

# 13TH INTERNATIONAL CONFERENCE OF YOUNG SCIENTISTS THE YOUNG SCIENTISTS FOR ADVANCE OF AGRICULTURE AGRISCI2024

ABSTRACTS





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The language of the abstracts has not been edited.

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#### **PLENARY SESSION**

#### DROUGHT TOLERANCE IN THE ERA OF CLIMATE CHANGE: ARE TETRAPLOIDS THE BETTER CHOICE?

# Edvinas Misiukevičius, Ingrida Mažeikienė, Monika Kurgonaitė, Viktorija Žukauskaitė, Birutė Frercks, Vidmantas Stanys

#### *Lithuanian Research Centre for Agriculture and Forestry*

Climate change has a significant impact on plant physiology, highlighting the necessity for water conservation and sustainable management practices in horticulture. The most of naturally occurring polyploids are found at the edge of habitat, where conditions are already unfavorable, these plants may be more adapted. However, it is still not clear why tetraploid plants are so superior and what genetic and molecular mechanisms appear in these plants. The aim of this research is to evaluate the genetic diversity, morphological, physiological and gene expression differences between diploid and tetraploid daylilies under drought stress. The methodology of this work was based on diploid and tetraploid daylily plants. Diploid and tetraploid plants were subjected to drought stress where morphophysiological and transcriptome analysis was performed. Overall review of diploid and tetraploid daylilies under severe drought revealed. Tetraploid daylily cultivars are more drought-tolerant than diploid cultivars. Tetraploids tend to hold higher relative water content in leaves, accumulate higher rates of reactive oxygen species, which potentially contribute to defense mechanisms. Diploid plants tend to have two times lower chlorophyll index, photosynthetic pigment changes determining more yellowing foliage with elevated water loss causing early leaf senescence and photosynthetic potential loss. Detailed studies of diploid and autotetraploid Hemerocallis spp. cv. Trahlyta transcriptome analysis reveals significant differences in gene expression patterns and molecular responses to drought stress. Tetraploids activate more signaling pathways (43) than diploids (19) under drought conditions. The most significant among them are phytohormones (abscisic, salicylic and jasmonic acid, auxin, cytokinin and ethylene), MAPK and photosynthesis signaling pathways. In tetraploids, the specific transcription factors - NAC, ERD9 and LOX4 - are activated in response to drought. Combined results show that tetraploid plants were more resistant to drought stress than diploids by activating alternative molecular pathways responsible for adaptation to drought stress.

# RELATIONSHIP BETWEEN LACTOSE CONCENTRATION AND RUMINATION QUALITY IN EARLY-LACTATION DAIRY COWS

# Samanta Arlauskaitė, Ramūnas Antanaitis, Karina Džermeikaitė, Justina Krištolaitytė, Akvilė Girdauskaitė

#### Lithuanian University of Health Sciences

In cows, lactose is the primary solid component of milk and is routinely measured in lactating cows worldwide as part of standard evaluation systems. Over recent decades, likely due to the growing availability of milk data from innovative technologies, lactose has been increasingly incorporated into scientific studies, alongside traditional traits like milk yield, fat, and protein concentrations. The goal of this study was to determine the connection between in-line lactose concentration and rumination quality. Research was conducted on a Lithuanian farm in 2023. A total of 502 cows were selected for the study. According to the literature (1) they were divided into two groups: A (lactose concentration < 4.7%) and B (lactose concentration  $\ge$  4.7%). The average milk yield was 12,500 kg per lactation. Milk lactose concentration was recorded continuously during each milking by the "BROLIS HERDLINE (Vilnius, Lithuania)" in-line milk analyzer, which was integrated into a milking robot. Rumination quality was monitored using the RumiWatch sensor (Itin + Hoch GmbH, Liestal, Switzerland). Jaw movements during rumination showed a significantly higher number of rumination chews (p < 10.01), with a 13.84% increase in Group B (ML ≥ 4.70%) compared to Group A (ML < 4.70%). The average number of rumination chews in Group B was 1280.80 chews per hour (±52.28), while in Group A, it was 1103.58 chews per hour ( $\pm$ 23.37). Additionally, the results showed a significant increase (p < 0.001) of 16.70% in the number of cuds in Group B (ML  $\ge$  4.70%) compared to Group A (ML < 4.70%). The average number of cuds in Group B was 22.69 per hour (±0.92), while in Group A, it was 18.90 per hour  $(\pm 0.38)$ . Our study has shown that lactose concentration has a strong relation with rumination quality and may provide insight into cattle health status.

# TRANSCRIPTOME PROFILE ANALYSIS OF EUROPEAN PLUM RESPONSE TO FUNGAL *MONILINIA* FRUCTIGENA PATHOGEN

#### Raminta Antanynienė, Monika Kurgonaitė, Birutė Frercks

#### Lithuanian Research Centre for Agriculture and Forestry

The European plum is a commercially important stone fruit worldwide, belonging to Prunus genus, Rosacea family. The main criteria for plum breeding are fruit quality, productivity, nourishment value and disease resistance. The most important diseases affecting stone fruits are caused by fungal pathogens of the genus Monilinia, with Monilinia fructigena being the most common species in Europe. These pathogens cause brown rot, blossom blight and twig canker, which results in considerable losses in stone fruit production. The knowledge of molecular mechanisms that determine plant response to pathogens is essential for controlling the effects of the disease. The underlying genes and molecular mechanisms involved in the postharvest response of plum to *M. fructigena* infection have yet to be identified. Fruits of the 'Victoria' plum were collected at the Institute of Horticulture of the Lithuanian Research Centre for Agriculture and Forestry orchards. The fruits were wounded and artificially injected with the pathogen. During this study, the dynamics of the plum's transcriptomic response to *M. fructigena* infection was observed by analysing gene expression levels at three postinfection stages: 24, 48, and 72 hours. The highest number of differentially expressed genes (DEGs) was shown after 72 h of infection compared to the control. The number of up-regulated DEGs in the 72h infected sample was significantly higher than in the 24h and 48h exposed fruit samples, showing the tendency to involve more genes in the response to prolonged exposure to the pathogen. The plum initiates complex defence responses to the M. fructigena infection. According to Gene Ontology enrichment analysis, the highest number of infection-related pathways were activated after 24h of infection. As the duration of the infection increases, infection-related pathways decrease. The comparative transcriptome analysis of the European plum allows us to understand the molecular mechanisms behind pathogen-infection responses. This information is essential for European plum breeding programs to develop fungi resistant varieties in the future.

# APPLE ORCHARD SOIL MICROBIOTA AND POSSIBLE FUSARIUM SPP. CONTROL BY ALTERNATIVE MEANS

# Vytautas Bunevičius, Juozas Lanauskas, Audrius Kačergius, Alma Valiuškaitė, Neringa Rasiukevičiūtė Lithuanian Research Centre for Agriculture and Forestry

Apple replant problems, commonly known as apple replant disease, are faced in fruit-growing farms worldwide. Apple orchards are usually replanted on the same sites, leaving no time for natural recovery of soil microbiota. Growth and development of replanted apple trees are usually suppressed, leading to a decline in yield and fruit quality. It can also make the plants more susceptible to common diseases. Due to the complex etiology of the disease, only limited progress has been made in developing soil remediation measures. The EU target to reduce chemical pesticides by 50% by 2030 also reduced choice of chemicals for soil remediation. It's important to look for effective alternative means to restore soil properties. Previous research has shown that biotic factors, especially microorganisms, causes apple replant problems. According to research on the soil microbiota, replant problems may be caused by various fungal, oomycete, actinomycete, and bacterial taxa, which are present in the soil to varying degrees. Alternaria spp. together with Fusarium spp., Cylindrocarpon spp. and *Mortierella* spp., they can contribute to the occurrence of apple replant disease. This study aimed to determine the distribution of pathogens in the apple orchard soil and evaluate the inhibitory effect of bio-activator and peppermint (Mentha piperita L.) essential oil against Fusarium spp. Soil microbiota taxonomic composition was evaluated using the ITS region of their rRNA gene. The results showed that the most common fungi in soil were Fusarium spp., Penicillium spp., Cylindrocarpon spp., Cyberlindnera spp. and Mortierella spp. The inhibition of Fusarium spp. was evaluated using bioactivator and 1200 µL L<sup>-1</sup>, 1600 µL L<sup>-1</sup> and 2000 µL L<sup>-1</sup> of peppermint essential oil. Results showed that bio-activator and peppermint essential oil have an inhibitory effect against Fusarium spp. The effect of the studied measures on controlling apple replant disease should be studied in more detail.

### POTENTIAL CARBON SINK CAPACITY ACROSS LITHUANIAN REGIONS

### Žygimantas Kidikas, Gediminas Zdanavičius, Vilma Naujokienė

### Vytautas Magnus University, Lithuania

Climate change is presenting unprecedented challenges, and agriculture is recognized as a major emitter of greenhouse gases (GHGs) within global economies. However, agricultural activities also play a significant role in carbon sequestration, particularly through soil, which is considered one of the most effective carbon sink mediums for mitigating atmospheric GHG concentrations. Enhancing soil carbon storage offers a dual benefit—reducing GHG emissions while improving soil fertility and agricultural productivity. Despite the known potential of soils to sequester carbon, understanding the projected capabilities of soil as a carbon storage mechanism remains crucial for effective climate action. This study aims to assess the carbon sequestration potential of agricultural soils in Lithuania by collecting, updating, and validating soil monitoring data at local, regional, and national levels. A monitoring methodology was developed to collect open soil data, coordinate the acquisition of soil samples across various Lithuanian regions, and analyze them using both spectral sensors and conventional laboratory methods. The research focuses on determining key soil parameters and assessing the impact of different farming practices on carbon sequestration. Experimental research involved collaborating with local farmers applying diverse practices on different soil types, and scanning soil samples in situ using various spectral sensors. The results provide valuable insights into the relationship between farming practices and CO<sub>2</sub> absorption, offering actionable data for optimizing carbon sequestration strategies. The findings underscore the importance of comprehensive monitoring and the potential for scaling soil-based carbon capture through improved agricultural management practices.

## ROOT ROT FUNGUS GENE EXPRESSION AT EARLY INFECTION STAGES IN SCOTS PINE

### Vilnis Šķipars, Maryna Ramanenka

Latvian State Forest Research Institute "Silava"

Transcriptomes from stem-inoculated Scots pine saplings were analyzed to identify unique and enriched *H. annosum* transcripts in the early stages of infection. Comparing different time points since inoculation identified 131 differentially expressed *H. annosum* genes with p-values of  $\leq 0.01$ . Our research supports the results of previous studies on the Norway spruce – *Heterobasidion annosum* s.l. pathosystem, indicating the role of carbohydrate and lignin degradation genes in pathogenesis at different time points post-inoculation and the role of lipid metabolism genes (including but not limited to the delta-12 fatty acid desaturase gene previously reported to be an important factor). The results of this study indicate that the malic enzyme could be a potential gene of interest in the context of *H. annosum* virulence.

**Acknowledgement:** This research was funded by the European Regional Development Fund postdoctoral research aid (grant number 1.1.2/VIAA/4/20/686).

#### HORTICULTURE

# SUSTAINABLE GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM FERMENTED ORIGANUM VULGARE L. EXTRACT AND THEIR ANTIMICROBIAL, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL COMPOSITION

#### Syeda Hijab Zehra, Khadija Ramzan, Jonas Viškelis, Aistė Balčiūnaitienė

#### Lithuanian Research Center for Agriculture and Forestry

Green silver nanoparticles (AgNPs) were synthesized using plant extract in aqueous form as bio reducing and bio capping agents. Due to their therapeutic potential applications, various metallic nanoparticles (NPs) are extensively prone to use in nanomedicine. Silver nanoparticles are re-markable in their physical and chemical properties. This study aims to develop biosynthesized AgNPs made using fermented *Origanum vulgare* L. herb extract, having increased antioxidant and antimicrobial activities. The biosynthesized AgNPs were characterized by Transmission Electron Microscopy (TEM), Scanning Electron Spectroscopy (SEM-EDS) techniques, and different spectrophotometric measurements. Green silver nanoparticles enhance antimicrobial activity against both Gram-negative and Grampositive bacteria, as demonstrated by the Kirby–Bauer disk diffusion method. The significant increase in antioxidant activity can be attributed to the phenolic com-pounds present in the extract of *O. vulgare* herb samples. TEM analysis visually confirms the spherical shape and size of 10-20 nm. SEM-EDS was performed for green AgNPs and precise and uniform distribution. This study presents, for the first time, the application of fermented *O. vulgare* herb extracts in the synthesis of silver nanoparticles (AgNPs) and demonstrates an enhancement in both antioxidant and antimicrobial activities.

