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THE EFFECT OF HYPERTHERMIA AND HYPERTHERMIC CHEMOTHERAPY ON CELLS, MITOCHONDRIA AND THEIR ENZYMES (Biochemistry)

Giedrė Šilkūnienė

Scientific supervisor:
Prof. Habil. Dr Vida Mildažienė,
Vytautas Magnus University

Dissertation defended:
27 January 2020

The aim of the study was to determine the potential targets of hyperthermia (HT) and hyperthermic chemotherapy in mitochondria isolated from healthy tissues and in cancerous and non-tumorigenic cells. For the first time, mitochondrial functions, enzyme activities, and changes induced by HT were compared in mitochondria isolated from healthy rat brain and liver. Sexual dimorphism in the response to HT was evaluated in brain and liver mitochondria; the age dependency of this response was assessed in liver mitochondria. The obtained results revealed that mitochondrial response to HT depends on many factors: tissue, sex, and age of the animal. It was shown for the first time that one of the HT targets in brain and liver mitochondria was glutamate dehydrogenase (GDH) enzyme, which was strongly inhibited by HT. For the first time the response of GDH to HT was compared in different non-tumorigenic and different types of cancer cell lines. The obtained results revealed that GDH in non-tumorigenic cells was inhibited by HT (like in mitochondria from healthy tissues), however, in the cancer cell lines HT-induced changes in GDH activity was highly heterogeneous – inhibition, absence of effect, or even stimulation. The correlation was established between the extent of GDH inhibition by HT and loss of cell viability. The novel fact demonstrated was that HT potentiates cytotoxic effect of cisplatin in ovarian cancer cells, and that the combinatory action of HT and cisplatin may be explained by the downregulated GDH expression.

INVESTIGATION OF MOLECULAR MECHANISMS IN HUMAN MYELOID LEUKEMIA CELLS USING NEW EPIGENETIC AND METABOLIC REGULATORS (Biochemistry)

Aida Vitkevičienė

Scientific supervisor:
Prof. Dr Rūta Navakauskienė, Vilnius University

Dissertation defended:
6 March 2020

Leukemia is a blood cancer caused by genetic alterations in hematopoietic cells. In this study, we concentrated on myeloid leukemia, which is a very heterogeneous disease. Its treatments is usually challenging, thus the development of new therapy approaches is necessary. The aim of our study was to elucidate molecular mechanisms in human myeloid leukemia cells using epigenetic and metabolic regulators. We demonstrated that natural polyphenol EGCG causes anti-cancerous epigenetic changes in acute promyelocytic leukemia cells and induces cellular senescence in acute promyelocytic leukemia and chronic myeloid leukemia cells *in vitro*. EHMT2/G9A histone methyltransferase synthetic inhibitor BIX-01294 causes anti-cancerous down-regulation of various epigenetic regulators in acute promyelocytic leukemia and chronic myeloid leukemia cells *in vitro*. Also, we showed that histone methyltransferase inhibitor 3-deazaneplanocin A and histone deacetylase inhibitor belinostat enhance conventional treatment for acute promyelocytic leukemia *in vitro* and *ex vivo*. Moreover, we demonstrated that oxidative phosphorylation inhibitor metformin modulates expression levels of proteins taking part in metabolic pathways of therapy-resistant acute myeloid leukemia patients' cells *ex vivo*, which might be useful in combinations with other therapeutics.

TOWARDS UNDERSTANDING AMYLOID FIBRIL FORMATION AND SELF-REPLICATION (Biochemistry)

Tomas Šneideris

Scientific supervisor:
Dr Vytautas Smirnovas, Vilnius University

Dissertation defended:
16 March 2020

Millions of people around the world suffer from amyloid-related disorders. Alzheimer's disease alone afflicts more than 50 million patients worldwide. Despite continuous efforts, there are still no disease-modifying drugs available for the majority of amyloid-related disorders and the overall failure rate in clinical trials is very high. One of the main reasons for this is a lack of fundamental knowledge of amyloid fibril formation process. In the present work, aggregation processes of insulin, mouse prion protein, and amyloid-beta were studied. Herein it is demonstrated that monomer-oligomer equilibrium is the fundamental factor

governing the formation of distinct insulin fibril strains. Moreover, evidence of the possible direct role of oligomeric insulin forms in the amyloid fibril formation mechanism is provided. Also, in this work it is shown that environmental conditions define the inhibitory efficiency of anti-amyloid compounds in a very sensitive manner. Furthermore, new mechanistic insights into the processes of prion self-replication are presented. In particular, the fundamental role of secondary nucleation in the "strain switching" is demonstrated, and a new phenomenon, which emerges during self-propagation reaction of distinct prion strains under different environmental conditions, is described. Finally, herein it is demonstrated that high-resolution atomic force microscopy combined with microfluidic sample deposition platform enables the possibility to image all molecular species present during the aggregation time-course, and allows acquiring single-molecule quantitative data.

FUNCTIONAL ANALYSIS OF CANONICAL AND MIRTRONIC MIRNAS IN THORACIC ASCENDING AORTIC ANEURYSM TISSUES AND DIGESTIVE SYSTEM TUMORS (Biochemistry)

Stasė Gasiulė

Scientific supervisor:
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Dissertation defended:
16 April 2020

miRNAs are non-coding RNAs and are involved in the post-transcriptional regulation of gene expression. Usually, various pathologies are characterized by altered miRNA expression profiles. Therefore, miRNAs can be used as biomarkers for disease diagnosis. Hence, miRNAs have a huge potential for applications during clinical practice. We identified that miRNAs are differentially expressed in ascending thoracic aortic aneurysm tissues and blood plasma samples compared with healthy samples by high-throughput sequencing. We detected that differentially expressed miRNAs may regulate the TGF-β signaling pathway and the increase of transcription factor KLF4 expression in a part of cells. During the canonical miRNA biogenesis pathway, the miRNAs are cleaved by Drosha and Dicer ribonucleases. However, during alternative biogenesis pathway, miRNAs can be produced in Drosha or Dicer independent manner. It was shown that miRNA can be located in short introns, termed mirtrons. These mirtrons are spliced and appear to bypass Drosha cleavage. However, biogenesis of only few mirtrons was experimentally proven to be directly dependent on

splicing. We identified that the biogenesis of only a part of bioinformatically predicted mirtrons is dependent on splicing. Moreover, we determined that splicing factors SRSF1 and SRSF2 promote mirtronic miRNA biogenesis. Finally, we identified that mirtronic miRNAs were differentially expressed in the digestive system cancer cell lines and tumours.

