency, while increasing the concentration of several nutrients in leaves. Conversely, the physiological response and the nutrient composition at maturity were modulated by N fertilization. The higher N fertilization reduced  $g_s$  in Cabernet Sauvignon and altered the concentration of key elements in both cultivars, with Grenache showing a delayed response than Cabernet Sauvignon. Transcriptomic data revealed genotype and developmental stage specific patterns, with the activation of stress-related pathways under water deficit. Soil type and water availability altered the bacterial and fungal community composition, with reduced relative abundance of *Glomeromycota* and *Basidiomycota* under water deficit. These findings highlight the seasonal and cultivar-dependent interactions between water and N availability, underscoring the importance of integrated irrigation and fertilization strategies to support a more sustainable viticulture.

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## **Ozone-Enriched MAP: A new prototype for minimally processed fruit and vegetables preservation and packaging compatibility**

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**Introduction:** The demand for chemical-free methods to extend the shelf life of perishable produce is growing. While Modified Atmosphere Packaging (MAP) is a recognized solution for maintaining quality. This project focuses on enhancing MAP's efficacy by incorporating ozone, generated through an innovative approach: utilizing a novel cold plasma prototype for in-package ozone generation. This technology aims to create an ozone-enriched MAP environment through very short treatment durations. The primary objectives were to demonstrate the feasibility of generating ozone directly inside the package, to evaluate its potential for reducing microbial decay and maintaining product quality, and to assess its compatibility with packaging materials. This system holds immense potential for offering a direct, efficient, and potentially scalable postharvest preservation method.

**Methods:** The cold plasma prototype was used to generate high levels of ozone directly within sealed packages. The treatment, applying very short durations, was applied to fresh produce samples already packaged in a passive Modified Atmosphere (MAP), which were then cold-stored. Initially and after the treatment and the storage durations, chemical quality parameters of the produce were assessed. Additionally, tests were conducted on the plastic films used for packaging in order to evaluate the impact of the plasma treatment on the packaging material itself.

**Discussion and conclusion:** The rapid in-package ozone generation via the cold plasma prototype demonstrated the feasibility of creating an ozone-enriched MAP environment with minimal treatment duration. This approach is very promising for delaying microbial growth and appears to effectively reduce microbial load without significantly altering most quality parameters of minimally processed fruits and vegetables. This innovative technology could offer a highly efficient and integrated solution for enhancing food safety and extending the shelf life of packaged products. While initial observations are encouraging, further research is necessary to fully characterize the optimal application parameters and to assess the broader implications of this rapid, in-package ozone generation method across various perishable food items. This includes thoroughly investigating any potential subtle effects on product attributes not yet fully explored, considering also that a deeper exploration of the effects on the packaging material itself is crucial.

## **Towards Selective Detection of Artificial Sweeteners: Electrochemical and Docking Studies for MIP Sensor Development**

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**Introduction:** Artificial sweeteners (ASWs), such as saccharin, aspartame, rebaudioside A, and diketopiperazine (DKP, a degradation product of aspartame), are widely used in food and beverage industries, yet they are not fully regulated as environmental contaminants. Their presence in surface waters raises concerns due to persistence and potential ecological effects. Although considered safe for human intake, their detection at ultra-trace levels remains a challenge. This study combines electrochemical techniques and molecular docking to investigate ASWs' redox behavior and their interaction with functional monomers, with the aim of developing a selective electrochemical sensor based on molecularly imprinted polymers (MIPs). The final goal is to develop a platform/device for environmental monitoring of ASWs in surface waters.

**Methods:** ASWs were investigated mainly using square wave voltammetry (SWV) and alternate current voltammetry (ACV) with a hanging mercury drop electrode (HMDE), under different pH conditions. Measurements were carried out in acetate buffer (pH 4.5), phosphate buffer (pH 7.4), Britton–Robinson buffer (pH 7.4), and natural seawater. Saccharin was studied as a Cu<sup>2+</sup> complex, while diketopiperazine and rebaudioside A were analyzed via their adsorption behaviour on the mercury surface. Molecular docking simulations using AutoDock Vina, Avogadro, ChimeraX, were performed to assess the interaction between ASWs and functional monomers (e.g., apocynin, pyrrole), supporting the rational design of molecularly imprinted polymers.

**Discussion and Conclusion:** Electrochemical analysis revealed no oxidation signals for aspartame and L-phenylalanine on the mercury electrode under the tested conditions. In contrast, saccharin showed a well-defined peak around +0.3 V when complexed with Cu<sup>2+</sup> in Britton–Robinson buffer at pH 7.4. DKP and rebaudioside A exhibited strong adsorption behavior on the mercury surface when analyzed via ACV. However, the overall sensitivity remained limited, with signals appearing only at micromolar levels. To overcome this, molecular docking simulations were performed to guide the rational design of a MIP-based sensor. Docking simulations confirmed specific interactions between the ASWs and some functional monomers, suggesting the more profitable for MIP preparation. These findings contribute to the rational design of electropolymerizable MIPs. Overall, integrating electrochemical investigations and molecular docking provides a solid foundation for developing robust biosensors for the detection of artificial sweeteners in environmental waters.

## **A one-health approach: from feed to functionalized food**

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**Introduction:** Recent advancements in animal welfare and feeding strategies have significantly influenced the qualitative and functional characteristics of buffalo milk and on the other hand among the various strategies under investigation for mitigating T2D and its related cardiovascular complications, targeted dietary interventions in human patients are gaining prominence. Emerging evidence suggests that betainized compounds, either directly supplied through the diet or endogenously synthesized by gut microbiota and secreted in the buffalo milk, serve as effective modulators (functionalized milk) in the nutritional management of type 2 diabetes (T2D). This metabolic disorder is frequently associated with impaired homeostasis, a dysregulated metabolic profile, and elevated allostatic load (AL), all of which are key contributors to increased cardiovascular disease (CVD) risk. AL can be quantified using biomarkers (cortisol, DHEA-S and their ratio). Establishing cutoff values of these hormones may serve as indicators of an individual ability to maintain or not the physiological equilibrium (homeostasis) and resilience also in a T2D condition.

**Methods:** The hair (45mg) was sampled non-invasively and painlessly, on the *vertex posterior* region of the head, both from clinically healthy and unhealthy people. Steroid concentrations were determined from the 1 cm segment of hair closest to the scalp using an in-house Enzyme-linked Immunosorbent Assay (ELISA) validated for hair Pividori et al. (2024).

The statistical analyses were performed using R software ver. 4.0.4 (R Core Team, 2021) and SPSS software ver. 17 (SPSS Inc., Chicago, IL, USA).

**Discussion and Conclusion:** Under conditions of chronic medical illness, concentrations of DHEA-S and cortisol have been observed to become dissociated; cortisol secretion increased while that of DHEA-S decreased. Anyway, considering the synchronized synthesis of these two hormones and their opposing effects the cortisol/DHEA-S ratio may be more informative than the absolute hormone concentrations. The disruption of the dynamic balance of these two hormones, especially a higher cortisol/DHEA-S ratio may have an impact on diseases. Our evaluations reveal that a cut-off cortisol/DHEA-S ratio of  $>1.46$ , identified through comparisons between clinically healthy and unhealthy people, is synonymous with the breaking of an optimal ratio, mainly due to the inability of DHEA-S to counteract the effects of stress that could lead to various pathologies. The concentrations of biomarkers analyzed in hair allow for a retrospective, cumulative, and objective determination of an individual's condition.

The establishment of baseline endocrine parameters constitutes a critical prerequisite for the subsequent evaluation of the effects of the administration of functionalized buffalo milk in patients with T2D. In particular, the investigation on different hormones of the HPA axis could provide also precious information regarding the allostatic load and the patient's coping ability with different stressors linked to the pathology and diet restrictions.



## Microplastic Contamination in Meat and Internal Tissues of Cattle and Pork

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**Introduction:** Microplastics (MPs, <5 mm) originate from primary sources (industrial production) and secondary sources (degradation of larger plastics). MPs are widespread in agricultural systems due to multiple vectors such as plastic mulch films, biosolids, feed packaging, and irrigation. Livestock may ingest MPs via contaminated feed and water. Recent evidence confirms MP contamination in various animal-derived products (milk, fish, meat), with potential health risks through bioaccumulation and systemic toxicity. MPs can trigger oxidative stress, immune dysfunction, and endocrine disruption, particularly due to their capacity to carry environmental co-contaminants. The detection of MPs in tissues highlights the relevance of exposure in both animals and humans. Given the role of meat as a primary protein source, understanding contamination pathways is critical.

**Objectives of this study:** to detect MPs in internal tissues of cattle and swine; to characterize MPs by size, shape, and polymer type; to estimate human dietary exposure from contaminated meat.

**Methods:** A total of 40 tissue samples (muscle, stomach, liver, and ovaries from cattle and swine) were analysed. Samples were digested in 10% KOH at 60°C for 24 h, then filtered with boiled water and acetone using a glass fiber membrane (1.2 µm pore size) using a vacuum filtration system. Nile Red stain was applied to detect microplastics (1–5000 µm), which were identified via fluorescence microscopy based on shape, fluorescence, and edge definition. Particle dimensions were measured with ImageJ software. Data were compiled in Excel, and statistical analysis was performed using IBM SPSS Statistics.

**Discussion and Conclusion:** A total of 78 MPs were identified in 24 out of 40 tissue samples, with a median concentration of 0.83 MP/g. The variability in MP presence appears to be influenced by factors such as tissue distribution, individual exposure, feed type, and the geographical or environmental conditions that may affect contamination levels. Particle sizes ranged from 5.31 to 1076.93 µm, with 44.9% measuring between 10–50 µm. Notably, smaller particles (<150 µm) are more likely to be internalized and distributed across internal tissues. Most MPs were morphologically classified as fragments (64.10%).

The estimated daily intake (EDI) of MPs for the adult target population, based on the consumption of beef and pork edible tissue, was calculated at approximately 1–3 MPs/Kg body weight.

Although no definitive evidence currently links MP ingestion to human health risk, their presence in internal organs and blood, suggests the potential for MPs to cross the intestinal barrier and disseminate systemically. In conclusion, future research should focus on developing standardized methodologies and validated analytical protocols for the MP detection in animal and human matrices. Such efforts are essential to improve contamination assessment and enhance understanding of MP transport, accumulation, and potential toxicity within biological systems.

## **Poster Section**

## **Deep Learning in Agriculture: Empowering the Transition to Agriculture 4.0**

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**Introduction:** Agriculture is currently going through a transformative revolution with the use of Artificial Intelligence, especially Deep Learning (DL), marking the start of Agriculture 4.0. Under the pressure of growing global demand for food, unpredictable climates, and shortage of resources, the era of yesteryear's conventional farming is being overcome by data-infused precision agriculture. DL, using automatic learning of complex patterns in multimodal fashion, is encouraging innovations in crop monitoring, livestock monitoring, optimization of resources, as well as autonomous agriculture. With the capacity of overcoming the boundaries of human-driven feature handcrafting, DL systems consistently outperform traditional methods in applications such as classification of disease, estimation of yield, as well as detection of behaviors of livestock. However, key issues remain, pertaining to scarcity of information, faith in model explainability, as well as limitations of fielding in real-world environments. Innovations within Explainable AI, Federated Learning, Generative AI, and Edge AI hold solutions for these issues, allowing development of an efficient, resilient, as well as sustainable global supply of food.