# MANAGEMENT OF MINERAL NUTRITION TO CONTROL THE QUALITY AND SAFETY OF POST-HARVEST LEAFY VEGETABLES

# Darius Jermala, Neringa Rasiukevičiūtė, Alma Valiuškaitė, Kristina Bunevičienė, Viktorija Vaštakaitė-Kairienė

#### Lithuanian Research Centre for Agriculture and Forestry

Fungal diseases cause up to 70% of crop yield reductions and post-harvest losses. While chemical fungicides are used for the purpose of controlling plant diseases, there are numerous hazards related to the development of pathogen resistance to chemicals as well as concerns regarding the environment. The connection between mineral nutrition and the control of plant diseases is known; however, the interactions between mineral nutrients and pathogens in leafy vegetables, particularly during post-harvest storage, have not been well reported.

In this study, we evaluated the influence of different concentrations of Ca (40, 60, 80 ppm), Mg (20, 40, 60 ppm) and N (80, 120, 180 ppm) on fungal pathogens *Alternaria* spp. and *Botrytis* spp. spread on lettuce (*Lactuca sativa* L.) grown in hydroponic systems. The lettuce leaves were artificially inoculated with 5 mm fungi discs and kept at 4°C and 22°C. The measurements of pathogen's spread were done after 2, 4, and 7 days after inoculation (DAI).

At 4°C, Mg concentrations did not significantly affect *Alternaria* spp. infection, with infection percentages remaining consistent across time points. For *Botrytis* spp., significant effects were observed at 2 and 4 DAI, but not at 7 DAI. At 22°C, Mg showed no significant impact on *Alternaria* spp., although infection increased overall with time. For *Botrytis* spp., Mg concentrations significantly influenced infection at 2 DAI, with the highest infection at 40 ppm after 7 DAI. Ca concentrations showed no significant impact on *Alternaria* or *Botrytis* spp. infection at 4°C across all time points. At 22°C, Ca significantly influenced Alternaria infection at 4 and 7 DAI, with lower concentrations (80 ppm) resulting in reduced infection. For *Botrytis* spp., significant effects were observed only at 2 DAI, with 80 ppm associated with lower infection rates. N concentrations did not significantly influence *Alternaria* or *Botrytis* spp. infection at 4°C. No both fungi. For *Alternaria* spp., infection at 4°C. However, at 22°C, N had a significant impact on both fungi. For *Alternaria* spp., infection rates at all time points, with higher concentrations resulting in elevated infection rates at all time points, with higher concentrations resulting in elevated infection rates at all time points, with higher concentrations resulting in elevated infection at 7 DAI (81.2% at 80 ppm). Mg and Ca had limited effects, particularly at lower temperatures. N consistently influenced fungal infections, especially at 22°C, highlighting its role in fungal susceptibility under warmer conditions.

Acknowledgement. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-23-20.

### THE EVALUATION OF SWEET CHERRY CULTIVAR FROST TOLERANCE BY REL METHOD

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Frost tolerance is important in the selection of sweet cherry cultivars, but small frost damage cannot be visually detected. The relative electrolyte leakage (REL) method can determine the effect of frost on the permeability of cell membranes, which is not visually detectable. In the result of frost, the cell membranes are damaged, increasing their permeability. Ions from the plant sample leak through the damaged membranes in water, increasing the electrical conductivity (EC) of the solution. However, REL indicates also the dormancy status of plant and increases by de-acclimation. Goal: to evaluate the frost tolerance of sweet cherry cultivars 'Iputj' and 'Arthur' using REL method. The cultivars were tested in two different orchards. Orchard 1 was planted in 2016 using the rootstock P. mahaleb. Orchard 2 was planted in 1994 using the rootstock *P. avium*. The method was applied to annual shoots from natural field conditions (control) and after freezing at -25°C in February and at -15°C in March. REL% was calculated as the relation of sample liquid EC before freezing and maximum EC. In orchard 1, the cultivars showed significant differences in REL% in natural conditions in February: 'Iputj' had lower REL% than 'Arthur'. In March, there were no significant differences between the cultivars, either under natural conditions or after freezing. In orchard 2, the cultivars had no significant differences in February. In March, significant difference was observed for the cultivars' Iputj', with REL% being significantly lower than' Arthur' after freezing. In both orchards and cultivars, REL% increased after freezing in February indicating increased cell membrane permeability due to frost damage. However, in March, a decrease in REL% was observed after freezing indicating a reduction in cell permeability. This suggests adaptation to frost during the re-acclimation.

# THE IDENTIFICATION OF THE BACTERIAL ORIGIN ANTAGONISTS FOR THE MANAGEMENT OF STONE FRUIT MONILIOSIS

### Saulė Raklevičiūtė, Augustina Kolytaitė

### Lithuanian Research Centre for Agriculture and Forestry

Moniliosis is a fungal disease that mainly affects stone fruits such as plums, peaches, and cherries. It is a destructive disease that occurs worldwide and damages fruits, blossoms, leaves, and shoots. Moniliosis is caused by *Monilinia* spp., more specifically *M. laxa*, *M. fructigena*, and *M. fructicola*. Moniliosis can appear as blight blossom in the spring, which affects the blossoms, and as brown rot, which damages the fruits. Infected blossoms will turn brown and will spread the infection to shoots, where it will start producing spores. Brown rot will cause fruits to rot and mummify, where the fungi can overwinter and continue spreading its' spores in spring. If weather conditions are favorable, brown rot disease can result in significant fruit losses within the orchard and post-harvest periods.

Some management tactics include regularly removing infected fruits as well as inspecting collected harvests. However, chemical control, such as the use of fungicides, is more reliable and effective in disease management. The start of fungicide usage should be in the early spring, before any flower buds appear, and applied regularly. Tree pruning should also be part of the treatment routine to lessen the possibly favorable weather conditions for fungal growth. Despite the reliability of this control method, there is a high risk of the development of fungal resistance to fungicides. It can also cause significant side effects on human health and the environment. Therefore, it is important to develop alternative methods to fight this disease.

The use of biological control agents against fungal pathogens could be a great strategy for managing such diseases. In this research, bacteria have been collected from plum tree phyllosphere and rhizosphere and their antagonistic properties have been tested against *M. fructigena*. The bacteria which inhibited the growth of this pathogen have been selected for identification.

# THE BIOLOGICAL AND GENETIC MECHANISMS OF FRUIT DROP IN APPLE TREE (*MALUS* × *DOMESTICA* BORKH.)

# Tautvydas Gurskas, Aurelijus Starkus, Šarūnė Morkūnaitė Haimi, Edvinas Misiukevičius, Vidmantas Stanys, Birutė Frercks

### Lithuanian Research Centre for Agriculture and Forestry

The apple tree (Malus × domestica Borkh.) is one of the most popular fruit trees for growing us a food tree worldwide. Global apple fruit production has grown rapidly over the past two decades. It has increased by 54%. The most popular is fresh fruits of high quality, which depend on different internal and external fruit parameters, like size, colour, acidity, sweetness, and maturity of the fruits.

Yield self-regulation in apple trees helps to have high quality of crop. Only 5–10% of blooms can develop into marketable-quality fruits. Self-regulation is a complex biological process that is influenced by environmental and genetic factors. Too much blooming and overcropping are big problems for apple growers. Over cropping determines many small and low-quality fruits.

Apples and other fruit trees have a natural system of fruitlet self-elimination. The natural optimal selfelimination of apple fruitlets is rare, and only a few apple tree varieties naturally thin fruitlets up to one fruit per inflorescence. In most cases, apple trees do not eliminate enough fruitlets to produce a qualitative fruit. The genetic programming within the tree determines how many of these buds will develop into fruit-bearing structures and how many immature fruitlets will be dropped.

Regulation of the fruitlets' number on the tree is an essential technological aspect in horticulture, as flower or fruitlet thinning is used to optimise the yield. This can be conducted by hand, mechanically, or by using chemical substances.

The biological and genetic mechanisms of yield self-regulation in apple trees are important. The internal factors like plant physiology, genetics, and hormones, as well as external influences like environmental cues and management practices. The internal factors are the focus of this review.

### **GENERATIVE DEVELOPMENT OF PERENNIAL ALLIUM PLANTS**

### Vaida Čepulienė, Rasa Karklelienė, Danguolė Juškevičienė, Jonas Viškelis

### Lithuanian Research Centre of Agriculture and Forestry

The Allium genus, part of the Amaryllidaceae family, includes many species that thrive in the Northern Hemisphere, often valued for their culinary and medicinal properties. The field experiments with *Allium schoenoprasum* L., *A. angulosum* L., *A. nutans* L., *A. fistulosum* L., and *A. ursinum* L. were carried out at the Lithuanian Research Centre of Agriculture and Forestry in 2022 - 2024.

Perennial *Allium* species are adapted well to their growing environment and survive in varying conditions, with bulbs allowing them to endure adverse climates and regrow each season. Long-term observations showed that flower and inflorescence morphology, size, and colors differ among the perennial *Allium* plant species. *Allium* plants usually form significantly more inflorescences in the 3rd and 4th growing years. After the first year of planting, *Allium* species normally form inflorescences of only single plants. Data from the literature show that the vegetation duration of the discussed *Allium* species is similar and lasts 195-220 days, except for *A. ursinum* L. Its vegetation duration was observed up to 110 days. Our results showed that the beginning of flowering and seed maturity period ranged from 33 to 54 days for tested species.

This research aimed to evaluate the complex effect of abiotic and biotic factors on the generative development of perennial *Allium* plants.

# IDENTIFICATION OF FUNGAL PATHOGENS NATURALLY OCCURRING ON FRUIT TREES OF THE ROSACEAE FAMILY

#### Akvilė Paliulytė, Raminta Antanynienė, Birutė Frercks, Monika Kurgonaitė

#### Lithuanian Research Centre for Agriculture and Forestry, Lithuanian University of Health Sciences

Fungal diseases, such as *Anthracnose, Fusarium, Botrytis* and *Monilinia*, are the major pests of fruit trees, affecting fruit quality and size. Monilinia affects blossoms, shoots and fruits. Anthracnose causes sandy, black, or brown spots on fruits and leaves, which can later spread to the branches. Another fungal disease, fusarium, is characterized by drying and yellowing leaves and can cause the plant 's shoots to wilt. Gray mold, also known as botrytis, primarily attacks fruits. Morphological characteristics alone are insufficient for determining diseases, as one disease can overshadow another or be easily confused with others. Therefore, the most accurate method for identifying microorganisms is molecular techniques. In this study the genetic diversity of fungal diseases on plum fruits was investigated using internal transcribed spacers (ITS1 and ITS2). The standard protocol for identification of four main fungal diseases by using multiplex PCR was developed.

#### IDENTIFICATION OF THE CAUSATIVE AGENTS OF BROWN ROT ON STONE FRUIT PLANTS

#### Rugilė Bartašiūtė, Raminta Antanynienė, Monika Kurgonaitė, Birutė Frercks

#### Lithuanian Research Centre for Agriculture and Forestry

Brown rot is caused by species of pathogenic fungi from the genus *Monilinia*, including *M. fructicola*, *M. fructigena*, *M. laxa*, and *M. polystroma*, *M. aucupariae*, *M. vaccinii* – *corymbose*. The pathogens infect pome fruits (such as apples and pears) and stone fruits (such as cherries, plums, sour cherries, apricots, nectarines, and others). In orchards, the fungus can affect blossoms, leaves, and annual shoots, with fruits being the most vulnerable. Initially, brown rot appears as a small, circular, slightly sunken spot and over time, it spreads covering the entire fruit. Studies indicate that brown rot can lead to losses of up to 90% of the harvest in some regions. *M. laxa* and *M. fructigena* are widespread in Europe, *M. polystroma* is found in Japan and Poland, and *M. fructicola* is present in North America and Australia. *M. vaccinii* – *corymbose* is widely distributed across North America and is considered one of the most serious diseases affecting blueberries. *M. aucupariae* is a fungal pathogen primarily affecting hawthorn fruits.

The aim of an investigation is to identify primers for accurately distinguishing all six *Monilinia* species. In this study, the ITS sequences and DNA sequences of hypothetically pathogenesis – related proteins of *Monilinia* spp. were analysed and primers were generated using ClustalX2 and Primer3Plus programs.