SUBSTRATE-DEPENDENT FATE OF STEM CELLS: INSIGHTS INTO ARTIFICIAL BONE FABRICATION (Biochemistry)

Milda Alksnė

Scientific supervisor:
Dr Virginija Bukelskienė, Vilnius University

Dissertation defended:
5 June 2020

Cells in the organism are surrounded by an environment that is both chemically and mechanically complex and distinct. There, they constantly interact with the extracellular matrix (substrate), which sends signals to the nucleus in a multitude of ways, activating the expression of genes responsible for cell migration, proliferation, differentiation, or apoptosis. Using the knowledge about such biological contacts, various artificial surfaces are manufactured, which solely by their physical, chemical, and mechanical properties stimulate the desired response and thus enable to control the fate of cells. One of the practical areas where such cell-substrate interaction studies can be used is the construction of artificial tissues. In this work, we analysed the impact of various FFF 3D-printed polylactic acid (PLA) scaffold modifications on the fate of dental pulp stem cells (DPSC) for creation of functional artificial bone tissue. Rat DPSCs were selected as a model. PLA scaffolds were modified by altering their topography, chemical composition, and surface coating with proteins in order to determine the impact of each substrate modification on cell adhesion, proliferation, and differentiation. The main study focus was the control of DPSC osteogenesis process; to this end, studies into molecular cell differentiation and analysis of cell-formed, bone tissue-specific extracellular environment were performed. Moreover, the manufactured constructs (DPSC-substrate) were evaluated as possible artificial bone tissue: their impact on the recipient in case of implantation was determined. This scientific work significantly contributes to the progress of bone regenerative medicine.

INVESTIGATION AND MODELING OF COMPLEX BIOCATALYTIC AND BIOELECTROCATALYTIC PROCESSES (Biochemistry)

Audrius Laurynėnas

Scientific supervisor:
Prof. Habil. Dr Juozas Kulys, Vilnius University

Dissertation defended:
29 June 2020

The goal of the thesis was to develop a method for the calculation of reaction rate parameters of complex homogeneous biochemical reactions and to apply it for the investigation of oxidation/reduction processes catalyzed by enzymes in homogeneous media and on surfaces. In the first part of the thesis, a method is developed for a calculation of reaction rate parameters from experimental data. In the second part, this method is applied for the investigation of the catalysis mechanism of laccase from *Didymocrea* sp. J6 in homogeneous media and on the surface. It was found that this laccase is capable of unusual direct electron transfer from electrode to the T2/T3 active centre, and mechanism of inhibition with fluoride ions was deduced. In the third part of the thesis, a reduction mechanism of the compound II of the peroxidase from *Coprinus cinereus* was investigated. It was found that in the first step of the catalysis, electron is transferred and is followed by a proton transfer. The reaction rate constants were explained in terms of Marcus electron transfer theory and nonadiabatic proton transfer theory.

ELUCIDATION OF THE COMPLEXITY OF MOLECULAR MECHANISMS OF CANCER CELL DRUG RESISTANCE TO ENHANCE TREATMENT EFFICIENCY (Biochemistry)

Nadežda Dreižė

Scientific supervisor:
Dr Mindaugas Valius, Vilnius University

Dissertation defended:
1 July 2020

We investigated the two most prevalent types of breast and colorectal cancer tumours. We used a triple-negative breast cancer cell line MDA-MB-231 as a model system for the study of resistance to experimental RH1 drug. Analysis of the RH1-resistant cell line revealed the enrichment of cancer stem cells, which are often associated with resistance and cancer renewal. Moreover, the activation of the c-KIT-AKT-mTOR signaling axis is crucial for the expansion of the CSC-like population. Therefore, inhibition of the c-KIT receptor along with RH1 can prevent resistance development. As a model system for colorectal cancer, we used colorectal carcinoma cell line HCT116 made resistant to widely used chemotherapy drugs, 5-fluorouracil (5-FU) and oxaliplatin (Oxa). In the case of 5-FU resistance, we showed changes in 5-FU metabolism proteins resulting in diminished DNA damage response, whereas the resistance

mechanism to Oxa was more complex and emphasized the importance of cancer cell plasticity through the enrichment of partial EMT phenotype bearing cells. Moreover, Oxa-resistant cells had an inactivating p53 mutations rendering Oxa treatment even more ineffective. This highlights the importance of mutational analysis of tumour suppressor genes and oncogenes before the selection of treatment.

DEVELOPMENT OF BIOACTIVE SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS (Biochemistry)

Evaldas Balčiūnas

Scientific supervisors:
Dr Daiva Baltriukienė, Dr John George Hardy,
Vilnius University

Dissertation defended:
11 September 2020

Tissue engineering is an interdisciplinary field aimed at constructing artificial tissues and organs that could be used for transplantation. The aim of this work was to investigate strategies and techniques for bioactive scaffold fabrication for both hard and soft tissue types. For hard tissue, a novel hybrid organic-inorganic material containing Al has been developed and structured in 3D using direct laser writing. The material was then compared to other similar materials containing Ti, V, and Zr in terms of its biocompatibility *in vitro*. Results show that the newly developed polymer is suitable for tissue engineering applications and improves collagen secretion in rat myogenic stem cells. Soft tissue engineering strategies were based on polydimethylsiloxane (PDMS). We demonstrated that its mechanical properties can be tuned by incorporating silk. In addition to that, a selection of PDMS block copolymers was screened in terms of their biocompatibility, some of which are promising for further biological applications. Surface biodecoration using collagen and RGD tripeptides significantly increased the number of attached rabbit myogenic stem cells and their metabolic activity.

INDIGO-PRODUCING ENZYMES: SELECTION AND APPLICATION (Biochemistry)

Mikas Sadauskas

Scientific supervisor:
Dr Rolandas Meškys, Vilnius University

Dissertation defended:
11 September 2020

Progress in the development of metagenomic libraries has led to the creation of rich libraries with various origins DNA fragments. However, selection of target genes and their encoded proteins from metagenomic libraries remains a significant challenge. The aim of this work was to use indigo biosynthesis as a marker for selection of metagenomic libraries. Four different indigo-producing enzymes

were selected and characterized. Icm enzyme was used as an auxiliary enzyme for the construction of enzyme-based screening system, where an enzyme of interest converts a substrate to indole-3-carboxylic acid, which is then oxidized to indigo by Icm, visually indicating the presence of the desired enzyme. In addition to Escherichia coli, this system was also found to be applicable in a gram-positive host, *Rhodococcus erythropolis*. *E. coli* cells with the recombinant Hind8 oxygenase as a bioconversion platform were used for the synthesis of new indigoid compounds with hydroxymethyl-, aminomethyl-, carboxy-, and carboxaldehyde groups. Although indole detoxification function has been suggested for the enzyme IifC, no functions for other Iif proteins have been assigned. By identifying that Iif proteins catalyze the initial steps of indole biodegradation, this study elucidates the long-searched genes and proteins responsible for indole catabolism, suggesting that indole can be detoxified by certain bacteria through Iif-mediated assimilation. Following the elucidation of bacterial indole biodegradation, several important aspects of bacterial degradation of indole-3-acetic acid (IAA, a plant growth hormone) have been addressed.