**Methods:** We methodically reviewed articles from 2020-2025 on Deep Learning technologies for farming. We have grouped the developments under four general categories: precision crop management, smart livestock production, water and soil resource optimization, and autonomous farm systems. We presented architectures of model (like CNN, LSTM, YOLO) with modalities of information (RGB, multispectral, thermal) and performance indicators (like accuracy, RMSE, mAP) per each. We presented future technologies of Explainable AI, Federated Learning, Generative AI, and Edge AI, that remove deployment problems. Paper selection was on relevance, scientific contribution, and usability with realistic farm systems.

**Discussion and Conclusion:** Deep Learning, 2020–2025, grew from a future promise to an underpinning technology transforming agriculture. In crop precision systems, DL enabled accurate detection of disease in weeds as well as fruit quality estimation. In livestock systems, non-invasive monitoring with computer vision has enabled individual recognition and welfare estimation. Soil and water management were enhanced using DL-driven precision water irrigation and digital soil mapping. Automated agronomic systems combined perception and action using DL-centric robots in real-time. Even such technologies exist, real-world implementation remains hindered by model obfuscation, scarcity of data, and rural infrastructure. Such hiccups can be overcome with emerging technologies being conceptualized: Explainable AI instills explainability as well as trustiness; Federated Learning provides privacy as well as group learning enablement; Generative AI bridges data scarcity; and Edge AI ensures low-latency, in-field inference. Additionally, hybrid DL–process-centric models allow scientific rigor as well as generalizability. To allow future development, systems marrying autonomous operation with efficacy as well as trustworthiness need to be integrated. Researchers should work on robust benchmarks, cross-domain datasets, as well as cross-disciplinary research to consolidate research achievements into scalable impacts within agriculture. Finally, DL is not optimizing discrete things in silos but is empowering an intelligent, sustainable agriculture ecosystem capable of solving the world's food security as well as environmental resilience dilemma.

## Drip Irrigation Performance with Reclaimed Wastewater

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**Introduction:** The reuse of treated wastewater in agriculture offers a strategic opportunity to enhance water resource availability and climate resilience in Mediterranean regions. However, its efficient distribution requires microirrigation systems capable of maintaining high hydraulic performance despite higher clogging risks associated with TWW. This study evaluates the technological performance of drip irrigation systems supplied with secondary-treated domestic effluents, with and without UV disinfection, in an open-field horticultural production. Trials were carried out in 2025 at the Valle dei Margi farmhouse (Grammichele, Sicily), where a constructed wetland treatment plant provide TWW for irrigation. The performance of sand filtration enhanced with additives, in combination with innovative emitter technologies designed to limit biofilm formation, is also evaluated in relation to the use of TWW.

**Methods:** A field experiment was carried out in winter-spring 2025 on an experimental irrigation system using three water sources: groundwater (GW), constructed wetland-reclaimed water (RW), and UV disinfection-RW disinfection (RW+UV). The irrigation network consisted of surface drip laterals (Ø16 mm, 11 mil thickness, selected to reduce damage from entomofauna observed in previous configurations) with in-line pressure-compensating emitters (nominal discharge  $1.1 \text{ L h}^{-1}$  at 1 bar), spaced at 0.30 m along the lateral. Each sector was connected to a filtration unit composed of disk filters (120 mesh), sand filters with additives, and pressure regulators (1 bar), ensuring proper operating conditions. Drip laterals included control emitters (green, CTR) and emitters treated with antimicrobial compounds (named yellow and blue). Hydraulic performance was assessed following the methodology proposed in terms of emission uniformity (EU) and flow reduction factor ( $R_d$ ).

Visual inspections complemented these measurements to assess biofilm deposition and sediment accumulation in emitters.

**Discussion & Conclusion:** Across all treatments, yellow and blue laterals maintained  $\text{EU} > 90\%$ , peaking at 98%, indicating excellent distribution uniformity. In contrast, CTR laterals (green) displayed significant degradation under RW and RW+UV irrigation, with EU dropping to 82–85% and  $R_d$  increasing up to 37%. Treated laterals (yellow and blue) maintained  $R_d$  between 5–14% and discharge rates between  $0.94\text{--}1.01 \text{ L h}^{-1}$  that decreased to  $0.87\text{--}0.99 \text{ L h}^{-1}$  in the control. Visual assessments confirmed that emitters with antimicrobial compounds exhibited reduced biofilm formation and sediment accumulation. These results demonstrate that the integration of constructed wetlands, enhanced sand filtration with additives, UV disinfection, and antimicrobial-treated emitters significantly improves the hydraulic reliability of drip irrigation systems using RW. This integrated approach reduces clogging risks, improves maintenance efficiency, and supports the safe, long-term adoption of RW in horticultural production under semi-arid Mediterranean conditions.

## Effects of Extreme Low-Oxygen Conditions on Volatile Compound Production in 'Pink Lady' Apples

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**Introduction:** The increasing demand for sustainable postharvest technologies has highlighted the potential of low-oxygen storage as a promising strategy to reduce food loss while maintaining fruit quality. However, effectively monitoring fruit metabolism under the stressed gas conditions remains a significant challenge. The primary objective of DCA is to elevate oxygen levels in response to signals of occurring fermentation, to restore aerobic respiration and prevent permanent damages to the tissue. Among the methods for detecting the quality state of apples in a DCA is the detection of volatile fermentation compounds including ethanol and acetaldehyde. This study investigated the effects of extreme low-oxygen conditions on the volatile compound profile and physicochemical characteristics of 'Pink Lady' apples.

**Methods:** A total of 400 apples were stored at 1 °C under four controlled atmosphere conditions: (T1) anoxic (100% N<sub>2</sub>), (T2) 0.5% O<sub>2</sub> + 95% N<sub>2</sub>, (T3) 1% O<sub>2</sub> + 99% N<sub>2</sub>, and (T4) air as control. After 10 days of storage, key quality parameters were evaluated, including respiration rate, firmness, total soluble solids (TSS), titratable acidity (TA), color and volatile compounds profile. Volatile profiles were assessed using solid-phase microextraction (SPME) followed by gas chromatography–mass spectrometry (GC-MS). A Hierarchical Cluster Analysis (HCA), combined with heatmap visualization, was used to explore similarities among treatments based on their volatile signatures.

**Discussion and Conclusion:** This approach enabled the visualization of relationships among samples based on their volatile profiles, aiding in the identification of treatment-specific effects. Additionally, Partial Least Squares Discriminant Analysis (PLS-DA) was performed to classify samples according to treatment and to identify the most influential volatile compounds differentiating the four treatments. Preliminary results indicated that specific volatiles particularly ethyl acetate, 1-butanol, 1-butanol-2-methyl, and several short-chain esters such as ethyl butanoate and ethyl propanoate were significantly affected by low-oxygen storage conditions, demonstrating to be strong potential biomarkers for differentiating aroma profiles across varying oxygen levels. The findings underscore the pivotal role of oxygen availability in regulating volatile biosynthesis and highlight the importance of precise oxygen control in dynamic controlled atmosphere (DCA) storage systems to extend shelf life while maintaining the sensory quality of the fruit.

## **Lethal and behavioural side effects of insecticides on the whitefly parasitoid *Eretmocerus eremicus***

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Introduction: Synthetic and bio-derived insecticides are routinely used to manage whitefly infestations in horticultural crops such as tomato. However, their potential non-target effects on whitefly natural enemies are often poorly investigated. Behavioural responses related to biological control services play a crucial role in evaluating the compatibility of these chemicals with biocontrol agents. This study assessed the non-target impacts of synthetic and bio-derived insecticides on the parasitoid *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), a key biological control agent of the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), with the aim of identifying insecticide options compatible with sustainable tomato production.

Methods: Under controlled laboratory conditions, both lethal and sublethal effects of six insecticides commonly used against *B. tabaci* in tomato crops at their field recommended rates were assessed on *E. eremicus*. The tested compounds included acetamiprid, azadirachtin, *Allium sativum* essential oil, chlorantraniliprole, deltamethrin, flupyradifurone and the entomopathogenic fungus *Beauveria bassiana* ATCC74040. Acute toxicity was investigated through topical exposure on pupae and residual contact on emerged adults over 48-hour period. The host-seeking behaviour of surviving adults females from these trials was investigated using a dual-choice olfactometer assay.

Discussion and conclusion: The survival and olfactory capacity of *E. eremicus* significantly varied among the tested insecticides. Chlorantraniliprole caused minimal mortality on both the exposed pupal and adult stages, indicating a high level of selectivity towards *E. eremicus*. Similarly, *B. bassiana* ATCC 74040 resulted safe on the parasitoid. *E. eremicus* females that emerged from pupae treated with chlorantraniliprole and *B. bassiana* retained their host-seeking behaviour, suggesting that these treatments preserve the ecological service of *E. eremicus*. Albeit azadirachtin showed moderate toxicity, its botanical origin may still make it preferable to conventional insecticides in certain contexts. The toxic effects of acetamiprid, deltamethrin, flupyradifurone and *A. sativum* essential oil highlight the need for further investigation into their non-target impacts. These results provide evidences of carefully evaluating side effects of insecticides before their inclusion into tomato Integrated Pest Management programs. Future research should focus on field-scale validation of insecticides and explore their effects on a broader range of beneficial arthropods to optimize biocontrol strategies in tomato cropping systems.

## **MetaBench: assessment of performance of metagenomic approaches**

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**Introduction:** Rapid advances in Next-Generation Sequencing (NGS) technologies along with a significant drop in sequencing costs have revolutionized the field of metagenomics changing our understanding of microbial ecosystems. By directly interrogating the community composition in a culture-independent manner, metagenomic sequencing is able to investigate structure, functionalities, and ecological interactions of microbial communities with their environment or host. However, the diversity of sequencing platforms, together with the ever-growing number of rapidly evolving bioinformatics tools, and the lack of standardization across microbial databases, requires researchers to make complex choices, especially given the lack of consensus on best practices. Therefore, this PhD project aims to assist end users in the decision-making process through the comparison of different NGS technologies, denoising algorithms, classification software, and reference databases.

**Methods:** This study leverages soil samples previously sequenced as part of an unpublished work, where V3-V4 regions of the 16S rRNA gene obtained from 36 soil samples were amplified and sequenced using three distinct NGS technologies: Illumina MiSeq, Illumina NovaSeq, and Element Biosciences Aviti. As part of this PhD, bioinformatic analyses have been performed using four software tools: two denoising algorithms, DADA2 and Deblur, and two taxonomic classifiers, Kraken2 and Centrifuge. Additionally, two reference databases have been employed: SILVA and Greengenes2.

**Discussion and Conclusion:** The comparison of software tools across multiple sequencing technologies and reference databases shows that the number of detected taxa at each taxonomic level is generally consistent regardless of the combination used. The only exception is Kraken2, which reports a higher number of species when used with Greengenes2 database. In terms of  $\alpha$ -diversity, several metrics have been calculated at the Family and Genus levels, including observed taxa, Chao1, Shannon, and Simpson indices. These indices vary across different combinations of sequencing technologies and software tools and tend to show higher values with Greengenes2 compared to SILVA database within each combination. Notably, both observed taxa and the Chao1 index exhibit a positive correlation with the total number of reads per sample, while Shannon and Simpson indices are robust to deviations in sequencing depth.  $\beta$ -diversity has been also investigated using NMDS plots based on Bray-Curtis dissimilarity, which revealed that all technologies are capable of differentiating soil samples, with Aviti and Novaseq showing a similar pattern. The preliminary findings of this benchmarking study begin to address the complex task of identifying the optimal combination of approaches and motivate us to leverage sample metadata to gain deeper insights into their strengths and limitations.