For primers effectiveness analysis, fruits from stone and pome fruit plants showing signs of brown rot were collected at the Lithuanian Research Centre for Agriculture and Forestry orchards. DNA was extracted and *Monilinia* spp. pathogen species were identified through a multiplex polymerase chain reaction (mPCR) method. Generated primers enable to distinguish *Monilinia* spp. through a single multiplex polymerase chain reaction.

# IMPACT OF RASPBERRY BUSHY DWARF VIRUS ON FRUIT QUALITY OF THREE RED RASPBERRY CULTIVARS

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Raspberry bushy dwarf virus (RBDV) is the most widespread virus in many regions of Ukraine affecting raspberry plantations. This study aimed to assess the impact of the RBDV on the yield and quality of three red raspberry cultivars: 'Brusvyana', 'Joan J', and 'Zyugana'. Research was conducted in two raspberry plantations in the Kyiv region. We collected fruits at the same time from both RBDV-infected and virus-free plants and analyzed them for various parameters, including the number of fruits per bush, size (number of drupelets), weight, and chemical composition (soluble solids content, pH, titratable acidity, total sugars, vitamin C, total phenols, and anthocyanins).

Results showed that RBDV reduces the number of fruits (by up to 33%) and significantly affects size and weight (by 46.1–61.3%). This impact was valuable across all three varieties, with significant deformities noted in fruits from infected plants. The content of total sugars and soluble solids content decreased the most in infected fruits of 'Brusvyana' by 11.9% and 12.5%, respectively. While vitamin C content dropped to 9.6%, this decrease was not statistically significant. RBDV did not result in significant changes in pH.

The total content of anthocyanins and phenolic compounds was higher in fruits from RBDV-infected plants (by up to 61%), although this effect was not observed in 'Joan J'. Overall, the findings confirm that RBDV negatively impacts the chemical composition and quality of raspberry fruits.

#### AGROBIOLOGY, AGROECOLOGY, AGRICULTURAL ENGINEERING

# ADAPTIVE AGROTECHNICAL MEASURES FOR THE STABILITY OF PRODUCTIVITY UNDER CHANGING CLIMATE

#### Justinas Gegeckas

### Lithuanian Research Centre for Agriculture and Forestry

The EU Agriculture sector for the last couple of decades has faced these main challenges: overuse of mineral fertilizers and plant protection products, changing climate patterns and soil degradation. To address these issues the EU policy, green course, besides more published aims, has the target by 2030 to reduce 50% of chemicals and 20% of fertilizers usage.

The aim of this thesis is to find out if the chosen agrotechnical solutions could help to adopt to climate change and in the short term compensate lost yields when smaller quantities of fertilizers will be applied. The chosen agrotechnical measures are foliar nutrition, biological seed treatment and hydrogel, which will be tested in most common crop rotation in Lithuania: peas followed by winter wheat, winter oil seed rape and spring wheat.

These alternatives will be compared to typical agriculture practices: NPK usage based on recommended quantities and seeds treatment with chemical fungicides. To evaluate the differences between different trial plot outcomes these main means were selected: yield, hectolitre weight, 1000 grain mass, root development, leaf size, SPAD index, chlorophyll fluorescence, plant sap analysis and soil Haney analysis.

In 2024 season, at LAMMC field testing facilities, in pea crop, above mentioned agrotechnical measures were implemented. The current obtained results showed that according to Duncan's statistical method there was no significant difference in yield among eleven trial treatments. However, the highest yield (2,722 t) were equally the same for the control (the same nitrogen quantity and no additional means) and for the treatment, which combined foliar nutrition and chemical seed treatment. Moreover, the same treatment showed the highest values in leaf's chlorophyll content (43,69) and pea's protein level (25,05). And finally, the highest chlorophyll fluorescence (0.584) was seen in the treatment in which 20% more mineral nitrogen fertilizers were applied. The primary results indicate that chosen agrotechnical measures could compensate for lower nitrogen applications in the future.

Acknowledgement: Supervisor Dr Virmantas Povilaitis.

# IMPACT OF ACCOUNTING SYSTEMS FOR GHG EMISSIONS AND CO2 ABSORPTION IN AGRICULTURE ON CLIMATE CHANGE MITIGATION

### Gediminas Zdanavičius, Žygimantas Kidikas, Vilma Naujokienė

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The main goal of this work is to analyse accounting systems for GHG emissions and CO<sub>2</sub> absorption in agriculture and to evaluate their effectiveness in reducing environmental pollution. Analysing the elimination of GHG emissions from the agricultural sector, when pollutants during various processes and activities are widely spread, noticeable and underground water, accumulates in the soil and around so in or, which as a result requires negative climate change and temperature fluctuations.

This paper analyses the contribution of GHG emissions from the agricultural sector to climate change and temperature changes. The focus is on pollution caused using chemicals, vehicles and electrical equipment in the sector, there are many resources that need to be consumed in large quantities, crossing the need for production, turning them into agricultural and meat farms that leave an important pollution footprint. It mainly focuses on three greenhouse gases, viz. carbon dioxide ( $CO_2$ ), methane ( $CH_4$ ) and nitrous oxide ( $N_2O$ ).

Based on the latest data from the Ministry of Environment and other institutions, the period 1990-2022 is presented. assessment, which makes it clear that the agricultural sector is the third most important source of GHG emission pollution in Lithuania and the fourth at the global level. Methods and innovative farming solutions are proposed that can reduce greenhouse gas emissions and CO<sub>2</sub> absorption processes. According to the EU agreement, there are targets until 2050. EU member states, including Lithuania, are obliged to strive to reduce the amount of GHG emissions to a neutral climate effect and at the same time to make decisions on how to compensate for GHG emissions that have already been emitted, which indicates a greater capacity to absorb CO<sub>2</sub>.

To assess the situation in farms, the assessment methodology of the construction cycle and the International Commission on Climate Change is chosen, as well as the accuracy and efficiency of their application. The evaluation of GHG emission and CO<sub>2</sub> absorption accounting systems, the possibilities of using calculators, the EU's aspirations and progress in creating a carbon dioxide certification system are analysed.

# ENHANCED-EFFICIENCY NITROGEN FERTILIZERS APPLICATION TO REDUCE AMMONIA VOLATILIZATION AND N $_2$ O EMISSIONS

#### Samar Swify, Romas Mažeika

#### Lithuanian Research Centre for Agriculture and Forestry

Recently, the application of enhanced-efficiency nitrogen fertilizers (EENFs) such as urease inhibitors and coated controlled-release urea has been extensively advocated for agricultural lands. These fertilizers are essential for optimizing nitrogen utilization efficiency and augmenting crop yield through the regulation of nitrogen transformations and reducing nitrogen losses via ammonia volatilization and N<sub>2</sub>O gas emissions. A greenhouse experiment under conditions of 60% humidity and 20°C temperature was performed for 28 days at the Agrobiology laboratory in 2021, aiming to study the effect of enhanced-efficiency urea fertilizers to reduce nitrogen losses and improve urea efficiency. Five treatments were used including control, urea, urease inhibitors (NBPT), urea coated with potassium humate, and urea coated with fulvic acid in the same dose 170 kg N ha<sup>-1</sup>. The barley (*Hordeum vulgare* L., Ema DS cultivar) was tested to evaluate the effect of these fertilizers on green mass. The plant is cultivated to an elevation of 40 cm, at which point, the biomass produced in each experimental vessel is excised and subsequently weighed. The biomass is subjected to drying at a temperature of 40 °C until a constant mass is achieved, after which it is ground and analysed for total nitrogen content. Furthermore, the concentrations of mineral nitrogen in the soil samples were quantified. Dräger tubes were utilized to assess the concentrations of ammonia (NH<sub>3</sub>) present within the soil containers, while samples of nitrous oxide gas were collected using a 20-cc syringe through the pierceable rubber septum located atop the cylindrical groove that enveloped the containers. Our findings demonstrate that the application of urease inhibitors markedly diminished the emissions of  $N_2O$  and the volatilization of NH<sub>3</sub> in comparison to the comparative treatments. The application of coated urea with potassium humate followed this. Conversely, urea resulted in significantly elevated greenhouse gas emissions, succeeded by the treatment involving urea coated with fulvic acid. In contrast, urea coated with fulvic acid displayed a nonsignificant higher green mass yield than conventional urea. Our results underscore the critical role that enhanced efficiency nitrogen fertilizers (EENFs) play in mitigating nitrogen emissions within agroecosystems while simultaneously enhancing the efficiency of urea utilization.

# THE IMPACT OF WINTER WHEAT CULTIVARS MIXTURES ON DISEASES AND YIELD AFTER DIFFERENT PRE-CROPS

#### Simonas Saikauskas, Jūratė Ramanauskienė

#### Lithuanian Research Centre for Agriculture and Forestry

Adapting to varied climate conditions during the growing season is essential for effective crop management, especially with the unpredictable weather linked to climate change. By cultivating diverse winter wheat varieties with varying adaptive traits, farmers can better cope with these fluctuations. This approach has gained traction, particularly as agricultural practices increasingly adopt reduced soil tillage and continuous sowing methods. However, growing wheat on the same land for 2-3 years consecutively can encourage disease development, underscoring the importance of crop rotation. Winter wheat mixtures integrate well into rotation systems, helping to disrupt disease cycles and maintain soil health for subsequent crops like corn or soybeans. This study investigates the spread of fungal diseases in genetically diverse wheat mixtures and examines the economic and sustainability benefits of these mixtures. Disease control remains a significant challenge for farmers, as it directly impacts yield. The overuse of chemical fungicides, however, has reached a critical point, caused environmental harm and threatening human health. Wheat mixtures offer a promising alternative by enhancing natural disease resistance, thus lowering the need for chemical treatments. Studies indicate that mixing wheat cultivars could reduce fungicide applications by up to 67% compared to monoculture systems. These mixtures also improve crop resilience, boosting yield quality and reducing disease prevalence. Key factors, such as canopy structure and diverse stem and leaf characteristics, contribute to the effectiveness of these mixtures. Recent research across multiple trial sites revealed a 70% reduction in azole fungicide resistance in cultivar mixtures, underscoring the potential of this approach. Even under variable weather, winter wheat mixtures have consistently shown positive impacts, with yield improvements of up to 0,5 tons per hectare compared to monocultures. This evidence suggests that mixed winter wheat cultivars offer a sustainable, resilient, and economically viable solution for modern agriculture.

### ANALYSIS OF COMPOST PRODUCTION FROM GREEN WASTE

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The analysis of compost production shows that compost is the product of the composting process, produced by the decomposition of organic waste by micro-organisms, soil protozoa and an aerobic environment. The phases of composting have been distinguished: incubation (from - 4 to 20 °C), mesophilic I (30 to 45 °C), thermophilic (45 to 65 °C, but there are differences of opinion between authors, others emphasizing 50 to 70 °C), mesophilic II (45 to 20 °C), maturation (close to ambient temperature), the essential difference being in the temperature ranges and the micro-organisms that dominate in these temperature ranges. Important parameters of the composting mixture are identified: C:N ratio, moisture content, particle size of the organic material, porosity, bulk density; monitoring parameters of the process: moisture content, pH, total oxygen consumption; the parameters identifying the quality of the final product must comply with the parameters laid down in the applicable legislation. The analysis of meat bone meal as a potential compost additive has shown that it can contribute to the promotion of soil biodiversity and, due to its slow decomposition, act as a long-lasting organic fertilizer. The equipment needed for the composting process is presented, which can contribute effectively to the efficient running of the composting process. The analysis of the environmental assessment of compost production has shown that the composting process, when carried out properly, is not harmful to the environment.

# QUANTITATIVE ASSESSMENT OF SOIL-BORNE PATHOGENS IN RELATION TO TILLAGE PRACTICES AND COVER CROP (WHITE MUSTARD) USE

# Neringa Matelionienė, Nazerke Torekhanova, Gražina Kadžeinė, Inga Tamošiūnė, Danas Baniulis, Skaidrė Supronienė

### Lithuanian Research Centre for Agriculture and Forestry

Soil-borne pathogenic fungi are major contributors to crop diseases, threatening agricultural productivity and soil health. Sustainable farming practices, such as conservation tillage and the use of cover crops, can potentially reduce the prevalence of these pathogens by altering the soil environment and promoting beneficial microbial communities. Our study investigates the effect of tillage methods (conventional tillage, reduced tillage, and no-till) and cover crop management (with white mustard and without) on the abundance of soil-borne pathogenic fungi (*F. culmorum*, *F. graminearum*, *V. longisporum*). Root zone soil samples were collected during the flowering stage of spring wheat in June 2024 and analyzed using quantitative real-time PCR. Preliminary results indicate that reduced tillage practice reduces the abundance of cover crops showed no significant effect on pathogen suppression but reduced total fungal DNA in the soil. These findings suggest that conservation tillage and cover crop combinations can create soil conditions that inhibit pathogen proliferation, possibly through enhanced competition from beneficial microbes and changes in soil physical structure.

