Croatian 59 2024 19 International Symposium on Agriculture

# Book of Abstracts

# Zbornik sažetaka

**11 – 16 February 2024, Dubrovnik, Croatia**

59
Croatian
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# Book of Abstracts

59
Hrvatski
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# Zbornik sažetaka

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**Sekcije . Sessions**

# Contents

Session 4 | **Vegetable Growing, Ornamental, Medicinal and Aromatic Plants**

Session 5 | **Crop Production**

Session 6 | **Fisheries, Wildlife Management and Apiculture**

Session 8 | **Viticulture, Enology and Pomology**

Session 9 | **Plant Protection**

59 Croatian 2024 19 International Symposium on Agriculture

Session 0

# Book of Abstracts

Plenary session

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Plenarna izlaganja

# From Disease to Depression to Dementia: Plant-derived Compounds Provide Solutions to Enhance Human Resilience, Vitality and Healthspan

Mary Ann Lila

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**Summary**

Unlike most pharmaceutical drugs, which usually feature just one or two active ingredients, plant extracts (herbal medicines or botanical drugs) typically contain a broad, multifaceted and overlapping range of interactive phytochemical compounds (phytoactives). The abundance, diversity and complexity of accumulated bioactive chemicals in natural plant-derived extracts allows them to interact (additively or synergistically) to counteract human disease symptoms and enhance human metabolism and immune function. While the powerful benefits of plant-based medicinals have been realized almost since the dawn of mankind, modern science is only now beginning to unravel the mechanisms by which plant-derived chemicals interact with human therapeutic targets to prevent and treat chronic disease and optimize health status. The recent availability of powerful 'omics technologies – metabolomics, transcriptomics, genomics, proteomics, lipidomics, nutrigenomics and more – has allowed science to finally begin to identify which plant species and which groups of metabolites are most efficacious, and to decipher the most efficient dosages, frequencies of intake, and product formats to help consumers proactively protect their own health. Berry fruits, which are already well-recognized as pharmacologically-active functional foods and which feature rich and varied phytochemical profiles dominated by flavonoids, can be used to illustrate common themes about the health-protective benefits of bioactive plant extracts. For many decades, it was an enigma to reconcile the observed diverse health benefits of berry dietary flavonoids with their (apparently) very poor bioavailability. Only recently, scientists determined that most (>90%) of these flavonoid compounds are not actually absorbed in the upper gastrointestinal tract, but, instead are biotransformed by commensal intestinal microbiota into smaller molecular weight phenolic catabolites. While some deglycosylation and chemical degradation occurs in the small intestine, it is the colonic catabolites (from lower-intestinal digestion) that subsequently enter circulation in sufficiently high concentrations to exert health-relevant bioactivities. Flavonoids that ultimately reach the human colon have a dual impact on human health. First, as prebiotics, they have a dramatic influence on the composition and the functionality of the gut microbiome. Ingestion of prebiotic flavonoids favorably alters gut microbial community structure, supporting beneficial commensal bacteria and reducing levels of opportunistic species. Prebiotic flavonoids and their metabolites quell inflammation in the gut, improving the epithelial barrier's integrity and activating tight junctions. Second, the biotransformation of dietary flavonoids by the intestinal microbiota drives the ultimate delivery of active metabolites into circulation where they elicit health-protective effects. Thus, the actual bioavailability of these flavonoids is largely dependent on their catabolism at the gut microbiome level and subsequent secondary xenobiotic biotransformation in the liver before entering circulation in the form of bioactive phenolic metabolites.

In addition, as illustrated by berry fruit preclinical and clinical studies, *color* is generally a reliable biomarker of health activities, *wild* species (with their highly diverse phytochemical profiles) can demonstrate some of the highest potencies, and *physical exertion* (movement) is a critically-important trigger that accelerates the bioavailability of circulating active metabolites. These common themes will be demonstrated in research probes on 1) phytoactives and human immune protection, 2) phytoactives and cognitive health in aging, 3) phytoactives and resistance to metabolic syndrome, and 4) phytoactives for skin resilience and protection.

How can the consumer best take advantage of health-protective plant compounds? Since plant-

derived active chemicals tend to be unstable after extraction, some novel ingredient formulations, such as microencapsulation in edible protein matrices, have been incorporated into convenient functional food products. These innovations are designed to ensure safe, stable delivery of compounds through the gastrointestinal tract and to the gut microbiome, to maximize digestibility, bioavailability and efficacy of phytoactives in the human system.

**Keywords**: berry fruits, plant extracts, flavonoid compounds, omics technologies

# Potential of Cannabis-originated Products in Chicken and Turkey Nutrition

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**Summary**

Poland is currently the leader in the European Union in terms of turkey and chicken meat production. For this reason, a big challenge for feed producers is to ensure the appropriate quantity and quality of feed raw materials (mainly sources of feed protein) to cover the growing demand in the production of broilers. One of the protein raw materials that could be used in feeding turkeys and chickens is hemp cake. In recent years, due to the growing interest in products derived from hemp (*Cannabis sativa* L.), their production has been increasing dynamically in Polland and around the world. Hemp cake is a product obtained during the process of pressing oil from hemp seeds, so it is a by-product that often has no further use and is considered as waste for producers. Research on broiler chickens models also indicates its possibility of being used in the diet. However, there are no feeding experiments using the turkey model, and due to significant differences in "feeding physiology" between chickens and turkeys, the results obtained from chickens cannot be directly transferred to turkeys. For this reason, there is a need to conduct nutritional studies to determine the nutritional value of hemp cake for turkeys. Another product obtained from hemp that may have practical application in turkey and chicken nutrition is cannabidiol (CBD), which has an effect that supports the functions of the digestive tract (impacting mechanically, humorally, neurologically and immunologically on the digestive system, including the modification of microflora). Although research on the potential of CBD has not yet been fully elucidated and mainly concerns human medicine or *in vitro* conditions, our previous research in the broiler chicken model, showed that CBD can have a beneficial effect on the processes that determine the functioning of the intestines and can support mechanisms related to immunity in birds, especially under conditions of induced stress. As in the case of hemp cake, the properties of CBD have so far been studied in poultry, mainly in the broiler chicken model. In the case of rearing turkeys for fattening, most health problems are related to disorders of the digestive tract, especially in the first periods of rearing (approx. 8 weeks), which determines the health status of the birds throughout the rearing period (usually up to the 20th week of life). For this reason, the effects of CBD may also positively support the health status of turkeys, but this requires verification. The aim of this study was to assess the nutritional value and properties modulating the functional state of the digestive tract of the hemp seed cake and properties of CBD in modulating birds' response to different stress conditions. A set of experiments was run to investigate turkey and chicken response to dietary inclusion of hemp seed cake and CBD regarding performance, gut integrity, gut microbial activity, selected indices of immune response and verification of potential axis occurrence. In the first phase, a performance response of chickens and turkeys to the dietary hemp seed cake inclusion was investigated. In the second phase, potential bioactive properties of CBD were verified in birds reared in optimal or applied to challenge conditions. In feeding trials, chickens were fed diets with inclusion level of up to 20% of hemp seed cake or up to 0.02% CBD, whereas, turkeys received diets with up to 20% of hemp seed cake or the same dose of fermented rapeseed cake. In the second phase, a set of experiments involving CBD was run: birds in the control group (CON) were fed a basal diet and were not challenged. Birds in the CBD group received a CON diet supplemented with 30 g/kg of *C. sativa* extract, while birds in the CON + *C. perfringens* and CON + LPS groups (positive control groups) were fed a basal diet and subjected to a *C. perfringens* or LPS challenge. Birds in the CBD + *C perfringens* and CBD + LPS groups received the same diet as the CBD-treated group, except that they were subjected to appropriate challenges. Briefly, on days 21 and 22 of life, birds from the

CON + LPS and CBD + LPS groups were orally (*per os*) administered LPS (*Escherichia coli* serotype O55:B5), while birds from the CON + *C. perfringens* and CBD Groups + *C. perfringens* was infected orally (*per os*) with 1 ml of inoculum containing approximately $10^8$ CFU/ml of *C. perfringens* type A strain 56 bacteria, according to a previously approved protocol. Body weight gain (BWG) on days 9–23 and 24–35 of the feeding periods did not differ between experimental groups ($P>0.05$), while BWG calculated for the entire period of feeding with the experimental diet was significantly lower in challenged birds, positive CON group, and CBD group than in the unchallenged negative CON group ($P<0.01$). *C. perfringens* challenge resulted in significantly lower collagen content in intestinal tissue than that observed in untreated chickens. CBD treatment significantly increased the mRNA expression of genes such as glucagon-like peptide-2 (GLP2), Toll-like receptor 4 (TLR4), and junctional adhesion molecule 2 (JAM2) (all $P < 0.001$). Zonula occludens-1 (ZO-1) gene expression was increased in all experimental groups compared with the unchallenged CON negative group and CON positive groups ($P<0.001$). Both *C. perfringens* challenge and dietary treatment resulted in changes in bacterial enzyme activity. Dietary treatments significantly influenced the activity of enzymes repairing DNA damage, but did not affect the rate of oxygen consumption in platelet mitochondria. A lower number of necrotic muscle fibers was found in the groups of chickens infected with *C. perfringens* after the addition of CBD compared to the CON positive group. The results showed that birds challenged with *C. perfringens* or LPS had significantly lower collagen content in the pectoral muscle. Our data showed that hemp seed cake is well tolerated by turkeys and chickens up to 20% of dietary inclusion. It has also been proven that CBD can influence birds' response to *C. perfringens* infection or LPS stimuli, which may allow time for effective treatment. The beneficial effect of CBD was manifested in the support of intestinal barrier function through increased expression of genes maintaining intestinal integrity. Current research has also shown that there is an axis between gut bacterial activity and chicken meat volatile compounds (VOCs), mediated by the production of short-chain fatty acids (SCFAs). Dietary supplementation with CBD reduced the level of formation of selected VOCs responsible for meat spoilage in challenged birds. Furthermore, dietary CBD down-regulates the production of putrefactive fatty acids, which reduces the concentration of spoilage-causing VOCs in breast meat. Moreover, our findings showed that the association between VOCs and SCFAs varied depending on the stress conditions (*C. perfringens* or LPS), however, CBD showed a more effective impact on the meat VOC profile of *C. perfringens* than the LPS challenge. Our results also indicate that there may be a potential relationship between the condition of the intestinal mucosa and histopathological changes in the superficial pectoral muscle under the influence of induced inflammation, and in this respect, CBD has a soothing effect due to protection against mucosal DNA damage. Further research in this area is needed to elucidate the mechanisms of action of CBD on skeletal muscle and the overall host response to pathogen challenge.

**Keywords**: hemp, cannabidiol, gut health, turkey, chicken

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# Achieving sustainable productivity in agriculture through beneficial microbial associations

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**Summary**

The availability of nitrogen and phosphorus is a major limitation to crop productivity and this is currently addressed primarily through application of inorganic fertilisers to augment these limiting nutrients. Use of such fertilisers contributes the greatest cause of pollution from agriculture in high and middle-income countries, while access to inorganic fertilisers is extremely limited for farmers in low-income countries. In natural ecosystems many species of plants acquire nitrogen and phosphorus through associations with beneficial fungi and bacteria, but the use of these beneficial microbial associations is currently very limited in agriculture. Through a detailed understanding of how plants associate with beneficial microorganisms, we are attempting to broaden their use in agriculture to facilitate sustainable productivity, accessible to all of the world's farmers.

**Keywords:** pollution from agriculture, inorganic fertilisers, fungi and bacteria, microbial association

# Revolutionizing African Agriculture: WACCI's Role in Sustainable Crop Improvement for Food and Nutrition Security

Eric Danquah[1], Samuel Offei[1], Kwadwo Ofori[1], Pangirayi Tongoona[1], Naalamle Amissah[1], Daniel Dzidzienyo[1], Agyemang Danquah[1], Beatrice Ifie[1], John Eleblu[1], Theresa Ankamah-Yeboah[1], Pearl Abu[1], Mavis Osei-Wusu[1], Rodomiro Ortiz[2], Ronnie Coffman[3] and Rufaro Madakadze[4]

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**Summary**

The projected increase in the global population to 9.7 billion people by 2050, with a substantial portion of this growth in sub-Saharan Africa, presents critical challenges and opportunities for food production. This demographic expansion is expected to increase demand for food by 50%, emphasizing the need for resilient and sustainable agricultural practices. This scenario is exacerbated by factors such as climate change, regional conflicts and pandemics that severely impact food systems. Despite a growth rate of 4.1% from 2000 to 2020, African agriculture is impeded by outdated agricultural practices. In response, the West Africa Centre for Crop Improvement (WACCI) at the University of Ghana spearheads innovative research and capacity development initiatives to unlock the agricultural potential of the continent.

WACCI has trained 111 plant breeders from across Africa, significantly transforming the continent's plant breeding programs. Its mission is to counteract the brain drain by training Africans within the continent and has evolved into a sustainable, research-driven institution. WACCI has developed and commercializes high-yielding hybrid maize and tomato varieties.

The Centre is advancing agro-entrepreneurship and youth empowerment, especially through its Kofi Annan Enterprise Hub for Agricultural Innovation. In addition, the WACCI is launching the African Academy of Agricultural Sciences (TAAAS) project in collaboration with the Cambridge Crop Science Center and other strategic partners. This initiative aims to nurture world-class scientists and create a foundation of partnerships that enhance higher agricultural education and research, thereby catalyzing agricultural transformation in Africa.

In this presentation, we will highlight our journey thus far, focusing on notable research outputs and outcomes. Additionally, we will outline a strategic pathway aimed at maintaining and enhancing our excellence into the future.

**Keywords**: food systems, climate change, plant breeders, brain drain, agricultural education and research

# Emerging invasive species threats to biodiversity and agriculture

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**Summary**

Globalization, including travel and trade, has resulted in the introduction of non-native species to new ranges worldwide, with more than 13,000 non-native plant species established across the globe, some of which cause profound impacts on natural and agro-ecosystems. The accumulation of established non-native species has increased steadily for most taxa and regions over time and shows no sign of slowing down. As a result, most ecosystems are now subject to multiple invasive species, including plants, pest, and pathogens. Although only a fraction of introduced species become problematic invaders, those that do can spread rapidly and have severe effects on natural areas, biodiversity, and agricultural systems, for example row crops, turf, and forestry. Furthermore, the ecological stress caused by the interaction of invasive species and other global change drivers such as climate change, anthropogenic disturbance, emerging pathogens, and other factors can have synergistic effects that are difficult to predict and mitigate. Although invasive plant species and other ecological stressors often occur simultaneously, how they interact to impact managed natural areas and agroecosystems is not well understood.

Improved forecasting and management of invasive species relies on better data from reliable field studies and experiments and conceptual and modelling advances. This presentation will focus on first explaining the general problem of invasive species, patterns in non-native species introductions, and potential impacts. Then, the results of three projects where interactions between plant invaders and other global change drivers were evaluated will be summarized, including: 1) A longer-term field experiment testing how drought and invasion (by the non-native perennial grass *Imperata cylindrica*) interacted to determine effects of a third stressor (fire) on longleaf pine (*Pinus palustris*), which is the foundation tree species for a threatened fire-dependent ecosystem in the Southeast USA that is also an important timber species; 2) A field experiment and modelling to test the effects of an emerging foliar fungal pathogen on the abundance and competitive effects of the invasive annual grass *Microstegium vimineum* in Indiana, USA and the potential of the invasive grass to act as a conduit for spillover of pathogens to agroecosystems; and 3) A field study and growth chamber experiment to assess if urbanization has caused evolution of the invasive forb *Erigeron annuus* in Croatia to better tolerate future climate conditions and potentially spread northward and into surrounding systems under climate change.

Altogether, these projects show that global change drivers, including climate change + fire, emerging pathogens, and urbanization can each enhance or alter the effects of invasive plants on native communities and ecosystems. They also highlight the need for longer-term, multi-factorial, manipulative studies that can generate data to accurately forecast ecological outcomes of interacting drivers of global environmental change for natural areas, biodiversity, and agricultural systems. Generating better predictions of invasive species establishment, spread, and impacts will enhance management efficiency and reduce the negative effects of invasions, thereby preserving biodiversity, maintaining ecosystem functions, and enhancing agricultural productivity.

**Keywords**: agroecosystem, climate change, ecological stressor, emerging pathogens, fire, synergy, urbanization

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Session 1

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# Zbornik sažetaka

Agroekologija, održiva poljoprivreda i klimatske promjene

# With soil from agricultural to applied life sciences: Basic soil map of Croatia sixty years later

Ferdo Bašić

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**Summary**

This year, 2024, is the sixtieth year since the beginning of the scientific inventory of soil in former Yugoslavia started in 1964 as a federal and ended in 1986 as a republic project "General soil map (GSM) of Croatia 1:50 000". The project has been burdened with contradictions since the beginning, and making a map does not solve the old, on the contrary, a bunch of new contradictions, resulting from progress world and our science on the genesis and evolution of soil, as a result of the new nomenclature (terminology) and soil classifications. Modern science sees the soil as a component of the ecosystem, with effects covered by the term soil effects in the ecosystem, which legitimate soil sciences as applied life sciences. These changes are followed by the inclusion of computers and new, more powerful techniques in research and interpretation, changes in the postulates of research, presentation and interpretation of results. The crown of it all is the change in the paradigm of soil as a unique natural resource and in soil management, ultimately and political changes the former state. It is therefore no wonder that this project has not been fully completed to this day. It is even more strange that no requests have yet appeared for the correct completion of the project. This work claims exactly that role. The basic pedological map - GSM 1:50 000 has been completed, but is not (yet) finished. The paper will propose actions necessary for the completion of the project, so that GSM can be transformed from "dead intellectual capital into which enormous effort, knowledge (hopes) and money have been invested. GSM have to be a valuable contribution to the socially, economic and environmentally sustainable soil management in *Lijepa Naša*. According of global trends that is the best way of soil sciences into applied life sciences.

**Keywords**: GSM of Croatia, sustainable soil management, applied life sciences

# Influence of biomass ash fertilization on soil pH, CaCO3, K2O and P2O5 under *Miscanthus x giganteus*

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**Summary**

The cultivation of energy crops, as well as the utilization of biomass ash are part of sustainable energy management and have a major impact on the environment. Biomass ash due to its chemical composition, have been used as source of fertilizers as well as in the form of soil conditioners and have been found to favorably affect the physicochemical properties of soils. As its importance is very high, the study regarding its influence on individual soil properties in different agroecological conditions is important to determine its scope and limitations for use. Therefore, the research aimed to determine the influence of different ash doses (2 and 5 t/ha) on the soil pH, carbonate content ($CaCO_3$), plant available phosphorus ($P_2O_5$) and potassium ($K_2O$) content under the perennial energy crop *Miscanthus x giganteus*. Study was conducted at AGR experimental station Šašinovec during 2016-2019. The study found that the application of ash had no significant effect on soil pH, however had on $CaCO_3$, $P_2O_5$ and $K_2O$. For both studied ash doses, there was significant decrease in the $P_2O_5$ and significant increase in $CaCO_3$ while the $K_2O$ content significantly increased only at the higher applied ash dose compared to the control plot. Biomass ash has proved to be a good soil conditioner, in terms of $CaCO_3$, $P_2O_5$ and $K_2O$ content under the studied agroecological condition. Further research on different types of agroecological conditions including soil types, vegetation, and climate will provide more insight on the effective utilization of biomass ash. Similarly research of biomass in combination with other organic substances like biochar could be more effective in terms of substitutes with chemical fertilizers and for soil amendments and lead to improve sustainability.

**Keywords**: biomass ash, soil reaction, calcium carbonate, soil conditioner, sustainability

# Soil quality across different land use in a peri-urban area of Zagreb (Croatia)

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**Summary**

Urban soils are threatened by urbanization, making the soil delivery of ecosystem services (e.g., climate regulation, biomass provision for food, water flow regulation, and recreational value) endangered. The Horizon project "InBestSoil" aims to co-create a framework for investment in the conservation and recovery of soil health by developing an economic valuation system of the ecosystem services delivered by healthy soil and the impacts of soil interventions. To achieve this, a lighthouse was established in Zagreb (Croatia) in peri-urban soils to assess the impact of land use and soil management practices. The topsoil (0-10 cm) properties (carbon stocks, bulk density - BD, soil water content - SWC, mean weight diameter - MWD, water-stable aggregates - WSA, infiltration, and $CO_2$ emissions) in 5 different land uses: forests – natural Quercus robur (FOR), grasslands – semi-managed (GRASS), abandoned agricultural land – afforested (AFFOR), cropland (CROP) and a grass-covered apple orchard (ORCH) were determined during Spring 2023. Land use had a significant impact on all -soil properties investigated. The results showed that the SWC value was significantly higher in GRASS and CROP than AFFOR and FOR land uses. Cropland and ORCH land use had significantly higher BD than other land uses, whereas AFFOR had significantly the lowest BD. The MWD has significantly higher values at CROP, GRASS, and AFFOR land uses than FOR land use. The FOR, AFFOR, and ORCH land uses showed significantly higher WSA values than the CROP land use. The FOR and ORCH land uses obtained a significantly higher infiltration than the CROP land use. Soil CO2 emissions registered significantly higher at GRASS than at other land uses. Significantly higher CO2 emissions were noted at ORCH than at AFFOR land use. Finally, carbon stocks were significantly higher in FOR than in other land uses. The AFFOR, ORCH, and GRASS land uses recorded significantly higher carbon stocks than CROP land use. Such results indicate that forestland, grassland, and agricultural land use with conservation agricultural practices improves carbon stocks, soil quality, and flood retention capacity in peri-urban areas.

**Keywords**: carbon sequestration, flood retention, soil degradation, soil management, InBestSoil

**Acknowledgments:** This work was supported by the European Commission Horizon 2020 project "InBestSoil - Monetary valuation of soil ecosystem services and creation of initiatives to invest in soil health: setting a framework for the inclusion of soil health in business and in the policy making process" (grant agreement 101091099).

# Practices on enhancing favourable habitat for natural enemies in potato protection – a review

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**Summary**

Sustainability in agriculture belong to the greatest global challenges. European Green Deal aims to reduce the use and risk of chemical pesticides, as well as the use of the more hazardous pesticides. Therefore, non-chemical solutions are needed in agriculture. One of options farmers have, is enhancing of habitats for natural enemies of plant pests. The potato production is endangered with: wireworms (*Agriotes spp.*), different species belonging to family *Noctuidae* (*Agrotis ipsilon* Hufnagel, *Agrotis segetum* Denis & Schiffermuller and *Autographa gamma L.)* and Colorado Potato Beetle (*Leptinotarsa decemlineata* Say.). Specific natural enemies of the above mentioned pests are very rare. However, the complex of generalist predators that attack different pest species may reduce their population and contribute to their control. Among many generalist predators, species belonging to family Carabidae and to order Heteroptera are the most important. Many scientific papers are dealing with the implementation of practices that maintain and enhance the efficacy of natural enemies as one of the main components of conservation biological control. In this review paper we analyzed all available practices on enhancing favourable natural habitat for natural enemies in potato production, as are: soil maintenance, planting the wildflowers next to the potato fields, creation of infrastructures of non crop vegetation, sowing flower strips, companion planting, mulching, cover cropping etc. All the practices analyzed, which promote the presence of natural enemies, contribute significantly to reducing the pest population. Since the plant protection products (PPPs) also have a significant impact on the number of natural enemies, successful conservation of natural enemies can be achieved by combining all available practices with the careful application of PPPs, implementing of IPM approaches, diverse crop rotation, cover cropping and biological insecticide use.

**Keywords:** integrated pest management (IPM), sustainability, potato production, natural enemies, biodiversity

# Analyzing Soil Erosion Dynamics in Permanent Plantations in Croatia: Examining the Influence of Management, Geography, and Soil Typology

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**Summary**

In the time of critical global changes, many studies have been conducted to understand the real problems of soil degradation. This study included 4 different sites in Croatia, which explained the soil erosion dynamics in permanent plantations. In orderto understand the influence of management, geography and soil type, few variables were selected to show what influences them the most. At each site, soil bulk density (BD), soil organic matter (SOM) and sediment loss (SL), were investigated under two management types (Grass and Tilled). Same methodology for soil sampling, laboratory analysis and rainfall experiment (30 minutes at an intensity of 58 mm $h^{-1}$) were used. Soil bulk density was 46% higher on Anthrosol in Istria than on Calcic Fluvisol in Dalmatia regardless of soil management. As for the SOM values, higher were noticed in the Northwestern Croatia on the Grass managed plantation on a Vertisol, compared to Slavonia region which had up to 324% higher values. While the SL did not vary between soil types and sites under grass cover, SL values were higher in the Continental region, than in the Mediterranean regions, where losses were up to 1575% for a single simulated rainfall. This study provided a clear insight into the influence of management, geography and soil type on the behavior of BD, SOM and SL, with the Vertisosls of North-Western Croatia showing a large influence on the physical and chemical properties of the soil, thus directly affecting soil hydrology.

**Keywords:** soil erosion, physicochemical properties, soil hydrology, soil loss

**Acknowledgments:** This work was supported by the Croatian Science Foundation through the project "Soil erosion and degradation in Croatia" (UIP-2017-05-7834) (SEDCRO).

# Effect of nitrogen, phosphorous, and potassium deficiency on winter wheat root morphology

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**Summary**

To achieve high and stable yields, winter wheat (*Triticum aestivum* L.) requires high levels of fertilizers, especially nitrogen (N), phosphorus (P), and potassium (K), but intensive use of NPK fertilizers poses environmental problems like soil degradation, eutrophication of the ground and surface waters, salt burns, excessive growth, and increasing carbon footprint in crop production. As a result, it is critical to apply fertilizers such as NPK in an effective manner to decrease loss and enhance nutrient utilization efficiency. One of the important functions of roots is resource acquisition, hence understanding the root system characteristics and how they interact with the soil environment can aid in the development of agricultural practices that optimize nutrient absorption and promote healthy plant growth, which results in higher crop yields and sustainable agriculture. The aim of the study is to quantify morphological changes of winter wheat root traits grown under different N, P, and K availability. Two winter wheat varieties (cv. OS-Olimpija and cv. Sofru) were grown under different N, P, and K availability, and root morphology was assessed by scanning and analyzing the root system during early plant development. Data of the extracted root traits, such as width, depth, length, surface area, diameter, volume, number of tips, etc., are statistically analyzed and discussed. Although cv. OS-Olimpija developed, on average, a bigger root system; both cultivars responded similarly to nutrient deficit treatments. The earliest (visible already after three days of growth) and most pronounced reduction in root growth (length, surface area, volume) was found for plants grown in K deficit treatment, followed by P and N deficit treatments. In P and K deficit treatments, the average root diameter increased, whereas it decreased in N deficit treatment, showing the different adaptive mechanisms of the root systems under different nutrient deficiencies.

**Keywords**: winter wheat, fertilizer, resource acquisition, root system characteristic, root morphology

# Rapid methods for assessing the efficiency of chickpea bacterization

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**Summary**

The research has been conducted to ascertain the efficacy of pre-sowing bacterization of local chickpea populations seeds with Nitrogen-Fixing Bacteria and solubilizing P bacteria. Trials was set up using the split plot randomized complete block design with four replications. Plot size was 3.5 m x 2.5 m. Solubilizing bacteria i.e., PGPR (P1) or its absence (control P0) were used for main plot treatment and 4 strain of N fixating bacteria (referent strain-X, autochthonous strain-30b, introduce strain 17a and ISC11) and control-B0. A field experiment was set up at location Popovo polje municipality Ravno, Bosnia and Herzegovina in 2022 growing season. The measurement of chlorophyll fluorescence and SPAD values was conducted two times in the vegetation, before and after flowering. The N content and dry matter values of aboveground biomass were also ascertained. Considering the Fv/Fm value as the most important parameter evaluated using a fluorimeter, the range of values determined for this research was 0.6-8.7, while lower values of 0.83 used as an indicating for a specific level of plant stress. The treatment P1/17a, which had 37.5% dry matter, and P0/ISC11, which had 2.95% N content, showed the greatest values of these two parameters. The treatment P1/ISC11 had the highest average yield (24.5 g/plant), according to the results. The SPAD index ranged from 21.1-45.8 for the local populations of chickpea from Herzegovina used in this research, with the differences due to a varied period of assessment, which decreased significantly with plant aging. The chlorophyll content, varied between 0.568 and 0.715 mg/g of fresh plant material. Measured parameters of the photochemical processes in the chickpea show a strong correlation, as does the relationship between chlorophyll a and b, while a somewhat milder correlation was established between the N content and yield, and a modest and almost non-existent correlation was established between laboratory measurements of chlorophyll and measurements obtained by rapid diagnostic methods.

**Keywords**: rapid diagnostic methods, SPAD index, chlorophyll fluorescence Fv/Fm, bacterization

# Carbon dioxide emissions from the soil in winter wheat (*Triticum aestivum* L.): effects of soil management

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**Summary**

Sustainable agricultural practices that aim to maximize carbon sequestration and reduce greenhouse gases releases are essential for mitigating the agricultural impact on climate change. The aim of this work was to determine the influence of different soil management practices on C-$CO_2$ flux in winter wheat field during one vegetation season (2023). The research was conducted in Križevci (Croatia) on Gleysol. The experimental field consists of three factors: i) tillage (CT-conventional tillage; CTD-conservation system deep; CTS-conservation system shallow); ii) liming (CN-without liming; CY-with liming); and iii) fertilization (FR-according to recommendation [with basic macronutrients NPK]; GFR-according to recommendation + biophysiological soil activator [GeO2]), 12 treatments in total. The average annual C-$CO_2$ flux ranged from 16.5 kg $ha^{-1}$ $day^{-1}$ at CTS-GFR-CN to 23.0 kg $ha^{-1}$ $day^{-1}$ at CT-FR-CN. Depending on the effect of fertilization in different tillage treatments, the study showed that the addition of FR had a greater effect on the annual C-$CO_2$ flux than the addition of GFR at all studied tillage treatments. Treatments with CTS tillage also showed lower flux values than the treatment with GFR addition. Comparing the different tillage treatments with the same liming and fertilization (FR-CN), the results showed that the lowest average annual C-$CO_2$ flux was recorded for the tillage treatment CTS with 18.8 kg $ha^{-1}$ $day^{-1}$, while the highest average annual C-$CO_2$ flux was recorded for CT with 23.0 kg $ha^{-1}$ $day^{-1}$. Comparing treatments with different liming but the same fertilization and tillage, certain changes occurred. Higher average annual C-$CO_2$ values were found in the treatment without liming and in the treatment with CT and CTD tillage. Higher average annual C-$CO_2$ values were found in the treatment with CTS tillage than in the treatment with liming.

**Keywords**: C-$CO_2$ flux, conservation tillage, fertilization, liming, agroecosystem

**Acknowledgments:** This work has been fully supported by Croatian Science Foundation under the project "Assessment of conservation soil tillage as advanced methods for crop production and prevention of soil degradation – ACTIVEsoil" (IP-2020-02-2647)

# Presenting the concept of low-input farming to Croatian agronomic community

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**Summary**

The aim of the paper is to present the concept of low-input farming in Croatian agronomic community. Modern crop farming is characterized by the use of powerful diesel-powered tractors with implements that have brought human labor efficiency to unprecedented levels. Slow but steady yield increases are achieved through the widespread use of agrochemicals (mineral fertilizers and pesticides). However, the sustainability of current intensive farming methods is questionable due to several reasons: excessive soil compaction by heavy machinery, degradation of soil structure and microbiome due to excessive use of mineral fertilizers and lack of organic fertilizers dependence on depleting fossil energy resources for farm operations and fertilizer production with impacts on climate, and excessive emissions of toxic compounds from pesticide application and resulting biodiversity loss and negative impacts on human health. To mitigate the negative impacts of modern intensive agriculture, some more sustainable options have been developed in recent decades, such as organic farming, ecological agriculture, integrated agriculture, conservation agriculture, precision agriculture, regenerative agriculture, and low-input agriculture. Currently, low-input agriculture receives the least attention and is largely unknown to the public and even the majority of farmers. Low-input farming systems aim to optimize the management and use of on-farm resources and minimize the use of off-farm inputs, such as purchased fossil fuels, chemical fertilizers, and pesticides. Since animal-power based agriculture relies on the feed produced on the farm as a source of clean and carbon-neutral bioenergy, it fits perfectly into the concept of low-input agriculture.

**Keywords**: low-input farming, sustainability, pollution, climate, energy

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# Influence of *Trichoderma atroviride* on the biodiversity of nematode community in olive groves

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**Summary**

Soil is most diverse ecosystem. As a substrate, it serves life of numerous organisms. Their mutual interactions maintain biological productivity. Most abundant group consists of nematodes, which, due to their trophic diversity, reflect not only their own but also state of bacterial and fungal communities in soil. Fungi from genus *Trichoderma* sp. they are successful colonizers of all habitats. Their role in soil is reflected in protection of plant roots. Purpose of research is to determine influence of fungus on structure of nematode community. Is carried out in project "Development of innovative methods for increasing yield of olives and classification of olive oil" in olive groves of Istria (Vodnjan) and Dalmatia (Nadin) on four varieties: Buža, Istarska Bjelica, Lastovka and Oblica in three treatments (control, $10^6$ and $10^8$) and four repetitions for each variety. Aim of research is to determine impact of mycorrhization on biodiversity of nematode genera in olive groves. *T. atroviride* species was isolated from soil in Skradin area and confirmed by molecular method. For implementation of research, *T. atrovioride* was prepared in concentrations $10^6$ and $10^8$. Control sampling to determine initial state of nematode genera was carried out in spring of 2022, before addition of fungus, and 48 genera were determined at Nadin location and 55 genera at Vodnjan location. In autumn of 2022, after first addition of *T. atroviride*, total number of nematodes at Nadin location was determined to be 54 genera in control, in $10^6$ 61 genera, in $10^8$ 61 genera, in Vodnjan location in control 59 genera, $10^6$ 57 genera and $10^8$ 63 genera. Based on inferential statistical analysis performed on two separate two-way analyzes of covariance (ANCOVA), number of genera after application of fungus in initial research conducted in 2022, regardless of variety, treatment and location, did not significantly statistically differ.

**Keywords:** biodiversity, fungi, olive, nematodes, soil

# Assessment of soil organic matter in post-fire period using VNIR spectroscopy

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**Summary**

Wildfires have a profound impact on ecosystems and soil organic matter (SOM), a critical factor in soil quality and carbon cycling. The aim of this research was to assess the impact of wildfire severity on SOM and the potential of visible-near infrared spectroscopy (VNIR) spanning the 350 - 1050 nm wavelength range for monitoring SOM in a post-fire landscape using two modeling approaches (i) Partial Least Squares Regression (PLSR) and (ii) Artificial Neural Networks (ANN). Following a comprehensive two-year investigation in Zadar County, Croatia, where a 13.5 ha mixed forest was moderately to severely affected by a wildfire, spectral reflectance analysis revealed that SOM content strongly influenced soil reflectance. High severity samples exhibited the lowest reflectance compared to those with moderate severity and the control group. The critical region for SOM information in post-wildfire soil estimation models was found to be between 500 and 700 nm. ANN consistently outperformed PLSR, achieving ratio of performance to deviation (RPD) values from 1.74 to > 2.5, whereas PLSR achieved values between 1.62 and 2.29, demonstrating ANN's capability to provide accurate predictions of SOM content in the conditions of complex post-fire SOM dynamics. In conclusion, VNIR spectroscopy, particularly coupled with ANN based models, offers a reliable and non-destructive method for assessing SOM content in post-fire environments, facilitating informed land management decisions for ecosystem recovery.

**Keywords**: wildfire, hyperspectral data, linear modeling, nonlinear modeling

# Spray inoculation of *Pseudomonas syringae* pv. *tomato* on *Arabidopsis thaliana* mutants impaired in methylation

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**Summary**

*Pseudomonas syringae* is a highly adaptable rod shaped, Gram-negative, hemibiotrophic bacterium with flagella, which elicits blights, leaf spots and galls on cultivar plants. *Pseudomonas syringae* survives on the leaf surfaces of plants as an epiphyte before it enters into the intercellular space, to initiate the infection process. *P. syringae* pv. *tomato* DC3000, the main model strain for studying this interaction, is the causing agent of bacterial speck in tomato plants, and it causes disease in the model plant *Arabidopsis thaliana*. Upon detection of bacteria, the plant triggers stomata closure as part of the innate immunity response. These initial steps of the bacteria-plant interaction are bypassed when plants are inoculated by infiltration. However, natural infection, can be mimicked through dip or spray inoculation. These means of inoculation are less efficient but can provide additional information about the relevance of the early steps on the different outcomes of the plant pathogen interaction. Since, previous reports have demonstrated that the loss of DNA methylation enhances resistance to bacteria (in an unspecific manner), it was of interest to analyse how the methylation status of the host plant (*Arabidopsis thaliana* Col-0) affects the interaction with spray-inoculated bacteria. We spray-inoculated plants with bacterial strains carrying mutations on genes relevant to the early steps of the infection process, *i.e.* a mutant defective in flagellin or coronatine production, as well as with wild type and wild type expressing the avirulence determinant AvrRpt2, as controls. We analysed their interaction with plants carrying each one of the following mutations: *met1-3*, *ago4-2*, *ddc*, and *ros1-4*. Symptom development was monitored after spray-inoculating 1 x $10^7$ cfu/ml. When bacterial growth was analysed in *met1-3* plants, we observed a significant decrease in bacterial symptoms in comparison to that found in wild-type plants.

**Keywords**: *Pseudomonas syringae*, methylation, *Arabidopsis thaliana*, spray inoculation

# Influence of gamma radiation on traditional common bean landraces

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**Summary**

Gamma rays are the most energetic form of electromagnetic radiation. In plant improvement, seed irradiation can induce genetic variability and alter physiological traits of plants, allowing plant breeders to select new genotypes with improved traits. The aim of this study was to determine the effect of 2 doses of radiation on chlorophyll fluorescence and multispectral traits of 3 different bean genotypes. The experiment was conducted in a greenhouse. Seeds of the traditional bean genotypes 'Trešnjevac', 'Biser' and 'Zelenčec' were irradiated with gamma rays at a dose of 100 and 150 Grey (Gy). Chlorophyll fluorescence and multispectral imaging were performed on the second generation of the plants. All traits of chlorophyll fluorescence were increased in Biser and Trešnjevac under 100 Gy irradiation compared to 150 Gy irradiation, except for the maximum quantum yield of PSII (Fv/Fm) and the quantum yield of non-regulated non-photochemical energy loss in PSII (ϕno), where the higher radiation dose increased these traits. In the case of Zelenčec, 150 Gy increased all chlorophyll fluorescence traits compared to 100 Gy, except for Fv/Fm, where lower radiation dose was significant. Of the multispectral traits, saturation was the only one that responded, and an increase in saturation was observed at 150 Gy in Trešnjevac and Zelenčec. Chlorophyll fluorescence has proven to be a valuable tool for evaluating radiation effects on different bean genotypes.

**Keywords**: ionizing radiation, electron transport rate, reflectance, chlorophyll fluorescence

# Spatial and temporal variability of soil compaction on different conservation tillage treatments

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**Summary**

Measuring of penetration resistance is known as one of the best, and also one of the fastest visible indicator of soil compaction. The experiment with the measurement of soil penetration resistance on different conservation tillage treatments was carried out during three experimental years. The experiment was set up in 2021 on two different soil types, Gleysol (Križevci site) and Stagnosol (Čačinci site). Tillage treatments were: ST (plowing), CTD (conservation deep, loosening up to 30 cm with a minimum of 30% of crop residues on the surface), and CTS (conservation shallow, tillage up to 10 cm with a minimum 50% of crop residues on the surface). Grown crops (same on both sites) were as follows: maize (2021), soybean (2022), winter wheat (2022/2023), and soybean as a second crop after winter wheat (2023). To determine soil compaction, each year penetration resistance was performed three times per vegetation year (beginning, middle, and end of vegetation). The penetration resistance was measured according to geolocated square grid design. The values of penetration resistance found in the soil roughly ranged from 1.0 up to 10.0 MPa. These high variations depend on a few factors, but primarily on tillage treatments and soil status (basically soil moisture). The main conclusion can be stated as: penetration resistance increased with the depth, penetration resistance is inversely proportional to conservation level (as crop residues on soil surface increase, penetration resistance decreases).

**Keywords**: conservation soil tillage, soil compaction, crop rotation, penetration resistance, crop residues

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# Isolation and characterization of plant growth promoting rhizobacteria and their potential role in bioremediation

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**Summary**

Soil pollution generally causes huge losses in the worlds agricultural output and therefore soil pollution control is essential in agriculture crop production system. For soil pollution management we usually reduce the use of chemical fertilizers and pesticide. The continuous use of those products without safe disposal leads to soil pollution. Bioremediation of soil pollution is an alternate eco-friendly method for soil pollution management in which plant growth promoting rhizobacteria (PGPR) are used in alleviating the contaminated soil. These PGPR have been found to bioremediate the polluted soil by using various types of mechanisms promoting beneficial plant-microbe symbioses. The aim of this study was the isolation of PGPR from 3 different locations in the city of Zagreb: Maksimir forest, agricultural area of the Faculty of Agriculture and polluted meadow in Maksimir. Phenotypic characterization of isolates included testing the tolerance to unfavorable soil conditions such as high levels of NaCl, different pH values, temperatures, intrinsic antibiotic resistance, biochemical characterization and screening for PGP properties. All isolates were negative for the phosphate solubilization test, while indole-3-acetic acid was produced by all isolates. Half of all isolates had the ability to synthesize amylase and protease enzymes. Only one isolate had the ability to produce exopolysaccharides. Some differences were found between isolates regarding their phenotypic characteristic, especially resistance to low pH and high temperatures which is extremely important due to climate change. PGPR characterization determined that strain SM4 produces IAA, exopolysaccharides, lytic enzymes which directly promotes plant growth and development. This strain should be included in further investigation in order to select high quality strain for application as biofertilizer for sustainable different plants production and the bioremediation process.

**Keywords**: PGPR, bioremediation, sustainable agriculture, phosphates solubilization, ecological characterization

# Fingerprinting of soil organic matter composition by FTIR-ATR spectroscopy

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**Summary**

In the face of the climate crisis, human-induced land degradation poses a serious threat to future generations. Aligning with several of the Sustainable Development Goals (SDGs) established by the United Nations, ensuring soil security holds great significance in achieving global sustainability. Hence, the advancement of soil assessment techniques is imperative for the protection of soil resources. Soil is a vital component of ecosystems, and plays a critical role not only in food production but also in carbon sequestration and climate regulation. Therefore, monitoring soil properties is crucial for maintaining soil health amid agroecosystem disturbances. In particular, soil organic matter (SOM) is an important indicator of soil quality and plays a multifaceted role in soil functionality. However, the heterogeneous nature of SOM makes it highly vulnerable to climate change. Climate change and soil management can affect the composition and stability of SOM, which in turn affects carbon and nutrient cycling in different soil types, depending on their properties. Infrared spectroscopy (IR), as a non-destructive method, is of great importance for detecting changes in soil dynamic properties such as SOM. Fourier transform infrared - attenuated total reflectance spectroscopy (FTIR-ATR) spectroscopy is a powerful tool for SOM characterization, providing insights into SOM functional groups and their degree of humification. The aim of this study is to evaluate the applicability of FTIR-ATR spectroscopy in characterizing SOM in different soil types in Croatia. The studied soil types, which differ in various soil indicators (pH, texture, clay content, etc.) and exhibit variations in SOM quantity and quality, are analyzed and discussed: Gleysols vertic, Regosols acric, Chernozems, Luvisols (terra rossa) and Dystric stagnosols (pseudogleys).

**Keywords**: climate change, Croatia, soil quality, soil degradation, SOM

# Development of an MIP-OES method for determining total microelement and heavy metal content in soils

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**Summary**

Accurate elemental analysis of soils is extremely important to determine the need for fertilizer application in case of nutrient deficiency and for environmental monitoring, since an elevated content of heavy metals is a considerable concern to human health and agricultural industry. The aim of this study was to develop and validate a method for simultaneous determination of total microelements (Fe, Mn, Zn, Cu) and heavy metals (Cd, Cr, Mo, Ni, Pb, Co) in soil samples, using the microwave induced plasma optical emission spectrometry (MIP-OES) technique. Sample preparation was performed by means of microwave digestion in aqua regia. Measurements of metal concentrations in sample solutions were performed on Agilent 4210 MP-AES. Multiple available wavelengths were tested for each element, but ultimately one was chosen, based on four criteria: optimal intensity of analytical lines given the expected concentration range, number of possible interferences, linearity and accuracy. Some instrumental parameters, such as torch alignment and nebulizer gas flow were also optimized. The accuracy of the method was determined by measuring reference materials obtained during a proficiency test. Repeatability was defined as %CV of the data obtained when 10 replicates of the reference sample were analyzed in the same run. Reproducibility was defined as a %CV of data obtained from different runs done by different analysts. LODs and LOQs were also determined after measuring the calibration blank sample in 10 replicates. After statistically analyzing the data obtained for each element, it has been found that all validation parameters were in accordance with acceptance criteria. Therefore, the robust and accurate MIP-OES method can be reliably used for monitoring soils, micronutrient content and heavy metal contamination.