METHODS FOR THE SELECTION OF HYDROLASES BY APPLYING *E. COLI* URIDINE AUXOTROPHIC STRAIN AND SYNTHETIC NUCLEOSIDES (Biochemistry)

Nina Urbelienė

Scientific supervisor:
Dr Rolandas Meškys, Vilnius University

Dissertation defended:
22 September 2020

The diversity of living conditions of microorganisms predict that the microbiota is a huge source of new biocatalysts. Metagenomic libraries are used for the screening and selection of metagenomics enzymes. In this study, selection methods for the screening of esterases, amidohydrolases, and glycosidases from metagenomic libraries were developed. To develop the selection methods for hydrolases, synthetic nucleosides were chosen as the sole source of uridine, supporting the growth only of those recombinant clones, which encode hydrolases that complement the uridine auxotrophy of the *E. coli* DH10B ΔpyrFEC strain by hydrolysis of uridine analogues. The analysis of selected hydrolases showed, that the developed methods are suitable for the selection of metagenomic enzymes with unknown function, and selected biocatalysts are very diverse in their sequences and catalytic properties.

THE ROLE OF *ACINETOBACTER BAUMANNII* BFMRS SYSTEM IN ENVIRONMENTAL FITNESS AND INTER-BACTERIAL COMPETITION (Biochemistry)

Renatas Krasauskas

Scientific supervisor:
Prof. Dr Edita Sužiedėlienė, Vilnius University

Dissertation defended:
25 September 2020

Acinetobacter baumannii is a Gram-negative bacterium that has spread in health-care institutions worldwide. It targets individuals with critical conditions and those who have a compromised immune system. The success of the pathogen comes from its highly regulated ability to persist in the environment. In bacteria, gene regulation is commonly performed via two-component systems. Additional mechanism that allows *A. baumannii* to persist in the environment is the presence of secretion systems. However, very few studies have been performed elucidating the regulatory circuits of the latter. The understanding of how they are regulated and how beneficial they are for the interaction with the environment might aid in the design of potential treating strategies. Here, we showed that the two-component BfmRS system from *A. baumannii* is required for pellicle formation while at the same time it inhibits motility. Also, we determined that BfmRS represses inter-bacterial competition via contact-dependent growth inhibition system. Moreover, we determined that due to the secretion of a toxic component, which causes target cell growth arrest, *A. baumannii* does not require a cell-cell contact for the competition. Lastly, we present evidence that capsule plays a protective role against this mechanism. In sum, this work shows that the BfmRS system plays a central role in the ability of *A. baumannii* to persist in the environment as well as its ability to perform the inter-bacterial competition.

HIGH-THROUGHPUT SINGLE-CELL SEQUENCING AND ANALYSIS (Biochemistry)

Juozas Nainys

Scientific supervisor:
Prof. Dr Linas Mažutis, Vilnius University

Dissertation defended:
25 September 2020

In 2015, a major technological breakthrough for the first time allowed to perform high-throughput single-cell transcriptome analysis and ushered in a new age of complex organism biology research. This thesis describes efforts to optimize the inDrops method for single-cell transcriptome analysis, as well as its application for studying complex biological systems. Optimizations of the separate

steps of the inDrops workflow allowed for up to ten times more efficient capture of unique transcripts from the individual cells. The second part of this thesis describes how Epithelial-Mesenchymal Transition was characterized at single-cell resolution for the first time using HMLE cell model. One drawback of single-cell transcriptional profiling, however, is the sparse nature of the data. Because of this, weak gene-gene correlations can be obscured during analysis. We have shown how this limitation can be addressed using imputation algorithms based on data diffusion. The final part of the thesis focuses on breast cancer. In collaboration with MSKCC (USA) clinicians, an atlas of immune cells was constructed for the first time, combining over 62000 individual immune cells isolated from eight patients. This atlas revealed a vast diversity in immune cells of both the adaptive and innate immune systems. Results presented in this thesis indicate that the complex signaling and local niches in tumour microenvironment play a significant role in shaping the host immune response.

STUDIES OF REDUCTION MECHANISMS OF QUINONES AND NITROAROMATIC COMPOUNDS BY FLAVOENZYMES DEHYDROGENASES-TRANSHYDROGENASES (Biochemistry)

Benjaminas Valiauga

Scientific supervisor:
Habil. Dr Narimantas Čėnas, Vilnius University

Dissertation defended:
29 September 2020

In this work two flavoenzymes dehydrogenases-transhydrogenases are investigated – *E. coli* nitroreductase A (NfsA) and *T. maritima* thioredoxin reductase (TmTR). Recently, nitroreductases have acquired more interest concerning problems with biodegaration of nitroaromatic compounds and gene-directed enzyme prodrug therapy. TmTR-catalyzed reactions are also relevant due to interest of application of hyperthermophylic organisms in the synthesis of fuel and industrial chemicals. It was determined that two-electron reduction of quinones and nitroaromatic compounds by NfsA follows "ping-pong" kinetics and the rate-limiting step is the oxidative half-reaction. The experimental data are most consistent with a single-step (H-) hydride transfer mechanism in the reduction of quinones by NfsA. The obtained data argue for the major role of direct reduction by NADPH in the reduction of intermediate nitroso compound. The determined E07 value of FMN cofactor of NfsA (–0.215 V) is close to that

of *E. cloacae* nitroreductase (–0.190 V). TmTR catalyzes mixed one- and two-electron reduction of quinones and nitroaromatic compounds. Reaction follows „ping-pong" kinetics with a rate-limiting oxidative half-reaction. Accumulation of FAD semiquinone (FADH●) during the TmTR-catalyzed reduction of quinones indicates that the oxidation of FADH● may be a rate-limiting step in this reaction. The redox potential of FAD of TmTR, –0.230 V, is close to redox potentials of other low molecular mass thioredoxin reductases.