## **Formulation of a symbiotic blend based on germinated brown rice and probiotics with promising health features**

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**Introduction:** The growing interest in natural well-being promotion strategies has highlighted that germinated brown rice (GBR), rich in insoluble fibres, is a promising source of prebiotic compounds. The combination of GBR and probiotics exerting health-related functions, increase bioavailability of molecules such as GABA and  $\gamma$ -orizanol. The aim of the present study was to evaluate the ability of GBR to support the viability and functionality of probiotics with promising health features. In addition, antioxidant activity, impact on cells viability, anti-inflammatory and estrogen-like potential, exerted by the symbiotic blend, were *in vitro* tested.

**Methods:** Four probiotic strains (*Lactocaseibacillus rhamnosus* CA15, *Limosilactibacillus fermentum* CS57, *Lactiplantibacillus plantarum* IMC510 and *Bifidobacterium animalis* subsp. *lactis* BCL1) were tested for lysozyme tolerance, different pH and bile salts concentrations and the survival to the simulated gastrointestinal tract, both single and in combination as a blend. Subsequently, the selected probiotics were inoculated into three different concentrations of GBR (GBR1, 40%; GBR2, 30%; GBR3, 20%) and tested for antimicrobial activity against several pathogens. Cytocompatibility of the probiotics and GBR was evaluated on cell lines, alongside antioxidant activity, anti-inflammatory potential, and glycogen release assays to assess functional bioactivity.

**Discussion and Conclusion:** The results obtained revealed a good survivability of the strains within the *in vitro* simulated gastrointestinal conditions. In addition, both all the strains and the blend exhibited good fermentation ability on all the tested GBR concentration, with the highest cell density at lower GBR concentration, supported by the lowest pH value. Furthermore, all probiotic strains demonstrated good antagonistic activity against the main pathogens. The symbiotic blend significantly enhanced cell proliferation and glycogen release and showed good anti-inflammatory effect, confirmed by decreased IL-6 and increased IL-10 and SIRT-1 expression. In conclusion, the symbiotic blend based on GBR and probiotics represent a promising formulation for human wellbeing.



## **Towards predictive and biological control tools for fungal pathogens of Mediterranean crops**

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**Introduction:** Fungal diseases pose a growing threat to olive and citrus crops in the Mediterranean region, exacerbated by climate change and increasing globalization. Rising temperatures and humidity create favorable conditions for fungal pathogens. These changes also compromise the effectiveness of traditional disease control strategies. Relevant pathogens include *Venturia oleaginea*, *Colletotrichum* spp., and *Phytophthora* spp. on olive, and *Alternaria*, *Penicillium* spp., and *Phytophthora* spp. on citrus. These organisms are associated with major yield losses, quality reduction, and postharvest decay. The effectiveness of disease management strategies relies heavily on early diagnosis and accurate prediction of infection risk periods. This study aims to develop predictive models based on historical climate data using machine learning and deep learning algorithms to support integrated disease risk management. In parallel, the research will validate highly specific and sensitive molecular diagnostic protocols and isolate lactic acid bacteria (LAB) strains with antifungal activity, in order to explore alternative biocontrol approaches.

**Methods:** Plant tissue samples will be collected monthly from high-risk areas in both Sicily and Malta and analyzed by real-time PCR using species-specific primers to detect target fungal pathogens. In parallel, local meteorological data will be collected systematically to support the development of predictive models. Historical and current climate datasets from Mediterranean regions will be processed and used to train machine learning and deep learning algorithms. LAB strains will be isolated from olive and citrus leaf surfaces and tissues, screened *in vitro* for antifungal activity, and subjected to metabolic profiling. Selected strains will undergo fermentation optimization to enhance the production of bioactive metabolites. This integrated approach combines epidemiological forecasting, molecular diagnostics, and microbial biocontrol within a framework designed for field applicability and sustainability.

**Discussion and Conclusion:** This research adopts an integrated approach combining predictive modeling with microbial biocontrol to enhance and innovate management strategies of plant disease in Mediterranean agroecosystems. The application of artificial intelligence to long-term climatic datasets is expected to support decision-making by identifying critical periods of disease risk and informing targeted interventions. At the same time, the use of rapid and sensitive molecular diagnostics, such as real-time PCR, strengthens the capacity for in-field pathogen detection and monitoring. The study also explores the potential of indigenous LAB communities associated with olive and citrus plants as biocontrol agents. The *in vitro* selection of antifungal strains, coupled with metabolic characterization and optimized fermentation protocols, represents a foundational step toward the development of microbial-based formulations. This study is situated within a broader effort to promote sustainable, integrated plant protection strategies aimed at reducing chemical inputs and enhancing crop resilience in the face of climate change. The synergistic use of predictive tools and biological agents may offer a robust framework for future phytopathological risk mitigation in key Mediterranean crops.

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## **Evaluation of feeding behaviour in growing bulls with an ear-tag accelerometer**

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**Introduction:** Precision farming relies on the availability of data collected automatically by sensors to increase the overall farm production efficiency. Feeding behaviour in cattle is one of the most monitored aspects, and many studies associate this trait with production, welfare, and health parameters.

**Methods:** This study aimed to evaluate whether the CowManager ear sensor (CowManager B.V.) is suitable for monitoring the eating time of growing bulls. For this purpose, 23 Italian Simmental bulls were equipped with an ear-tag accelerometer and video recorded for 5 days (HDR-AS50, Sony Corp.). For each day and for each pen, the three non-consecutive hours with the highest number of accesses to the feed bunks were selected. A trained operator visualised roughly 350 hours of records and annotated eating behaviour using Boris software (Behavioral Observation Research Interactive Software, v.8.19.1), an appropriate tool for live observation.

**Discussion and Conclusion:** The data recorded by the accelerometer were compared with visual observations with a 2-sided paired t-test, and a significant difference ( $p < 0.001$ ) was found with an average difference of 1.83 min/h. The Pearson coefficient (0.55,  $p < 0.01$ ) denoted a moderate positive correlation between the ear-tag accelerometer and visual observation according to the manual "Applied Statistics for the Behavioural Science" (5th edition). The concordance correlation coefficient of 0.52 confirmed this finding, suggesting that the results seem promising despite the difference between the data recorded. However, a more thorough evaluation of CowManager is needed to detect accurately the eating behaviour in growing bulls.

## **Influence of Spectral Characteristics of LED Lighting on Nutritional Quality of Vegetable Products: A Systematic Review**

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**Introduction:** Light plays a crucial role in driving plant growth and metabolism. Some light wavelengths promote the biosynthesis of secondary metabolites, increasing the concentration of phytochemicals and enhancing the nutrient and nutraceutical quality of the product. Hence, artificial lighting with light emitting diodes (LEDs), widely adopted in vertical farms, can significantly improve the quality of vegetables. By modulating the quantity, duration, and quality of light—specifically luminous intensity, photoperiod (the duration of light exposure, expressed in hours), and spectrum (wavelength composition)—it is possible to improve the chemical composition of functional foods. To understand the effects of LED lighting in vegetables grown in vertical farming, a systematic literature review (SLR) was conducted.

**Methods:** The SRL was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) methodology. To identify relevant studies, two major scientific databases—Web of Science (WoS) and Scopus—were utilized. A temporal range of 10 years, from 2015 to 2024, was applied to include the most recent and innovative research in the field. The following search string was explicitly designed to align with the objectives of the review:

TITLE-ABS-KEY ( ( LED OR "LED light" OR "vertical farm\*" OR wavelength ) AND ( "baby leaf" OR "edible flower\*" OR microgreen\* ) AND ( "bioactive compounds" OR "functional foods" OR "health benefit" OR nutraceutical\* OR osmoprotectant OR phytonutrient OR antioxidant OR "ascorbic acid" OR carotenoid OR chlorophyll OR glucosinolates OR "mineral composition" or nitrates OR phenolic OR pigment\* OR polyphenol OR "secondary metabolites" ) ) AND PUBYEAR > 2014 AND PUBYEAR < 2025 AND ( LIMIT-TO ( DOCTYPE , "ar" ) )

**Results and Conclusion:** The initial search retrieved 276 records. After removing 103 duplicates and screening titles and abstracts for relevance, 107 studies were selected for full-text analysis. The analysis shows a growing interest in this field over the last decade, with WoS consistently yielding more articles than Scopus. A thorough review of the articles is currently underway. So far, it has been noted that the primary focus is on microgreens, which account for over 70% of the collected articles. There are few references on edible flowers and baby leaves. The Brassicaceae family warrants special mention, as it appears to be one of the most studied taxonomic groups, represented in nearly 40% of the reviewed articles. The emphasis is clearly on understanding the role of LEDs in altering the quality and health profile of these products, as well as the potential mechanisms of action.

## Exploring the Genetic Diversity and Population Structure in three Sicilian horse breeds

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**Introduction:** Sicily still preserves some of the oldest European horse breeds. The presence of such diversity in a relatively limited territory which includes various environmental realities, constitutes a precious resource of genetic variability. Sicilian equine heritage is represented by Sanfratellano (SAN), Siciliano (SIC) and Purosangue Orientale Siciliano (PSO) breeds, known for their resilience and adaptability to a wide range of climatic conditions, sometimes even challenging. In recent years, a rapid erosion of animal biodiversity has been highlighted due to the development and spread of a few breeds with specialized aptitude; this process has consequently determined a drastic numerical contraction of the local breeds. In this study the analysis of genetic relationships between breeds aims to investigate the genetic variability, the origin and evolution of Sicilian horse breeds, to define strategies for the protection, management and valorization of this genetic heritage.

**Methods:** A total of 234 Sicilian horses (64 PSO; 63 SAN; 93 SIC) were genotyped with the Illumina Equine 80K array. Chromosome assignment and position for each marker were updated on the equine EquCab 3.0 genome assembly. After the quality control process, using PLINK 1.9, a total of 220 subjects and 58,642 SNPs were kept for the subsequent analyses. The within-population genetic diversity coefficients ( $H_o$  and  $H_e$ ) and the contemporary effective population size ( $cNe$ ) were estimated. The genomic structure was investigated using ADMIXTURE software, and represented by the circle plot obtained using BITE R package. Neighbour-Net was constructed from the estimated genetic distances using SplitsTree4 software. A multidimensional scaling analysis using SNPs genotyping data from public repositories was employed to frame Sicilian breeds within the diversity of the species.

**Discussion and Conclusion:** The SNP genotyping matrix of Sicilian horses was analyzed to identify the level of intra and inter breed diversity. Diversity indexes showed the highest diversity in SIC ( $H_e = 0.331$ ,  $N_e = 74.2$ ,  $F_{is} = -0.013$ ), while the lowest value was reported in PSO ( $H_e = 0.288$ ,  $N_e = 31.3$ ). The admixture analysis showed the differentiation between PSO and SAN reflecting the careful mating plans, and at the same time, the influence of PSO and SAN breeds on the structure of SIC. The aforementioned population structure was confirmed by Neighbor-Joining tree, which showed a clear distinction of PSO from other analyzed samples, as well as a consistent group of individuals belonging to SIC derived from a phylogenetic node common to the SAN. The multidimensional scaling analysis, based on identity by state distances, revealed a partial overlap between Siciliano and Sanfratellano breeds in a consistent group of saddle horses, while the Purosangue Orientale Siciliano was close to Arabian horses as expected. These are preliminary results that will be used to conduct further analyses to describe and characterize Sicilian horses, an essential and necessary requirement for the development and subsequent choice of strategies and techniques for the conservation of local breeds.