**Keywords**: soil analysis, soil microelements, heavy metals, MIP-OES

# Functional quality of wheatgrass grown from seed biofortified with selenium and zinc

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**Summary**

Wheatgrass (*Triticum aestivum* L.) is rich in vitamins, minerals, amino acids, and phenolic compounds and is therefore considered a functional food. The aim of the experiment was to determine the influence of biofortification with selenium and zinc and the day of cutting on the content of biologically active compounds in wheatgrass juice. Wheat was grown under field conditions and biofortified with 1.5 kg Zn/ha and 10 g Se/ha in the flowering phase (Se and Zn 1), i.e. with the same amount of selenium and zinc in the flowering and milk ripening phase (Se and Zn 2). The wheat grain obtained after harvesting was used in the experiment conducted under controlled conditions. The wheatgrass variety Srpanjka was grown under controlled conditions, and cutting was carried out on the 6th, 8th, and 10th day after harvest. The juice was extracted from the young seedlings using a wheatgrass juicer. The content of chloroplast pigments, vitamin C, total phenols, flavonoids and the antioxidant activity by DPPH method, were determined in the wheatgrass juice. Biofortification with selenium and zinc had no significant effect on the content of chloroplast pigments and the antioxidant activity determined by the DPPH method. The highest value of total phenolics content was determined in wheatgrass juice (Se and Zn 2) on the 6th day of cutting and was 147.34 μg GA /100 mg F.W. The lowest phenolic content in juice was determined in wheatgrass grown from the seeds of non-biofortified plants (Se and Zn 0) and mowed on the 8th (122.55 μg GA /100 mg F.W.) and 10th (121.42 μg GA /100 mg F.W.) day after sowing. The juice obtained on the 10th day after cutting and Se and Zn 2 biofortification treatment contained the lowest amount of vitamin C (51.04 mg vitamin C /100 g F.W.). The results confirm that biofortification and the day of mowing significantly influence the nutritional composition of wheatgrass juice

**Keywords**: juice of wheatgrass, selenium, zinc, vitamin C, phenols

# The influence of seaweed on soil respiration

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**Summary**

Soil respiration is an indicator of soil health, i.e. biological activity. It can be an indicator of healthy soil, capable of decomposing organic residues and distributing the nutrients necessary for crop growth. The aim of this research is to determine the effect of the addition of seaweed to the soil on the intensity of respiration. Seaweeds were added to the soil at different concentrations (2% and 4%) and the respiration intensity was measured over 21 days using the titration method, i.e. the amount of $CO_2$ released was measured by "capturing" the released carbon dioxide in a template with NaOH. Fluvisol and two types of seaweed from Norway were used in the experiment, and the results were expressed as mg $CO_2$ /g dry matter/day. A statistically significant effect of seaweed added to the soil on the intensity of respiration was determined. On the day of the first measurement, seaweed1 (0.018), seaweed2 (0.078), and soil (0.145) had the statistically significant lowest values of respiration intensity. Significantly higher values than these were found in the soil combined with seaweed1 and seaweed2, and the highest respiration intensity was found in the soil mixed with seaweed1 (1.911), respectively seaweed2 (1.837) in a concentration of 4%. The highest values of the sum of respiration intensity on day 21 were determined in the combination of soil and both seaweeds (seaweed1 5.615 mg $CO_2$ /g dry matter) (seaweed2 5.750 mg $CO_2$ /g dry matter) at a concentration of 4%, while the soil alone had 0.565 mg $CO_2$ /g dry matter.

**Keywords**: fluvisoil, respiration, seaweed, $CO_2$

# Characterization of plant growth-promoting seed-borne endophytes, associated with mustard, basil, and lettuce seeds

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**Summary**

This study addresses the different contributions of seed-borne endophytes associated with mustard (*Sinapis alba* - SA), basil (*Ocimum basilicum* - OB), and lettuce (*Lactuca sativa* - LS). We used both culturable (a variety of media was used to isolate endophytic bacteria) and non-culturable approaches (16S rRNA gene run on the NovaSeq 6000) to comprehensively investigate the diversity of endophytic bacteria in these seeds. Our assessments of the selected isolates included a range of traits that promote plant growth. The results showed that SA seeds had high relative abundance (RA) of taxa such as *Clostridium*, *Mycobacterium*, *Shinella*, *Rhizobiales*, and *Microbacteriaceae*. LS seeds were characterized by *Sphingomonas*, *Clostridium*, *Rhizobium*, *Devosia*, and *Pseudonocardia*, while OB seeds had high RA of *Sphingomonas* and *Micrococcaceae*. The analysis of alpha diversity (Shannon index) showed that OB seeds had the highest diversity and SA seeds the lowest. *Priestia megaterium* (SA) showed remarkable salt stress tolerance and growth ability at 0.025% cobalt and 0.1% lead, while *Stutzerimonas stutzeri* (SA) was the strongest producer of IAA, showed growth tolerance at 0.01% mercury concentration and excellent nitrogen fixation abilities. These two strains were also identified as efficient phosphate solubilizers. *Bacillus cabrialesii*/*Bacillus inaquosorum* (OB) stood out as the best producer of siderophores and showed remarkable simultaneous production of proteinase, xylanase, gelatinase and pectinase. In conclusion, our study provides a comprehensive understanding of the diversity of endophytic bacteria in mustard, basil, and lettuce seeds and their potential to promote plant growth, which is valuable for sustainable and healthier agricultural practices.

**Keywords**: endophytic bacteria, amplicon sequencing, alpha diversity, sustainable agriculture, biological control

# Influence of the parent rock on "Montado" soils in three locations of Portugal

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**Summary**

"Montado" is a Portuguese term that refers to a traditional agroforestry system commonly found in Portugal and Spain and is characteristic of the Iberian Peninsula's Mediterranean ecosystems. It involves the cultivation of cork oak and holm oak trees alongside grazing livestock, primarily pigs and cows. This study aimed to characterize the soils of "Montado" in three different geological units in the regions of Évora (Ediacaran migmatites, ~590 Ma), Grândola (Devonian phyllites, metasiltstones and quartzites, ~370 Ma) and Chamusca (Quaternary fluvial conglomerates, ~2,5 Ma). The texture of the soils is loamy sand (Évora and Chamusca) and sandy loam (Grândola) and the pH is strongly acidic (Grândola and Chamusca) and moderately acidic (Évora). An elemental analysis through an X-ray fluorescence analyzer showed significant ($p \leq 0.05$) higher levels of Zn, Ca, S, Mn, Fe and P in the Évora soil (the latter three being similar in the Grândola soil and significantly higher than the Chamusca soil). Cu, K and Mo levels were not significantly different ($p \leq 0.05$) among the three fields. Also, colorimetric parameters (through the CiaLab system) showed significant differences, with Grândola field presenting higher values for L (brightness) and Évora significantly higher values for a and b (respectively, red and yellow). Chamusca soil revealed higher moisture, whereas similar levels of organic matter content were found in the three soils. In addition, elemental leaf analyses of the cork oak trees in the three soils showed the same trend concerning mineral elements, except for lower Ca in Évora, probably due to interactions with other chemical elements.The differences in the chemical composition of the three soils are related to the different geological substrates (parent rock). Soils developed in older geological units show higher nutrient content. The possible association of such soil differences with ecophysiological traits related to C-assimilation performance of young and old cork oak trees is also under analysis.

**Keywords**: oak trees, soils, parent rock, Portugal

# A comparative analysis of K, Ca, S and P accumulation in leaves of three cork oak forests of Portugal

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**Summary**

The landscape of the Portuguese mainland is characterized by cork oak forests in the south. his iconic landscape, with a vegetation cover dominated by cork oaks, is the result of the interaction of cultural and natural factors over time and develops in a region characterized by poor soils and a Mediterranean climate. In this context, macronutrients are crucial for the functioning of plant metabolism. In view of the observed decline of cork oak forests, a tool to support decision-making and the management of cork oak forests will be developed to ultimately strengthen the competitiveness of companies by performing a micronutrient analysis on mature cork oaks. The content of K, Ca, S and P was determined in mature cork oak leaves from three Portuguese experimental fields in Azinhal / Grandola (GPS: 38.1071851, -8.4419802), Casal da Balsa / Chamusca (GPS: 39.3620909, -8.3843477) and Cabanas / Évora (GPS: 38.5493728, -8.0642313), on February 17, April 14 and July 25, 2023. The average values of K, Ca, S and P in the three experimental fields were: on February 17$^{th}$, 11400, 9500, 2100 and 0.1100 ppm; on April 14, 2.14, 10200, 2200 and 1400 ppm; on July 25, 12400, 7700, 1800 and 5500 ppm. The results obtained will be analyzed considering the climatic and geographical variables of the 3 cork oak forests. The results obtained are analyzed considering the climatic and geographical variables of the 3 cork oak forests. The results obtained are presented and discussed in detail, considering the climatic and geographical variables of the 3 cork oak forests. However, an important conclusion was that the accumulation of macronutrients in the leaves of cork oaks can develop synergistic and antagonistic interactions depending on the edaphoclimatic conditions of the experimental fields.

**Keywords:** agroforestry ecosystems, Cork oak, macronutrients;

# How microplastics modify the effects of agrochemicals – changes in reproduction success of two Collembola species

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**Summary**

Soil is the basis of all terrestrial ecosystems and is the most important source of resources for plant and animal life in the environment. In the modern world, various types of pesticides and, more recently, microplastics are among the most common contaminants of agricultural soil. In this study, a reproduction test was conducted with two ecologically different springtail species, *Folsomia candida* and *Sinella curviseta*. The species were exposed to different concentrations of fungicides (Quadris), microplastics (PVC 63 – 125 μm) and their mixture. The aim was to investigate the individual effect of the pollutants, and to determine whether microplastics modify the toxic effect of the fungicide used. Quadris caused a significant inhibition of reproduction in both springtail species at the same concentrations. The analysis of the dose-response curve shows that the use of Quadris poses a higher risk for *F. candida*. Microplastics also caused significant inhibition of reproduction in both species, but the inhibition occurred - in *F. candida* at lower concentrations, suggesting that it is more sensitive. The equitoxic mixture of Quadris and microplastics showed an additive effect on *F. candida*, while the effect on *S. curviseta* - was synergistic. The results show how important it is to investigate the interactions of pesticides, microplastics and their mixtures on soil organisms in order to better assess ecological risks and predict harmful consequences for the environment.

**Keywords**: polyvinyl chloride, azoxystrobin, fungicide, soil pollution, mixture toxicity

# Soil fertility and agrochemical indicators in Republic of Croatia in 2022

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**Summary**

Agricultural producers in the Republic of Croatia are obliged to control the agrochemical properties of the soil at least every 4 years. Accordingly, a total of 78,766 samples have been analyzed in the last 4 years, of which almost 75 % (59,029) in the last two years. In 2022, 31,313 samples from a total of 96.712 ha of arable land were analyzed. The average results were very similar in all four years, as about 42-52 % of the soils were either low in humus (SOM) or low in available phosphorus or too acidic. In 2022, less than 2 % SOM was determined in 41.4 % of the samples, i.e. on a total of 42,101 ha (43.5 % of examined areas), which is very similar to the previous year with 41.8 % of the samples with SOM < 2 % whereby the average SOM content in the mentioned soils was similar in both years (1.68 and 1.61 %). The proportion of acidic and highly acidic soils is even higher, as $pH_{KCl}$ <5.5 was determined in 47.01 % of the samples (46.4 % of the analyzed areas, i.e. 44,884 ha), which is similar to 2021 (49.9 %) and 2020 (46.0 %). However, the availability of phosphorus in the soil is also very worrying, as 52.6 % of the analyzed samples (45,979 ha) belong to the worst soil classes (classes A and B). It is also significant that an increasing proportion of soil samples from the two worst phosphorus supply classes were identified in the four- year series (50.7 % in 2021, 44.7 % in 2020 and 37.3 % in 2019). The supply of the soil with available potassium is significantly more favorable, as 55.0 % of the samples (53,548 ha, i.e. 55.37 %) were found to be in the medium supply class, and only 24.2 % of the samples were poorly supplied with potassium. The results of the four-year analyzes clearly show that the SOM content, the acidity of the soil and the availability of phosphorus individually limit soil fertility in Croatia. An additional problem is that more than one factor of unfavorable intensity is often present in the soil at the same time. For example, low SOM content and low phosphorus availability were found on 20.3% of arable land (19,667 ha), low phosphorus availability and excessive acidity on 25.4% (24,518 ha), and low SOM content and excessive acidity on 25.9% of arable land (25,076 ha). In addition, 13.3% of arable land (12,873 out of a total of 96,712 ha) was found to have low SOM content, low phosphorus availability and excessive soil acidity at the same time. As in previous years, the results of the analysis in 2022 show that low SOM content, low P availability and excessive acidity are the greatest threats to the fertility of arable land in Croatia.

**Keywords**: acidity, phosphorus, potassium, soil pH, SOM

# The occurrence of aflatoxin B1 in maize (*Zea mays*, L.) in an experimental field in Osijek

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**Summary**

Maize (*Zea mays*, L.) is a globally significant arable crop, serving as a vital food and feed source worldwide. The presence of aflatoxigenic fungi and the consequent contamination of corn with aflatoxins represent a significant hazard to, both, human and animal health. Aflatoxins are mycotoxins with proven harmful effects on mammalian cells. Regulations in the EU and many countries limit the concentration of these toxins in food and feed, which can lead to significant economic losses. In this study, corn cobs were collected in an experimental field in Osijek, 2 weeks prior to the seasonal harvest, during 3 vegetation seasons (2021-2023). Each year, 15 cobs visibly contaminated by fungi, were collected to assess the presence and determine the concentration of aflatoxin B1 (AFB1). The collected corn cobs were crowned, and AFB1 was extracted from grains by chloroform/methanol solution. The results revealed that while conidia of aflatoxigenic strains were detected in all study years, the presence of AFB1 varied throughout the research period. In the first year of the research, none of the examined cobs presented AFB1, in the second year two cobs were contaminated, and in the third year, three cobs were contaminated with AFB1. Additional evaluation will consider factors like climate, mycotoxigenic fungi vectors, and preventive measures to clarify their link with AFB1 contamination. This data will inform recommendations for preventing AFB1 contamination in maize.

**Keywords:** maize, mycotoxin, AFB1, food safety

# Beneficial bacteria associated with chickpea: their diversity and the influence on yield

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**Summary**

The objective of this study was to isolate and identify the plant growth promoting rhizobacteria (PGPR) and rhizobia associated with chickpea and to assess their phenotypic characteristics in laboratory conditions. Symbiotic efficiency and the influence of selected isolates on chickpea's yield was tested under field conditions. The results of *rrs* gene sequencing revealed considerable diversity within isolates which includes alfa, beta and gamma proteobacteria as well as actinobacteria. Phenotypic characterization indicates their high resistance to environmental stress conditions (low temperatures and pH, high NaCl concentrations). Most of the isolates were able to synthesize exopolysaccharides. Phosphate solubilization ability was shown by 17 isolates and some isolates were characterized by high IAA production. In the experimental field of Institute of Agriculture and Tourism, Poreč, the highest values for both N content and seed protein content were obtained for plants inoculated with indigenous rhizobial strain 30b. In experimental field of Faculty of Agriculture, Zagreb, inoculation with rhizobial strains ISC11 and 47b significantly increased the nodule number and nodule dry weight as well as the N content in plants in comparison to the reference strain. In the third location, family farm in Zadar, the application of PGPR strain (SGS1, *P. fluorescens*) had no significant effect on measured parameters. Our results show that in Mediterranean part of Croatia (Zadar), significant influence of inoculation on the chickpea yield was generally not determined, probably due to presence of indigenous rhizobia in the soil. As opposed to that, significant influence of inoculation in continental part (Zagreb), where chickpea is not a frequently cultivated, indicates the potential of PGPB application as biofertilizer.

**Keywords**: PGPR, rhizobia, chickpea, inoculation, phenotypic characteristics

# Smart management of land and water resources in agriculture in Bosnia and Herzegovina: implementation of H2020 SMARTWATER project

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**Summary**

Nowadays we are faced with the intensive degradation of important resources in agricultural production (land, water etc.). Processes like erosion, pollution and excessive tillage practices (land resources), unsustainable use and inadequate irrigation or drainage management (water resources) are becoming more intense. SMARTWATER project funded by the European Commission (EC) and coordinated by the University of Banja Luka (BiH) started in 2021 with main objectives to: i) reinforce the networking, research and innovation capacities of the University of Banja Luka (UNI-BL), University of Sarajevo (UNSA) and other BiH institutions in the field of sustainable agricultural water management and ii) increase the competences and fund-rising skills of UNI-BL and UNSA for successful participation in EU projects. SMARTWATER implementation includes several activities: advanced courses, summer schools, joint experimental studies, academic exchanges, stakeholders' meetings, dissemination and the development and promotion of smart water management tools. SMARTWATER network includes different target groups: teachers, students, early-stage researchers (PhD students), farmers, policy makers, final users, general public etc. Four main project topics include: (i) cloud-based smart technologies, (ii) new generation of satellite remote sensing data, (iii) water-energy-food nexus optimization and (iv) climate change impact in agriculture. Also, joint experimental studies are organized in three years and at two locations in BiH, being the aim of this task to investigate maize productivity under different water and nitrogen treatments. Most of these activities are already finished, but project team will continue to contribute to sustainable land and water management in BiH agriculture by implementing remaining tasks.

**Keywords:** SMARTWATER, twinning, networking, maize, irrigation

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# Pedogenic carbonates in Calcocambisol: a case study from a location on the North Dalmatian plain

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**Summary**

Pedogenic carbonates are secondary carbonate deposits that form in soils in arid and semi-arid climates. Their formation is a result of the dissolution and reprecipitation of existing geogenic, biogenic, or pedogenic carbonates and depends on a multitude of factors, but is mostly controlled by soil water drainage and the concentration of soil air $CO_2$. We studied a 0.95 m deep Calcocambisol soil profile located in the North Dalmatian plain, focusing on the distribution of pedogenic carbonates in the profile at 5 cm resolution. Thus, soil particle size distribution, pH, $CaCO_3$, soil organic carbon content, and nodule morphometry were analysed. The profile consists of A-Bw-Bt-Bk-R horizons, having typical properties of Red Mediterranean soils. The top of the soil profile (up to 20 cm) does not contain any carbonate particles. The majority of pedogenic carbonates in the form of nodules can be found in the Bt and Bk horizons. Here, pedogenic carbonates comprise up to 72% of the total mass of soil (in average, 37.6% in Bt and 64.6% in Bk horizon). Nodules are characterized by spherical or irregular morphologies, but fine needle morphologies also occur. The size of carbonate nodules increases with depth, up to 60.4 mm in diameter in Bk horizon. Formation of pedogenic carbonates is characteristic for more arid climates, and therefore their presence in the soil profile can be used as indicator of past climatic changes.

**Keywords**: pedogenic carbonates, carbonate nodules, Calcocambisol, North Dalmatian plain

# Three-year investigation of tillage and organic amendment management on the crop and biomass yields in Croatia

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**Summary**

Proper land management is a crucial factor for - sustainable agricultural production, which has - a positive effect on the stability of crop yields. This research aims to assess the impact of two soil management methods (Conventional tillage – Ct; Reduced tillage – Rt) with an application of organic amendments (Farmyard manure – FYM; Biochar - BC; Control - CK) on crop yields in different cropping systems (monoculture and rotation). The experiment was carried out in Marija Magdalena (North Western Croatia) on loamy Stagnosol. In the maize monoculture system in 2021, yield did not significantly differ between Ct and Rt treatments (7.04; 7.63 t $ha^{-1}$), while yields were significantly higher in the FYM treatment (8.39 t $ha^{-1}$) than in the BC treatment (5.98 t $ha^{-1}$)-. Maize yields in the 2022 - did not differ significantly, regardless of tillage and amendment. In 2023, the Ct treatment (13.69 t $ha^{-1}$) had a significantly higher maize yield than the Rt treatment (9.72 t $ha^{-1}$). However, there were no significant differences between the CK, FYM, and BC treatments. In a crop rotation system, there were no significant differences in yields for the maize yield in 2021, winter wheat in 2022, and ryegrass in 2023, regardless of the treatments and tillage. Maize yield in the crop rotation system was higher on the Rt (9.19 t $ha^{-1}$) treatment than on the Ct (9.00 t $ha^{-1}$). Furthermore, the yield was highest in the BC treatment (9.79 t $ha^{-1}$), followed by the CK treatment (9.04 t $ha^{-1}$), and the lowest was in the FYM treatment (8.45 t $ha^{-1}$). The yield of winter weatwas higher on Ct (6.47 t $ha^{-1}$) compared to the Rt (6.46 t $ha^{-1}$) treatment, while CK (6.25 t $ha^{-1}$) showed the lowest yields and FYM (6.67 t $ha^{-1}$) the highest. In the third year, ryegrass had higher biomass yields in the first two swaths on the Ct (41.14; 24.39 t ha-1) treatment compared to the Rt (38.92; 20.09 t $ha^{-1}$) treatment, and BC treatment had the highest yields followed by CK and FYM. On the third swath, Rt treatment showed a higher biomass yield than Ct, while CK had higher yields than FYM and BC treatments. It was observed that the crop yields were partially affected by the type of tillage or amendment used. However, the positive effects of reduced tillage, organic amendments, and crop rotation on soil properties, as well as their ability to control the spread of pests and diseases, should be considered as significant factors for crop production.

**Keywords**: conventional tillage, reduced tillage, farmyard manure, biochar, yield

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# Invasive alien species on the abandoned agricultural land

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**Summary**

Agricultural areas contain numerous alien species, some of which we characterized as invasive alien species (IAS). In agricultural production, invasive alien species are often considered weeds. The spread of the IAS on abandoned and active agricultural lands occurs unintentionally, whether by spontaneous dispersal (without intervention) or through the transportation of seeds, plant material, soil (as contamination) or as stowaways on machinery and equipment. In this paper, a spatial analysis of the data collected within the project "Mapping of alien and invasive plant species", other relevant data from the Flora Croatica database and data collected in the framework of natural habitat research in the years 2019 to 2023 is carried out. For the selected alien (invasive) species, the frequency of records on agricultural land in Croatia is analyzed. In lowland areas, the spread of *Amorpha fruticosa*, Solidago gigantea, Reynoutria x bohemica through watercourses (rivers, streams and canals) and floods, the overgrowth of abandoned (wet) grassland with *Solidago gigantea* and *Sorghum halepensis* is conspicuous, while in some (hilly) areas the overgrowth of agricultural land with acacia (*Robinia pseudoacacia*) has been recorded. In the (sub) Mediterranean region, *Ailanthus altissima* is a widespread IAS whose spread is favored by an unclear protocol for the removal of species.

**Keywords:** inavasive alien species, IAS pathways, code of conductc for IAS

# Screening for drought tolerance in five plum rootstocks based on physiological and biochemical parameters

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**Summary**

Climate change and increasingly frequent droughts have a significant impact on fruit production. Rootstocks are becoming increasingly important in orchard management for fruit production, as they can significantly influence growth, productivity, and fruit quality in terms of physical traits and chemical compositions, as well as resistance to various abiotic and biotic stresses. In recent years, grafting onto stress-tolerant rootstocks has been suggested as an effective strategy to cope with various stresses. The aim of this research was to study the physiological and biochemical leaf characteristics of different plum rootstocks under drought conditions and identify the most drought-tolerant. The greenhouse experiment included five plum rootstocks ('Wavit', 'Adesoto', 'St. Julien', 'Penta', 'Torinel') and was conducted in 2023 at the Agricultural Institute Osijek in Croatia. The findings reveal that the selected rootstocks exhibited varying mechanisms of tolerance when subjected to the examined drought stress conditions. It was observed that 'Torinel' had the highest maximum quantum efficiency of photosystem II ($F_v/F_m$ 0.85), photosynthetic performance ($PI_{ABS}$ 2.55) and relative water content (RWC 63%) in drought conditions. Additionally, 'Torinel' demonstrated the lowest increase in hydrogen peroxide ($H_2O_2$ 1.53 μmol/g FW), malondialdehyd (MDA 16.95 nmol/g FW), and proline content (1.92 μmol/g FW). These results indicate that 'Torinel' rootstock exhibits greater drought tolerance compared to the other rootstocks.

**Keywords**: chlorophyll fluorescence, drought, rootstock, MDA, proline

# Multiyear Miscanthus biomass production at the marginal land: case of Chomutov, the Czech Republic

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**Summary**

*Miscanthus* × *giganteus* (*M*×*g*) is a second-generation energy crop known for its good adaptability in the diverse soil and environmental conditions. With high biomass yield and cellulose content, *M*×*g* holds promise as a valuable resource for the biofuel and various bioproducts manufacturing. Cultivating of *M*×*g* on the slightly contaminated or marginal soils presents environmental and economic advantages, including potential for soil remediation and carbon sequestration. The multiyear research has been conducted at the post-mining marginal land in Chomutov, the Czech Republic. Prior establishing of *Mxg* plantation the soil was treated by various soil amendments, i.e.: biochar in two dosages: 5% and 10% (BD1 and BD2), digestate (D), sewage sludge (SS), and hemicellulosic waste (HW). *M*×*g* rhizomes were originally taken from the deposit field of Miscanthus d.o.o. in Popovača, Sisak-Moslovina county, Croatia and planted at the spring of 2021 to the depth 10-12 cm. The growth parameters were monitored over three vegetation seasons. Results indicated that in the second and third growing seasons the number of *M*×*g* plants per plot was the highest in the soil amended with BD2 whilst the lowest numbers were fixed in the SS amended and HW amended soils, respectively. Plant height showed differences in the second growing season, with BD2 treated soil yielding the tallest plants and SS treated soil showing the shortest plant. The dry weight (DW) measurements revealed that in the second vegetation season *M*×*g* grown in D amended soil had the highest DW value followed by BD1 and BD2, other soil amendments showed much less DW value. The study demonstrates the potential of *M*×*g* cultivation in the post-mining land and positive effect of digestate and biochar amendments to the crop's growing parameters.

**Keywords:** energy crop, biochar, soil amendments, growth parameters, biomass

# Effect of wildfire on the spread of invasive plant species in the southwestern area of Karlovac County

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**Summary**

The number of invasive plant species continues to increase in Europe and poses a risk to biodiversity by spreading rapidly into new ecosystems. Fire is known to facilitate the invasion of many invasive plant species. The aim of this study is investigated a possible relationship between wildfires and the prevalence of invasive plant species. The study was conducted in the fire affected forestry unit „Bosiljevac" (3381.66 ha) in SW part of Karlovac County. The study area was affected by fires in 2012, 2019 and 2022, which burned an area of approximately 500 ha. The fire spread from agricultural areas to young forest areas. The possible directions of IAS expansion and the proportion of the area covered by surrounding pastures and unrestored forests were recorded using an unmanned aerial vehicle and georeferenced images. The data analysis and recent field visits revealed a weak correlation between burned areas and the spread of invasive plant species. The fire did not lead to an increase in the pace of invasion into the burned areas, primarily due to its frequency, so the IAS could not spread either by seed or vegetatively, and also due to the large coverage with fern *Pteridium aquilinum*. Invasive alien plant species such as *Bidens frondosa* L., *Erigeron annuus* (L.) Pers., *Phytolacca americana* L, *Robinia pseudoacacia* L. and *Solidago* sp. were found on unburned areas of the study site. The study will help in monitoring of impact of invasive plant species on the succession patterns of the studied areas after a fire.

**Keywords**: IAS, wildfire, succession, fire management, ecosystem restoration

# Effect of different green manure strategies on the yield of maize

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**Summary**

According to the Green Deal, the members of the European Union are to achieve a 55% reduction in greenhouse gas (GHG) emissions by 2030 and reach climate neutrality by 2050. These commitments represent a major challenge for the member states, and in order to achieve the targets set, it is necessary to use effective instruments. In the EU-27 agricultural sector, agricultural soils and the right choice of nutrient management methods account for more than 55% of the climate protection potential. In addition to sequestering atmospheric $CO_2$, using green manure plants improves the structure of the soil, contributes to increasing biodiversity, and can provide a sustainable way of ensuring nutrients for the subsequent crop. We set up our experiment in 2022 in Hungary on humic sandy soil. In the experiment, we applied green manure plant species individually and in mixtures at different sowing times, to determine the biomass yield, as well as their forecrop effect on maize. The treatments were compared with fertilized (N80 kg $ha^{-1}$) and control treatments, and were applied in 3 replications in a randomized design. Our results showthat green manure plants used in a reasonably selected mixture produced a significantly higher biomass yield. In the experiment, the lupin produced 3.3 t $ha^{-1}$, the common vetch 4.6 t $ha^{-1}$, the oil radish 11 t $ha^{-1}$, and the buckwheat 2 t $ha^{-1}$ of above-ground biomass, but with a suitable combination of varieties we obtained 56.5 t $ha^{-1}$ of biomass as a result of the combination of oil radish and common vetch. In terms of maize yield, the time of the incorporation had a significant role in favor of autumn ploughing. Concerning the forecrop effect, green manure mixtures resulted in a higher maize yield compared to the solo-cropped green manure species. Common vetch and oil radish green manures sown independently resulted in the same effect of fertilization, while green manure mixture of oil radish and common vetch proved to be the most effective in terms of maize yield.

**Keywords**: green manure, fertilization, yield, maize, biomass

# Peri-urban land use planning and their implication on soil contaminant concentrations

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**Summary**

In EU 60% - 70% of soils are unhealthy. The European Commission set up the goal, thought the Mission 'A Soil Deal for Europe,' to establish 100 living labs and lighthouses to lead the transition towards healthy soils by 2030. One of the many lighthouses in Europe is established near Zagreb, Croatia (Šašinovec). This research aimed to assess peri-urban soil quality based on heavy metals aggregation. Lighthouse site Šašinovec covers 63 ha and is divided in five land uses (apple orchard, grassland, cropland, forest and abandoned agricultural land). Soil sampling was conducted in spring 2023, and eight composite soil samples (0-30 cm) was collected from each land use. In total, 40 soil samples were prepared and analysed to obtain heavy metal contents (Cr, Mn, Ni, Cu, Zn, As and Pb) using portable X-ray fluorescence, with additional determination of soil reaction and organic matter (OM) quantification. The one-way ANOVA and Tukey post hoc test results indicate significant variation of accumulated metals in soil within the different land uses. In the orchard, significantly higher amounts of Mn, Ni, Cu, and As were recorded, while the statistically lower contents of Cr, Mn, Ni, Cu, Zn and As were determined in forest. Although the quantified values of Cu, Zn, As, and Pb do not excite threshold values according to the national and international legislation, results reveal that peri-urban soils were affected by urbanization and soils were contaminated by Cr and Ni. Chromium content ranged from 103 mg Cr/kg in acid forest soil up to 205 mg/kg in alkaline soils of orchard, grassland, cropland and abandoned agricultural land. Accumulated content of Ni excited national (75 mg/kg) and international (50 mg/kg) threshold values and varied from 108 mg/kg to 135 mg/kg in all alkaline land-use soils. Correlation analyses reveal significant and very strong correlation of pH and OM on Cr accumulation indicating that peri-urban land-use planning and soil management on each land-use class can affect the overall soil quality and contamination level.

**Keywords**: soil health, heavy metals, lighthouse, agricultural land, forestland

**Acknowledgments:** This study has been funded by the Horizon Europe Project InBestSoil (GA 101091099) "Monetary valuation of soil ecosystem services and creation of initiatives to invest in soil health: setting a framework for the inclusion of soil health in business and in the policy making process".

# Soybean seed yield in response to sowing date and inoculation in conventional and organic production systems

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**Summary**

In organic farming systems, choosing the right combination of variety, maturity group, planting date and specific inoculum could extend production time and maximize soybean yield. Most of the commercial soybean varieties developed in Serbia have been tested in conventional systems, but rarely in organic farming. The aim of this study was to investigate the potential of locally developed soybean varieties for organic farming under the conditions of different sowing dates and rhizobial seed treatment. The trials were conducted on conventional and organic fields at the Zemun Polje site in 2023. Soybean varieties of maturity group 0 (Lela and Selena) and maturity group I (Laura and Jasminka) were investigated. Two sowing dates and a microbial preparation - „Biofor soya" - were used. At the second sowing date, inoculation contributed to a yield increase of 4.6 % in organic and 8 % in conventional cultivation compared to the control. At the first sowing date, inoculation only led to better yields in organic cultivation compared to the control, while in conventional cultivation the yield without inoculation was higher than the yield of the inoculated varieties. The average yield of all varieties across all treatments and sowing dates was 4.13 t/ha in conventional cultivation, while it was only 3.24 t/ha in organic cultivation. In organic farming, the average yield of all varieties across all treatments was 21.9 % higher at the second sowing date than at the first, while in conventional farming the seed yield at the second sowing date was only 1.46 % higher than at the first. The sowing date appears to be an important factor for the productivity of the variety and the efficiency of the inoculation treatment.

**Keywords:** soybean, organic farming, yield, sowing date, microbial fertilizer

# Macroelements in French beans on the market in the city of Zagreb

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**Summary**

French beans (*Phaseolus vulgaris* L.) are annual herbaceous plants belonging to the legume family (Fabaceae). They have high nutritional value and serve as a source of essential nutrients, including minerals. When purchasing, consumers lack access information about their mineral composition. The aim of this research was to determine the quantity of specific macroelements in the French beans available on the market in the city of Zagreb. Samples were collected from three markets, three retail chains, and three organic product stores. French bean samples were subsequently ground, homogenized and analyzed fresh and dried at 105 °C. Nitrogen was determined using a modified Kjeldahl method, while after digestion with concentrated $HNO_3$ and $HClO_4$ in a microwave oven, phosphorus was determined using a spectrophotometer, potassium by flame photometry, and calcium and magnesium by atomic absorption spectrometry. Dry matter (DM) in French bean samples ranged from 8.18% to 10.38%. The determined amounts of macroelements in the dry matter (DM) of the beans were as follows: 3.62-3.71% N DM, 0.45-0.47% P DM, 2.19-2.87% K DM, 0.29-0.34% Mg DM, and 0.37-0.7% Ca DM. Values in fresh matter (FM) were: 307.84-379.04 mg N/100 g FM, 37.97-47.94 mg P/100 g FM, 197.81-225.79 mg K/100 g FM, 27.59-32.69 mg Mg/100 g FM, and 36.97-66.42 mg Ca/100 g FM.

**Keywords**: minerals, nutrients, *Phaseolus vulgaris* L., pods, vegetable

# Developing an advanced *Miscanthus x giganteus* phytotechnology for post-military land revitalization

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**Summary**

*Miscanthus spp*. is recognized among the prospective agent for phytoremediation, and species *Miscanthus* × *giganteus* (*Mxg*) shows the highest biomass yield and immense lignocellulose content, including in the pure soils. Its cultivation requires less input compared to other energy crops and promotes carbon sequestration potential. The phytoremediation with *Miscanthus spp.* has been proved when the soil was contaminated by trace elements, pesticides, oil products, and a mixture of xenobiotics of organic and inorganic origins. When the process has been supported by soil amendments, isolates, plant priming and plant growth regulators, *Mxg* grows sufficiently in the nutrient-poor and marginal soils and demonstrates an ability to enhance the soil health. *Mxg* can be proposed for the revitalization of the post-military lands, including recovering of the Ukrainian contaminated/damaged territories after the war. Economic returns can be obtained from the revitalization of the contaminated land with the further option to use it in agriculture and with a market value of biomass processing to biofuels and bioproducts. The Lab observations on effect of soil amendments, microbial isolates, plant priming and plant growth regulators has now been under the consideration of the field scale experiments established at the military land in Ukraine. The utilization of waste accompanied the process (contaminated plant's parts and non-conditioned biomass) by pyrolysis with receiving of biochar has been effective. The incorporation of biochar and its mixture with wood waste to the contaminated/damaged soil can support phytotechnology, thus reducing the availability of contaminants to move to the biomass, and enhance crops' productivity, decrease plant stress, and prolong the vegetation period serving as a carbon capture storage method, thus contributing to the reduction of the carbon emissions.

**Keywords**: military contaminated/damaged land; trace elements and hydrocarbons; phytoremediation parameters; biochar, Ukraine

**Acknowledgments:** The research is supported by NATO SPS MYP G6094 "Mitigation of climate change through advanced phytotechnology for military lands".

# Self-sustainable, smart module for city green infrastructure in climate change adaptation – Mod4GrIn project in theory and practice

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**Summary**

Our cities are not sufficiently greened and their inhabitants feel the lack more and more. Green roofs and walls are elements of green infrastructure where native plant species resistant to adverse climatic conditions (temperature fluctuations, drought) can be used. Equipping green infrastructure with a network of sensors, a smart irrigation system and power supply through photovoltaics also makes it possible to reduce the expenses of running this infrastructure. Their use can lead to cost optimization in a relatively short time, not only in financial terms, but also in material and environmental terms. Such solutions increase the retention of rainwater, the thermal insulation of buildings, improve the microclimate and aesthetics of a given place, thus enhancing human well-being and the overall quality of life rating in the city. An improved understanding of the interactions and feedbacks between urbanisation, ecological processes and climate change, together with evidence-based guidance, is crucial for the development of appropriate solution-oriented strategies for cost effective ecosystem based climate change adaptation and mitigation strategies, and for local biodiversity conservation measures. The aim of the project was to develop a self-sustaining smart module system for urban green infrastructure. This module consists of green roof, green wall and ground level vegetation solutions for buildings covered with stress-resistant native plant species and equipped with a water circulation system and monitoring devices configured based on the Internet of Things (IoT) and powered by solar energy. The implementation of the proposed concept will help mitigate climate change, restore ecosystem functions destroyed during the urbanisation process by mitigating the urban heat island (UHI) effect (attenuating local temperature and humidity), improving water retention while preventing excessive surface runoff, improving air quality and supporting urban biodiversity and natural capital.

**Keywords:** biodiversity, urban green infrastructure, climate change adaptation, native plant species

**Acknowledgments:** The project "Self-sustainable, smart module for city green infrastructure in climate change adaptation - Mod4GrIn" has been funded by the Norway Grants 2014-2021 through the National Centre for Research and Development (grant agreement number NOR/POLNOR/Mod4GrIn/0013/2019-00).

# Agroecological principles in aquaculture: a case study from East Africa

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**Summary**

Agroecology in East Africa is gaining momentum due to its potential benefits for food security, the environment, and climate, supported by research, policy, and economic drivers; meanwhile, integrated aqua-agriculture, as one of the promising practices for agroecological transition, confronts challenges like infrastructure and expertise hurdles. The PrAEctiCe project addresses these by unveiling three distinct "living labs" in East Africa. In Kisumu (Kenya), the lab focuses on the synergy between aquaculture and intercropping, utilizing aquaculture wastewater for irrigation and converting aquaculture sludge into fertilizer. The Namulonge (Uganda) lab delves into aquaponics, experimenting with varying combinations of fish and vegetables to optimize water, energy, and nutrient dynamics. In contrast, the Morogoro (Tanzania) lab integrates fish and poultry systems, utilizing fishpond wastewater for vegetable irrigation and combining aquaculture sludge with poultry manure to enrich the soil. Living labs are designed following general agroecological principles adopted for aquaculture. By providing tangible demonstrations and fostering knowledge sharing, they contribute crucial data for the ongoing development of agroecology-tailored indicator framework for aquaculture and the decision support tool for smallholder farmers, with the goal of charting a promising agroecological path of African agriculture.

**Keywords**: agroecology, integrated aquaculture, living labs, East Africa, smallholder farmers

**Acknowledgments:** This summary is based on the activities and results of the PrAEctiCe project (Potentials of agroecological practices in east Africa with a focus on circular water-energy-nutrient systems, 101084248), funded by the European Union.

# Impact of waterlogging stress on soil microorganism

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**Summary**

In the term of predicted climate change, our goal was to see how the waterlogging conditions will alter structure of soil microorganisms with the emphasis on plant growth promoting (PGP) microorganisms. For this purpose, we settled a greenhouse experiment using white cabbage as a model plant with two different waterlogging-regime treatments (once or twice during the growth stage). For community structure analysis total DNA was extracted from soils, sampled once at different time points (in regard to treatment regime), and submitted to amplicon sequencing. PGP properties of fungi and bacteria were tested on array of specific selective media. Identification of isolates was performed by sequencing the ITS2 or 16S gene regions. Waterlogging was shown to effect both structure of the total bacterial and fungal community as well as the several PGP traits (organic acid production, amylase and protease activity). Within PGP community *Bacillus, Peribacillus* and *Pseudomonas* represented dominant members. The waterlogging affected the abundance of the *Stenotrophomonas* population, yet some of the genera like *Brevibacterium, Ensifer, Micrbacterium* disappear after being subjected to such conditions. Waterlogging had a positive effect on the *Pseudomonas* and *Bacilllus*, which are rich in species with PGP properties. Some of the isolates showed an antagonistic effect against 3 tested fungal pathogens. PGP fungi were mostly represented by genera *Penicillium* and *Aspergillus*. Two consecutive waterlogging had a negative effect on the PGPF community. Few isolates were identified as *Mortierella elongata,* a species that has been intensively investigated due to its potential in the fight against extreme climatic conditions. Eventually, we selected 11 isolates of PGP bacteria as those with high potential for application as bioinoculum for promoting the plant growth in extreme climate conditions.

**Keywords**: PGPB, PGPF, community structure, waterlogging, climate change

# Prediction and visualization of phosphorus, potassium and humus in the soils of Osijek-Baranja County

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**Summary**

Geographic information technologies (GIS) are ubiquitous in agriculture, and their use in agricultural soil analysis is constantly evolving. The new GIS technologies offer numerous advantages. GIS technologies can be used to define soil zones based on their properties as determined by laboratory analysis. The aim of the research is to compare the values of soil fertility parameters (soil reaction, humus content) determined by prediction in GIS based on previous soil analysis and field validation of data using classic soil analysis. In the period from 2021 to 2022, 12,250 soil samples were analyzed in the area of Osijek-Baranja County to a depth of 0-30 centimeters. Based on the soil analysis, a prediction map was created using the Kriging geostatistical tool. The analysis of the soil samples showed that the average pH value of the soil in the county is 6.07. The minimum pH reaction was 3.05, and the maximum was 8.12. The average humus content in the soil was 2.08%. The minimum humus content was 0.17%, and the maximum was 35.6%. The average phosphorus content in the soil was 20.90 mg/kg. The minimum phosphorus content was 0.3 mg/kg, and the maximum was 40 mg/kg. The average potassium content in the soil was 21.94 mg/kg. The minimum potassium content was 2.56 mg/kg, and the maximum was 51 mg/kg. The predicted values were validated using 100 control soil samples from 2023. By comparing the results of the humus content in the soil samples, no significant changes were found in the soil class of humus content compared to the prediction values. By comparing the results of the soil reaction in the soil samples and the prediction values, significant differences were found in both the pH values and the interpretation values according to the soil classes. When comparing phosphorus and potassium, significant differences were found in the predicted values of soil supply in certain areas.

**Keywords**: soil analyses, Kriging interpolating methods, spaltal distribution.

# Sulphur in air, soil and vegetation at Molve Field

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**Summary**

The discovery of the Molve gas field in 1974, the largest gas field in Croatia to date, and the beginning of natural gas exploitation in these areas (1981) created the need for monitoring possible environmental pollution from the plant's operation. The aim of this study was to examine and present the results of measurements of gaseous sulphur compounds in air ($H_2S$, mercaptans (R-SH), and sulphur dioxide ($SO_2$)) and total sulphur content in soils and plants carried out over several years (2011 – 2020) at Molve Field. Samples of air, soil and plants were collected at five locations in the vicinity of the central gas station (CGS) Molve and boreholes Molve 9 (M9), Molve 10 (M10), Molve 11 (M11) and Molve 12 (M12). Sampling of $H_2S$, R-SH and $SO_2$ in air was done twice a year, in a 30-day period during the warmer season (summer time) and during the colder season (winter or late autumn). Surface [(0-3) cm] and subsurface [(3-8) cm] soil samples as well as the leaves and stems of ribwort plantain (*Plantago lanceolata* L.) were also collected twice a year, in spring and autumn. Depending on the sampling location and season, the average monthly mass concentrations of $H_2S$ in air varied between 0.28 μg $m^{-3}$ and 5.58 μg $m^{-3}$, R-SH between 0.19 μg $m^{-3}$ and 4.55 μg $m^{-3}$, and $SO_2$ between 0.43 μg $m^{-3}$ and 3.00 μg $m^{-3}$. Exceedances of the prescribed limit value (LV) for $H_2S$ (5 μg $m^{-3}$) were less than 7 times during the monitoring period 2011-2020; however, during the winter periods of 2015, 24-hour R-SH mass concentrations exceeded LV (3 μg $m^{-3}$) at the M11 (9 times) and M12 (10 times) locations, respectively. Since 7 exceedances of the limit value are allowed during the year, the air quality at these monitoring stations was considered unsatisfactory, i.e. the results indicated that the air quality was of the 2nd category (polluted air) with regard to R-SH. During the ten-year monitoring of $SO_2$, the LV (125 μg $m^{-3}$) was never exceeded for winter and summer periods. Mean values of total sulphur in soil ranged between 10 mg $kg^{-1}$ and 2960 mg $kg^{-1}$ depending on the soil type, sampling depth and location. Depending on the sampling location and season, the mean values in ribwort plantain ranged between 1120 mg $kg^{-1}$ and 5100 mg $kg^{-1}$. The content of sulphur in the plantain and soils ranged within tolerable values. Occasional exceedances of $H_2S$ and R-SH limit values indicated the need for further continuous monitoring of air quality in the area of Molve Field.