VIBRATIONAL SPECTROSCOPY STUDY OF MEMBRANE ANCHORING MONOLAYERS AND ADSORBED BIOMOLECULES (Biochemistry)

Martynas Talaikis

Scientific supervisor:
Prof. Habil. Dr Gediminas Niaura, Vilnius University

Dissertation defended:
25 November 2020

The structure and function of biomolecules are closely linked. Therefore, understanding the processes that govern the changes in the molecular structure is of fundamental and practical importance. Here we analyze tethered bilayer lipid membranes (tBLM) and laccase enzymes at the electrode surface in water and controlled electric potential environments. Water is crucial in maintaining the native state of biomolecules, while the electric methods are widely used in studying such biomolecules. To realize how these forces affect the structure and orientation of surface-adsorbed tBLMs and laccase enzymes in-situ, we applied the surface-enhanced Raman spectroscopy (SERS). Anchoring monolayer is an integral part of tBLMs. Our data suggest that molecules in the anchoring monolayer are prone to aggregate into hydrophobic clusters due to the water and/or electric potential, in this way corrupting the functional properties of tBLMs. We also propose a conventional spectral indicator for the quality of the anchoring monolayer. The information on how the structure of surface-adsorbed enzymes is altered due to the changing external electric potential is still scarce. Presented in our study is the link between the electrode potential and the tertiary structure of the laccase enzymes and their functional properties. We show that at a negative potential, enzymes adopt an extended-flat configuration, while at more positive electrode potential, they acquire "native" vertical configuration and its activity.

KNEE JOINT FUNCTIONAL ALTERATIONS BEFORE AND AFTER ANTERIOR CRUCIATE LIGAMENT RUPTURE SURGERY DURING DIFFERENT REHABILITATION PROGRAMS (Biology)

Dovilė Kielė

Scientific supervisor:
Doc. Dr Nerijus Masiulis,
Lithuanian Sports University

Dissertation defended:
6 February 2020

The aim of this study was to determine the changes in knee extensor and flexor muscle torque, torque variability, balance, and functional test results before and after anterior cruciate ligament surgery using different rehabilitation programmes. The results of the study suggested that after ACL rupture and surgery, muscle torque, balance, and RFD measurements should be taken to assess neuromuscular system damage and contractile functions, and the symmetry index as well as the hamstring to quadriceps ratio (H:Q) are expedient in assessing the effect of ACL rupture. After ACL rupture and surgery, decreased bilateral functional capacity of legs is observed, and therefore rehabilitation and physiotherapy should be performed on both legs. The findings and conclusions of the study will be useful to rehabilitation practitioners at various stages of patient treatment. Understanding neuromuscular dysfunction after ACL rupture enhances theoretical knowledge and allows for more effective modification of rehabilitation programs. The conclusions may contribute to the improvement of rehabilitation programs in the presence of ACL rupture and after ACL surgery.

POLYMORPHISMS OF GENES RELATED TO THE REFRACTION DISORDERS AND HSA-MIR-328-3P EXPRESSION ASSOCIATIONS WITH MYOPIA (Biology)

Edita Kuncevičienė

Scientific supervisor:
Prof. Dr Alina Smalinskienė,
Lithuanian University of Health Sciences

Dissertation defended:
6 May 2020

The aim of this study was to evaluate the role of refraction-related gene polymorphisms and hsa-miR-328-3p in myopia. Using a twin study, the genetic and environmental effects of myopia were evaluated. So far, the heritability of myopia has not been studied in Lithuania. Also, single-nucleotide polymorphisms (SNPs) of *GJD2* rs634990, *RASGRF1* rs8027411, *PAX6* rs662702, *COL2A1* rs1635529, and *COL8A1* rs13095226 genes have been linked to myopia and the significance of these polymorphisms with respect to myopia and sex has been evaluated. The paper presents data on identified combinations of genotype variants that

increase the risk of myopia in individuals. In this study, non-invasive studies of hsa-miR-328-3p in peripheral blood of myopia and healthy subjects, due to limited ocular tissue availability, to date no changes in expression of hsa-miR-328-3p have been investigated *in vivo*. The association of PAX6 gene SNPs rs662702 and hsa-miR-328-3p with retinal pigment epithelial density was evaluated.

GENETIC DIVERSITY OF *DERMACENTOR RETICULATUS* TICKS IN EUROPE (Biology)

Matas Galdikas

Scientific supervisor:
Prof. Dr Algimantas Paulauskas,
Vytautas Magnus University

Dissertation defended:
8 May 2020

In this study, a large-scale genotyping of *D. reticulatus* from 31 sampling sites, across its distribution range in Europe, was performed for the first time. Investigations of genetic diversity and genetic population structure were performed by adapting microsatellite genetic markers. Genetic diversity values were determined by using mtDNA 12S, 16S rRNA, and nucleus ITS2 region genetic markers. In Europe, the distribution of *Dermacentor reticulatus* into two genetic subpopulations was depicted for the first time and assigned to Western and Eastern Europe with an overlap in Central Europe. However, fixation of one genetic variant on the borders of distribution range was only observed in Spain populations. Unique haplotypes were found in Latvia and Croatia. It was shown that the British Isles are not isolated and are linked to German and Polish populations; furthermore, links between the populations of Poland and France was observed.

SALINOMYCIN AND DICHLOROACETATE SYNERGISTICALLY INHIBIT CANCER CELLS *IN VITRO* AND *IN VIVO* (Biology)

Aistė Skeberdytė

Scientific supervisor:
Prof. Dr Sonata Jarmalaitė, Vilnius University

Dissertation defended:
22 May 2020

Combination therapy is the cornerstone of cancer treatment. The simultaneous application of cytotoxic drugs potentiates their efficacy compared with monotherapy, because it targets the key pathways in a synergistic or an additive manner. Such therapy is likely to diminish drug resistance, while simultaneously providing cytotoxic benefits, such as inhibition of tumour growth, decrease of cancer stem cell population, reduction of metastatic potential, and induction of apoptosis. In the present study, we investigated the effects of two drugs – dichloroacetate and salinomycin and the combination of both agents on the colorectal as well as lung cancer cell lines *in vitro* in 2D and 3D cell culture models. We have also tested the therapeutic potential of this combination therapy *in vivo* in an allograft C57BL/6 mice model. *In vitro* studies were dedicated to investigate a potential mechanism of salinomycin+dichloroacetate action, and with *in vivo* studies we analysed the effects of this therapy on tumour growth, metastatic site formation, and the expression of tumour progression markers.