# CHARACTERIZATION OF MICROPLASTICS ISOLATED FROM SEWAGE SLUDGE OF DIFFERENT SCALE WASTEWATER TREATMENT PLANTS

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Microplastics, defined as plastic particles ranging from 1  $\mu$ m to 5 mm, are highly durable and mobile, making them difficult to eliminate from the environment. The persistent release of plastics, which take hundreds of years to decompose and gradually fragment into smaller particles, poses a significant and unpredictable environmental risk. While research on microplastics has extensively covered aquatic systems, studies addressing soil contamination remain understudied, and methods in this field is still emerging.

Wastewater treatment plants (WWTPs) act as key collection sites for microplastics. Although advanced treatment processes can remove up to 99.9% of these particles from water, a large portion still accumulates in sewage sludge, presenting environmental concerns. For instance, in Germany, concentrations can reach as high as 24,000 particles kg<sup>-1</sup> dry sludge. This is a concern, especially since roughly half of this sludge is applied as agricultural fertilizer. Consequently, this study aimed to investigate and detail the characteristics of microplastics found in WWTPs of varying scales.

Sewage sludge was collected from a WWTP in Kaunas (large scale) and Šilalė (small scale), Lithuania. Fenton's reagent was used to remove organic matter (OM) from sewage sludge, later saturated NaBr solution was used to perform density separation. Qualitative characteristics: size, shape and color were evaluated using a stereomicroscope.

It was found that regardless of the scale of the WWTP, the dominant shape of the particles was fragment, the color – transparent, and as the size of the particles decreased, their percentage increased. However, differences in size between small and large WWTP low density (<1 g cm<sup>-3</sup>) MP particle were observed. Sewage sludge from small-scale WWTP contained approximately 12.71 % more MP particles ranging in size from  $0.03\mu$ m to 1mm compared to large-scale WWTP. These findings raise a new research question about the influence of WWTP size, equipment and treated wastewater volume on microplastic fragmentation.

#### ENHANCING CARBON ACCUMULATION IN ACID SOIL BY COMBINATION OF LIME AND MANURE

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### Lithuanian Research Centre for Agriculture and Forestry

Soil organic carbon (C) is a key component in regulating ecosystem production and agricultural sustainability. Soil plays an important role in mitigating climate change, focusing on increasing organic carbon (SOC) in the soil system. Also, permanganate oxidizable carbon (POXC) has been widely utilized as a soil condition indicator due to its correlation with biological indicators and sensitivity to management effects over relatively short time periods. In this study, we examined the effects of different management practices on soil physical separation into different aggregates and C accumulation in these fractions. This experiment was initiated in 1949 at the west part of Lithuania, where soil of the experimental site is the Retisols (texture moraine loam). This study was comprised of four treatments: (T1) Unlime (naturally acidic soil), (T2) Farmyard manure (FYM) 60 tha<sup>-1</sup>, (T3) Lime 2.0 tha<sup>-1</sup>, and (T4) Lime 2.0 tha<sup>-1</sup> + FYM 60 tha<sup>-1</sup> with three replications. The lime and farmyard manure were applied every 5 years interval into the soil. Soil samples were collected from a depth of 0-10 and 10–20 cm. The soil aggregates were separated into eight fractions by Elliot method. The proportion of organic carbon, permanganate oxidized carbon, fulvic acid and humic acid were calculated. Our study showed the organic carbon was much higher in T4 19.28% especially in silt clay fraction as compared to naturally acidic soil. The permanganate oxidized carbon content was higher in mesoaggregate (57%), and silt clay fraction (46%) as compared to other aggregate fractions respectively. While the fulvic acid decreased 35% in silt clay fraction of lime and manure treated soil in both soil layers. These results indicate that the aggregate associated carbon in different aggregates can help us to better understand carbon storage in acidic soil and help to reduce the risk of carbon emission to the atmosphere.

# ASSESSING THE EFFECT OF INTERCROPPED WINTER RYE SERVICE CROPS ON GRAIN LEGUME AND SOIL PROCESSES

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#### Lithuanian Research Centre for Agriculture and Forestry

The integration of winter rye (Secale cereale L.) as a service crop in intercropping systems offers potential benefits for enhancing soil quality and grain legume (Fabaceae) performance, yet its impacts on soil processes and legume yield stability are not fully understood. This study evaluates the relay intercropping system of grain legumes and winter rye in organic farming conditions. A field experiment was performed in 2023 - 2024 at the Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture. The treatments comprised relay intercropped winter rye with pea, lentils, chickpea and their corresponding monocropping with four replicates involved. Soil chemical properties: mineral nitrogen (Nmin) (0-30 and 30-60 cm), water extractable organic carbon (WEOC) and humic substances (HSs) (0-25 cm) were analyzed. Samples for soil chemical properties analysis were taken after the legume harvest. The yield of winter rye was evaluated in 2024. The highest mineral nitrogen concentration at 0 – 30 cm depth was observed in spring pea, grown as the sole crop, compared to the winter rye spring sown control. The same tendency of mineral nitrogen concentration was observed at 30 – 60 cm depth. The highest mobile humic acid concentration was observed in relay intercropped winter rye and lentil. The highest organic carbon concentration was observed in winter rye spring sown. The highest yield of winter rye was after chickpea pre-crop. Data from one year shows that monocrops of legumes create good conditions for pre-crops of winter cereals to grow. However, the results indicate that a higher Nmin amount after legumes can lead to N losses (there can be too much nitrogen for cereals due to their low N needs in the fall). In plots with legume and rye intercropping, a lower Nmin level was found, and the excess N for rye in the fall is managed effectively.

# THE ROLE OF MODERN TECHNOLOGIES IN ENHANCING SUSTAINABLE DEVELOPMENT GOALS IN AGRICULTURE AND FORESTRY

### Yanal Alkuddsi, Marius Aleinikovas, Benas Šilinskas, Mohammad Almogdad, Mindaugas Škėma

#### Lithuanian Research Centre for Agriculture and Forestry

This research explores the pivotal role of modern technologies in promoting sustainable development goals (SDGs) within the agriculture and forestry sectors. As global challenges like climate change and food security become increasingly pressing, innovative solutions are essential for achieving sustainable practices. Genetically modified crops are highlighted as a significant advancement in agricultural technology. These crops are engineered to enhance desirable traits such as pest resistance, drought tolerance, and improved nutritional value. By increasing crop yields and reducing reliance on chemical pesticides, GMOs contribute to food security and sustainable farming practices. The adoption of GMOs aligns with the SDGs by promoting responsible consumption and production patterns. The research discusses the role of nano-fertilizers in improving agricultural efficiency and sustainability. Unlike traditional fertilizers, nano-fertilizers provide nutrients to plants in a more controlled manner, reducing waste and minimizing environmental impact. This technology enhances soil health, boosts crop productivity, and decreases the need for chemical inputs, supporting the SDGs related to sustainable agriculture and environmental conservation. The study also examines the potential of bioethanol produced from wood waste as a renewable energy source. Utilizing by-products from forestry not only addresses waste management challenges but also contributes to reducing greenhouse gas emissions. By converting tree residues into bioethanol, this approach promotes sustainable energy production, aligning with the SDG to ensure access to affordable, reliable, and sustainable energy for all. The integration of modern technologies such as GMOs, nano-fertilizers, and bioethanol production from wood waste plays a crucial role in enhancing sustainable development goals in agriculture and forestry. These innovations not only improve productivity and efficiency but also promote environmental sustainability, thereby contributing to a more sustainable future.

# EFFICACY OF THREE EPN SPECIES ON ADULT MORTALITY OF THE INVASIVE PEST OTIORHYNCHUS SALICICOLA

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*Otiorhynchus salicicola* is a broad-spectrum polyphage weevil that has recently spread massively in Europe, causing significant damage to plants, particularly to raspberries and strawberries. One of the best environmentally friendly means for controlling economically important insect pests is entomopathogenic nematodes (EPNs). Although EPNs are suggested for the biocontrol of some weevils, no evidence of the efficacy of these products on *O. salicicola* is available. In this study, we evaluated the effectiveness of three EPN species on adult weevil mortality, and the best-performing EPN species in terms of concentration and timing were determined. The two most efficient EPN species were also tested for possible synergistic or antagonistic effects of infection. Therefore, our results contribute to a better understanding of EPN biopesticide effectiveness on *O. salicicola* adults.

# EFFECT OF DIFFERENT GRANULAR ASH ON NUTRIENT BALANCE AND ASSESSMENT OF HEAVY METAL POLLUTION

### Gabija Žilytė, Kristina Bunevičienė, Urtė Mečionė, Aušra Bakšinskaitė

#### The Lithuanian Research Centre for Agriculture and Forestry

It is well-known that the current make-use-dispose approach is unsustainable and negatively impacts the environment, economy, and public health. In response to these concerns, governments worldwide are seeking scientific methods to recycle various types of waste. Biofuel ash is an alkaline waste rich in minerals and nutrients, capable of addressing the need to enhance soil fertility. However, it is important to note that ashes will likely contain potentially hazardous substances. Utilizing this ash for fertilizer production can play a significant role in tackling waste management challenges, but proper assessment and management of its composition are essential to ensure safety and environmental protection. This study aimed to investigate heavy metal accumulation and mobility in the plants and soil while examining changes in soil nutrient levels. The field experiment was carried out for three years (2019-2021). For the experiment, researchers used ash and three granulated ash products. The fertilizing products were applied on the surface and incorporated into the soil before the first year's sowing. During the experiment, physicochemical analyses of fertilization products, soil, and plants were conducted. Two indicators were selected to evaluate the contamination of grains and straws with heavy metals: the contamination factor (CF) and the pollution load index (PLI). Soil pollution was low in all treatments. Grains and straw accumulated the highest amount of Ni. The analysis of Cd, Cr, Cu, and Zn in the grain showed a moderate contamination factor. It is also worth noting that fertilization with ashes had a different effect on the nutrient balance.

**Acknowledgment:** This study was supported by the long-term research program "Productivity and sustainability agrogenic and forest soils", implemented by the LAMMC.

# PLANT MICROBIAL BIOSTIMULANTS ENHANCE THE PRODUCTIVITY AND QUALITY OF CARROT ROOT CROPS AND EFFECT AGROCHEMICAL SOIL PROPERTIES

#### Božena Ignotienė, Sigita Jurkonienė, Virgilija Gavelienė

#### Nature Research Centre, Lithuania

Application of microbial biostimulants is growing as sustainable agriculture develops. This research aimed to assess the effects of two commercial plant microbial biostimulants ProbioHumus and NaturGel on carrot growth, yield, and quality. The study was conducted in controlled laboratory conditions and field settings. Two factors were used in the study: ecological - in organic plots and nonecological – in nonorganic plots. Carrots were treated at 9 leaves stage using 2 L ha<sup>-1</sup> of preparations in organic farms and, using the same volume of preparations combined with application of mineral fertilizers in nonorganic farms. Biometric measurements and chemical analyses were conducted at maturity. The average weight of carrot roots increased by 17 g in organic farms and 20 g in conventional farms when treated with ProbioHumus compared to the control. Both ProbioHumus and NaturGel positively impacted carrot quality in organic and conventional farms, promoting higher levels of monosaccharides, ascorbic acid, carotenoids, phenols, and boosting antioxidant activity. Nitrate analysis indicated that carrots from organic farms had about half the nitrate content of those from conventional farms, and the probiotics did not significantly affect nitrate levels. Moreover, ProbioHumus and NaturGel were effective even at low doses. The use of microbial biostimulants is recommended as part of sustainable cultivation practices. These probiotics also enhanced the soil's agrochemical properties, including total and nitrate nitrogen, total and available phosphorus, organic carbon, humic acid, and humus content. In summary, plant probiotics serve as an ecological alternative for cultivating carrots while improving soil health.