**Keywords**: hydrogen sulfide, mercaptans, *Plantago lanceolata* L., sulphur dioxide

# Development of a GIS model for mapping soil water erosion in cork oak groves in Portugal

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**Summary**

Soil is a scarce, valuable resource whose importance has been increasing exponentially due to the rapid degradation processes observed in recent decades, mainly enhanced by global climate changes, leading to the redefinition of current soil policies to monitor and avoid its degradation. The aim of the research is to develop a GIS tool for mapping the potential water erosion areas in three agricultural cork oak groves located in central Portugal in order to monitor its soil conservation ecosystem services at a macro scale. It used the revised universal soil loss equation (RUSLE) implemented in Arcgis PRO software version 3.1, using model builder tools alied to the geospatial data of each oak grove in raster format with a pixel size of 30 metres. The agriculture oak groves were classified into five erosion classes: class 1 – no erosion; class 2 – very low erosion (0-1,4); class 3 - low (1,4-10); class 4 – moderate (10-25); class 5 – high erosion (>25), (ton.ha$^{-1}$.yr$^{-1}$). Results were validated by in situ field observations, and the first preliminary conclusions suggested: (i) to perform a sensitive analysis using maximum precipitation values from the last ten or five years instead of from the last 30 years; (ii) to improve the spatial resolution of the digital terrain model for scale studies higher than 1:25000; (iii) to adopt neutral value for the parameter related to agriculture soil conservation practices in RUSLE equation, since the cork oak areas mainly enclose forest management actions instead of agricultural management actions. Once validated and automated, the model allows the zoning and comparision of distinct cork oak groves in terms of potential for water erosion, giving support to soil conservation monitoring and sustainablemanagment.

**Keywords**: soil erosion, oak groves, ecosystem services, GIS

**Acknowledgments:** The work was developed under the scope of Compete project nbr.181618 - ESA-EcoSystemAdapt and the Projet GeoBioTec (UIDB/04035/2020).

# Effectiveness of sewage sludge and sevage sludge based fertilizers on different soils

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**Summary**

Sewage sludge digestate, compost and vermicompost produced from the same digestate were tested on acidic sand and calcareous loamy soils in a pot experiment. In the 75 days long trial perennial ryegrass (*Lolium perenne*) was used as a test plant. The organic amendments were applied in 1 and 3 % doses. On acidic sandy soil the positive effect of the applied materials on plant biomass increased in the following order: vermicompost, compost, digestate. The 1 % doses doubled, the 3 % doses tripled the biomass compared to the control. On calcareous loam the same order was: compost, vermicompost and digestate however, there was no significant difference between the materials. The average biomass increment was 50 % compared to the control. The average agronomic efficiency of the materials (biomass increment, g / applied N, g) increased in the following order: compost (3.4), vermicompost (4.2) and digestate (6.8), which indicates that during a short period of time the less stabilized material may provide more nutrients for plants. Regarding the plant available (ammonium-acetate + EDTA soluble) potentially toxic element contents, the higher doses of each material significantly increased Cu, Ni and Zn concentrations on both soils and the lower doses used on the calcareous loam also caused a significant increase in the Ni concentration. The data confirm that sewage sludge and sewage sludge-based materials can be used effectively as fertilizers, but the risks of their use must be taken into account.

**Keywords**: sewage sludge digestate, compost, vermicompost, biomass, toxic elements

**Acknowledgements:** The work was financed by the Hungarian Ministry of Agriculture (AKGF/10/2021_10) and by theWALUE—Waste to Value—GINOP-2.2.1-15-2017-00080 as well as 2021-1.2.4-TÉT-2021-00035 project.

# Soybean increases grain Zn and protein of winter wheat grown in the three-crop rotation

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**Summary**

Crop rotation systems provide various benefits to sustainable agriculture. The inclusion of legume crops in rotation enhances the nutrient cycle in the soil-plant system, and especially the nitrogen (N) nutritional status of wheat. On the other side, N is involved in increased zinc (Zn) deposition in grains. Therefore, it is hypothesized that the inclusion of soybean in cereal-cereal rotation (e.g. maize–winter wheat) can increase both Zn and protein contents in wheat grains. The study was conducted at the experimental site in Rimski Šančevi, Serbia, as a part of a long-term crop rotations field trial established on a Chernozem in 1946. The trial included maize–winter wheat and maize–soybean–winter wheat crop rotations. To our best knowledge both cropping systems didn't receive fertilization since the beginning of a long-term trial. Bread winter wheat cv. Zvezdana was harvested in 2022. Wheat plots of both crop rotation systems were split into three subplots, and each represented one replication. Results of the *t*-test revealed that grain yield in three-crop rotation of 2.12 t $ha^{-1}$ was significantly higher than 1.10 t $ha^{-1}$ obtained in maize–winter wheat rotation, as well as grain Zn concentration (24.8 mg $kg^{-1}$ and 20.6 mg $kg^{-1}$, respectively) and grain protein (10.8% and 9.7%, respectively). Our results indicate the high potential of soybean to increase Zn and protein content in wheat grain under long-term conditions without NPK fertilization.

**Keywords:** crop rotation system, soybean, wheat grain, zinc, protein

# A comparative analysis of Zn, Cu, Mn and Fe accumulation in leaves of three cork oak forests of Portugal

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**Summary**

The study of geographically dispersed agroforestry ecosystems in mainland Portugal poses enormous challenges for companies if they want to ensure that they are sustainable and resilient. Indeed, growth is highly dependent on nutritional parameters, as micronutrients which are essential for the physiological functioning of plants, especially in the mobilization of assimilates. In this context, a tool will be developed to support decision-making and management of cork oak forests in order to strengthen the competitiveness of companies by performing micronutrient analysis in mature cork oaks in relation to photosynthesis rates. The levels of Zn, Cu, Mn and Fe were determined in mature cork oak leaves from three Portuguese experimental fields in Azinhal / Grandola (GPS: 38.1071851, -8.4419802), Casal da Balsa / Chamusca (GPS: 39.3620909, -8.3843477) and Cabanas / Évora (GPS: 38.5493728, -8.0642313) on February 17, April 14 and July 25, 2023. The average values of Zn, Cu, Mn and Fe in the three experimental fields were: on 51, 20, 733 and 800 ppm (February 17); 53, 22, 800 and 933 ppm (April 14); 51, 22, 600 and 330 ppm (July 25). The results obtained are presented and discussed in detail, taking into account the climatic and geographical variables of the three cork oak forests. However, the main conclusion is that, depending on the edaphoclimatic conditions, different antagonisms and synergistic interactions prevail in the accumulation of the studied micronutrients.

**Keywords**: agroforestry ecosystems, cork oak, micronutrients

# Innovative biodegradable waste treatment technology: case study with sludge from wastewater treatment plants

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**Summary**

An innovative technology for the processing of biodegradable material/waste has been developed, which decomposes and processes in an extremely fast time (in hours). In that short period, the material losses it unpleasant smelt, if there was any. Depending of input composition, the quality level of output as soil improver could be achieved. The advantage of the technology is that, unlike commercial batch systems, it is a continuous system, which increases the processing capacity. The innovation consists in ensuring processing in thermophilic conditions and in the way of maintaining aerobic conditions in whole processing material. Depending on the shape of the material, in the case of larger pieces of leftover food and the like, coarse shredding is preferable to speed up the process. Occasional excess moisture in part of the input material does not pose a problem. The paper will present the results of the treatment of different types of biowaste, including the treatment of sludge from wastewater treatment plants under real conditions in Mostar, Split and Poreč.

**Keywords**: innovative technology, biodegradable waste, sludge from wastewater treatment plants, thermophilic process

# Assessment of nematodes as bioindicators of soil health in integrating *Miscanthus x giganteus* phytotechnology

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**Summary**

Nematode community structure and diversity are closely linked to soil ecological processes, such as nitrogen cycling and decomposition. Nematode composition is often used as a bioindicator of soil health, but its potential to assess agronomic practices was not fully explored in *Miscanthus x giganteus* phytotechnologies. This study aimed to investigate the effect of soil amendments, i.e.: biochar, sewage sludge, biogas digestate, and hemicellulose waste on nematode community composition under the *Miscanthus x giganteus* phytotechnologies. The research goal also was to estimate the nematode community response in soil exposed to four levels of lead contamination in the crop's plantation. The investigation found that the incorporation of organic amendments affected the nematode food web and suppressed plant parasitic species. Soil contamination with lead was decisive in transforming the nematode community, but only at the highest level of contamination. The study underscores the need for continued research in the sensitivity of nematodes to the state of soil health at *Miscanthus x giganteus* phytotechnology with biomass production.

**Keywords**: nematodes, organic amendments, soil contamination, soil health

# Increasing the potential for improving morphological characteristics of soybean seeds (*Glycine max* L.) through an ecological approach

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**Summary**

Soybean is one of the most important oil and protein crops in the world. Soybean accounts for 30% of the world's edible oil production. A major problem affecting soybean production is low seed viability. One treatment that promotes uniform and rapid seed germination and increases resistance to adverse environmental conditions is seed hydropriming. The objective of this study was to determine the importance of hydropriming treatment on seed morphological and physiological characteristics. Three soybean varieties: Lela, Selena and Laura were used for the experiment. After a period of immersion in water (T0-control, T1-2h, T2-4h, and T3-6h), the length, and weight of the roots, stems, and seed germination were measured in a laboratory setting. Compared to untreated seed, germination was not significantly altered by seed treatment. This characteristic was primarily variety dependent, with seed of the cultivar Laura having the highest germination rate (98%) when treated with T0 and T1. The morphological characteristics of the seedlings differed significantly after hydropriming. After T1 treatment, the variety Laura had the longest root with 15.822 cm. In the T1 treatment, the variety Lela had the longest stems (9.033 cm). Seed treatment did not significantly increase root and stem weight.

The results of the study show that seed treatment increases the ability of seeds to withstand stressful conditions, especially during prolonged droughts.

**Keywords**: eco-friendly priming, phenotype, seeds, soybean

# Cave microbes – undercover biocontrol agents

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**Summary**

Increasing global food demand necessitates the development of sustainable agricultural practices that not only improve plant yield and quality, but also suppress soil-borne and endophytic pathogens without resorting to synthetic chemicals. Caves, which harbour a surprising diversity of distinctive microorganisms, are characterised by harsh oligotrophic conditions. These conditions reinforce fierce competition between microbes and promote the production of unique antimicrobial metabolites that are potential source of novel biocontrol agents. *Pseudomonas* spp. for example, are known producers of a wide range of bioactive compounds that suppress various plant, animal and human pathogens. However, their occurrence and diversity in caves are still poorly understood. The aim of this study was to determine the diversity of cave microbiota, isolate the *Pseudomonas* strains and characterise their biocontrol activity. Sediment and water (n=19) collected from a karst cave in Šibenik-Knin County were used to analyse microbial diversity by 16S rRNA NGS sequencing and to obtain pure isolates (n=322). *Pseudomonas* spp. was found to be an important part of the cave microbiota and accounted for 25.4% of all reads. *Pseudomonas* spp. isolates (n=65) confirmed by MALDI-TOF and sequencing of 16S rRNA gene, were genotyped by repPCR and their antimicrobial potential against selected pathogens (n=9) was assessed. *Pseudomonas* strains exhibited the strongest inhibitory effect, ranging from very strong to complete inhibition, against *B. cereus*, *L. innocua*, *E. amylovora*, *A. tumefaciens*, *B. cinerea*, highlighting their immense potential as a biocontrol inoculant in plant production.

**Keywords**: karst cave, microbial diversity, NGS, *Pseudomonas* spp., biocontrol

# The effect of zinc nanoparticles on the cherry plum germination

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**Summary**

Biocorridors are fragments of autochthonous vegetation in agricultural landscapes that connect individual biocentres. In addition to the autochthonous vegetation, other non-native species also invade these communities. *Prunus cerasifera* Ehrh. (cherry plum) is invasive in some parts of the world. It is a diploid species with very good generative reproduction, producing abundant flowers and vital pollen. *P. cerasifera* is part of the thicket biocorridor at the edges of lowland agricultural soils. Fertilizers based on nanoparticles draw attention to the safety of using these products. Pollen is a suitable bioindicator for various environmental pollutants. The aim of our study was to determine the toxicity of zinc nanoparticles (ZnNP) on pollen grains of *P. cerasifera*. The experiment was conducted with three treatments and control. ZnNP treatments at concentrations of 2.8, 28 and 280 mg.l$^{-1}$ and a control without ZnNP addition were used in the study. The effect of ZnNP on the germination of pollen grains was investigated. The results show that 81.15 % germinated pollen grains were observed in the control sample. However, at a concentration of 2.8 mg.l$^{-1}$ ZnNP, the percentage of germinated pollen grains was considerable lower at 59.97%. Furthermore, at a concentration of 28 mg.l$^{-1}$ ZnNP 47.42 % of the pollen grains germinated, while at a concentration of 280 mg.l$^{-1}$ ZnNP only 33.51 % of pollen grains germinated. The results indicate an unfavorable effect of ZnNP on the germination of *P. cerasifera* pollen when the concentration of zinc nanoparticles increases.

**Keywords**: zinc nanoparticles, pollen germination, cherry plum, *Prunus cerasifera*

# Comparison of methods for calculating potential evapotranspiration in different climate types in Croatia

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**Summary**

Evapotranspiration is an important part of the hydrologic water cycle as well as an important component in irrigation planning. The aim of this research was to compare the amounts of potential evapotranspiration (PET) calculated by different methods, in different climate types, as well as to determine the correlation between them. Potential evapotranspiration (mm) was calculated by 3 methods (Thornthwaite, Blaney-Criddle and Ivanov) for 4 meteorological stations (Osijek, Zagreb - Maksimir, Parg and Hvar). According to Köppen, the meteorological station Osijek has a Cfa climate type, humid subtropical climate. Zagreb-Maksimir and Parg weather stations have a Cfb climate type, temperate oceanic climate, while the Hvar meteorological station is located in the area of a Mediterranean climate with hot summers (Csa climate type). In the Croatian lowlands with climate types Cfa and Cfb, the average amount of PET was the lowest according to the Blaney-Criddle method (709.30 mm), and the highest according to the Ivanov method (859.95 mm). For the Parg station in mountainous Croatia, Cfb climate type, the average amount of PET was the lowest according to the Ivanov method (553.01 mm), while in this case the highest amount of PET was measured according to the Blaney-Criddle method (620.29 mm). On Hvar, Csa type climate, the smallest amount of PET was calculated by Thornthwait's method (891.57 mm), and the highest by Ivanov's method in the amount of 1368.55 mm. The highest correlation in the amount of PET was found between the Thornthwait and Blaney-Criddle methods (r=0.97*; Spearman; *p<0.05) and the lowest in the amount of PET was determined between the Thornthwait's and Ivanov's methods (r=0.59*).

**Keywords**: climate types, PET, Thornthwaite, Blaney-Criddle, Ivanov

# Do microplastics affect the reproductive success of beneficial soil organisms?

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**Summary**

Microplastics are small plastic fragments that are widespread in both the aquatic and terrestrial environment. Due to its versatile uses and chemical properties, the production of plastic products has steadily increased over the last decade. Considering the wide range of plastics and their highly varying chemical properties, it is important to assess possible negative impacts on soil ecosystems. Two Collembola species, *Folsomia candida* and *Sinella curviseta*, were exposed to two different microplastic particles and reproduction success was observed. Tire abrasion microplastic significantly affected both adult survival as well as reproduction success in *Sinella curviseta*. In Folsomia candida, the effects were similar, although the effects of both microplastic particles were less pronounced. Overall, this study showed adverse effects of two types of microplastics on beneficial soil organisms indicating potential harmful effects on the soil ecosystem. To gain a deeper understanding of the effects of microplastics on the soil ecosystem, future research should include a broader range of microplastic particles, encompassing different types, compositions, shapes and sizes. Further research should consider the use of a range of biomarkers to better understand the mechanisms behind the observed toxicity of microplastics.

**Keywords**: microplastics, soil, Collembola, reproduction, risk assessment

# Physiological responses of apricot leaves to drought

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**Summary**

This study evaluated physiological responses in the leaves of two apricot cultivars (cv.), Sungiant and Crvena kajsija to severe drought stress. Apricot plants were exposed to drought by withholding water in greenhouse pot experiment. Biochemical parameters (dry matter, proline and phenolic content) and chlorophyll *a* fluorescence (ChlF) were evaluated at the beginning of the experiment, 5$^{th}$ and 7$^{th}$ day after stress (DAS) initiation. Significant increase in dry matter (DM) and proline at 5 and 7 DAS confirmed drought stress in both cultivars, while cv. Sungiant had smaller increase of DM and greather increase of proline content compared to cv. Crvena kajsija. Phenols content in leaves of cv. Crvena kajsija increased during drought progression but in the leaves of cv. Sungiant phenols had similar values as in control plants. According to ChlF parameters, in the leaves of cv. Crvena kajsija drought caused decrease of photosynthetic efficiency already at 5 DAS, while the same change was seen in the leaves of cv. Sungiant at 7 DAS. Considering all presented results, it can be presumed that the cv. Crvena kajsija was more susceptible to drought stress than cv. Sungiant because despite the activation of defence mechanisms seen as activation of phenolics, the most sensitive process in the plant, i.e. photosynthesis, was violated. In contrary, cv. Sungiant activated proline more efficiently and initiated antioxidative defence mechanisms, probably enzymatic components, more effectively.

**Keywords**: chlorophyll *a* fluorescence, dry matter, phenolic content, photosynthesis, proline

# Estimation of nitrogen concentration in winter wheat leaves using multispectral imaging

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**Summary**

Nitrogen (N) fertilization is key for maintaining high and stable yields. However, the nitrogen use efficiency (NUE) of winter wheat (*Triticum aestivum L.*) is only 30-50 %. To ensure sufficient yield, some farmers overfertilize their fields. The excess N causes pollution and an increase in costs. For optimal fertilization, the plant N status should be monitored. Most of the existing methods are destructive and time-consuming. Multispectral imaging is a promising technology to estimate the N content of crops by using the leaf spectral reflectance. This study aims to assess the efficiency of multispectral imaging in the determination of N content in winter wheat leaves. A field trial was set up with a split-plot design in four replications. N-treatments, N0 (21 kg N/ha) fertilized with 300 kg NPK (7:20:30) applied at seeding, and N1 (80 kg N/ha), 300 kg NPK applied at seeding + 130 kg of Urea (46 % N) split into three applications, representing a main plot. The subplot was represented as 10 wheat varieties. During a heading stage (GS50) ten flag leaves have been collected per N-treatment x wheat variety x replication. Multispectral images in 6 different wavelengths were acquired, and different vegetation indexes were calculated for each leaf. Total leaf N was determined using the Kjeldahl method. Data were analysed using a general linear regression model with multispectral data as a predictor and N leaf content as a response. The Highest relationship for leaf N content was found with a Chlorophyll Index, ($R^2$= 0,7), Anthocyanin Index ($R^2$= 0,78), and Normalized Difference Vegetation Index ($R^2$= 0,64). This study shows that N content in leaves can be estimated using multispectral imaging. Nevertheless, further data must be provided to develop a more accurate model.

**Keywords**: multispectral imaging, precision farming, vegetation indexes, nitrogen fertilization, nitrogen use efficiency

# Introduction to proficiency testing and quality control in analytical laboratory for soil fertility

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**Summary**

Proficiency testing (PT) serves to demonstrate the reliability of the analytical results, in addition to identifying failures and enabling corrective or preventive actions to be taken. According to the legislation of the Republic Croatia and the HRN EN ISO/IEC 17025, accredited/authorized laboratories must participate in PT in order to ensure the quality of their analysis. The method provides independent evidence that the laboratory produces technically valid and reliable results and evaluate the actual analytical performance of a laboratory. There are many factors which can influence the quality of the analysis, some of them are: used test method and laboratory procedures, the staff's operation and the laboratory equipment. Internal quality control (IQC) is one of a number of concerted measures that analysts can take to ensure that the data produced in the laboratory are fit for their intended purpose. IQC comprises the routine practical procedures that enable the analyst to accept a result or group of results or reject the results and repeat the analysis. This is important for the quality of analytical data and is a final check of the correct execution of all of the procedures that are prescribed in the analytical protocol. The practical approach to QC is determined by the frequency with which the measurement is carried out, the size and nature of each run and is the principal recourse available for ensuring that only data of appropriate quality are released from a laboratory. In order to prevent inappropriate interpretation, it is important that a measurement uncertainty should accompany analytical results or be readily available to those who wish to use the data. When PT used in conjunction with IC and management committed to quality, it helps to promote knowledge of the analysis processes and guarantees the reliability of the results.

**Keywords:** proficiency testing, quality assurance, IQC, HRN EN ISO/IEC 17025, soil

# Summer surface temperatures of agricultural crops in the agro-ecological regions of the Republic of Croatia under current climatic conditions

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**Summary**

Under today's climate conditions, extreme meteorological events have an increasing impact on agricultural production. The aim of this study is to determine the surface temperatures of different crops in three agricultural regions of the Republic of Croatia. Three local government units were selected for the study: the city of Vinkovci in the Eastern Pannonian subregion and the city of Sv. Ivan Zelina in the Western Pannonian subregion, and the city of Šibenik in the Central Adriatic subregion. Surface temperatures at all three locations were calculated using Landsat 8 satellite images in the last decade up to July 2022, the warmest period in all three subregions. Surface temperatures ranged from 21°C to 65°C at all three locations. The warmest areas were in the city of Šibenik, where a fire occurred in mid-July. In addition to these areas, agricultural areas without plants (bare ground) were the warmest at all three sites. High temperatures were also measured in densely built-up areas in the city of Šibenik. In all three cities, the highest temperatures were measured on the bare soil of agricultural areas. Agricultural areas where plants were growing at the time of the survey had slightly lower temperatures. This is the first study of its kind to indicate high temperatures on agricultural land surfaces during the summer. Further research is needed to determine the duration of high temperatures, their impact on soil and yields, and ways to mitigate their effects.

**Keywords:** agricultural regions of Croatia, climate change, agricultural crops, surface temperature, Landsat

# Influence of physiologically active substances on the mineral composition of cherry leaves

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**Summary**

Physiologically active substances are compounds which have a direct physiological effects in various plants, such as promoting plant growth and development, influencing their nutritional properties, crop yield and response to environmental stress. The aim of this study was to determine how the treatments with different physiologically active substances affects the mineral composition of sweet cherry leaves. The research was conducted in 2022, with Regina variety, on two locations in Zadar County: Murvica and Ninski stanovi. All treatments (5) in 3 repetitions were carried out by foliar application 3 times in the phase of fruit growth and development. Treatments were: K - no treatment, only water, T1 – Ca nutritional supplement, T2 - biostimulator (extract *Ascophyllum nodosum* L.), T3 - proline solution, T4 - salicylic acid solution. Leaf samples were collected for the analysis of the following macroelements: total carbon (TC), total nitrogen (TN), calcium (Ca), magnesium (Mg) and potassium (K). Plant sampling was made before and after the foliar application, randomly on each side of the canopy, from five trees per repetition. Statistical analysis showed significant differences between locations and between the initial and final analysis of TN, Ca and Mg, however with insignificant differences among the applied treatments.

**Keywords**: leaf, mineral composition, physiologically active substances, Regina, sweet cherry

# Correlation between nitrogen and chlorophyll content in maize (*Zea mays* L.) leaves

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**Summary**

Physiological examination of nitrogen (N) concentration and the correlation between N and chlorophyll content in the leaves of maize (Zea mays L.) was carried out in two consecutive years at two growth stages of maize. Eight fertilization treatments were applied in four replicates including organic and mineral fertilizers and a control treatment. Leaves were measured by portable chlorophyll meter in the field (Opti-sciences CCM-200) through vegetative stage V4 (four fully developed leaves) and reproductive stage R5 (biomass). Furthermore, leaves were also analyzed in laboratory for N concentration. In addition, the highest correlation coefficient in 2018 was 87.8 % at the V4 stage. At the R5 stage, the correlation coefficient was lower at 57.6 % probably because the weather in June caused hail, which may have partially affected the lower green biomass yield. In 2019, the correlation coefficient was 61.9 % in V4 stage and 87.1 % in R5 stage. As showed, leaf chlorophyll content was positively correlated with leaf nitrogen concentration in both year and during maize growth stages.

**Keywords**: correlation, nitrogen, chlorophyll, leaf, maize

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Session 2

# Book of Abstracts

Agricultural Economics and Rural Development

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Agrarna ekonomika i ruralni razvoj

# The influence of production technology and origin on consumer preferences for apples

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**Summary**

This study explores how information about the production technology (organic and conventional) and the origin (local and non-local) of apples influences consumer preferences. The survey was conducted as part of a broader study of a sample of 103 consumers from the City of Zagreb. A hedonic scale with nine levels of liking was used to assess preferences. Initially respondents visually rated two apples that differed in production method and origin based on their photos, followed by assessments with additional information about production technology and origin. After the purely visual presentation, respondents liked the conventional apples more ($F(1,204) = 5.203$, $p = 0{,}024$). However, after learning about production technology, preferences shifted significantly towards organic apples ($F(1,204) = 40.720$, $p < 0{,}000$). Concerning origin, preferences for organic apples after information are higher for locally grown apples ($F(1,101) = 8.787$, $p < 0{,}004$).

**Keywords:** organic, conventional, local, hedonic scale

# Modeling predictors of behavior of consumers suffering from food intolerances or food allergies

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**Summary**

Food intolerances, food allergies and celiac disease are atypical, hypersensitive reactions of the body that appear after consuming certain products and are referred to as adverse reactions to food. One fifth of the population in Western countries believes that they have symptomatic reactions to food but the real prelevance to adverse reactions is still unknown. In recent years food not containing potentially harmful ingredients have become more popular as food sensitivity has long become a social trend. Therefore the aim of the study is to understand this issue by identifying predictors of buying intentions and behavior for people suffering from adverse reactions to food. For this purpose, the authors used three well-established behavioral models – the Theory of Planned Behavior (TPB), the Health Belief Model (HBM) and the Protection Motivation Theory (PMT). In addition, another model was created based on these constructs and all their strengths. The survey was conducted on the Forthright online platform among 1001 respondents using the CAWI (Computer-Assisted Web Interview) method. The article brings a new quality to science as it is the first one to take into account predictors of consumer intentions and behaviors related to food products suitable for people with food intolerance or allergies and analyzes the indicated predictors based on different theoretical models.

**Keywords:** food allergy, food intolerance, celiac disease, adverse reaction to food, diet,

# Index Insurance in Agriculture- Current Experiences and New Knowledges

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**Summary**

The original idea of an index in risk transfer was published in the middle of the last century. With the development of digital technology and the economy, indexed insurance (II) is gaining traction. II overcomes the problems of traditional insurance such as systemic risk, asymmetric information (moral hazard and adverse selection), high administrative costs and premiums, and the need for political support. In the case of II, the value of the objective measure (index) at a particular location is used as the trigger. Most attention is currently paid to weather-based index insurance, which uses precipitation or air temperature as an index. The weather data is frequent, of high quality, objective and readily available and therefore reliable. This paper presents trends in the development of II, the hype behind it and critically examines the prospects of II in addressing climate change. In Croatia, there have been few attempts to introduce II in the insurance sector. Some anecdotal evidence and the media question the marketability and economic sustainability of II in Croatia. We used semi-structured interviews with insurance companies and secondary data to examine why the theoretical expectations of index insurance have not been fully met in practice. The preliminary results indicate a baseline risk and a low level of knowledge and understanding of the mechanisms of II. The findings provide a starting point for the possible future design of II in Croatia, the EU and globally.

**Keywords**: index insurance, agriculture, risk, risk management, Croatia

# Consumers' willingness to pay for Criollo meat

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**Summary**

This article investigates consumers' willingness to pay for Criollo meat in a large representative sample of US consumers. We implement a fully incentivized condition within a geographically dispersed sample of consumers in which bids for a criollo steak elicited by a BDM mechanism are realized with certainty and the products are priority-shipped in dry-ice coolers. The fully incentivized condition is compared to Between-Subject Random Incentivized Schemes (BRIS), in which only a fraction of subjects realize their choices. We tested two treatments with a 10% probability framed as a percentage or absolute number of subjects, one treatment with 1% probability, and a purely hypothetical reference condition. The results reveal that BRIS with 10% and 1% payment probabilities are effective in eliciting valuations that are statistically indistinguishable from the fully incentivized scheme. All incentivized conditions mitigate hypothetical bias, resulting in lower product valuations than the purely hypothetical condition. We find that consumers have a premium of around $1.80 for Criollo steaks over regular steaks which is around 36 cents lower than the one elicited from the hypothetical treatment.

**Keywords**: Becker-DeGroot-Marschak mechanism, Between-Subject Random Incentivized Scheme (BRIS), criollo beef, hypothetical bias, large-scale experiments, random lottery incentives, sustainable beef, willingness to pay

# Local Istrian breeds as providers of ecosystem services

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**Summary**

The loss of animal genetic resources, primarily caused by the modernization of agriculture, is considered a significant threat to the world's biodiversity in general. Local livestock breeds possess genetic material that can be extremely useful in providing food in increasingly evident climate changes. However, their value is much broader as they provide numerous additional ecosystem services that are often not sufficiently recognized. Ecosystem services are benefits people obtain from nature, provided by habitats or individual species, and there are four types of them: provisioning, regulating, cultural, and supporting services. The County of Istria has already made great efforts to preserve and promote local livestock breeds (Istrian cattle, Istrian donkey, Istrian sheep, and Istrian goat). However, they have yet to be recognized as ecosystem service providers. The first step in valorizing local breeds as providers of ecosystem services should be to list the services they provide. Using the method of expert interviews, we compiled a list of the services provided by the four Istrian local breeds included in this research. Secondly, using the analytical hierarchical process (AHP), we asked 13 experts divided into two groups (breeders and non-breeders) to evaluate each Istrian local breed as a provider of ecosystem services and to evaluate the importance of ecosystem services provided by these breeds using pairwise comparisons. The results show that breeders prioritize supporting services most, while another group of experts prioritizes provisioning services. As the most important provider of ecosystem services, non-breeders consider Istrian cattle, while breeders consider it the Istrian goat.

**Keywords**: ecosystem services, analytic hierarchy process, Istria, local breeds

**Acknowledgments**: This research has been supported by Croatian Science Foundation (Genetic, Economic and Social Interactions of Local Breed Conservation Programs, GGD LocBreed; IP-2020-02-4860) and the European Union's Horizon 2020 research and innovation programme under grant agreement No 952303 (AgriFoodBoost).

# Models of mass communication in the service of the development of rural areas

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**Summary**

After the Second World War, mass media became one of the key segments of the development of society, because they contributed to the movement along the traditional-modern continuum. This was recognized by the world superpowers, which competed in the field of development and development policies, while they dichotomously divided the world during the Cold War into the capitalist West and the communist East. Consensus was reached only in the application of the mass communication model as a catalyst for the development of rural areas. This gave birth to three main paradigms of the rural development and mass communication models formed in accordance with them: the Western Modernization paradigm, the Eastern Paradigm of dependence and the hybrid Multidimensional paradigm. They were later combined into the concept of Development Communication. Considering that, as it grew over time, it turned into a scientific branch widely applied within agricultural and communication sciences, the aim of this paper is to give a concise account of it origin and development. Understanding the basics of Development Communication is important for the future steps in the development of Croatian rural areas. Namely, contemporary criticism of this discipline is directed towards the need to revise the dualism of development in order to not ignore the transition. Therefore, the proposed solution is the introduction of trichotomy and Digital Agriculture, a transitional concept increasingly popular in Croatia as well. Scientific controversy in this regard is that some consider this concept, due to the application of Social Media, only an upgrade to Development Communication, while for others, due to the complexity of its subsystems, it is new and independent.

**Keywords**: mass media, rural development, development communication, digital agriculture

# The Impact of Riparian Zones along Rivers under the New CAP in Slovenia

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**Summary**

This research primarily aims to investigate the New Common Agricultural Policy (CAP) 2023-2027 implementation measures in Slovenia, focusing on preserving and restoring riparian zones along rivers. Agricultural landholders near watercourses are obligated by the Water Act with the DKOP4 standard to establish riparian areas as protective strips, with precise widths of 15 meters for first-order watercourses, 5 meters for second-order watercourses, and 3 meters for drainage ditches exceeding 2 meters in width. These protective strips are limited to vegetation comprising grass, clovers, alfalfa, indigenous plants, shrubs, or trees, with strict prohibitions on organic and mineral fertilizer use and plant protection products. Slovenia has approximately 13,471 hectares of riparian lands mainly with intensive agricultural use. The most extensive cultivatable riparian areas along primary and secondary watercourses are predominantly found in Slovenia's northeastern and northwestern regions. The case study focuses on a farmer cultivating land in the riparian areas adjacent to the Sotla River. The Sotla River, located at Croatia's border, is classified as a first-order watercourse. For the case study crop yield reduction and land area loss during the New Common Agricultural Policy (CAP) 2023-2027 were calculated.

**Keywords**: riparian zones, CAP, agriculture land, Slovenia

# Market perspectives of berries in the Republic of Croatia

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**Summary**

Berries play an important role in human nutrition due to their chemical composition, which is naturally enriched with numerous nutritional and bioactive compounds. Berries mostly include strawberries, raspberries, blackberries, blueberries, currants and aronia. As a result of market demand, a high level of berry production has been recorded in European countries in recent decades. The Republic of Croatia is not self-sufficient in the production of berries. The FAOStat database was used to present the situation of the berry market. In the period 2012-2021, berries were produced on an average of 1,600 ha, of which blueberries were produced on 43% of the area, strawberries on 39% of the area, and raspberries on 19%. In the same period, the average production of berries amounted to 4,806 t, of which the majority refers to the production of strawberries (80%), while the production quantities of blueberries and raspberries amount to 14 and 6%, respectively. The foreign trade balance indicates negative trends, as an average of 6,226 thousand dollars was imported, and an average of 1,034 thousand dollars was exported. Strawberries (53%) and blueberries (28%) participated the most in the value of imports, and blueberries (46%) and strawberries (35%) in the value of exports. Therefore, it can be concluded that perspective and the interest of producers in the production of berries exists, and recently (since 2018) there has been an evident increase in interest in blueberry production. Some of the suggestions for improving the situation in the sector are investment in infrastructure (storage facilities, specialized machinery, processing, packaging), further linking of producers due to a joint presence on the market, as well as producer education on innovations in technology, production, processing and marketing.

**Keywords:** berries, strawberry, blueberry, production, foreign trade exchange

# Open spatial data in animal protein production in Croatia

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**Summary**

EU Data Governance Act prescribes the measures for boosting open data (OD) re-use aimed at creating additional value for citizens and small producers. Agricultural and environmental data stand out among the five most important sectoral data defined in this Act as drivers of digital transition in primary food production. The market OD is the collection that contributes the most to the empowerment of food producers (the Open Data Charter assessment). Moreover, the Implementing Act on high-value data sets as part of the OD Directive emphasizes the necessity of providing OD of the name, functional status and address of the registered business headquarters. Since the spatial data on animal protein production is not available as OD from the Central Register of Livestock (animals or farms), the aim of this research was to assess the current availability level of such open data by geocoding available data of different aggregation levels and using basic centrographic statistics and map visualizations using R spatial packages. Based on the type of data and the data aggregation level the following can be concluded: the data that is collected, produced or paid for by public bodies is freely available for anyone to re-use for any purpose but only in the limited form and aggregated predominately at the county level. Open availability as individual-level data of these registers has a potential to provide added value for more than 42% of Croatian population living and producing in rural areas.

**Keywords**: open data, animal production, digital transition, centrography

# Educational opportunities and challenges for the study program Agroeconomics

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**Summary**

Formal education should provide certain knowledge, skills and competences that correspond to social, personal and cultural needs in the labor market. The success of the real sector is reflected in the conditionality and mutual functioning of the education system and the labor market. The specific educational opus of the master's degree in agricultural engineering (majoring in agricultural economics) includes technological knowledge, but also knowledge in agricultural economics (accounting, market, marketing, rural sociology, entrepreneurship, rural development, agrarian policy, etc.). The employment of this profession is reflected in various forms of business, both in private business and running a family farm, consulting and in state institutions. At the Faculty of Agrobiotechnical Sciences Osijek, various activities are systematically carried out that form student competencies through teaching, scientific, organizational and promotional activities. During the analysis of the education system, available data from the Quality Assurance Committee and the Office for Quality Assurance and Improvement of Education at the Faculty of Agrobiotechnical Sciences Osijek, as well as data from the Unified University Student Survey, were used. The goal of this research was to analyze and compare the results of the conducted surveys and examine the effectiveness of the implemented measures. The results of the research indicate an increasingly strong influence of dual education, a greater interaction of teachers through adapted teaching methods, as well as the involvement of students in extracurricular activities. The paper provides guidelines and measures necessary for effective quality management in higher education.

**Keywords:** education, agro-economics, labor market, quality management

# Use of the Health Belief Model to examine willingness to use omega-3-enriched eggs

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**Summary**

The market for functional foods is relatively young and growing, mainly due to increased consumer awareness of the importance of nutrition for human health. Omega-3 fatty acids are essential fatty acids that help to reduce the risk of heart disease and maintain normal blood cholesterol levels. The production of omega-3 fatty acid-enriched eggs requires a modification of the poultry feed, which usually involves the addition of fish and/or flaxseed oil and flaxseed. The aim of this study was to use the Health Belief Model (HBM) to determine the factors influencing the willingness to consume omega-3 enriched eggs. The data was collected from 159 respondents (egg purchasers) using a validated questionnaire. Multiple linear regression in the SPSS statistical package was used to analyse the relationships between the HBM constructs. The results showed that perceived benefits, severity, cue to action and self-efficacy had a significant impact on the willingness to use omega-3 enriched eggs. However, perceived barriers, health motivation and perceived susceptibility had no effect on willingness to use omega-3 enriched eggs. Promoting omega-3 enriched eggs by doctors and as tasty, easy available and beneficial for heart health is likely to trigger the willingness to use omega-3 enriched eggs.

**Keywords**: omega-3 enriched eggs, consumers, survey, health belief model

**Acknowledgments:** This research has been supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 952303 (AgriFoodBoost).

# The impact of mergers of large broiler companies on prices and costs

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**Summary**

Mergers of large companies require regulatory approvals. The approval decision always weighs benefits in terms of cost synergies against the costs due increased market power and higher prices to consumers. This paper analyzes the impact of a merger between the third and the seventh largest broiler producers in the United States on costs and chicken prices. We first develop a model of a Cournot oligopoly with a competitive fringe, and then apply merger simulation to predict the welfare effects. We fit our theoretical framework to the pre-merger data under various assumptions about the number of Cournot players ($N^r$). The results suggest that the merger between Sanderson Farms (#3) and Wayne Farms (#7) will increase the market price of chicken meat, but the magnitude of the effect is sensitive to the number of oligopoly players. Under the assumption that $N^r$=6, Wayne Farms belongs to the competitive fringe, and the merger happens between a strategic firm and a competitive firm. Under the assumption that $N^r$=7, Wayne Farms is also part of the oligopoly, and the merger happens between two strategic players. The gain in profits for the oligopoly also depends on the size of the oligopoly: the source of it is the induced cost synergy when the newly merged firm shifts production from less efficient plants to more efficient ones. The aggregate welfare, defined as the sum of consumer surplus and oligopoly profit, would increase by 1.15% when $N^r$=6 or decrease by 0.15% when $N^r$=7.

**Keywords**: horizontal merger, merger simulation, competitive fringe, cost synergies

# Models for horticultural farms in Adriatic Croatia to adapt to climate change

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**Summary**

Climate change is becoming an increasingly pronounced phenomenon that poses a challenge for agriculture when it comes to ensuring the required quantity and quality of produce as well as a satisfactory and stable income for farmers. Parts of the Mediterranean region, including Adriatic Croatia, are increasingly exposed to climate risks, as rainfall and temperature fluctuations and the occurrence of extreme weather events are becoming more pronounced. The aim of the research is to determine the models of horticultural farms in Adriatic Croatia and to propose measures for their adaptation to climate change. The production and economic characteristics of olive, vineyard and vegetable farms, which are the most represented in this area, are described at the level of the region and individual counties. The largest share of farms in Zadar County (45%) is engaged in olive growing, but the average olive-growing area per farm is largest in Split-Dalmatia County (1.71 ha). The highest gross income from olive growing per farm is achieved in Istria County (€ 73,690). Istria County has the largest average area of vineyards per farm (1.50 ha), but the highest gross income per farm from this production is achieved in Zadar County (€182,224). Vegetable production is most strongly represented in Istria County (on 63% of farms), where it generates the highest gross income per farm (€33,296). For the entire production in almost all counties, the most pronounced climatic changes are the uneven distribution of precipitation and the increasingly hot summers. The most important adaptation measures are the focus on organic farming, the development of an irrigation system and a more favorable insurance policy. In these directions, the resilience and adaptation of farms to climate change should be strengthened.

**Keywords**: climate change, adaptation, olive growing, viticulture, vegetable growing, Adriatic Croatia

# Agricultural policy development of Bosnia and Herzegovina and EU approximation process

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**Summary**

The paper presents the results of the analysis the latest follow-up of agricultural and rural development policy in the Bosnia and Herzegovina and its approximation with the EU CAP, focusing on the years 2020 to 2022. The paper provides an overview of the current national policy framework both at the level of the state of BiH and at the level of its entities, the analysis of the support budget for the agriculture and rural development sector, and the state of the agricultural sector of BiH on the way to the Green Agenda. APM (Agri-Policy Measures tool) was used for agricultural policy analysis and represents a combination of the EU concept of agricultural policy pillars and the OECD PSE classification. The year 2020-2022 is the period for the drafting of strategic documents for the development of agriculture and rural areas at all levels, either in the stage of the already adopted document or in the draft phase and in the process of adoption. With EUR 134.3 million, the total allocations for the agriculture sector were higher by 14.1% compared to 2021 (EUR 117.7 million), i.e. by 55% compared to 2019. The structure of the total budget in 2022 is such that market price policy measures and direct payments to producers are still dominant with 75.9% (101.9 million EUR): Pillar II and structural and rural development measures participate with 25, EUR 1 million or 18.7%, while general measures in agriculture are the least represented, making up only 5.4% (EUR 7.2 million) of the total agricultural budget. The approximation process is slow and more declarative. The total support is insufficient, it mainly refers to direct payments (especially payments based on output), without major allocations for structural changes and agro-environmental issues. There are a large number of measures similar to those in the CAP, but either they are not applied or the allocations for them are very modest. Bosnia and Herzegovina is at the very beginning of fulfilling its obligations under the Green Agenda.

**Keywords**: Agricultural policy, rural development, EU CAP, Bosnia and Herzegovina, Green Agenda

# NOVAFERT project - SWOT and PEST analysis of alternative fertilising products in Continental Croatia

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**Summary**

Prolonged global political and economic turmoil has significantly impacted the agricultural sector. Supply chains have been disrupted, significant losses have been recorded and the price of key products, including fertilisers, has risen. It is therefore necessary to raise awareness of the need for new, innovative fertilising products based on locally available resources. Some of these needs are the subject of the NOVAFERT scientific research project, which aims to demonstrate the ecological, economic, and technical feasibility, as well as the safe production and application, of a broad portfolio of alternative fertilising products containing recycled nutrients from different waste streams (bio-waste, bio-products, digestate, sludge, wastewater, treated manure, etc.). This would contribute not only to reducing the use of mineral fertilisers but also to reducing the negative environmental impact, i.e. the dependency on agricultural nutrients in representative European countries. One of the final objectives is also to formulate a policy to overcome existing obstacles at the local and European Union level. Considering the above, an analysis was carried out at the regional level within the 7 target regions, based on the waste streams allocated according to their availability in the target region. A socioeconomic characterisation of the situation in Continental Croatia was carried out using simple but powerful tools, SWOT (Strengths, Weaknesses, Opportunities, Threats) and PEST (Political-Legal, Economic, Social, Technological factors) analysis, to design an effective strategy to overcome the identified obstacles, influence the adoption of policies, but also the possibility of association and cooperation of different stakeholders with the NOVAFERT project. Specifically, in Continental Croatia, this analysis covered the following waste streams: biowaste, treated manure and digestate. IPS Konzalting, as part of the NOVAFERT project, presented the results of the SWOT and PEST analysis in the form of a spider diagram, trying to answer the questions of economic profitability, environmental sustainability, and legal readiness. The results obtained will help to formulate an action plan that will pave the way for the use of alternative fertilisers.

**Keywords:** NOVAFERT project, alternative fertilising products, SWOT and PEST analysis

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# FERTIMANURE project - development of business models for more sustainable manure management

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**Summary**

FERTIMANURE is a project that develops, integrates, tests and validates innovative nutrient management strategies to efficiently recover mineral nutrients and other products with agronomic value from manure. The goal is to produce reliable and safe fertilisers that can compete in the EU market. To understand the business environment and the constantly changing market, the project collected opinions from relevant stakeholder groups. Effective communication with stakeholders through various workshops, surveys and SWOT analysis was crucial for the development of business plans and models, providing insights into the economic profitability and sustainability of producing bio-based fertilisers (BBFs) from manure. Business models for farmers will illustrate the fundamental elements of operations and structure the idea of BBF production coherently. In the development of the Dutch business model, one of the key statements for ensuring added value relates to the potential for more precise fertilization in terms of quantity and quality. This could potentially increase the efficiency of nutrient use, accompanied by reduced emissions and environmental impact, as well as replacing the use of mineral fertilisers and reducing costs. The ability to better utilize manure directly on the farm is crucial. Furthermore, the main channels are focused on liquid flows/products, enabling distribution in the nearby region and concentrated products that ensure distribution over longer distances. Using the FERTIMANURE project as an example, the importance of business planning and modeling, as well as the impact of quality identification and continuous interaction with the target stakeholder group on research outcomes and product positioning in the market, will be demonstrated.

