

# Agritech – Spoke 2

# ORAL

# PRESENTATIONS

Please note that the abstracts are sorted by the last name of the presenting author.

## A new integrated solarization system to control soil borne pathogens in strawberry plots

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The development of new physical methods provides an alternative to synthetic agrochemicals, offering tools for sustainable plant growth and crop protection. This study introduces a novel solarization system involving the application of biochar onto the soil surface before applying a solarization film (Solin<sup>®</sup> method). Through emulation of solar panels, this technique is based on a black matrix which enhances solar radiation absorption, resulting in increasing of soil temperatures. To evaluate the effectiveness of this method in controlling soil-borne pathogens of strawberry, small polyethylene bags (0.02 µm membrane sun-bags) containing fungal pathogens belonging to the genus *Neopestalotiopsis*, and temperature probes were buried in soil plots covered with different films. The experimental design encompassed four treatment plots: i) bare soil (control); ii) standard solarization with PET film; iii) solarization with multilayer thermal film (Polysolar); and iv) Solin<sup>®</sup> method. Soil samples were collected for metabarcoding analysis of microbial communities, targeting fungi, bacteria, and oomycetes. The experimental results confirmed the enhanced effectiveness of this new approach, as a significant increase in soil temperature was observed. Temperature assessment, survival of fungal pathogens, and analysis of resident microbial communities across the treatment plots demonstrated the potential for shorter solarization treatment, while maintaining efficacy in controlling target pathogens, with limited disruption of the soil microbiome dynamics.

## Belowground features in agrosystems: task 2.1.2 activities

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Soil and belowground plant structures play a key role in resilience, productive and environmental functions of agroecosystems. Among activities of task 2.1.2 ecosystem services of hypogean plant structures and soil microbial communities are addressed by the University of Basilicata unit through microcosm, mesocosm and field experiments and methodological work on segetal species.

Five mesocosm experiments were set up with the specific aims of investigating: root architecture and the capability of roots to produce sheaths at the soil-plant interface as a trait useful for wheat resilience and rhizosphere relations in unreliable water supply conditions; screening belowground traits diversity for soft wheat breeding; soil physical-chemical properties related to earthworms; root architecture and exudates of pure and consociated legumes and grasses (collaboration within T2.1.2 units with the university of Catania). A collaboration with University of Napoli Agritech units is aimed at ongoing characterizing belowground plant products with <sup>13</sup>C-CPMAS NMR in solid and liquid state mass spectrometry and gas/liquid chromatography.

We conducted methodological work on rhizosheath, and published a method aimed at incorporating root features in the agronomic evaluation of organic materials: we propose root hairs as indicators of fertilizer and amendment quality and we developed a GIS (Geographic Information System)-based procedure to this end.

We also worked on two field experiments, one specifically set up on indicators of belowground features of crops and pollinator-strip herbaceous plants, the other for the study of bioindicators based on soil microalgae and cyanobacteria (SM&C) communities in an olive orchard managed with sustainable ( $S_{mng}$ ) or conventional ( $C_{mng}$ ) techniques for 22 years. Among results the  $S_{mng}$  vs.  $C_{mng}$  soils had significantly higher microalgae ( $2.210 \cdot 10^4$  vs.  $0.872 \cdot 10^4$  g<sup>-1</sup> soil), and cyanobacteria ( $0.408 \cdot 10^2$  vs.  $0.240 \cdot 10^2$  g<sup>-1</sup> soil). Dominant species detected by light microscopy and 16S/18S/ITS rDNA metagenomics were *Trebouxia*, *Euglena*, *Chaetophora* green algae genuses, and the diatom *Cymbella* in  $C_{mng}$  and *Anabaena* cyanobacterial genus, *Oedogonium* and *Scenedesmus* green algae, and the diatoms *Navicula* and *Pinnularia* in  $S_{mng}$ . Soil management caused different profiles of intra- and extracellular SM&C metabolites, with an up-modulation in  $S_{mng}$  of the biosynthetic pathways of secondary metabolites, hormones, fatty acids and lipid, some with growth-promoting properties. Results will aim at providing management guidelines, indicators for agrosystem evaluation and criteria for the choice of crops and wildplant/pollinator strips based on belowground ecosystem services. Activities contribute to key exploitable results of Spoke 2 such as improved knowledge on soil biodiversity and functionality, and protocols and bioindicators for evaluating the impact of agroecological strategies on farmland biodiversity.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

### TASK 2.1.2

## "Bactrocera oleae" management: a pipeline from data to process-based model and spatial distribution

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*Bactrocera oleae* poses a significant threat to olive groves, leading to considerable losses in production. In response to this challenge, a modeling tool was developed to predict the population dynamics of the olive fly.

Real data on the phytophagous presence was collected both from dataset of males captured by feromone traps in previous studies, and in 2023 by new automated camera traps, that allow to monitor from remote the number of adults collected by the device. The data was used to calibrate a process model. In particular, a literature-based model, validated with temperature as the only environmental parameter, has been expanded to incorporate the impact of relative humidity. The simulations, encompassing both theoretical scenarios for understanding model behaviours and real data simulations, were conducted using a numerical tool. The developed process model will be integrated into a novel monitoring approach that utilizes Species Distribution Models (SDM) and Geographic Information Systems (GIS). This methodology aims to enhance accuracy in comprehending the spatial distribution and ecology of *Bactrocera oleae*.

4

This collaborative activity was undertaken within the framework of task T2.3.2, involving the participation of partners IBF, Unina, and UniCt.

## Phenotyping the genotype X environment interactions by image analysis

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Plant phenotyping has gained a broad consensus: to support genetic crop improvement, test new bioactive molecules, and study physiological responses to biotic or abiotic stresses and other complex plant traits. High-throughput plant phenotyping (HTPP) at robotized platforms or affordable (low-cost) phenotyping solutions can speed up the study of genotype x environment interactions through proper algorithms of image analysis, row data extraction, and transformation data to the plants of crop traits.

Here, various phenotyping solutions have been used in studying some morphological, anatomical, and physiological plant traits.

A simple low-cost Speed Breeding Technology (SBT) was assembled in 2023 and tested using different lentil genotypes by comparing an extended photoperiod treatment (20 h light and 4 h dark at 20 °C) in a growth cabinet to (i) a field-grown conventional control treatment and to (ii) glasshouse condition.

HTPP, in combination with traditional physiological measurements, was used to study the effects of different kiwifruit rootstocks on the physiological responses of the grafted yellow-fleshed cultivar under short-term waterlogging stress.

In field conditions, the 'Aglianico' grapevine cultivar grafted onto 7 rootstocks was investigated to develop new algorithms from RGB images, for fast and digital recognizing vegetative, reproductive growth and developmental phenological stages. In addition, the influence of rootstock on water transport was examined analysing the anatomy of woody structures (e.g., size, counting and density of xylem vessels) and starch content.

Results have shown that SBT, in lentil, significantly shortened the number of days from sowing to reaching the targeted phenological stages, having the possibility to compare multiple generations per year with respect to open field and greenhouse conditions and to substantially accelerate genetic gain in lentil, and for rapid development of high yielding, biotic and abiotic stresses resistant and climate change resilient lentil lines.

In kiwifruit HTPP, significant leaf gas exchange activity declines were observed in G3/Hayward and G3/D1 grafting combinations. Moreover, parameters obtained through the plant phenotyping image acquisition, such as projected shoot area, plant height, solidity, and colorimetric indices, will be analyzed in the upcoming months. Combined with RGB images of 'Aglianico,' these may help discriminate waterlogged from water deficit symptoms in field conditions.

An affordable image-based method for woody starch estimation was validated in 'Primitivo' and 'Aglianico' grapevine cultivars.

In conclusion, after the first growing season of the project, UniBAS has developed new solutions to study complex plant traits.

### TASK 2.3.1

## Multiple-families analysis for the identification of QTLs linked to tolerance to mal secco disease in lemon

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Mal secco disease is a severe tracheomycosis representing the most limiting factor for lemon (*Citrus limon* (L.) Burm. f.) cultivation in the Mediterranean and Black Sea area, where almost the 50% of lemon worldwide production takes place. The causal agent of mal secco disease is the mitosporic fungus *Plenodomus tracheiphilus*; after penetrating the host through wounds, it colonizes the xylem vessels and causes the progressive desiccation of the whole plant. Recently, a marker-trait association study was employed for the identification of molecular markers linked to the resistance towards mal secco. To this extent, two lemon segregating populations have been developed by crossing the mal secco tolerant genotypes lemon 'Interdonato', and *C. latipes* (female parents) with the lemon 'Femminello Siracusano 2kr' (male parent), which produces superior quality fruits, but is highly susceptible to mal secco. The behaviour of these full-sib populations towards mal secco infection has been monitored both through *in planta* and in field assays. Taken together, the 1% of the segregating genotypes do not show any symptoms in both trials. Moreover, in a preliminary characterization of xylem vessel features, the xylem vessel density was demonstrated to be correlated with susceptibility in a subset of citrus genotypes with known behaviour towards the disease. A functional genomic study is under progression together with the phenotyping. Taking advantage from the recent *de novo* sequencing of lemon, the full-sib population will be genotyped through the Single Primer Enrichment Technology (SPET) approach, and QTL analysis on selected SNPs will lead to the identification of genomic regions (thus molecular markers) significantly associated with resistance to mal secco. This tool will pave the way for Marker Assisted Selection (MAS) for the selection of novel varieties coupling high fruit quality and tolerance to the disease.

## Foliar deposit estimation in plant protection treatments

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The effectiveness of a spray application of Plant Protection Products (PPPs) is primarily influenced by the size of the droplets, the extent of target surface coverage, and the deposit of active substance on the target. The objective of this research was to estimate the foliar deposit as a function of droplets size and surface coverage of the target, assessed under laboratory conditions. As deposit is typically assessed using complex procedures, in this study a linear regression analysis is proposed, which allows the estimation of unit foliar deposit ( $\mu\text{L}/\text{cm}^2$ ) as a function of mean characteristic diameters of the spray and the percentage of covered surface measured on Water Sensitive Papers (WSPs). Experimental tests were carried out by using a custom-designed test-bench. Simultaneous measurement of drop size spectrum on Petri dishes, surface coverage on water sensitive papers, and foliar deposit on orange leaves were performed in triplicate by using four nozzles (TP 11001-SS, TP 11003-SS, TP 11006-SS, and TP 8008-SS) at the nominal pressures of 450, 300, 200, and 220 kPa, respectively. The corresponding flow rates were 0.49, 1.17, 1.94, and 2.71 L/min. Measured Volumetric Median Diameters (VMDs) increased from 122  $\mu\text{m}$  (TP 11001) to 268  $\mu\text{m}$  (TP 11003), 413  $\mu\text{m}$  (TP 11006), and 508  $\mu\text{m}$  (TP 8008). Percentage of covered surface on WSPs increased from 30.11 % (TP 11001) to 81.97 % (TP 8008) due to the increase in nozzle flow rate. Foliar deposit also showed an increasing trend from 0.66  $\mu\text{L}/\text{cm}^2$  (TP 11001) to 1.50  $\mu\text{L}/\text{cm}^2$  (TP 11003), 2.09  $\mu\text{L}/\text{cm}^2$  (TP 11006) and 4.33  $\mu\text{L}/\text{cm}^2$  (TP 8008). Foliar deposit increased quite linearly with percentage of covered surface and it was positively correlated with all diameters, with coefficient of correlation ranging from 0.31 (Numeric Median Diameter) to 0.91 (VMD). The multiple linear regression between foliar deposit (dependent variable) and percentage of covered surface and spray drop diameters (predictors) allowed the assessment of foliar deposit with high coefficient of determination ( $R^2 = 0.91$ ). This result holds great potential as it enables the calculation of the deposit using quantities that are either easily measurable (such as the fraction of surface covered on WSPs) or already known (such as the drop diameters produced by a nozzle, given by the nozzle manufacturer). However, further studies are necessary to handle different working conditions such as other spraying pressures, different nozzles, and varying values of percentage of covered surface.