# OPTIMIZATION OF CELLULOLYTIC ACTIVITY CONDITIONS OF SOIL BACTERIA AND EVALUATION OF THEIR INFLUENCE ON SEED GERMINATION

#### Egidija Satkevičiūtė, Arman Shamshitov, Skaidrė Supronienė

#### Lithuanian Research Centre for Agriculture and Forestry

Cellulolytic bacteria have great potential for application in various industrial fields and can become an effective tool for increasing the output of agricultural products, but due to the extremely high abundance and diversity of species, these microorganisms are relatively little studied. As a result, five cellulolytic bacteria isolated from the soil of central Lithuania were analyzed in this work: B. pumilus, B. mobilis, M. chalcea, S. canus and S. achromogenes. The aim of the research was to determine the cellulolytic activity of mentioned soil bacteria, optimize its conditions and evaluate the effect of cellulolytic bacteria on seed germination. In this work, the gravimetric method was used to investigate how the cellulolytic activity of the tested bacteria varied depending on the type of substrate (wheat straw, rapeseed straw and their mixture). It was observed that B. pumilus, B. mobilis, M. chalcea and S. achromogenes bacteria decomposed wheat straw most efficiently, and S. canus bacteria – a mixture of wheat and rapeseed straw. The strongest decomposition of wheat straw, rapeseed straw and their mixture were characterized by S. canus bacteria. The Congo red method was used to optimize the conditions most suitable for the cellulolytic activity of the bacteria under study. It was found that the most optimal conditions for B. pumilus bacteria were 25°C temperature and pH 7, for B. mobilis bacteria – 10°C temperature and pH 5, for M. chalcea bacteria – 20°C temperature and pH 4, S. canus and S. achromogenes bacteria – 30°C temperature and pH 6. Evaluation of the germination of wheat inoculated with the tested bacteria was carried out. It was determined that B. pumilus, B. mobilis, S. canus and S. achromogenes bacteria inhibited wheat germination, while M. chalcea bacteria stimulated wheat germination by 3%.

#### TRANSMISSION OF SARCOCYSTIS SPP. IN LITHUANIAN LIVESTOCK FARMS

#### Agnė Baranauskaitė, Aistė Stundžėnaitė, Petras Prakas, Elena Servienė, Živilė Strazdaitė-Žielienė

#### Nature Research Centre, Lithuania

About 15,000 species of single-celled protozoa are known in the world. To date, most studies of these parasites have been conducted by analyzing animal carcass samples. However, parasitic protozoa can be transmitted by cysts through contaminated water or food. It is possible that there are more transmission routes, but so far, no further research has been done in this area. In general, the most studied protozoa belong to the phylum Apicomplexa. One of them is the causative agent of sarcocystosis. Therefore, the aim of this study was to determine the prevalence of Sarcocystis species infecting domestic animals in farmlands in Lithuania and to investigate possible routes of transmission of these parasites. During the research, environmental (water, hay, and soil) and insect samples were collected from 12 livestock farms in Lithuania. Prior to genomic DNA isolation, sporocysts of Sarcocystis spp. in environmental samples were collected and concentrated by filtration. Meanwhile, the insects were first washed with sterile water by shaking for 1 hour, then they were crushed, strained, and the resulting liquid was used to collect the sporocysts by filtration. A nested PCR using specific or universal primers targeting COX1 or 28S rRNA was performed to detect Sarcocystis DNA in the samples. The obtained results showed that 8 species of Sarcocystis infecting domestic animals were found in environmental samples, including zoonotic S. hominis. Regardless of the type of environmental sample, the most common species detected was S. cruzi infecting cattle. Furthermore, analysis of the results from the insect washes also revealed the presence of S. cruzi species. To conclude, it can be said that various insects, especially those that feed on animal feces, can be one of transmission routes of parasitic protozoa in livestock farms.

Acknowledgement: The project has received funding from the Research Council of Lithuania (LMTLT), agreement (S-MIP-23–7).

#### EVALUATION OF BIOCOATING EFFICACY IN MITIGATING AMMONIA EMISSIONS FROM MANURE

### Ieva Knoknerienė, Rolandas Bleizgys

### Vytautas Magnus University, Lithuania

Ammonia (NH<sub>3</sub>) is a primary gas responsible for the acidification of precipitation, which can subsequently cause significant damage to ecosystems. These gaseous emissions not only contribute to atmospheric pollution but also pose risks to both livestock in confined environments and human workers. A significant portion of these harmful gases is released during the microbial degradation of proteins in cattle urine and manure. The intensity of gas emissions during manure storage is influenced by the type of biocoating applied to the manure. To assess the efficacy of various biocoatings in reducing ammonia emissions, a series of studies were conducted using materials such as chopped straw, sawdust, hemp leaves, and peat. Ammonia concentration was measured using the GME700 gas analyser, which employs laser spectroscopy for continuous or cyclic measurement in automatic mode. The experimental data were normalized to determine the ammonia emission intensity per unit area of manure surface and the ventilation intensity per unit area. The results indicated that the reduction in ammonia emissions was most pronounced when manure was covered with bio-organic coatings characterized by lower porosity and higher liquid absorption capacity. Specifically, the study found that ammonia emissions were minimized with a biocoating thickness of more than 10 cm of straw, 8 cm of crushed hemp leaves, 5 cm of coniferous sawdust, and 3 cm of peat. These findings suggest that the use of bio-organic coatings with specific material properties can significantly reduce ammonia emissions from manure storage.

#### FORESTRY

# FORESTRY DEER POPULATIONS ON THE RISE: INSIGHTS FROM HUNTING DATA IN THE BALTICS AND CENTRAL EUROPE

# Mindaugas Bakševičius, Darius Hardalau, Michal Manton, Gediminas Brazaitis, Kastytis Šimkevičius, Artūras Kibiša

#### Vytautas Magnus University, Lithuania

This study explores the evolution of deer populations in the Baltics, Central, and Eastern Europe through the analysis of hunting bag data, which represents the total number of specimens harvested annually. By utilizing hunting bag statistics reported by wildlife managers and hunters, this research aims to provide a more reliable indicator of population trends compared to traditional wildlife monitoring techniques, which may suffer from issues such as double counting and underreporting. The study focuses on red, roe, and fallow deer, drawing data from national statistics databases over a decade-long period from 2012 to 2022. While acknowledging the impact of factors such as poaching, predation, and vehicle collisions, the analysis centres on hunting bag figures as a primary metric. The findings indicate that hunting bag data can serve as a dependable basis for understanding population dynamics and trends, offering valuable insights for wildlife management and conservation strategies in the region. This research underscores the significance of integrated approaches in assessing cervid populations and their ecological roles, contributing to the broader discourse on biodiversity and ecosystem health.

# FREE GROWTH IN YOUNG NORWAY SPRUCE (PICEA ABIES) PLANTATIONS

### Simonas Šilingas

### Vytautas Magnus University, Lithuania

The study was carried out between 2020 and 2022, assessing the occurrence of secondary growth of Norway spruce in natural habitats. The study assessed the phenology and dendrometric characteristics of the individuals but focused on the secondary growth events.

The results showed that, depending on the season, between 23 % and 50 % of the trees were free growing. The mild and warm conditions in August and September favoured free growth. Among the trees aged 6-9 years, 82-84% of the trees without free growth maintained this status over the following two seasons. Silleptic growth decreased with age and proleptic growth increased. Over the seasons, individual trees were more consistent in maintaining proleptic growth than sylleptic growth. Trees growing on a wet site had significantly more free growth in all seasons than trees growing on a normally irrigated site. Norway spruce individuals showing signs of secondary growth were significantly taller than those without signs. We conclude that selecting trees based on overall height, especially those with weak free growth, can exploit the advantages of free growth without significantly increasing the risk of autumn or winter frost damage.

### ENHANCING RIPARIAN ZONES THROUGH FOREST MANAGEMENT AND INFRASTRUCTURE

### Toms Štāls, Līga Pentjuša, Zane Lībiete

### Latvian State Forest Research Institute "Silava"

Forested riparian zones along smaller streams are essential for local ecosystem health and have significant conservation value. These areas serve as natural buffers, filtering water, stabilizing banks, and reducing erosion. They also support the hydrological cycle by regulating flow, preventing floods, and contributing to groundwater recharge, while providing recreational spaces and adding scenic value.

This study examines a 1.4-kilometer stretch of the Tora River, running through forested land in northcentral Latvia. The river, averaging 2-4 meters in width, is bordered by a 50-meter-wide riparian protection zone. Historically, forest management along the river has overlooked considerations for water quality and nearby ecosystems, leading to densely populated, even-aged spruce stands close to the water. However, the riparian area also supports valuable habitats and structures vital for biodiversity, such as old oak trees and standing or fallen deadwood, which provide habitats for rare species (identified by experts).

To enhance water quality, preserve natural values, improve environmental accessibility, and educate wider audience, green and blue infrastructure initiatives in study site were introduced in late 2023 and early 2024. These efforts included diversifying the riparian area by thinning dense spruce growth and encouraging broadleaf species, constructing a peak flow control structure in the ditch before it discharges into the river, and creating a nature trail with informational exhibits about ongoing research and riparian processes. Environmental monitoring, initiated three years before these actions, will continue for several years.

This monitoring includes tracking various parameters across the entire territory, such as physical and chemical indicators of ground, precipitation, and river water. It also involves monitoring groundwater levels and air temperature on the riverbank (to assess shading) and analysing the volume and composition of litter. Long-term monitoring efforts yield valuable data for understanding ecosystem dynamics, guiding future conservation strategies, and assessing the effectiveness of restoration interventions.

### USAGE OF REMOTE SENSING TECHNIQUES IN FOREST FIRE ANALYSIS

# Sadig Zeynalov, Gediminas Brazaitis

### Vytautas Magnus University, Lithuania

Forest fires, while often perceived as destructive forces, play a crucial role in maintaining the health and diversity of ecosystems. In many ecosystems in the world, fire forms an inseparable part of the system. Regular wildfires are needed for the continuity of African savannas, Mediterranean maquis or pine forests in many regions of the world. In these ecosystems, called fire-dependent ecosystems, many species adapt to the existing fire regime and maintain their continuity in the system.

Remote Sensing (RS) and Geographic Information Systems (GIS) provide a superior approach to fire monitoring and assessment. Especially in the prevention, damage analysis and monitoring stages, images obtained by remote sensing satellites provide a synoptic data set containing rich spectral and spatial information. When evaluated in terms of forest areas with high fire risk factors, the ability of a geographic information system to process key features such as meteorological, physical, ecological and logistic data together creates the opportunity for the development of prevention and early intervention plans, as well as for the early analysis of damage that will occur because of a possible fire. Post-fire vegetation recovery can be assessed by using different indices through RS techniques. In literature, various of methods have been used before. The Normalized Difference Vegetation Index (NDVI), the Enhanced Vegetation Index (EVI), and the Normalized Burn Ratio (NBR) are the NIR-based spectral indices that are most frequently used to monitor PVR among those derived from RS imagery. Many studies have been done regarding post-fire vegetation recovery rate, and recovery driven patterns. The monitoring of post-fire vegetation recovery is important since it provides valuable data for assessing ecosystem resilience, determining landscape dynamics, and forest management.

#### **BIODIVERSITY INDICATORS IN HEMI-BOREAL FORESTS: TREE-RELATED MICROHABITAT RICHNESS**

### Gailenė Brazaitytė, Vitas Marozas, Žydrūnas Preikša, Gediminas Brazaitis

### Vytautas Magnus University, Lithuania

Tree-related microhabitats (TreMs) are established biodiversity indicators that provide habitats for a variety of animal, plant and fungi species. Microhabitats are typically associated with old-growth forests, but can occur in commercially managed stands providing shelter, food, and breeding areas. In this study, we aimed to survey TreMs covering mature commercially managed hemi-boreal forests of Lithuania. We observed stands with different dominant tree species including Norway spruce (Picea abies), Scots pine (Pinus sylvestris), silver birch (Betula pendula), black alder (Alnus glutinosa), European aspen (Populus tremula), pedunculate oak (Quercus robur), small-leaved linden (Tilia cordata) and Norway maple (Acer platanoides). In 132 plots (500m2), we surveyed tree species, the number of microhabitats per tree, and their types. Regardless of the species, TreMs on average occurred on 23.2% of the trees, of which 18.8% had single microhabitats, and 3.4% had two microhabitats. We determined that TreMs were on 51.1% of pedunculate oaks, 46.2% of Norway maple, 43.4% of black alder, 26.8% of European aspen, 21.2% of silver birch, 15.4% of small-leaved linden. The lowest occurrence of TreMs was observed on conifers – 8.0% of Norway spruce and 1.0% of Scots pine. The most common microhabitat types were deformation and growth forms (in 7.72 out of 100 trees), and epiphytes (in 7.28 out of 100 trees), while the least abundant were nests (in 0.27 out of 100 trees), and bark microhabitats (in 1.38 out of 100 trees). This suggests that biodiversity in commercial forests can be supported by retention of trees bearing microhabitats, prioritizing trees with two or more TreMs and uncommon types on various tree species.