SCREENING, CHARACTERIZATION AND FUNCTIONALITY ANALYSIS OF HETEROPOLYSACCHARIDE DEGRADING ENZYMES (Biology)

Raimonda Petkauskaitė

Scientific supervisor:
Prof. Dr Nomeda Kuisienė, Vilnius University

Dissertation defended:
25 May 2020

Starch and pectin are among the most important heteropolysaccharides for biosphere. Nature and biotechnology relies directly on possibilities to exploit these biopolymers. Full exploitation of starch and pectin would be impossible without the possibility to disrupt these polymers enzymatically. The available variety of amylases and pectinases remains unsatisfactory in the context of biotechnology challenges. Novel amylases and pectinases were screened using soil metaproteomic and enrichment culture methods. Enzymes were detected by zymography from soil microorganisms. Sequences of detected enzymes were identified by mass spectrometry. Thermostable and thermoactive pullulanase AmyA1 and pectate lyase PL47 from geobacilli were characterized *in vitro*. Intracellular type I pullulanase AmyA1 hydrolyses pullulan, glycogen, and soluble starch. Constitutively secreted endopolygalacturonate transeliminase PL47 was active against polygalacturonic acid, pectin, and pectin

from apple pomace. Under suboptimal reaction conditions, AmyA1 and PL47 were able to generate long-chain oligosaccharides, which are valuable for biotechnology. Immobilization of AmyA1 and PL47 increased thermostability. AmyA1 activity was increased by immobilization, but immobilized PL47 was less active. Isolation of *Geobacillus thermodenitrificans* DSM 101594 strain from soil and genome sequencing ensured *in silico* characterization of AmyA1 and PL47 operonic organization and characterization of functional importance of the enzymes. AmyA1 is important for basic nutritional strategy of *G. thermodenitrificans* DSM 101594 ability to utilize starch; PL47, obtained by horizontal gene transfer, ensures ability to utilize pectin, which reflects strain adaptation to econiche.

GENE EXPRESSION RESEARCH IN PROSTATE ADENOCARCINOMA TISSUES (Biology)

Rita Bandariavičiūtė

Scientific supervisor:
Prof. Dr Sonata Jarmalaitė, Vilnius University

Dissertation defended:
29 May 2020

This study aimed at identification of new prognostic biomarkers of prostate adenocarcinoma in order to develop molecular diagnostic tools for prostate tumour sub-typing into aggressive and slowly growing, indolent prostate cancer. In this work, an innovative assay approach was used that includes application of modern molecular tools: microarray and quantitative PCR. For the first time, detailed analysis of the expression of 50 different ABC transporters in prostate cancer biosamples was performed. The influence of metallothionein genes on the development of prostate cancer has been poorly studied, and there is no data on the expression of the *MT1E* gene in prostate adenocarcinoma tissue. For the first time, we showed an association between the *MT1E* gene expression and prostate cancer progression. Moreover, we assessed the mechanism of gene down-regulation by promoter DNA methylation. New potential molecular biomarkers have been studied that can assist in prediction of disease progression and reliable discrimination of tumour tissues from non-tumour ones.

ANALYSIS OF RNA IN BODILY FLUIDS FOR PROSTATE CANCER DIAGNOSIS AND OUTCOME PREDICTION (Biology)

Kristina Stuopelytė

Scientific supervisor:
Prof. Dr Sonata Jarmalaitė, Vilnius University

Dissertation defended:
29 May 2020

Although local PCa treatment is quite effective and aggressive disease develops for a minority of patients, such PCa cases are associated with worse prognosis and shorter survival; also, there is a lack of reliable biomarkers for the evaluation of response to treatment. Blood- and urine-circulating RNA can be used as lowly-invasive biomarkers for diagnostic and prognostic purposes in PCa. We found that miR-148a, -365, -375, and -429 are PCa-specific and have significant associations with clinical-pathological parameters of PCa. We also demonstrated that the combination of urinary miR-148a and miR-375 is a sensitive molecular tool for non-invasive diagnosis of PCa, while the combination of miR-19b and miR-19a/b specifically predicts biochemical recurrence. Castration-resistant prostate cancer (CRPC) remains incurable. Due to the heterogeneous nature of CRPC, approximately 30% of patients are already resistant to these new drugs. In patients treated with abiraterone acetate (AA), urinary miR-148 predicts progression-free survival, while the combination of miR-148a and miR-375 – overall survival. One of the resistance mechanisms could be AR splicing variants (AR-Vs) which lack a ligand-binding domain and are constitutively active. We demonstrated that a higher level of AR-FL and -V1 transcripts in AA-treated CRPC patients' blood predicts progression-free survival and overall survival.

WINTER OILSEED RAPE COLD RESISTANCE CHARACTERISTICS AND THEIR ALTERATION AFTER TREATMENT WITH POLYAMINES AND PROLINE (Biology)

Elžbieta Jankovska-Bortkevič

Scientific supervisor:
Dr Sigita Jurkonienė, Nature Research Centre

Dissertation defended:
7 August 2020

Low temperature is an abiotic factor limiting the spread and cultivation of plants in many areas in the world. The cultivation of economically important winter oilseed rape (*Brassica napus* L. ssp. *oleifera biennis* Metzg.) in temperate regions is problematic due to acclimation-preparation for wintering and wintering in harsh and varying conditions, and its physiological-biochemical characteristics of acclimation and reaction to cold have not been thoroughly investigated. Studies have shown that plant stress response can be modified by physiologically active substances. The aim

of this study was to investigate the physiological and biochemical characteristics of winter oilseed rape resistance to cold and the possibility of metabolism control with polyamines and proline. A model simulating autumn-winter conditions was developed for the study. The genotype-dependent patterns of changes in physiological-biochemical characteristics (H+-ATPase activity, ethylene content, and proline accumulation) under acclimating and increasing cold were determined. The study validates the suitability of the studied characteristics and the developed autumn-winter conditions simulating model to investigate the cold resistance of plant varieties, the level of acclimation and the effect of physiologically active substances. It has been found that under laboratory and field conditions, polyamines (spermidine, spermine, putrescine) and proline modified the response of winter oilseed rape to cold, increased survival under cold stress conditions, had a positive effect on the formation of productivity elements, and can be used in oilseed rape growing technologies.

PREVALENCE OF HUMAN PAPILLOMAVIRUS AMONG COUPLES WITH INFERTILITY PROBLEMS IN LITHUANIA (Biology)

Vilma Jeršovienė

Scientific supervisor:
Dr (HP) Dalius Butkaukas, Nature Research Centre

Dissertation defended:
10 August 2020

Infertility is a worldwide problem which affects about 10–30% of couples of reproductive age. One of its causes is sexually transmitted diseases. HPV viruses are associated with epithelial lesions and cancers. HPV infections have been shown to be significantly associated with many adverse effects in the reproductive function. For the first time in Lithuania, the prevalence of HPV infection among couples undergoing assisted reproduction procedures was studied. Cervical swabs were used to test the virus in women and semen remaining after IVF was used to detect HPV infection in men. It was found that the prevalence of HPV infection among couples with infertility issues in Lithuania reaches 33%. The results of this study confirm that HPV infection found in men with infertility problems has a significant impact on their sperm quality. A statistically significant relationship was found between HPV infection and sperm motility and vitality. The results of studies conducted in the world show that in couples, HPV concordance ranges from 6% to 84.6%. Meanwhile, our results showed that the same high-risk types of HPV were detected in 14% of the infected couples.