## **Attitude and perceptions of dairy cattle herders towards the compensation system for wildlife damages in North-Eastern Italy.**

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**Introduction:** Human-wildlife conflicts contribute to biodiversity decline and the abandonment of traditional grazing practices. Conserving biodiversity is crucial for maintaining healthy ecosystems, and it offers advantages, both economic and related to ecosystem services. Meanwhile, mountain dairy farming also provides ecosystem services (e.g., landscape preservation, mitigation of natural hazards). The negative interactions can manifest in different ways, from trophic competition with wild ungulates to livestock predation. Strategies to reduce these interactions include both prevention (e.g., fences) and mitigation measures, such as post-damage compensation. While compensation schemes are widely used, their effectiveness is often hindered by bureaucratic complexities, delayed payments, and failure to evaluate indirect damage. Such damages include lost services and increased stress for both animals and livestock herders (herein, herders), in turn diminishing herders' tolerance towards wildlife. Understanding herder attitudes and perceptions on these themes is therefore essential to develop proper management strategies aimed to enhance long-term human-wildlife coexistence in shared landscapes.

**Methods:** The study was conducted between 2024 and 2025 in the Autonomous Province of Trento, and data were collected through structured interviews with local dairy cattle herders. A stratified random sampling approach was employed to minimize potential biases and obtain a more representative understanding of the situation in the area. The frequency distribution of herder responses regarding their attitude and perceptions was analysed using R.

**Discussion and Conclusion:** The results of the interviews revealed that slightly more than half of the sample experienced wildlife damages from large predators, wild ungulates, or both. About livestock predations, the majority of herders highlighted the lack of genetic evaluation of lost animals (e.g., endangered breeds) as a key concern in compensation requests. With regard to indirect damages, almost all respondents considered the deterioration of herders' quality of life due to stress as a key underestimated damage. These findings underline a general dissatisfaction among dairy cattle farmers within the Autonomous Province of Trento concerning the existing post-damage compensation system, primarily attributable to the inadequate evaluation of indirect damages. Based on the results achieved, a collaboration between research institutions, wildlife policymakers, and local herders is essential to identify the most effective strategies to counteract the shortcomings derived by the non-evaluation of indirect damages. Furthermore, there is a need to further explore interactions between domestic and wild ungulates, particularly concerning trophic competition, which appears to be a topic requiring a review to assess the current state of knowledge. In conclusion, these findings not only provide insights for decision-makers but will also help guide our upcoming fieldwork and research activities.

## **Recovery of bioactive compounds and in particular eriocitrin from citrus processing wastes**

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**Introduction:** Nowadays, especially in the circular economy, the valorization of citrus by-products, as added value for companies, is emerging as a symbol of a sustainable and economically advantageous approach, reducing, or eliminating wastes and increasing profits. Eriocitrin is a natural compound widely present in the plant world and represents a molecule belonging to the class of polyphenols. It's a flavonoid found in citrus fruits and mainly in lemons and their by-products such as peels, residues after pectin extraction, molasses, washing water and finisher residues. Molasses come from an intermediate stage in the processing of the peel. At this stage, the peel is treated with lime and then pressed; the liquid obtained from pressing is rich in bioflavonoids and in eriocitrin. The extraction and recovery of this molecule is interesting because it has significant antioxidants, antidiabetic, antitumoral properties and for the development of functional ingredients, nutraceuticals as supplements and anti-browning agent.

**Methods:** Extraction can be carried out using solvents such as ethanol, methanol, dimethyl sulfoxide, and water-ethanol mixtures in powders derived from dried peelings and finisher residues, or in the fresh product. To improve extraction efficiency, this technique can be integrated with innovative methods such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE) or Pressurized Hot Water Extraction (PHWE) using only water as a solvent. For liquid by-products and mixed powders, specific adsorption resins are used to bind the bioflavonoids, which are then stripped by regeneration in ethanol. Qualitative and quantitative analyses are predominantly carried out using High Performance Liquid Chromatography.

**Discussion and Conclusion:** The valorization of eriocitrin from citrus processing wastes shows the use of innovative extraction techniques, such as ultrasound, and microwave treatments, which improve the yield of the process by degrading the cell wall of the starting matrix or facilitating contact with the solvent in the case of powdered by-products. The solvent is often a water-ethanol mixture, because eriocitrin, does not prefer ethanol absolute, as a polar molecule. The US patent (2002) shows that after mixing 200 g of FMC lemon powder with 1 L of water, centrifugation and elution on specific resin, a recovery of 96.8% is obtained with 70% ethanol, equal to 0.388 g of eriocitrin. The extraction of 1 g of lemon residues powder with UAE and 40 ml of a mixture of Et-OH and 50% water gave encouraging results of 20.71 mg/g dry weight of eriocitrin recovered. PHWE at 160°C for 5 minutes, pressure (10.24 Mpa) and water as solvent, shows how from 10 g of powder of dried lemon peel yields 30.41 mg/DW of eriocitrin. The design of low environmental impact plants for the recovery of eriocitrin could be a starting point for reducing citrus wastes and increasing the sustainability of the business.

## **Regenerative Agriculture in Mediterranean cropping systems: a long-term multi-source assessment**

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**Introduction:** Regenerative agriculture (RA) is a key strategy for climate change mitigation through soil carbon sequestration, improved water use efficiency, and sustainable land management. RA practices (minimal soil disturbance, permanent soil cover, and crop rotations) enhance soil health and resource efficiency, especially in vulnerable agroecosystems. Long-term assessments are essential to validate the agronomic and environmental benefits of RA and to support its broader adoption. This study evaluates the long-term effects of two tillage systems - minimum tillage (MT) and no-tillage (NT) - on a durum wheat cropping system over ten years (2014–2024) at the "Manfredini" experimental farm (CREA-CI, Foggia). The aim is to understand how these systems affect crop performance and resilience under varying climatic conditions by integrating field measurements with remote sensing tools.

**Methods:** The experiment compared MT and NT in a durum wheat cropping system on 1 ha plots with 4 replications over ten years. Agronomic data, including yield, phenology and grain quality, were collected annually. In later years, Sentinel-2 imagery was analysed using Google Earth Engine to derive NDVI, NDWI, and GNDVI indices. These remote sensing indicators were correlated with field data to assess crop vigour and soil–crop interactions under different tillage regimes. Agrometeorological variables and soil water availability were also considered to interpret seasonal and interannual variations in crop performance.

**Discussion and Conclusions:** Satellite derived indices showed good correlations with field observations, validating remote sensing as a reliable tool for long-term monitoring of RA systems. NT consistently exhibited higher NDVI values during terminal drought periods, particularly from stem elongation to grain filling, indicating improved crop vigour and water stress resilience compared to MT. This was attributed to better soil moisture retention and reduced evaporation under NT, enhanced by surface residue cover and improved soil structure. Yield gaps between NT and MT narrowed significantly in dry years, highlighting NT role in stabilizing yields under climate variability. Preliminary analysis suggests that soil organic carbon accumulation and improved water conservation are key drivers of RA benefits over the decade. The integration of satellite and field data provided valuable temporal and spatial insights, supporting a holistic evaluation of soil, plant and climate interactions. These findings underscore the potential of RA for climate-resilient agriculture, particularly in Mediterranean environments. The study highlights the value of integrated monitoring frameworks to inform sustainable land management policies and precision agriculture strategies. Overall, RA, when properly monitored and adapted, could contribute to food security, environmental protection, and long-term agricultural sustainability.

## Physiological responses of plants to abiotic stress in alpine environments

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**Introduction:** Climate change is putting increasing pressure on alpine ecosystems, particularly in the European Alps, where rising temperatures and changing precipitation patterns are altering key ecological processes. One of the most notable manifestations of this is the 'greening' phenomenon, characterised by shrub encroachment and changes in species composition, which threatens the structural integrity of alpine grasslands. These ecosystems, which are shaped by extreme climatic conditions and short growing seasons, are highly sensitive to climate variability. Rising temperatures coupled with declining summer precipitation could accelerate compositional changes, reducing endemic biodiversity and facilitating the dominance of competitive, generalist species. Against this backdrop, this project seeks to assess the combined impact of reduced precipitation (through rainfall exclusion) and increased temperatures on alpine grassland communities. This approach will allow us to study the impacts of these climate stressors, providing critical insights into the resilience and vulnerability of high-altitude grassland ecosystems in future climate scenarios.

**Methods:** The study takes place in an alpine grassland at ~2,000 m a.s.l. in Sappada, Friuli Venezia Giulia. The experimental design includes a control (no cover) and three treatments, using transparent plastic shelters, that reduce rainfall and increase temperature: (1) full-season cover, (2) early-season cover, and (3) late-season cover. Three key species (*Homogyne alpina*, *Carex ornithopoda*, *Sesleria caerulea*) were sampled to assess morphometric traits, pigment content (chlorophylls and carotenoids), and total biomass. Multispectral images were acquired using drones and close-range sensing to calculate vegetation indices, providing insights into plant physiological responses under different climate manipulation scenarios.

**Discussion and Conclusion:** We expect that temperature increase and reduction in moisture induced by the treatments will significantly impact the morphometric parameters of the analysed species. This would lead to a decrease in leaf area and a reduction in chlorophyll concentration, as well as an increase in carotenoids. Total biomass is also expected to decrease, particularly in treatments where plants are subjected to greater water and heat stress. However, cover could have a positive effect in the first part of the growing season by mitigating the harsh climatic conditions typical of high altitudes. In treatments where plants are covered only at this stage, opposite responses to those observed in other treatments could be seen. Multispectral images collected by drone and proximity sensors should show a decrease in NDVI as the physiological stress on the plants increases. Comparing images acquired at different scales will allow us to verify whether the drone-collected data are sufficiently representative. If so, aerial remote sensing could become an effective tool for rapidly monitoring the health of large areas of alpine grassland, helping to assess the impact of climate change on these sensitive ecosystems.



## **Pomegranate (*Punica granatum* L.) by-products extracts with high antioxidant and antimicrobial action as food additives in a circular economy and environmental sustainability perspective – "From waste to wealth" objective**

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**Introduction:** The increasing prevalence of resistance to conventional preservatives among foodborne bacteria represents a growing concern for global public health and food safety, driving the scientific community toward the identification and development of novel, effective, and sustainable control strategies. In this context, natural bioactive extracts obtained from food industry by-products—often considered waste—are gaining attention for their content of antioxidant and antimicrobial compounds. These compounds offer a promising and eco-friendly alternative to conventional chemical food additives, which are increasingly questioned due to health concerns and demand for natural alternatives. This PhD project focuses on the valorisation of pomegranate (*Punica granatum* L.) peels (PPs), a by-product representing approximately 50% of the whole fruit's weight, as a rich and sustainable source of natural antioxidant and antimicrobial agents. The aim is to optimize the extraction protocol, characterization, and functional application of these bioactive compounds in the context of food preservation and human health.

**Methods:** Enzyme-Assisted Extraction (EAE) and Ultrasound-Assisted Extraction (UAE) will be used to efficiently recover high-value compounds from PPs. Total phenolic content, antioxidant activity (via Folin-Ciocalteu and DPPH assays) and phytochemical profiling will be used to characterize the extracts. Their antimicrobial activity, using agar-well diffusion assay, will be evaluated against different pathogens. Interaction between the most promising extract and the human gut microbiota will be studied to understand the metabolic fate of the extract once ingested. Finally, a novel natural food additive incorporating PPE will be applied to a selected food to evaluate its effects on shelf-life, microbial stability, and overall quality.