### PINE NEEDLE PATHOGEN LECANOSTICTA ACICOLA IN LATVIA

### Selita Rancāne, Dārta Kļaviņa, Baiba Krivmane, Keitlīna Krastiņa, Zane Striķe, Tālis Gaitnieks

Latvian State Forest Research Institute "Silava"

*Lecanosticta acicola*, the causal agent of brown spot needle blight, is a known pine needle pathogen. During the last decades, it has spread in Northern Europe, including the Baltic countries, where it has mainly infected exotic pine species. This study presents novel data on the occurrence of the pathogen in introduced *Pinus* spp., as well as on the further spread of the pest to the native pine species in Latvia. In the autumn of 2022, infection of *L. acicola* was discovered on several young native Scots pine (*Pinus sylvestris* L.) stands throughout Latvia; infection extent was monitored during the following seasons. The transmission of the pathogen to the local pine populations could accelerate spread of the pathogen and decrease the vitality of pines in the Baltic region in the future. The fungus was identified by molecular PCR-based methods as symptom severity was still low (mostly latent infection); conidia were found only on a few pines in surveyed stands. Nevertheless, the observed behavior of the pathogen implies the establishment and presumed invasion in the native forest areas, and thus the emergence of additional risks for forest management.

# INFLUENCING PUBLIC ACCEPTANCE OF PRESCRIBED FIRE AS A FOREST MANAGEMENT TOOL IN LITHUANIA

### Šarūnas Bujakovski – Kukcinavičius, Michael Manton, Charles Ruffner

### Vytautas Magnus University, Lithuania

The use of prescribed burning is largely accepted and employed in North American as useful tool for reducing fuel loading and improving forest health. Whereas in Europe prescribed fire is considered as a destructive element that strikes fear within society. For instance, Lithuania forest law has prohibited the use of prescribed fire or any other fire. The development of new forest policy in Europe calls for "Closer-to-nature" forest management that emulates natural forest patterns and processes. This includes prescribed fire.

The aim of my project contains several aspects:

- Identify the forest fire history of the Lithuanian forest landscape.
- Identify the benefits of prescribed burning for sustainable forest management.
- review policy on prescribed fire.
- improve the general lack of understanding about prescribed fire through education.

# DNA MARKER-BASED FOREST GENETIC MONITORING (FGM) SYSTEM IN LITHUANIA: CASE STUDY ON QUERCUS ROBUR

#### Mindaugas Ilčiukas, Darius Danusevičius

#### Vytautas Magnus University, Lithuania

Pedunculate oak (Quercus robur L.) is an important and valuable forest tree species throughout Europe. Oaks are a key biodiversity species which provide habitats for many other species. At the same time, it is valued for its durability and longevity in the forestry and construction industries. Ongoing climate change and overexploitation are recognized as the main threats to oak trees and their ecosystems. This challenge requires the assessment of genetic variability towards defining sustainable management strategies. Thus, genetic diversity is of paramount importance and serve as a base for forests tree species evolution and adaptation to climate change. The aim of Forest Genetic Monitoring (FGM) is to assess the status of genetic resources and quantify relevant changes at a temporal scale, to preserve long-term adaptive evolutionary potential. European forest genetic recourses program (EUFORGEN) has emphasized the importance of FGM and presented revised FGM methods. Furthermore, EUFORGEN underlined that the system for FGM of the forest genetic conservation units would be an invaluable tool for conservation and sustainable use of forest genetic resources. Therefore, our study is presenting DNA marker-based case study of FGM of pedunculate oak in two forest genetic reserves (Dzirmiškis and Didžiagiris) in Lithuania. In total we genotyped 368 trees: 194 adults (92 DZIR\_T and 92 DZIR\_T) and 194 saplings of natural regeneration (92 DZIR\_V and 92 IGNA\_V). Our results based on 12 microsatellite markers shows how DNA markers can be employed for DNA based FGM system in Lithuania. The FGM project and case study on pedunculate oak will be presented.

# THE ASSESSEMENT OF VEGETATION DEPENDENT ECOSYSTEM (DIS)SERVICES ALONG FOREST ROAD VERGES

### Līga Pentjuša, Linda Gerra-Inohosa, Zane Lībiete, Agnese Skabe

### Latvian State Forest Research Institute "Silava"

The construction and renovation of forest roads create a multifunctional landscape that supports various ecosystem services and disservices. In a managed forest landscape forest roads provide habitat for plants and animals, thus supporting biodiversity. At the same time, roads cause landscape fragmentation and serve as a potential distribution pathway for invasive alien species.

We studied vegetation-dependent ecosystem service changes along forest road edges in a conventionally managed forest area in Latvia by conducting repeated ground vegetation assessments. We analyzed four different ecosystem service indicators - forest specialist plants, invasive plant species, usable plants and plants important for pollinators - one year before, as well as two and seven years after road building or reconstruction.

Study period was 2016 to 2024. In total, five forest roads were surveyed. Along each road, ground vegetation composition was assessed in a 1 km long road section where 20 3x10 m vegetation survey plots were established.

We found significant changes in species composition and richness across the surveyed four indicator groups over time. Our results suggest that the construction of new roads creates environmental conditions favorable for non-forest specialists and can potentially contribute to the spread of invasive plant species in the forest landscape. However, constructed roads create habitat for higher diversity of light demanding species including plants important for pollinators, thereby contributing to both ecosystem services and disservices.

# CORD-FORMING BASIDIOMYCETES: THEIR ANTAGONISM TO HETEROBASIDION SPP. AND ARMILLARIA SPP. IN PEAT SOIL

#### Keitlīna Krastiņa, Dārta Kļaviņa, Tālis Gaitnieks

#### Latvian State Forest Research Institute "Silava"

Effective management of *Heterobasidion* root-rot in spruce forests requires exploring biological control options, particularly cord-forming fungi with antagonistic properties. This study aims to evaluate the effects of *Resinicium bicolor* and *Hypholoma* spp.—cord-forming basidiomycetes known to inhibit *Heterobasidion* spp.—against *Heterobasidion* root-rot and *Armillaria*. We evaluated the competitive ability of *Resinicium* bicolor and *Hypholoma* spp. against pathogenic fungi *Heterobasidion* spp. and Armillaria spp. under laboratory conditions in peat soil using wood blocks. New isolates of *Resinicium* bicolor and *Hypholoma* spp. against for growth in laboratory conditions. Additionally, we tested their antagonism in preventing *Heterobasidion* spore infections in spruce wood. Results indicate that *Resinicium bicolor* and *Hypholoma* spp. exhibit competitive interactions with pathogenic fungi in controlled settings, suggesting their viability as biocontrol agents in peat soils.

FOOD SAFETY AND VETERINARY MEDICINE

# IN VITRO STUDY OF FERMENTED BOVINE MILK DIGESTION: VALIDATED METHOD FOR THE ISOLATION OF INDIGENOUS PROBIOTICS

# Agnė Vasiliauskaitė<sup>1</sup>, Elvidas Aleksandrovas<sup>1</sup>, Ida Rud<sup>2</sup>, Lina Laučienė<sup>1</sup>, Mindaugas Malakauskas<sup>1</sup>, Loreta Šernienė<sup>1</sup>

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Probiotic bacteria have gained popularity due to increasing scientific evidence supporting their health benefits. However, although some lactic acid bacteria (LAB) demonstrate good survival in vitro during digestion simulations, their survival is influenced by various factors and may decrease when incorporated into food products.

Therefore, in this study, in vitro digestion simulations of fermented bovine milk and acid-curd cheese samples (control and those with isolated *Lacticasei bacillus paracasei* A11) were performed using the standardized static method. The counts of A11 were analyzed in cheese pre- and post-digestion to assess survival. The probiotic properties of the strain were evaluated in acid (2.5 pH; 0, 3 h) and bile salt (0.3, 0.5, and 1%; 0, 24 h) conditions. Counts of this strain in cheese samples were determined using 16S rRNA gene amplicon sequencing (MiSeq, Illumina).

Results indicated that A11 tolerated acid (8.61  $\pm$  0.06 log CFU/g after 3 h) and bile salts (6.66  $\pm$  0.2 log CFU/g after 24 h with 1% bile salts), highlighting its probiotic potential. A significant decrease in LAB counts was observed in cheese without A11 post-digestion (approximately 4.46 log CFU/g), while cheese with A11 maintained higher LAB stability (around 6.08 log CFU/g) post-digestion (p < 0.05). Predigestion, A11 counts were 6.95  $\pm$  0.05 log CFU/g on day 1 and 6.89  $\pm$  0.03 log CFU/g on day 10. Post-digestion, counts remained stable at 6.7  $\pm$  0.44 log CFU/g on day 1 and 6.84  $\pm$  0.1 log CFU/g on day 10, indicating a 98% survival rate.

This study demonstrated that A11 is highly tolerant of acid and bile salts, confirming its probiotic potential. Cheese without A11 showed a significant reduction in LAB counts post-digestion, while cheese with A11 maintained greater stability. The strain exhibited a 98% survival rate during digestion, supporting its viability as a probiotic.

# ANTIMICROBIAL RESISTANCE OF ESBL AND/OR AMPC PRODUCING ESCHERICHIA COLI ISOLATED FROM FIVE-DAY-OLD BROILERS FLOCKS IN LITHUANIAN POULTRY FARM

# Beatričė Kasparavičienė, Jurgita Aksomaitienė, Neringa Kašėtienė, Jūratė Stankevičienė, Mindaugas Malakauskas, Aleksandr Novoslavskij

### Lithuanian University of Health Sciences

The high prevalence of ESBL and/or AmpC producing Escherichia coli in broilers can lead to human infection and increasing antimicrobial resistance (AMR) cause a risk for consumer health.

Our study of three broiler flocks revealed a high prevalence of ESBL and/or AmpC *E. coli* in a 5-day-old broilers cloacal samples (up to 57.5%) and environmental swabs (up to 25%). One hundred two ESBL/AmpC *E. coli* isolates (92 ESBL positive/AmpC negative and 10 ESBL positive/AmpC positive) were tested against 13 antibiotics with E-test strips to determine MIC according EUCAST (2024) and CLSI (2024) guidelines. All examined isolates were sensitive only to meropenem, fosfomycin and colistin, but resistant to at least two of the tested antibiotics. A total of 23 different antimicrobial resistance profiles were observed with the most common profiles (17.65%) as CRO/AMS/AUG/CIP/SXT/TE and a combination of CRO/CIP. Multidrug resistance was confirmed in 80.39% of the tested ESBL and/or AmpC *E. coli* strains. The high resistance rates of ESBL and/or AmpC E. coli were observed for ceftriaxone (93.1%, MIC range from 2 mg/L to >256 mg/L) and ciprofloxacin (97.1%, MIC range from 0.19 mg/L to >32 mg/L). E. coli isolates from cloacal and environmental samples showed similar AMR profiles to all tested antibiotics except that environmental isolates were 5.9 times frequently resistant to gentamicin in comparison to cloacal isolates (p < 0.05).

A high prevalence and antimicrobial resistance of ESBL and/or AmpC producing *E. coli* in 5-day-old broilers emphasizes the need for strategies to control their spread in the early stages of broiler production.

**Acknowledgement:** This research was funded from the Research Council of Lithuania (LMTLT) grant No. 022-PRO-00037 under the framework of the Joint Programming Initiative on Antimicrobial Resistance.