MONITORING CARDIOVASCULAR SYSTEM IN EXERCISERS FOR HEALTH (Biology)

Živilė Kairiūkštienė

Scientific supervisor:
Prof. Habil. Dr Jonas Liudas Poderys,
Lithuanian Sports University

Dissertation defended:
25 August 2020

Physical exercises are used to address four major medical challenges: treatment; rehabilitation; prevention; and health promotion. On the other hand, it is emphasized that just as with a medicine, if it is used too little it is ineffective and if used too much it is harmful. Therefore, understanding of the response and long-term adaptation of the human body to various types of workloads is a relevant issue in physiology, medicine and sports science. The aim of this study was to determine the dynamics of cardiovascular parameters and their interaction during dosed and maximal exercise tests while monitoring exercising for health. It has been shown that dynamic interaction during exercise is indicative of a better functional compatibility of many physiological mechanisms, and their faster recovery after a dosed exercise test demonstrates that the cardiovascular system of participants is better adapted to workload than it was before training. It is shown that the inter-dynamics of changes in the ratio between RR and JT intervals of ECG allows the assessment of the accumulation of fatigue caused by exercise load in training sessions and also during the time periods of exercising.

APPLICATION OF FLUXOMIC METHODS TO STUDY TUMOR CELL METABOLISM (Biology)

Ieva Ceslevičienė

Scientific supervisor:
Dr Sergio Bordel Velasco
Lithuanian University of Health Sciences

Dissertation defended:
27 August 2020

The aim of the study was analysis of metabolic and transcriptional differences in healthy and tumour cells and search of antitumour targets using fluxomic methods. The results of the study show that most cancer cells are characterized by the enhanced aerobic glycolysis, the Warburg effect. The Warburg effect consists in the lactic fermentation of glucose even at high oxygen concentrations. It was initially believed to be due to defects in cancer cell mitochondria; however, this hypothesis has proven to be wrong, as mitochondria have been shown to be functional and cancer cells cannot survive in fully anaerobic conditions, which proves that they are still dependent on oxidative phosphorylation. Recent studies have shown that the mitochondria of cancer cells function well and both oxidative phosphorylation and lactic fermentation are important for energy production.

THE EFFECT OF CHROMOSOME 10, MYOSTATIN AND AGEING ON MORPHOPHYSIOLOGICAL TRAITS UNDER DIFFERENT FEEDING CONDITIONS (Biology)

Mindaugas Kvedaras

Scientific supervisor:
Prof. Dr Aivaras Ratkevičius,
Lithuanian Sports University

Dissertation defended:
14 September 2020

Whereas, an excessive loss of skeletal muscle mass negatively correlates with muscle strength, strategies that can maintain skeletal muscle mass during states of negative energy balance are required, particularly for those who require rapid weight loss, but are unable to exercise. In addition, malnutrition is considered to be a major contributing factor to ageing-related loss of muscle mass or sarcopenia. Mstn null animals showed significant increase in muscle mass (up to two-fold) and decrease in fat tissue compared to the wild type. Thus myostatin dysfunction might lead to improvements in health that are achieved by CR if muscle mass plays the major role in them. The main aim of our studies was to examine effects of chromosome 10 (Chr 10), myostatin dysfunction, and ageing on skeletal muscles of mice under different feeding conditions. Our results showed that chromosome 10 of the A/J mouse strain contributes to the reduced citrate synthase activity and endurance performance. In addition, we found out that myostatin dysfunction does not protect from muscle wasting during CR in mice and 48 h fasting is associated with reduction in peak and specific force of soleus muscle in old but not young mice.

SARCOCYSTIS SPECIES RICHNESS IN MEMBERS OF THE FAMILY CERVIDAE (Biology)

Eglė Rudaitytė-Lukošienė

Scientific supervisor:
Dr Petras Prakas, Nature Research Centre

Dissertation defended:
22 September 2020

Parasites of the genus *Sarcocystis* are characterised by an obligatory two-host life cycle. *Sarcocystis* spp. form sarcocysts in the muscles of intermediate hosts, which can cause acute infections. Previous studies have revealed a high prevalence of *Sarcocystis* parasites found in deer muscle, but the species composition of the parasites in these hosts has not been thoroughly investigated. The aim of this study was to examine sarcocysts infection and species diversity of *Sarcocystis* parasites in five Lithuanian deer species (fallow deer, moose, red deer, roe deer, and sika deer) using morphological and molecular analysis. The results of the research allowed identifying and describing four new species, *S. pilosa*, *S. frondea*, *S. nipponi*, and *S. entzerothi*. The results showed that *cox1* is suitable for identifying closely related species of *Sarcocystis*.

The highest number of different *Sarcocystis* species was found in the red deer and the sika deer; seven *Sarcocystis* species were confirmed. *Sarcocystis* species composition in the sika deer was established for the first time. New intermediate hosts have been confirmed for five *Sarcocystis* species. Half of the *Sarcocystis* species (9/18) found in Lithuanian deer use more than one intermediate host. Also, species using three (*S. ovalis*) and four (*S. entzerothi*) different intermediate hosts were verified for the first time. *Sarcocystis* species were characterised by a different level of intraspecific genetic variability, depending on the parasite species.

COMPARATIVE STUDIES ON THE EXPRESSION OF TYPE I AND II INTERFERONS AND THEIR PRODUCING CELLS IN AUTOIMMUNE RHEUMATIC PROCESSES (Biology)

Ieva Varanauskienė

Scientific supervisor:
Dr Irena Dumalakienė,
Centre for Innovative Medicine

Dissertation defended:
24 September 2020

Sjögren's syndrome, or autoimmune epithelitis, is a systemic autoimmune disease that affects the salivary and lacrimal glands. Much attention is being paid to research of this disease due to the diverse clinical manifestations of Sjögren's syndrome, a predisposition to lymphoproliferative and disease prevalence. However, despite these efforts, the true cause of this disease is still unknown. It is believed that one of the main factors causing an autoimmune disease is a viral infection. The aim of this work was to evaluate the quantitative changes of the immune cell populations, closely related to viral infections, and type I and II interferons produced by these cells in rheumatic processes and to analyse the possible (probable) persistence of epidemic parotitis virus particles in minor salivary glands. Genetic (PCR) testing methods were used to search for epidemic parotitis virus particles. A detailed quantitative analysis of peripheral blood cells by flow cytometry was also performed. We analysed saliva samples and although we did not find viral RNA in them, the obtained results allow us to make assumptions for further detailed research in salivary gland sample biopsies. However, this work is the first attempt to prove that one of the most likely causes of Sjögren's syndrome may be a persistent epidemic parotitis virus. Correlations established between clinical signs and quantitative changes in cells allow assumptions to be made about disease activity, progression, and severity of symptoms.