**Discussion and Conclusion:** Pomegranate peels contain high levels of phenolic compounds, particularly hydrolysable tannins like punicalagin, ellagic acid, and anthocyanins, with punicalagin as the most abundant compound in the peel; it is known for its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The aim of this PhD project is to extract these valuable compounds to obtain high-value extracts as promising alternative to chemical additives, in particular preservatives. Enzyme-Assisted Extraction and Ultrasound-Assisted Extraction will be applied to maximize the yield and functionality of the extracted compounds, with particular attention to process sustainability and green chemistry principles. Extracts will be optimized especially against Gram-negative bacteria, which are typically more resistant than Gram-positive ones and pose a serious threat to food safety. Studying the interaction between PPE and gut microbiota is important to better understand the real safety of the extracts, including the biotransformation of some ellagitannins into other bioactive and more powerful compounds with promising antioxidant, estrogenic, anti-inflammatory, and anticarcinogenic activities. Finally, the integration of PPE into a specific food matrix will be examined to evaluate its practical feasibility, effect on shelf life, influence on microbial safety and on product quality. These objectives are perfectly in line with environment sustainability and growing demand for health-oriented products.

## **Arbuscular mycorrhizal fungi inoculation as sustainable tool to improve yield and phytochemical value of *Cynara cardunculus* L.**

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**Introduction:** *Cynara cardunculus* L., a species native to the Mediterranean region, encompasses both globe artichoke (var. *scolymus*) and cultivated cardoon (var. *altilis*), crops of increasing interest due to their ability to accumulate high levels of bioactive phytochemicals. Owing to their richness in polyphenols, flavonoids and sesquiterpene lactones, these crops are being widely explored as multipurpose plants for nutraceutical, pharmaceutical and bio-based applications. However, in Mediterranean agroecosystems, their sustainable cultivation is challenged by limited water availability, declining soil fertility and the need to reduce agrochemical inputs. The application of Arbuscular Mycorrhizal Fungi (AMF) may play a pivotal role in enhancing nutrient uptake, Water Use Efficiency (WUE), and plant tolerance to abiotic stress. Within this context, the present PhD project aims to investigate the contribution of AMF symbiosis to improving yield performance and phytochemical accumulation in *C. cardunculus* under the characteristic edaphoclimatic conditions of eastern Sicily.

**Methods:** Two field trials were conducted on an organically managed farm in the Gela district (CL, Sicily). The first, on globe artichoke (cv. Romanesco G1), followed a completely randomized design with three replicates to assess three AMF treatments (Rizocore®, AEGIS IRRIGA®, and an untreated control). The second, on cultivated cardoon (cv. Altalis 41), followed a split-plot design, testing two planting densities (2 vs. 4 plants/m<sup>2</sup>) as the main factor and AMF application (AEGIS IRRIGA® vs. untreated control) as the subplot factor. Plant physiological traits, biomass yield and phytochemical accumulation were evaluated, as well as soil AMF colonization and water content.

**Discussion and Conclusion:** This research highlights the role of arbuscular mycorrhizal fungi in enhancing the sustainability and functional traits of *Cynara cardunculus* under field conditions. In globe artichoke, preliminary data suggest that different AMF treatments can influence secondary metabolite production, particularly under water-limited conditions. In cultivated cardoon, the combination of mycorrhizal inoculation and optimized planting density promoted plant regrowth following repeated cuttings and improved both biomass and phytochemical yield. Ongoing analyses will further elucidate AMF colonization patterns and their relationship with morphological traits and phytochemical profiles. Overall, the results contribute to advancing sustainable and resilient *C. cardunculus* cultivation in Mediterranean agroecosystems.

## **Assessing the Value of Sustainability in the Citrus Supply Chain**

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**Introduction:** Agriculture is the main pillar for ensuring food security in a context of a growing global population, but it is responsible for the consumption of natural resources and emissions, so sustainable practices could be the key to improve agri-food supply chains, such as that of citrus fruits. Producers are the first link in the supply chain, responsible for implementing more sustainable cultivation and production practices. So, a TPB (Theory of Planned Behaviour) questionnaire was implemented to evaluate the awareness of citrus farmers about sustainability issue and propensity to join to voluntary sustainability certifications.

**Methods:** This study adopted three socio-psychological constructs, Attitude (ATT), Subjective Norm (SN), and Perceived Behavioral Control (PBC), derived from the Theory of Planned Behavior (TPB). The TPB questionnaire has been implemented on Google Forms, open-ended, multiple choice, or Likert scale questions were included. Then the responses were collected directly through individual interviews by organizing face-to-face meetings or via phone calls.

**Discussion and Conclusion:** The three pillars of sustainability have been evaluated. From an economic point of view, sustainable practices require higher initial costs for certification, for some companies it represents a constraint, in particular for smallholders, although subsequently higher market prices would ensure economic returns, also favored by consumers' willingness to pay more for certified or local products. Certifications also ensure social sustainability, able to enhance rural employment and to avoid the rural land abandonment, as well as to have certainly environmental benefits. To increase producers' bargaining power is fundamental the collaboration into cooperatives and organization, to reduce inequalities along the supply chain. In this context, the role of public institutions is crucial, policymakers support can accelerate the adoption of sustainable models, through initial economic incentives especially for smallholders.

## **Enhancing red raspberry (*Rubus idaeus*) breeding with molecular tools: QTLs for fruit quality and powdery mildew resistance**

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**Introduction:** The cultivation of red raspberry (*Rubus idaeus*) have experienced significant growth over the last two decades, driven by the development of new cultivars and increasing consumer interest in healthy diets. However, the inherently delicate nature of raspberries leads to a short shelf life, posing a major challenge to their marketability. Modern breeding programs aim to develop varieties with improved pomological traits-such as firmness, color and brightness-that remain stable post-harvest and throughout the supply chain. In recent years, breeding efforts have also expanded to incorporate resistance to diseases which are becoming more prevalent due to climate change, like raspberry powdery mildew. This research aims to identify quantitative trait loci (QTLs) and candidate genes associated with desirable fruit quality traits and disease resistance. The ultimate goal is to develop molecular markers that can support marker assisted selection (MAS) in raspberry breeding programs.

**Methods:** For each trait of interest, a F<sub>1</sub> mapping population will be developed through targeted crosses between selected raspberry genotypes. The resulting plants will be clonally propagated and evaluated over three growing seasons. Phenotypic traits to be measured include: fruit firmness, color, brightness and tolerance/resistance to powdery mildew. Genomic DNA will be extracted from young leaves and genotyped using double digest restriction site-associated DNA sequencing (ddRAD-seq). High quality SNP (Single Nucleotide Polymorphism) markers will be generated and used to construct genetic linkage maps. QTL analysis will be conducted using the R package R/qtl, employing a custom script to identify genomic regions associated with the measured traits.

**Discussion and conclusions:** Although the mapping population derived from a cross between the cultivar 'Glen Moy' and 'Latham' has been widely used in the past for SNP discovery in raspberry, this study utilizes four novel F<sub>1</sub> population to broaden the genetic basis for QTL mapping. This multi-population approach enhances the power to detect loci associated with key pomological and resistance traits, enabling the identification of robust markers and facilitating the comparisons with results obtained by other research groups investigating genes involved in raspberry ripening and tolerance/resistance to emerging diseases. In addition, the use of the ddRAD-seq technique offers the potential to improve marker quality compared to the previously used GbS (Genotyping by Sequencing) method. The findings from this research are expected to significantly contribute to the discovery of QTLs, the assessment of their effects and the identification of associated markers, which can be utilized in marker assisted selection. Ultimately, this will enhance the efficiency and precision of raspberry breeding, providing new molecular tools for the development of high quality and disease resistant cultivars better suited to meet both growers' needs and market demands.

## **Social Networks and Sustainable Innovation in the Etna DOC**

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**Introduction:** This PhD project explores the adoption of sustainability-oriented innovations (SOIs) in the Etna DOC wine sector, focusing on the role of territorial organizations, particularly the Etna DOC Protection Consortium, as enablers of innovation in contexts dominated by micro and small enterprises. In response to pressing sustainability challenges, the study aims to understand how innovation processes are embedded in local production systems and how formal and informal networks support knowledge exchange. By investigating the interplay between innovation and social capital, the project highlights how territorial identity, shared rules, and collaborative practices foster the transition toward more sustainable and resilient wine systems. The Etna DOC Consortium is analyzed as a key broker in coordinating strategies, promoting open knowledge circulation, and enhancing the cultural and economic value of the wine territory.

**Methods:** The research applies a multi-method approach combining systematic literature review, social network analysis (SNA), and participatory tools such as stakeholder mapping, focus group and semi-structured interviews. The project also includes clustering methods to identify functional subgroups and patterns of innovation diffusion and cooperation among local enterprises. Empirical work focuses on the Etna DOC area, examining how the Consortium influences relational structures and supports the uptake of sustainable innovations across the local wine cluster.

**Discussion and Conclusion:** The project aims to identify the key factors that influence the adoption of SOIs in the Etna DOC wine sector. SNA will be used to map the relationships among producers and between enterprises and institutions, highlighting patterns of interaction, centrality, and cohesion. Special attention is devoted to the Etna DOC Protection Consortium, examined in its role as an intermediary actor fostering collaboration, knowledge circulation, and strategic alignment.

The study investigates how formal and informal ties among micro and small enterprises affect access to information, learning processes, and innovation uptake. Clustering methods will help identify functional subgroups within the local wine system, offering a more detailed understanding of cooperation dynamics and innovation diffusion. By combining network analysis with qualitative insights, the research seeks to assess how territorial organizations contribute to strengthening local governance and collective strategies. While grounded in the specific context of the Etna DOC, the project aims to build a transferable analytical framework applicable to other wine-producing regions. Ultimately, it contributes to broader discussions on rural innovation systems, GI governance, and the role of intermediary institutions in supporting sustainable development pathways in agri-food clusters.

## **The Exploitation of Nanotechnology in Herbicides and Bioherbicides: A Novel Approach for Sustainable Weed Management**

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**Introduction:** By 2050, the global population is expected to surpass 9.7 billion, requiring a 70% increase in agricultural productivity to ensure food security. Agriculture, already occupying half of the world's usable land, faces major challenges such as rising temperatures and water scarcity. Weeds are the leading biotic factor reducing crop yields - by 20–50% annually - causing economic losses of up to USD 32 billion and threatening biodiversity. Despite various control methods, synthetic herbicides remain dominant, with 1.7 million tons used each year. Their overuse leads to herbicide resistance, environmental pollution, and harm to non-target organisms. In response, integrated weed management strategies, supported by policies like the European Green Deal, promote reduced reliance on hazardous chemicals. Bioherbicides based on allelochemicals offer a sustainable alternative but are limited by low stability and efficacy. Nanotechnology offers a promising solution by enhancing bioherbicide performance through controlled release, improved plant penetration, and reduced environmental impact.

**Methods:** The methodology adopted for this review followed PRISMA guidelines. A preliminary Google Scholar search defined the core idea. Subsequently, comprehensive bibliographic searches were conducted on Scopus and Science Direct databases, using the following keywords inside the research string: "nanotechnology" AND "weed management" OR "herbicide" OR "bioherbicides". Strict exclusion criteria were applied: non-English articles, non-original research (e.g., reviews, book chapters), and unrelated topics were excluded. A second screening further removed studies focusing on environmental detoxification or ecotoxicological profiles of nanoparticles. Ultimately, only 30 documents containing quantitative data on nanoherbicide and nanobioherbicide efficacy were selected for analysis.