7

### TASK 2.3.3

## Weed communities identification in tender wheat: a multiscale approach

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Weeds are one of the main causes of yield loss, and chemical management with herbicides is the only choice for an effective weed control. The heavy dependence on chemical control is somehow associated with scarce understanding of weed dynamics and ecological crop-weed interactions in the field of weed science. The goal of selectively identify weed species in the field at different spatial scales is therefore pivotal to cope with the patchiness of weed distribution in the field, being the main bottleneck for a throughout understanding of weed dynamics. This has been one of the main reasons of the oversimplification of agricultural landscapes, that needs to be reverted to reduce the footprint of agricultural production. By using a multiscale approach thanks to image acquisition, neural networks weed recognition, and clustering of weed patches, our goal is to give the basis for a more sustainable weed control that can reduce the need of herbicides in agriculture.



## Use of dynamic data (DSS) for improving crop protections

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The objective of trials performed to verify IPM plans of the main diseases on durum wheat, wine grape and industrial tomato; the objective of the trial was to verify the application of Integrated Pest Management (IPM) lines using the phenological phases of the crops under trial, while the comparison was made with the same IPM lines following the indications of the DSS . The use of DSS has shown that it has been possible to reduce the number of treatments with good crop protection results.

## The setting of agrochemicals transport monitoring and modeling in unsaturated and saturated zones for the assessment of specific groundwater vulnerability: the pilot site of Torre Lama (southern Italy)

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The intensive use of agrochemicals in the modern agricultural practices represents an increasing and world-wide environmental problem, causing the contamination and quality degradation of groundwater resources. Consequently, several European Directives, including the Groundwater Directive (Directive 2006/118/EC) and the Pesticide Directive (Directive 2009/128/EC) have been issued for groundwater protection and control of pollution from agrochemicals. These issues are within the main focus of the branch “Crop health: a multidisciplinary system approach to reduce the use of agrochemicals” (Spoke 2), of the project “National Research Center for Technology in Agriculture”, whose aim is the assessment of crops health and the development of environmental protection models. In such a framework, the assessment of groundwater vulnerability, which is considered as the potential level of groundwater contamination depending on natural attenuation processes of pollutants occurring in the unsaturated zone, from the source to the saturated zone (groundwater), is a crucial point for a complete assessment of agrochemicals effects on natural systems. To achieve these goals, the research aims at assessing the specific groundwater vulnerability to agrochemical pollutants of a shallow aquifer by coupling the monitoring of pesticides and/or nutrients transport through the unsaturated and saturated zones. The research will be carried out at Torre Lama Agricultural University Company (Bellizzi, SA), located in a flat alluvial landscape, which is cropped with wheat. This site was selected due to its representativeness in term of typical hydrogeological settings occurring in alluvial plains, which represents the prevailing feature of the most of world intensive crops. A preliminary hydrogeological characterization of the area was reconstructed by using the two and three-dimensional stratigraphic model derived from 65 stratigraphic and piezometric surveys, collected and made available by ISPRA and gathered by the municipal urban plan of Bellizzi municipality. At the local scale, a complex hydrogeological setting was recognized as formed by a multi-layered aquifer (gravel, sandy gravel and sand), separated by less permeable horizons (clay, sandy clay, silt and sandy silt). A preliminary groundwater flow model was also reconstructed, showing a variability of the piezometric levels from upstream towards the coastline. Based on the pedological and hydrogeological features of the test area, a monitoring station of transport and fate of agrochemicals in the unsaturated and saturated zones was designed at it's going to be installed. According to the local stratigraphic setting, multilevel probes will be set in the unsaturated zone to measure water content, soil temperature and water salinity. Moreover, lysimeters will be installed at different depths in the unsaturated zone to extract samples of capillary water to be analysed for their chemical content. While, in the saturated zone, multiparametric probes will be set in a piezometer from which water samples will be taken for chemical analysis.

Measurements and characterizations obtained will be used to calibrate a numerical model of agrochemical transport in unsaturated/saturated conditions. This will allow to model the specific groundwater vulnerability to agrochemical, thus, to evaluate the potential pollution grade suffered from the aquifer system under given crop conditions and agrochemical uses. This model is expected to be exported in other similar hydrogeological and crop conditions and used as a tool for crop and agrochemicals management.

**Keywords:** *Specific groundwater vulnerability assessment, water quality, agrochemical pollution, crop growth model, alluvial aquifer.*

### TASK 2.3.2

## New tools and strategies for insect control

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The need to reduce pesticide use and their negative impacts on the environment and on human health has promoted intense research efforts towards the identification of ecologically and economically alternative tools and strategies for crop protection.

The study of the interactions between insects and other organisms has allowed the identification of new molecules and genes that can be used as natural insecticides or to enhance plant defense mechanisms or natural antagonists killing activity. Here the most promising tools and strategies identified will be presented.

The exogenous application on plant of peptides involved in the systemic activation of defence gene triggers effective resistance barriers against pathogens and pests. Other promising tools were identified by studying the virulence factors encoded by insects' natural antagonists (predators and parasitoids), some of them encode proteins with direct killing activity, which are good candidate molecules for the development of new bioinsecticides. The study of the molecular targets of these virulence factors has allowed the identification in lepidoptera of a gene involved both in the embryonic development and in the immune response. Its silencing, mediated by RNA interference, induces embryonic mortality and a strong larval immunosuppression, which is observed also in larvae feeding on tomato plants colonized by beneficial fungi. The induction of immunosuppression results into an enhanced susceptibility to entomopathogens and, more in general, to increased ecosystem services by natural antagonists.

## Management variability of major agricultural systems across socio-environmental gradients

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Depending on the environmental and socio-economic context, there is usually a large variability in management within both organic and conventional farming. This variability makes it difficult to generalise their benefits and drawbacks as well as to guide the development of policy instrument and decision support systems. Using a large existing datasets generated within “Climvit” a project promoted by the SEI-SEA, we analysed the agricultural practices in vineyards across Italy. Most agronomic practices did not differ between conventional and organic vineyards and, in both systems, climate and landscape were strong predictors of agronomic practices, pest management and yield. Conventional vineyards yielded higher outputs than organic ones, only in cold-wet climates, suggesting that organic vineyards may become more financially viable in the face of future climate warming. Next, we plan to deepen our investigation considering some of the major Italian crops. Besides tailoring crop management, to create sustainable agricultural landscapes, it is essential to assess and account for different stakeholder values and views of ecosystem services such as crop production, pollination, biocontrol and cultural services. Here, we developed an on-line questionnaire to quantify stakeholders’ interests in crop production vs. other ecosystem services and identify potential competing priorities or conflicts. Our results will foster a multi-actor collaboration to promote the transition to a more sustainable agriculture.

## Development of the Agritech Spoke 2 DSS Platform

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A three-tier Geospatial Cyber-Infrastructure designed to enhance decision-making and reduce the use of agrochemicals will be presented. This system integrates:

1. A data tier, ensuring robust and scalable management of diverse datasets.
2. A logic tier, where computational models and algorithms process data to generate valuable output data related to pest status and crop management.
3. A presentation tier, tailored for various user experiences, ranging from a classic WebGIS interface for interactive data exploration and tool application, to a Dashboard providing precomputed data for immediate consumption by end-users. In addition, a web-API for advanced users and automatic requests will be implemented too, enabling direct data access and tools manipulation.

This structure aims to deliver accessible, efficient, and user-centered tools for agricultural decision support.

## Image registration of a multispectral camera in close-range application

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Multispectral imaging (MSI) for agricultural applications is playing a key role in plant stress assessment. However, one of the main problems especially in close-range applications is the misalignment of spectral bands provided by these instruments. The approach proposed in this study considers various distances from the target (500 mm to 1500 mm with a step size of 100 mm) and applies corrective shifts to achieve accurate registration among bands. Through a comparative evaluation of two alignment methods, Checkerboard (CB) and Discrete Fourier Transform (FT), this research aims to provide an effective solution for accurate image registration by facilitating reliable spectral analysis. Specifically, the proposed method involved the analysis of alignment-related offsets among the tested methods. In addition, the study explored the extraction of vegetation spectral indices for vegetation analysis and discrimination between healthy and diseased plants, and evaluated their relationship with the quality of alignment obtained at different heights. The results confirmed the trends in the changes in offsets as the target distance varies, showing satisfactory accuracy in the alignment of raw spectral images at different distances, with an error of about 1 pixel. Among the vegetation indices used, the Normalized Difference Vegetation Index (NDVI) proved to be capable of discriminating between healthy and nonhealthy leaves. The study aims to establish a framework applicable to remote sensing and agricultural monitoring, providing a valuable tool for monitoring plant health.

14

**Keywords:** Image alignment, Multispectral image, Image processing, Precision agriculture, Crop health status, Variable height.

### TASK 2.3.3

## Multipurpose bioformulations from the lab to the field

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Innovative strategies for the development of product alternatives to synthetic chemical pesticides and fertilizers in agriculture include the use of antagonists of disease agents and pests that damage crops, and the arsenal of bioactive compounds that they naturally produce. Plant beneficial microorganisms used as biological control agents and or stimulants of plant growth, such as numerous fungi belonging to *Trichoderma* and *Bacillus* rhizobacteria, are putative bioformulation components. Designing microbial consortia with compatible and complementary isolates requires targeted selection and testing to determine adaptability for the practical field applications. Furthermore, technologies that integrate and reduce doses of presently used chemicals, or assist in the monitoring and provisional management of farming systems are important in the passage to eco-sustainable agoecosystems.

## Biogeochemical and microbial indicators for a multivariate analysis of kiwifruit decline

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Kiwifruit decline is a syndrome responsible for severe economic losses in Italy which is one of the major kiwifruit producers worldwide. Causes and origins of this syndrome have not yet been fully clarified, but from the studies carried out to date, it is the result of biotic and abiotic components, which seem altered by crop intensification practices such as, for example, high irrigation frequency due to a high-water requirement by this crop. Multidisciplinary research was planned in the task 2.1.2 of AGRITECH aiming at building up a dataset of soil biological and chemical parameters to search for identifying a range of indicators to predict plant stress in kiwifruit groves using a multivariate approach.

A survey was carried out in four kiwifruit orchards in a specialised growing area in the Lazio region affected by this syndrome. Soil samples (0-25 cm deep) were taken in three zones along the rows and strips of each orchard. Kiwifruit plant tissues were collected from two plants per sampling zone. Soil samples were characterized for physico-chemical (pH, water content, water holding capacity, bulk density and total concentration of Al, As, Ca, Cd, Cr, Fe, K, Mg, Mn, Na, Ni, Pb, Cu, V and Zn), biological (microbial respiration, hydrolase, dehydrogenase,  $\beta$ -glucosidase and urease activities) and ecotoxicological (*L. sativum* L. and *S. saccharatum* L.) properties. Taxonomic qualitative analysis of soil fungal communities by using long reads sequencing approach is currently in progress. Quantitative analysis of soil fungi (total fungi, ascomycetes and basidiomycetes) and bacterial functional population (Actinomycetes, *Pseudomonas* and *Bacillus*) were carried out with digital PCR and quantitative PCR. Microarthropod communities were evaluated using both taxonomic and molecular tools in parallel. Plant stress response was analysed in term of catalase (CAT) and phenylalanine ammonia-lyase (PAL) enzyme activities and proline content. The whole dataset currently completed at the 80% has been subjected to first explorative analysis such as Principal Component Analysis and multiple variable correlation.

Preliminary analysis showed that the functional fungal and bacterial populations and biochemical soil features varied between treatments and groves; whilst microarthropod community (density and biodiversity) differently varied in planted and strips. Enzymatic activities, such as CAT and PAL, increased significantly in plants showing decline symptoms in comparison with healthy plants; proline content showed a similar trend.



## Electromagnetic fields in agricultural applications

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Aim of the presentation is to introduce electromagnetic field applications as environment-friendly methods to improve crop productivity (i.e. enhancing seed germination, pest prevention). The devices for microwave, electric and magnetic field treatments available at the Non Ionizing Radiation Lab for test at lab scale and on field levels are presented. Their design and realization to perform accurate experimental setup are described.