# SILICA MONOLITHS MODIFIED IN-SITU WITH PENTAFLUOROPHENYL LIGANDS FOR TOCOCHROMANOL SEPARATION BY HPLC

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#### Institute of Horticulture, Latvia

Silica-based monoliths offer higher separation efficiency per unit pressure drop compared to particlepacked columns. However, the narrow range of commercially available chemistries restricts their application. This study includes the modification of silica-based monoliths with pentafluorophenyl (PFP) ligands to contribute to agricultural research by allowing efficient separation of essential plantderived compounds. The in-situ modification of these monoliths enables hydrogen bonding, dipole,  $\pi$ - $\pi$  and hydrophobic interactions, which are critical for the precise separation of tocochromanols compounds important to plant nutrition and human health. Three Chromolith silica monoliths were modified with three different PFP-moieties. The silica monolith was successfully modified in-situ with 3-(pentafluorophenyl)propylmethyldichlorosilane and the separation behavior was compared to a particle packed pentafluorophenyl column and a C18 monolith for the separation of tocochromanols (Vitamin E related compounds). The modified PFP monoliths demonstrate successful separation of Vitamin E-related compounds under optimized conditions (water-methanol 22:78, v/v; flow rate 1.45 mL/min; temperature 15°C), outperforming traditional C18 monoliths while maintaining lower backpressure. Additionally, backpressure and Van Deemter comparisons were performed and demonstrated the main PFP monolith advantages were obtained near the pressure maximum of the PFP particle packed column at 3.5 mL/min, comparable efficiencies were obtained, and the PFP monolith decreased the backpressure by one-third. The obtained monolith can be utilized for the separation of various agriculture-related compounds, and the method demonstrated is adaptable for modifying silica monoliths with different ligands, suited to specific compound separations.

**Acknowledgment:** This research was funded by the Latvian Council of Science, project "Dicotyledonous plant families and green tools as a promising alternative approach to increase the accessibility of tocotrienols from unconventional sources", project No. lzp-2020/1–0422".

# DEVELOPING MICROBIOLOGICALLY SAFE AND VALUE-ADDED KOMBUCHA VIA SOLID-PHASE EXTRACTION OF SEA BUCKTHORN LEAVES

### Juozas Girtas<sup>1</sup>, Ingrida Mažeikienė<sup>1,2</sup>, Antanas Šarkinas<sup>1</sup>, Karolina Almonaitytė<sup>1</sup>

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Sea buckthorn is a valuable plant that contains almost 200 nutrients and biologically active components, mainly in berries and leaves. The process of harvesting these berries, which involves cutting the branches, freezing and shaking, leaves a lot of biomasses, which is usually composted, but has the potential to be used again. Considering the requirements of EFSA and the lack of scientific publications, it is important to evaluate the genetic parameters of sea buckthorn secondary raw material products. This would increase the possibilities of using the remaining biomass after separating the sea buckthorn berries.

This study examines the secondary utilization of sea buckthorn biomass for the development of new food products with enhanced nutritional value. This study aims to optimize the extraction of bioactive compounds from these secondary raw materials using an innovative solid-phase extraction method based on the creation of a pressure gradient between solid and liquid matrices. The compounds extracted were evaluated and applied to food matrices, such as in the production of kombucha, to create value-added products. The study includes shotgun sequencing for comparative metagenomic analysis of the *Medusomyces gisevii* culture used in Kombucha to understand their effects on fermentation and product quality. This new approach is in line with the European Green Deal initiatives for the sustainable use of resources and the development of a circular economy, promoting the sustainable use of biomass and waste reduction in the food industry.

# INVESTIGATIVE STUDIES ON *BABESIA CABALLI* AND THEILERIA EQUI PIROPLASMS WITHIN THE HORSE POPULATION IN LITHUANIA

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Equine piroplasmosis is a globally distributed, tick-borne disease affecting horses, donkeys, and other equids, caused by the protozoan parasites *Theileria equi* and *Babesia caballi*. While infected animals often show few signs—like fever, jaundice, pale mucous membranes, or decreased performance – some can be asymptomatic, complicating detection and control. The epidemiology and prevalence of this disease in Lithuania remain largely unreported. To bridge this gap, a study collected 152 serum samples and 83 whole blood samples from clinically healthy horses, varying in age, sex, and breed, across 13 municipalities in Lithuania.

The study employed two diagnostic methods: Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). ELISA, which may not effectively identify early-stage infections or differentiate between active and prior infections, indicated that  $7,24\% \pm 4,2\%$  of serum samples tested positive for *Theileria equi*, while one sample tested positive for *Babesia caballi*. PCR, known for its heightened sensitivity and specificity, especially in detecting low parasite levels and identifying pathogens even in chronic stages, determined that  $27,7\% \pm 9,5\%$  of the blood samples tested positive for piroplasms. Statistical analysis indicated a significant relationship between *Theileria equi* infection and the municipality where horses were kept, though factors like age, sex, and breed showed no significant correlation when tested with ELISA.

Further PCR analysis, which included DNA primers specific to piroplasms, found a statistically significant association between breed and infection rate. Additionally, PCR results highlighted a significant correlation between piroplasm infection and the municipality of residence, while no notable correlation was observed with age or sex.

Although sample size was limited by available resources, this research confirms the presence of *Theileria equi* and *Babesia caballi* in Lithuania's equine population. As climate change progresses, it is anticipated that the prevalence of these pathogens may rise, underscoring the need for ongoing surveillance and management in affected regions. It is essential to consider appropriate prevention measures and the implementation of an EP surveillance program to reduce and control infection.

# CROSS-SECTIONAL STUDY ON THE PREVALENCE OF TICK-BORNE ENCEPHALITIS IN HORSES IN LITHUANIA

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Tick-borne encephalitis virus (TBEV) is an emerging zoonotic pathogen of growing concern in Europe, particularly in endemic regions like Lithuania. While various animal species have been studied for their potential as TBEV sentinels, the role of equine as indicator of TBEV circulation remains incomplete. The present study evaluated TBEV prevalence dynamics in Lithuanian horses. A serosurvey targeting TBEV-specific IgG antibodies and direct virus detection analysis of selected horses (n=82) were performed in 7 districts in Lithuania. Results showed high seroprevalence, 54 (65.9%, 95% CI 55.1 – 75.2) horses from 7 districts of Lithuania were positive for TBEV IgG. To assess active infections, the whole blood samples were tested using PCR for the presence of TBEV RNA. This screening identified 7 horses (8.5%, 95% CI 4.2 - 16.6) as viremic. These positive samples were subsequently analyzed by digital PCR for precise quantification of viral load. The total viral load varied from 0.91 to 18.72 viral copies/µL. Additionally, comparative analysis was made to compare the results of TBEV antibodies distribution in 2019 and 2023 years. TBEV seroprevalence among horses significantly increased from 2019 (35.8%, 95% CI 26.2 - 46.7) to 2023 (71.4%, 95% CI 60.0 - 80.7), (p<0.01). This increase may indicate intensified TBEV circulation within equine environments in recent years due to climate change or more frequent contact between horses and ticks because of expanding tick habitats. Overall, the findings indicate widespread exposure of TBEV infections in Lithuanian horses. This highlights the potential of horses as sentinel animals for monitoring TBEV circulation in endemic areas. Regular surveillance in equine populations could strengthen early detection of TBEV activity, aiding public health efforts to mitigate transmission risks to both animals and humans.

### ANIMAL WELFARE IN INTENSIVE FARMING

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### Vytautas Magnus University, Lithuania

The welfare and well-being of animals in intensive farming have become a significant ethical and social concern, open to investigation in both political and scientific settings; Despite the European Union's (EU) extensive efforts since the 1960s to safeguard animal welfare in agricultural and industrial operations, it has failed to address all contributing elements, including the underlying issues that drive the intensification of animal husbandry. To investigate and comprehend important concerns in the previously stated topic of animal welfare further, a theoretical analysis and a comprehensive literature evaluation are carried out in this work. In this context, "animal welfare" and "animal well-being" are used interchangeably to refer to the physical and mental well-being of animals, including their general health, comfort, natural behaviors, overall quality of life, and lack of discomfort. These factors provide insight into how industrially raised animals' lives deviate from their natural course. Even though the current rules and policies take noticeable measures toward protecting the mental and physical health of animals, more thorough studies are required to improve animal management, handling, and care techniques. Particularly intensively bred farm animals that are one of the main targets of industrialized economies. Public education and awareness must also make progress alongside scientific discoveries for these research insights to truly have an impact, and to guarantee that the results significantly enhance animal welfare. Creating a welfare system that truly considers animals' psychological, physical, and behavioral needs, along with their basic needs, relies not only on stronger policies but also on raising public awareness and apprehension. When people are more informed and ethically aware, animal welfare can become a priority and concern in everyday choices, customer behavior, and public values, not just in policy considered for legal reasons. This social support is essential for driving meaningful, lasting improvements in how animals are treated and kept.

### EFFECTS OF PLASMA AND PLASMA-ACTIVATED WATER (PAW) TREATMENT ON RADISH SEEDS

### Sara Asna Ashari

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This study explores the effects of cold plasma treatment and plasma-activated water (PAW) on the germination and early development of radish seeds, aiming to advance sustainable agricultural methods. Cold plasma and PAW introduce reactive oxygen and nitrogen species to seeds and plants, promoting various physiological responses beneficial to plant growth. Plasma technology, primarily used for disinfection and food processing, is being repurposed in agriculture to enhance seed germination, growth vigor, and stress resilience. The research compares germination rates and seedling vigor of radish seeds exposed to either cold plasma or PAW against untreated controls. The methodology in this study explores the effects of cold plasma and plasma-activated water (PAW) on radish seed germination and early growth, with flexibility to incorporate additional experiments. Using a dielectric barrier discharge (DBD) plasma system, radish seeds are exposed to reactive oxygen and nitrogen species to enhance germination and seedling vigor; parameters like plasma intensity and exposure duration are adjustable for further trials. PAW is created by treating water with atmospheric plasma, which introduces reactive species, and is then applied to seeds, offering a chemical-free growth boost. Key metrics, including germination rate, root and shoot length, and chlorophyll content, are recorded, with the flexibility to test PAW effects at various concentrations or under different stress conditions. This approach allows for future experiments that could involve diverse crop types, stressor simulations, or the evaluation of plasma and PAW effects in field settings to fully explore their agricultural potential. Preliminary findings indicate that cold plasma treatment significantly enhances seed coat permeability, facilitating water uptake and nutrient mobilization. This, coupled with the biochemical properties of PAW, leads to improved germination rates, faster seedling growth, increased plant vigor, and enhanced stress tolerance. These results highlight the potential of plasma-based technologies as sustainable and eco-friendly alternatives to chemical treatments, offering a promising avenue for enhancing crop resilience and reducing reliance on synthetic inputs in modern agriculture.

#### AGRONOMY

### WHEAT GRAIN INFECTED BY ASPERGILLUS FLAVUS MODIFIES BEHAVIOUR OF YELLOW MEALWORM, TENEBRIO MOLITOR L.

#### Gabrielė Bumbulytė Žukevičienė, Ieva Lučinskaitė, Dovilė Čepukoit, Vincas Būda

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The reduction of insecticide application is crucial to mitigate the risks posed to human health and the environment. To control stored product pests, innovative and eco-friendly alternatives are essential, supported by a deep understanding of pest ecology and behavior. This study explores the behavioral responses of the yellow mealworm (*Tenebrio molitor* L.), a globally significant pest, to volatile organic compounds (VOCs) emitted by wheat grain contaminated with the mold fungus *Aspergillus flavus*.

The investigation employed a two-choice pit-fall test, utilizing vials containing control grains (no stimulus) alongside vials containing Aspergillus flavus-infected grains at different ages (0, 5, 10, 20 days). A Petri dish served as the experimental arena.

Additionally, the study evaluated the effects of *Aspergillus flavus* compounds reported in the literature. Behavioral experiments were conducted in Petri dishes using a two-choice assay. Beetle behavior was meticulously tracked using the computer program EthoVision XT 12 (developed by Noldus, the Netherlands). Observations spanned 5 minutes, with 10 replications performed for each tested compound. Chemical compounds were applied to five spots on the side of the Petri dish (2  $\mu$ L each, totaling 10  $\mu$ L).

Through video analysis and behavioral data processing, significant sex-based differences were observed. Female mealworms showed sensitivity to mold contamination from the onset of infection, whereas males exhibited noticeable responses only after a five-day period. VOC analysis identified compounds with both repellent and attractant properties. Hexanal acted as a repellent for both sexes, while 1-octen-3-ol repelled males but was neutral for females. In contrast, 1-hexanol attracted females and repelled males at higher concentrations, and 3-methylbutan-1-ol attracted females only at elevated concentrations, with no significant effect on males. Given its repellent properties, hexanal is recommended for further evaluation as a potential candidate for grain storage pest management.