**Discussion and Conclusion:** Nanotechnology offers a promising approach for enhancing weed control efficacy and sustainability. Nanoparticles' unique characteristics, such as reduced size, high surface area, and chemical properties, enable controlled release, improved plant tissue penetration, and reduced environmental impact. Nanoformulations of conventional herbicides can boost their effectiveness and limit dispersion, while nanobioherbicides, derived from natural substances like essential oils or plant extracts, present a viable, eco-friendly alternative. For instance, silver nanoparticles (AgNPs) synthesized from plant extracts show significant potential as bioherbicides. However, a thorough risk assessment is essential for agricultural nanotechnology. Future research must focus on evaluating nanoparticle bioaccumulation in soil and plants, their toxicity to non-target organisms, and long-term impacts on human health and the environment. There is an urgent need for field experiments to confirm efficacy and selectivity under real-world conditions. Additionally, technical aspects like application methods and the stability of nanoformulations over time require further investigation by scientists and the agrochemical industry to develop marketable products.

## **Toward modern pesticide use reduction strategies in advancing precision agriculture: A bibliometric review**

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**Introduction:** In a scenario in which sustainability, productivity and environmental responsibility converge, the reduction of agrochemical inputs—specifically Plant Protection Products (PPPs)—represents a pivotal challenge for modern agriculture. While Integrated Pest Management (IPM) has guided efforts to contain pesticide use, the emergence of Precision Agriculture Technologies (PATs) offers a transformative opportunity to optimize the application of PPPs through highly targeted, data-driven interventions. The article under analysis provides a rigorous and systematic bibliometric review of the scientific production (2015–2024) on this theme, aiming to map how PATs are contributing to the reduction of pesticide usage, what technologies are at the core of this innovation process, and how the research community is evolving in terms of collaborations, topics and methodological focus.

**Methods:** A structured search was performed using Scopus and Web of Science databases, resulting in a final dataset of 1,115. To ensure relevance and comprehensiveness, queries were formulated using Boolean operators, wildcards, and exact phrase matching. Only journal articles in English were included. The records were cleaned through deduplication and metadata normalization to generate a coherent dataset. These were then analysed using citation analysis, co-authorship mapping, co-citation networks, and keyword co-occurrence methods. Articles were categorized into seven major technology areas: Artificial Intelligence, Sensors, Data Processing, Drones, Robots, Variable Rate Technologies and Pulse Width Modulation. Some articles did not find a match and have therefore been included in the “General Topic” category. The classification was non-exclusive, allowing individual articles to belong to multiple categories to reflect their interdisciplinary nature. Visualization and statistical analysis were conducted using *VOSviewer* and *RStudio*.

**Discussion and Conclusion:** Results revealed a rapid growth in scientific output, particularly after 2020, with China, the USA, and India as the top contributing countries. The co-citation analysis further identified three main thematic clusters: AI and vision systems, robotics and mapping for weed management, and precision spraying technologies. AI (497 articles), Sensors (415), and Data Processing (360) were the most prevalent topics, forming the technological core of pesticide reduction strategies. Over 50% of the articles addressed multiple technologies, highlighting the integrative nature of current research. AI appeared strongly linked to other domains, especially Sensors and Robots, suggesting its centrality in modern digital farming. By contrast, PWM remained a niche topic with limited integration into broader automated systems. This review not only maps the current state of the art but also highlights the growing need for PATs to become increasingly standardized and interconnected, while addressing their economic impacts on farms. Future research should improve PAT interoperability with open-source platforms and study socio-economic and environmental impacts on biodiversity, soil, and water for sustainable farming.

## **Integrative Assessment of Citrus Genotypes under Deficit Irrigation: Physiological and Biochemical Perspectives**

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**Introduction:** The global demand for increased food production underscores the need for agricultural systems that are both high-yielding and environmentally sustainable. In Mediterranean agroecosystems, where water scarcity is a major limiting factor, improving water-use efficiency and enhancing stress resilience through genetic and agronomic strategies are critical priorities. The determination of crop water status (CWS) is crucial for promoting sustainable irrigation and precision agriculture. Water stress in plants can be evaluated through plant-based measurements, such as non-automated methods for measuring leaf or stem water status, or even through biochemical indicators, employing quantitative techniques capable of detecting subtle molecular changes within biological tissues. This study explores the physiological and biochemical responses of different *Citrus sinensis* (L.) Osbeck rootstock-scion combinations to deficit irrigation. The experimental design aimed to evaluate the genotype dependent variability in response to water stress, revealing the adaptive potential of rootstock-scion interactions.

**Methods:** Deficit irrigation (DI) was supplied under field conditions in Sicily, Italy, during the 2024 growing season. Full irrigation (FI) served as the control. Deficit irrigation treatment supplied a water deficit of 60%, compared to control treatment (FI), which provided the full level of irrigation (100% ETc). Water stress levels were identified using an integrated approach combining in-field assessment of crop water status through stem water potential (SWP) measurements by Scholander pressure chamber, and spectrophotometric quantification of biochemical markers of oxidative stress (hydrogen peroxide H<sub>2</sub>O<sub>2</sub> and malondialdehyde MDA).

**Discussion and Conclusion:** Significant genotype-dependent variations were observed in both physiological and biochemical parameters, highlighting the importance of rootstock selection in modulating drought response. The factors analyzed included the water regime (WR), day of the year (DOY), and rootstock-scion interactions. Notably, a significant genotype × WR interaction was identified for the Carrizo-MELI and Carrizo-TDV genotypes (used as controls) in relation to SWP. Likewise, significant differences in H<sub>2</sub>O<sub>2</sub> and MDA content were also found among the M5761-MELI, Carrizo-MELI and Carrizo-TDV genotypes. These results contribute to the identification of citrus genotypes with improved water stress tolerance and support breeding strategies aimed at enhancing the resilience of fruit tree crops under climate change scenarios. Integrating physiological and biochemical measurements can significantly enhance the accuracy and functional relevance of CWS detection, particularly when aiming to optimize irrigation scheduling under water-limited conditions, as often occurred in Mediterranean cropping systems. Biochemical indicators offer a sensitive means to detect early or subtle responses to water stress, which may not be visually or spectrally evident. These evaluations will be further integrated with transcriptomic analyses aimed at elucidating the gene expression patterns associated with the response to water stress, in order to better understand the molecular mechanisms underpinning stress adaptation.



## **Integrated management of *Albugo occidentalis* in spinach: climate-driven emergence, varietal resistance, and sustainable control.**

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**Introduction:** Climate change is profoundly affecting Mediterranean agriculture, increasing the spread and severity of fungal diseases. In the Capitanata area (Apulia, Southern Italy), spinach cultivation is particularly threatened by white rust, caused by *Albugo occidentalis*. Rising temperatures and relative humidity, exacerbated by extreme weather events, promote the formation of microclimates favorable to disease development, especially in high-density crops irrigated by sprinklers. White rust leads to significant yield and quality losses, with growing economic impact. At the same time, European regulations call for a drastic reduction in chemical pesticide use by 2030, encouraging sustainable alternatives. The goal is to identify resistant spinach cultivars and evaluate the effectiveness of alternative biological products under open-field conditions, in order to support an integrated strategy for disease management.

**Methods:** In greenhouse conditions, several spinach cultivars were tested by artificial inoculation with *A. occidentalis* and maintained under high humidity using a humidifier. Disease incidence was monitored through regular assessments and the calculation of the McKinney index. In parallel, various biological products were applied under open-field conditions, including formulations based on antagonistic microorganisms and resistance inducers. Assessments were conducted weekly, recording plant responses to treatments starting from the natural onset of the disease, which was triggered by rising temperatures. All collected data were compared to identify the most effective management strategies.

**Discussion and Conclusion:** The results confirm that climate change promotes the development of diseases such as *A. occidentalis*, upsetting phytosanitary balance in open-field conditions and reducing the effectiveness of traditional strategies based solely on chemical control. High humidity and elevated temperatures – environmental conditions that are occurring more frequently – enhance the incidence and severity of white rust, leading to yield losses that can exceed 30%. The study revealed significant differences among cultivars and treatments. Under controlled conditions, the cultivars “Houston” and “Warthog” showed good resistance to infection. In the field, the formulation based on *Bacillus subtilis* proved to be the most effective among the tested biological products. These findings support the validity of an integrated pest management (IPM) approach, combining genetic selection, biocontrol, and agronomic practices to reduce reliance on synthetic fungicides. The adoption of IPM strategies aligns with the goals of the European Green Deal, which promotes more sustainable agriculture aimed at protecting both the environment and public health.

## **Sustainable control of fungal soil-borne pathogens on tomato crops by phytocompounds**

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**Introduction:** The use of chemical fumigants against soilborne fungi in agriculture represent a serious problem concerning the environmental impact, health of humans and animals. The European laws promote the sustainable use of pesticide by Directive 2009/128/EC, applied in Italy with D.L. 150/2012, to reduce the chemicals in agriculture. Recently, scientific research is addressed to study microorganisms as biological control agents and plants rich of putative bioactive phytocompounds. The current study aims to find putative botanical fungicides as sustainable alternatives from spontaneous plants capable to control the fungi. The study has been based on plants containing putative compounds bioactive against soilborne fungi. The spontaneous plants selected were *Matricaria chamomilla*, *Raphanus sativum* and *Solanum lycopersicum*, and the fungi *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Athelia rolfsii*. Further objective was to assay the effectiveness of phytocompounds with potential antifungal activity by extraction in water (WEs), ethanol (EtEs) and methanol (MetEs), *in vitro* and *in vivo*.

**Methods:** The plants used in this study have been collected, dried and grinded into powders and used for extraction in water, ethanol and methanol (1:10; w:v). The extracts included in PDA were tested against the fungi at four concentrations (25%, 50%, 75%, 100%). Measurements of perpendicular diameters were taken every 48 hours. During the greenhouse trial, pots of 1L were inoculated with the three fungi and treated with WEs, then tomato seedlings were transplanted. After 50 days, data of disease severity, measurements and biomass analyses and mycological analyses were carried out. Statistical ANOVA was performed to analyze the dataset obtained.

**Discussion and Conclusion:** This study highlighted that plant extracts are useful means to inhibit fungal soilborne pathogens. The *in vitro* trials showed that *A. rolfsii* was significantly inhibited by 100%-WEs of all botanical species used, while the 100%-EtEs and 100%-MetEs were never able to reduce the mycelial growth. The same results were obtained from the other fungi, demonstrating that the water extraction was more effective than ethanol and methanol for extracting important bioactive phytocompounds able to significantly limit the fungal growth of all fungi assayed. For these reasons, WEs have been chosen to perform the greenhouse trials, that confirmed to be effective to control fungi inoculated. The mycological isolation carried out on roots and collars showed that the tomato plants treated with WEs of all three plants, appear to be more vigorous and healthier with very low fungal infections. These topics are part of a broader doctoral project that aims to search and identify plant metabolites with antifungal activity; to assay *in vitro* their putative phytotoxicity; to study the response of plants treated to ascertain potential SAR (systemic acquired resistance) and SIR (systemic induced resistance) mechanisms; and to understand which genes are involved in resistance mechanisms.