## Self-DNA inhibition for the biological control of *Orobanche ramosa* in tomato: evidence from metabolomic study

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The inhibitory effect of extracellular self-DNA of a pathogen, parasite or weed species has been suggested as a potential solution for novel approach of pest biocontrol. Experimental field applications demonstrated the efficacy of this concept when applied to the biocontrol of broomrape (*Orobanche ramosa*) in tomato cultivation. Metabolomic studies showed that the exposure to a biofertilizer harnessed with broomrape DNA, while positively affecting health and some minor metabolites in tomato, produced a strong inhibitory effect on key metabolic functional processes of the parasite plant, significantly reducing its vigour and leading to desiccation of the turions. The outcome is very promising in terms of possible new management scenarios aimed to pesticide reduction in agriculture.

## Improving stress resilience and agroecosystem services exploiting beneficial microbes

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Plant genetic improvement has long been employed as a crucial strategy to address environmental stresses and enhance traits essential for human needs. However, a drawback of the continuous selection of genomic plant traits, coupled with the substantial inputs required to maintain the selected genotypes, is the current adverse impact on the biodiversity of plant-associated microbes. It is noteworthy that these microbes constitute an extended genome, referred to as the hologenome, playing a pivotal role in plant adaptation to various stresses. In recent years, researchers have increasingly directed their focus towards isolating, characterizing, and preserving plant-associated microbes, collectively termed the holobiont. To this end, a collection of endophytic bacterial strains was directly obtained from tissues of grapevine and eggplant under drought and low N condition, respectively, aimed at their characterization and at creating tailored inocula with potential effectiveness in contrasting the two stresses. In grapevine, the selected strains were tested in biocontrol assays against major pathogens and, concurrently, the top-performing ones were characterized for their plant growth-promoting (PGP) traits. Similarly, strains isolated from different eggplant lines were assessed for their nitrogen-fixation capabilities and PGP traits. In the second step, grape plants and eggplant seedlings were inoculated with a specifically designed microbial synthetic community (SynCom), and their progress was monitored throughout the season. Additionally, grape plants were subjected to water limitation, and the stress was monitored using ecophysiological parameters, showing that plants inoculated with the SynCom showed an improved ability to cope with water limitation. Root and leaf samples were collected and analyzed by means of targeted metabolites quantification, RNAseq and microbiome analysis. Conversely, eggplants were exposed to three different levels of nitrogen (N) supply, and their growth and ecophysiological parameters were recorded, demonstrating that SynCom inoculated plants displayed an improved ability to cope with N limitation. At the end of the season, root and leaf samples were collected from control and SynCom inoculated plants to assess molecular responses and the impact on the composition of the microbial community to contrast low N conditions.

## Resistance to pyrethroids in the predatory mite *Amblyseius andersoni* (Acari: Phytoseiidae)

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*Amblyseius andersoni* (Chant) is a generalist predatory mite of importance for European orchards and vineyards, where it is considered a key biological control agent of spider mites, in particular. Its conservation is fundamental for successful pest management strategies. Pesticide use, particularly pyrethroid insecticides, threatens predatory mite survival and persistence of the population of this predatory mite in the agroecosystems. For this reason, in European vineyards and orchards in an integrated pest management framework, the use of insecticides belonging to this group was limited. Currently, pyrethroids have been increasingly used in fruit orchards and vineyards to manage the key pests *Scaphoideus titanus* and *Halyomorpha halys*, raising some concerns for spider mite management. Identifying predatory mite strains with a low susceptibility toward pyrethroids can enhance biological control strategies under the current pest management scenario. Under laboratory conditions, we investigated the effect of pyrethroids on different *A. andersoni* strains. We evaluated the dose-mortality response, the effect of deltamethrin on the fecundity, and the effects of field dose application of etofenprox and spinosad on survival and fecundity of the predatory mite. Results showed that a different susceptibility to a pyrethroid insecticide characterized the investigated strains. Comparing dose-response curves highlighted that some strains showed resistance to pyrethroids insecticides with a high resistance factor. Differential susceptibility to spinosad was also found in resistant strains. The implications for IPM programs in perennial cropping systems of pesticide resistance in *A. andersoni* are discussed.

## Target and non-target effects of essential oil-based insecticides

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Plant-derived insecticides, with essential oils (EOs) playing a central role, are a promising tool for sustainable control of agricultural pests because their applications can reduce the negative environmental impact of conventional management systems (e.g., synthetic insecticides). Although their practical implementation is hampered by various drawbacks such as phytotoxicity, stability and degradation patterns, innovative and advanced nanotechnology could help to overcome such limitations. However, the non-target impact of nanoformulated EOs on plants and biological control agents has been poorly investigated. We evaluated in the laboratory whether different nanoformulated EOs from four botanical families (i.e., Apiaceae, Asteraceae, Liliaceae, and Lamiaceae) are effective in controlling key tomato pests with different feeding strategies (i.e., a chewer, *Tuta absoluta*, and a sap sucker, *Phenacoccus solenopsis*). The non-target activity of EOs was also evaluated on plant and entomophagous insect species used in biological control applications. Most of the EOs evaluated caused significant mortality on insect pests, and the estimated lethal concentrations varied greatly. Fennel and anise EOs caused significant mortality on *T. absoluta*, while garlic was the most effective compound against *P. solenopsis*. Most of the EOs had negligible toxicity on tomato plants and they showed no lethal and sublethal effects on the tested predator *Cryptolaemus montrouzieri*. Our studies provide a basis for the future development of crop protection strategies within the framework of Integrated Pest Management. However, further toxicological studies are needed to evaluate the lethal and sublethal effects of EOs on other agricultural pests and their natural enemies.

21

Keywords: botanicals, greenhouse pest, horticultural crops, IPM, nanotechnology, plant protection

### TASK 2.2.4

## Protein hydrolysates from Brassicaceae oilseed by-products as potential biostimulants

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The use in agriculture of products such as biostimulants can help achieve sustainable production with minimal environmental impact.

In this context, the repurpose of agricultural by-products into new bioproducts, following a circular economy approach, is highly valuable. Defatted seed meals (DSMs), by-products of seed oil extraction, were therefore here exploited as raw materials to produce protein hydrolysates rich in free amino acids and small peptides, molecules known for their biostimulant effect on plants. Among oilseeds, crambe (*Crambe abyssinica* Hochst.) and rocket (*Eruca sativa* L.), two species of the family Brassicaceae, were chosen because their oils show interesting characteristics for industrial applications, and the valorization of their DSMs is essential to create a value chain and may help introduce these minor crops into the cropping system.

These DSMs were subjected to a mild two-step enzymatic protein hydrolysis, reaching 40% hydrolysis degree and obtaining hydrolysates with 10-15% w/w of free amino acids and peptides with molecular weight lower than 30 kDa. The hydrolysates were characterized for macro and micronutrients, amino acid composition, total phenolic content, antioxidant activity and the presence of other potentially bioactive compounds, such as glucosinolates.

Preliminary tests were performed to evaluate the biostimulant properties of the hydrolysates in laboratory bioassays and on maize in the early stages of growth in hydroponic culture, showing effects on nitrogen accumulation, SPAD index and root architecture. In vitro tests on tomato germination and root development also showed promising activities. Potential effects in the resistance of tomato to saline stress are currently under study.

## Meteo, pest risk, and treatments analysis using multidimensional cubes

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The OLAP paradigm has been proven successful, during the last three decades, in supporting decision makers when analyzing their business and scientific data. The basic idea of OLAP is to let users, non-skilled in IT, create exploration paths within multidimensional cubes of data by interactively changing the focus of analysis via a small set of intuitive operators. Within the Agritech project, we have started to design some multidimensional cubes to be fed with data flows sent from the different partners. Specifically, these cubes will contain data related to meteo conditions, pest risk, and treatments, associated with temporal and geographical information. The goal of this talk is to introduce the pillars of the multidimensional model and to demonstrate the effectiveness of OLAP using data that describe the captures of brown marmorated stink bugs in traps scattered across a territory.

## Lepidopteran diversity across agricultural landscapes at multiple spatial scales

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Intensive management of agro-ecosystems has led to a decline in pollinating insects, weakening one of the most important ecosystem services. To understand the relationship between pollinators and landscape management in an agricultural context, we analysed the diversity and abundance of Lepidoptera. Our studies focused mainly on olive groves, one of the main permanent crops in the Mediterranean basin, studying communities at landscape and farm scale. Monitoring was carried out in cultivated and semi-natural areas to provide information for the proper management of agro-ecosystems and individual farms. Observational studies were conducted with field activities to collect original data and synthesis studies were carried out using available data sets not generated within Agritech. The observational studies concerned the monitoring of diurnal Lepidoptera in 10 landscapes within an olive-growing area in the Crotona province, choosing a cultivated and a nearby semi-natural area for each landscape. The observations started in March 2023 and will continue in 2024. Sampling took place biweekly for 15 minutes in each site and species were mostly identified in the field. The number of individuals observed was counted. Few specimens were identified in the laboratory using classical and molecular techniques. Until now 2,778 individuals belonging to 65 species have been recorded. Landscape level analyses using GIS methods are underway. During 2024, research on nocturnal lepidoptera will also be carried out at farm level in a vineyard in the Crotona area. Synthesis studies were performed using two datasets available for nocturnal lepidoptera in cultivated and natural landscapes. The first dataset consisted of lepidoptera collected in the Sila Massif forests; the second consisted of moth abundance data collected in an olive farm. In the first dataset, where forests were divided into linear and compact according to the patch shape, we found that compact forests hosted richer and more abundant communities and that community composition was influenced by patch shape. In the second dataset, consisting of 7,569 specimens belonging to 332 species, beta-diversity analyses showed that the distribution of some species were not barycentric in semi-natural patches, cultivated patches having a not negligible role in sustaining beta-diversity at farm scale. We were also able to link the presence of rare species in cultivated and urbanised landscapes to the persistence of small semi-natural areas, highlighting their importance for pollinator diversity.



## Assessing pollination deficit in pear orchards: the role of pollinator community, landscape context and farm management

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Pear (*Pyrus communis* L.) is an important perennial crop highly dependent on pollination service. Several insect pollinator species have been observed on pear flowers but, due to the low sugar concentration in nectar and frequent harsh conditions during bloom, this crop usually shows low pollinator abundance and suffer potential pollination deficit. The aim of this study was to quantify pollination deficit in 16 pear orchards in the Emilia-Romagna region, which plays a pivotal role in the national pear production, and to assess the role of pollinator community, landscape context and farm management on yield. Our results showed an overall pollination deficit of 21% with strong variations among farms; at the same time, high pesticides input was detected in all orchards. Seed production, which is linked to pollination deficit, was positively influenced by honey and wild bee abundance, and was negatively related to pesticide risk. On the contrary landscape factors did not affect pear production and pollinator community. Our results highlight a lack of an optimal pollination service in most Italian pear orchards and the need to reduce the pesticide pressure on insect pollinators.

## Drivers and barriers to increase the uptake of IPM plans

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In recent years, the European Commission has promoted more sustainable agricultural production processes by encouraging the adoption of more sustainable practices through the Farm to Fork strategy. Indeed, agriculture is widely regarded as a major source of environmental pollution due to the overuse of pesticides and chemicals, resulting in a loss of functional biodiversity, land degradation, and contamination of air, water, and soil (Jhariya et al., 2019). This is especially true in the wine industry, where disease and pest pressure force winegrowers to use pesticides extensively (Marsala et al., 2020), slowing the adoption of more sustainable production systems. Winegrowers are being pushed to adopt more sustainable production systems in order to limit the environmental and social damage caused by the rapid spread of viticulture (Galletto and Barisan, 2018). However, even though several environmentally friendly viticulture practices exist, and numerous political incentives provided by public policy interventions such as Rural Development Programs have pushed the adoption of more sustainable production protocols, several factors, such as farmers' personal traits, environmental variability, and economic and political aspects (Raimondo et al., 2021), slowing the adoption of more sustainable viticultural practices.