**Keywords:** FERTIMANURE project, BBFs, business models, manure

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# Book of Abstracts

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# Zbornik sažetaka

Očuvanje bioraznolikosti, genetika i oplemenjivanje

# Genetic variability of domestic potato germplasm

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**Summary**

Production of potato in Croatia is adopting European trends in branding and designation of geographical origin. An important prerequisite for branding is a detailed evaluation of local germplasm on genetic level. The goal of this research is the analysis of genetic variability of nine accessions of traditional Croatian varieties of potato, and their comparison with the variability of five foreign commercial varieties cultivated in Croatia, using six pairs of AFLP markers. Analysis of molecular variance revealed statistically significant genetic variability between and within the studied cultivars, with higher variability found within accessions of traditional cultivars. Cluster analysis showed three main groups of varieties. One group consists of variety 'Krasno' (IND00087), 'Žuti krušni' (IND00088), and 'Lipički žuti' (IND00075). The second group consists of only one variety, 'Istra' (IND00076). The third group consists of the accessions of Croatian traditional varieties and foreign varieties, grouped into four sub-groups. The former two groups are statistically significantly separated from the latter. These results represent a contribution for the initiation of potato breeding program, branding of traditional varieties, and the conservation of domestic potato germplasm within the framework of the National Programme for Conservation and Sustainable Use of Plant Genetic Resources.

**Keywords**: *Solanum tuberosum* L., traditional varieties, AFLP, intra-varietal variability, conservation

# GEI decomposition of yield performance in maize multi-environment trials in Türkiye and Croatia

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**Summary**

Maize is one of the most important crops in the world. Its significance generates many new research and development strategies in order to cope with recent climate changes. One of the strategies in maize breeding is using multi-environment trials (MET) to evaluate the relative performance of genotypes in target environments. In statistical analysis of MET the key point is the analysis of genotype and environment interaction (GEI). GEI analysis provides a better understanding of how different crops respond to different environmental conditions. In this study, several locations in Türkiye are selected along with two locations in Croatia thus comprising two sets of environments. A total of 22 hybrids across 17 environments were used in cycle 0 (2010-2011), and 7 hybrids across 19 environments were used in cycle 1 (2015-2019). GEI was decomposed by the Additive Main effect and Multiplicative Interaction (AMMI) model, and partitioned to signal ($GEI_S$) and noise ($GEI_N$) based on ANOVA. Results for cycle 0 were stratified into four classes of GEI response in AMMI1. Hybrid 16 showed above-average grain yield (GY) and positive response to environmental quality ("work-horse hybrid" with static stability). GY of hybrid 20 was above average, however, this hybrid was the best only in specific environments ("race-horse hybrid" with dynamic stability). After selection, mostly standard hybrids remained in design for cycle 1 where two hybrids represented typical "work-horse" vs "race horse" characteristics. This study shows the ability to efficiently carry out hybrid breeding for very diverse growing areas (hot, water managed vs. temperate, rainfed) based on breeder's expertise combined with sufficient genetic diversity and proper GEI analysis.

**Keywords**: maize, yield, GEI, MET

# Identification of local Slovenian grapevine varieties (*Vitis vinifera* L.) of the Primorska region using SSR markers

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**Summary**

Grapevine is an economically important fruit crop in Slovenia. In 2020, grapevine occupied an area of 15,260 hectares and the annual production of grapes was 103,637 tones. The Primorska wine-growing region accounts for 42 % of Slovenia's total vineyard area. Besides widely known varieties such as 'Refošk', 'Malvazija', 'Merlot' and 'Chardonnay', there are also local varieties such as 'Vrtovka', 'Pokalca', 'Planinka', which are adapted to the local climate (hot and dry summers) and are therefore interesting for breeding new varieties. However, for these local varieties, only few genetic analyses were done in the past to identify varieties, to evaluate their synonyms and homonyms and their potential relatives. In this study, we genotyped 197 different varieties of *Vitis vinifera* L., located in the Primorska region, using nine simple sequence repeats (SSR or microsatellites), a core set selected from the GrapeGen06 research European Project. The analyzed microsatellite profiles were compared to data from the Vitis International Variety Catalogue (VIVC). We confirmed some previously assumed synonyms and discovered several new ones, for example, 'Fzunek' = 'Muscat Petite Grain' and 'Kanarjola' = 'Tribjan' = 'Zdenka' = 'Trebbiano Toscano', which are named differently despite having the same microsatellite profile. We identified several homonyms, for example different types of 'Zunek', 'Sevka' and 'Pika', which differ in several microsatellite loci. By comparing the microsatellite profile of the homonyms with the data from VIVC, we were able to identify 50 % of the homonyms. For the other half of the homonyms and for the varieties whose names and profile have not yet been published in VIVC, a parentage analysis was done. The results of this study have solved many dilemmas related to synonyms, homonyms and the origin of local Slovenian grapevine varieties, which will help to identify true-to-type material and also to develop new varieties adapted to the influence of climate change, such as drought and heat.

**Keywords**: Grapevine, SSR, synonyms, homonyms

# NATURA 2000 and agriculture in the area of Ravni Kotari in Zadar County, state and challenges

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**Summary**

The Natura 2000 is the ecological network of the European Union which includes areas of interest for the conservation of endangered species, habitat types, and related corridors between them. Ravni Kotari is registered as an area important for the conservation of endangered species and habitat types in Zadar County extends from Town of Nin and the Karin Sea in the northwest to the Krka River in the southeast, Bukovica region in the northeast and the City of Zadar in the southwest. It is an area characterized by agricultural production, especially in fruit and vegetable growing and more recently in viticulture, winemaking and olive growing. The aim of this research was to collect and analyse scientific data and other sources, and to determine the state and challenges in conservation and agricultural use. For Natura 2000 Ravni kotari, two target habitat types have been established; Mediterranean tall moist grasslands (*Molinio-Holoschoenion*) and caves and pits closed to the public, and seven target species. Also, we determined 18 target bird species, of which the Golden crow (*Coracias garrulus*) has the largest recorded population in Croatia in this area. The goal is to preserve a mosaic of agricultural areas with sustainable production as suitable habitats for the conservation of target species. It is necessary to establish monitoring of the state of target species and their habitats and target habitat types for which there is insufficient data, and for their preservation it is necessary to implement the conservation measures.

**Keywords**: agriculture, habitats, Natura 2000, Ravni Kotari, target species

# Variability of Fe and Zn in wheatgrass juice

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**Summary**

Due to its pronounced antioxidant activity and bioactive components, numerous studies have shown that wheatgrass juice helps in the prevention of various diseases and contributes to improved tolerance of long-term therapies in chronic patients. Under controlled conditions, the cultivation of wheatgrass typically lasts up to 15 days. However, considering the mineral composition of wheatgrass, it is important to determine the optimal duration of cultivation. Therefore, this research aimed to determine the total and *in vitro* bioavailable concentrations of Zn and Fe in the juice of wheatgrass grown for 6, 8, and 10 days. Simulation of *in vitro* digestion was carried out using the standardized method according to Minekus et al. (2014), while total concentrations were determined by the ICP-OES technique. The study found that the highest average total and *in vitro* bioavailable concentrations of Fe and Zn were recorded in wheatgrass juice of 6-day-old plants. In both cases, concentrations decreased as the cultivation period extended. For Fe, there was a 17% lower bioavailability in wheatgrass juice on the 10th day of cultivation compared to the 6th day, and for Zn, there was a 34% lower bioavailability on the 10th day compared to the 6th day of cultivation. Based on this research, it can be concluded that for individuals using wheatgrass juice as a dietary supplement with an aim to increase their iron and zinc intake, it is optimal to grow wheatgrass for 6 days after germination.

**Keywords**: wheatgrass, *in vitro* digestion simulation, mineral composition, bioavailability

# The impact of sound on the growth and antioxidant enzymes activity in basil

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**Summary**

Being sessile, plants, continuously deal with their dynamic and complex environment, identifying important cues and responding with appropriate responses. Consequently, the sensitivity of plants has evolved to perceive a variety of external stimuli, which ultimately ensures their successful survival. Research over the past few centuries has shown that plants respond to environmental factors such as light, temperature, moisture, and mechanical disturbances (wind, rain, touch, etc.) by modulating their growth and development accordingly. Sound vibration as a stimulant has attracted attention only recently. However, little information is available on the effects of sound on plant growth, physiological and biochemical processes. The aim of this study was to determine the effects of the sound of different frequencies (0 Hz, 250 Hz, 500 Hz) on growth and antioxidant enzymes activity in the model plant basil. The study was conducted in growth chambers in which three-week-old basil seedlings were exposed to sound of a specific frequency for 10 days. Sound treatment of frequencies of 250 and 500 Hz had a positive effect on the growth of basil plants, as well as on the fresh and dry weight of the plants. In addition, sound treatment at the same frequencies significantly affected the protein content in basil leaves and the activity of antioxidant enzymes, superoxide dismutase and peroxidase.

**Keywords**: sound treatment, plant length, SOD, POX, basil

# Coleoptile length and Rht alleles in Croatian wheat varieties

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**Summary**

Coleoptile length is an important determinant of the sowing depth in wheat, and it is of particular importance in conditions of reduced water availability in the soil. The aim of this research was to examine the variability of coleoptile length of Croatian wheat varieties as well as the distribution of Rht alleles that are associated with coleoptile length. The research was conducted on 25 varieties of hexaploid winter wheat. The coleoptile length was examined in laboratory conditions, where filter paper was used as a growth medium. The experiment was set up in three repetitions where 50 grains were used per variety and repetition. The length of the coleoptile was measured after 10 days of growth in the growth chamber. DNA was extracted from all varieties using the CTAB method. Primers specific for the Rht-B1 and Rht-D1 loci were used for the PCR analysis. PCR products were analyzed using horizontal electrophoresis. Coleoptile length ranged from 3.78 cm to 9.54 cm, with a mean value of 6.08 cm. The analysis of variance revealed statistically significant differences ($p < 0.001$) between the examined wheat varieties in terms of coleoptile length. At the Rht-B1 locus, most varieties (68 %) had the Rht-B1b allele, while at the Rht-D1 locus, most varieties (92 %) had the Rht-D1a allele. Varieties with Rht-B1a allele had the highest average coleoptile length, and varieties with Rht-B1b allele had the lowest average coleoptile length.

**Keywords**: wheat, coleoptile length, Rht genes

# Elimination of potato virus M and potato virus S from four traditional potato cultivars

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**Summary**

Potato (*Solanum tuberosum* L.) is the third most important agricultural crop in the world. Numerous factors influence its yield and quality, viruses being one of the most important. Traditional Croatian cultivars such as Plitvički (accession no. IND00086), Lipice žuti (IND00075), Poli (IND00062), and Žuti krušni (IND00088) play a crucial role in biodiversity conservation and can serve as a source of genes for future breeding programs. In order to ensure the use, conservation and exploitation of their full genetic potential, the aim of this work was to free the above-mentioned traditional potato cultivars from potato virus M (PVM) and potato virus S (PVS), with which they were infected. Micropropagated infected plants were used as starting material. Three different treatments were used for virus elimination: ribavirin chemotherapy (RIB), electrotherapy followed by RIB (ET + RIB), and thermotherapy followed by RIB (37 °C + RIB). After treatment of *in vitro* plants with a temperature of 37 °C or with electrotherapy, as well as when treated with ribavirin alone, vegetative tips were isolated from axillary buds and cultured for a total of 95 days on MS medium supplemented with ribavirin (50 mg/l). ELISA confirmed the success of all three treatments in eliminating PVM and PVS. The treatment at 37 °C + RIB achieved the highest percentage of plants free of both PVM (95.4%) and PVS (86.1%). As for the cultivar, the highest percentage of elimination of both viruses was achieved in the Lipice žuti cultivar, reaching 92 %.

**Keywords**: traditional potato cultivars, potato virus M and S, chemotherapy, electrotherapy, thermotherapy

# Trials on anther cultures in *Vicia faba* L.

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**Summary**

Leguminous species, including *V. faba*, are considered recalcitrant to most *in vitro* approaches including haploidization. To date, there are very few studies dealing with anther (AC) or isolated microspore cultures (IMC) in *V. faba*. Response in anther culture was restricted mainly to anther browning and dying and sporadically to callus development. In isolated microspore cultures rarely microspore division occured and no development was observed. No plant regeneration from AC nor IMP was reported for *V. faba*. These findings together with the progress in haplopidization protocol development in other legumes i.e., pea and chickpea were the assumption to undertake the study on the trials of induction of androgenesis in *V. faba.* The aim of this study was to analyse the effect of culture media and different temperature treatments applied both on floral buds and on anthers in culture. Two cultivars of *V. faba* were used in this study. DAPI (4'6-diamidino-2 phenylindole) staining was applied to determine the developmental stage of the cells in anthers. Anthers containing predominantly microspores (mostly in medium and late stage) were selected for the study. Both flower buds as well as anthers in the culture were pretreated with cold pre-treatment (4°C) for 1-3 days. Anthers were isolated on two solid media containing growth hormones (2,4-dichlorophenoxyacetic acid, 1-napthaleneacetic acid and kinetine). After 30 days of culture we observed several following changes in the cultures: callusing on anthers, anthers with embryo-like structures (ELS), as well as swelling and browning of anthers. Browning of anthers was connected with tissue dying and those anthers extracted phenolics to the medium. Callus and ELS production was stimulated predominantly by cold pre-treatment (4°C per 1 day) of floral buds. Obtained samples of callus tissue developed on cultured anthers were subjected to ploidy analyses, which showed presence of haploid tissue.

**Keywords:** androgenesis, faba bean, ploidy, haploidization

# Phenolic acids profiling of drought-tolerant and susceptible maize inbred lines in response to long-term water deficit

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**Summary**

The development of drought-tolerant genotypes capable to reconfigure their metabolic pathways to cope with water deficit is of utmost importance for plant breeding. Further progress in maize performance under drought is expected by combining marker-assisted breeding with metabolite markers. Due to the fact that grain is more susceptible to water stress than vegetative tissues, the prediction of grain yield from the biochemical parameters of the leaves is a challenge. Hence, by evaluating five maize inbred lines differing in drought tolerance, the aim of this study was (i) to determine whether the tolerance/susceptibility to water stress can be attributed to the level of phenolic acids in maize leaves and (ii) to evaluate the relationship between these metabolites and grain yield as an indicator of plant productivity. Water deficit caused the reduction of protocatechuic, caffeic, and sinapic acid in all inbreds evaluated. However, the highly pronounced increase of ferulic and especially cinnamic acid content under water deficit conditions indicated a possible crucial role of these secondary metabolites in preventing the harmful effects of water deficit stress, making them quite promising metabolic markers for maize breeding, as those in the well-watered condition were correlated to grain yield in water deficit conditions, allowing selection under optimal growth conditions.

**Keywords**: drought, grain yield, HPLC analysis, secondary metabolites, *Zea mays* L.

# Quantitative and qualitative changes in the yield of winter wheat with regard to the year of recognition

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**Summary**

The Variety List of the Republic of Croatia is an official list of varieties of plant species that are recognized on the territory of the Republic of Croatia. There are currently 100 registered varieties of wheat on the Variety List of the Republic of Croatia, which are recognized based on testing for Distinctness, Uniformity and Stability (DUS) and testing the value for cultivation or use (VCU). After the variety is deleted from the variety list, it is stored in the collection of the National Program for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture in the Republic of Croatia.

In this work, 42 winter wheat varieties known in the Republic of Croatia from 1905 to 2020 were conducted on quantitative and qualitative characteristics at the testing field of the Croatian Agency for Agriculture and Food in Osijek in the 2023/24 growing season. Of the tested varieties, 27 are registered in the Croatian database of plant genetic resources (CPRGD) and 15 varieties are registered in the Variety List of the Republic of Croatia.

The trial was sown with a seeder Winterstaiger in three replications on plot of 11.25 $m^2$ with a sowing rate of 200 grains/$m^2$. The average weight of 1000 grains was 30.5 grams, and it ranged from 22.8 grams to 41.2 grams. The average starch content was 64.56%, and it ranged from 58.8% to 68.6%. The protein content was on average 14.42%, and ranged from 12.2% to 18.3%. The average gluten content was 28.63%, and it ranged from 23.0% to 40.5%. The conducted parameters significantly varied among varieties according to the time of recognition of varieties.

**Keywords:** wheat, protein, starch, gluten, genetic resources

# Testing maize inbred lines and their crosses for resistance to fusarium ear rot

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**Summary**

Fusarium ear rot, caused by the pathogenic fungus *Fusarium verticillioides*, is one of the most significant diseases of maize. Its presence in maize reduces yield and quality due to contamination with mycotoxins, which poses a threat to human and animal health. The aim of this research is to compare the resistance of inbred lines and their crosses to this disease pathogen. Experiments were set up in a split-plot design in two replications, one with inbred lines, and the other with crosses. The main factor in the experiments was genotype (inbred line/cross), with treatment (natural and artificial infection) as a subfactor. ANOVA for infection rating in inbred lines showed a significant effect of line and treatment, while the line × treatment interaction was not significant. ANOVA for infection rating in crosses showed a significant effect of cross and treatment, while the cross × treatment interaction was not significant. Under natural conditions, an average infection rating of 1.92 was obtained, ranging from 1.28 (L4) to 3.30 (L11) for inbred lines. Artificial ear infection resulted in an average ear infection rating of 4.08, ranging from 2.76 (L15) to 6.27 (L11). For crosses under natural conditions, the average infection rating was 1.88, ranging from 1.35 (C5) to 2.41 (C4). Artificial infection resulted in an average ear infection rating of 3.67, ranging from 2.63 (C12) to 4.48 (C10). The correlation coefficient between ratings under natural and artificial infection for inbred lines was 0.86**, whereas for crosses, the correlation was not significant.

**Keywords**: maize, inbred line, cross, fusarium ear rot, *Fusarium verticillioides*

# Mapping of the invasive species *Ailanthus altissima* (Mill.) in the area of the Telaščica Nature Park

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**Summary**

A rapid spread of the invasive alien species *Ailanthus altissima* (Mill.) has been observed in the Telašćica Nature Park. Therefore, two field surveys were conducted in July 2023 on 17 surveyed plots (10 x 10 m) in two localities: 1. Vela Sestrica island and 2. Veli vrh (Smrčevica) in Čuh polje. The aim was to determine the number of female trees based on the presence of flowers/fruits. The number of trees with a girth less than and more than 20 cm, the height and diameter of the tree and the extrapolated number of female trees for the entire area were also made. The collected GPS coordinates of the species distribution were transferred to the geographic information system QGIS. In locality 1., nine plots were investigated, and the identified infested area is in the northwestern part of the island. The first female mother tree and a total of 21 female trees were found here. Two endemic species were recorded as part of the rock vegetation: *Campanula pyramidalis* and *Corydalis acaulis*. At locality 2., eight plots were investigated where *A. altissima* is part of the typical Mediterranean vegetation of dry grasslands developing on rocky soil. Two endemic taxa were recorded here: *Seseli tomentosum* and *S. montanum* subsp. *tommasinii*. Not a single female tree was observed here, so it is assumed that *A. altissima* spreads vegetatively. The infestation here is recent and no more than 15 years old, as inferred from the large number of young and low trees.On total area of 1700 $m^2$, 1094 individuals of *A. altissima* were recorded. The abundance of the species was estimated at 35.737 individuals on total area of all known sites at 44.367 $m^2$. The average density of occurrence of individuals is 0.66/$m^2$, and the estimated total number of female individuals of *A. altissima* is 299. The average density of occurrence of female individuals is 0.023/$m^2$. These results can therefore serve as a baseline for monitoring of the spread of the invasive species *A. altissima* in the Dugi otok area.

**Keywords**: Dugi otok, invasive species, Nature Park, GIS

# An automated remote monitoring system for endangered, protected, and indicator wildlife species in the Nature Park Kopački Rit

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**Summary**

The technology of bioacoustic remote monitoring represents an efficient and precise tool for tracking biodiversity on a broader scale. It is a non-invasive approach, that enables real-time species tracking, creating the possibility for swift intervention in biodiversity protection. Also, it enables the collection of a large amount of data and information on the presence and behavior of wild animal species without directly disturbing their natural habitat. By deploying offline sound recording devices (AudioMoth), video traps and standard monitoring methods, we will record, track, and analyze data, sounds and video footage of various wildlife species, including birds, amphibians, insects, mammals, and other taxonomic groups over three years. The goal of the project WatchOut is to monitor approximately 50 different species of wild flora and fauna within the Nature Park Kopački Rit. We are developing regional convolutional neural networks (CNN) for specific species to facilitate their long-term monitoring with a particular emphasis on endangered, protected, and indicator species. Through the analysis of spatial and temporal changes in the ecological state over the last decade, we will establish a framework for assessing the distribution of natural habitats and the stability of terrestrial, aquatic, and marsh ecosystems in Kopački Rit. The key to successful conservation lies in the establishment of an automated, cost-effective, and comprehensive remote monitoring system for species presence over time.

**Keywords**: bioacoustic monitoring, AudioMoth, remote sensing technology, Convolutional Neural Networks, stability of ecosystem

# Genotypic differences in nitrogen use efficiency of winter wheat

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**Summary**

An experiment with four nitrogen (N) fertilization levels added as topdressing, 0 kg N/ha (N0), 40 kg N/ha (N40), 80 kg N/ha (N80), and 120 kg N/ha (N120) and 25 winter wheat genotypes was carried out in six environments. The aim of the study was to determine the influence of N fertilization on N use efficiency for grain yield (NUEgy), N use efficiency for grain N yield (NUEgN), and their components, N uptake efficiency (NUpE), N utilization efficiency (NUtE) and N harvest index (NHI) of the tested genotypes. Analysis of variance revealed significant effects of environment (E) and genotype (G) for all traits and fertilization (F) for all traits except NHI. The F×E interaction was significant for NUE, NUpE and NUEgN, the G×E interaction for all traits except NUpE, and the G×F interaction for NUE and NUtE. Mean NUEgy decreased with increasing N fertilization level and was 46.4, 46.7, 36.1, and 32.3 kg of grain dry matter per kg available N, for fertilization levels N0, N40, N80, and N120, respectively. Mean NUEgN also decreased with increasing N fertilization level and was 0.78, 0.72, 0.68, and 0.65 kg grain N per kg available N for the same four N fertilization levels. The contribution of each component to NUEgy and NUEgN depended on the level of N fertilization, but in general NUtE contributed more to NUEgy, while NUpE was more important for NUEgN. Correlation coefficients between N fertilization levels varied from 0.77 to 0.90 for NUEgy and from 0.47 to 0.76 for NUEgN.

**Keywords**: wheat, nitrogen use efficiency, genotype, nitrogen fertilization

# C3 and C4 photosynthetic performance in cold stress

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**Summary**

A tendency for early sowing to avoid summer water and heat stresses from occurring during the most susceptible growth periods poses a considerable risk of cold stress for warm-season crops in Croatia, among which soybean and maize play a crucial role in the agricultural sector. To address the mentioned issues, a study was conducted investigating the difference between C3 and C4 photosynthetic performance and biomass reduction due to cold stress early in soybean (C3) and maize (C4) vegetative growth. The findings revealed significant low-temperature effect on the minimum fluorescence ($F_0$), the maximum fluorescence ($F_m$) and the maximum quantum yield of PSII photochemistry ($F_v/F_m$), with notable distinctions between C3 and C4 photosynthesis. Low temperatures had the most pronounced effect on the average performance index of overall photochemistry ($PI_{total}$). The only consistent significant difference between C3 and C4 photosynthesis was in $PI_{total}$. C4 $PI_{total}$ was higher than C3 $PI_{total}$ by 41.5%, 34.4% and 42.9% on day one, two and three in the optimal ambient conditions, and by 141.9%, 108.4%, 51.7% on day one, two and three of the imposed low-temperature treatment respectively. Although initially, C3 $PI_{total}$ decreased more compared to C4 $PI_{total}$ as a result of cold stress, at the end of the low-temperature treatment the difference equalized. In conclusion, C4 maize had higher ability for energy conservation than C3 soybean, but the ability for energy conservation in C3 soybean was more resilient to low temperatures.

**Keywords**: $PI_{total}$, photosynthesis, soybean, maize, climate change

# Portable spectrometer-based cold stress detection in C3 and C4 plants

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**Summary**

The early detection of stress in crop plants is crucial yet challenging. The exploration of stress responses through advanced non-destructive techniques becomes instrumental in identifying stress indicators. In the context of C3 and C4 plants, understanding their unique responses to stress is crucial for devising targeted strategies. The primary focus of this work is to identify cold stress in soybean (a C3 plant) and maize (a C4 plant) to uncover specific indicators that differentiate the stress responses of C3 and C4 plants. Portable hyperspectral sensor devices were used as a non-destructive and high-throughput approach to detect stress-related processes in the early stages, often invisible to the human eye. The plant experiment was set up in growth chamber in fully controlled conditions and included 12 soybean cultivars and 14 maize parental lines owned by Agricultural Institute Osijek. Numerous vegetation indices were associated with the stress response. Phytochemical reflectance index (PRI) and structure insensitive pigment index (SIPI) were recognized as general cold stress response indicators in both crops. The Zarco-Tejada and Miller Index (ZMI) has demonstrated its efficacy as a stress indicator in maize but not in soybean. Conversely, for soybean, the greenness index (G) has emerged as a specific stress indicator. Identifying, quantifying, and capturing stress responses in early stages of plant growth holds significant advantages for both breeding and crop management.

**Keywords**: cold stress, C3 and C4 plants, stress detection, maize, soybean

# Quality of winter wheat in post-registration trials in the 2022/2023 growing season

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**Summary**

In the Republic of Croatia, winter wheat occupies the largest area, and in 2021, 147,390 ha were harvested. The aim of the research was to show yield and quality parameters; protein content, sedimentation value according to Zeleny, gluten content and starch content in winter wheat grains of post-registration trials. Quality parameters were determined using the NIR FOSS INFRATEC ™ 1241 Grain device. The trial was set up according to a random block layout at the Osijek location (N 45°52´, E 18°67´), and consisted of 31 genotypes in three repetitions with the application of standard agricultural techniques for winter wheat. According to the conducted research, the yield of the researched genotypes ranged from 5.1 t/ha to 9.3 t/ha with an average value of 7.5 t/ha. The highest average yield was achieved by genotype 10, which originates from a foreign breeding program. Regarding the quality parameters, the gluten content ranged from 20.7% to 31.4% with an average value of 26.1%, and the sedimentation value from 24.5 to 58 with an average value of 41.3. For these parameters, the highest value was achieved by domestic genotype 3. The protein content in wheat grain ranged from 10.8% to 14.6% with an average value of 13%, and the highest content was achieved by genotype 1. In accordance with the Ordinance on Quality Parameters and Qualitative Classes, 7 genotypes (22.5%) belong to the first class, 17 genotypes (55%) to the second class, and 7 genotypes (22.5%) to the third class. It can be seen that the largest number of genotypes for the 2022/2023 season belongs to second class.

**Keywords**: wheat, post-registration, protein, sedimentation value, quality

# Use of molecular methods during the certification procedure: cereals and brassicas example

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**Summary**

Seed certification procedure at import and/or during the growing season of material for seed production raises the problem of identifying varieties of atypical plants, especially in cereals and brassicas. The usefulness of genetic identification is demonstrated in the analysis of seed quality, where morphological characteristics of the seed or plant can cause ambiguity in varietal purity and germination testing, leading to uncertainty about plant variety, species and even genus level. There are some practical applications using different molecular tools to successfully distinguish: I) different phenotypes within specific barley variety; II) among different wheat varieties; III) between wheat, rye and triticale; IV) authenticity of seed phenotypes for targeted oat variety; V) among oat and naked oat seed phenotypes; VI) among rapeseed and oilseed rape; VII) between oilseed rape and white mustard seed phenotypes; VIII) authenticity of phenotypes within selected buckwheat variety. In these cases, genetic comparisons were carried out at the binary and/or co-dominant level, depending on the relatedness of genotypes and the polymorphisms of molecular markers.

An implementation of the molecular approach in certification process provides a fast, efficient, reliable and financially acceptable tool for the inspection service, ministries, farmers, seed importers and breeders themselves.

**Keywords**: molecular markers, genetic distinguishing, related species, authenticity, certification service

# Correlations of micro and macro elements in wheatgrass juice and powder

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**Summary**

Wheatgrass represents wheat plants at the end of the tillering stage, commonly used as a natural dietary supplement in the form of fresh juice or powder. Wheatgrass leaves have a complex nutritional composition, and among other nutrients, they are rich in micro and macro elements. The aim of this research was to evaluate correlations between different micro and macro elements as well as correlations between total and *in vitro* bioavailable concentrations of examined elements in wheatgrass juice and powder. In general, the highest significant positive correlations were found between total Mg and total Ca concentration in juice and powder. In juice, a correlation between total and *in vitro* bioavailable concentrations of Se is significant, positive, and high, while a correlation between total and *in vitro* bioavailable concentrations of Zn is low. Correlations between total and *in vitro* bioavailable concentrations of all elements are lower in powder than in juice (the exception is K) indicating higher bioavailability in juice than in powder. Also, the bioavailability of Zn is the lowest among other elements indicating that Zn bioavailability is under the effect of different factors and not only the total concentration of Zn. Correlations between elements are complex, and further research to elucidate those connections should be done.

Keywords: wheatgrass, *in vitro* bioavailability, correlation, biofortification, microelements

# The application of genomic predictive modeling of combining abilities in the selection of maize breeding lines

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**Summary**

The use of genomic data in the predictive modeling of maize yields has already become state-of-the-art in the breeding industry. Nitrogen (N) fertilization is a key component of maize agronomic practice, but N depletion can limit yields, especially in low-input agricultural areas of Southeast Europe. There is a significant variability in maize responses to N, with some genotypes showing break-points in agronomic performance at lower N doses. In addition, limiting N fertilization could have beneficial effects on the agroecosystem, including preventing nitrate leakage and reducing greenhouse gas emissions while retaining agronomic performance. In this research, our aim is to construct a model for genomic prediction of nitrogen use efficiency using genotypic and phenotypic data and to identify the best-performing genotypes. We test-crossed 120 densely genotyped inbred lines of dent maize with two testers from the Iodent and B37 heterotic groups. The inbreds were genotyped using the Illumina Infinium Maize SNP50K array, resulting in ~49,000 filtered (MAF <0.05, missing <0.05) and imputed positions for further analysis. 207 hybrids were phenotyped in field trials in 2023 with three levels of N fertilization, 0, 69 and 136 kg N/ha. Our results promise to contribute to the development of sustainable maize production systems that balance high yields with environmental and economic considerations.

**Keywords:** maize genomics, predictive modeling, quantitative genetics, maize breeding

# Hyperspectral responses of maize inbred lines to water withholding

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**Summary**

Maize is an important agricultural crop that is vulnerable to drought stress. To assess plant's performance and tolerance to harsh conditions, a proper set of tools is required. Precise phenotyping offers an insight into current physiological state of plants, producing quick and precise results. In this research one such high-throughput phenotyping method, hyperspectral imaging, has been used with the aim of assessing difference in response to drought stress in different maize inbred lines in the early stages of its growth and development. 168 maize inbred lines were grown in growth chamber with controlled conditions and measurements were taken several times throughout the growing period of 28 days by two PolyPen RP 410 handheld spectrometers with spectral response half width of 8nm (380-790nm and 640-1050nm). The treatment was conducted by withholding water 10 days after sowing. Preliminary results have shown a difference in hyperspectral responses between the treatment and the control and also between different genotypes. The different responses linked to different genetic structure of specific inbred lines serve as an identifying characteristic for the given trait and offer a valuable tool in creating a model for prediction of production under drought stress conditions. This information may prove to be of great value for breeders and producers, allowing them to plan the production in a more efficient manner with better allocation of their resources and fewer losses.

**Keywords:** maize, phenotyping, hyperspectral, drought, stress

# Monitoring drought impact on spectral indices in legumes

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**Summary**

The spectral reflectance of the leaves of large-seeded legumes (*Phaseolus vulgaris* L., *Pisum sativum* L., *Vicia faba* L., *Cicer arietinum* L. and *Vignia unguiculata* L.) was evaluated in order to investigate whether plant leaf reflectance responses to stress depending on the intensity and length of the drought. The research was conducted under controlled conditions with consistent temperature and humidity to rule out any other stressors. Fundamental indicators of drought intensity were relative water content (RWC), stomatal conductance, and water binding pressure in the soil. Reflectance at visible wavelengths increased consistently in the leaves of stressed plants after six days of drought due to reduced pigment absorption. Visible reflectance was particularly sensitive to stress in the wavelength range of 460 to 750 nm. Infrared radiation reflection was comparatively insensitive to stress. Therefore, the most reliable indicator of plant stress was reflectance at visible wavelengths. Stress reactions in visible reflections were very comparable across species. In all species, the Flavonol Reflectance Index (FRI), Chlorophyll Green (CLG), and Chlorophyll Index RedEdge 710 (CLR) were on a consistent pattern. It was also shown that there was an increase in the ratio of carotenoids to chlorophyll, as well as in the concentration of flavonoids and carotenoids.

**Keywords**: Legumes, drought, spectral reflectance

# LiveSeeding project - Insights into the opportunities and bottlenecks in organic seed and plant breeding sector

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**Summary**

While European legislation already requires the use of organic seeds in organic agriculture, the continued granting of derogations for non-organic seeds in the organic sector discourages entrepreneurship in organic seed. European Union has set ambitious targets for the organic seed sector in the Farm2Fork Strategy and EU organic regulation, and to reach 100 % organic seed use in organic farming by 2036, a six-fold increase in the production of organic seeds in the next 10 years is needed. LiveSeeding project is a Horizon Europe Innovation Action that brings together leading organisations in the sector with the aim of fostering the growth of the organic sector and transition towards more sustainable local food systems by delivering high quality organic seed of diverse cultivars adjusted to organic farming for a wide range of crops. However, organic seed and plant breeding sector is facing many challenges from the technical (e.g., yields, land, infrastructure), economic (e.g., seed and certification cost) and regulatory (e.g., derogations, poor interaction with policymakers) point of view. These challenges are discouraging for seed enterprises willing to increase their efforts and engagement in the organic seed production and breeding. As part of the LiveSeeding project, IPS Konzalting has conducted Survey on the European organic seed and breeding sector to detect technical, economic and regulatory challenges that organic seed producers face, and to analyse bottlenecks and opportunities of the organic seed and cultivar testing network market. This survey was widely distributed among relevant stakeholders in Europe, and it serves as a starting point for the assessment of the obstacles that block the horizontal proliferation (scale-out) and scale-up of the organic seed and cultivar testing. The survey will provide potential solutions for development of business models and plans for SMEs to boost horizontal proliferation, scaling-up and consequently profitability of the organic seed and breeding sector, including cultivar testing.

**Keywords:** organic seed, organic plant breeding, organic agriculture, sustainable local food systems, LiveSeeding project

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59
Croatian
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19
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# Book of Abstracts

Vegetable Growing, Ornamental, Medicinal and Aromatic Plants

59
Hrvatski
19
Međunarodni
Simpozij
Agronoma

# Zbornik sažetaka

Povrćarstvo, ukrasno, ljekovito i aromatično bilje

# LIFE OrnamentalIAS - new project in Croatia

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**Summary**

This paper presents the new LIFE project OrnamentalIAS - Prevention and Management of Adverse Impact of Ornamental Invasive Alien Plant Species on Endangered Habitat Types and Species of EU Importance. The project will be implemented in cooperation with 10 partners from Slovenia and Croatia over six years (2023-2029). University of Zagreb Faculty of Agriculture and Zeleni Prsten are responsible for the activities in Croatia. The remaining eight partners are responsible for the activities in Slovenia (Zavod Republike Slovenije za varstvo narave, RA SORA, Slovenian Water Agency, KGZ Slo, KGZS-Zavod Celje, Notranjski regijski park, Arboretum Volčji potok, Simbio). The main objectives of the project are to limit the spread of ornamental invasive alien plant species (IAS) from cultivated and urban areas into endangered natural habitats, to develop adapted techniques for the disposal of IAS residues and to test their potential for circular economy, to improve and establish an Early Warning and Rapid Response system (EWRR) for water management and agriculture, and to improve the conservation status of species and habitats of EU importance. This project will influence the perception of invasive ornamental plants and increase the interest of the general public and professionals in planting non-invasive alternatives to prevent the introduction of IAS into natural habitats through long-term cooperation between Slovenian and Croatian partners.

**Keywords**: invasive alien plant species, ornamental species, non-invasive alternatives, circular economy

# Biological activity and biflavone content of ginkgo (*Ginkgo biloba* L.) – ornamental, medicinal and food plant

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**Summary**

Ginkgo (*Ginkgo biloba* L.) is known in our region mainly as an ornamental tree with its characteristic yellow leaves in autumn. Its young green leaf extract is used worldwide to improve cognitive functions, and in Asian countries ginkgo seeds are used in gastronomy. Here, we investigated its biological activity, antioxidant activity, and inhibition of acetylcholinesterase and tyrosinase. Inhibition of acetylcholinesterase has been associated with a possible beneficial effect on neurological diseases such as Alzheimer's disease, while inhibition of tyrosinase may prevent melanogenesis. We also determined the biflavone content in different Ginkgo samples by HPLC-DAD. Yellow leaves showed stronger biological activities than green leaves. In samples of ginkgo leaves, we identified five biflavones - amentoflavone, bilobetin, isoginkgetin, ginkgetin, and sciadopitysin, with sciadopitysin being the most abundant (2.4 mg/g dw). In contrast to the leaves, we did not detect biflavonoids in the seeds, which are used as food, but the seeds showed tyrosinase inhibitory activity. The biological activity and content of biflavones in ginkgo depend on the type of tissue studied. For the use of ginkgo in medicine, we should choose the right type of tissue and harvest the plant at the stage when it accumulates the most bioactive ingredients.

**Keywords**: ginkgo, biflavonoids, enzyme inhibition, HPLC-DAD, sciadopitysin

# *Amanita muscaria* (L.) Lam. - a mushroom with unused pharmacological potential

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**Summary**

*Amanita muscaria* (L.) Lam. a mushroom better known as the red fly agaric, is usually stigmatized in the literature as a (deadly) poisonous mushroom. *A. muscaria* is an ectomycorrhizal mushroom that belongs to the division Basidiomycota, to the order Agaricales and to the family Amanitaceae. The diameter of the mushroom cap is usually between 4 and 21 cm, but can reach 50 cm. It is characterized by convex to flat shapes and usually has a bright red color with white spots. This visually striking mushroom possesses a unique chemical composition, mainly featuring ibotenic acid and muscimol which are isoxazole compounds that have been utilized for their psychoactive effects over millennia alongside many other compounds contained within this mushroom, most of which hold potential for pharmaceutical applications. There are numerous historical records and anecdotes of the beneficial effects of *A. muscaria* species nowadays, particularly in the area of alleviating anxiety, pain, inflammation, and mitigating chronic neurodegenerative diseases such as Parkinson's and Alzheimer's disease, as well as the effects of traumatic experiences. Research on ectomycorrhizal mushrooms is complex because it is technically impossible to fully mimic the complexity of plant-fungal interactions in order to grow the fungus in the laboratory. However, as the biology and ecology of these microorganisms continue to be explored and documented, new insights into the complexity of symbiotic interactions between plants and fungi are emerging. In addition, with the development of new techniques, new opportunities for laboratory cultivation of mycorrhizal mushrooms are emerging that could potentially enable the production of extremely valuable compounds.

**Keywords:** Fly agaric, ethnomycology, pharmacology, ibotenic acid, muscimol

# Collecting and preservation of *Brassica incana* Ten. accessions from south Croatia

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**Summary**

*Brassica* genus is a key genus in the *Brassicaceae* family, and it comprises many of the economically significant species of vegetable, condiment and oilseed crops. It is believed that *Brassica* crops have originate from the Mediterranean area. There are several wild *Brassica* species native to the Mediterranean area, one of them being *Brassica incana* Ten. which is a member of the Croatian flora. This crop wild relative potentially has agronomically useful traits, such as biotic and abiotic stress resistance. This work describes two collecting missions set on southern Croatian islands and islets to collect *B. incana* for long-term conservation. These missions were also an opportunity to monitor the population status of previously mentioned populations. During the first collecting mission, the loss of two previously recorded habitats due to anthropogenic influences was found. Some of the collected seeds and/or vegetative organs were planted in the experimental greenhouse of the Institute of Agriculture and Tourism, Poreč, Croatia. Morphological description of successfully grown *B. incana* accessions was carried out. Monitoring, collecting, preserving and multiplying this important genetic resource is of great value for future breeding programs and the ever-increasing demands of food production.

**Keywords**: crop wild relative, cabbage, genetic resources, biodiversity, conservation

# Effects of deficit irrigation on ornamental traits of *Salvia farinacea* Benth.

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**Summary**

The aim of this work was to determine the influence of three irrigation treatments (100%, 80% and 60% of field water capacity (FWC)) on morphological parameters and flowering of *Salvia farinacea* Benth. plants. Deficit irrigation treatments (60% and 80% FWC) reduced stem length, leaf length and width, peduncle length, inflorescence length, the number of secondary branches with inflorescence bud, and, in the 60% FWC treatment, stem diameter and the number of primary branches with inflorescence bud. Plants grown under deficit irrigation also showed reduction of inflorescence length and inflorescence stem length. Although the first open flowers appeared at the same day in all treatments, moment when 50% of the plants had open flowers in both variants of deficit irrigation was slightly delayed, specifically two days later than in the control variant. Despite the fact that reduction of the inflorescence length and the number of branches with inflorescence is an undesirable effect, the less irrigated plants, due to their smaller height, had a more compact and balanced habit. The results suggest that deficit irrigation can be applied to *S. farinacea* production as an alternative or supplementary method of height control and used to produce plants of commercially satisfactory quality with less water consumption.

**Keywords**: Mealycup sage, reduced irrigation, morphological characteristics, flowering, height control

# Changes in phenolic content and antioxidant capacity during processing of black garlic (*Allium sativum* L.)

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**Summary**

Black garlic is a form of regular garlic that undergoes a unique aging process, resulting in a distinct black color, sweet taste, and soft texture. The transformation occurs through a slow and controlled thermal process over several weeks, during which the garlic's oligosaccharides break down and react with amino acids into new compounds (Maillard reaction), such as melanoidins, responsible for the dark hue. Despite its name, black garlic does not have the pungent odor associated with fresh garlic. Instead, it boasts a rich, molasses-like sweetness with subtle umami undertones. Widely appreciated for its complex flavor profile and potential health benefits, black garlic has gained popularity. Black garlic exhibits higher antioxidant activity compared to fresh garlic due to the process that transforms its chemical composition. The aging process enhances the presence of antioxidants, making black garlic a potent source of free-radical-fighting compounds when compared to its raw counterpart. In this work, fresh garlic was treated with elevated temperature (75$^0$C) and constant humidity for 40 days to produce a new product – black garlic. Total phenolic content and antioxidant capacity measured by Ferric Reducing Antioxidant Power as well as the reduction of 2,2-diphenyl-1-picrylhydrazyl radical was evaluated at the beginning, during and after the thermal process. The total phenolic content significantly increased during the course of the process with the final product having over 10 times the phenolic content compared to the initial value. Similarly, antioxidant capacity also significantly increased in both antioxidant tests during the thermal process yielding an increase in antioxidant capacity over 100 times compared to fresh garlic.

**Keywords**: FRAP, DPPH, aging, Maillard reaction, bioactive compounds

# Non-native plant species in the Croatian nursery production

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**Summary**

This paper analyzed the characteristics of the nursery production of horticultural material in Croatia with the aim of determining the extent of the diversity i.e. the ratio of autochthonous and non-native as well as invasive plants in the production process. A total of 729 taxa from 11 nurseries from the coastal and continental part of Croatia were analyzed. The production structure was analyzed. It was determined that in the total offer of nurseries in the coastal part of Croatia, 15.6% are autochthonous, 84.4% are allochthonous, of which 1.3% are invasive plant species. In the continental part, 20% are autochthonous, 80% allochthonous, of which 4.3% are invasive species. It is necessary to accentuate presence of certain invasive plant species in Croatian nurseries, which is not in compliance with legislative frame: *Acer negundo* L., *Robinia pseudoacacia* L., *Oxalis pes-caprae* L., *Parthenocissus quinquefolia* (L.) Planchon, *Carpobrotus edulis* (L.) N.E.No. in Phillips, *Bidens frondosa* L., *Broussonetia papyrifera* (L.) Vent. and non-native species *Pistia stratiotes* L. which is listed as quarantine pest according to EPPO, and in Croatia recorded since 2021. We conclude that Croatian nursery production characterize higher proportion of non-native plant species compared to autochthonous ones, and that indigenousness should be encouraged in Croatian plants production.

**Keywords:** nursery production, Croatia, non-native plants, autochthonous plants, invasive plants

# Assessing leaf functional traits of *Salvia farinacea* Benth. under deficit irrigation

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**Summary**

The aim of this research was to determine the effects of deficit irrigation on the anatomical and physiological characteristics of leaves of *Salvia farinacea* Benth. (mealycup sage) transplants. Greenhouse-grown *S. farinacea* plants were subjected to three irrigation treatments, a control (irrigation to container capacity) and two deficit irrigation treatments (80% and 60% of the control), and analyzed at the time of beginning of flowering. In plants grown under deficit irrigation, leaves showed a decrease in the thickness of the adaxial epidermis and an increase in the thickness of the palisade parenchyma. Stomatal density did not differ between the treatments, but water deficit led to a decrease in stomatal size, on both sides of the leaf. The antioxidant activity measured by ABTS assay did not differ between the treatments, but ferric reducing antioxidant power (FRAP) decreased in plants irrigated to 80% of container capacity, as compared to the control. The content of total phenolic compounds decreased in both deficit irrigation treatments. Water deficit conditions also caused a reduction in the content of photosynthetic pigments, while the chlorophyll content index increased. The chlorophyll fluorescence parameter $F_v/F_m$ slightly decreased with increasing water deficit, yet average values remained above 0.80 in all treatments.