FUNCTIONAL CHARACTERISTICS AND REGULATION OF INTRACELLULAR CALCIUM ION SIGNALLING IN HUMAN MESENCHYMAL STEM CELLS OF DIFFERENT ORIGINS AND CHONDROCYTES (Biology)

Ilona Uzielienė

Scientific supervisor:
Dr Eiva Bernotienė,
Centre for Innovative Medicine

Dissertation defended:
25 September 2020

The aim of this dissertation was to compare functional characteristics and regulation of intracellular calcium ion signalling in human menstrual blood-derived mesenchymal stem cells with bone marrow mesenchymal stem cells and chondrocytes. To the best of the knowledge, this is the first time MenSCs functional properties, including phenotypical characteristics, differentiation potential, and iCa^{2+} levels, were studied and compared to classical BMMSCs and chondrocytes. Differences in surface marker expression, osteogenic/adipogenic/chondrogenic differentiation and iCa^{2+} level in MenSCs have been determined. Chondrogenic differentiation of both, MenSCs and BMMSCs, was stimulated using additional growth factor – Activin A, which has never been used to stimulate chondrogenesis in MenSCs, whereas the obtained results suggest its distinct role on the function of those cells. Therefore, this study highlights the similarities and differences between MenSCs and BMMSCs properties and the importance of molecular mechanisms that control stem cell differentiation. Moreover, this is the first time iCa^{2+}-dependent mechanisms were studied in MenSCs and compared to BMMSCs and chondrocytes, using antihypertensive drug nifedipine and VOCC agonist BayK8644. The study with nifedipine includes novel information regarding its role in regulation of BMMSCs and chondrocyte energy metabolism, iCa^{2+} and chondrogenesis, which is important for understanding the molecular mechanisms regulating those processes and may also lead to the development of further therapeutic strategies.

PREVALENCE AND GENETIC DIVERSITY OF VECTOR-BORNE PATHOGENS IN SMALL RODENTS (MURIDAE, CRICETIDAE) (Biology)

Dalytė Mardosaitė-Busaitienė

Scientific supervisor:
Prof. Dr Algimantas Paulauskas,
Vytautas Magnus University

Dissertation defended:
25 September 2020

The aim of the study was to investigate the prevalence and genetic diversity of vector-borne pathogens *Babesia* spp., *Rickettsia* spp., and *Bartonella* spp. in various rodent species. A total of 1180 rodents belonging to eighth species – *Apodemus flavicollis* (*n* = 499), *A. agrarius* (*n* = 82), *Mus musculus* (*n* = 12), *Micromys minutus* (*n* = 77), *Myodes glareolus* (*n* = 396), *Microtus oeconomus* (*n* = 69), *M. agrestis* (*n* = 28) and *M. arvalis* (*n* = 17) – were captured with live or snap

traps in 19 locations from coastal and continental areas of Lithuania from 2013 to 2017. The overall prevalence of *Bartonella* pathogens in rodents was 54.8%, *Rickettsia* 28.3%, and *Babesia* 2.8%. Small rodents in Lithuania harbour multiple *Bartonella* species belonging to six genogroups, specifically *Bartonella grahamii*, *B. taylorii*, *B. tribocorum*, *B. coopersplainsensis*, *B. doshiae* and *B. rochalimae*. The highest *Bartonella* infection rates and the highest species diversity are detected in rodents from coastal areas. The present study represents the first evidence that *Babesia* pathogens circulating in *A. flavicollis*, *M. minutus*, *M. glareolus*, *M. oeconomus*, and *M. agrestis*, and the presence of zoonotic *Babesia microti* 'Jena/Germany' strain potentially pathogenic to humans. The high prevalence of *Rickettsia helvetica* pathogens in *A. flavicollis*, *M. minutus* and *M. glareolus* detected in the present study suggests that these rodents may play an important role as potential reservoir hosts and thus in the maintenance of this pathogen in Lithuania. The findings of this study demonstrated the importance of small rodents as potential reservoirs of zoonotic pathogens in Lithuania.

PREVALENCE OF TICK-BORNE PATHOGENS IN MIGRATORY BIRDS AND THEIR TICKS (Biology)

Vesta Jonikė

Scientific supervisor:
Prof. Dr Jana Radzijevskaja,
Vytautas Magnus University

Dissertation defended:
25 September 2020

In this work, for the first time, a large-scale of study was performed to assess the infection rate of migratory birds and their transmitted ticks with tick-borne pathogens. For the first time it was found that *Hyalomma marginatum* ticks reach the territory of Lithuania together with the migratory birds; also, this study represents the first description of infestation of *Ixodes lividus* ticks in sand martin nests. For the first time in Europe, six different *Ixodes lividus* genotypes were identified. Using different molecular methods, *Rickettsia helvetica*, *Rickettsia monacensis*, *Rickettsia aeschlimannii*, and *Borrelia valaisiana* were detected in ticks from migratory birds for the first time in Lithuania. The present study is the first reported *Rickettsia vini* in ticks from sand martin nests in the Baltic region, with different *Rickettsia vini* genotypes identified for the first time in Europe. This study represents the first detection of *Borrelia* spp., *Rickettsia* spp., and *Anaplasma phagocytophilum* in bird tissues in the Baltic region. The results of the study allow assessing the potential risk to animals and human health in Lithuania.

INTEGRATED APPROACH OF TEAM SPORTS PERFORMANCE ASSESSMENT (Biology)

Bruno Figueira

Scientific supervisor:
Doc. Dr Nerijus Masiulis,
Lithuanian Sports University

Dissertation defended:
8 October 2020

The general aim of this study was to explore and validate different tools that allow studying and assessing several performance variables in basketball and association football during both training and match environments. To fulfil this purpose, the data were collected from both training tasks and competitive scenarios. The participants were players from the age groups of under-15, under-17, under-19, under-21, professionals and amateur. Several techniques such as linear and non-linear processing, time-motion, cognitive, physical, and physiological analysis were applied to explore the effects of several training conditions and match scenarios on sporting performance. The different tools used allowed assessing sport performance from a global overview by validating an indoor tracking system, going through a more specific analysis by validating tools that assess physical, physiological and tactical performance, and to a more detailed analysis by validating a method that assessed the cognitive complexity of the task. Future applications focused in the promotion of higher-level performance may benefit from a complex performance approach that integrates several factors. Thus, manipulating the task constraints according the present results may provide relevant information for practice task design in order to promote a functional collective behaviour regarding the physical, physiological, tactical, and cognitive demands.