Recent research emphasizes the significance of psychological/behavioral dimensions in influencing farmers' propensity to adopt sustainable agricultural practices (Despotovic et al., 2021). Environmental awareness (EA) is regarded as the first important prerequisite that people should acquire in order to re-orient their behaviors in a pro-environmental/social direction since it reflects people's concern for and knowledge of the environmental consequences of their actions, (Fu et al., 2020). So far, several studies have been conducted over the last few decades to investigate the relationship between environmental awareness and behavior for various groups of people, including students, project managers, citizens, households, consumers, and heads of manufacturing companies. Despotovic and colleagues (2021) have recently provided a theoretical and methodological framework for measuring environmental awareness as a multidimensional concept, with a focus on farmers' environmental awareness and its role in adopting cleaner agricultural practices.

However, no studies have tested in Italy environmental awareness as a multidimensional concept, particularly in a specific sector, such as viticulture, where more sustainable production systems are desperately needed. As a result, an empirical study will be accomplished among winegrowers in the Conegliano Valdobbiadene Prosecco PDO, a DOCG (Controlled and Guaranteed Designation of Origin), which is historically Italy's most important area for the production of Prosecco's sparkling wines (Pomarici et al., 2019). The relationship between the EA of Prosecco winegrowers and the adoption of agricultural systems can be useful in understanding whether and to what extent environmental awareness influences the adoption of sustainable production systems. As a result, once environmental awareness is measured as a multidimensional concept, following the framework of Despotovic et al., (2021), the current study attempts to answer the following two research questions: RQ1: "Which EA dimension (environmental knowledge, environmental attitudes, connectedness with nature, environmental behavior, and biospheric concern) best describes Italian winegrowers' environmental awareness?" and RQ2: "Does winegrowers' environmental awareness in the Conegliano Valdobbiadene Prosecco Protected Designation of Origin area influence their adoption of integrated pest management protocols?" By using Structural Equation Modeling and a Multinomial Probit Model, this paper empirically tested the predictive power of environmental awareness in influencing winegrowers' likelihood of adopting organic and integrated pest management protocols. Results provide valuable insights into potential drivers for the adoption of more sustainable production systems in Italian viticulture.

### TASK 2.3.4

## Bioactive compounds from alfalfa (*M. sativa*): evaluation of their potential use as biopesticides against tomato pathogens

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In the frame of Task 2.2.4 – Spoke 2 of Agritech project, bioactive compounds from plant biomasses were tested for potential application as new eco-friendly biopesticides. Extracts were characterized, and their effectiveness was evaluated towards pathogens infecting *Solanum lycopersicum*. In this work we report preliminary results on three extracts from *Medicago sativa*.

Phenolics and saponins were extracted and purified from alfalfa. Prosapogenins were obtained by alkaline hydrolysis of saponins. From UPLC characterization phenolics showed a high amount of apigenin glycosides, saponins were composed by bidesmosides of medicagenic and zanhic acids, while prosapogenins were rich in monodesmosidic derivatives.

Saponins and prosapogenins were evaluated for *in vitro* activity (antigerminative and growth inhibition) against *Fusarium oxysporum*, *Alternaria alternata* and *Botrytis cinerea*. Prosapogenins were effective in inhibiting mycelial growth and conidia germination. Thus, this extract was assayed against alternariosis and grey mold of tomato, showing significant control efficacy in a dose-dependent manner.

In antiviral activity tests *in vivo* against tomato spotted wilt orthotospovirus (TSWV), plants were inoculated with TSWV and treated with extracts. Plants were sampled weekly and TSWV titer was measured by real-time RT-PCR. The best performing extract was the phenolic mixture, decreasing the virus titer significantly at 17 and 31 d.p.i.

## Novel monitoring techniques for wild flora and arthropods

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The aim of this research is to improve the monitoring of wild flora and arthropods by developing cost-effective and expeditious biomonitoring tools and protocols for indicators of agroecosystem health using non-invasive methods. First, we applied DNA metabarcoding to assess weed soil seedbank biodiversity in comparison to the traditional germinable seedbank assessment technique. Soil samples were collected in rice fields and margins to allow for seed germination in a greenhouse. The emerged weed seedlings were identified and counted. Preliminary DNA metabarcoding analysis was conducted on the same soil samples to develop a reliable protocol for weed species identification. Further analysis on weed distribution, frequency, and dominance will cover both methods. Second, the criteria, design, and functioning of artificial flowers taking in consideration visual, acoustic, vibrational, and olfactory cues as sensory impacts were developed as non-invasive non-destructive method for monitoring insect pollinators. The preliminary version of this bioacoustics-based monitoring tool has been developed in collaboration with the University of Bologna. Laboratory/field testing of this artificial flower will start involving key bee species once the prototype will be built. Third, the viability of immunolabeling/capture technique applied to insect pests monitoring was tested for pest monitoring of the polyphagous phytoplasma vector *Orientus ishidae*. Data from previously obtained field experiments, involving labelling with non-harmful substances such as chicken egg whites or whole fat cow milk, followed by ELISA tests in laboratory, were analysed. The markers retention was tested on field-collected adults caged together with labelled plants, indicating a retention for up to two weeks.

## Optical and electrochemical biosensors for environmental DNA detection

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In recent years, the environmental DNA (eDNA) has emerged as a powerful tool for species detection, biomonitoring and surveillance, offering greater sensitivity and efficiency compared to traditional survey methods.<sup>1,2</sup> Current eDNA detection relies on Polymerase Chain Reaction (PCR), but its cost and complexity necessitate simpler solutions for real-time monitoring. Therefore, developing a simple and sensitive detection system for real-time eDNA monitoring is meaningful and highly desired. Here, we propose two innovative approaches: an Electrochemical Impedance Spectroscopy (EIS)-based biosensor and a colorimetric biosensor utilizing gold nanoparticle aggregation.<sup>3</sup>

Our EIS-based biosensor demonstrates effective functionalization of gold electrodes with antibodies, facilitated by a custom microfluidic cell.<sup>4</sup> Optimization of single-stranded DNA (ssDNA) probe functionalization is underway, showing promising results in detecting concentrations of *S. cerevisiae*-derived ssDNA within the range of 100-1 nM. The colorimetric biosensor relies on competitive aggregation of gold nanoparticles in the absence of the target eDNA. Two distinct batches of functionalized gold nanoparticles (f-AuNPs) with complementary ssDNA sequences are synthesized, allowing for aggregation in the absence of the target. Preliminary results indicate detection of 10 nM of *S. cerevisiae*-derived DNA within 30 minutes. These approaches present efficient, sensitive, and potentially cost-effective methods for real-time eDNA monitoring, offering significant advancements in environmental surveillance and biomonitoring.

29

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### TASK 2.1.3

## An up-scaling approach for studying soil and biomass variability in annual and perennial crops

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Plants are dependent on the environment for their growth and functions. A comprehensive toolbox is available to study plant-environment interactions at different spatiotemporal scales.

We evaluated ground-based soil and vegetation parameters in different annual crops (tomato, winter wheat, and maize) and perennial crops (grapevine) that are important for interpreting, evaluating, and validating UAV and satellite-derived vegetation indices.

First, the LAIR R package was developed after compiling a library of Normalized Differences Vegetation Index (NDVI) – to – Leaf Area Index (LAI) conversion formulas by Bajocco et al. (2022).

During the 2023 growing season, soil variability was studied applying proximal sensing techniques, including electro-magnetic induction (EMI) measures of soil apparent electrical conductivity (ECa), and LAI (estimated from LAIR with data of NDVI of Sentinel 2 satellite) was used to assess winter wheat growth and development variability in an experimental field near Foggia (South-East of Italy). In cooperation with Spoke 7 (T7.1.1.) at a sloping marginal 'Chardonnay/157-11 and 'Primitivo/157-11C' irrigated vineyard located in Matera province, EMI, plant-based vegetative, and reproductive measurements were also used to evaluate NDVI values measured from the Sentinel 2 satellite.

Results showed a correlation between soil variability and winter wheat and grapevine crop growth. For perennial crops with discontinuous canopy, sentinel 2 data were less accurate than ground-based measurements in establishing a relationship between ECa – vine growth - NDVI, mainly due to the presence of turf. Based on preliminary results, a relationship between ECa and grape quality traits might be envisaged.

In conclusion, establishing a collaborative environment among Institutions is a sturdy key in obtaining satisfactory field data for calibration and monitoring crop growth at different spatiotemporal scales.

## Pathogenicity mechanisms and exploitation of pathogen derived molecules

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With the increasing need of alternative tools and strategies for plant protection against pathogens, the identification of new bioactive molecules is mandatory to improve sustainable management of agricultural practices. In this context, plant pathogen-derived molecules represent an almost unexplored strategy. In a previous work, we found that *Fusarium oxysporum* (*Fo*), one of the most destructive pathogens affecting a wide variety of plant crops, regulates its growth and development by using peptide pheromones (A and  $\alpha$ ) and their cognate receptors (Ste3 and Ste2). In this class of fungi, pheromone recognition modulates different aspects of fungal physiology and pathogenicity, including germination, chemotropism and quorum sensing. The aspartyl protease Bar1 is required for  $\alpha$ -pheromone cleavage and signalling desensitization leading to a precise regulation of the transition process between quiescence to proliferative growth. Understanding how Bar1-pheromone interaction occurs is important to develop more active  $\alpha$ -pheromone variants to be used as *Fo* germination blockers. Here, to identify the cleavage site of Bar1 in *Fo*  $\alpha$ -pheromone (WCTWRGQPCW), we performed an HPLC-Q-TOF-MS analysis of synthetic  $\alpha$ -pheromone incubated in presence of fungal cells. Our results show that  $\alpha$ -pheromone treatment with wt, but not with Bar1 $\Delta$  fungal germlings, produced two complementary sub-products deriving from pheromone cleavage between Thr<sup>3</sup> and Trp<sup>4</sup> residues. Alanine-scanning of the Bar1 putative cleavage site showed that the mutated version of  $\alpha$ -pheromone had an increased anti-germinative and chemotropic activity. Collectively, our results represent a novel framework for the generation of pathogen-inspired biocontrol molecules.

# Agritech – Spoke 2

# POSTER

# PRESENTATIONS

Please note that the abstracts are sorted by the last name of the presenting author.  
Additionally, at the bottom of the page, you will find the reference code of the  
poster, which is displayed on the grid.



## UPTraps: Artificial Intelligence in Remote Pest Surveillance and Descriptive Modeling for Sustainable Agroecosystems

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Recent climate changes pose challenges to phytosanitary management, notably in pest control. Fluctuating temperatures and irregular precipitations disrupt the life cycle of acclimated species. Among them, the Mediterranean fruit fly (medfly), *Ceratitis capitata* surged its population in recent years leading to increased damage to fruit crops. Past large-scale capture and pesticide strategies are now ineffective against this pest; however, these treatments are no longer effective or sustainable within the framework of the new concepts of more eco-sustainable agriculture. In addition, human activities like trade have facilitated the introduction of exotic species, as seen with the recent invasive process of Oriental fruit fly, *Bactrocera dorsalis*, in Southern Italy. Given these challenges, it is crucial to develop new advanced methodologies to better plan phytosanitary activities. Artificial Intelligence can play a key role in this scenario to involve innovative and efficient monitoring and control methods in pest management. The development of sophisticated and effective control techniques using Artificial Intelligence is crucial for the creation of innovative monitoring to better plan activities in Integrated Pest Management. The use of highly specific electronic tools in preventive pest monitoring can enhance phytosanitary management significantly by offering more precise control measures. UpTraps project focuses on studying *C. capitata* to update information about its life cycle and to assess medfly response to *B. dorsalis* invasion. A monitoring network was set up using both traditional prototypes of electronic traps; different deep-learning approaches will be applied to improve the automated recognition system of the e-traps. Monitoring activities are carried out in 6 infested fields and also include the use of smart control units for high-frequency agrometeorological and soil trend measurements. Through the interpolation of agrometeorological and entomological data, different mathematical models based on matrix and Ordinary Differential Equation (ODE) models will be developed to describe the phenology and the population dynamics of both pests in the Campania Region. Several Machine Learning approaches, including Artificial Neural Networks, will be tested using agrometeorological data and pest presence/absence as input to create a predictive model as a tool for implementation in Decision Support Systems. From a preliminary analysis of degree days and by calculating them with different threshold temperatures available in the literature, a shift in the life cycle of *C. capitata* occurred, as well as a delay of the first appearance of *Bactrocera dorsalis* in 2023, compared to the expected outcome. These preliminary results emphasize the importance of updating the data on the life cycle of *C. capitata* and suggest several hypotheses on the probable new invasions of the Oriental fruit fly.