**Keywords**: mealycup sage, water deficit, leaf anatomy, stomata, antioxidant activity

# Se content in fresh edible parts of vegetables as a result of Se-enriched irrigation

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**Summary**

The effect of Se-enriched irrigation water was studied on six different vegetable plants in a greenhouse experiment. The investigated plants were cabbage, potato, tomato, bean, pea and carrot. The treatments included 0, 100 and 500 μg $L^{-1}$ Se concentration in the irrigation water in the form of Na-selenate. The aim of the experiment was to investigate the possible agricultural biofortification of vegetable plants with Se and test the usability of irrigation water with a high Se content.

One aspect of the evaluation may be the amount of Se in 100 g of fresh edible vegetable part. The 100 μg $L^{-1}$ treatment resulted in almost four times the recommended daily intake of 55 μg of Se in 100 g of fresh cabbage head and green pea seed, however, in the case of dwarf french beans, potatoes, tomatoes and carrots, the edible parts contained Se corresponding to 104, 78, 67 and 92%, respectively, of the recommended daily intake. As a result of the 500 μg $L^{-1}$ treatment, the amount of Se measured in 100 g of fresh edible parts of all vegetables far exceeded the recommended daily intake, and in the case of cabbage and green peas, it exceeded the daily tolerable upper intake level of 450 μg by almost three times.

Based on the results, the enrichment of irrigation water with Se could be a possible method for agronomic biofortification of vegetable crops, but the Se concentration must be chosen carefully, because there is a large difference between crop species.

**Keywords**: irrigation, biofortification, selenium, recommended daily intake, vegetables

**Acknowledgements:** This work was funded by the National Research Development and Innovation Office, project number: 2021-1.2.4-TÉT-2021-00035 and NVKP_16-1-2016-0044.

# Impact of cultivation technology on the glucosinolate profile of kale microgreens (*Brassica oleracea* L. var. *acephala*)

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**Summary**

Glucosinolates are sulfur-containing compounds found in plants of the Brassicaceae family (cruciferous vegetables). The main role of glucosinolates in the plant is participating in the defense against biotic and abiotic stress, while these compounds also have significant health benefits for humans. The glucosinolate profile refers to the composition and diversity of glucosinolates present in the plant. Their concentration can vary depending on the variety, age, environmental factors, and plant cultivation technology. Kale microgreens represent the early growth stage of kale (produced in a short time frame) known for an intense taste similar to mature vegetables with increased concentrations of nutrients and glucosinolates. This research was conducted as a two-factor experiment to determine the impact of harvest time and mechanical stress on the glucosinolate profile, identifying and quantifying different compounds present in kale microgreens. Detection of the glucosinolate profile was carried out using liquid chromatography in tandem with mass spectrometry (LC-MS). Fourteen glucosinolates were detected in kale microgreens, with sinigrin, glucobrassicin, and glucoiberin being the most prevalent. In early and mid-harvest compared to late harvest, 10 out of the 14 detected glucosinolates showed significantly higher concentrations. Mechanical stress treatment showed a significant effect on the concentration of glucoiberin and glucoraphanin, while the interaction between harvest time and stress significantly influenced the concentration of neoglucobrassicin. The determined concentrations of glucosinolates in kale microgreens exhibit good potential for their valorization as a food product with added value for human health.

**Keywords:** bioactive compounds, cruciferous vegetables, LC-MS, microgreens, secondary metabolites

# Influence of microalgae microparticles on the chemical composition of tomato fruits in hydroponic cultivation

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**Summary**

Hydroponic cultivation is increasingly used in Croatia, due to better management of factors during the growth and development of plants with the aim to obtain fruits of higher quality, higher yields and a longer harvest period, so that fresh tomatoes can be offered throughout the year. To reduce the use of mineral fertilizers and pesticides, it is necessary to offer a product that provides nutrients to the plant throughout the growing season. One of the methods to achieve this goal is encapsulation, which allows to protect active substances such as nutrients and microorganisms from external influences and the controlled release these substances over time. The objective of this research was to determine the effect of liquid microalgae substrate, calcium ion carrier microparticles, and microalgae carrier microparticles on yield, total carotenoid and total polyphenol content, and antioxidant activity of tomato fruit grown hydroponically. The treatments were applied in the cultivation of three tomato cultivars: 'Fiorentino', 'NUN 09231 TOF' and 'Zagrebačka rana'. The results showed a positive influence of the microparticles on 23% higher yield, but also on the chemical composition of tomato fruits (50% increase in lycopene and polyphenol content).

**Keywords:** *Solanum lycopersicum* L., encapsulation, lycopene, polyphenols

# The impact of biostimulants application during vegetation on pepper seeds germination

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**Summary**

Pepper (*Capsicum annuum* L.) is an annual herbaceous plant of the Solanaceae family, which is one of the most important agricultural crops due to its exceptional economic importance, the nutritional value of its fruits and its confirmed antioxidant activity. The aim of this work was to determine the influence of the application of biostimulants during the growing season on the germination of pepper seeds. During the 2019 growing season, two pepper cultivars (bell peper - Šorokšari and horn pepper - Kurtovska kapija) were treated with the biostimulants Eco Green and Zeogreen. At the end of the growing season, 300 seeds from each cultivar were collected (100 seeds from plants treated with each of the two biostimulants and 100 seeds from plants that had not been treated during the growing season - control) and placed for germination under controlled conditions at a constant temperature of 25 $^{0}$C and a light regime of 16/8 h day/night. At the end of the experimental period, the seed germination parameters were calculated. It was found that the application of the Eco Green biostimulator had a positive effect on the germinability, mean germination time (day), germination index, uncertainty of the germination process and mean germination rate, while there was no statistically significant positive effect on the germination parameters when treated with the Zeogreen biostimulator.

**Keywords**: *Capsicum annuum,* cultivar, Eco Green, germination parameters, Zeogreen

59 Croatian 2024 19 International Symposium on Agriculture

Session 5

# Book of Abstracts

Crop Production

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Ratarstvo

# Influence of seeding density and sowing date on agronomic properties of *Camelina sativa* L.

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**Summary**

Camelina or false flax is an ancient oilseed crop belonging to the Brassica family. Recently, there has been renewed interest in the cultivation of camelina due to the unique value of the oil, which can be used for human nutrition and as livestock feed, but also due to its adaptability to a wide range of environments. The aim of this study was therefore to determine the influence of sowing date and seeding density on seed yield, oil yield, and yield components (number of siliques per plant, number of fertile branches per plant, number of seeds per silique and 1000 seed weight) of camelina under agro-ecological conditions in north-western Croatia. The trial was conducted in 2023 on an experimental field at the University of Zagreb Faculty of Agriculture. The field trial was carried out according to the split-block design in three replicates. The trial included two sowing dates: March 23, 2023 and April 19, 2023, and three seeding densities: 250 germinable seeds/$m^2$, 500 germinable seeds/$m^2$ and 750 germinable seeds/$m^2$. The results showed that both sowing dates and different seeding densities had a significant influence on seed yield, oil yield, and number of siliques per plant. In addition to these traits, sowing density also influenced the number of fertile branches per plant. An earlier sowing date resulted in a higher seed yield, a higher number of siliques per plant, and a higher oil yield. Sowing densities between 500 and 750 germinable seeds/$m^2$ achieved the highest seed and oil yield, but also significantly decreased the number of siliques per plant and the number of fertile branches. The obtained results lead to the conclusion that under our conditions an earlier sowing date and sowing densities between 500 and 750 germinable seeds/$m^2$ can be recommended for a high seed and oil yield.

**Keywords**: camelina, sowing dates, seeding density, seed yield, oil yield

# The influence of the environment on the yield and quality of sugar beet in VCU trials

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**Summary**

Official variety trials are set up to test the value for cultivation and use (VCU trials) for the purpose of registration varieties. In accordance with the Ordinance on the registration of varieties of agricultural plants (Official Gazette 99/08, 100/09, 109/10, 77/13 and 57/18), the following data are determined for sugar beet varieties during the growing season, among others: sowing and emergence dates, date of leaf appearance; severity of attack by leaf spot disease (*Cercospora beticola*); the number of early and late bolters and the lifting date. During lifting, the following are determined on each plot: root weight (gross and net), sugar content, potassium, sodium and amino nitrogen content. Data were used in the study from VCU sugar beet trials in the growing years 2018, 2019, 2020, 2021 and 2022 at the locations Tovarnik, Osijek, Beli Manastir, Virovitica and Kutjevo. The trials were arranged in a randomized complete block design with four replications.Objective of this study was to examine the influence of precipitation on root weight, sugar, potassium, sodium and amino-nitrogen content and to express it with correlation coefficients. No statistically significant correlation was found between precipitation and roots weight, content of sugar, potassium, sodium and amino-nitrogen. Further comprehensive analyses of sugar beet VCU trials should be performed to see the effects of air temperature and other agronomic factors on the mentioned properties.

**Keywords**: sugar beet, VCU trials, yield, content of sugar, potassium, sodium and amino-nitrogen

# Effect of period of application and amount of nitrogen fertilizer on the protein content of wheat grain in vegetation 2022/2023 year

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**Summary**

The area under winter wheat occupies the largest share in the Republic of Croatia. Given that we are witnessing large fluctuations in the price of mineral fertilizers, it is extremely important to apply the most optimal supplement with nitrogen fertilizer in order to optimize the costs of agricultural production. The aim of the research was to determine which method of applying nitrogen fertilizers gives the best results in terms of yield and protein quality in winter wheat grains. In the growing season 2022/2023. field research was conducted on the yield and seed quality of winter wheat with different periods and amounts of Nitrogen fertilization application. The experiment was set up according to RCBD schema in CAAF Osijek (N 45°52′, E 18°67′) with seven variants of nitrogen fertilizer application in four repetitions with the applied of standard agrotechnics for winter wheat. The results of the research showed that there is a statistically significant difference at the $P<0.05$ level in the yield of treatment 1 (control - 150 kg/ha of KAN in tillering) compared to all other treatments. The protein content in wheat grain ranged from 14.68 in variants 1 and 4 to 15.20 in variant 3, but there was no statistically significant difference between the treatments. The sedimentation value and starch content are responsible for the quality of the bakery strains, and it should be noted that they were the worst in variant 1 (control). The obtained research results will contribute to the correct selection of the time and amount of nitrogen fertilizer application on winter wheat at the Osijek location.

**Keywords**: wheat, yield, protein, starch, nitrogen fertilizer

# Evaluating some morphological traits of 3 chickpea varieties under rainfed conditions in Debrecen, Hungary

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**Summary**

Chickpea (*Cicer arietinum* L.) is one of the main legumes produced under several climatic conditions due to the high content of proteins, in addition to the amino acid composition it has. As a legume, however, it is affected by drought stress, which is reported to continuously increase in several regions where chickpea is grown, leading to serious losses in yield. Thus, it would be of major importance to evaluate the response of this legume to episodes of drought stress. However, such research is lacking in many regions in Europe, with Hungary and Poland as some examples. Together with our partners in Poland, a study was conducted to evaluate the influence of rainfed cultivation of newly sown chickpea genotypes in Debrecen, Hungary. Three chickpea genotypes were sown in a randomized complete block design with 4 replicates. Half of the seeds of each genotype were inoculated and compared to a non-inoculated control. Results showed that number of branches, plant height and flower number was higher in the inoculated plants in all 3 genotypes as compared to the non-inoculated counterparts. However, inoculation had no significant effect on the root volume and length of any of the studied genotypes. Results also revealed significant differences among the 3 genotypes in terms of the studied traits, regardless of inoculation. It could be concluded that inoculation is recommended in the studied area as a supporting treatment for a better morphology of the studied chickpea genotypes under the current rainfed conditions in the study area. Future research will use these findings to evaluate the effects of 2 major abiotic stresses; namely, drought and salinity stresses, on the physiological and the biochemical traits of chickpea, and their probable correlation with the final seed yield and seed composition under the agroclimatic conditions in both locations of Poland and Hungary.

**Keywords**: agroclimatic conditions, *Cicer arietinum*, drought stress, inoculation.

**Acknowledgment:** This research was funded by National Research, Development and Innovation Fund of Hungary, grant number TKP2021-NKTA-32, financed under the TKP2021-NKTA funding scheme. This study was also supported by the National Research, Development and Innovation Office of Hungary under grant: RRF-2.3.1-21-2022-00008.

# Agronomic properties of brown mustard (*Brassica juncea* L.) in dependency on seeding density

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**Summary**

Brown mustard seeds are commonly used as a spicy condiment and base material for producing spicy hotdog mustard. Besides this use, it can also be cultivated as an alternative spring oilseed crop. The aim of this study was therefore to determine the influence of seeding density on seed yield and yield components of brown mustard, as well as oil yield and fatty acid composition under agroecological conditions in the north-west of the Republic of Croatia. Four seeding densities were included in the trial: 50, 70, 90 and 110 germinable seeds/m$^2$. The field trial was conducted on the experimental field of the University of Zagreb Faculty of Agriculture during one growing season. Significant differences were found among the seeding density regarding seed yield, yield components (plant height, number of fertile branches and siliques) and oil yield. The average oil content varied from 30.95% in the seeding density of 70 seeds/m$^2$ to 34.01% in the seeding density of 90 seeds/m$^2$. The oil was dominated by erucic acid (22.4%), oleic acid (19.05%), linoleic acid (22.9%) and linolenic acid (13.2%). Thus, according to the achieved results, seeding densities between 90 and 110 seeds/m$^2$ can be recommended under our conditions to obtain a high seed and oil yield. As far as the fatty acid composition is concerned, the oil extracted from brown mustard seeds is not suitable as an edible oil because of its high erucic acid content. Still, it is well-suitable for industrial purposes.

**Keywords**: brown mustard, seeding density, seed yield, oil yield, fatty acid composition

# Yield and protein content of spring barley as affected by nitrogen topdressing

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**Summary**

Spring barley malting variety (Quench) and its response to environmental conditions and topdressing were investigated in 2-years experiment (2021 – 2023). The aim of this study was to determine the yield and protein content of spring barley by applying different amounts of nitrogen as topdressing in order to investigate their effect on the achievement of maximum yields and protein content in 3 locations in continental Croatia (Zagreb, Kutjevo and Osijek). The study was set up according to a randomized complete block design in 4 replication and included 3 variants of topdressing with nitrogen and control: $N_0$ = control without topdressing, $N_1$ = 40, $N_2$ = 80 and $N_3$ = 120 kg/ha N. Applied in the form of calcium ammonium nitrate (KAN – 27% N) at the stage of tillering of barley plants. It is not recommended to surpass 120 kg/ha N as it offers no advantages to the plant and carries the potential for environmental harm. On all locations conventional and uniform agricultural techniques were applied. The study findings indicated that there was no significante difference in yield among the treatments. The highest yields of grain were achieved in Zagreb at $N_3$ rate; Kutjevo at $N_3$ and Osijek at $N_1$. However, there was a significant disparity in protein content among the different treatment groups, at a significance level of $P<0.05$. Highest being at $N_3$ rate an all locations. This result is expected as the amount of available nitrogen strongly influences grain yield and protein content. It has been noted that elevated nitrogen levels can lead to the transfer of an excessive amount of nitrogen from vegetative parts to grains, consequently leading to higher protein content. Further investigation is needed as to finding optimal nitrogen topdressing for acquiring quality and yield of spring barley, in respect to environment and economical viability.

**Keywords**: spring barley, nitrogen fertilization, yield, protein content

# Influence of sowing rate, nitrogen fertilization rate and harvesting phase on the productivity of teff

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**Summary**

The objective of the study is to determine the effect of the sowing rate, nitrogen fertilization and phase of harvesting on the yields of biomass and DM (dry matter) of teff (Eragrostis tef (Zucc.) Trotter). Teff is an annual wheat crop with ancient character. The experiment was conducted in the period 2021 - 2023 in the region of Central South Bulgaria. Three sowing rates - 10, 15, and 20 kg $ha^{-1}$ and four levels of nitrogen fertilization - 0, 30, 60, and 90 kg $ha^{-1}$ were studied in four replications. The yields of Teff biomass and dry matter (DM) were established in two phases of the development of teff: the milk stage and the dough stage. The results obtained about the yields have been processed with multifactor Univariate ANOVA analysis with the Post Hoc LSD test. Higher yields of dry matter are realized in the dough maturity phase. Fertilizing with increasing rates of nitrogen increased yields of green mass and dry matter. The highest productivity was found when fertilizing with 90 kg $ha^{-1}$ of nitrogen at all three sowing rates. The sowing rate of 15 kg $ha^{-1}$ was found to be the most appropriate for the conditions under which tef was grown. The yields of green mass and DM were influenced the most by the climate conditions of the experienced years and nitrogen rates ($p<0,001$), and a little by the sowing rates ($p<0,05$).

**Keywords**: *Eragrostis tef* (Zucc.) Trotter, productivity, nitrogen fertilization rate, sowing rate, harvesting phase

# Influence of nitrogen fertilization on production parameters and mycotoxin contamination of spring barley

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**Summary**

One of the ongoing tasks of the European Food Safety Agency is to evaluate potential hazards related to the occurrence of novel mycotoxins in food and feed. The objective of this study was to investigate the influence of soil fertility, as influenced by different levels of nitrogen fertilization, on the occurrence of mycotoxins in spring barley grains. A field trial was conducted at three locations (Zagreb, Kutjevo, Osijek) during the 2023 growing season to investigate the effects of four levels of nitrogen fertilization (0, 40, 80, 120 kg/ha) on yield, moisture, and hectoliter weight. In addition to production parameters and protein content of spring barley, mycotoxins (total aflatoxins, aflatoxin B1, deoxynivalenol - DON, zearalenone, T2 toxin, and ochratoxin) were determined. For the production parameters, the different location had a significant effect on all traits ($p \leq 0.001$). In addition, a significant difference ($p \leq 0.001$) was observed in protein content, which increased from 11 g/kg ST to 13 g /kg ST in barley fertilized with 120 kg/ha mineral fertilizer. Mycotoxin contents in the studied samples differed statistically significantly ($p \leq 0.05$) with respect to fertilization and location, only for the content of T-2 mycotoxins. A significant difference ($p \leq 0.001$) was also found for the content of DON, but the mycotoxin itself was detected only at the Kutjevo site. Regarding fertilization, the change in mycotoxin content was not linear; the highest level of contamination was detected with fertilization at 120 kg/ha and the lowest without treatment fertilization. In conclusion, different nitrogen fertilization affects the protein content of spring barley, but the most important variable in spring barley production is location. Regardless of the mycotoxin levels detected, all values determined are below the permissible limits according to the EU regulation, and all samples can be considered safe for use as food for humans or animals.

**Keywords**: spring barley, fertilization, mycotoxin

# Morphometric analysis of the stomatal apparatus of selected species of weedy grasses: a possible way for determination?

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**Summary**

The aim was to determine whether the weedy taxa *Panicum miliaceum* subsp. *ruderale*, *P. miliaceum* subps. *agricola* and *Echinochloa crus-galli* can be distinguished from each other based on stomata parameters. In the vegetative stage they are difficult to determine. One possibility is to use the microrelief (replica) method. We compared the length and width of the stomata, the length and width of the stomata pore and the stomata number per 200 000 μm. Based on Tukey's HSD test, we found that the stomata length on the upper leaves side is significantly different among all three species. On the lower leaves side, we found that there is a significantly difference in the stomata length between *P.m*. subsp. *ruderale* and *P.m*. subsp. *agricola* and also between *P.m*. subsp. *ruderale* and *E. crus-galli*. There was a significant difference between *E. crus-galli* and the two *Panicum* taxa in the stomata width on both the upper and lower leaf sides. The stomatal pore length on the upper leaf side was significantly different among all species and on the lower side there was a significant difference between *P.m*. subsp. *agricola* and *E. crus-galli* and also between the two *Panicum* taxa. In the case of stomatal pore width on the upper leaf side, we found a significant difference between *E. crus-galli* and both *Panicum* taxa, and on the lower side we found an insignificant difference between all species. When assessing the stomatal abundance, we found that the upper leaf side is significantly different among all three taxa and on the lower leaf side there is a significant difference between subsp. *ruderale* and subsp. *agricola* and also between *E. crus-galli* and *P.m*. subsp. *ruderale*. It was confirmed that the method mentioned above is suitable for the determination of *E. crus-galli* and both *Panicum miliaceum* taxa vegetative stages.

**Keywords**: *Panicum miliaceum* subsp. *ruderale*, *Panicum miliaceum* subsp. *agricola*, *Echinochloa crus-galli,* weeds, stomata apparatus

# BC maize hybrids yield in performance trials in 2023

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**Summary**

A total of 89 trials were processed in 2023 with an average of eight BC maize hybrids per location. After two unfavourable years, 2023 was more favourable for maize production. Because of too much precipitation during spring planting was more difficult than usual, and as a consequence lower plant densities were achieved. During flowering period conditions were more favourable for maize, which resulted in good fecundation. In August and September there was not too much heat stress and distribution of precipitation was better than last two years, which resulted in higher grain yields. The results show average grain yield (AGY) of 10,61 t/ha, average grain moisture (AGM) at harvest of 18,12% with an average plant density of 70.011 plants/ha. The analysis of the results shows the difference between maize yields from trials in Slavonia in relation to the yield from trials in western part of Croatia. Thus, in four eastern counties BC323, a hybrid from FAO 300, achieved AGY of 10,01 t/ha with AGM at harvest of 14,44%, while in the other production counties it achieved AGY of 11,43 t/ha with AGM of 17,34%. Furthermore, BC415 in the four most eastern counties had AGY of 9,95 t/ha with AGM of 15,69%, and in other counties had AGY 11,77 t/ha and AGM of 19,36%. The best trial result in 2023 was obtained at the location Belajske Poljice (Karlovac county) with hybrid BC323 (17,18 t/ha).

**Keywords**: production trials, maize, hybrids, grain yield

# Self-defence of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) – Different strategies

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**Summary**

Chemical self-defence based on the synthesis and accumulation of naturally bioactive compounds is a very successful and ubiquitous strategy in the plant world. This summary and proposed poster summarize the results on chemical self-protection in maize and wheat. Its aim is to draw attention to the diversity of the compounds involved and the desirability of testing them simultaneously, since in most cases the investigations carried out have been limited to a single group of chemicals. Chemical defence compounds in wheat and maize are: Cyclic hydroxamic acids which are toxic to many insects and are effective deterrents against herbivores. Volatile organic compounds (VOCs) protect plants directly by repelling or poisoning herbivorous animals. Phytosterols which can interfere with herbivore digestion and nutrient absorption, making it more difficult for herbivores to extract nutrients from the plant. Salicylic acid and its derivatives which contribute to the development of local and systemic resistance. Phenolic compounds in maize act as inhibitors, interacting with the proteins of the target herbivore organisms. Maysin is a C-glycosyl flavone that is produced in constitutively high amounts in maize silk and correlates with increased resistance to insect pests. Two groups of terpenoids, the kauralexin class and the zealexin class are the specific metabolites associated with defence in maize. Oxylipins isolated from maize suppress the growth of pathogenic fungi and herbivores. The effectiveness of chemical self-defence can vary depending on the variety/hybrid, the environmental conditions and the presence of individual pathogens or pests. Modern agricultural practices - the use of plant hormones and elicitors - and genetic modification have been used to enhance natural chemical defences and improve resistance to pests and diseases.

**Keywords**: chemical defence, wheat, maize, plant resistance

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# The effect of different soil types on chickpea yield under conditions in south-western Poland - a preliminary research

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**Summary**

Chickpeas are one of the oldest and most valued crops. As a legume, it provides food for a growing global population, and its importance will continue to grow as the climate changes. Numerous environmental and climatic factors influence the development and yield of chickpeas. In 2023, a study was launched to assess the suitability of chickpeas as an alternative crop for cultivation under the conditions of south-western Poland, the Lower Silesia region. The three chickpea genotypes (Amorgos, Elmo and Orion) were sown on two different soils: a very heavy, loamy soil and a light, sandy soil. The following yield components were determined: plants density per 1 $m^2$, weight of seeds from 1 plant and 1000-seed weight. After harvest seed yield was determined at 15% moisture content. Soil type significantly affected all traits examined, except 1000-seed weight. The cultivation of chickpeas on heavy soil has proved to be more favourable. All parameters tested were genotype-dependent. The most beneficial values of analysed traits were observed in the Orion cultivar (kabuli type). The interaction of factors significantly affected weight of seeds from 1 plant and seed yield. Cultivation of the Orion on heavy soil showed the highest yield of seeds from 1 plant, and the highest seed yield per ha, but similar yields were obtained also from the same soil from the Elmo cultivar. In conditions of south-western Poland cultivation of chickpeas on heavier soils seems to be more beneficial. The experiment should be continued in subsequent years to obtain confirmed results.

**Keywords**: heavy and sandy soil, moderate climate, *Cicer arietinum*, yield components, cultivar

# Effect of biotic stress on antioxidant enzyme activities and malondialdehyde content of sweet maize (*Zea mays* L.)

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**Summary**

Global warming is a major problem for crop production. This phenomenon accelerates the spread of pathogens such as the corn smut (Ustilago maydis), and the host plant's defence system is weakened. The aim of this study was to investigate the effects of corn smut infection on antioxidant enzyme activities (asorbate peroxidase (APX), guaiacol peroxidase (POD) and superoxide dismutase (SOD)) and malondialdehyde (MDA) content of maize (*Zea mays* L. cv. Desszert 73), as maize is a very important crop worldwide. The experiment was conducted under field conditions in the garden of the University of Debrecen. The infection material was prepared under laboratory conditions. The treatments were: Control (0 spoidium nunber); 2500, 5000 and 10 000 sporidium number/ml concentrations. The antioxidant enzymes activities and malondialdehyde content were measured using a spectrophotometer. There are two sampling times: 7 and 14 days after infection. The different concentrations of corn smut infection significantly increased the APX, POD and SOD activities and MDA content at both sampling times. The experiment proved that the concentration and sampling time play an important role in corn smut infection, because the higher the concentration, the more the mentioned parameters increased. Prevention is very important to avoid maize blight infection. The sweet corn hybrids are very suspicious for the corn smut infection.

**Keywords**: maize, biotic stress, antioxidant enzyme activity, malondialdehyde

# Evaluation of plant parameters and forecrop effect of common vetch (*Vicia sativa* L.) under different agrotechnical conditions

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**Summary**

In our experiment, we investigated the seed production parameters of common vetch (*Vicia sativa* L.) in a crop rotation system in case of solo and mixed (oats and common vetch) cropping with different nutrient supply levels (control, 80 kg $ha^{-1}$ N, 80:96:96 kg $ha^{-1}$ NPK), and its forecrop effect on corn. We set up our experiment in 2022 in Hungary on humic sandy soil. In 2022, a year of extreme drought, the pod number was not affected by nutrient supply in the case of mixed sowing with oats, and pod number decreased in the case of optimal nutrient supply, whereas a marked effect of nutrient supply on pod number was observed in the case of solo cropping. Similar trends were observed for seed number and seed weight. In the extreme drought year, the yield of common vetch in the mixed sowing was minimal (0.02 - 0.06 t $ha^{-1}$), while in the pure sowing the yield was 0.40 - 0.47 t $ha^{-1}$. In 2023, which was a favorable year for common vetch, pod number was not influenced by sowing method and nutrient supply, except in the case of optimum nutrient supply (NPK 80:96:96 kg $ha^{-1}$) in mixed sowing, where pod number decreased significantly, similar to the seed weight per plant. Common vetch yields were higher in case of solo cropping (0.91 - 1.18 t $ha^{-1}$) than in the mixed treatment (0.78 - 0.99 t $ha^{-1}$), but the difference between treatments was not significant. In both cropping methods, the control treatment had the highest yields. In the year 2023, no significant differences in corn yield were observed between the vetch forecrops grown with different seeding and nutrient treatments, with yield averages ranging from 11.85 to 13.50 t $ha^{-1}$. In terms of seed production, common vetch seed yield in a drought year competes with oats in mixed cropping, thus reducing the seed yield of common vetch. As a forecrop, common vetch also has a considerable positive effect on yield in a favourable year for corn.

**Keywords**: common vetch, forecrop, yield parameters, fertilization, corn

# Influence of intercropping maize with climbing bean on forage yield and nutrient composition of silage

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**Summary**

Corn silage is a high-yielding, voluminous forage rich in energy but with insufficient protein content for high-producing cattle. Since legumes have low dry matter yield, acceptable forage yield and quality can obtained from intercropping cereals and legumes compared with their sole crops. In this research, corn (*Zea mays* L.) and climbing beans (*Phaseolus vulgaris* L.) were sown in different sowing densities in order to determine the best ratio in the intercropping system of corn and beans to achieve high yield and good nutritional composition of silage. A pure maize crop (75000 grains $ha^{-1}$) and maize in intercropping with beans were sown: 75000 grains $ha^{-1}$ of maize and 37500 grains $ha^{-1}$ of beans, 75000 grains $ha^{-1}$ of maize and 50000 grains $ha^{-1}$ of beans and 75000 grains $ha^{-1}$ of corn and 75000 grains $ha^{-1}$ of beans. The highest yield of dry matter was determined with the highest proportion of beans (21.6 t $ha^{-1}$), and the lowest yield with 75000 plants $ha^{-1}$ of corn and 37500 plants $ha^{-1}$ of beans (19.4 t $ha^{-1}$). All intercrop had higher crude protein values in dry matter 86 g $kg^{-1}$ for the 37500 plants $ha^{-1}$ of beans, 94 g $kg^{-1}$ for the 50000 plants $ha^{-1}$ of beans and 101 g $kg^{-1}$ for the 75000 plants $ha^{-1}$ of beans than the monocrop maize (74 g $kg^{-1}$). Based on the yield and nutritional composition of silage, we can conclude that the intercropping with the highest proportion of beans had the highest yield and the best nutritional value of silage.

**Keywords:** intercropping, maize, climbing bean, yield, silage

59
Croatian
2024 Sa
19
International
Symposium on
Agriculture

Session 6

# Book of Abstracts

Fisheries, Wildlife Management and Apiculture

59
Hrvatski
19
Međunarodni
Simpozij
Agronoma

# Zbornik sažetaka

Ribarstvo, lovstvo i pčelarstvo

# Revisiting the sex determination and sexual differentiation of African catfish (*Clarias gariepinus*)

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**Summary**

The molecular background of sex determination (SD) and early sexual differentiation of African catfish (*Clarias gariepinus*) was investigated. Controversial information is available about the genetic SD mechanism of this species; some authors suggested XX/XY, others ZZ/ZW system, and recently it was proposed that the two SD systems might even co-exist. We have validated a male-specific DNA marker (CgaY1) in over 600 individuals spanning across three generations and found that the efficiency of the marker is over 96%. According to our results, Hungarian stocks of this species possess XX/XY sex determination mechanism only. Temperature-induced masculinization was also suggested by others, but no such effect was detected in our lines. The early expression profile of *vasa*, *foxl2*, *cyp19*, *sox3*, *sox9*, *pten*, and *mark2* genes was analyzed in the head and trunk of genetically sexed larvae and the first sign of sexual differentiation was detected at 15 days post-hatching and presumably factors originating from the head (possibly the brain) play an important role in this process. Sexually dimorphic expression was present in *sox9* and *pten*. Novel sex-associated SNP markers were isolated by ddRADseq and their sequence alignment pointed to chromosome 24.

**Keywords**: African catfish, *Clarias gariepinus*, sex determination, sexual differentiation

**Acknowledgments:** The work was supported by the iFishIENCi (Horizont 2020, No 818036), 2017-2.3.3-TÉT-VN-2017-00004 projects, the Frontline Research Excellence Grant (KKP 140353 to LO) and the New National Excellence Grant (MATE-K/3037-7/2023).

# Settlement of European flat oysters on spat collectors during spawning season of 2022, in Bistrina, Mali Ston bay

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**Summary**

Mali Ston bay in Croatia is the largest aquaculture site of European flat oyster *Ostrea edulis* (Linnaeus, 1758) in the Mediterranean. Farming is currently based exclusively on spat collected from the wild, but settlement is not monitored. This study analyzed settlement of *O. edulis* on commercial spat collectors (coupelles) during the Spring spawning season in Bistrina. The collectors were suspended on floating longlines at a depth of 7 - 8 m, 1 m above the sea bed. Each week from the 19 April to 13 June 2022 (9 weeks total), a triplicate of collectors was deployed and left in the sea for four months, then analyzed over the course of 9 consecutive weeks, respectively. The abundance of spat and largest shell length were analyzed on subsamples of 45 coupelles from each collector. The number of oysters on collectors placed from the 19 April (1.9 per coupelle) to the 17 May (10.6 ind. per coupelle) increased, and then retained similar values towards the end of the spawning season (collectors placed on 13 June; around 7.5 ind. per coupelle), with collectors placed on the 31 May having highest observed abundance (11.5 ind. per coupelle). All collectors had oysters of different size categories, ranging from 0.57 mm to 70.43 mm in shell length, confirming a continuous recruitment throughout the summer months. Nevertheless, a consecutive increase in average shell length was observed in oysters on collectors placed from 19 April (18,13 ± 7,06 mm) to 10 May (35,39 ± 9,89 mm) and then a mild decline of size on collectors placed towards 13 June (29,84 ± 9,03 mm). Thus, collectors should be placed on 10 or 17 May depending if you prefer spat abundance or size, respectively. Further research will be made to correlate these results with abiotic parameters and larval abundance.

**Keywords**: *Ostrea edulis*, juvenile, larvae, coupelle, Chinese hat, Adriatic

# Is there a benefit of using hook with an appendage in a small scale coastal Adriatic Sea longline fisheries?

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**Summary**

In many recreational fisheries around the world, fish are often released alive right after capture. This practice is commonly known as catch and release fishing. The survival of the released fish depends on many factors, among which the anatomical hooking location is the most important one. Deeply hooked fish have little chance of survival upon release compared to other hooking locations. A study was conducted in the eastern Adriatic Sea, where a standard hook was replaced with a modified hook equipped with a wire appendage to potentially reduce the incidence of deep hooking. The catch efficiency of the hooks was also investigated. The modified hook was tested in a coastal demersal longline fishery where both hooks were fished in parallel. Results showed decreased probability of deep hooking for the two most abundant species in the catch *Sparus aurata* and *Diplodus annularis*. The presence of a wire appendage significantly reduced the catch efficiency of the modified hook compared to a standard hook. The results suggest that a modified hook can reduce the probability of deep hooking, however the reduced catch efficiency of the modified hook compared to a standard hook likely represents a barrier for its use in the analysed fishery.

**Keywords**: hook selectivity, longline, Adriatic Sea, capture mode, catch comparison

# Changes in riverine fish communities as a result of renewed rip rap shoreline protection

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**Summary**

River modifications are one of the major threats to freshwater biodiversity. They often include construction of rip-rap banks, changing the natural state of the river habitats. Stretch of the Sava River between Slovenia and city of Zagreb in Croatia was regulated since mid-20th century, with most banks modified to rip rap zones. One part of this rip rap modification, near Medsave village, was renewed in the late 2017. The aim of this study was to determine the possible changes in ichthyocenosis as a result of renewed rip rap riverbank. Fish community data (abundance of individuals) collected as monitoring data from 2003 to 2022 were divided into three groups: before renewal (2003-2017), first year after renewal (2018), second year after renewal and after (2019-2022). Data were analyzed by between-group principal component analysis and similarity percentages. Several changes in species presence and abundance were detected. *Cyprinus carpio*, *Esox lucius* and *Gymnocephalus cernua* were not detected until the renovation, and abundance of *Carassius gibelio*, *Pseudorasbora parva*, *Abramis brama* and *Chondrostoma nasus* was much higher after renovation. *Rutilus virgo* and *Barbus balcanicus* were relatively abundant before but were absent after renovation, as were some less abundant species such as *Leuciscus aspius*, *Leuciscus idus*, *Barbatula barbatula* and *Romanogobio uranoscopus*. The impact of the Brežice hydroelectric power plant dam, located just few kilometers upstream and in operation since late 2017, may also be the cause of change in fish community, as the reservoir may be the source of several lentic species that use renewed rip rap bank as preferred microhabitat. The solitary effects of this renewed shoreline protection on fish assemblage is not clear, but the case of the Sava River in this area is further evidence that multiple anthropogenic stressors are likely causing changes in natural freshwater fish communities that should be carefully monitored in the future.

**Keywords**: Sava river, river alterations, fish abundance, anthropogenic impact

# African swine fever of wild boar in Europe and Croatia - yesterday, today, tomorrow

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**Summary**

African swine fever (ASF) is a highly contagious viral infectious disease of wild boar and domestic swine. The virus is extremely resistant in nature, harmless to humans, but mortality in pigs can be up to 100%. The first official case of ASF was described in Africa in 1921 in Kenya (although it is considered that it appeared for the first time in 1907), while in Europe it was first reported in Portugal in 1957. Today, ASF is endemic in sub-Saharan Africa, where the disease was originally discovered. However, in 2007 ASF outbreaks occurred in Georgia, and the disease spread to the neighbouring countries, affecting wild boar and domestic pigs. The first cases in the EU have been recorded 2014 in wild boars from the Baltic States and Poland. Since then, the disease has spread to other EU countries and neighbouring countries outside the EU. From the beginning of year 2023 until October 13, 2023, 3.961 foci (in 16 countries) of ASF in domestic pigs and 6.505 cases (in 20 countries) of ASF in wild boars were reported to the EU disease notification system (Animal Diseases Information System – ADIS). The first cases in Croatia were reported on June 26, 2023, when the Croatian Veterinary Institute confirmed ASK in blood samples of sick domestic pigs and in organ samples of dead domestic pigs taken from two facilities in the area of Posavski Podgajci in Vukovar-Srijem County. These are two smaller buildings where pigs were kept outdoors. Since then, according to ADIS data, until October 13, 2023, in Croatia the disease was proven in 1.019 domestic pigs (25 % of the total positive registered in 16 countries in ADIS), and until September 27, 2023, in 9 wild pigs (0,14 % of the total positive registered in 20 countries in ADIS). Most of the countries recorded by ADIS in which ASF has been recorded have a (significantly) higher number of positive wild pigs, while Serbia, Bosnia and Herzegovina, Romania and Croatia have a (significantly) higher number of positive domestic pigs. It is to be expected that due to the implementation of eradication measures, the number of positive domestic pigs will decrease in the near future in these countries, including Croatia, and the number of positive wild pigs will increase.

**Keywords**: African swine fever, Croatia, domestic swine, wild boar,

# Assessment of grass carp culture as a candidate for future sustainable aquaculture production in Europe

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**Summary**

Feeding an expected global population of nine billion by 2050 presents a challenge that is engaging all members of the food producing industry. Fish and other aquatic products are expected to play an important role in fulfilling this demand. For this reason, and due to overfishing, aquaculture became the fastest-growing protein production system, increasing annual production by more than 600% from 1990 to 2020. However, this increase is still insufficient to meet expected needs. Some of the challenges are climate changes and various environmental protection issues. This resulted in increased research into the introduction of new species into aquaculture, and the possibilities of increased farming of herbivorous and climate resilient species that are already cultivated. While the introduction of new species takes a long and complicated research, the intensification of already farmed species presents a much easier path. An example of such a species that still poses a great potential in Europe is grass carp, *Ctenopharyngodon idella*. In China, its homeland, it is the most important freshwater aquaculture species with annual production of 5.76 million tons in 2021. Unlike China, in Europe it is mostly cultivated in small quantities (1500 to 2000 tons per year), mainly in polyculture with common carp, and is difficult to find on the market. Its' fast growth, good meat quality, tolerance to a wide range of temperatures, salinity, and low oxygen levels, developed technology in different farming systems (pond polyculture and monoculture, in-pond partitioned system, and closed recirculation system) make this species an ideal candidate for increasing aquaculture production in Europe. With regard to EU legislation related to non-native species, known cultivation technology can help to overcome the associated environmental barriers. In this paper, all possibilities of intensification of grass carp production in areas where it has been declared non-native species are analyzed.

**Keywords**: *Ctenopharyngodon idella*, climate resilient species, farming technology, non-native species

# Cryopreservation of European flat oyster (*Ostrea edulis*) larvae

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**Summary**

European flat oyster (*Ostrea edulis*) larvae were cryopreserved using two protocols previously described for other oyster species following several modifications. Culture of the European flat oyster suffered several setbacks due to parasitic outbreaks, thus, assisted reproductive techniques such as cryopreservation can help in the conservation and farming of the species. Oyster larvae (veligers) were diluted in two simple extenders (0.4 M trehalose or 0.4 M sucrose) with the addition of ethylene glycol (EG), dimethyl-sulfoxide (DMSO) or propylene glycol (PG) in concentrations of 10–20% with or without supplementation of polyvinylpyrrolidone 40,000 average MW-PVP-40- (PVP). Larval suspensions were aspirated into 0.25-ml or 0.5-ml straws and frozen in a controlled-rate freezer using two slow cooling profiles. Assessment of larval survival 20 minutes or 24 hours post-thaw revealed that the cooling profiles and the type of extender used did not have a significant effect on survival rates. On the other hand, the type of cryoprotectant, straw volume and the time of assessment had a significant main effect on larval survival percentages ($p<0.001$ in all three cases). Survival percentages of 70–80% were observed 20 minutes post-thaw which decreased to 40–60% at 24 hours. Generally, EG and DMSO resulted in higher post-thaw larval survival than did PG when larvae were cryopreserved in 0.25-ml straws, whereas, these differences were not detected when 0.5-ml straws were employed. Results of these experiments will allow later studies on long-term survival of cryopreserved European flat oyster larvae.

**Keywords**: European flat oyster, larvae, cryopreservation, cryoprotectants, controlled-rate freezer

**Acknowledgments:** The work has been supported by the Ministry of Science and Education of the Republic of Croatia and the National Research, Development and Innovation Office of Hungary (project 2019-2.1.11-TÉT-2020-00247) as well as the KKP program of the Hungarian University of Agriculture and Life Sciences.

# Invasive species vs. thermophilic species that expand their range

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**Summary**

According to the EU Regulation N. 1143/2014, "Alien Species" or Non-Indigenous Species (NIS) means "any live specimens of a species, plants, animals, fungi and micro-organisms, that have been introduced outside its natural range". Invasive Alien Species (IAS) means an "alien species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services". Cryptogenic and crypto-expanding species are included separately from the validated NIS. Due to the high degree of uncertainty regarding their origin and mode of introduction, the designation of species as cryptogenic was inconsistent among national experts in many cases. Crypto-spreading species would under this definition be species with some evidence of their non-indigenous status but uncertain due to an unclear mode of introduction from their natural domain, including natural range expansion. Regarding introduction status, range-expanding and partly native (i.e., native in one country/subregion but introduced in another) species in the Mediterranean were excluded at Pan-Mediterranean scale. However, species partly native in a subregion were included at the subregional scale. This paper tries to define the problem and the main difference between species that expand their natural range due to climate change (and belong to the cryptogenic species group) and NIS, IAS, and the rest of the cryptogenic species group. Namely, the expansion of the area of a certain species as a result of climate change necessarily includes changes in the ecosystem and the expansion, i.e. the movement of the area of at least part of the other species in that ecosystem, so we must investigate this phenomenon as a change of the entire ecosystem, not just as the introduction of a new species. The paper also raises some questions about risk assessment and proposed treatment of identified NIS and IAS, and particularly discuss their consequences for fisheries and aquaculture.

**Keywords**: cryptogenic species, crypto-expanding species, climate changes, aquaculture, fisheries

# Effect of brood interruption methods on infestation of honey bee (*Apis mellifera* L.) colonies with Varroa destructor

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**Summary**

The aim of this study was to evaluate three different treatment of the ectoparasitic mite *Varroa destructor* in honey bee (*Apis mellifera* L.) colonies: brood interruption by queen caging (QC group), queen confinement to a single isolator frame where queen was free to lay eggs (ISO group), and the extended release of oxalic acid through the glycerox strips (GLX group). These methods were compared to the control group which was treated using CheckMite+ strips (C group). Parameters measured included number of worker bees and brood cells, honey yield during the sunflower nectar flow and infestation of adult worker bees with *V. destructor* mite. The study was carried out in the Baranja region (Croatia) in summer and autumn of 2023 on 32 colonies. At the beginning of the study (5.7.2023.) colonies had on average 23.672 bees and 22.865 brood cells, with no significant differences between the groups (p>0,05). At the measurement on 13.10.2023., colonies on average had 11.880 bees and 11.076 brood cells, also with no differences between groups (p>0,05). At the first measurement, average infestation with *V. destructor* was 1,71%, with the lowest infestation of GLX group (1,26%) and highest in ISO group (2,01%), but without significant differences (p>0,05). However, at the measurement on 13.10.2023. there were significant differences (F(3,23)=6,267, p=0,004) with GL and C group having higher infestation (4,84% and 6,04%) comparing to the IS and QC group (2,27% and 2,41%). Finally, the average amount of extracted honey was 18,03 kg with no significant differences between groups (p>0,05). Our study showed that the brood interruption during main summer nectar flow does not have a negative effect on colony development until winter and honey production, while the *V. destructor* infestation was significantly lower comparing to the control and glycerox group.