# NITROGEN FIXATION EFFICEINCY OF BEST PERFORMING RGIZOBIUM IN CONTROLLED CONDITIONS UNDER ABIOTIC STRESS

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Soybean (Glycine max L. Merr.) cultivation is one of the main agricultural practices in many countries around the world. The latest research has focused on soybean cultivation in cool climate states, like Germany, Sweden, Denmark and Norway. The Baltic States are not a typical region for growing soybeans. Therefore, soybean growth, development and environmental conditions should be investigated to gain knowledge about the optimum soybean possibilities of production and nitrogen fixation at high latitude. To this end, we have screened naturally occurring endophytic and exophytic bacteria from soybean plants grown at the LAMMC Greenhouse during 2022-2023. Out of 130 screened microbes, the best-performing strains from a previous experiment were chosen for their potential to increase nitrogen fixation in soybeans. Subsequently, another greenhouse experiment (GE2) was conducted between February and April 2024 to evaluate the impact of these endophytes on soybean production and their contribution nitrogen fixation efficiency. Five treatment groups were established: 1. Control, 2. Arthrobacter pascens LEP 5, 3. B. japonicum, 4. Arthrobacter pascens + B. japonicum, and 5.A combination of Arthrobacter pascens LEP 5, B. japonicum, and Dynocarb. Three factors such as best performing microbes, temperature + 14 °C Night +14 °C day , +18 °C night +20 °C day and moisture variability substrate wet (500 ml/week), substrate dry (200 ml/week) were mainly focused on in this experiment. Once the plants reached a certain height, we utilized Phenospex Smart Plant analysis using Hortcontrol 3.9 software. This allowed us to obtain multiple measurements from a single screening, such as plant height, saturation, digital biomass, leaf area index, canopy lightness, surface angle, and voxel volume distribution. By analyzing these parameters statistically, we obtained highly promising and significant results for the selected microbes and biostimulants. Experiments will be conducted in field and growth chamber by using same best performing microbes for further evaluation.

# SHRIMP SHELLS VALORIZATION FROM BIOWASTE TO BIOPROTECTION: SUSTAINABLE FUNGICIDE DEVELOPMENT, AND ANTIFUNGAL ACTIVITY PREDICTION WITH ARTIFICIAL NEURAL NETWORKS

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Valorizing shrimp shells for chitosan recovery and synthesizing chitosan-based nanoparticles offers a sustainable, eco-friendly approach to fungicide development. This study evaluated the antifungal potential of chitosan nanoparticles derived from shrimp shells and synthesized using various methods, targeting Fusarium graminearum, a significant fungal pathogen in agriculture. Using in vitro assays, the fungal growth inhibition rates were measured across different concentrations (200 ppm, 500 ppm, 1000 ppm) for each sample over one week. A neural network-based dose-response model was developed to predict EC50 and EC90 values for each sample accurately. MIC values were determined based on the lowest concentration achieving significant inhibition, while EC50 and EC90 values were calculated to assess half-maximal and near-complete inhibition potency, respectively. Among the tested samples, preparations L10 and DP4 demonstrated the strongest antifungal activity with the lowest MIC values, requiring minimal concentrations to inhibit fungal growth effectively. These two preparations also showed the lowest EC50 and EC90 values, achieving both half-maximal and nearcomplete inhibition at reduced concentrations, highlighting their potency and efficiency compared to other samples like AS1 and LH1, which required significantly higher doses. The findings suggest that chitosan-based nanoparticles, particularly L10 and DP4, exhibit strong antifungal properties with promising potential as natural fungicides in agriculture. Their lower MIC, EC50, and EC90 values indicate that these preparations can effectively inhibit Fusarium graminearum at reduced concentrations, making them cost-effective and environmentally friendly alternatives to synthetic fungicides. Further studies are recommended to assess field applicability and long-term efficacy under agricultural conditions. In conclusion, L10 and DP4 stand out as the most promising candidates for agricultural fungicide development, with the potential to enhance crop protection against fungal pathogens while supporting sustainable farming practices.

**Acknowledgment:** This research was funded by the Research Council of Lithuania (LMTLT), under agreement No. S-MIP-23-6.

### INFLUENCE OF HEAVY METAL ON SEED GERMINATION

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The study investigates the effects of cadmium (Cd) and copper (Cu) on the germination and early growth of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) seeds. Germination is a crucial phase in plant development, heavily influenced by environmental factors. However, contamination from heavy metals like Cd and Cu poses potential risks to seed viability and growth, impacting agricultural productivity and ecosystem health. This study aims to elucidate how these metals affect germination rates and the morphological characteristics of seedlings.

In controlled laboratory conditions, barley and wheat seeds were exposed to 200  $\mu$ M concentrations of Cd and Cu. Parameters assessed included germination success, shoot and root lengths, and fresh and dry biomass weights. The results showed that both Cd and Cu affected germination and growth, though the extent of impact varied between metals and plant species. For barley, germination rates remained similar across treatments, but Cu significantly reduced shoot and root lengths, with Cd having a comparatively lesser effect. Fresh weight differences indicated that Cu influenced water content more than Cd, though dry weights were consistent across treatments.

For wheat, Cu and Cd led to reductions in shoot and root lengths, with no substantial differences in germination rates. Fresh weights were reduced in both metal treatments, with Cu causing more pronounced effects in root weights than Cd. However, as with barley, dry weights remained unaffected, suggesting that metal toxicity primarily impacted water content rather than biomass accumulation.

These findings contribute to understanding the environmental risks posed by heavy metal contamination in agricultural soils. They highlight that while germination rates may not always be significantly impaired, early seedling growth is highly susceptible to heavy metal stress, especially with Cu exposure, which could limit plant establishment and development.

# ASSESSMENT OF BRASSICACEAE COVER CROPS AND TILLAGE PRACTICES ON MYCORRHIZAL COLONIZATION INTENSITY IN CEREAL-BASED CROPPING SYSTEMS

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Soil management practices, particularly tillage methods and cover crop management, might have detrimental effect on arbuscular mycorrhizal fungi (AMF) colonization, which is critical for soil health, nutrient uptake, and plant resilience. It's well known that mycorrhizal fungi form beneficial symbiotic relationships with plants, but certain agricultural practices, such as conventional tillage, are known to disrupt mycorrhizal networks in the soil by breaking apart hyphal structures. Brassicaceae plants, including white mustard, due to secretion of compounds which can inhibit AMF colonization considering their antimicrobial properties, potentially impacting the following crop's mycorrhizal associations. Therefore, this study aims to evaluate the effects of different tillage practices on mycorrhizal colonization of barley roots within a cereal-based cropping system, focusing on the influence of Brassicaceae cover crop integration. The study was carried out in 2024 as a part of a longterm field experiment. A sample of volunteer barley plant roots was performed in October 2024 during tillering stage. For the AMF colonization intensity assessment methyl blue staining method was used, further with root fragments placed on slides, fixed with Canada balsam, and observed under a light microscope. Preliminary results variation across treatments, with mycorrhizal colonization intensity ranging from 35.08±7.31% to 57.27±4.69%. Statistical results showed that no-till practices significantly enhanced AMF colonization compared to conventional tillage and reduced tillage (p = 0.0067), suggesting that tillage reduced colonization due to soil disturbance. Preliminary findings also indicate that prior presence of white mustard as a cover crop further influenced colonization of the following barley, with reduced colonization observed, likely due to their non-host status and antimicrobial exudates. These findings highlight the importance of selecting appropriate tillage practices to support crop associations with AMF, which is detrimental for supporting soil sustainable agrosystems.

# EFFECTS OF INOCULATION OF SOYBEAN SEEDS WITH *BRADYRHIZOBIUM JAPONICUM* IN ORGANIC FIELDS IN LITHUANIA

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Soybeans are crops with great importance, as a source of high amounts of protein. Moreover, cultivation of this plant can improve soil fertility through increased nitrogen fixation, optimize nutrient cycling, and enhance crop productivity. In Baltic countries, there is a growing interest in boosting local protein production and reducing reliance on imports. Therefore, soybean cultivation can become a promising option. However, there is a lack of understanding how effectively cultivate soybeans in cold regions absent from *Bradyrhizobium* strains in soils, given their high capacity for biological nitrogen fixation (BNF).

This study investigated the inoculations effects of soybean N2-fixation using commercial mixes containing *Bradyrhizobium japonicum* strains. Field experiment was carried out in Lithuanian Research Centre for Agriculture and Forestry (LAMMC) in Akademija (55°24′ N, 23°51′ E), Kėdainiai district. Inoculation significantly enhanced atmospheric nitrogen fixation in soybeans (Glycine max) grown above the Northern boundary of their distribution. However, two selected soybean varieties (Merlin and Laulema) exhibited slightly different nitrogen utilization patterns. Selected commercial biostimulants had different impacts on biological nitrogen fixation of soybeans with "Bactolife" and "Rhizofix 10" having the highest nitrogen fixation efficiency. This study enhances soybean cultivation practices for sustainable N management in Lithuania and highlights the importance of *Bradyrhizobium* inoculation strategies to replace inputs of N-fertilizers.

**Acknowledgments:** This work was a part of postdoctoral research supported by the Research Council of Lithuania No. S-PD-24-45.

# CRISPR-BASED MODIFICATIONS OF ROOT ARCHITECTURE IN TOMATOES FOR ENHANCED PHOSPHORUS UPTAKE: A REVIEW

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Phosphorus (P) is an essential nutrient for every plant as it plays a significant role in the development and growth of plants. However, we have low phosphorus reservoirs in soil. Phosphorus deficiency negatively affects plant yield, and its deficiency often led to plant death. Developing plants that have a strong root architecture that can response well to low phosphorus availability is needed. Globally Tomato (Solanum lycopersicum L.), is the second most highly cultivated vegetable crop. They are a rich source of antioxidants and lycopene which are associated with many health benefits, making tomatoes a pivotal component of our diet. Considering its nutritional importance, scientists are interested in targeting its root system architecture at a very early stage (seedling) for good phosphorus uptake using CRISPR technology. PHOSPHATE TRANSPORTER 1 (PHT1) and PHOSPHATE RESPONSE 1 (PHR1) have been targeted for good phosphorus uptake. The observed signs of phosphorus starvation like decreased shoot fresh weight, increased root fresh weight, and an elevated root-to-shoot ratio, were demonstrated using CRISPR induced mutants. Along with increased soluble phosphorus concentration in the roots and decreased phosphorus content in the shoots, these mutants also displayed greater anthocyanin accumulation assuring CRISPR efficiency in tomatoes. Members of the SPX-MFS subfamily, such as VPT1 and OsSPX-MFS1-3, controls vacuolar phosphorus transport within the vascular tissues, whereas PHO1, a transporter from the SPX-EXS subfamily, controls phosphorous transport from roots to shoots. Additionally, these mutants showed a changed distribution of soluble phosphorus, with higher levels in roots and lower levels in shoots, as well as increased anthocyanin accumulation in shoots. This abstract review focuses on the importance of CRISPR in improving root system architecture to increase phosphorus uptake under limited phosphorus reservoirs.

# DEVELOPMENT OF DROUGHT TOLERANT TOMATO PLANTS BY KNOCKING-OUT MIRNAS USING CRISPR/CAS9 SYSTEM

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Solanum lycopersicum (tomato) is a major crop worldwide, suffering great yield losses due to drought, intensified by climate change. Shortage of water availability is one of the main sources of affecting plant growth and reducing the productivity of tomatoes. Recent studies highlighted microRNA as the key regulators of gene expression under the abiotic stresses. Unfortunately, their role under drought stress remains under-investigated. This research aims to investigate the role of microRNA in drought tolerance and to develop potential drought tolerant tomatoes by employing CRISPR/Cas9 system. In this study, two drought-responsive miRNAs (sly-miR5302b & sly-miR9477) were selected from previously present transcriptomics data. Single guide RNAs (sgRNAs) specific to these miRNAs were designed and inserted into the final expression vector pHSE401 using golden gate cloning system. The designed plasmid was transferred to Agrobacterium tumefaciens (GV3101) using the electroporation method. Ten days old cotyledon leaves of tomato variety Crocker were transformed using Agrobacterium-mediated transformation method. After the treatment, the explants were cultured on the selection media to induce callus formation. The regenerated shoots were then transferred to rooting media for root induction. The plants were transferred to greenhouse in pots to acclimatize to external environment and fruit formation. After confirmation of transgenic lines by PCR and sanger sequencing, three transgenic lines were selected to further analyze drought tolerance and gene expression. Drought stress was applied at the fifth leaf stage for 2 weeks by reducing the field capacity to 40 percent. The response of these plants to drought stress will be observed and documented. Data will be collected on morphological, physiological, biochemical, and histological parameters. Gene expression levels will be examined by qPCR analysis in transgenic plants grown outdoors. This study will provide critical insights into miRNA function under drought stress, offering potential breakthroughs for developing drought resilient tomatoes.