## Bioactive peptides for plant protection and stimulation

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In the last two years, at least 138 extreme weather events were recorded in Italy over land (Source: ESWD). This affects, for instance, diseases caused by seasonal pathogens [1], striking plantations during periods incompatible with the natural vegetative cycle. Diverse biosafe approaches have been developed to support plant protection and stimulation. In this respect, bioactive peptides, obtained from both natural and synthetic sources, represent a valid alternative thanks to their high selectivity of action and their low average residence time in the field [2]. To date, although there is considerable literature about their formulation and delivery in precise biomedicine, little is known in the agrotechnological field. Here, two different peptide-based approaches are explored. Firstly, the formulation of a previously reported bioactive peptide with four different counterions is described [3]. We show that its hygroscopicity, self-oxidation half-time, and solubility in water may vary significantly according to its formulation. Secondly, a protective hydrogelating biomaterial (PHB) for controlled biostimulant delivery has been designed. We show that the first generation of such materials can be precisely tuned to make its aggregation responsive to proton and metal concentrations. We then performed the rational design of a second generation, hopefully overcoming the limitations of the previous series. In perspective, the study will be devoted to on-field tests to check both formulation and delivery capabilities of both approaches.

34

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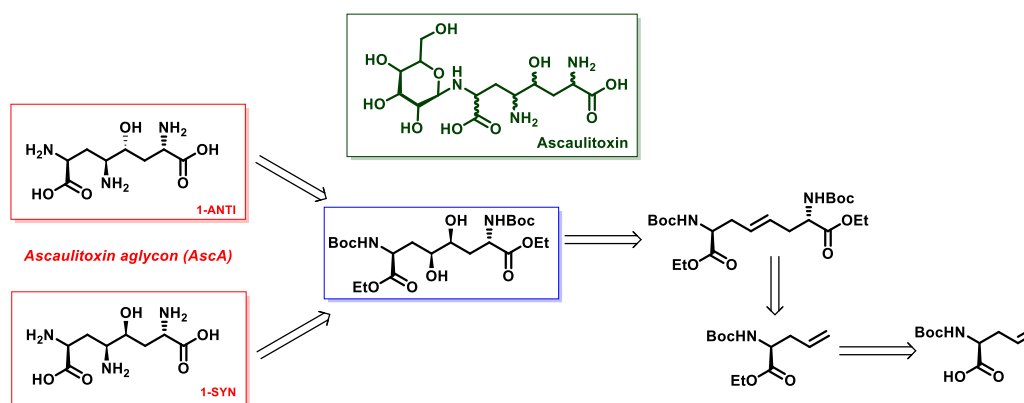
## Approach to the stereoselective synthesis of ascaulitoxin aglycon and phytotoxicity of its intermediates.

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Agriculture faces a significant challenge in safeguarding crops against weed competition while minimizing their spread. To this end, biological agents, like phytotoxins from plant pathogens, offer promising traits: they have low persistence in the environment and highly targeted modes of action. This specificity minimizes pest resistance issues and decreases toxicity to non-target organisms. Among these biological agents, ascaulitoxin and its aglycone, derived from the *Ascochyta Caulina fungus*<sup>1</sup>, stand out as potent phytotoxins with potential for controlling weeds.<sup>2</sup> However, uncertainties remained about its relative and absolute configuration. Therefore, a study aimed at synthesizing all possible stereoisomers of ascaulitoxin aglycone was undertaken to determine the absolute configuration of the natural compound and to enable structure-activity relationship studies, providing quantities necessary for large-scale applications. This report details an initial approach to the diastereo- and enantioselective synthesis of ascaulitoxin aglycone. The synthesis of *threo* isomers of the central amino and hydroxy groups was carried out by an asymmetric dihydroxylation-Mitsunobu azidation sequence, while the *erythro* isomers were obtained through direct asymmetric aminohydroxylation. Both methods originate from the same olefin, readily prepared from commercially available Boc-L-allylglycine through self-metathesis of its aminoester. Furthermore, the phytotoxic activity of *N*-BOC protected methylesters of ascaulitoxin aglycone diastereomers, together with the diol precursors, was tested by leaf-puncture assay on host and non-host weed plants. Radical growth assays on *Lepidium sativum* and *Solanum lycopersicum* were also carried out.

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Scheme 1.

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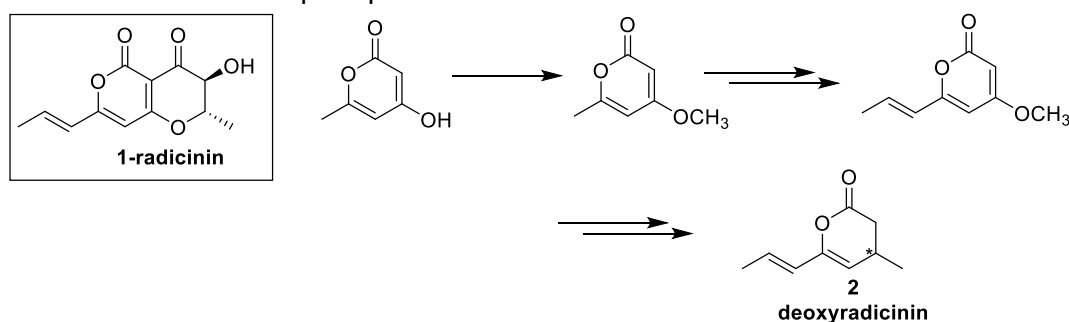
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### 3-deoxyradicinin as biopesticide: efficient synthesis and mechanism of action

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Fungal phytotoxins constitute an essentially endless source of bioactive metabolites. Their large structural diversity reflects in a wide variety of bioactivities and potential applications in both medicine and agriculture. One of the most appealing application of fungal phytotoxins is in the development of bioherbicides, which show lower or nil toxicity, and then a lower environmental and ecological impact, than the traditional synthetic pesticides. In this context, the synthesis of *deoxyradicinin*, the immediate precursor of the more well-known fungal metabolite *radicinin*, can be addressed. In the 1950s it was isolated from *Stemphylium radinum* and its structure was determined in 1964 by means of chemical and spectroscopic comparison studies<sup>1</sup>. Recently has been synthesized the biosynthetic precursor of Radicinin, the fungal metabolite deoxyradicin, in in racemic form, and the unsaturated derivative of the latter, dehydroradicin, much simpler to prepare than the asymmetric radinin. (±)-Deoxyradicinin and dehydroradicin were tested by bioassay leaf puncture on *Buffelgrass*<sup>2</sup>, an invasive perennial grass of the African continent, and their activity was compared with the activity of optically active natural radicinin. The studies have shown that the activity of the racemic deoxyradicin, obtained synthetically, is comparable to that of the natural radinine being only lower, while that shown by the synthetic unsaturated is about one third lower than the first two. This confirmed that (±)-deoxyradicinin can be used as an alternative to Radicinin as a possible bioherbicide against *Buffelgrass*<sup>2</sup>. The possibility of using, with similar results, racemic or non-chiral compounds in place of the enantiopure ones is a great advantage for their preparation. Furthermore, a racemic compound is much easier to prepare on a large scale than an optically active one. Recently, starting from the consideration that the synthetic procedures of deoxyradicinin in racemic form reported in the literature<sup>2</sup> presented various limitations, such as the high cost and toxicity of many reagents used, the high number of synthetic steps, the low yields of the individual steps and the difficult reproducibility on a large scale, in the same laboratory where this thesis work was carried out, a new synthetic strategy of this molecule on a large scale, simpler and with low environmental impact, was developed (Scheme 1). This methodology, which involved the use of a low-cost commercial lactone as the starting substrate, allowed deoxyradicinin to be obtained in 6 steps, 4 of which were conducted in the absence of solvent, limiting the extractions and purifications to isolate the pure product.



Scheme 1.

## Rapid colorimetric biosensor for environmental DNA detection

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Over the past decade, environmental DNA (eDNA) has emerged as a promising tool for studying biodiversity, utilizing genetic markers to deduce the presence of various species.[1] Currently, eDNA detection is carried out using different approaches based on the use of Polymerase Chain Reaction (PCR). This technique is expensive, time-consuming and requires specialized personnel.[2] In this scenario, it is highly desirable to develop a rapid colorimetric biosensor for the detection of eDNA, which can also be employed for real-time analysis. Our approach is based on the competitive and specific aggregation of gold nanoparticles induced by the absence of the target (eDNA). To this aim, we synthesize two distinct batches of functionalized gold nanoparticles (f-AuNPs), each having a different and complementary single-stranded DNA (ssDNA) sequence. In the absence of the target analyte, mixing the two batches of f-AuNPs together will result in aggregation due to the interaction between their complementary ssDNA sequences. On the contrary, aggregation will not be visible when the target analyte is recognized by one of the two batches of f-AuNPs. The preliminary results obtained using ssDNA sequences derived from *S. cerevisiae* are encouraging since have been detected 100 nM of DNA in only 30 minutes.

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## Analysis and classification of satellite images to determine melliferous and pollen potential for predicting ecosystem services

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The importance of honey bees (*Apis mellifera* L.) and other *Apoidea* species is of a broad consensus, with a high value of pollination services crucial to producing fruits and vegetables. As a result of the significant relationship between plants and pollinators, honey bees' activity contributes to biodiversity conservation. However, honey bee colonies have been dropping in recent years due to numerous environmental factors, such as climate change, pesticides, pathogens and loss of foraging lands, which would hopefully be predictable.

In this context, differential skills, such as remote sensors and Artificial Intelligence (A.I.), could be helpful to elaborate multiple data to monitor and predict ecosystem services for crop production. This research project (HiveTechSpace) aims to implement a framework that can predict the melliferous and pollen potential by exploiting heterogeneous information sensed by in-hive sensors and satellite images. A remote sensing infrastructure for data and image collection will be built, and proper image classification and A.I. algorithms will be enacted. After validation, the procedure will be embedded in a D.S.S. provided by the 3Bee S.R.L. Company to enhance the support provided to farmers and beekeepers. HiveTechSpace project will improve the pollination service and food quality by optimizing the data interpretation for monitoring the flowering status (quantified through the data collected), suggesting the best place to locate hives to maximize honey production. The results of the HiveTechSpace project can be translated into a concrete application to improve and implement prevention, diagnosis, and decision-making methods in the food production system.

## How can honeybee health be monitored? Novel techniques for field application

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The first aims of the research activity are focused on 1) developing a panel of biomarkers to monitor the health and nutritional status of honeybees; 2) testing the efficacy of the panel in field conditions. These research activities are preparatory to the implementation of smart hives equipped with innovative sensors, to discover connections between the biomarkers and data obtained from the sensors.

A panel of nutritional and health biomarkers was developed and tested in the field in different environmental conditions at the colony level. This panel included hemolymph proteins representative of key metabolic processes, immunity and nutritional status (total proteins, apolipoprotein I and II, vitellogenin, transferrin, and hexamerin 70a). These proteins made it possible to evaluate health and nutritional status of the colonies during the productive season. The data obtained are confirmatory that long-living winter bees have higher concentrations of hemolymph total proteins than summer bees. Vitellogenin, transferrin and hexamerin 70 showed a significant increase from April to November, mirroring the physiological changes of honeybees across the seasons. Preliminary results of 2023 research suggest correlations between the number of Varroa mites and some hemolymph components.

The second step consists of the design of an embedded IoT system to monitor the hive's microclimate, along with key indicators such as TVOC and vibrations that can provide insights into the hive's health status. Gathering comprehensive data is crucial for developing an innovative IoT sensor system designed for remote monitoring of both the hive and the ecosystem associated with it.