**Keywords**: *Apis mellifera*, *Varroa destructor*, biotechnical methods, infestation, honey production

# Growth performance of different airbreathing (Clariidae) catfish lines

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**Summary**

Clariidae catfish are important species in intensive aquaculture production in various African, Asian, and some European countries. However, there are big differences in the growth performance of the different species, hybrids, and lines. Our objective is to investigate the early growth performance of different catfish lines from the Clariidae family and the analysis of the full production period of F4 generations of an African catfish (*Clarias gariepinus* Burchell, 1822) line selected for more efficient feed utilization with low fish meal content. Additionally, an alternative algae-derived protein sourced feed was also tested. During the preliminary larvae rearing tests, the Vietnamese African catfish showed the highest specific growth rate (14.5%) compared with a Hungarian, a Vietnamese *Cl. macrocephalus*, and a hybrid catfish (*Cl. gariepinus x Heterobranchus longifilis*). In the F4 generation of the selected African Catfish lines a higher growth rate was observed compared to the non-selected genotype using four different diets (Control 1 - CD, Control 2 - AC, low fish meal - ED, and algae meal containing - AD) in a flow-through system. The direct selection gain for the body mass was 21% in the case of ED, 19.3 % with AC, 26% with CD, and 21.3% with AD. While in Recirculating Aquaculture System (RAS) the selection gain was 33% with ED, and 12% with CD, respectively. Additionally, a significant feed-specific selection gain of 21% was detected between ED and CD in the RAS system. The algae complementation had a minimal effect on the meat color in the yellow range, but there was no effect on smell and taste.

**Keywords**: catfish, Clariidae, selection, growth

**Acknowledgments:** The work was supported by the National Research Development and Innovation Office (NKFIH) Hungary, grant number 2017-2.3.3-TÉT-VN-2017-00004, the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16) projects and by iFishIENCi project (European Union's Horizon 2020 research and innovation program under grant agreement No 818036) and a New National Excellence Grant (MATE-K/3037-7/2023). The project is co-financed by the European Union and the European Social Fund.

# Shellfish fouling community on larvae mesh bag collectors in two Natura 2000 sites, Pantan and Cetina estuary

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**Summary**

The paper presents research of the shellfish fouling community on shellfish larvae collectors placed in two Natura 2000 sites, Pantan (HR3000430) and Cetina estuary (HR3000126) in a year 2022. The collectors were installed with the purpose of recording recruitment of critically endangered shellfish *Pinna nobilis*, Linnaeus 1758 species, while the aim of this research was to collect data on the biological diversity of shellfish fouling community on the collectors, including the determination of Mediterranean invasive and endemic species of marine organisms. During the analysis of the fouling community, a total of 19 species of bivalves from 9 families were determined. During the laboratory analysis, no invasive species of bivalves where determined, however, an invasive species of sea squirt *Styela plicata* was found.

**Keywords:** biodiversity, Natura 2000, shellfish larvae collector, invasive species, *Styela plicata*

# Sustainable management of fisheries and aquaculture discards and side-streams

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**Summary**

By some definitions, sustainability refers to a process-driven path of continuous improvement that aims to create more resource-efficient products that preserve functional ecosystems. Upcycling of underutilized or discarded marine materials can be a sustainable strategy for achieving a circular bioeconomy with the production of high value-added materials. The definition of "discards" includes many fish species or bycatch products that have little or no commercial value, undersized or damaged commercial species, and species that have commercial value but are not caught in sufficient quantities to warrant sale. Proper treatment and handling of this biomass would result in the conversion of waste to rest raw materials (RRM) or by-products. Depending on the species processed in fisheries and aquaculture industry, solid RRM may consist of low-grade whole fish, fish skeletons including heads and tails, viscera, skin and filet trimmings, and shell material in the case of shellfish and crustaceans. More than 20 million tonnes of food grade fisheries side-streams are generated, and between 7 and 10 million tonnes of commercial fisheries catch are discarded at sea each year. The EU's Common Fisheries Policy aims to put an end to this wasteful practise, which is why a specific measure was adopted – the 'Landing Obligation," which was introduced in 2015 and has been fully in force since January 2019. Some of the landing obligation rules require that all catches of species subject to catch limits or minimum sizes must be landed and counted against fishermen's quotas. In addition, undersized fish caught and landed should not be used (sold) for direct human consumption, but for products such as animal feed, fish meal and pharmaceuticals. EU countries are also required to help fishermen by making it easier to store undersized fish and find potential outlets. Nowadays, there is an increasing trend to use fishery by-products as material for the production of fish meal and fish oil. Also, many efforts have been made to use them for animal feed, fish silage, fertilizers, and biofuels, and to utilise more of them for human consumption. The use of various side-streams and discards in food remains a challenge in terms of proper handling and cold storage to maintain food grade quality. Developing new ingredients or new products in various forms based on aquaculture and fisheries RRM and by-products is a potentially viable alternative to add value to products, avoid economic losses, reduce environmental impact, and provide consumers with nutritious, cost-effective, and convenient foods with longer shelf lives. Development of technology for upcycling of discards and side-streams in the aquaculture and fisheries industry is promising with and is likely to make significant progress in the future. The research is part of a project Bilateral Cooperation in the Field of Sustainable Upcycling of By-products in Aquaculture and Fisheries (SAFE) and is co-funded by Iceland-Liechtenstein-Norway-Grants.

**Keywords**: fishery by-products, rest raw materials, landing obligation, circular bioeconomy

# Molecular genetic analyses of growth selected african catfish (*Clarias gariepinus*) lines

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**Summary**

Hungary is at the forefront of intensive breeding of African catfish (*Clarias gariepinus)* in Europe. However, for their intensive production to be economical and sustainable in the future, we must use methods that can reduce expenditures. The most expensive component of the intensive fish breeding systems is the feed and its expensive, high protein-containing fish meal and oil ingredients. If these are replaced with plant-based components, the efficiency of production decreases. We created catfish lines which are selected for better utilization of decreased fish meal content feed (supplemented with soy meal) through four generations. The effects of selection were investigated by ddRAD sequencing (SNP markers) on genomic level, and by digital PCR and quantitative Real Time PCR on gene expression level. Sixteen SNP markers were identified with favorable Fst (fixation index) values. We are currently testing them on the F2 and the F4 generation. The digital PCR and qPCR results suggesting correlation among the lines and the feed at the growth-related genes (growth hormone, growth hormone receptor, insulin-like growth factor I and II, myostatin, follistatin-alpha, somatolactin, activin receptor type I and type II, somatostatin). In the future we would like to examine our lines using RNA sequencing to identify more differentially expressing genes among the selected and control fish and feed groups.

**Keywords**: African catfish, fish feed, gene expression, growth

**Acknowledgments:** The work was supported by the by iFishIENCi project (European Union's Horizon 2020 research and innovation program under grant agreement No 818036), and by the National Research Development and Innovation Office (NKFIH) Hungary, grant number 2017-2.3.3-TÉT-VN-2017-00004.

# Is the axis deer *Axis axis* (Erxleben, 1777) really an invasive species in Europe?

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**Summary**

Invasive alien species are considered by public and private environmental institutions to be one of the greatest threats to biodiversity worldwide. Today, there are a large number of species that have been classified as invasive alien species. The European Union published the third updated in August 2022. One of the 22 species in the new updated list is the axis deer (*Axis axis*). According to the available scientific literature, this species is also on the list of worst alien species for Europe in terms of impact. The axis deer is a South Asian ungulate that was introduced into Europe at the beginning of the 20th century. There are records of introductions in the Czech Republic, France, Ireland, Slovenia, and the United Kingdom, but without success. The only successful introductions in Europe were in Croatia, where three free-ranging populations have been established on Brijuni, Rab and Dugi Otok. How invasive this ungulate species really is in Europe was assessed using the Terrestrial Animal Species Invasiveness Screening Kit (TAS-ISK). The result of the assessment showed that the risk for Europe is classified as "medium". Unfortunately, it can be concluded that the current classification as an invasive species for this type of ungulate is incorrect, and that further research is needed to determine impacts in particular. The scientific knowledge should dominate in any management decisions related to invasive alien species.

**Keywords**: Europe, chital, medium risk, spotted deer, TAS-ISK

# Pollination research in the flora and vegetation of northern Croatia with utility analysis for *Apis mellifera*

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**Summary**

The proportions of insect, wind, self and water pollinated plant species as well as species useful for *Apis mellifera* in northern Croatia were determined. The study included 507 plant taxa belonging to 95 plant families and covering the flora and all vegetation (habitat) types in the study area of the Bedekovčina settlement. The flora and vegetation studies were carried out in the field, and the data on the pollination types of plant species and their usefulness for *A. mellifera* were taken from the literature and available databases. The results show that most plant species depend on insect pollination (73.6%), followed by self-pollination (30%), wind (25%) and water pollination (0.6%). However, different plant species have different pollination strategies, some have one, two, three and even four types of pollination: the largest group consists of pure insect pollination (43%), followed by both insect and self-pollination (27%), pure wind pollination (22%), insect and wind pollination (2.6%), etc. In total, 54% of the plant species useful for *A. mellifera* were found, of which 51% provide pollen, 47% nectar and 4% honeydew. The results suggest that *A. mellifera* could be a potential pollinator for about half of the flora. Of the traits analyzed, habitat types and plant family affiliation have the greatest influence on the distribution of pollination types. Most insect-pollinated plant taxa are found in grassland, forest and ruderal habitats, suggesting that these habitats are the most important for pollinators. Given the global decline of insects, the results provide a basis for conservation, selection of best management practices and beekeeping.

**Keywords**: pollination, flora, vegetation, *Apis mellifera*, Croatia

# Occurrence of parasit *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) in *Uranoscopus scaber* (Perciformes: Uranoscopidae) from the southern Adriatic Sea

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**Summary**

During the study of population dynamics of the stargazer *Uranoscopus scaber* Linnaeus, 1758 in the southern Adriatic Sea, which also included the diet of the stargazer, the presence of parasites was observed. A total of 360 specimens of the stargazer were collected over a period of one year, and in analyzed stomachs 44 parasites of *Hysterothylacium aduncum* (Rudolphi, 1802) were recorded in 22 infected stomachs, 13 females and 9 males. The total length of the parasites ranged from 3.87 to 11.04 cm (6.78 ± 1.73 cm) and the weight from 0.002 to 0.33 g (0.06 ± 0.07 g). Infestation intensity ranged from 1 to 6 individuals with an average of 2 individuals per fish. Previously, the stargazer from the Black Sea was reported as a host for the parasites *H. aduncum* and *H. fabri*, while only *H. fabri* was detected in the individuals from the Ionian and Adriatic Sea. This study presents the first report of *H. aduncum* in stargazer from the Adriatic Sea.

**Keywords**: *Uranoscopus scaber*, *Hysterothylacium aduncum*, Adriatic Sea, host, parasite

# Adriatic sprat in the trap – possible consequences of global warming

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**Summary**

The Adriatic sprat, *Sprattus sprattus* L., is one of the small pelagic fish species. It belongs to the boreal species in the Adriatic Sea and therefore prefers colder than warmer habitats, especially during its spawning season. The goal of the research is to describe the importance of sprat in the Adriatic Sea in the past and present, and to predict what its future might look like if global warming continues. In the past, sprat was one of the three most important small pelagic fish species in Adriatic fisheries, along with anchovy and sardine. In the Adriatic, sprat occurred in considerable quantities in mixed catches of small pelagic fish, mainly in the northern part of the Adriatic, but also further south in the Dalmatian region. As it was an economically important species, its catches were recorded separately from other pelagic fish species in official fishery statistics. Since then, sprat catches have declined, and recently sprats are caught only occasionally, usually mixed with sardines, in small quantities without significant economic value. As a result, sprats are losing their own category in official fisheries statistics and are only included in the "other species" group. Therefore, scientific acoustic surveys are the only source of recent information on sprat in the Adriatic Sea. Analyses of these acoustic survey's data, which focused on habitat suitability in the Adriatic, revealed that sprat habitat is highly threatened by climate change, suggesting that sprat may disappear from the Adriatic by the end of this century if global warming continues. In addition, warming sea favors the spread/invasion of thermophilic fish species that will increase the natural mortality of sprat due to predation and competition for available ecosystem resources.

**Keywords**: sprat, Adriatic Sea, climate change

# Neighbour or enemy: Attitudes of hunters towards the wolf (*Canis lupus* L.) in Croatia

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**Summary**

A survey was carried out to determine the attitudes of the hunters towards wolves (Canis lupus L.) in Croatia. Nowadays, wildlife conservation is as much a social issue as it is part of natural sciences. Species conservation depends on effective management practices; however, these are often influenced by public opinion. A person's attitude toward a species affects hers or his acceptance of that species, and the associated management actions. Concerning large carnivores, hunters represent one of the most relevant stakeholders. This stakeholder group is not well represented in general population surveys. The purpose of this survey was to determine the most recent attitudes and opinions of Croatian hunters towards wolves, to predict the feasibility and focus points on implementation of planned management activities in the future. A total of 254 hunters from all over Croatia were surveyed. The largest number of respondents (55.1%) were between the ages of 35-54. Out of the total number of respondents, 28.7% live in the wolf's distribution area. An 86.6% of respondents believe that the number of wolves has increased in the last 10 years and 62.6% of them considers it is good to have wolves in Croatia. 36.6% hunters agreed with the statement that in areas where wolf attacks on livestock are frequent, wolves should not be protected even if it meant that livestock farmers would not be able to claim or receive compensation for damages. Hunters believe that it is important to protect wolves in Croatia for future generations (39.4%) and when we asked about the number of wolves there are in Croatia, the majority of respondents believe that this number is around 400 individuals. Based on the aforementioned facts, we may conclude that that the attitude of the hunters towards the wolf in Croatia is actually positive. Although hunters expressed rather positive attitudes toward wolves, their willingness to hunt and to decrease wolf numbers should be kept in mind and followed in the future.

**Keywords**: wolf, hunters, attitudes, management

# Communicative discourse of hunting

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**Summary**

The communication segment, which contains much needed information for the smooth functioning of hunters associated in hunting societies and hunting associations of counties united in the national umbrella organisation Croatian Hunting Federation, has changed over time. Considering that the Lovački vjesnik is the only constant in informing members, as it was published long before the establishment of the national umbrella organisation, the development component and the speed of reaching the desired information in the 132 years of publishing the members' newsletter is interesting. In accordance with the electoral changes within the organisation as well as the regime and state changes in these areas, the flow of information was not prevented even under wartime conditions. Therefore, we can conclude that the somewhat archaic way of accessing information via printed media is still the only constant in the flow of correct information to the end user in terms of hunting. Supported by a digital system, the printed edition gives an insight into reality without fake news. This leads to the conclusion that the only continuous and most objective way for hunters to obtain information is the Lovački vjesnik, regardless of the time imbalance in reaching the desired information.

**Keywords:** Croatian Hunting Federation, hunters, information, Lovački vjesnik

# Settlement of benthic organisms on oyster spat collectors in Mali Ston Bay, Adriatic Sea

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**Summary**

The farming of the oyster *Ostrea edulis* Linnaeus, 1758 in the Mali Ston Bay is based on the collection of wild spat; nowadays, plastic discs on poles are used. Since the substrate submerged in the sea provides a habitat for various benthic organisms, the growth and development of the juvenile oysters settled in the same habitat may be affected. Since April 6, 2022, three sets of collectors have been sunk weekly. The last, ninth set, was released on June 13, 2022. Weekly sampling of collectors exposed to settling organisms from the water column for four months began on August 30, 2022, and the final sampling took place on October 25, 2022. Organisms were carefully sampled from the top and bottom of the discs separately. A total of 11.95 kg of fouling was measured in 156 examined samples. The fouling process was more intense on the lower side of the collectors - the total mass on the lower side was 2.15 times greater than the mass measured on the upper, light-exposed side. A total of 41 different taxa from 10 phyla (Rhodophyta, Porifera, Cnidaria, Platyhelmintes, Annelida, Bryozoa, Arthropoda, Mollusca, Echinodermata, Chordata) were recorded. The most numerous phylum was the Mollusca with 20 taxa, including the most abundant species *O. edulis*. Non-native species, the serpulid worm *Hydroides elegans* (Haswell, 1883), the sponge *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, and the bryozoan *Amathia verticillata* (delle Chiaje, 1822), were also common; *H. elegans* was identified in every sample, regardless of immersion time or light exposure.

**Keywords**: European flat oyster *Ostrea edulis*, collectors, oyster spat, biofouling

# Chemical diversity of headspace volatiles of selected bee pollen from Croatian flora

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**Summary**

The aim of this study was to investigate the chemical composition of volatile organic compounds (VOCs) in selected bee pollen from the Croatian flora. Selected multifloral and unifloral bee pollen samples from different regions of Croatia were collected in 2023., including Quercus ilex (Otočac), Prunus spinosa (Gornji Poloj) pollen and pollen originating from various botanical sources (Zagreb). The differentiation and sorting of bee pollen of the same botanical origin was based on the visual appearance and further microscopic examinations. The headspace (HS) volatiles from whole (native) bee pollen grains were extracted using headspace solid-phase microextraction followed by gas chromatography and mass spectrometry analysis (HS-SPME/GC-MS). In this preliminary study, extraction was performed at 50°C for 40 minutes using a divinylbenzene/polydimethylsiloxane (DVB/PDMS)-coated fiber. The HS-SPME method, developed in Laboratory for metabolomics in Institute for Adriatic Crops and Karst Reclamation was modified and applied for the first time for the study of VOCs in bee pollen. The main volatile metabolites identified in Quercus ilex pollen were aliphatic aldehydes hexanal, heptanal and nonanal, as well as some other derivatives such as alcohol and ketone derivatives. The main VOCs of multifloral pollen-grains headspace profile were acetic acid (tentatively identified), octane and benzeneethanol, while the headspace profile of bee pollen from Prunus spinosa was more reach and diverse in terms of the main components including hexanal, isomers of lilac aldehydes and isomers of lilac alcohols, including also some other aldehydes and alcohols. The differences in haedspace VOCs profiles as well as difference in main compounds significantly contribute to the differences in bee pollen aroma and directly reflect the botanical source. In general, volatile content of bee pollen is rarely studied, but the identification of bee pollen VOC metabolites and their diversity can make an important contribution to creation of VOCs databases for metabolomic studies and help in traceability research of VOCs in other honey bee products.

**Keywords**: bee pollen, headspace solid-phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS), headspace volatile

59 Croatian 2024 19 International Symposium on Agriculture

Session 7

# Book of Abstracts

Animal Science

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Animalne znanosti

# ELISA milk pregnancy test as a tool for fertility management in sheep - pilot project on Croatian farms

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**Summary**

Optimizing reproduction is pivotal for successful ruminant farming, particularly in the dairy industry. In 2023, there are a total of 258 registered dairy sheep producers in Croatia. According to current knowledge, the aforementioned breeders do not use modern methods of early pregnancy detection in ewes, at least not to a greater extent. Introducing an innovative pilot project for early pregnancy diagnosis in sheep aims to enhance reproductive efficiency and foster effective improvements. One of the most used methods for early detection of pregnant sheep is determination of pregnancy-associated glycoproteins (PAGs) which appear in maternal blood from day 20 of gestation onward. By using milk instead of sheep blood, the tests showed a sensitivity of 100% from day 60 of gestation onward, so milk is a good alternative to plasma for early pregnancy diagnosis in sheep. The aim of this study is to recognise the challenges that arise under real production conditions in determining pregnancy status in sheep using a laboratory enzyme-linked immunosorbent assay (ELISA) based on the determination of PAGs in milk samples in Croatia. Pregnancy status can be tested for a maximum of 184 samples in approximately 4 hours using standard laboratory equipment and ready-to-use reagents. In this way, open animals can be identified as early as 60 days after fertilization to ensure timely rebreeding and maximise milk production and profitability. Based on the preliminary results, further research needs to be conducted to establish good fertility management of dairy sheep.

**Keywords**: sheep, milk, ELISA, PAG, reproduction

# The forage quality of indigenious semi-natural pasture as affected by nitrogen fertilization

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**Summary**

Nitrogen (N) fertilization affects grassland production and forage quality. Very little information is known about the effect of N fertilization on forage quality of semi-natural pastures, particularly in light of the trend of extensification and reduction of N fertilizer application in order to preserve biodiversity. A three-year study of the influence of N fertilization (35, 100 and 150 kg N $ha^{-1}$) on the forage quality was conducted on a semi-natural pasture of the community *Arrhenaretheretum medioeuropaeum*. For this study, adult Charolais sheep were used in high-intensity rotational grazing (119 animal units (AU)/ha during a maximum of 24 hours). The influence of N fertilization was determined ($P<0.05$) only on forage crude protein content (CP), while he organic matter (OM), neutral detergent fiber (NDF) and acidic detergent fiber (ADF) content was not affected by N fertilization ($P>0.05$). The insignificant interaction of year × N fertilization for all quality indicators indicates that the influence of N fertilization on forage quality was similar in all investigated years. Reducing the amount of N fertilizer from 150 to 100 kg N $ha^{-1}$ $yr^{-1}$ did not affect the CP content ($P>0.05$), while reducing the amount of N from 150 to 35 kg $ha^{-1}$ $yr^{-1}$ decreased ($P<0.01$) forage CP content, on average by 9.5%. It can be concluded that in conditions of reduced N application, farmers will be faced with a significant change in the forage quality, and ultimately a potential decrease in animal productivity.

**Keywords**: forage quality, nitrogen, semi-natural grassland, grazing

# The effect of sward suppression and the red clover sowing rate on the success of grassland renovation in the sowing year

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**Summary**

The aim of this paper was to determine the effect of sward suppression (SS) (no herbicide vs application of 900 g glyphosate $ha^{-1}$) and red clover sowing rate (RC; 0, 8, 16, 24 and 32 kg $ha^{-1}$) on the sward height, number of RC seedlings, dry matter yield (DMY) and the proportion of RC in the herbage of the renovated sward in the sowing year. The SS resulted in a significant decrease in the sward height ($P<0.001$), an increase in the number of RC seedlings ($P<0.001$) and the proportion of RC in the herbage compared with no herbicide application, except when sowing 32 kg RC $ha^{-1}$ ($P=0$ ,0566). The number of RC seedlings increased linearly with the increase in the sowing rate at both levels of SS ($P<0.001$). Herbicide application reduced total DMY 60 days after sowing by 36% ($P<0.001$) compared with no herbicide application. RC sowing increased grassland DMY by 21% compared to the control, without significant differences among sowing rates ($P>0.05$). Averaged for all sowing rates, RC proportion in the herbage 60 days after sowing with the herbicide application was 11.9% in DM, while without the herbicide application the proportion of RC was only 1% in DM ($P<0.001$). The effect of the sowing rate on the RC proportion in the herbage depended on the level of SS ($P<0.001$). The results indicate that sowing of RC into existing sward without effective control of the competitiveness of the existing vegetation carries the risk of poor sowing success, except when very high seeding rates are used.

**Keywords**: grassland renovation, competition, sward suppression, red clover, sowing rate

# Analysis of birth weight factors in calves from selected Slovak Spotted Cattle farms

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**Summary**

Calf growth traits such as birth weight (BW) are of major economic importance for cow-calf producers and for farmers in general, as other authors have shown. The goal of this study was evaluate the selected factors for BW in different breeding conditions of the Slovak Spotted calves. The data of herds of Slovak Spotted breed was provided from the database of agricultural enterprises of Central and Eastern Slovakia. A total of 1,160 calves born 2020 to 2022 were observed during evaluation. The basic statistical, variability and linear model were evaluated using the SAS. The value of statistical significance, the value of the F-test and the $R^2$of the above mentioned BW were evaluated with its relation to: herd, years of birth, period of birth, breeding type, sex and sire. The average value of BW of calves was 40.0±7.1kg, ranging from 20 to 65kg. During the years 2020 to 2022 were as follows: the highest number of calves was analysed during 2021 (767), where the average BW of calves was 38.7±5.3kg. The linear model to represent $R^2$ = 0.555% in case of BW of calves for all fixed effects. According analyses of the effect to BW of calves were most influenced by the sire $R^2$=0.476% after that the effect of herd $R^2$ = 0.438% and the effect of breeding type $R^2$=0.222% (P<0.001). This study suggests that the birth weight of the calves studied, which were kept under different conditions in Slovakia, was significantly influenced by the sire, herd, breeding type, sex and period of birth.

**Keywords**: cattle, calves, birth weight, factors, coefficient of determination

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# Distribution of particle structure in corn silage on dairy farms in the Republic of Croatia

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**Summary**

Corn silage is not important only in meeting the nutritional and energy needs in the rations of dairy cows, but it is also significant in a physiological sense because it represents an important source of physically active fibers during the breakdown of food in the rumen, the aim of this study was to determine to what extent corn silages meet the quality of this physical indicator in the overall evaluation of silage. For this study of the distribution of the structure of corn silage particles, three-year studies were conducted in ten counties of the Republic of Croatia on 270, 256, and 231 samples in the years 2020, 2021, and 2022, respectively. The distribution of the particle structure of corn silage was measured by the method of sieving over three sieves, and according to this method, the physical quality of the corn silage was evaluated based on the target values of particle distribution per sieve. Differences in the distribution of corn silage particles between individual counties were statistically significant ($P<0.05$) only between Međimurje and Varaždin counties in 2022. Following the values of the particle structure by individual sieves, in all three years the best values were recorded on sieve 2, which was also statistically significant compared to sieve 1, sieve 3, and sieve 4 (box at the bottom). The values of the particle structure between individual classes of silage confirmed statistically significant differences ($P<0.05$) only in the year 2021. Based on the obtained results related to the distribution of the particle structure, it is evident that the silage storage technique needs to be improved.

**Keywords**: corn silage, particle structure, dairy cows, dairy farms, sieves

# Milking cluster removal strategies for dairy cattle

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**Summary**

Automatic cluster removers (ACR) have been developed since 1970 to increase the automation of the milking process in conventional milking parlours, decreasing labor costs, and improving milking parlor efficiency. Once the milk flow drops below preset milk flow threshold, milking is terminated. Basic scientific knowledge on the removal of the milking cluster, demonstrates that optimal cluster removal settings for conventional milking of cows can be of more than 400 g/min, measured at the outlet of the claw, without having a negative effect on milk yield. Recent studies showed that increasing ACR settings even up to 1.2 kg/min decrease individual milking duration without decrease in milk production and composition. To achieve optimal detachments at high flow rates cows should have good and consistent premilking teat preparation, short but consistent interval until attachment, proper cluster alignment, and remain stress-free. Conventional milking machines remove the entire cluster at the same time, resulting in some quarters being more over-milked than others. Recent research and field experience suggests that leaving a moderate amount of milk in an udder after milking, in a modern dairy animal, does not reduce milk production or increase mastitis risk.

**Keywords**: cluster removal, dairy cow, milking parlour, milking, milk flow

# Wool disposal practices of sheep breeders in Croatia

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**Summary**

The aim of this study was to investigate, through a questionnaire survey, what breeders in Croatia do with the wool after sheep shearing. For this purpose, 517 or 2.65 % of registered sheep breeders in 17 Counties were surveyed in 2017. It was found that most breeders are men (76.77 %), over 55 years old (78.91 %), have higher education (54.20 %) and their family farm has two active members (57.08 %). The sample studied was dominated by farms with flocks of up to 100 animals (65.89 %), with only one breed of sheep (82.33 %) kept in an intensive production system (82.14 %), mainly for meat production (66.07 %). Most sheep in Croatia are sheared once a year (96.10 %), most often in May (34.07 %), without any preparation before shearing (74.90 %). Sheep breeders usually shear their sheep themselves (68.56 %), using hand shears and electric sheep shearing machines in equal proportions (48.82 %: 47.65 %). At about 85 % of farms less than 3 kg of wool per sheep is sheared, with a predominance of farms producing up to 1.5 kg of wool on average (47.53 %). Furthermore, sorting and washing of wool is not a common practice among sheep breeders in Croatia (4.30 % and 2.36 %). Only 22.06 % of sheep breeders manage to sell the wool, while most of them (77.94 %) dispose of it in various ways, often harmful to the environment. Thus, most of the breeders burn the wool (29.86 %) or dispose it in the landfill (27.49 %), which is a great danger for the environment, so it is necessary to find a suitable and sustainable solution for the disposal of sheep wool.

**Keywords**: wool, shearing, sorting, disposing, environment

# Inbreeding depression and inbreeding load for litter size in Black Slavonian pig

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**Summary**

Despite the relatively high number of individuals in the population of Black Slavonian pig, studies showed low genetic diversity in terms of absolute inbreeding coefficients and inbreeding rates. One of the known consequences of inbreeding is depression, which leads to a reduction in the phenotypic yield of fitness-related traits and other economically important traits. The genetic basis of inbreeding depression is the high degree of homozygosity in inbred individuals. This results in the presence of recessive allele pairs and the loss of the advantage of over-dominance at heterozygous loci. However, inbreeding depression in a population is highly variable and depends on the genotype of individuals that produce inbred individual. This variability can be attributed to a specific hidden individual inbreeding depression load. The aim of the study was to analyze the inbreeding depression for number of piglets born alive in 18470 litter records of 5642 sows and evaluate and predict the inbreeding loads for inbred individuals in the population. The pedigree contained 6199 triads. The total number of inbred animals was 1826. We used Mendelian decomposition of inbreeding and multivariate analysis with direct additive and inbreeding depression load effects. Small, but not significant effect of inbreeding on number of piglets born alive was found in Black Slavonian pig. However, mating plans should be created considering these results and the application of optimal contribution selection is an adequate tool to control inbreeding in the population.

**Keywords**: inbreeding depression, inbreeding load, litter size, Black Slavonian pig

# The relationship between birth order and teat order of piglets in highly prolific sows: a preliminary study

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**Summary**

Litter management is an important task in the production of highly prolific sows. There is no simple answer to the question of what breeders should rely on to foster/mix piglets effectively. Among other things, teat order significantly determines the behavioural profile of piglets and thus their ability to cope with competition in the litter. In addition to knowing the behavioural profile, it is important to determine the suckling position of the piglets as early as possible in order to adapt cross-fostering to the conditions in the potential foster litter, e.g. to (un)occupied teats. As the teat order is only formed a few days after birth, it is important to find an early clue to predict the suckling position of the piglets. One could be the order of birth, which also determines the time of first contact with the mother's udder and suckling. Therefore, the aim of this preliminary study was to find out whether birth order (BO; determined by direct observations) and teat order (TO; determined by video analysis) are related. In addition, we also analysed body weight in relation to BO. A total of 65 piglets from 4 litters were studied under farm conditions. Although the first suckling usually occurs on the posterior udder, piglets born earlier tended to establish their later suckling position towards the front udder (over ¾ on the anterior and middle udder) and late-born piglets occupied teats on the posterior udder significantly more often (over 53%). Birth weight tended to be positively correlated with BO (r = 0.23; i.e. heavier piglets were born late), while weaning weight showed no correlation with it (0.06). Our preliminary results suggest that birth order is important information for breeders to rely on when planning litter management. However, recording birth order is not straightforward and methods for its verification, including the use of modern technologies, should be further considered to improve litter management on farms with highly prolific sows.

**Keywords**: birth order, teat order, large litter, litter management, highly prolific sow

# The prevalence of subclinical and clinical mastitis and its effect on milk yield of dairy cows bred in various region

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**Summary**

In this study, the prevalence of subclinical (SCM) and clinical (CM) mastitis and its impact on subsequent milk production among Holstein and Simmental cows bred in different breeding regions (Eastern, Central, and Mediterranean) were analysed. The analysis included over 8 million test-day records and used somatic cell count (SCC) as an indicator of mastitis. SCC below 200,000/ml indicates healthy cows, 200,000/ml to 400,000/ml indicates subclinical mastitis, and exceeding 400,000/ml signifies clinical mastitis.

The obtained results showed that both breed and breeding region significantly influenced the prevalence rate of SCM and CM. The highest prevalence of SCM (15.11%) and CM (24.69%) was in Holsteins bred in Central. In Simmental cows, the highest prevalence of SCM (14.47%) was determined in Central, while the highest prevalence of CM (20.07%) was determined in the Mediterranean region. Furthermore, the lowest daily milk yield was observed on the day when SCM or CM was detected in both breeds and all regions (22.05 kg/day at SCM, and 20.02 kg/day at CM in Holsteins in the Mediterranean; as well as 16.58 kg/day at SCM, and 14.89 kg/day at CM in Simmentals in Central region. However, milk yield increased in subsequent recordings, and the extent of the increase varied based on breed and breeding region. Holstein cows in the Eastern region (41.73 kg) and Simmental cows in the Mediterranean region (39.64 kg) showed the highest total increase in milk production.

**Keywords**: milk yield, (sub)clinical mastitis prevalence, Holstein, Simmental, breeding region

# Possibilities and challenges of substituting soybean meal with full-fat black soldier fly larva meal (*Hermetia illucens* L.) in broiler nutrition

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**Summary**

The aim of our work was to examine the effects of a partial (50%, 75%) and total replacement of soybean meal with full-fat meal black soldier fly (*Hermetia illucens* L.) in broiler chickens' diets on the internal organs morphology and intestinal morphometry. Histopathological evaluation included: liver, kidney, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, bursa of Fabricous, spleen, and lungs. Morphological analysis of gut included height, width of the villi, crypt depth, and thickness of the muscular layer. Main histopathological lesions were diagnosed in liver, alimentary system and lungs. Glycogenic degeneration of the hepatocytes, infiltration of lymphoid cells around blood vessels and bile ductules, and presence of proteinaceous material in sinusoids shown significant differences compared to control group in liver. In the lungs infiltration of lymphoid cells and granulomatous pneumonia were more frequent diagnosed. In the gut, irregular surface of the villi, Goblet cells hyperplasia and undigested feed were observed mainly in experimental groups. Morphometric analysis of the gut revealed differences between experimental and control group in villi height and crypt depth in duodenum, ileum with higher values in experimental groups. All experimental groups show thickening of the intestinal muscular layer. Addition of more than 50% of the full-fat meal black soldier fly in diets has negative impact on intestinal morphology, and change morphology of the internal organs.

**Keywords**: black soldier fly, broiler, internal organs, morphology

# Effect of dietary supplementation of the industrial hemp leaves on physical properties of broiler chicken meat

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**Summary**

The aim of the study was to determine the effect of dietary supplementation of the industrial hemp leaves (*Cannabis sativa* L.) on the physical properties of broiler chicken meat. The study was conducted on 100 male Ross chickens, which were divided into 4 experimental groups. The control group (K-0) was fed without hemp leaves, while groups P-1, P-2 and P-3 were supplemented with 10 g/kg, 20 g/kg, and 30 g/kg of industrial hemp leaves, respectively. The animals were slaughtered at 42 days of age. The physical properties of chicken meat (thawing loss, pH value, color, cooking loss) were determined on the 3 breast muscle samples (*M. pectoralis superficialis*) per group. The results of the present study show that the b* color parameter was significantly higher ($p<0.05$) in group P-1 than in the other groups. No statistically significant differences were found between the other investigated physical properties of broiler meat ($p>0.05$). Dietary supplementation of the industrial hemp leaves did not affect the investigated physical properties of the chicken meat. Nevertheless, it is important to consider the positive results of previous studies on the effects of different hemp products (such as seeds, oil, or cake) on chicken meat quality, as well as trends in the poultry industry. In this sense, it is necessary to consider the possibility of conducting research based on the supplementation of different hemp products in chicken feed, larger sample size, and numerous other meat quality attributes.

**Keywords**: hemp (*Cannabis sativa* L.), physical properties, chicken meat

# The rib-eye area in cattle determined by different methods

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**Summary**

The rib-eye area (REA) is an important indicator of cattle carcass composition since it is positively correlated with carcass conformation. The aim of this study was to determine the correlation between the REA of cattle measured with different methods and estimate (dis)advantages of these methods. The REA from 131 young bulls was traced on a transparent foil on the cross-section between the 12$^{th}$ and 13$^{th}$ rib. Polar planimeter (used as referent method), adjusted grid method, two-point method and AutoCAD software were used to determine REA. Before analysis in AutoCAD, traced areas were scanned and saved in JPG format. The smallest ($p<0.05$) REA was determined with AutoCAD (89.22 cm$^2$) compared to other methods (90.44, 90.76, 91.15 cm$^2$ with a planimeter, two-point and adjusted grid method, respectively). Strong positive ($p<0.0001$) correlation coefficients were found between areas measured with a planimeter and other methods (0.954, 0.947 and 0.978 for the two-point, adjusted grid method and AutoCAD method, respectively). Determining the REA with a planimeter is relatively fast but requires high precision and concentration. In AutoCAD, if any mistakes are made, they can be quickly corrected, and the surface is automatically recalculated. However, this method can lead to underestimation of REA. The adjusted point grid method subjectively seems to be the fastest but requires much experience in counting points. The two-point method is the simplest, but suitable for elliptical, not for irregularly shaped REA.

**Keywords**: rib-eye area, planimeter, grid method, two-point method, AutoCAD

# Effects of climate change on sheep flock health

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**Summary**

The aim of this study was to determine the effects of extreme weather conditions caused by climate change on the health of sheep in Croatia. The study was carried out on 27 flocks of sheep in continental, mountainous and coastal areas. Anamnestic data were used for the study, followed by clinical examinations, coprological and serological examinations and a necropsy. It was found that extreme weather conditions affect the health of sheep flocks in two ways - indirectly and directly. Indirectly, the negative impact of prolonged dry and rainy periods on feed quantity and composition is reflected in reduced feed intake, poor condition and a decline in the general resistance of the sheep. Changes in meal composition due to the lack of certain feeds can cause digestive and metabolic disorders that directly affect flock productivity, especially in dairy sheep. The direct impact is manifested direct on the animal, such as the impact of high temperature on the appearance of heat stress. High environmental temperatures lead also to a decrease in food consumption and reproductive disorders. It was also found that long periods of rain accompanied by above-average ambient temperatures increase the risk of mass invasion of ticks, especially in herds on the islands. In addition, the occurrence and spread of bluetongue in Croatia is interpreted as a consequence of global warming, while long periods of rain or drought are associated with rot and listeriosis. Based on this research, it can be concluded that, due to climate change, farmers will have to change the technology of food preparation and sheep farming, especially in dairy herds, as well as veterinary preventive measures in the long term in order to adapt to diseases that occur as a result of weather extremes.

**Keywords:** climate change, sheep, flock, health

# Factors affecting intramuscular fat content in pigmeat

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**Summary**

The intramuscular fat (IMF) content, especially in pigs, significantly impacts its meat quality. In the context of genetics, this fat level varies across species, breeds, and even between different types of muscles within a breed. Environmental factors like nutrition significantly contribute to this variation, as well as the factors like gender and age. The amount of fat is closely linked to the number and size of fat cells in the muscles, influenced by the rate of muscle growth. Early developmental stages, such as the growth and differentiation of fat cells and the surrounding connective structure, can influence individual differences in fat content in pig carcass. Increasing the muscle mass and carcass leanness, which was the trend in selection schemes for decades also reduced overall fat content in the muscles of pigs. However, in recent selection practices, more focus was given to increase IMF content in pigmeat. These efforts to increase fat content have explored genetic approaches using ultrasound measurements on live animals and identifying DNA markers influencing IMF distribution. Advances in genomics, including transcriptomics and proteomics, have improved our understanding of fat accumulation in various muscle tissues. Controlling the IMF content in pig carcasses through nutrition has proven to be challenging compared to genetic strategies. Overall, the biological mechanisms explaining IMF content variability differ between genetic and nutritional factors. The objective of this study was to provide extensive overview of literature findings and current knowledge about IMF content, and finally to provide guidelines in future pig breeding programs.

**Keywords**: intramuscular fat content (IMF), pigs, meat quality, selection

# Genome-wide signatures of selection and gene ontology analysis of East Adriatic sheep

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**Summary**

Sheep breeding boasts a rich history in the Croatian Mediterranean region, where it thrives in traditional systems devoid of targeted breeding programs for carcass or milk yields. Among the 9 local breeds registered in Croatia, 8 find their roots in the Mediterranean region. These breeds display a diverse array of phenotypes, encompassing variations in size and colour. However, their overarching characteristic lies in their multipurpose background, serving roles in milk, meat, and wool production. Robustness and resilience to environmental conditions unite these breeds. Recent advancements in high-density genome-wide SNP data have empowered a more precise identification of selection signatures, thus enabling a cross-examination between studies. In this study, we genotyped East Adriatic sheep breeds using HD Illumina Infinium 600K SNPchip. Genomic regions displaying strong selection signals, along with candidate genes pinpointed through extreme ROH (Runs of Homozygosity) island approach, extended haplotype homozygosity (EHH), and composite likelihood ratios (CLR), underwent further evaluation via gene ontology. This approach proves invaluable in grouping genes with similar biological functions. The analysis uncovered genomic regions and potential candidate genes pivotal in the local adaptation of sheep in the East Mediterranean area, shedding light on their distinctive production types.

**Keywords**: Genomic selection signatures, East Adriatic sheep, extreme ROH islands, gene ontology, extended haplotype homozigosity

# Determining the mineral composition of „Paški sir“ cheese using inductively coupled plasma mass spectrometry method

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**Summary**

The primary objective of this study was to investigate how the technological process impacts the mineral composition of „Paški sir“ cheese. The mineral composition of „Paški sir“ cheese samples (n=18) was determined using inductively coupled plasma mass spectrometry (ICP-MS) method. For the purposes of the study, the cheeses were divided into three groups: (1) control group (n=6), cheeses produced with natural commercial rennet (Bioren, Christian Hansen, Denmark) and commercial microbial cultures (Di Prox, Bioprox, France); (2) group (n=6), cheeses produced with lyophilized indigenous lamb rennet and microencapsulated microbial cultures; (3) group (n=6), cheeses produced with innovative microcapsules containing indigenous lamb rennet and microbial cultures. The mineral profile of the samples included the determination of the content of Ca, Cu, Fe, K, Mg, and Na. The average mineral contents (mg/kg) depending on the group (group 1, 2, 3) were: Ca (9492.48, 8728.02, 8021.89), Cu (0.42199, 0.67070, 0.66707), Fe (8.04236, 10.98963, 14.58159), K (1426.09, 1247.86, 1361.19), Mg (606.50, 620.20, 579.05), and Na (17678.07, 16018.07, 16678.38). Analyzing the mineral composition of cheese can aid producers in upholding product quality and investigating its nutritional aspects.

**Keywords**: “Paški Sir” cheese, minerals, nutritional aspects

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# The effects of different dietary protein inclusion levels on capons growth performance

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**Summary**

The aim of this study was to compare selected growth performance traits in capons fed diets with different protein content at different ages. The experiment was performed on 288 commercial crossbred dual-purpose Rosa 1 male chickens. At 8 weeks of age, the cockerels were surgically castrated (EC No. 543/2008). Until 9 weeks of age, capons were randomly assigned to 3 dietary treatments; dets containing 15% (group 1), 18% (group 2) and 21% (group 3) protein. In all groups, isocaloric diets were composed of the same major ingredients. The birds were raised to 16, 20 and 24 weeks of age. Both age (P>0.000) and diet (P = 0.004) exerted significant effects on the body weight (BW) of capons. An interaction between the experimental factors was also observed (P = 0.044). At 16 weeks of age, the BW of capons was lower in groups 1 and 2 than in group 3 ( P> 0.05). At 24 weeks of age, no significant differences in the BW of capons were found between groups. There was no effect of diet on daily feed intake (DFI) in any of the age groups. Diet had a influence on the feed conversion ratio (FCR). At 16, 20 and 24 weeks of age the FCR was lower in group 3 than in groups 1 and 2. At the end of rearing period (24 wk), FCR in groups 1, 2 and 3 was respectively; 4.24 kg/kg, 4.34 kg/kg and 4.11 kg/kg (P> 0.05). In conclusion, dietary protein inclusion levels had a significant effect on BW in capons to 20 weeks of agee. Capons fed a diet containing 21% protein were characterized by the lowest FCR.

**Keywords**: capon, feed, dietary protein inclusion, growth performance

# Genetic markers and fatty acids in sheep meat: exploring links to sensory properties

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**Summary**

The incorporation of sheep into diverse agricultural contexts has given rise to the development of distinct sheep breeds, each exactly adapted to thrive in varying climatic conditions, with a specific emphasis on optimizing meat production. This paper researches the intricate connections between candidate genes, fatty acid composition, and sensory properties in sheep meat. Within this analysis, the focus is directed towards selected genes that have garnered substantial research attention in recent years, each intricately correlated with many fatty acids linked to the sensory properties of meat. Improvements in meat flavour, tenderness, and juiciness can be achieved through augmenting intramuscular fat content and optimizing fatty acid profiles. The dynamics of lipid accumulation, degradation, and the transportation of fatty acids play a crucial role in governing the deposition of intermuscular fat. This deposition is primarily orchestrated by a confluence of genetic and dietary factors. In addition to exploring the molecular and biochemical aspects of meat quality, this study places a crucial emphasis on sensory analysis. Sensory attributes encompassing flavour, tenderness, juiciness, and overall eating quality, are subsequently correlated with the fatty acid composition of the meat. By unravelling the genetic underpinnings that dictate the fatty acid composition of sheep meat and understanding their implications for sensory attributes, this research aims to lay the groundwork for strategies focused on producing meat products with superior nutritional and sensory characteristics.