## **Global pattern and thematic evolution in robotic applications for the transformation of the food manufacturing sector**

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**Introduction:** The food industry is undergoing a swift transformation due to the integration of robotics that enhances efficiency, consistency, and safety in food manufacturing and processing. Robots are utilized in manufacturing to execute regular activities such as packaging, sorting, and palletizing, hence improving operational efficiency and reducing labor costs. In food processing, robots can facilitate complex operations such as cutting, chopping, kneading, cleaning, mixing, and cooking with exceptional accuracy. This guarantees uniform outcomes and reduces unnecessary waste, making them suitable for various culinary applications. Robotic arms equipped with sensors enable precise quality control and help maintain food safety standards. The integration of robotics with IoT, AI, and big data is driving further innovation in food manufacturing, supporting sustainable practices by optimizing resource use and reducing environmental impact.

**Methods:** This study provides a comprehensive statistical analysis and critical interpretation of recent advancements in robotics in the food processing sector, offering a detailed evaluation of current research trends and existing knowledge gaps. A bibliometric analysis of research pertaining to robotics within the food sector was conducted, aiming to identify key authors, significant studies, and emerging themes for researchers, scientists, and industry stakeholders to stimulate new investigations and practical applications in robotics solutions for food manufacturing.

**Discussion and Conclusion:** The results demonstrate a significant and rapid increase in academic productivity, evidenced by a marked rise in publications and citations, particularly since 2020. The findings indicate that robotics applications in the food sector can be classified into three primary categories: (1) robotics technologies for automation tasks, (2) deep learning enabled robotics for image processing, and (3) a multidisciplinary cluster integrating artificial intelligence, Internet of Things, big data, and blockchain technologies. Many studies focus on post-harvest operations such as harvesting, transportation, and image segmentation. In the meat industry, robotics is primarily used for slaughtering and processing plant operations. Examining thematic evolution from 2000 to 2025 reveals a shift in research focus from general terms like 'automation' to more specific themes such as 'deep learning' and 'robotics', highlighting the development and diversification of robotics applications in the food sector. The global collaboration patterns assess the field's evolution; for instance, collaboration was mostly within a continent rather than between continents. The growing global engagement of scientists in this field validates its evolution as a rapidly expanding and important area. However, much of the existing research remains experimental, and further work is required to enable widespread industrial adoption.

## **Greenhouse Gas Emissions from Soil and Strategies for Their Mitigation in the agroecosystems in Mediterranean environment**

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**Introduction:** Global demand for agricultural products is expected to increase by approximately 50% by 2050, posing a significant challenge in the context of climate change and environmental degradation. Conventional farming practices, characterized by intensive input use and poor soil management, are accelerating soil degradation, threatening food security, and significantly contributing to greenhouse gas (GHG) emissions. Agriculture accounts for approximately 10–14% of total anthropogenic GHG emissions, being responsible for a considerable share of methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and carbon dioxide (CO<sub>2</sub>) emissions. Therefore, sustainable soil management is central to climate mitigation and adaptation strategies. The aim of this study is the evaluation of the effects of conservative soil management practices, namely crop rotation, soil tillage and fertilization managements on agro-environmental outcomes, including crop development and yield, greenhouse gas emissions at the soil-atmosphere interface, soil organic carbon storage, soil water capacity and crop water use efficiency.

**Methods:** The study, conducted as an open-field experiment, focuses on crop rotation, fertilisation and soil tillage. The first factor compare monoculture versus crop rotation schemes including durum wheat (*Triticum turgidum* subsp. *durum*) and a set of rotational winter crops, faba bean (*Vicia faba*), safflower (*Carthamus tinctorius*), and crambe (*Crambe abyssinica*), selected for their contrasting agronomic traits and potential to improve soil health. The second factor regards conventional tillage versus reduced or no-tillage systems. The third factor compare mineral versus organic fertilization and non-fertilised control. Crop growth and yield will be assessed with plant measurements of leaf area index, aboveground biomass, and grain production and they will be related to remote sensing (NDVI). Greenhouse gas emissions at the soil-atmosphere interface will be continuously monitored using a photoacoustic gas analyzer (Innova 1512, Lumasense), connected to a 24-channel multipoint sampler (1409, Lumasense) and 18 flow-through chambers. Soil moisture will also be tracked to assess the water-saving potential of the tested practices.

**Discussion and Conclusion:** Reducing greenhouse gas (GHG) emissions from agricultural soils is essential for a sustainable agriculture. Agronomic practices such as the use of organic fertilizers, reduced soil tillage, conservative agronomic management, can lower emissions without compromising productivity. However, their effectiveness depends on climatic and pedological conditions, requiring site-specific strategies and standardized monitoring systems. Further research will be necessary to define optimal use methods of agronomic inputs and adoption of conservation agriculture techniques. This study outcomes will help to identify the most suited soil and crop management through the support of crop modelling.

## **Study of the ecophysiological and productive response of Nerello Mascalese subjected to four different canopy management techniques**

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**Introduction:** In the face of ongoing climate change, viticulture is experiencing significant challenges, particularly regarding the alteration of grape quality parameters. Among the most evident effects are shifts in ripening dynamics, sugar accumulation, and acidity loss, which threaten the typicality and balance of wines. These trends call for a critical reassessment of traditional vineyard management practices, especially those related to canopy architecture and source–sink balance. In this context, the present study explores four distinct canopy management strategies as potential adaptive tools. The research is being conducted under the unique pedoclimatic conditions of Mount Etna, a high-altitude volcanic environment increasingly exposed to extreme weather events. The objective is to investigate how these practices influence the eco-physiological behavior, yield components, and grape composition of *Nerello Mascalese*, the principal native red variety of the Etna region.

**Methods:** The study began in 2025 and is being carried out over two consecutive seasons in a commercial vineyard located on the northern slope of Etna, where four agronomic practices are being applied: late winter pruning (BBCH 12), early shoot trimming (BBCH 73), late shoot trimming (BBCH 80), and late defoliation (BBCH 80). Phenological performance is being monitored by examining the main vine developmental stages, such as bud break, flowering, veraison, and ripening. Water potential and gas exchange, as well as quantitative and qualitative production parameters, are being assessed on a monthly basis.

**Discussion and Conclusion:** It is believed that the fruit ripening dynamics can be modified through the calibrated carbon limitations induced by canopy interventions. The forward shift in phenology should delay sugar accumulation and organic acid degradation throughout the season, while simultaneously promoting a satisfactory phenolic and aromatic profile.

The effectiveness and practical relevance of the proposed canopy management techniques will be evaluated in relation to modern commercial quality standards, with the aim of identifying vineyard strategies capable of enhancing resilience to climate change while safeguarding varietal identity and market competitiveness. The results are expected to support a selective, context-specific application of canopy interventions as a promising approach to adapt Mediterranean viticulture to increasingly challenging environmental conditions.

## **Dissection of genetic bases of plant salt response through the study of halophyte plants as model species and application to Mediterranean crops**

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**Introduction:** Climate change is intensifying the exposure of crop species to several abiotic stresses, such as high soil salinity, thus compromising yield and quality. This project aims to identify the molecular mechanisms involved in response to such stress using *Cakile maritima*, a halophyte naturally adapted to extreme environments, as a model species to identify candidate genes (CGs) associated with both short and long-term responses to salinity. The most promising CGs will be evaluated in durum wheat (*Triticum turgidum* var. *durum*) to assess their expression profile under saline stress. CGs will be studied in a panel of tetraploid wheat genotypes endowed with different levels of salt tolerance, to identify any variation in gene sequences or gene expression correlating with different performances under stress conditions. Finally, the project aims to develop suitable molecular markers to be employed in MAS (Marker Assisted Selection) of resilient varieties to be ultimately employed in more sustainable Mediterranean agriculture systems.

**Methods:** Putative CGs involved in physiological and metabolic mechanisms of salt adaptation were searched for in the model halophyte *Cakile maritima* by a targeted literature review. Leaf samples were collected from *Cakile* plantlets grown under control (0 mM) and treated (100, 400 mM NaCl) conditions, at 0, 6, 24, 48, 72 hpt. Total RNA was extracted and retrotranscribed into cDNA. CGs sequences were retrieved from *C. maritima* draft genome, available on phytozome database, and used to design specific primer pairs to run gene expression studies by Real-Time PCR. Transcriptome was also undergone RNAseq for large-scale identification of DEG (*Differentially Expressed Genes*) between control and treated plants.

**Discussion and Conclusion:** Genetic analyses were conducted on *Cakile maritima* leaf and root samples, collected at different levels and durations of NaCl supply. Sequences of CGs putatively involved in plant response to salt were retrieved from recent scientific literature related to both halophytes and *Arabidopsis thaliana*. Although a glycophyte, *Arabidopsis* is a model species with a fully annotated genome sharing a high similarity level with *Cakile*. By means of suitable bioinformatic tools, CGs sequences were isolated from *Cakile* draft genome and used to design primer pairs for gene expression study by Real-Time qPCR. Primers were checked for specificity and amplification efficiency on both gDNA and cDNA extracted from *Cakile* plants grown under unsalted and salted conditions, and further optimized for RT-qPCR reactions. Results are expected to shed light on specific gene expression patterns related to early or late plant response to salt, thus validating data from scientific literature. Moreover, RNA-Sequencing run on the same samples is enabling a broader gene profiling at the transcriptome level, thus unveiling large-scale differentially expressed genes between control and treated plants and detecting additional salt molecular targets. Preliminary results on model *Cakile* will pave the way to the dissection of genetic bases of salt response in durum wheat.

## **Innovative strategies for oxidation protection in pistachio products**

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**Introduction:** The most significant challenge facing the pistachio industry is the reduction in shelf life due to fungal contamination and the consequent possible presence of aflatoxin B1, hull browning and lipid oxidation during the postharvest period. Fat oxidation and the formation of hydroperoxides in unsaturated fatty acids, during the storage of pistachio powder directly impact its quality level.

No compound has yet been identified in scientific literature that acts as a marker for identifying, in qualitative and quantitative terms, the bitter and/or spicy flavour of pistachios or pistachio-based products. Several compounds may be responsible for bitterness and considering that most nuts are roasted at high temperatures, roasting can induce complex chain reactions, including the Maillard reaction, caramelization, and lipid oxidation. Based on the literature and cross-checking among the chemical compounds responsible for bitter flavours, evidence of flavours attributable to the spicy/bitter/pungent attribute has never been found in pistachios.

**Methods:** The PhD thesis project (founded by BMC srl) has the aim to identify the quality marker in pistachios responsible for the occurrence of an abnormal flavour that can be described as spicy, pungent, or bitter. Chemical analysis will be conducted to assess the degree of oxidation of pistachio products over their shelf life. This analysis will use official methods to determine free acidity, the peroxide number, and the anisidine value. Additionally, linoleic acid content will be monitored using high-performance liquid chromatography (HPLC) as a potential indicator of abnormal flavours in the product.

**Objectives of the PhD project:** Studying the kinetics of lipid oxidation and the products of the Maillard reaction could help identify the marker responsible for bitter flavours in the raw material and pesto. Once identified and quantified, a correlation could be established between the intensity of flavour perception and production technology. Additionally, innovative strategies will be developed to control and mitigate lipid oxidation, potentially maintaining the quality characteristics of farm products throughout their shelf life. In this context, we will test bioactive compounds and aromatic plant extracts to evaluate their effectiveness in counteracting lipid oxidation.