## Highly sensitive impedimetric biosensor for environmental DNA detection

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In recent years, the environmental DNA (eDNA) has emerged as a powerful tool for species detection, biomonitoring and surveillance, offering greater sensitivity and efficiency compared to traditional survey methods.<sup>1</sup> Therefore, developing a simple and sensitive detection system for real-time eDNA monitoring is meaningful and highly desired. In this context, a biosensor emerges as an exemplary solution due to its manifold advantages. This is particularly true for impedimetric biosensors, as they offer elevated sensitivity, selectivity, real-time detection, and cost-effectiveness. For this purpose, we are developing an Electrochemical Impedance Spectroscopy (EIS)- based biosensor for eDNA detection. In our previous work<sup>2</sup>, we successfully demonstrated the functionalization of the gold screen printed electrodes (AuSPEs) with antibodies. This achievement was possible through the design of a customized microfluidic cell, enabling the exclusive interaction of different solutions with the working electrode. Currently, we are optimizing the functionalization of the AuSPEs with the single-stranded DNA (ssDNA) probes. Following the functionalization, the ssDNA-modified electrode is brought into contact with a solution containing the target ssDNA, leading to hybridization and the formation of double-stranded DNA (dsDNA) on the electrode surface. This interaction is monitored through EIS, yielding highly promising preliminary results. In fact, using ssDNA derived from *S. cerevisiae*, concentrations within the range of 100-10 nM are easily discernible, with excellent prospects for further improving the detection limit.

40

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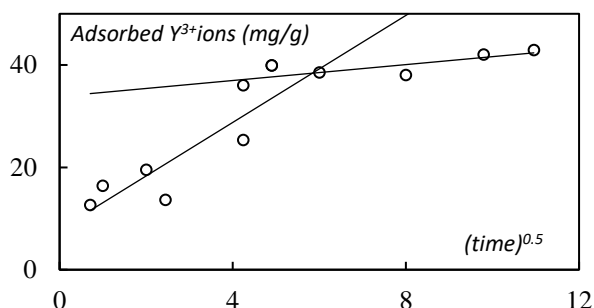


## Adsorption of lanthanides cations onto pyrolysed biochar obtained from livestock waste

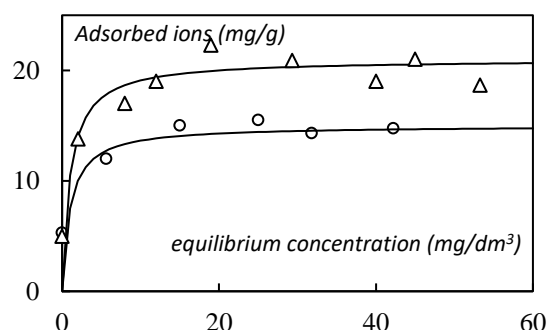
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Lanthanides are part of the group of rare earth elements (REE), which constitute elements from lanthanum to lutetium, also including yttrium. Due to their use in advanced solid state technologies, they constitute emerging pollutants that are toxic to human health. There is a growing interest in the application of adsorption, in the removal of lanthanides ions from different wastes. This work investigates adsorption isotherms of  $Y^{3+}$ ,  $La^{3+}$ ,  $Ce^{3+}$ ,  $Pr^{3+}$  and  $Nd^{3+}$  ions, on pyrolyzed biochar<sup>1</sup> obtained from livestock waste. The uptake behaviour of lanthanides is of special interest because their behaviour is similar to that of the trivalent actinides  $Pu^{3+}$ ,  $Am^{3+}$  and  $Cm^{3+}$ , which has serious consequences for remediation of the environment. Solid material (livestock waste) was pyrolyzed in an Ar(g) atmosphere. Thermal treatment applied consists of heating up to 700°C (with a speed of 10°C/min) for 2 h. The study of the acid-base properties of the biochar surface is conducted at 25°C, on suspensions containing 0.1 M NaClO<sub>4</sub>. Potentiometric data are in agreement with the presence of acidic and basic sites. It is possible to assign the acid sites to phenolic-type groups. The nature of basic sites is still under investigation. Biochar has a point of zero charge (PZC), which is the pH value at which the surface charge is zero, equal to  $5.0 \pm 0.1$ . Kinetic measurements indicate that process follows the pseudo-second order kinetic model, as well as the intraparticle diffusion model<sup>2</sup>. Adsorption isotherms<sup>3</sup> were utilized to evaluate the removal efficiency of lanthanides from solution. Experimental data are in agreement with the Langmuir model.



Amount of adsorbed  $Y^{3+}$  ions per gram of biochar as a function of the square root of time at 25 °C in 0.1 M NaClO<sub>4</sub> (solid concentration of 3.5 g/dm<sup>3</sup>).



Amount of adsorbed  $La^{3+}$  ions (triangle) and  $Ce^{3+}$  ions (circles) per gram of biochar, as a function of equilibrium concentration (mg/dm<sup>3</sup>) at 25 °C in 0.1 M NaClO<sub>4</sub> (solid concentration of 3.5 g/dm<sup>3</sup>).

Distribution coefficient values of  $La^{3+}$ ,  $Ce^{3+}$ ,  $Pr^{3+}$  and  $Nd^{3+}$  ions in function of atomic number present a discontinuity for the  $Ce^{3+}$  ion. This effect, also found in other solids, is known as "tetrad" effect<sup>4</sup> and results from increased stability at quarter, half, three-quarter, and complete filling of the 4f electron shell of lanthanides. Tetrad effect was originally recognized in the partitioning of organic complexes of lanthanide elements between organic and aqueous phases, where the partition coefficients of lanthanides complex against atomic number form four contiguous curves, each curve consisting of four elements; La–Ce–Pr–Nd, Pm–Sm–Eu–Gd, Gd–Tb–Dy–Ho and Er–Tm–Yb–Lu.

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## Effects of vineyard management on leafhoppers with emphasis on *Scaphoideus titanus*

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The effect of vineyard management was evaluated on *Scaphoideus titanus*, vector of Flavescence dorée phytoplasma, and other arthropods inhabiting vineyards in two areas located in the Veneto region (Padova and Treviso provinces) during the 2023 growing season. In the Padova area, organic and conventional vineyards, the latter managed according to voluntary IPM regional guidelines, were compared. These vineyards comprised Glera or Pinot gris varieties. Vineyard management did not influence the abundance of *S. titanus* nymphs, probably because their densities were low. There were more *S. titanus* adults on traps of organic vineyards, but only on one sampling date. Flavescence dorée incidence was not affected by vineyard management. Additional effects of vineyard management were noticed on two other leafhoppers. *Hebata vitis* was more abundant in organic vineyards of Glera variety. *Zygina rhamnii* was also more abundant in organic vineyards of both varieties. Interestingly, vineyard management affected predatory mite abundance. In particular, *Amblyseius andersoni* was more abundant in conventional than in organic vineyards. The study carried out in Treviso province involved organic and conventional vineyards of Glera variety. There were no differences between organic and conventional orchards in terms of *S. titanus* abundance before mandatory insecticide applications. Later, adult captures were higher in organic than in conventional vineyards. These results are likely influenced by pesticide use, i.e., systemic insecticides and pyrethroids in conventional vineyards while pyrethrins in organic vineyards. Despite the higher impact of conventional insecticides on leafhopper populations, *S. titanus* numbers appeared to be relatively low, showing that Flavescence dorée can be managed successfully in organic vineyards.

## Polysaccharide micro- and nano-carriers for tunable and responsive release of pesticides and biostimulants

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In the last years, the choice to use non-toxic natural substances in the place of synthetic ones is an increasing trend in many different fields. In particular, new technologies for precision farming are being developed with the goals of preserving the biodiversity and respecting the environment by reducing the use of chemicals. This approach applies to both active molecules with fertilizer, stimulating and/or pesticide properties, and carriers [1]. In this framework, this research line aims at designing an eco-sustainable and versatile platform for the delivery of different natural actives.

Sodium alginate hydrogels are promising carriers because of their low toxicity, high biodegradability and biocompatibility, the low costs of raw materials, and, last but not least, the ease of preparation and scaling-up by means of eco-sustainable methods [2].

The hydrogel properties can be tuned in terms of stiffness and dimensions, realising a variety of possible release mechanisms, to meet a specific way of application or targeting necessity [3,4]. In this respect, the necessary requirement is the definition of structure-function relationships based on a thorough physico-chemical characterization of the alginate hydrogels, which is carried out by analysing the inner local organization and dynamics by means of Electron Paramagnetic Resonance (EPR) spectroscopy, as a function of both the gelation conditions and the environmental variables (temperature, pH, presence and nature of ions). These results will be related to the hydrogel capability to encapsulate and release different natural actives, evaluated by means of spectroscopic techniques, to put the basis for the use of alginate hydrogels as a versatile precision farming technology in the agrochemical industry.

43

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## Impact of improved tomato genotypes and soil pathogens on non-target microbial species

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Microbial communities play a fundamental role in the bionetwork function of soils maintaining the fertility of agricultural soils driving biogeochemical cycling and protecting plants against soil-borne plant pathogens. This research aims to assess the structure of the root-associated microbiota of two tomato genotypes and to evaluate how the presence of a fungal plant pathogen on plant roots affects the composition and diversity of soil and root-associated microbial. Two different tomato genotypes, Money Maker (susceptible host) and Motelle (resistant host), were inoculated with the plant pathogen *Fusarium oxysporum* f.sp *lycopersici* isolate 4287 race 2 and grown in a greenhouse. Non inoculated plants were used as controls. Ten days after inoculation, total genomic DNA was extracted from rhizosphere soils and root samples and used as template for high-throughput sequencing. Bioinformatic analysis of variance of the Shannon index showed that microbial diversity was affected by habitat (root and rhizosphere;  $P < 0.001$ ). The PCoA of the weighted UniFrac community distance showed a marked difference between the bacterial communities of the two different tomato genotypes in both the rhizosphere and root samples. Taxonomic analysis of the bacterial communities at the order level showed that among endophyte populations identified in the roots of the Money Maker genotype, Pseudomonadales was a dominant taxon in uninoculated plants, and notably enriched when the pathogen was present.

This study increases the basic scientific knowledge on the impact that improved tomato genotypes might have on non-target microbial species.

## Permeabilization of *Ceratitis capitata* (Medfly) embryos for the delivery of bioactive molecules

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*Ceratitis capitata* (Medfly) is an agricultural insect pest affecting more than 25 plant species worldwide. The sterile Insect Technique (SIT) is an eco-friendly genetic approach to control this insect population and other Tephritidae species, which requires the massive production of male-only progeny (sexing) before sterilization of release. Because of the limitations of the current sexing methods, which rely on embryonic female-specific lethality induced by chromosome and genetic mutations, we investigated novel approaches of sexing applicable to wild-type Medflies and easily transferable to other species, such as the invasive *Bactrocera dorsalis*. A complete and irreversible masculinization of XX embryos can be produced in Tephritidae by microinjecting dsRNA targeting the female-determining *transformer* (*tra*) gene function (Saccone, 2022; Pane *et al.*, 2002), resulting in competitive XX males compared to XY males (manuscr. in prep). Sexing based on masculinization would avoid differential lethality and double the production of male-only progeny. We are exploring 1) other smaller RNAi-inducing molecules, including siRNAs (small interfering RNAs) and FANA-aso (stable antisense nucleotides), and 2) embryo-permeabilization chemical methods of delivery alternative to manual microinjection, including bleach, Triton X-100, Tween 20 and Citrasolv®-Heptane solutions (Rand *et al.*, 2010). Preliminary experiments using as permeabilization indicator fluorescent Rhodamine B and modified FANA-aso (green), led us to observe more efficient delivery into embryos using Triton permeabilization. Ongoing delivery experiments, that also include electroporation approaches and a combination of chemical and physical treatments, will further improve this promising delivery technology.

## Advancements in precision weed management: autonomous robot and predictive model

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Site-specific weed management is well-known for its potential to reduce herbicide application in agriculture, but it is just one aspect of precision that requires attention. Additionally, the timing of weed control operations is crucial. While contemporary agricultural technologies prioritise site-specific precision, the advancement of emergence predictive models strives for temporal precision. In this context, this research explores the potential of an autonomous robot with a precision weeder, comparing its efficacy with traditional tractor mechanical weeding and exploring the best operation timing. The goal is to advance precise and environmentally friendly weed control practices in agriculture, aligning with the evolving landscape of sustainable farming.