**Keywords**: candidate genes, fatty acids, sheep meat, sensory attributes, *Ovis aries*

# Morphopmetric characteristics and carcass cuts of the Pag sheep

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**Summary**

The study aimed to assess the carcass and viscera weight, as well as various morphometric measures in 5 to 7-year-old culled Pag sheep. These morphometric measures included the length of carcass and halves, depth and width of chest, width of pelvis, circumference and width of buttock, lower leg length, and subcutaneous fat thickness. Additionally, the study aimed to establish models for estimating the weight of high-quality carcass cuts (buttock, loin, back, and shoulder; BLBS) using morphometric traits through multiple linear regression. The average slaughter weight of the Pag sheep in the study was 15.6 kg, with a range from 12.45 to 21.65 kg. The coefficients of variability were highest in kidney and pelvic fat weight (163%) and subcutaneous fat thickness (121%). In contrast, morphometric traits showed low variability, ranging from 2.77% to 9.30%. This indicates external uniformity despite differences in fatness levels. Notably, the variability of carcass cuts in Pag sheep ranged from 12.53% to 38.02%, with the buttock accounting for the largest proportion of the carcass at 25.35%. The study highlighted three models for estimating BLBS yield, all of which consistently showed a high adjusted coefficient of determination, ranging from 0.9486 to 0.9868. Among these models, the one comprising carcass weight, lower leg length, buttock width, and chest depth was identified as the most suitable for estimating the weight of high-quality carcass cuts in Pag sheep.

**Keywords**: Pag sheep, carcass, morphometric traits, carcass cuts, linear multiple regression

# Convergent Genomic Signatures of Adaptation in Livestock Guardian Dogs and Gray Wolves

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**Summary**

Livestock Guardian Dogs (LGDs) and Gray Wolves (*Canis lupus*) are contrasting but related pillars within the family *Canidae*. LGDs, bred to protect livestock, bear the marks of both natural and artificial selection, while Gray Wolves remain untamed apex predators shaped solely by natural selection. Despite these disparities, both species share common genetic threads reflecting their adaptation to ecological niches and interactions in regions where LGDs protect livestock from Gray Wolves. We conducted a comprehensive genomic analysis using advanced methods such as eROHi, iHS, nSL and HRiD to identify positive selection signatures in LGDs and Gray Wolves, while genetic differentiation is assessed using the $F_{ST}$ approach. Our study revealed shared and case-specific positive selection signatures indicating convergent evolution in response to common ecological challenges. Our analysis reveals 26 regions with 161 candidate genes in LGDs and 43 regions with 552 candidate genes in Gray Wolves, identified by multiple approaches. In addition, three shared candidate regions with 12 candidate genes indicate possible convergent evolution in response to common ecological challenges. This research sheds light on the genetic adaptations underlying the unique traits and behaviors of LGDs and Gray Wolves, and improves our understanding of their co-evolutionary dynamics.

**Keywords**: Livestock Guardian Dogs, Gray Wolves, genomic signatures, adaptation

**Acknowledgements:** This study was supported by project ANAGRAMS-IP-2018-01-8708 funded by the Croatian Science Foundation and by the project KK.01.1.1.04.0058 funded by the EU Operational Programme Competitiveness and Cohesion in the period 2014–2020.

# Estimation of genomic inbreeding ($F_{ROH}$) in Croatian Holstein cattle population

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**Summary**

More precise estimates of inbreeding coefficients in a population can be obtained using genotypic data involving long stretches of homozygous genotypes known as runs of homozygosity (ROH). Knowledge of ROH length also provides the possibility to distinguish between distant inbreeding (short segments) and recent inbreeding (long segments). The objective of the study was to estimate inbreeding using ROH ($F_{ROH}$) in the Croatian Holstein cattle population. The study was conducted on 90 genotyped cows (Bovine 50K SNP BeadChip), but after quality control of genomic data (call rate per SNP 0.95 and animal 0.90) using the PLINK software (v1.09), 85 animals and 45,661 SNPs were retained in the inferential statistical analysis. PLINK toolset was also used for estimation of lengths of the ROH segments of different size. The estimation of genomic $F_{ROH}$ () was conducted in R programme based on the following criteria: the minimal number of SNPs in ROH was calculated by the L-parameter, the maximal gap between SNPs was set to 1 Mb, the minimum SNP density was set to 1 SNP every 150 kb, no heterozygotes allowed, and the minimum length that constituted the ROH was set to 1 Mb. A total of 3,116 autosomal ROHs ranging from 1.0 to 59.5 Mb were detected. On average, there were ~37 ROHs per animal with an average length of ~6.94 Mb. Individual $F_{ROH>1\ Mb}$ ranged from 4.33% to 19.74% with an average of 10.25%. The frequencies of $ROH_{1\text{-}2\ Mb}$, $ROH_{2\text{-}4\ Mb}$, $ROH_{4\text{-}8\ Mb}$, $ROH_{8\text{-}16\ Mb}$, $ROH_{>16\ Mb}$ were 2.7%, 33.9%, 37.0%, 19.3%, and 7.1%, respectively. The estimated $F_{ROH1\text{-}2\ Mb,}$ $F_{ROH2\text{-}4\ Mb,}$ $F_{ROH4\text{-}8\ Mb,}$ $F_{ROH8\text{-}16\ Mb,}$ $F_{ROH>16\ Mb}$ were 0.07%, 1.50%, 3.06%, 3.15%, and 2.47%, respectively. These results suggest that majority of the inbreeding events occurred within 20 generations ago.

**Keywords:** Holstein, SNP data, inbreeding coefficient, matting

# Hygienic strategies of farm animal welfare development

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**Summary**

Animal welfare is an important attribute of the "food quality concept" because its lowering causes greater exposure of animals to environmental hazards, increasing the risk of diseases (especially infectious) and, consequently, increases the probability of drug/antibiotics residues in food of animal origin. The aim of our research was to develop hygienic strategies to improve the livestock welfare. We have tried to determine if essential oil mist might reduce the concentrations of volatile compounds (greenhouse gases, ammonia, odour compounds), dust fractions and microbes in broiler houses. We have noted that the essential oils misted in poultry houses have a potential to reduce respirable dusts, bacteria, fungi, carbon dioxide, ammonia, methyl mercaptan, o-cresol and aniline. In the next study we have determined the hygienic effect of classic straw and six alternative bedding materials (light pellets, medium sawdust, peat, chopped straw, flax, hemp) in calf housing. Considering the total counts of aerial bacteria, the best quality characterized peat, light pellet, and sawdust. The most contaminated air was registered using traditional straw. Whereas, in the case of fungi, chopped flax and hemp had the best impact on the high quality of air. The fungal contamination levels were the highest using light pellet and peat. The lowest concentrations of aerial particulate matters, ammonia and hydrogen sulfide were registered in boxes with light pellet and sawdust. We have concluded that above described hygienic strategies significantly influence livestock welfare improvement. Therefore, it is worth continuing research in this direction to improve described methods.

**Keywords**: animal welfare, hygiene, essential oil, bedding material

# The emerging concern of microplastics and nanoplastics in animal health

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**Summary**

In recent years, the pervasive presence of microplastics and nanoplastics in the environment has raised concerns about their animal health impact. These tiny plastic particles, often under 5 millimeters, infiltrate ecosystems, potentially harming a wide range of species. Microplastics stem from plastic item breakdown, while nanoplastics can come from various products. They've been found in animals' digestive systems, tissues, and bloodstream, raising bioaccumulation concerns. Microplastics and nanoplastics enter domestic animals through inhalation, ingestion, or skin contact. Inhaled microplastics, originating from dust and materials like textiles and tires, are common. Ingestion via the food chain and water is also prevalent. Although skin is generally impermeable, entry is possible through wounds, sweat glands, or hair follicles. Studies reveal physical harm from ingestion, including tissue damage, blockages, and nutrient absorption reduction. These particles may carry toxic chemicals, leading to health issues like endocrine disruption, immune problems, and increased disease susceptibility. Addressing this concern is crucial for animal and human well-being. Interdisciplinary research is needed to understand these impacts and develop mitigation strategies. Immediate attention, research, and collaboration are essential to minimize the impact of microplastics and nanoplastics on animal health.

**Keywords:** microplastics, nanoplastics, animal health, bioaccumulation

# Artificial Intelligent (AI) technologies in livestock production systems

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**Summary**

The influence of the Fourth Industrial Revolution on agriculture, often referred to as Agriculture 4.0 or Smart Agriculture, extends to livestock production systems, transforming them in significant ways. This ongoing wave of change builds upon the advancements of industrialization witnessed in the First, Second, and Third Industrial Revolutions.

However, the Fourth Industrial Revolution represents a new era, introducing a range of cutting-edge technologies that have revolutionized livestock production systems. Artificial intelligence (AI), machine learning, cloud computing, and smart sensors have become an integral part of managing and enhancing these systems. In this context, the realm of Big Data Analytics has emerged as an essential player, aiding in the interpretation and utilization of the vast amounts of data generated by these systems. Within intensive livestock production systems, such as those in dairy, pork, and poultry, digital and AI-monitored automated milking and feeding systems, estrus detection equipment, and animal behavior monitors have gained prominence. These technologies enable a more precise assessment of animal behavior and vital activities, leading to increased production efficiency and improved animal welfare. "Smart" technology and equipment are also employed to control environmental conditions, encompassing ventilation, temperature, humidity, and lighting, ensuring optimal production conditions are maintained within manageable parameters. Overall, Agriculture 4.0, with its AI technologies and other cutting-edge advancements, has revolutionized livestock production. By embracing and harnessing the power of Agriculture 4.0, the industry can pave the way for a more productive, sustainable, and animal-friendly future.

**Keywords**: artificial intelligence, smart farming, livestock production, technologies

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# Book of Abstracts

Viticulture, Enology and Pomology

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Vinogradarstvo, vinarstvo i voćarstvo

# Effect of timing of leaf removal on volatile composition and content of *Vitis vinifera* cv. Merlot grapevines

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**Summary**

Leaf removal in the cluster zone is one of the commonly applied viticultural practices used to increase light penetration and to decrease humidity in dense foliage. The timing of leaf removal plays a major role in the synthesis of volatile compounds in grapes, since they are synthesized at different times during berry ripening.

A two- year study (2018/2019) has been carried out in the vineyard in the continental part of Croatia, Zagreb vineyard hills, to study the effects of different timings of leaf removal in relation to the volatile profile of grape variety 'Merlot' (*Vitis vinifera* L.). The yield and the ratio of leaf area to yield was not affected by the leaf removal, while eary leaf removal (before flowering) reduced grape and berry weight. Regardless of the timing, leaf removal reduced total acidity in the must, while only leaf removal carried out at the beginning of the veraison led to an increase in the sugar content in the must. Leaf removal reduced the content of C6 volatile compounds, that give negative green and vegetal aromas.

Modification of the canopy microclimate can signifficantelly improve volatile composition of the grapes, and therefore contribute to the grape quality.

**Keywords**: grapevine, leaf removal, grape volatile compounds

# Role of non-*Sacchoromyces* yeasts in the aromatic profile of cv. Babić red wines

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**Summary**

The research analyzed the influence of different non-*Saccharomyces* yeast species on the aromatic profile of red wines made from Babić grape variety. The grape originated from the Middle and South Dalmatia, from two wine-growing hills Šibenik (position Jadrtovac) and Primošten (position Široke), vintage 2020. The aim of the study was to determine the differences between the control treatment with *Saccharomyces cerevisiae (Sc)* strain and the sequential inoculation treatments with *Lachancea thermotolerans (Lt) x Sc* and *Toraluspora delbruecki (Td) x Sc* on the concentration of individual and total esters and terpenes. Both sequential inoculations with *Sc* were implemented 72 h after the non-*Saccharomyces* strains. The maceration process lasted for eight days with the daily manual punching-down of the pomace cap, while the alcoholic fermentation lasted for 14 days in total. Volatile aromatic compounds in final wines were determined by the SPME-Arrow- GC/MS method. The results showed a significant difference between the control treatments and sequential inoculations in Babić wines from both wine-growing positions regarding the *cis*-linalool oxide, geraniol, neric acid, and nerol, terpenes that contribute to sensory profile with floral and rose-like aromas. Significant differences were shown between some ethyl esters e.g. ethyl-furoate, -hexanoate, -lactate, -2-hydroxy-3-methylbutanoate, -3-hydroxybutanoate; diethyl-glutarate and -succinate, which contribute to the aromatic profile with fruity, buttery, overripe or aging aromas.

**Keywords**: ethyl esters, GC/MS, *Lachancea thermotolerans,* terpenes, *Toraluspora delbruecki*

# Valorization of the 'Plavac mali' variety in the period from 2015 to 2023.

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**Summary**

'Plavac mali' is the most significant indigenous red grape variety in Croatia. With a share of approximately 8% in the total vineyard areas and a 5% presence in the domestic wine market, this variety plays a pivotal role in the Croatian wine industry. It has undergone a process of clonal selection, resulting in the registration of five clones, and certified planting material of this variety is available on the market. Its importance is further emphasized through two protected designations of origin, 'Dingač' and 'Potomje,' which confirm its irreplaceable role in the Croatian viticultural heritage. To support its quality, it's worth noting that 92% of the total wine produced from this variety is labeled with a protected designation of origin, and 22% is classified as premium wine according to traditional standards (KZP). This paper provides an overview of the trends in the production of 'Plavac mali,' its regional representation, quality guidelines, as well as the physico-chemical and sensory characteristics of the wine during the considered period.

**Keywords**: *Plavac mali, wine, market, Dalmatia, indigenous variety.*

# Pollination effects on fruit and seed characteristics of 'Golden Delicious' apple trees

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**Summary**

The aim of the work was to determine pollination effects on certain characteristics of 'Golden Delicious' (*Malus domestica* Borkh.) fruit and seeds. Field experiment was carried out as part of PRIMA SafeAgroBee (Horizon 2020) project in 2022 on trees in 4 apple orchards in location Baštica, Zadar County. Experiment was set up on five trees with four inflorescences (treatments) per orchard. Treatments that were applied to inflorescences were open pollination (O), open pollination with hand pollination (OH), excluded pollination with hand pollinations (EH) and excluded pollination (E). Before flowering inflorescence in treatments E and EH was closed with an anti-insect mesh. During flowering, flowers in the inflorescence in treatments OH and EH were hand-pollinated with pollen of a compatible cultivar., Before thinning, initial fruit set is determined as a percentage of fertilized flowers. Furthermore, fruit weight and diameter, symmetry index, sugar content, skin aspect and number of developed seeds were observed. It was determined that the closing of inflorescence with mesh had a significant effect on the percentage of fertilized flowers. Specifically, highest percentage of fruit set was in treatment O with 87.7% of fertilized flowers while in treatment E only 36.3% of flowers were fertilized. Results showed differences in fruit weight, diameter and skin aspect between treatments, but these differences were not significantly different. However, the number of seeds in the fruit was significantly affected by treatments. Average number of seed per fruit was highest in treatment OH (8.16) and lowest in treatment EH (5.22). In conclusion, the lack of pollinators significantly affects flower fertilization and seed development in apple fruit.

**Keywords:** apple, fertilization, fruit traits, pollination, seed development

# Vine load as an important factor in the quality of must and wine of the Graševina variety

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**Summary**

The research was conducted during the growing seasons of 2021; 2022; and 2023 at the viticulture and wine-growing experimental station owned by Faculty of Agrobiotechnical Sciences Osijek located in Mandićevac, wine-growing, sub-region of Slavonija, Đakovo vineyard. The experiment was set up as a randomized block design with six treatments in four replications. Treatments were pruned with: 8, 12, 16, 20, 24 and 28 buds. The goal of the research was to examine the influence of yield on some qualitative parameters of must and wine of the Graševina variety, since yield is an important factor in the economics of grape and wine production. Some of the parameters that were measured were the following: number of bunches, average yield per vine, total soluble solid, total acidity, alcohol content and relative density. Based on the measured results, an ANOVA was made. A statistically significant influence of vine load level on the number of vine bunches was determined, and the results also differed between years. Although other parameters differed between years, no statistically significant influence of applied treatments was found. The increase in vine load (number of buds) did not show a negative impact on the qualitative parameters of must and wine. The conducted research provides good guidelines for continuing the study of the influence of vine load on grape wine quality.

**Keywords**: must, wine, Graševina, buds, load level

# Morphological characteristics of the fruit of strawberry tree (*Arbutus unedo* L.) and myrtle (*Myrtus communis* L.) in Zadar county

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**Summary**

Strawberry tree (*Arbutus unedo* L.) and myrtle (*Myrtus communis* L.) are wild fruit species that are widespread throughout the Adriatic region. Both species grow in the form of a bush or a small tree, and have edible fruits (berries) that make these two wild species interesting. Aim of this work is to determine the morphological characteristics and basic chemical composition of strawberry tree and myrtle fruits in order to assess the differences between individuals of natural populations. Fruit collection was carried out in 2022 on the island of Ugljan and included 13 myrtle and 11 strawberry tree bushes at the stage when the fruits have reached full maturity and before the fruit drop. Obtained results of the average fruit weight in strawberry tree show a range from 13.65 g to 4.32 g, while in myrtle weight ranged from 0.13 to 0.38 g. The average dry matter of the fruit ranged from 23.34 to 33, 35% in strawberry tree, while in myrtle this range was higher, 33.65 to 48.85%. In both species, a large variability was observed between the investigated individuals. Since the research was carried out on wild individuals in different locations, this may be one of the reasons for such variability, but genetic predisposition may also affect the investigated properties, so individuals with larger fruits may certainly be interesting for further research or selection.

**Keywords:** dry matter, length, Mediterranean, width, weight, wild fruit species

# Protein hydrolysates as green tools in sustainable olive production

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**Summary**

Biostimulants are compounds which have a positive impact on plant fitness and resistance to pests without harming the environment and represent a green alternative to synthetic chemicals used in plant nutrition and protection. The bilateral HR-SLO HRZZ-IP-2022-10-8305 project "PROGRESS" aims to design a novel biostimulant originating from olive leaves, which are by-products of the olive oil industry, and to use them in sustainable olive production. Particularly, two main experiments will be performed. In the first, protein hydrolysates' (PHs) effect on the growth of olive seedlings and the chemical composition of their tissues will be evaluated. In the second experiment, PHs will be applied on mature olive trees and plants' performance will be monitored through the differences in composition of minerals and primary and secondary metabolites and the amount and quality of olive oil will be determined. Furthermore, the penetration depth of foliar applied PHs will be studied using molecular imaging (Attenuated Total Reflection-Fourier Transform Infrared microscopy and MeV-Secondary Ion Mass Spectrometry) which will, accompanied by mineral distribution imaging (Micro-Particle-Induced X-ray Emission), enable resolving the mode-of-action of the PHs. Finally, PHs effect on olive spot disease will be tested, where redistribution of elements around the symptomatic necrotic spots will be determined. The project will engage an interdisciplinary team to deliver knowledge required by plant biologists, agronomists, farmers and nutritionists.

**Keywords:** Biostimulant, proteins, enzymatic hydrolysis, fungal infection, plant resistance, olive oil quality.

# Silicon foliar fertilization modulates oleuropein concentration in olive leaves

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**Summary**

The olive leaves are the potent source of different phenolic compounds with secoiridoid oleuropein as the most dominant one. Plant phenolic profile can be modulated with foliar fertilization and silicon (Si), as a beneficial element, has been proven to increase plant strength to different biotic and abiotic stresses.

Thus, our study aimed to determine the effect of silicon foliar fertilization on the olive leaves mineral and phenolic concentrations. The field experiment was carried out as a randomized block design with two foliar treatments (Si+ and Si-) in four replicates. The treatments were applied on sixteen 5-year-old olive trees of the 'Leccino' cultivar during the spring period.

At sampling time, 15 days after treatment, results have shown higher oleuropein as well as ole-aglycone concentrations of the Si+ plants compared to Si– ones. Micro-particle induced X-ray emission (micro-PIXE) analysis has shown higher Si, potassium (K) and phosphorous (P) bulk concentration in the Si+ leaves in contrast to the control treatment. Due to our results foliarly applied silicon was mainly allocated in the leaf epidermis.

**Keywords**: Leccino cv., phenolic, micro-PIXE, phosphorous, potassium.

# Assessment of storage capacity of Golden Delicious apple variety in Zagreb County

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**Summary**

Factors such as soil, variety and production technology greatly affect the mineral composition of the fruit, and thus its storage capacity. In the Republic of Croatia, the Golden Delicious variety is one of the most important varieties and is second in terms of planted areas, also is the second most important variety in Zagreb region. In addition to the economic importance of this variety, it is necessary to emphasize the year-round demand for its fruits on the market. For this reason, fruits intended for storage must satisfy internal and external quality parameters. During the 2020 growing season, leaf and fruit were sampled at four locations within Zagreb County, and their mineral composition was determined. Sampling was carried out at two key moments to determine the susceptibility of fruits to the occurrence of physiological disturbances during storage - in the second half of July and at the time of harvest. Also, at the time of technological maturation or harvest, in addition to the analysis of the mineral composition of leaves and fruits, the following physicochemical analyzes of fruit quality were performed: hardness, soluble dry matter, starch index, Streif index and malic acid. After harvest the fruits were stored and physicochemical analyzes of the fruits were performed once a month. This research was conducted with the aim of determining the mineral nutrition content of leaves and fruits and the storage capacity of the Golden Delicious variety in the Zagreb region.

**Keywords**: apple, Golden Delicious, fruit, leaf, mineral composition, storage

# Quantitative and Qualitative Indicators of the Rosé Wine Market in Croatia: Analysis of Trends 2015. - 2023.

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**Summary**

Rosé wines in Croatia have experienced significant changes in their production, reflecting the increasing demand at both the global and local levels. This phenomenon, internationally referred to as "ROSEEVOLUTION", has also taken hold in the domestic wine market over the past decade. Their share in the overall domestic wine market has doubled, approaching a share of nearly 4%, representing substantial growth over the observed period. From the regions of Istria and Kvarner, as well as Slavonia and Croatian Danube, comes over 70% of rosé wines introduced to the market in the country. Given the changes in market demand, where there is a gradual decline in demand for red wines and a growing interest in light and fresh white wines, rosé wines have positioned themselves as a key component that allows producers to adapt to these trends while preserving the existing assortment. The quality is underlined by the fact that 85% of rosé wines are protected designation of origin wines, within which those traditionally expressed as quality wines KZP are dominant. This paper provides a detailed insight into production trends, varietal and regional representation, qualitative trends, as well as the physicochemical composition and sensory characteristics of rosé wines over the observed period.

**Keywords:** grapevine, rosé wine, market, traditional term, variety

# Variability of pomological and physico-chemical properties of fruits of different apple cultivars from the Zagreb County

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**Summary**

The variability of pomological and physico-chemical properties depends on the genetic characteristics of the cultivar, the area and the cultivation system, the age of the fruit tree, pomo- and agrotechnics, weather conditions, etc. The aim of the work was to determine the pomological and physico-chemical properties of the fruits of the traditional cultivars - TC ('Bobovec', 'Božićnica', 'Kanada', 'Siva Jesenska', 'Zelenika', 'Šampanjka', 'Mašanka', 'Krivopeteljka' and 'Johnatan') and to compare them with the commercial cultivars - CC ('Fuji', 'Jonagold', 'Idared', 'Granny Smith' and 'Golden Delicious'). The fruits were harvested in 2022 in a private orchard with traditional and commercial apple cultivars in Zagreb County. After the laboratory analyzes, it was found that the weight of the fruits ranged from 93.4 g to 217.6 g for TC and from 167.6 g to 208.2 g for CC. The fruit shape index ranged from 0.7-1.0 (TC) and 0.8 to 0.9 (CC). The firmness of the TC fruit ranged from 3.5 to 8.1 kg/cm$^2$ and that of the CC fruit from 3.6 to 7.3 kg/cm$^2$. The soluble solids content was between 10.2 and 17.1 °Brix (TC) and 14.3-15.0 °Brix (CC), the total acid content between 0.5 and 0.9 (TC) and for CC between 0.3 and 0.6 % as malic acid. The results show that CC has less variation in the values of the investigated properties, and has more uniform fruit compared to TC, while some TC have pronounced positive fruit quality properties (e.g. fruit firmness, soluble solids content, total acids, etc.), indicating their potential for fresh consumption, preservation or processing.

This research was funded by the Croatian Science Foundation (UIP-2020-02-8461).

**Keywords:** *Malus domestica* Borkh., traditional cultivars, commercial cultivars, fruit quality

# Grape berry quality parameters of Prokupac variety - Comparison between biodynamic and conventional management

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**Summary**

This study examined influence of two different vineyard management practices – biodynamic (BIO) and conventional (CON), applied in a vineyard of a red wine variety Prokupac, (Serbia, 43°18' 29" N 21°23'15"E), on grape berry quality parameters: soluble solids (SS), pH, total acidity (TA), total phenolic content (TPC), radical-scavenging activity (RSA), and total anthocyanin content (TAC). Both investigated vineyard management approaches were combined with two different winter pruning levels (the bud number per vine was 10 and 20, respectively). Soluble solids content was not influenced by different managements, but very significant differences were found in different bud loads treatments. SS showed significantly higher accumulation in berry in lower bud load treatment, 10 buds (23.8 $^0$Brix) compared to the 20 bud load (21.7 $^0$Brix). On contrary, TA showed significant differences between managements, BIO (6.65g $L^{-1}$) and CON (6.44g $L^{-1}$). There were differences in TPC between BIO and CON treatments (4.86 and 4.16 mg GAE g-1 respectively). While TPC was significantly higher in the BIO management treatment, bud load treatments were not affected this characteristic. Total anthocyanin content (TAC) was affected only by the management practices and was significantly higher in CON management treatment. Mean value for TAC were 0.10 mg mal-3-glu $g^{-1}$ (BIO), 0.19 mg mal-3-glu $g^{-1}$ (CON). TPC, RSA and TA were significantly higher in the biodynamic management treatment compared to the conventional, whereas the pruning level did not affect these attributes. SS content was not influenced by different managements, but different bud load has had. There were not significant differences in pH between applied treatments. These results indicate that the Prokupac has achieved favorable results under the biodynamic management, in terms of quality of wine grapes. In both trailed pruning level treatments, the lower bud number per vine (10 buds) was more favorable. These results clearly justify further research of this topic, with the overall aim to get a full picture of the effect of biodynamic vineyard long management practice on grape quality in Prokupac variety.

**Keywords**: biodynamic management, Prokupac variety, SS, TA, berry polyphenols

# Monitoring and genetic characterization of local grapevine varieties (*V. vinifera* ssp.) from Styria in Slovenia

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**Summary**

Knowing the characteristics and requirements of each grapevine variety is crucial to successful grape production. In the past, many viticultural experts (Clemente, von Vest, Rath, Trummer…) dealt with identification of vine varieties and laid the modern ampelography (the science of grapevine variety description). In the 19th and at the beginning of the 20th century, itinerant teachers played a very important role in this field, organizing in this way various specialized trainings, tested the grapevine varieties in experimental farms and thus created better realistic conditions for variety description. At the beginning of 19th century in Styria around 300 grape varieties were under cultivation. First introduction of new varieties and later also the appearance of phylloxera contributed to the loss of many varieties. The purpose of this project was to find old varieties that existed in this area before phylloxera and to determine them through genetic analyses. Directly collected samples (340 grapevine accessions) from old vineyards within the Slovenian wine-growing region of Styria were analyzed using 24 SSR markers. A total of 56 different genotypes were detected between 2020-2022. After comparison to the available databases, 27 old varieties and 38 unique genotypes belonging to autochthonous accessions were found out. Several parameters were calculated to assess the usefulness of the chosen loci in this work and results representing the genetic similarities among the accessions was constructed using the neighbor joining method to investigate possible parentage relationships in the sample. The unique genotypes will have to be described ampelographically and technologically evaluate in the future.

**Keywords**: *V. vinifera L.*, local varieties, genetic characterization, Styria, Slovenia

59 Croatian 2024 19 International Symposium on Agriculture

Session 9

# Book of Abstracts

Plant Protection

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Fitomedicina

# Plant parasitic nematodes of *Miscanthus* × *giganteus* and their effect on the crop's production

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**Summary**

*Miscanthus* × *giganteus*, a commercial crop for bioenergy and lignocellulosic products was assesed for the occurrence of plant parasitic nematodes and their distribution in Poland and Ukraine. The study found 53 species of the plant feeding nematodes, belonging to 22 genera. Two genera might contribute to the yield losses in both countries: *Longidorus* – vectors of plant pathogens and *Pratylenchus,* ranks among the world list of the most economically important harmful species responsible for lesion disease. For the first time in the rhizosphere of *Miscanthus* × *giganteus* the species *Amplimerlinius macrurus, Rotylenchus agnetis, Rotylenchus pumilus,* and *Paratylenchus nanus* were recorded. This fact indicated that yield loss might be expected during upscaling cultivation of *Miscanthus* × *giganteus* caused by the plant parasitic nematodes, potentially initiating the crop damage. The distribution of the nematode species was evaluated using the non-metric multidimensional scaling which revealed the spatial heterogeneity of sampling points and community dynamics. The results suggested that nematode species indicators of time-sensitive clusters could be considered as indexes of the nematode community dynamics, while those of spatial-sensitive clusters could be considered as pointers of the soil types.

**Keywords**: plant parasitic nematodes, biomass yield, spatial-temporal distribution, *Miscanthus* × *giganteus*

# The brown marmorated stink bug (Hemiptera: Pentatomidae) - a major threat to European crop production

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**Summary**

The brown marmorated stink bug (*Halyomorpha halys* Stål, 1855) is an alien heteropteran species of East Asian origin that was introduced to Europe in 2004. It has easily adapted to climatic conditions and has become a highly invasive pest. *H. halys* is an extremely polyphagous stink bug that attacks more than 300 plant species, preferring tree fruits, field crops, vegetables, and ornamentals. Both nymphal and adult stages cause characteristic damage to host plants by feeding on fruits, pods, buds, and stems. This leads to deformation, especially in fleshy fruits. Later, suberification of the fruit tissue and formation of necrotic spots occur. Stink bugs can also cause indirect damage by transmitting plant pathogens that cause fruit rot, which in turn contributes to economic losses in crop production. This makes this pest a major problem for agriculture worldwide. Effective control measures based on insecticide treatments have been used in practice under integrated pest management, but to reduce the environmental impact in production, new sustainable measures should be used. Recently, biological control by natural predators has received the most attention. In order to prevent further spread and evaluate the effectiveness of control measures, monitoring programs are being implemented in the field. This paper reviews the distribution, biology and ecology of the pest, host plants, damage, monitoring and control measures.

**Keywords**: distribution, biology and ecology, monitoring, control strategies

# Efficacy of different insecticides treatments in controlling brown marmorated stink bug (*Halyomorpha halys*)

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**Summary**

The brown marmorated stink bug (*Halyomorpha halys* Stål) is an invasive polyphagous pest, native to eastern Asia. In recent years, it has been causing more and more damage to a large number of agricultural crops. Among the fruit species in Croatia the greatest damages were recorded on pears. The aim of this study, conducted at Agricultural Institute Osijek, was to evaluate the efficacy of topical application eight active substances in controlling adult forms of brown marmorated stink bug considering that the adults are much more resistant to the insecticidal action compared to the nymphs. Active substances used in treatments were acetamiprid, spinetoram, deltametrin, spirotetramat, azadiractin, nettle and willow oil extract, kaolin, and silicone polymers. Adult forms of the pest were collected with entomological catcher in pear orchard of Agricultural Institute Osijek and placed in plastic containers. Each treatment had three replicates. Treatments with highest mortality (100% after 48 h) that showed the statistical significance were spinetoram, acetamiprid and silicone polymers. It is very important to make the best choice of preparation according to insights on its efficacy, as well as based on its impact on environment and human health.

**Keywords**: brown marmorated stink bug, insecticide efficacy, adult forms, pear

# The influence of temperature on the germination of herbicide-sensitive and herbicide-resistant common windgrass

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**Summary**

Seed germination is a critical stage in the life cycle of plants, especially annual weeds. This research hypothesizes that the herbicide resistance trait affects the germination pattern of an annual winter weed – common windgrass (*Apera spica-venti* (L.) P. Beauv). The experiment aimed to analyze the germination rates of herbicide-sensitive and herbicide-resistant common windgrass biotypes *in vitro,* depending on the germination temperature. The dish experiment was carried out in autumn 2018 and spring 2019, using a free-standing phytotron chamber. The tested seeds germinated at the constant temperatures of 8, 18, or 28 °C, with a photoperiod of 10/14 h day/night. The seeds of thirteen windgrass biotypes were used in the experiment: three sensitive and 10 resistant to various herbicides from the HRAC groups 1, 2, 3, or/and 7. The analyzed germination indices were the First Day of Germination, the Final Germination Percentage, the Mean Germination Time, the Germination Rate Index, the Coefficient of Velocity of Germination, and the Germination Index. The experiment was conducted in a completely randomized design, in two series (spring and autumn) and three repetitions in each series. It was found that temperature affects the germination intensity of sensitive and resistant broom biotypes and is a criterion for grouping the tested biotypes, which proves the similarities between resistant and sensitive biotypes in response to this factor. Seeds germinate better at 8 °C and 28 °C, and resistant biotypes achieve higher germination rates than sensitive biotypes.

**Keywords**: *Apera spica-venti*, germination dynamics, germination indicators

# Identification and subgrouping of cucumber mosaic virus isolate infecting New Guinea impatiens (*Impatiens hawkeri*) in Serbia

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**Summary**

New Guinea impatiens (*Impatiens hawkeri*) is a species of flowering plant in the family Balsaminaceae that is popular as a bedding and pot plant. In May 2020, impatiens plants showing chlorotic and necrotic concentric rings, mosaic, leaf malformation and filiformism were noticed in a greenhouse in Rača Kragujevačka, Šumadija District, Serbia. Collected leaves were serologically tested against impatiens necrotic spot orthotospovirus (INSV), tomato spotted wilt orthotospovirus (TSWV), cucumber mosaic virus (CMV), turnip mosaic virus (TuMV), and tobacco mosaic virus (TMV). Most of tested samples with chlorotic and necrotic concentric rings (81.81%) were positive for TSWV, while CMV was detected in four samples with mosaic, leaf malformation and filiformism. CMV ELISA-positive sample (106-20) was mechanically inoculated to five plants of *Chenopodium quinoa*, *Nicotiana debneyi*, and *N. glutinosa*. Local chlorotic lesions on *C. quinoa* and severe mosaic and leaf malformation on *N. debneyi* and *N. glutinosa* were observed 5 and 13 days post-inoculation, respectively. PCR fragments of all five genes were digested by following restriction enzymes: *Hind*III, *Sac*II (1a gene), *Mlu*I (2a gene), *Stu*I, *Sal*I (2b gene), *Bae*I (MP gene), *Sfc*I and *Hae*III (CP gene). Differentiation of CMV subgroups were conducted based on the restriction patterns obtained *in situ* RT-PCR-RFLP analyses and isolate 106-20 was classified into subgroup IA with haplotype IA; IA, IA; IA, IA. This study reports for the first time the presence of CMV on impatiens in Serbia and its occurrence on this widely cultivated ornamental in our country may have a destructive impact on its production.

**Keywords**: New Guinea impatiens, CMV, bioassay, RT-PCR-RFLP

# Preparation and characterization of copper alginate microparticles as a new plant protection agent

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**Summary**

Phytopathogenic fungi are the living pathogens, causing about 75% of plant diseases and causing great damage to agricultural production. They parasitize almost all parts of plants, especially when moisture is elevated. One of the new methods used in the suppression of phytopathogenic fungi is the encapsulation of compounds in biopolymer microparticles, achieving their timed release and prolonged action. The aim of this research is to prepare and characterize copper alginate microparticles produced by the process of ionic gelation using different initial concentrations of copper ions (2%, 3%, and 4%). Encapsulation of copper cations enables the release of copper ions in certain time intervals. Information about the intermolecular interactions between biopolymers and copper ions was obtained by Fourier transform infrared spectroscopy (FTIR). The main interaction between alginate and copper ions is electrostatic in nature, which is a consequence of different charges. The interaction is also influenced by the presence of hydrogen bonds. The *in vitro* copper cation release profile from different microparticle formulations was fitted to a Korsmeyer-Peppas empirical model. Fickian diffusion was found to be a rate-controlling mechanism of release from microparticles. The results obtained opened perspectives for the future use of copper alginate microcapsules in plant protection.

**Keywords:** alginate, copper, crop protection, microparticles, time release

# Simultaneous encapsulation of silver and zinc ions in biopolymer microparticles

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**Summary**

One of the biggest problems to the world's commercial crop and food production is plant pathogenic bacteria and fungi, which raise production costs dramatically and reduce crop productivity. The aim of this research is to prepare and characterize new formulations of microparticles: microspheres, encapsulating silver and zinc ions in biopolymer, sodium alginate, coating microspheres with a layer of polyelectrolyte complex of chitosan and alginate. Novel alginate microparticles (microspheres and microcapsules) loaded with $Zn^{2+}$, or simultaneously with $Zn^{2+}$ and $Ag^{+}$, were prepared and characterized (microparticle size and surface morphology, encapsulation efficiency, loading capacity, swelling). Investigation of molecular interactions in microparticles using FTIR spectroscopy revealed complex interactions between all constituents. The zinc and silver cations characteristics were not adversely affected by the encapsulation in biopolymer microparticles, and their activity remained quite high. The *in vitro* release profiles of $Zn^{2+}$ and $Ag^{+}$ ions were analyzed by the Korsmeyer–Peppas empirical model. Very low values of the exponent n revealed that Fickian diffusion was a rate-controlling mechanism for both cations, $Zn^{2+}$ and $Ag^{+}$. Lower values of the release constant k for microcapsules imply a slower release of $Zn^{2+}$ or $Ag^{+}$ ions than from microspheres. The outcomes demonstrated that prepared microparticles can potentially be used in plant protection and nutrition.

**Keywords:** alginate, microspheres, microcapsules, zinc ions, silver ions

# Influence of Biostimulants on Physiological Traits and Induced Plant Defense Responses in Tomato Production

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**Summary**

Greenhouse vegetable production is an intensive system with the potential to provide a year-round supply of high-quality vegetables. However, sustainability challenges, climate change, and growing consumer demand for safe food necessitate innovative approaches. Plant biostimulants, substances and microorganisms enhancing nutrient absorption, stress tolerance, and crop quality, hold the potential to support sustainable agriculture by reducing pesticide use and enhancing productivity. The objective of this 2023 research, conducted in a controlled greenhouse environment in Istria, Croatia, from March to August, was to investigate how biostimulants influenced the physiological characteristics of tomatoes and induced plant defense responses against common pests. This study aimed to enhance productivity and provide practical application guidelines. Three biostimulants were applied through both foliar treatment and a novel alginate microcapsule-based method with controlled release: two based on a unique combination of complex copper and phosphite or chelated copper with glutamine, and one based on seaweed extracts with elevated calcium content. Preliminary results indicate that, in comparison to the control variant, all treatments led to increased crop yields and improved yield per plant. However, the copper-based treatment efficiently reduced *Tuta absoluta* damage, albeit at the expense of phytotoxicity, resulting in a lower average fruit yield. This research underscores the ongoing importance of biostimulant studies in advancing sustainable vegetable production.

**Keywords**: biostimulants, foliar treatment, encapsulation, South American tomato moth

# Antifungal effect of tea tree, rosemary and thyme essential oil on development of phytopathogenic fungus *Alternaria alternata*

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**Summary**

A major problem in modern plant production is the limited number of available fungicides due to the resistance of phytopathogenic fungi. The resistance development is significant, especially in the species of the mycotoxigenic genus *Alternaria*, which cause mycoses on a large number of plants. The fungus *Alternaria alternata* (Fr.) Keissler is known as the causative agent of tomato early blight, which causes great damage in crops and in storage. Inhibition of this foliar pathogen is difficult due to the limited number of effective fungicides. Reduced application of fungicides with the same mechanism of action minimizes the emergence of phytopathogen resistance. In order to reduce the pressure of phytopathogenic fungi, biological control with essential oils is one of the innovative options. Accordingly, the antifungal effect of three essential oils (tea tree, rosemary and thyme) on the pathogenic fungus *Alternaria alternata* was tested in laboratory conditions. The results of testing the antifungal effect of essential oils in the volatile phase show that thyme oil significantly inhibited the pathogen by 91.3% and led to significant microscopic changes, while rosemary and tea tree oils did not exhibit a significant inhibition index (16.7% and 0.7%, respectively). Based on obtained results, volatile phase of thyme oil in a low concentration has the characteristics of a potential early blight control agent.

**Keywords**: *Alternaria alternata*, fungistatic effect, disc diffusion method, early blight, resistance

# Decontamination of *Escherichia coli* from fresh tomato fruits by phenylboronic acid

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**Summary**

Coliform bacteria are major contaminant of fresh vegetables, such as tomato fruits. Consequently, *Escherichia coli* infections caused by consumption of row tomato are on the rise in human population. To ameliorate the risk of *E. coli* infections, a safe and economic decontamination of fresh tomato fruits is sought for. Recently we reported phenyl boronic acid (PBA) as a potent antimicrobial compound against plant pathogenic bacteria, which is environmentally friendly, and it is virtually non-toxic to humans and other mammals. In this study we determined minimal inhibitory concentration (MIC) of 1 mg/ml and PBA killing kinetics against *E. coli in* vitro. Based on these results we treated fresh tomato fruits inoculated with *E. coli* by various PBA concentrations. Washing and incubation of contaminated tomato fruits in PBA solution reduced the growth of *E. coli* washed from tomato fruits in a dose- and time-dependent manner, while having no adverse effect on tomato fruits. We conclude that PBA is suitable for decontamination of *E. coli* from fresh tomato fruits.

**Keywords**: antimicrobial, antibacterial activity, microorganisms

# *Pseudomonas syringae* pv. *persicae*, the causal agent of cacterial dieback of peach in the Republic of Srpska

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**Summary**

*Pseudomonas syringae* pv. *persicae* (Psp) is a widespread and economically important stone fruit tree disease. *Pseudomonas syringae* pv. *persicae* was evaluated by the European Commission (EU COM) as Regulated non-quarantine pest (RNQP). In the Republic of Srpska, this national supervision program of the presence of Psp began in 2016 and it is continuously implemented all these years. The main aim is monitoring the presence of pests/entries in orchards and nurseries of peaches and nectarines. Monitoring was carried out in 7 different areas all over Republic of Srpska. Per year, more than 65 samples of stone fruit leaf tissue from symptomatic and symptomless host material were collected and analyzed in commercial production and in nurseries, but also on border crossings. Plant disease diagnosis is based mainly on microbiological cultural techniques on selective media, together with molecular diagnostic techniques (rep-PCR). Positive results were found both in nurseries and in orchards, so it is necessary to continue monitoring the presence of this pathogen in the future.

**Keywords**: *Pseudomonas syringae* pv. *persicae* (Psp), stone fruit, rep-PCR

# Monitoring and identification of fruit-fly species in mango trees in Cuanza Sul, Angola

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**Summary**

Fruit-fly (*Diptera-tephritidae*) pest is one of the primary causes of low fruit quality and substantial economic losses, especially for mango tree (*Mangifera indica* L.) growers worldwide. Hence, identification and monitoring of the main fruit-fly species remains a strategic requirement to reduce levels of infestation and achieve satisfactory economic returns. The purpose of this study was to identify fruit-fly species by monitoring their appearance on different varieties of mango trees planted at Quinta dos Pomares, Cuanza Sul – Angola in an effort to understand their ecology. There were three mango tree genotypes, covering 2 hectares each. Thirty McPhail traps were set up, spaced at eight mango trees intervals. Mango juice at 90% w/v was used as an attractant in the traps to capture fruit-flies. The experiment was carried out over a 6-month period, coinciding with the harvest time. A total of 3469 fruit-flies were captured, which were from three fruit-fly species: *Bactrocera carambolae*, *Anastrepha fraterculus* and *Ceratitis capitata*; the *Bactrocera carambolae* species was the largest population with 60.1% followed by *Anastrepha fraterculus* and *Ceratitis capitata* populations with 33.2% and 6.7%, respectively. The peak of all populations was in January, with significant differences between species. The rate of fruit larvae parasitization was higher in the Haden mango tree genotype. *Bactrocera carambolae* population was considered the main fruit-fly species affecting the fruit quality of mango trees at Quinta dos Pomares, Cuanza Sul – Angola. Therefore, there is the possibility to contribute to the reduction in infestation rates knowing their ecology.

**Keywords**: fly identification, *Mangifera indica* L., insect population, larva

# Onion thrips: a pest of economic importance and a model insect for research

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**Summary**

Onion thrips (*Thrips tabaci* Lindeman) is a serious pest of various cultivated plants, with three distinct lineages within a cryptic species complex. Despite the well-known significance of this pest, many attributes of these lineages are not yet fully understood. The aim of this paper is to call attention to some of these attributes. We recently produced a comprehensive review of plants in which evidence of breeding occurs among the lineages and the whole species complex. The results indicate that leek-associated 2 (L2) is the most widespread lineage by number of host plants and distribution; leek-associated 1 (L1) lineage is primarily found on onion crops and with localized distribution and the tobacco-associated (T) lineage is only reported from tobacco in few locations. We also performed no-choice-design cross-mating experiments under a controlled laboratory environment with virgin adult individuals from all three lineages in order to study the reproductive behavior of onion thrips. We found that the L1 and L2 lineages of this cryptic species complex are reproductively isolated from the T lineage; therefore, they represent different species. Divergence in the behavior of conspecific and heterospecific pairs became evident only after contact. We confirmed mating between thelytokous females (L2) and arrhenotokous males of the L1 lineage. The post-mating behavior of males indicated the presence and role of an anti-aphrodisiac pheromone.