## **Bio-products to mitigate the adverse effect of climatic changes on crop performance**

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**Introduction:** The global demand for food has recently grown significantly due to population increase, and the farming sector is under pressure to produce enough food to satisfy the world's food security. Climate change is intensifying the frequency and severity of abiotic stress such as drought, salinity, and high temperatures, with severe consequences on crop productivity. This project aims to evaluate the biostimulant potential of various bio-products derived from organic waste (FORSU), vermicompost, and microalgae extracts on crops under abiotic stress.

**Methods:** The products will be obtained by different kinds of extractions such as alkaline hydrolysis, solvent both organic and inorganic extraction. In order to evaluate their biostimulant potential on crop species such as tomato, pepper, and lettuce, the extracts will be tested at different concentrations on a laboratory scale. The best extract preparation and optimal concentration will be selected for field trials. Treatments will be applied via foliar spray or soil drench. Physiological and biochemical parameters, including growth rate, nutrient uptake, oxidative damage, and antioxidant responses will be monitored.

**Discussion and Conclusion:** The extracts from organic waste, vermicompost and microalgae extract are prepared. The first formulation tested was a microalgae-extract, applied as a foliar treatment on tomato plants under water stress conditions. This initial trial will assess the potential of microalgae extract in improving stress resilience. The result from this approach is expected to highlight sustainable biostimulants solutions that can significantly enhance crop productivity under stressful environmental conditions.



## Are Volatile Organic Compounds Biomarkers of Grape Trunk Diseases Infection?

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**Introduction:** Grapevine trunk diseases (GTDs) are among the most widespread and challenging diseases to manage worldwide. This disease complex involves a wide range of fungal pathogens, which often act as consortia that vary depending on the grape variety and environment. Cultivation practices, such as pruning, generate multiple wounds that act as the main entry point for these pathogens. However, the development of symptoms in young vines suggests that infections may originate in the field or nursery. Volatile organic compounds (VOCs) are metabolites derived from primary and secondary metabolism in all living organisms, and their ecological function has been widely studied. In recent years, VOCs have attracted interest as potential disease diagnosis biomarkers. The aim of this study was therefore to characterise the VOCs profiles associated with the causal agents of GTDs, and to identify specific compounds as infection biomarkers for an early detection system.

**Methods:** *Fomitiporia mediterranea*, *Phaeoacremonium minimum*, *Botryosphaeria* spp., and *Neopestalotiopsis clavispora* fungal strains were grown at 25 °C for 10 and 5 days respectively, on three different media: PDA, PDA supplemented with Cabernet Sauvignon and Sauvignon blanc sawdusts (10%) in sterile vials (10 mL). Non-inoculated media served as control. The VOCs released in the vials headspace were sampled by a fiber DVB/CAR/PDMS and analysed by SPME a combined with GC–MS technique.

**Discussion and Conclusion:** Numerous VOCs were detected in all samples. The four fungal species exhibited distinct VOCs profiles. In particular, *F. mediterranea* produced higher levels of esters and ethers, while *Botryosphaeria* spp. produced higher levels of alcohols and ketones. Both, *P. minimum* and *N. clavispora*, were characterised by the production of carboxylic acids. However, VOCs production and expression were greatly influenced by the growth substrate, although not for all fungi in the same way, probably due to the activation of specific fungal metabolic pathways. The ten most abundant compounds for each fungus were selected on the assumption that there may be potential disease biomarkers among them. These sets proved to be unique for each species, both qualitatively and quantitatively, suggesting potential diagnostic value. However, further research is needed to assess whether such compounds are also emitted by the plant during the infection process. These findings provide promising insights into the development of non-invasive diagnostic tools for the early detection of GTDs in nursery and vineyard.

## **Evaluation of removal efficiency in full-scale constructed wetlands for wastewater treatment and reuse: two Sicilian case studies for small settlements.**

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**Introduction:** The Mediterranean is highly vulnerable to climate change, facing water scarcity worsened by rising demand and both diffuse and point-source pollution of surface and groundwater. To meet rising water needs, treated urban wastewater (WW) is gaining attention as an “unconventional” but stable resource. Nature-based solutions, such as constructed wetlands (CW), have recently emerged as cost-effective, decentralized treatment options with low maintenance and environmental benefits. Despite their advantages, adoption in the Mediterranean remains limited. This study assesses pollutant removal efficiency (RE) and performance of two full-scale CWs for treatment and reuse in small settlements: one at the Al-Cantàra winery (Randazzo, Italy) and another at the IKEA store in Catania, where WW is reused for irrigation of olive trees (subsurface) and of green areas, respectively, besides for toilet flushing in the second system.

**Methods:** The Al-Cantàra Winery in Randazzo (Catania) produces up to 100,000 bottles annually and, since June 2024, treats its winemaking WW using a CW system. The CW system (~310 m<sup>2</sup>) consists of three units: horizontal flow (HF, 120 m<sup>2</sup>), vertical flow (VF, 150 m<sup>2</sup>), and free water surface (FWS, 40 m<sup>2</sup>) treating approximately 10 m<sup>3</sup>/day. The IKEA store in Catania, with ~300 employees and high weekend footfall, lacks access to a public sewer. Since 2014, its variable WW is treated on-site by a hybrid CW system (~1400 m<sup>2</sup>), after a screening and a sequencing batch reactor (SBR). The system handles up to 30 m<sup>3</sup>/day and includes an HF unit (400 m<sup>2</sup>) followed by two VF units (530 m<sup>2</sup> each) in series. WW samples were collected every 15–30 days at the inlet and outlet of each treatment. Analyses followed standard protocols (APHA et al., 2005) to determine chemical, physical, and microbiological parameters: TSS; BOD<sub>5</sub>; COD; TP; NH<sub>3</sub><sup>+</sup>-N; TN; NO<sub>3</sub><sup>-</sup>-N; NO<sub>2</sub><sup>-</sup>-N (mg/L), and *Escherichia coli* (cfu/100 ml). Monitoring was conducted from November 2024 to July 2025.

**Discussion and Conclusion:** The treated WW at the outlet of the Alcantara CW system respects the regulatory discharge limits throughout the monitoring period. However, to meet the more stringent standards required for WW reuse, the integration of a recirculation process within the system is necessary. The CW system at Al-Cantàra achieves a RE of approximately 75.4% for TSS, 92.4% for COD, 89.8% for NH<sub>3</sub>-N, 86.1% for TP while the CW system installed at the IKEA facility shows even higher removal rates, with 99.3% for TSS, 93.4% for COD, 88.4% for TN, 99.9% for NH<sub>3</sub>-N, and 54.9% for TP. In both systems, *Escherichia coli* removal reaches 99%, corresponding to a 2 log units' reduction. Both CW systems demonstrate high pollutant RE, confirming their potential as sustainable solutions for WW treatment and reuse in Mediterranean contexts.

## **Agri-Food By-Products for Climate-Resilient Sheep Farming: Nutritional Strategies to Enhance Welfare and Milk Quality Under Heat Stress**

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**Introduction:** Climate change represents one of the most pressing issues of our time. Monitoring and managing the welfare status, as well as the productive and reproductive efficiency of small dairy ruminants, has become increasingly challenging, particularly in the Mediterranean area. Sheeps exhibit notable resilience, enabling them to tolerate extreme environmental conditions, including elevated ambient temperatures. Within this context, diet plays a key role as a strategic tool to enhance nutrient utilization efficiency and to offset nutritional imbalances induced by thermal stress. Agri-industrial by-products (AIPB) are materials or waste resulting from the production, processing or transformation of food products that, while not destined for direct human consumption, represent valuable animal feeding sources. This study proposes a feeding strategy based on the supplementation with agri-food by-products, and on feed additives which could have a synergistic effect in mitigating the negative consequences of heat stress in sheep.

**Methods:** In order to investigate current and novel by-products or feed additives to sustain animal welfare and productivity and improve sustainability, a literature review was conducted using the Scopus and Google Scholar databases. Keywords were "*heat stress, Agri- industrial by-products, sheep, welfare, milk production, dietary supplementation*". Studies reporting enhancements in milk yield and immune function in sheep were included. Furthermore, the review examined the doses and methods of administration of various by-products and additives, with the aim of supporting the design of future *in vivo* experimental studies.

**Discussion and Conclusion:** Agro-industrial by-products provide bioactive compounds that could have beneficial effects for the environment, such as reducing excretion of enteric methane and nitrogen (e.g., tannins) or increasing the nutraceutical value of human food from animal sources (e.g., beneficial fatty acids, antioxidants, etc.). Moreover, AIBPs have well-documented antimicrobial and antioxidant properties, and their administration may induce systemic effects that can indirectly affect milk, and also, could help mitigating the dysfunctional inflammatory responses induced by heat stress conditions. The use of specific AIBPs can contribute to cytokine regulation due to immunomodulatory properties and improve the hygienic quality of sheep's milk. The choice of AIPB will be based on local and seasonal availability and the complete characterization of the nutritional and functional properties will be conducted in order to integrate AIPBs in dairy sheep diet. *In vivo* trials will be performed to assess the effect of selected AIPBs and feed additives on the immune response and productive performance in dairy sheep and on milk nutritional quality under heat stress conditions.

## **AI-BIM Integration for Cost and Time Optimization in Construction Project Management: A Review**

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**Introduction:** Planning and scheduling directly affect the duration, costs, and environmental impact of construction projects, thus necessitating the adoption of digital strategies and process automation. Recent European directives support building automation, fostering zero-emission digital buildings, thereby addressing challenges such as delays, cost overruns, material waste, and safety risks. Traditional construction management techniques lack the adaptability required by complex modern projects, highlighting the need for new approaches. In this contest, Building Information Modelling (BIM) offers a solution by integrating project data, facilitating coordination between stakeholders. Advances in computing have driven the integration between BIM and Artificial Intelligence (AI), which improves estimation accuracy and supports informed decision-making. A literature analysis was conducted to examine how BIM and AI support automation of construction processes, with a focus on time and cost optimisation. In this analysis, tools and methodologies currently used were identified, limitations and research gaps were analysed, and future research directions in construction planning and management were outlined.

**Methods:** State-of-the-art analysis followed PRISMA guidelines to ensure rigorous synthesis and reproducibility. A literature search was conducted in June 2025 using Scopus, focusing on integrating BIM with AI for construction time and cost management. Keywords such as "BIM," "Artificial Intelligence," "multi-objective," "cost," and "time" were used, initially identifying 1905 documents. Applying inclusion criteria (including only articles written in English, without applying any restrictions regarding publication year or geographical area) reduced the dataset to 890. Screening excluded studies unrelated to construction scheduling or cost control, or those using BIM or AI separately. After this process, 44 relevant articles were selected for the review.

**Discussion and Conclusion:** Results highlights BIM-AI integration advantages in construction project management, improving estimation accuracy, optimising resource allocation, and enhancing planning processes. Among the AI algorithms, artificial neural networks and genetic algorithms have proven to be the most effective for defining optimisation models in this context. Their application contributes to cost and time efficiency, while simultaneously minimising planning errors.

Within this framework, BIM (primarily implemented through Revit) serves as the central data environment, providing project information as input for AI analytical models. The level of detail in digital models directly impacts the accuracy of predictions: The higher the BIM models' detail is, the higher the accuracy of algorithms outcomes. Other software supporting this framework includes Microsoft Project (used for initial construction planning), Navisworks (for 4D simulations), and Dynamo (applied for data transfer and storage before optimization processes).

Key challenges identified by this study include limited interoperability between software platforms and coordination among project team members, and incomplete temporal analysis in construction project management. Future research should focus on developing detailed methodological frameworks and generalised models, enhancing interoperability across software and stakeholders, and applying these advanced methods throughout the entire project lifecycle.

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