The study compared the performance of the agricultural robot 'Robotti 150 D' (Agrointelli, Aarhus, Denmark) and a traditional tractor equipped with the precision weeder, Rotosark (OliverAgro s.r.l., Verona, Italy), for mechanical weed control in a maize field at the experimental farm of the University of Padova in northeastern Italy. Weeding operations were conducted at two velocities (5 km/h and 3 km/h) and weed presence over time was monitored using the emergence model AlertInf.

Results indicate that both the robot and the tractor achieved a weed control efficiency of approximately 95%, with no statistical differences observed between methods or velocities. Even at the species level, no significant differences were identified. Furthermore, the final weed biomass showed no significant variations between plots managed by the robot and tractor. The study also emphasizes the potential benefits of integrating the emergence predictive model with weed control operations, with ongoing data collection set to evaluate these advantages in the coming years. AlertInf emerges as a valuable tool for enhancing precision weed control. The linkage of AlertInf predictions with weeding operations has the potential to increase overall precision, reduce pesticide use, and improve both chemical and mechanical control efficacy. In conclusion, the similar efficacy of autonomous robotic weeding and traditional tractor-based methods highlights the potential for autonomous systems to deliver weed control results comparable to conventional practices. Moreover, autonomous robots, when coupled with predictive models, represent a promising solution for precision agriculture, demanding continued exploration for future applications.



## Exploring extracts from agricultural biomasses to obtain new biostimulants and biopesticides

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Phytochemicals for crop management can be a valuable alternative to the use of agrochemicals by improving the sustainability of cropping systems. Plant biomasses are rich in bioactive compounds that have potential application for both biostimulation and crop protection from pathogens and pests. A workflow on extracts and protein hydrolysates production from selected agricultural biomasses was designed with the aim of investigating their potential use in agriculture. The bio-based products, enriched in bioactive compounds, are obtained from: i) brassicaceae oilseed defatted meals and other biomasses enriched in glucosinolates and/or their hydrolysis products, and low molecular weight peptides and free amino acids; ii) other brassicaceous biomasses; iii) alfalfa material (saponins, phenolics); iv) olive chain waste and/or other biomasses by-products; v) essential oils of *Petroselinum crispum* and *Carlina acaulis*. The obtained extracts, divided into groups according to the expected activity, are currently or will be tested *in vitro* and *in vivo* on different model crops and target pests/pathogens. The models identified for the study are tomato host under abiotic stress or infected by tomato spotted wilt orthotospovirus (TSWV), fungi or infested by pinworm; olive host infected by *Verticillium dahliae* or infested by *Bactrocera oleae*, and *Tetranychus urticae*. The most promising extracts will be evaluated for their toxic and side effect on selected beneficial mites: *Phytoseiulus persimilis* and *Neoseiulus californicus*. The preliminary findings allowed to identify some bioproducts with different composition and purification levels, among which protein hydrolysates and extracts enriched in phenols, glucosinolates and saponins, to be further tested and developed in application protocols.

## Effect of sulfur on *Halyomorpha halys* (Hemiptera: Pentatomidae) in laboratory, semi-field and field experiments

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*Halyomorpha halys* (Hemiptera: Pentatomidae) is a pest native to Asia and invasive to the US and Europe, where is considered a major pest in fruit orchards. Being characterized by high polyphagia and mobility and subjected to low insecticide residual effects, *H. halys* is a challenging pest to manage. Current pest control programs require frequent insecticide applications to reduce pest damage. The use of repellents and deterrents could be a potential tactic in integrated pest management strategies against *H. halys*. In the laboratory, we assessed the mortality of *H. halys* induced by sulfur applications at different doses and its response to sulfur in choice experiments. Semi-field experiments were conducted to test the effect of sulfur on host plant choice. The effect of sulfur application on *H. halys* infestation and associated damage was assessed in pome fruit orchards. In the olfactometer and choice experiments, sulfur treated fruits were less preferred by *H. halys* as compared to untreated ones. A low infestation level was observed in sulfur-treated plants, with a significant reduction in pest damage. These results suggest that sulfur can affect *H. halys* behavior, representing a promising tool for *H. halys* management.



## ***Beauveria bassiana* metabolites involved in biocontrol activity against pathogens**

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Entomopathogenic fungi belonging to *Beauveria bassiana* (*Bb*), apart from being widely used in agriculture for the control of insect pests, they also play different roles in natural agroecosystems, including endophytism, plant growth promotion and disease control. Here, we compared the metabolome and the entomopathogenic potential or biocontrol activity of ten *Bb* isolates respectively on the insect pest *Spodoptera littoralis* or against the fungal plant pathogen *Fusarium oxysporum*. Metabolomic analysis (gas chromatography coupled to mass spectrometry, GC-MS) of *Bb* exo-metabolites revealed that *Bb* is able to produce a plethora of biologically active compounds. While some have proven antifungal, antibacterial and insecticidal properties, others (*e.g.* oxalic acid) may be involved in softening the insect cuticle and lowering environmental pH, a mechanism already described to inhibit fungal pathogen penetration in plant root tissues. Further experiments will be required to investigate the biological role that the different classes of metabolites produced by *Bb* play in plant-interaction and biocontrol.

## Root-associated microorganisms for optimizing biological control in tomato

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Tomato is one of the most widely consumed vegetable crops worldwide, but its production is constantly threatened by numerous pests. In the context of Integrated Pest Management (IPM), the induction of natural plant defense mechanisms mediated by soil microorganisms is considered a sustainable solution for pest control. Plant Growth-Promoting Fungi (PGPF) and Rhizobacteria (PGPR) are promising alternatives to synthetic chemicals, but their effects on plant-herbivore-natural enemies interactions remain largely unknown. We studied the olfactory response of two natural enemies (i.e., the predatory beetle *Cryptolaemus montrouzieri* and the parasitic wasp *Eretmocerus eremicus*) of tomato key pests to volatiles emitted by tomato plants previously inoculated with fungal and bacterial strains showing PGP traits in a two-way olfactometer, under laboratory conditions. Three fungal species, including commercial strains of two *Trichoderma* mycoparasitic fungi (i.e., *T. asperellum* and *T. harzianum*) and an entomopathogenic *Beauveria bassiana* strain (ATCC 7404), and six bacterial species (i.e., commercial strains of *Bacillus subtilis* and *B. amyloliquefaciens*, and laboratory isolates of *B. spizizenii*, *Pseudomonas fluorescens*, *P. veronii* and *P. gessardii*) were tested 3 and 7 days after inoculation, in comparison with untreated plants. In preliminary observations, *C. montrouzieri* showed significant attraction towards plants inoculated with *Pseudomonas* spp. and *T. asperellum*, 3 and 7 days after inoculation, respectively. By contrast, the other treatments generally induced a repellent effect with a significant preference by *C. montrouzieri* adults towards untreated plants. A similar trend was recorded for *E. eremicus*. This preliminary study can provide the basis for understanding tri-trophic interactions triggered by root-associated microorganisms in tomato cropping system. Future investigations including further pest-natural enemy combinations will help in understanding the role of Volatile Organic Compounds (VOCs) upon plant inoculation with PGPF and PGPR to optimize biocontrol strategies through the use of biostimulants.

50

Keywords: induced plant defenses, natural enemies, Plant Growth-Promoting microorganisms, tri-trophic interactions, VOCs

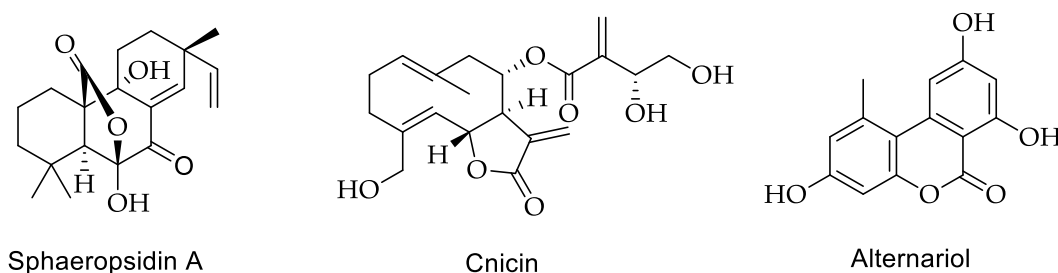
## Chemical and biological characterization of microbial and plant metabolites for the development of potential innovative pesticides

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One of the promising strategies for achieving sustainable agriculture is the adoption of integrated pest management (IPM). This approach involves a combination of control methods that consider the specific characteristics of the invasive species and the local ecosystem. Given the hazardous effects of synthetic pesticides, there is an urgent need for alternative strategies in managing the agricultural pests. Natural products based biopesticides could contribute to IPM strategies, helping to minimize the environmental impact of pest control practices.

Our ongoing work aims to discover natural compounds for the development of biopesticides suitable for IPM practices. Here we present the isolation bioactive metabolites from plants, endophytic and phytopathogenic fungi. In particular, strains of *Alternaria alternata*, *Dothiorella sarmentarium* and *Diplodia corticola* isolated from pear, grapevine and oaks, respectively, were selected as sources of secondary metabolites. In addition, the plant *Glechoma hederacea* has been identified as a potential biocontrol agent against *Plasmopara viticola*, a destructive oomycete that affects grapevines causing significant economic losses worldwide. Moreover, the plant *Centaurea cineraria* L. subsp. *cineraria* has been investigated as a potential source of inhibitors for broomrape (parasitic weeds) radicle growth. This communication reports on the chemical and biological characterization of low molecular weight metabolites isolated from selected sources exploring their potential as bio-inspired pesticides. The chemical structures of some of them are reported below.



**Figure** Bioactive metabolites isolated from selected sources.

## Usage of chitosan from *Hermetia illucens* as a preservative for fresh *Prunus* species fruits: a preliminary analysis

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Due to its properties, chitosan is suitable for use as a natural biopolymer coating material to preserve the quality and extend the shelf-life of fresh products. Chitosan-based bioactive films are successfully used in the packaging of fruits, vegetables, eggs, and meat against microbial contamination and spoilage. Currently, the main commercial source of chitosan are waste streams from the marine food industry (crustacean exoskeletons). However, issues of sustainability of crustaceans make insects, particularly bioconverters, a promising alternative source of chitin and chitosan. This work is focused on the exploitation of one of the side streams (pupal exuviae) generated from *Hermetia illucens* farming, which prove to be the best biomass of insect for the chitin extraction and the production of chitosan. Chitosan from pupal exuviae of *H. illucens* were used for the formulation of coating solutions to be applied to fresh fruit, apricots (*Prunus armeniaca*), yellow nectarines (*Prunus persica*) and yellow peaches (*Persica vulgaris*). Fruits were coated with 0.5% and 1% bleached and unbleached chitosan and stored at room temperature and 4°C. Changes during storage occurred in weight loss, total soluble solids content and pH. The effect of the chitosan-based coating on spontaneous development of mould on the skin of fruit was also assessed. The results of this investigation revealed that insect chitosan is as effective as or better than the commercially available crustacean chitosan in maintaining more stable some post-harvest physicochemical parameters. The effects are more evident on fruits stored at 4°C than on those stored at room temperature. No relevant differences are found between the two tested concentrations of chitosan, nor between bleached and unbleached chitosan. These preliminary results provide an encouraging starting point for validating pupal exuviae from particularly *H. illucens*, for the chitosan use in the agri-food industry.