**Keywords**: onion thrips, cryptic species complex, distribution, host plants, reproductive isolation

# Phytopathogenic fungi on olive tree identified for the first time in Croatia

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**Summary**

During field survey conducted in 2021 and 2022, while samples of olive branches showing symptoms of infection with species from the *Botryosphaeriaceae* were collected, olives with symptoms distinct from those caused by the previously mentioned species were observed. Samples were collected and fungal isolates were grown on PDA. Isolates were determined based on the morphological characteristics of mycelium and spores. After morphological determination, molecular identification of isolates was conducted using the PCR method carried out using primer pairs ITS4/ITS1, ITS5/ITS4, Bt2a/Bt2b, Btub2Fd/Btub4RD, and EF1-728F/EF1-986R. The PCR products were sequenced, and the sequences obtained were compared with relevant sequences from the GenBank® database. Through phylogenetic analysis of the sequences, the species *Cytospora pruinosa* Défago, *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai, and *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous were identified. Pathogenicity tests conducted on olive seedlings have confirmed the pathogenicity of these species. It was the first time the species *C. pruinosa* was identified as the cause of olive tree disease in Croatia, although it had previously been described as a cause of olive tree disease in South Africa and Spain. The symptoms it causes include branch and twig dieback, changes in bark color, and tissue necrosis. The species *N. gorlenkoana*, *N. osmanthi*, and *N. philosophiae-doctoris* were not earlier described as pathogens on olive trees. Among these, only *N. osmanthi* had been previously found in olive trees but had not been described as pathogenic. *N. philosophiae-doctoris* was previously never, since this research, identified as a plant pathogen. These species cause leaf spot symptoms on olive trees. The species *P. iranianum* is mainly associated with esca on grapevines and here it was found in olive trees for the first time worldwide. The symptoms it causes are like those described for the species *C. pruinosa*.

**Keywords**: leaf spot, molecular identification, *Olea europaea* L., olive branch dieback, phylogenetic analysis

# Allelopathic potential of ruderal plant species on tomato and lettuce

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**Summary**

The aim of the research was to evaluate the allelopathic potential of water extracts of several ruderal plant species on seed germination and seedlings growth of tomato and lettuce. Water extracts were prepared from dry above-ground biomass of plants in concentration of 5% and tested under controlled laboratory conditions using Petri dish bioassay. The results of the study showed that all tested species had allelopathic effect that was dependent on test species and measured parameter. According to their average inhibitory potential plant species can be ranked from the highest to the lowest as follows: *Melilotus albus*, *Portulaca oleracea*, *Verbascum phlomoides*, *Plantago lanceolata*, *Cichorium intybus*, *Rorippa austriaca*, *Vicia grandiflora*, *Eupatorium cannabinum* and *Polygonum aviculare*. The greatest negative effect was recorded in treatment with *M. albus* water extract which reduced germination, root and shoot length of seedlings, on average, by 43.9%, 93% and 89.4%. *P. oleracea* water extract exhibited a substantial stimulatory effect on both test species and promoted root and shoot length, on average, by 19.8% and 51.4%. Test species differed in their sensitivity to water extracts with tomato being more susceptible compared to lettuce. The pH of water extracts of the evaluated ruderal plant species ranged from 5.26 (*P. aviculare*) to 7.71 (*V. grandiflora* and *M. albus*), and had no effect on their inhibitory allelopathic potential.

**Keywords**: allelopathy, water extracts, germination, inhibition, ruderal plants

# New toxigenic species of the genus *Aspergillus* on maize kernels

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**Summary**

Species of the genus *Aspergillus* are distributed throughout the world but are primarily represented in subtropical and warm temperate climates. In warm, humid subtropical or tropical climates or even in temperate climates in which the growing season is unusually hot and dry, species of the genus *Aspergillus* can infect seeds in the fields. The movement of fungal species threatens food security and creates global public concern worldwide. Climate change is altering the distribution, incidence and intensity of plant pests that is moving north into more temperate zones of Europe. In recent years, Serbia has been facing climate changes accompanied by a warming trend, which is a cause for concern. A more frequent occurrence of *Aspergillus* species was observed in the years in which the weather conditions were characterized by high temperatures, lack of precipitation and pronounced drought conditions (2012, 2015, 2017). The intensive outbreaks of *Aspergillus* species were in 2012. In addition to the previously known species *Aspergillus flavus*, which was a storage pathogen, it also occurs in field conditions. We discovered a new species of *A. parasiticus* in Serbia, for the first time. During the 2015 and 2017 we identified new species of the genus *Aspergillus* section *Nigri*. In order to adapt to climate change, priority is given to the cultivation of drought-resistant maize hybrids. The introduced challenges require additional climate change adaptation strategies, including various changes in production systems, in order to maintain long-term productivity.

**Keywords**: *Aspergillus*, maize, climate change

# Trichothecene chemotypes of *Fusarium graminearum* species complex in Serbia

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**Summary**

The species *Fusarium graminearum* represents one of the most economically important factor that threaten agricultural production in the world. In addition to affecting yield reduction and grain quality, this species is of great importance due to its ability to synthesize mycotoxins. Knowing the toxicological profile of *F. graminearum* species is essential for agriculture and food industry, because trichothecene chemotypes (NIV, 3ADON, 15ADON) have different toxicological properties that directly affect human and animal health. In this study, 20 isolates of *F. graminearum* originating from maize and wheat were examined. DNA isolation was performed from the mycelium of the fungus using the commercial kit Dneasy Plant Mini Kit according to the manufacturer's instructions. Chemotype detection was performed using two sets of specific primers (TRI3 and TRI12) designed for trichothecenes by Ward *et al*. in 2002. After the polymerase chain reaction was completed, the products were electrophoretically separated on a 1% agarose gel, and visualization was performed by staining with ethidium bromide and observing under UV light. Amplified fragments were read visually, and a positive reaction indicated the presence of DNA fragment of the expected size. All isolates of *F. graminearum* examined in this study had products of size 610bp at TRI3 and 670bp at TRI12, which indicates that isolates originating from Serbia belonged to 15ADON chemotype.

**Keywords**: mycotoxins, NIV, 3ADON, 15ADON

# Classification of damage by cereal leaf beetles on winter wheat using machine learning, spectral reflectance, and vegetation indices

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**Summary**

Cereal leaf beetles (CLB) pose a threat to wheat crops, causing distinctive symptoms on the leaves in adults and larvae. This results in reduced chlorophyll content, tissue loss, and diminished photosynthetic efficiency, leading to yield losses of up to 40%. In recent years, the utilization of remote and proximal sensing systems has become pivotal in detecting stress factors in crops. These methods enable the rapid assessment of plant physiological responses to pest infestation, serving as a crucial part in site-specific pest management strategies. The study used a spectroradiometer to detect stress induced by CLB, with a focus on classification of damage levels in winter wheat. Extensive measurements were conducted in both field conditions and a climate chamber with artificial infestation, recording leaf spectra. The dataset encompassed 123 samples, categorized into four damage levels. Analytical approach involved clustering, PCA, and two machine learning algorithms - SVM and RF.

Addressing damage level challenges, various input data, incorporating leaf spectra (350-1400 nm), 9 vegetation indices (VI), and PCA-transformed data were explored. The SVM algorithm, utilizing VI as input data, achieved the classification accuracy of 83.88%, while the same algorithm using only PCA-transformed data exhibited the highest accuracy at 97.29%. Our research contributes to precision agriculture, enabling early pest detection and targeted crop management for optimal yield in a changing climate.

**Keywords**: cereal leaf beetles, remote sensing, spectroradiometer, classification, machine learning

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# Book of Abstracts

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59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Hrana i kvaliteta hrane

# Can pulsed electric field pretreatment improve the phenolic profile of virgin olive oil from Croatian varieties?

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**Summary**

Some significant research has shown that it is possible to improve the yield and chemical composition of virgin olive oil (VOO) by integrating the pulsed electric field (PEF) in the production line. However, the general conclusion from these studies is that the effect of PEF strongly depends on the olive variety used for oil production. The aim of this study was to investigate the effect of PEF as a malaxation pretreatment on the phenolic composition of VOOs from Rosulja, Istarska Bjelica, Levantinka and Oblica. The electric field strength was varied from 1 to 8 kV/cm and the treatment time from 30 to 102 s. Phenols were determined by the HPLC method with UV detection. The applied electric field strength had a statistically significant effect on the concentration of all individual and total phenols, while the treatment time had a statistically high effect on hydroxitirosol, tyrosol, oleacin, oleocantal and on total phenols. However, the results confirm that the olive variety has the greatest influence on the phenolic composition of the VOOs and that PEF pretreatment has different effects on the oil composition of the Croatian varieties studied. The strength of the electric field had a negative influence on the phenolic compounds of Istarska Bjelica and Oblica VOOs and no influence on the oils of the other two varieties. The duration of the treatment had a negative influence on the phenolic compounds only in the Rosulja variety, while it had no influence in the other varieties.

**Keywords:** virgin olive oil, pulsed electric field, malaxation, phenolic compounds, Croatian olive varieties

# Validation of meat products without added nitrites, nitrates or phosphates

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**Summary**

The challenge for the meat industry is developing meat products without added additives that have similar sensory properties and will persist throughout a product's shelf life with the same level of safety as traditional nitrite-cured products. The first phase of study was comparing new recipes with commercially available products. The new recipes were REC1, without added nitrates, nitrites or phosphates which are commonly used in industrial production, and REC2, products with food additive substitutes of natural origin. Also, the replacement of sodium ascorbate with the acerola plant (*Malpighia emarginata*) was studied. The second phase was validation of chemical, physical, microbiological and sensory parameters of novel meat products - cooked ham (as representative of thermally processed meat products) and fermented sausage. In the first phase, it was concluded that the new meat products had lower concentrations of nitrite and nitrate than the standard sample with added additives. The water activity values in meat products were comparable to those from the literature and the presence of pathogenic bacteria has not been proven. New recipes were evaluated through a test of difference for both meat products with positive marks, compared to the standard sample, and were considered acceptable prototypes for the next phase. Also, acerola has successfully replaced sodium ascorbate as a natural source of ascorbic acid in meat products. Furthermore, in the second phase, it was proved that the new meat products had concentrations of nitrite and nitrate below the method's limit of quantification during the entire shelf life period. Phosphates, comparable to those from raw meat, were not added in meat products. Using the weighed points method, cooked ham and fermented sausage were classified into the highest quality category, in terms of color, flavor, odor and texture during the entire shelf life, and were evaluated as microbiologically safe, according to the Guide for Microbiological Criteria for Foodstuffs (Ministry of Agriculture, March 2011).

**Keywords**: nitrites, nitrates, phosphates, acerola, meat products

# Application of medicinal mushroom extracts in bread and their effect on bread quality

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**Summary**

Bread is a basic food and in Slovakia its consumption, together with pastries, is around 63 kg per person per year. Therefore, the effort to improve the nutritional value of bread is understandable. One of the advanced ways is to design it to ensure the expected nutritional benefit. As part of the research, extracts from medicinal mushrooms shiitake and maitake to which excellent nutritional value is attributed, were added to composite flours. The amount of extracts added was 5%, 7.5% and 10%. As a result of these additions, the basic technological properties of the composite flours have changed. Compared to control flour, wet gluten content decreased only slightly, but gluten swelling, and gluten extensibility decreased significantly. Falling number, Zeleny sedimentation volume and crude protein (N x 5.7) also decreased. The experimental loaves were baked from composite flours in verified combinations and evaluated by Volscan Profiler volume analyser (Stable Mycrosystems, Surrey, UK). The following parameters were evaluated: weight of the bread (g), bread volume (mL), specific volume (mL/g), volume yield (mL/100g flour), aspect ratio of a middle slice. The weight of the experimental loaves decreased in proportion to the amount of addition of medicinal mushroom extract, by a maximum of 6.6% in the loaf with the addition of maitake extract in the amount of 10%. The volume and specific volume of the experimental loaf decreased with the addition of maitake extract in proportion to its amount, by at most 15.6% (volume) and 9.3% (specific volume). Interestingly, however, the addition of shiitake extract in the amount of 5% increased the volume and specific volume of the experimental loaf by 4.7% and 8.5%, respectively. It means that with such an addition, as well as the nutritional benefits, there was also an improvement in the technological quality of the bread. Higher additions (7.5%, 10%) reduced the evaluated parameters in the case of shiitake extract, by 13.6% and 7.8% respectively. Nevertheless, all additions were technologically acceptable, and the differences in technological quality of experimental loaves compared to control loaves were not important. These types of designed foods with nutritional benefits have potential for producers and are desirable by consumers.

**Keywords**: shiitake, maitake, composite flour quality, bread quality

# Nutritive value of albumin cheese made from whey after the production of trappist cheese

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**Summary**

The aim of this paper is to present the results of the research, on the nutritional values of albumin cheese made from the whey remaining after the production of Trappist cheese. The production of albumin cheeses is possible thanks to the particular characteristics of whey proteins, which do not react to the action of rennet enzymes, so they remain unchanged during the coagulation of the milk and turn almost completely into whey after the separation of the curd. The total solids content of albumin cheese was 27.9%, fat content 15.00% and fat content in total solids 55.17%. Protein content was determined to be 8% and the ash content 0.62%. The energy value of the albumin cheese was 181 kcal/752 kJ. The consumption of ricotta and albumin cheeses is a good basis for the new dietary trends that in recent years have been looking for products with a lower milk fat content, whatever you think of them. They are a good source of whey protein, easily digestible and with a lower energy content.

**Keywords**: albumin cheese, Trappist cheese, nutritional value, whey

# Fermentation characteristics of alfalfa silage during one week of aeration

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**Summary**

The aim of this study was to investigate the changes in lactic acid and volatile fatty acids (VFA) content in alfalfa silage ensiled without and with three different concentrations of lactic acid bacteria (LAB) inoculant during a seven-day silage aeration. Alfalfa silage was ensiled with three combined concentrations of homofermentative and heterofermentative LAB inoculant (T2: standard, T3: 1.5x standard and T4: 2x standard) and a control without LAB inoculant (T1) in vacuum bags (SmartVac, Status d.o.o.). All treatments were sampled in five repetitions. After 124 days of ensiling, silages were aerated for 7 days. Changes in silage temperatures were measured every 15 minutes throughout the aeration period and sampled on day 0, 3 and 7 to determine fermentation parameters. Throughout the aeration period, all silages tested remained aerobically stable. However, T1 and T4 had 3x higher lactic acid values compared to T2 and T3, indicating an unexpectedly pronounced homofermentative activity in T4. The application of the inoculant had a positive influence on the acetic acid content at the end of aeration compared to the control group (38 – 47 g/kg DM in T2 – T4 vs. 30 g/kg DM in T1) and on the propionic acid content (0.83 – 3.32 g/kg DM in T2 – T4 vs. 0.76 g/kg DM in T1). The highest butyric acid content was found in T3 (15.17 g/kg DM vs. 2.68 g/kg DM in the other three tretments). The results show that when using an inoculant, it is preferable to use the concentration recommended by the manufacturer, as this concentration allows the production of silage with an optimal content of lactic acid and VFA for aerobic stability.

**Keywords**: aerobic stability, alfalfa silage, LAB inoculant concentration, fermentation parameters

# Evaluation of the Elementary Composition of Plants used in Infusions, by X-Ray Fluorescence Analysis (XRF)

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**Summary**

The number of plant species used worldwide in infusions, other than the tea plant *Camellia sinensis* L., is increasing mainly due to the recognize of bioactive compounds with beneficial properties on human physiology. The elemental composition of these plants is often ignored and is fundamental to analyse their presence in the raw-material and in parallel evaluate the transfer to the brew. In this context, 36 different "tea" plants with scattered origin were analyzed using XRF. It must be stressed that the raw materials are freely sold in Portuguese market stores without any reference of the elemental composition in the label. All the analysis were made in triplicate and the average concentration values were expressed in mg $kg^{-1}$ on a dry weight basis. For expediency reasons we indicate the maximum concentration for each element followed by the average concentration: Mo (max.=13.9 in Lemon Grass, = 11.5), Zn (max.=163 in White Birch, =35.1), Cu (max.=49.8 in Corn Silk, =26.0), Fe (max.=1064 in Stonebreaker, =154.7), Ca (max.=49696 in Lichwort, =18875), K (max.=74579 in Elderberry, = 28750), S (max.=15409 in Horsetail, =3138.8), Si (max.=44595 in Horsetail, =2678.3), Cl (max.=63720 in Globe Amaranth, =154.7) and P (max.=15027 in Elderberry, =3985.0). Horsetail (*Equisetum arvense* L.) is recommended for cases of loss of connective tissue, hair and nails, whose Si, S and Ca concentrations with 44595, 15409 and 36462 mg $kg^{-1}$, respectively, can be a good contribution to the good maintenance of these tissues and bones. The medicinal claims that Elderberry (*Sambucus nigra* L.) must prevent and cure some colds and flu, could be well correlated with its Zn content of 54.3 mg $kg^{-1}$ although the highest value was observed in White birch (*Betula pubescens* Ehrh.). Further research regarding the transfer of the elements to the brew is needed and is currently under evaluation.

**Keywords**: Elementary Composition, Infusion Plants, Raw material, XRF

# Cereals whole grain as important source of biologically active ferulic acid

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**Summary**

Cereals are an important source of biological active phenolic compounds with numerous health-promoting effects. Ferulic acid (FER) is the most abundant phenolic acid in common cereals, representing up to 90% of total phenolic compounds. In this study, 38 whole grain samples of different types of cereals evaluated for FER content. Before conducting the HPLC analysis, the cell wall-bound FER acid was first released through alkali treatment and subsequently extracted by an appropriate organic solvent at a low pH condition. The mean value of FER was highest in the popcorn and corn samples (275.6 and 174.8 mg/100 $g_{dm}$, respectively), followed by winter and spring barley and triticale (61.0 – 56.8 – 51.9 mg/100 $g_{dm}$, respectively). The samples of wheat and hull-less barley possessed the similar FER value (44.8 – 48.3 mg/100 $g_{dm}$, respectively), while the lowest one found in husked oat (26.1 mg/100 $g_{dm}$). Like many bioactive components, FER found mostly in the outer layers of cereals, therefore the consumption of whole grains and their products is important in terms of a healthy diet. Consumption of 2 – 3 servings per day (about 48 g) of whole grains may reduce the risk of cardiovascular disease, cancer, and type 2 diabetes.

**Keywords**: cereals, whole grain, ferulic acid, HPLC

# The role of the national reference laboratory (NRL) in the coordination of official laboratories for pesticide residue analysis

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**Summary**

Member States shall appoint one or more national reference laboratories for pesticides (NRL). One national laboratory can be a reference for more than one type of NRL. dr Andrija Štampar Teaching Institute of Public Health is the national reference laboratory for fruits and vegetables, cereals and SRM. The obligations of NRL are described in Regulation 625/2017 and include cooperation with European reference laboratories, participation in trainings and inter-laboratory comparisons organized by these laboratories. One of the important activities of the NRL is the coordination of activities in official laboratories. NRL cooperates with official laboratories, passing on all the information obtained from EURL, but also conducting training. The work of official laboratories is monitored in such a way that their passing results on mandatory PT schemes are monitored. Official and reference laboratories are categorized by EURL into category A and category B laboratories. A laboratory enters category B when it either does not have a sufficient scope for pesticide residue analysis or produces unsatisfactory results. Such laboratories require special supervision in the form of the visit of the head of the NRL to those laboratories in order to investigate the problem. There are seven official laboratories in the Republic of Croatia (3 state and 4 private). The problems that official laboratories face are the lack of sufficiently educated staff and the lack of financial resources for basic work.

**Keywords**: national reference laboratory, pesticides, coordination

# Exploring the antiproliferative potential of select berry juices against late and early-stage colon cancer cells

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**Summary**

Colorectal cancer remains a formidable global health challenge, demanding innovative approaches to enhance therapeutic strategies. Considering this, our study aimed to investigate the potential antiproliferative effects of selected berry juices, namely dwarf elderberry (*Sambucus ebulus* L.), wild blackberry and raspberry (*Rubus idaeus* L.) juices, specifically targeting colon cancer cells. Our focus on two distinct colon cancer cell lines, SW1116 (representing an early-stage) and Colo205 (representative of late-stage cancer), reflects the diversity in stages of this malignancy. These cells were selected to gain a comprehensive understanding of how selected berry juices may exert their effects across different phases of colorectal cancer progression. Each berry juice harbors a unique composition of phenolic compounds, which have been associated with potential anticancer properties previously, so phenolic profiles of berry juices were evaluated. These results demonstrated that wild blackberry and raspberry juices exhibited significant antiproliferative effects on SW1116 cells (58% and 45%, respectively) at all tested concentrations, while dwarf elderberry juice had a modest antiproliferative effect (79%). Also, results revealed that wild blackberry and raspberry juices exhibited antiproliferative effects on Colo205 cells (35% and 29%, respectively) in contrast to dwarf elderberry juice which had no antiproliferative activity against these cells. Raspberry juices exhibited dose-dependent antiproliferative activity against both type of cells. Results of this study could help design novel colorectal cancer treatments. Additionally, they add valuable information to the established and evolving database of knowledge about the use of natural compounds in cancer treatments.

**Keywords**: colon cancer cells, dwarf elderberry juice, wild blackberry juice, raspberry juice, phenolic profile

# Effect of monosodium glutamate addition on physical and sensory properties of cooked sausages

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**Summary**

Monosodium glutamate (MSG) is a commonly used flavour enhancer in the food industry. It is now considered one of the strategies for reducing sodium content in meat products to address concerns about excessive sodium chloride intake. Consequently, this study aimed to explore the effects of MSG on the physical and sensory properties of cooked sausages with elevated MSG content. For this purpose, the cooked sausages were prepared under three treatments. Treatment A served as a control with no added glutamate, while treatments B and C had 0.15% and 0.30% MSG added to the ingredients used in the control treatment, respectively. Measurements of colour and water activity, along with sensory analysis conducted by both consumers and trained evaluators, were carried out on the cooked sausages. The results indicated that there were no significant differences in terms of colour, water activity, likability, and sensory profile among the treatments. However, the perceived saltiness and spiciness exhibited significant differences among the treatments, with treatment C showing an excessively pronounced character. This suggests that by incorporating MSG, sodium chloride content can be reduced while maintaining consistent sensory characteristics.

**Keywords**: monosodium glutamate, flavour enhancer, salt reduction, sensory analysis, colour

# Presence of Glyphosate, Aminomethylphosphonic acid and Glufosinate in fruits and vegetables on Croatian market

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**Summary**

Glyphosate (N-(phosphonomethyl)glycine) is a widely used broad-spectrum, non-selective, systemic, and translocating herbicide in various applications for weed control in agriculture, silviculture, and garden maintenance. It is one of the most commonly used herbicides in European agriculture, representing 33% of total herbicide sales in the EU. In the environment, it strongly adsorbs to most soils, with a half-life of microbiological degradation ranging from 7 to 60 days. The primary degradation product is Aminomethylphosphonic acid (AMPA), which has a longer half-life than glyphosate. Glufosinate (2-Amino-4-[hydroxy(methylphosphonoyl)]butanoic acid) is a non-persistent, broad-spectrum herbicide that inhibits amino acid biosynthesis in plants. It was banned in Europe since 2018.

In 2023, the European Commission extended the approval for glyphosate, but the safety profile of glyphosate and its commercial formulations remains controversial. An in-house method for the determination of glyphosate, AMPA, and glufosinate in plant-origin samples was developed and fully validated according to Sante/12682/2019. The samples were prepared using Solid Phase Extraction (SPE) after derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC-CL) and quantified using ultra-performance liquid chromatography with triple quadrupole mass spectrometry (UPLC-MS/MS) method.

The sample consisted of 220 pieces of various fruits and vegetables. Results showed that a total of six samples, or 2.7%, had residues of the examined herbicides. Out of these, four samples, or 1.8%, had Glyphosate residues, which were found in beans (ranging from 0.10 mg/kg to 0.30 mg/kg) and in strawberries (1.8 mg/kg). In two pear samples, AMPA residues were found (0.036 mg/kg and 0.24 mg/kg). The method's performance was confirmed by the satisfactory results (z-score <2) of the proficiency testing organized by EU Reference Laboratories for Residues of Pesticides. None of the examined fruits and vegetables at the point of analysis posed a direct health risk to consumers and comply with EU regulations.

**Keywords**: fruits and vegetables, pesticides residue, herbicide, UPLC-MS/MS

**Acknowledgments:** This work was carried out within the project "Food Safety and Quality Center" (KK.01.1.1.02.0004). The project is co-financed by the European Union from the European Regional Development Fund.

# Total polyphenol and flavonoid content and antioxidant properties of semi-hard goat cheese ripened in oil

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**Summary**

The aim of the study was to determine the content of total polyphenols and flavonoids, as well as the antioxidant properties of semi-hard goat cheese ripened in oil (mixture of Mljet's extra virgin olive oil and refined sunflower oil; 50:50). 5 batches of cheese were produced. The cheeses of the same batch were divided into 3 groups according to the ripening method: (i) ripening in air (control group), (ii) ripening in oil after 10 days of ripening in air, (iii) ripening in oil after 20 days of ripening in air. In the cheese samples taken at days 0, 10, 20, 30, 45 and 60 of ripening, the content of total polyphenols and flavonoids was determined, and the antioxidant activity was measured by the spectrometric method. Prolonged ripening in air before immersion in oil leads to greater water loss and the formation of a porous structure of the cheese through which the oil can penetrate. Thus, group 3 had significantly higher content ($p<0.05$) of total polyphenols and flavonoids than group 2. Their content increased during ripening of the cheeses of groups 2 and 3, while a decrease was observed in the control cheese after 45 days, indicating a different course of biochemical processes due to ripening in different media (oil and air). Cheese ripened in oil had significantly higher antioxidant activity ($p<0.01$) than the control cheese, suggesting that oil as a ripening medium has a positive effect on the nutritive value of cheese.

**Keywords**: semi-hard goat cheese, ripening in oil, polyphenols, flavonoids, antioxidant properties

# Impact of chemical parameters on patulin contamination in traditional and commercial apple cultivars

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**Summary**

This study aims to assess the quality of traditional and commercial apple fruits intentionally infected by *Penicillium expansum* and subsequent patulin occurrence. Several factors affect apple patulin production, including cultivar type, geographical location, climate, mechanical injury, storage conditions, pre- and post-harvest conditions, etc. However, the sugar, acid content, water activity and texture of apples play a significant role in determining the susceptibility of apples to *P. expansum* infection and subsequent patulin production. In this study, we have determined the effect of various chemical parameters, namely sugar content, acid content, water activity and texture, on the growth of *P. expansum* and patulin production on traditional ('Kandaska Reneta' and 'Božićnica') and commercial ('Fuji' and 'Granny Smith') apples. After the apples were inoculated with *P. expansum* (conidia suspension of $10^6$ CFU $mL^{-1}$), they were placed in a refrigerated (at +4°C) environment to simulate storage conditions. The produced patulin concentrations were determined by the multi-analyte 'dilute and shoot' LC-MS/MS method of Sulyok et al. (2020). Among the apple varieties tested, 'Granny Smith' exhibited the highest resistance to fungal growth for 72 days, followed by 'Božićnica' (57 days), 'Kanadska Reneta' (56 days), and 'Fuji' (47 days). Comparing the reducing sugar content of the four apple cultivars, it was observed that 'Fuji' had the highest amount (11.78 g/100g), followed by 'Kanadska Reneta' (9.33 g/100g). On the other hand, 'Božićnica' and 'Granny Smith' displayed lower values of 8.94 g/100 g and 8.92 g/100g respectively. This indicates that 'Fuji' has a higher level of sweetness compared to the other three varieties. When it comes to flesh firmness, 'Kanadska Reneta' displayed the highest value (244.60 g), indicating a firmer texture, whereas 'Fuji' had the lowest (274.79 g), suggesting it might be softer in texture. The pH values were relatively close for all cultivars, ranging from 3.37 to 3.70, with 'Božićnica' having the highest pH. 'Granny Smith' had the highest level of patulin (4723.20 µg/kg). The other cultivars had levels below the limit of detection (LOD). Based on the data presented, it can be concluded that there is no direct correlation between patulin content and the measured parameters in selected apple cultivars. This suggests that factors other than here enquired, such as storage conditions or inherent resistance to fungal contamination, may have an influence on patulin levels. This research was funded by the Croatian Science Foundation (UIP-2020-02-8461).

**Keywords**: apple quality, traditional Croatian apple cultivars, patulin

# Effect of high hydrostatic pressure pre-treatment on the properties of vacuum-dried avocado peel

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**Summary**

Avocado peels, often discarded as food waste, contain an abundance of nutrients and antioxidants, making them a valuable resource for various culinary and industrial applications. The research focuses on the treatment of avocado peels with high hydrostatic pressure at different levels (50, 100 and 150 MPa) and durations (4 and 8 minutes) followed by subsequent drying in a vacuum dryer (100 mbar at 60 °C). The statistical analysis shows a strong correlation between the antioxidant activity (measured by DPPH) and the two main process parameters: pressure and processing time. In particular, antioxidant activity shows a positive correlation with increasing pressure, reaching a maximum of 87.85% at 150 MPa. Conversely, processing time shows an inverse correlation with antioxidant activity, reaching the highest values at the shortest processing time. Both pressure and processing time under high pressure have a significant effect on total phenolic content. Increasing the pressure leads to an increase in gallic acid concentration in the dried avocado samples up to 922 μgmL-1. Given the combined consideration of phenolic compounds and antioxidant activity, it is evident that the optimal processing parameters for avocado peels are a pressure of 150 MPa applied for 4 minutes, followed by drying at 60°C under a vacuum pressure of 100 mbar to preserve valuable compounds and maximize antioxidant potential.

**Keywords**: high hydrostatic pressure, avocado peel, vacuum drying

**Acknowledgements:** This work was supported by means of the Croatian Science Foundation project IP-2019-04-9750 - HYDRYBY

# Assessment of technological characteristics in chicken sausages utilizing inulin for fat reduction

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**Summary**

Due to the high demand for low-fat, healthier meat products, alternative strategies are pursued to reduce fat content while maintaining the fundamental properties of traditional products. The research strives to obtain products with reduced fat content and caloric value with the addition of inulin. Four groups of cooked chicken sausages were tested: the control group without fat replacement, the second with 50%, the third with 75%, and the fourth with 100% fat replacement with inulin suspension. Sausages with a higher inulin content had a significantly ($p<0.05$) higher process loss and cooking loss than the sausages from the control group. The first and second groups had significantly ($p<0.05$) better emulsion stability. Reducing the fat content in sausages significantly affected ($p<0.05$) some sausages characteristics, such as moisture, L*(lightness), a* (redness), and b* (yellowness). As a result, the caloric value of sausages with inulin was significantly ($p<0.01$) impacted throughout all groups. In summary, the study demonstrates that incorporating inulin as a prebiotic dietary fiber enables the production of cooked sausages with reduced fat content and optimal physico-chemical properties. This innovative meat product not only offers lowered energy value but also holds exceptional nutritional value, representing a significant source of calories derived from reduced fat content supplemented with prebiotic fibers (inulin).

**Keywords**: inulin, fat, cooked chicken sausages, functional food

# Quality of cooked cheese with the addition of spices

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**Summary**

The aim of this study was to investigate the influence of the addition of spices (basil and chive) on the physico-chemical and sensory properties of cooked cheese as well as on its microbiological quality. The cooked cheese was made from full-fat cow's milk in 3 batches, and the cheeses produced from the same batch were divided into 3 groups: I) cooked cheese without spices (control), II) cooked cheese with added chive and III) cooked cheese with added basil. The physico-chemical analyses of the cheese included the determination of the content of dry matter, milk fat, protein, salt and pH value. To determine the microbiological quality of spices and cheeses, microbiological analyses were carried out to detect *Salmonella* spp., sulphite-reducing clostridia, *Escherichia coli*, *Listeria monocytogenes*, yeasts, moulds, as well as the total number of aerobic bacteria. The cheese samples for the microbiological analyses was taken immediately after production and after 45 days of storage in the refrigerator. A panel of 5 experts evaluated the sensory properties of the cheese using a scoring system with a maximum score of 20. The addition of spices had no influence on the physico-chemical properties of the cheese, but had a positive effect on the sensory properties of the cheese. The microbiological quality of the cheese was directly influenced by the addition of spices. The conditions during cheese storage in the refrigerator are suitable for the growth and multiplication of yeasts and moulds.

**Keywords**: cooked cheese, spices, physico-chemical properties, microbiological quality, sensory properties

# *Triticum dicoccum* as a source of nutritional valuable proteins and essential microelements in human nutrition

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**Summary**

*Triticum dicoccum* (Schrank ex. Schübl) is currently grown as an alternative type of wheat primarily in organic farming. In the work, we focused on the analysis of individual protein fractions, primarily of albumins and globulins, which are characterized by the content of essential amino acids, but also the proportion of prolamins, which cause celiac disease in predisposed person. From a nutritional point of view, we were also interested in the content of microelements in *Triticum dicoccum* grain. 7 genotypes of *Triticum dicoccum* obtained from RIPP Piešťany (PN) were analyzed: PN4-41, PN8-26, PN2-43, PN3-13, PN6-8, PN6-37, PN8-23. The crude protein content in the grain was 11.33-15.27%. From the results of the analysis of the protein fractions by the Kjeldahl method, it follows that the genotype PN4-41 had the highest content of the albumin and globulin fraction (26.55%), while the least amount of albumin and globulin was in the genotype PN8-23 (8.02%). On average, the fraction of albumins and globulins in *Triticum dicoccum* grain was 17.68%. Gliadins, which contain celiac active epitopes, were the most determined in the PN8-23 (69.94%). The least amount of gliadins was in the PN4-41 (54.31%). The average content of gliadins in *Triticum dicoccum* grain was 61.02%. The proportion of glutenins was from 19.13% (PN4-41) to 22.82% (PN2-43). The results of SDS-PAGE show that the majority of proteins are fractions with Mr 33 kDa - 96 kDa, corresponding to the fraction of gliadins. ELISA with monoclonal antibody G12 determined that the content of coeliatic active proteins was 47.86-83.45 g/kg of flour (average 62,65 g/kg). *Triticum dicoccum* is not suitable for patients with coeliac disease. The ICP-OES method was used to determine the content of microelements. Of the nutritionally important elements, we determined the content of Ca from 302 mg/kg (PN8-23) to 470 mg/kg (PN3-13), Cu from 4.41 mg/kg (PN8-23) to 5.06 mg/kg (PN6-37), Fe from 28.9 mg/kg (PN2-43) to 41.4 mg/kg ((PN6-37), K from 3647 mg/kg (PN4-41) to 4287 mg/kg (PN2-43), Mg from 1221 mg/kg (PN3-13) to 1321 mg/kg (PN 6-8), Mn from 29.7 mg/kg ((PN2-43) to 36.8 mg/kg (PN4-41) and Zn from 31 mg /kg (PN8-26) to 50.0 mg/kg (PN8-23). Na concentration was very low, from 8.1 mg/kg (PN4-41) to 35.3 mg/kg (PN6-8). Quality wheat for bakery use should have the highest content of protein fractions forming gluten. In *Triticum dicoccum*, the proportion of these proteins was 65.84–80.36%. From a nutritional point of view, the concentration of microelements sodium, magnesium, zinc and calcium is interesting. The alternative wheat type *Triticum dicoccum* is a suitable addition for the production of bakery products to diversify the assortment of cereal foods.

**Keywords**: *Triticum dicoccum*, protein fractions, microelements

# Yolk carotenoid content and deposition efficiency vary depending on the mineral form and vitamin A levels in the laying hen diet

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**Summary**

This study aimed to investigate the influence of the level of vitamin A supplementation (5000, 10000, 20000 IU/kg) and form of trace minerals (inorganic or organic) on the bioavailability of carotenoids, expressed as carotenoid deposition efficiency (CDE), in laying hens fed diets differing in two commercial maize hybrids. The *in vivo* experiment was conducted with 252 Lohmann Brown hens randomly allocated to 12 dietary treatments (2×3×2 factorial arrangement) with seven replicates each. After depletion, the experimental period lasted 6 weeks, during which the eggs were collected once a week for analysis. The CDE was calculated based on carotenoid content in diets and yolks, daily egg production and daily diet intake. The carotenoid contents in the diets and yolk samples were determined using the RP-HPLC method. The form of trace minerals and the level of vitamin A supplementation had no effect on the yolk contents of lutein, zeaxanthin and total carotenoids. However, the levels of α-cryptoxanthin and β-cryptoxanthin decreased ($P>0.01$) with increasing vitamin A levels in the diet, suggesting possible competition for absorption with provitamin A carotenoids. Consistent with this, the CDE for lutein, α-cryptoxanthin, β-cryptoxanthin and total carotenoids ($P>0.001$) decreased at higher dietary vitamin A levels. Overall, the results suggest that the form of trace minerals in the laying hen diet does not affect the bioavailability of carotenoids. However, a higher vitamin A levels in the diet have a negative effect on utilization and yolk deposition of dietary carotenoids, despite its beneficial effect on laying hen health.

**Keywords**: laying hens, egg yolk, carotenoids, vitamin A, trace minerals

This work has been fully supported by Croatian Science Foundation under the project ColourMaize (IP-2019-04-9063). The work of doctoral student Dora Zurak has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation.

# Egg quality parameters and yolk oxidative stability in laying hens fed diets differing in mineral form and vitamin A supplementation levels

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**Summary**

The aim of this study was to investigate the effects of different levels of vitamin A (5000, 10000 and 20000 IU/kg) and the form of trace minerals (Zn, Mn, Fe, Se; inorganic or organic) on egg quality and yolk oxidative stability in laying hens fed diets differing in two commercial maize hybrids. A total of 252 Lohmann Brown hens were randomly assigned to 12 dietary treatments in a 2×3×2 factor arrangement with seven replicates each (cage with 3 hens). The trial lasted nine weeks and the eggs were collected weekly for analysis after the third week. Egg quality parameters were determined using the Digital Egg Tester (DET 6000, Nabel, Kyoto, Japan) and a calliper. The oxidative stability of the yolks was determined using the TBARS method on eggs from the fifth week of the experiment as susceptibility to iron-induced lipid oxidation in the yolk emulsion. Supplementing the diet of laying hens with inorganic trace minerals and vitamin A led to an increase (P>0.05) in egg weight and length, eggshell weight as well as yolk colour and proportion in the egg. However, the TBARS values of the egg yolks (P>0.01) increased with increasing vitamin A level in the diet. Although the increase was numerically low (24.5 and 57.7 ng/g after 100- and 200-min incubation of the emulsions), it indicates a possible pro-oxidising effect of this fat-soluble vitamin at high concentrations. In conclusion, the results suggest that supplementing the diet of laying hens with inorganic trace minerals and vitamin A can improve egg quality parameters, ensuring a high-quality end product for consumers and minimizing losses in the commercial egg industry.

**Keywords**: laying hens, egg quality, TBARS, vitamin A, minerals

This work has been fully supported by Croatian Science Foundation under the project ColourMaize (IP-2019-04-9063). The work of doctoral student Dora Zurak has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation.

59 Croatian 2024 19 International Symposium on Agriculture

Session 11

# Book of Abstracts

Precision Farming

59 Hrvatski 19 Međunarodni Simpozij Agronoma

# Zbornik sažetaka

Precizna poljoprivreda

# Opportunities for reshaping European agriculture: Green technologies and the EU Green Deal

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**Summary**

The aim of this research is to explore the possibilities for transforming European agriculture by embracing green technologies within the framework of the EU Green Deal. The study investigates how sustainable practices and technologies can be integrated to achieve sustainability goals within the European agricultural sector. It also examines the impact of the EU Green Deal on farmers and rural communities and explores strategies to facilitate the transition to a more sustainable agriculture. This research underscores significant opportunities for reshaping European agriculture by adopting green technologies as part of the EU Green Deal. Sustainable agriculture can contribute to reducing environmental burdens, enhancing the competitiveness of farmers, and ensuring the viability of rural communities. Key to this transformation is the alignment of policies, allocation of resources, and educational efforts to support the shift away from traditional practices that have negative environmental impacts.

**Keywords**: agriculture, EU Green Deal, green technologies, environmental impact

# Biodegradability of *Arundo donax* during a continuous mesophilic anaerobic digestion

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**Summary**

Diversifying biogas feedstocks is a strategic approach towards sustainability as the EU attempts to deal with climate change and ensure energy independence. The objective of this study was to investigate *A. donax*, a perennial energy crop, and observe if it could be used as a substitute feedstock for continuous anaerobic digestion. This research closely investigated the biogas production and parameters like CH4, CO2, of *A. donax* from two feedstocks, *A. donax* and corn silage, and their mixtures in a continuous AD during 136 days under mesophilic conditions at 39°C. The results showed a biogas methane production from *A. donax* that are equivalent to those of corn silage; its high CH4 yield suggests that it has the potential to be a viable and long-term substitute feedstock for the biogas production. The highest biogas production was found to be from *A. donax* used as 100 % raw mateiral in AD reactor. The lowest biogas potential was found to be the mixutre of *A.donax* and corn silage in ratio 2:1. However, a 1:2 mixture showed an interesting increase in the amount of methane, highlighting the significance of the synergistic interaction between the feedstocks in the methane production. To determine the optimum mixing ratios of corn silage and *A. donax* that optimize the generation of biogas and methane, additional study needs to be done. It can be concluded that *A. donax* can be harvested in autumn, stored, and then used as a substrate in biogas production which makes this invasive plant a valuable source of energy.

**Keywords**: *Arundo donax*, biodegradability, biogas, biomethane

**Acknowledgements:** The research was financed by the OP "Competitiveness and Cohesion" 2014-2020, project KK.01.1.1.07.0078 „*Sustainable biogas production by substituting corn silage with agricultural energy crops*".

# Biogas production from *Arundo donax* in a continuous digestion system

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## Abstract

Although corn silage has historically served as the primary raw material for biogas production, there is an obvious shift towards more sustainable sources. Particularly, there has been interest in agricultural energy crops such as *Arundo donax*. This study evaluated the biogas/biomethane production of *A. donax* and a mixture of corn silage and *A. donax* in two ratios (1:2 and 2:1) through the process of anaerobic digestion with COD, DM content, VS, pH value, and FOS/TAC being continuously monitored. During this process, the comparative analysis accentuates the viability of both, *A. donax* and corn silage, as well as their mixtures as potent feedstocks for biogas production. The biogas yields ranged from 356.81 to 548.81 NL/kg VS. It was observed that a 100 % *A. donax* substrate exhibited a superior biogas yield initially, however, an intriguing boost in methane proportion was noted with a mixture at a 1:2 ratio, underlining the essence of the synergistic interaction between the feedstocks in methane production. Notably, the levels of $H_2S$ were within permissible limits, eliminating the need for further desulphurization procedures, which is important. Further studies are necessary to identify the optimal mixture ratios of corn silage and *A. donax* that maximize biogas and methane production and should also explore the feasibility of storing the crop, considering the continuous process of AD and the seasonality of feedstock, to ensure more consistent and efficient biogas production.

**Keywords**: agricultural energy crops, *Arundo donax*, biogas, biomethane

**Acknowledgements:** The research was financed by the OP "Competitiveness and Cohesion" 2014-2020, project KK.01.1.1.07.0078 „*Sustainable biogas production by substituting corn silage with agricultural energy crops*".

# Exploring the Potential of Near-Infrared Spectroscopy in Evaluating Soil Properties and Detecting Microplastics

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**Summary**

Diffuse reflectance spectroscopy in the visible, VNIR spectral range has increasingly been utilised in recent years to analyze physical, chemical and biological soil properties, providing a good alternative to conventional laboratory soil testing methods in terms of costs and chemical products consumption. This review aims to address the feasibility of using visible (Vis) (400-700 nm) and near-infrared (NIR) (700-2500 nm) reflectance spectroscopy for the prediction of soil properties and for a rapid assessment of microplastic (MP) concentrations in soils, in order to avoid extraction steps and directly quantify the amount of plastic in a sample. An overview will be provided on the soil properties directly (e.g. SOM, soluble salts) and indirectly (macronutrient concentrations, pH, microbial biomass) measured by VNIR, focusing on the possibility to quantify and characterize soils potentially contaminated with heavy-metals (Cr, Pb, Cd, Ni, Cu) and microplastics. The soil properties which were most strongly correlated with soil reflectance properties were SOC, SOM and soil texture. Exchangeable Ca and Mg and CEC were also satisfactorily predicted. Even though results suggest that microplastics can be identified and quantified in soil samples, the possibility to apply this technique to multiple scenarios is still being discussed. A comprehensive spectral library should be built pregressively in order to facilitate laboratory and field spectroscopy for identifying pollutants.

**Keywords**: microbial biomass, organic matter, pH, soil pollutants, VNIR spectral range

# Developing Technologies to Lower Environmental Impacts of Grain Storage and Processing

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**Summary**

The global reliance upon cereal grains continues to be critical to many countries' economies and food systems. All aspects of these supply chains have substantial environmental impacts, whether the intended end use is fuel, feed, or food. The objective of this study was to compile environmental impact data from a variety of published sources, synthesize this information to determine ecological trends, and identify areas where grain supplies chains can be improved. This study has determined that critical environmental shortcomings in the cereal grain industry occur both due to farming practices as well as factory processing. Transportation and consumer use had much smaller impacts. These findings were true for various countries. Specific technological approaches to improve farm-scale sustainability include regenerative practices such as no-till agriculture, field-scale bioreactors for water runoff treatment, and cover cropping. Additionally, precision practices such as seeding, fertilizing, spraying, and weeding will yield substantial impact reductions. To improve factory-scale processing, using variable frequency drives and improved motor control systems, high efficiency lighting systems, water reduction technologies, alternative sanitation approaches, and other equipment modifications can reduce environmental impacts. Because of the substantial emphasis on cereal grains as foundational ingredients for many aspects of societies, we must reduce their environmental impacts to make these supply chains more sustainable.

**Keywords**: cereals, grains, environmental impacts, life cycle assessment, precision technologies, efficiencies

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