## Usage of chitosan from *Hermetia illucens* as an innovative and sustainable preservative for strawberry

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Strawberries, widely favored fruits by consumers, are acknowledged for their popularity but are recognized as highly perishable fruits. The fruit structural integrity postharvest is typically sustained for only a few days under standard storage conditions. To mitigate the rapid deterioration of strawberries and prolong their shelf life, an alternative approach involves the treatment with a biodegradable and health-friendly compound, such as chitosan. Due to its antifungal and antimicrobial properties, this bio-coating would act as a protective barrier, offering defense against external agents as well as preventing the growth of molds and fungi that could contribute to the accelerated decay of the fruit. The potential of chitosan derived from *Hermetia illucens* pupal exuviae to slow down the deterioration of the native strawberry (*Fragaria x ananassa*) cultivar "Melissa" was investigated. Strawberries were stored at room temperature, at 4°C, and at mixed storage conditions (4°C+RT). The results showed that chitosan from *H. illucens* was more effective than commercial polymer in food preservation, stabilizing and improving some fundamental postharvest parameters. In particular, decolorized chitosan was most effective in containing physicochemical parameters (weight loss, pH, and soluble solids content) and in preserving treated strawberries from fungal decay. No decolorized chitosan, on the other hand, was more functional in preserving and enhancing the nutraceutical properties (total phenolic and flavonoid content, total anthocyanins and antioxidant activity) of the treated strawberries. The observed results validate the possibility of using insect chitosan as a substitute for crustacean chitosan in the preservation of post-harvest fruits, particularly deriving from *H. illucens* pupal exuviae.

## Preliminary investigation on the effect of insect-based chitosan on preservation of coated fresh cherry tomatoes

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Chitin is the main structural component of the arthropod exoskeleton and the cell wall of yeasts and fungi. Nowadays, the main commercial source of chitin (crustaceans' exoskeleton) is no longer a sustainable source. For this reason, insect-derived chitin has received increasing attention, as an alternative and more sustainable source. Among insects, the Diptera *Hermetia illucens* can be considered a very promising source of this biopolymer: while larvae can feed on organic waste and bioconvert it in larval biomass, the breeding waste (pupal exuviae and dead adults) can be used as source of chitin and chitosan. Due to its antimicrobial, antioxidant and film-forming capacity, chitosan is one of the most promising natural polymers for use as edible coating. Chitosan-based coatings reduce dehydration and retard microbial and fungal spoilage by acting as barriers, able to delay ripening and senescence. Coatings produced by chitosan crustaceans have already been successfully used on a variety of fresh fruit and vegetables. In this work, chitosan was produced from *H. illucens* pupal exuviae following two different types of deacetylation methods: heterogeneous and homogeneous and applied on cherry tomatoes (*Solanum lycopersicum*). The packaging of tomatoes is a key element in their post-harvest preservation, as they are perishable climacteric fruits. Indeed, tomatoes, in the post-harvest period, continue to undergo biochemical ripening and respiration mechanisms, which affect their deterioration and thus lead to their senescence. Chitosan was applied by spraying and by dipping at concentrations of 0.5% and 1 % and tomatoes were stored at room temperature and 4°C. The weight loss, physico-chemical parameters, total phenolics, total flavonoids and variation in antioxidant activity of the tomatoes were studied for a 30-day storage period. All parameters were kept stable with the use of heterogeneous chitosan, in comparison to the homogeneous one. Furthermore, the spraying method, compared to the dipping technique, was more effective in reducing weight loss and pH variation. Chitosan coatings were effective in reducing the pH variation of the tomatoes. No significant differences in the protective action between chitosan derived from *H. illucens* and commercial chitosan were detected. These preliminary results provide an encouraging starting point for new opportunities in the use of chitosan from *H. illucens*.

## Plant defence via bio-stimulant or antimicrobial peptides application

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The management of fungal and bacterial plant diseases mostly relies on chemical control and specifically on the application of copper-based products and/or synthetic fungicides that can determine negative effects on the environment and on the health of growers and consumers. Therefore, searching for new sustainable alternatives to chemical control is crucial.

A promising low-impact method for controlling plant diseases is the activation of plant defence mechanisms by treating with natural elicitors or antimicrobial compounds. For example, yeast cell walls contain molecules perceived by plant cell receptors as elicitors and thus able to activate the plant immune response. In this research we evaluated and compared the efficacy of different cell wall extracts from the yeast *Saccharomyces cerevisiae* for their capacity to induce grapevine tolerance to the fungal pathogen *Botrytis cinerea*, the causal agent of the grey mould disease. Symptoms development and molecular mechanisms activated in yeast-treated plants after pathogen inoculation have been investigated.

Trichogin GA IV is a non-ribosomal antimicrobial short peptide naturally produced by the fungus *Trichoderma longibrachiatum*. Its antimicrobial activity resides in the ability to insert into phospholipidic membranes and form water-filled pores, thus perturbing membrane integrity and permeability. With the aim of developing new sustainable biopesticides, we designed modified trichogin analogs, containing one to three Gly-to-Lys substitutions, to increase water-solubility and antimicrobial activity against plant pathogens. By in vitro and in vivo bioassays, we identified at least two peptide analogs of trichogin effective in reducing Fusarium Head Blight and powdery mildew of wheat and tomato bacterial speck disease under controlled conditions.



## Side effects of sweet orange essential oil on the parasitoid *Exorista larvarum*, selected as model non-target insect

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In recent years, there has been a growing use of biomolecules in agriculture. However, their impacts on non-target species remain insufficiently understood. The aim of this study was to assess both the direct (e.g., mortality rate) and indirect (e.g., fecundity) effects of sweet orange essential oil (EO) (the sole EO marketed in Italy as an insecticide) on the tachinid fly *Exorista larvarum* (L.). This species was selected as model non target insect due to its dual biological roles as both a parasitoid of pest lepidopterans and a pollinator. The experimental procedures were adapted from internationally recognized protocols used for studying pesticide toxicity on *Apis mellifera* L., a reference insect species in Environmental Risk Assessment. For our experiments, mated *E. larvarum* females were utilized. Both acute contact (a) and oral (b) toxicity were tested, by applying a single 1  $\mu$ L drop of EO suspension onto the fly thoraxes (a) and offering 10  $\mu$ L of OE suspension in a drinking trough to flies (b). Different concentrations (0.5%, 5%, 10%, 20%) were tested. The results revealed that sweet orange EO had no direct or indirect adverse effects on *E. larvarum* upon contact. It showed direct toxicity when ingested at high concentrations, but, interestingly, at these same concentrations, it also acted as a repellent for *E. larvarum*.



## Effects of microbial inoculants and their metabolites

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Formulations based on selected fungi belonging to *Trichoderma* genus are successfully used in agriculture due to their aptitude to act as biocontrol agents and/or to stimulate plant growth and resistance to biotic and abiotic stress. Several strains of *Trichoderma* are well-known producers of secondary metabolites involved in the beneficial interactions with the plants. A very effective way to discover novel bioactive compounds is the OSMAC (One Strain MAny Compounds) strategy, which is based on the application of a variety of growing environments on a selected microbial strain to induce biosynthesis of metabolites not produced under standard laboratory conditions. Through the application of this strategy, a modulation of the metabolite production in *Trichoderma harzianum* M10 has been registered. In particular, an up regulation of harzianic acid and siderophores was detected in 1/10 PDB; while the never isolated 5-hydroxy-2,3-dimethyl-7-methoxychromone was evidenced in full PDB under shaking condition. Moreover, the effects of two strains (*T. harzianum* M10 and *T. afroharzianum* T22) and the *Trichoderma* metabolite 6-pentyl- $\alpha$ -pyrone were evaluated *in vitro* and field trials for plant growth promotion activity. Harzianic acid and 5-hydroxy-2,3-dimethyl-7-methoxychromone were tested against *Rhizoctonia solani* and human colon adenocarcinoma cell lines (HCT116 – used for non-target effect). M10, T22 and 6PP-based treatments enhanced seed germination rate and increased seedlings emergence, whereas no significant differences on biometric parameters (i.e. plant height or weight) were observed during field experiments. Regarding the antibiotic activity, HA completely inhibited the growth of *R. solani* at 100  $\mu\text{g plug}^{-1}$  while at the same concentration the chromone derivative showed 49 % inhibition. The chromone derivative and HA reduced the viability of HCT116 cells in a concentration-dependent manner.

## Development and application of innovative microbial-based biostimulant formulations to enhance plant growth

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Biostimulants play a crucial role in ensuring crop yield and nutritional quality, providing a sustainable alternative to chemical fertilizers. Specifically, biofertilizers contain live microorganisms capable of colonizing the plant rhizosphere, enhancing nutrients availability, and promoting plant growth. This research aimed to develop novel microbial-based biostimulants and assess their effectiveness on maize plants. Three bacterial strains, *Bacillus megaterium* EL5, *Azotobacter chroococcum* 76A, and *Kosakonia pseudosacchari* TL13, were selected based on their plant growth-promoting activities. Antagonism tests were performed to ensure the absence of antimicrobial activity among the three selected strains, followed by microbial biomass production through liquid-state fermentation. Two different formulations, freeze-dried and hydrogel, were developed and experimental tests were conducted to evaluate the effectiveness of the innovative microbial-based biostimulants on maize plants cultivated under controlled growth conditions, including both optimal irrigation and water stress scenarios. Biometric indices were evaluated, affirming the efficacy of both freeze-dried and hydrogel formulations. Additionally, q-PCR analyses were conducted to quantify target genes associated to nitrogen (*nifH*, *narG*, and *nirK*) and phosphorus (*phoD* and BPP) biogeochemical cycles, along with the 16S rRNA gene to assess total bacterial population. The results indicated a significant impact of biostimulants on the soil microbial community increasing the copy numbers of target genes. These results highlighted the pivotal role of this microbial consortium in augmenting two fundamental soil activities making the selected bacterial strains promising candidates for the development of new biofertilizers for sustainable agriculture.

## In vitro antifungal activity and in vivo efficacy of insect-derived chitosan treatment against *Botrytis cinerea* in strawberry

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Strawberry is a perishable fruit, susceptible to development of rots by a range of fungi, in particular *Botrytis cinerea*. Chitosan represents an alternative to agrochemicals for improving shelf-life and fighting fungal pathogens. A chitosan-based formulation derived from pupal exuviae of *Hermetia illucens* has been used for improving strawberry shelf-life. The effects of a decolored (PEDEC) and not decolored (PEND) chitosan from the black soldier fly were evaluated and compared with commercial chitosans from crustaceans (CCs), *in vitro* and *in vivo*. An inhibition/reduction of fungal growth and a disturbance of normal fungal morphology were observed, being MIC of 0.5 and 1 mg mL<sup>-1</sup> and growth inhibition of 70 and 4% for PEND and PEDEC, respectively. Both chitosans showed equal or better potential application than CCs in controlling *B. cinerea* in strawberry post-harvest treated. Different effects for chitosans depended on their different molecular weight and deacetylation degree distributions, as well as the presence or absence of melanin pigments in their structure. PEND could act directly against the fungus, with effects predominantly associated with fungitoxic properties; PEDEC might principally provide viable alternatives, such as the elicitation of biochemical defense responses in fruits, for example through total phenols, in particular the flavonoids. Chitosan derived from *H. illucens* is undoubtedly a promising tool to control *B. cinerea* and, therefore, usable for sustainable production of high-quality strawberry.

## Improvement of RNAi-based biopesticide strategy that combines targeting *RNase 1*, *RNase 2*, and *ATPase* genes

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Although RNA interference (RNAi) is predominantly used as a reverse genetic tool, in recent years it has been demonstrated that this technique, thanks to its high sequence specificity, can be used to develop next-generation eco-sustainable bioinsecticides, limiting side effects on non-target species (Christiaens *et al.*, 2020). For field applications, one of the most promising delivery methods of dsRNA molecules is through adult feeding (Upadhyay *et al.*, 2011). However, intestinal nucleases can negatively impact gene silencing efficiency. This project aims to demonstrate that, by simultaneously silencing intestinal nucleases (*RNase1* and *RNase2*), it is possible to improve the RNAi technique targeting a vital gene (*v-ATPase A*) in Medfly adults as previously shown in *Bactrocera dorsalis* (Tayler *et al.*, 2019). The experimental results showed that it is possible to deliver dsRNA molecules by feeding flies with a water-sucrose solution, a condition that allows them to survive. The gene silencing analysis demonstrated that the expression of *v-ATPase A* is significantly reduced when flies are fed with a dsRNA solution against this and *RNase1* and *RNase2* genes, compared to flies treated with only *v-ATPase A* dsRNA solution. Furthermore, the gene silencing is associated with a very high mortality rate of treated flies compared to control groups, especially in the group where the nucleases were silenced. Our data pave the way towards alternative pest control applications, based on the use of the RNAi technique, targeting fundamental and specific genes for the insect.