INDEPENDENT AND COMBINED INTRACELLULAR ELECTROTRANSFER OF DNA AND SMALL MOLECULES (Biology)

Sonam Chopra

Scientific supervisor:
Prof. Dr Saulius Šatkauskas,
Vytautas Magnus University

Dissertation defended:
30 October 2020

Electroporation is a non-viral method for the delivery of small and large molecules that has been used in a variety of studies and applications. However, complete implementation of this technique in clinics is limited due to the low transfection efficiency, high cell mortality, and poor gene expression regulation. In this work, the interrelation of small and large molecules was investigated for the analyses of independent and combined electrotransfer efficiency. It was shown that the independent electrotransfer efficiency can be increased by increasing the plasmid concentration as the distance between

the electroporated cell and the nearest plasmid decreased. In combined electrotransfer, the electrotransfer efficiency of anticancer drug increases in the presence of plasmid DNA. Moreover, the combined electrotransfer allows transient control of the cell-death regulated gene expression. The duration and efficiency of gene expression can be regulated by changing bleomycin concentration. Based on our results, the presence of DNA increases the electrotransfer efficiency of small molecules. But in the case of oligos, the presence of DNA decreases the oligo's electrotransfer. These findings would potentially help to increase the efficiency and applicability of DNA electrotransfection in electrogene therapy and electrochemo-gene therapy.

DEER KEDS PARASITIZING LITHUANIAN AND NORWEGIAN CERVIDS AND THEIR INFECTION WITH PATHOGENS (Biology)

Kamilė Klepeckienė

Scientific supervisor:
Prof. Dr Jana Radzijevskaja,
Vytautas Magnus University

Dissertation defended:
27 November 2020

Cervids such as roe deer, red deer, and moose are parasitized by various ectoparasites, including deer keds, which are often found in the fur of these mammals. As blood-sucking ectoparasites, deer keds can transmit various types of pathogens. There are few studies related to the diversity of deer keds parasitizing Lithuanian and Norwegian cervids and their infection with pathogens. For the first time in Lithuania, research was carried out to determine the abundance of deer keds found in different cervid hosts and to comprehensively assess the prevalence of bartonellosis pathogens in deer keds and their hosts. Using molecular research methods, three different genotypes of *Lipoptena cervi* and *L. fortisetosa* deer keds were identified for the first time in Lithuania. The infection of moose with *L. fortisetosa* deer keds was described for the first time, and the infection of this species of deer keds with *Bartonella* bacteria in Europe was evaluated. *L. cervi* deer keds, which is widespread in Lithuania and Norway, and *L. fortisetosa* deer keds, abundant in Lithuania, have been found to be carriers of *Bartonella* strains combining *B. schoenbuchensis*, *B. chomelii*, *B. capreoli* species. The study results provide essential knowledge about deer keds, their species and genetic diversity, and their potential effects on the transmission of *Bartonella* pathogens to cervids in Lithuania and Norway.

PRE-mRNA SPLICING ASSOCIATED WITH ONCOLOGICAL DISEASES: a STUDY OF SPLICING FACTORS AND HYPOXIC MICROENVIRONMENT (Biology)

Inga Pečiulienė

Scientific supervisor:
Dr Arvydas Kanopka, Vilnius University

Dissertation defended:
27 November 2020

Oncological diseases remain one of the unsolved problems of the entire society as the oncology-related morbidity and mortality have been systematically increasing. As pre-mRNA splicing is a highly important process in regulating gene expression, it is not surprising that changes in splicing have been observed in many different diseases. Unfortunately, the reasons for the expression of disease-associated mRNA isoforms and their regulatory factors have not been completely understood yet. This study describes the research of splicing factors and the expression of tumour-associated mRNA isoforms in healthy and tumour gastrointestinal organ tissues and tumour cell lines and examines whether/how hypoxic microenvironment in tumour cell lines affects tumour-associated pre-mRNA splicing changes. Studies in this work have shown differences in the expression of SRSF1, SRSF2, U2AF65, U2AF35, and KHSRP splicing factors and tumour-associated Fas and Rac1 mRNA isoforms in tumour tissues and commonly used tumour cell lines. Our results indicate that reduced oxygen content in the cell environment (hypoxia) affects Fas pre-mRNA alternative splicing. The results obtained in this work provide valuable information about the processes initiating diseases in various systems.

INVESTIGATION OF THE FUNCTIONING OF dsRNA VIRUSES IN *SACCHAROMYCES* GENUS YEASTS (Biology)

Bazilė Ravoitytė

Scientific supervisor:
Prof. Dr Elena Servienė,
Nature Research Centre

Dissertation defended:
18 December 2020

The objective of this study was to investigate the effect of the inherent dsRNA virus on *Saccharomyces* spp. yeast gene expression and to elucidate mechanisms of long-lasting yeast-virus co-adaptation in nature. In the present study, transcriptomic alterations linked to dsRNA-based killer systems were analysed in two *Saccharomyces* genus yeast species, namely domesticated *S. cerevisiae* and wild *S. paradoxus*. High-throughput RNA-sequencing was employed for the investigation of *S. cerevisiae* dsRNA-virus-related transcriptional responses. The first study analyzing the impact of each dsRNA component (L-A and M) on the *S. cerevisiae* transcriptome was provided. Gene transcription alterations in *S. paradoxus* cells with different sets of dsRNA viruses

were evaluated. Transcriptional responses to the elimination of L-A-lus, M2, L-A-66, and M66 dsRNA viruses were described. Numerous differently expressed genes upon viral dsRNA loss in *Saccharomyces* spp. yeasts were identified, the majority of which have not been linked to dsRNA viruses previously. Comparison of transcriptomics results between virus-cured *S. cerevisiae* and *S. paradoxus* species were performed. The findings of this research expand the knowledge of the interplay between dsRNA viruses and their hosts providing links of virus-host co-adaptation strategies. The obtained results are important for research of fungal, plant, and other dsRNA viruses.

QUANTUM CHEMICAL INVESTIGATIONS OF THE FRAGMENTATION OF AMINO ACIDS BY LOW ENERGY ELECTRONS (Biophysics)

Laura Baliulytė

Scientific supervisor:
Dr Jelena Tamulienė, Vilnius University

Dissertation defended:
7 June 2020

Living organisms are continually affected by ionizing radiation emitted by natural sources, such as cosmic rays and radionuclides. Low-energy electrons are generated due to the interaction of gamma and Röntgen rays with biomolecules. These low-energy electrons damage molecules, including amino acids. In this dissertation, the fragments that are produced during L-threonine, L-glutamic acid, L-glutamine, L-leucine, and L-isoleucine fragmentation were identified. Additionally, the fragmentation process of these amino acids in vacuum and water was also examined. The results indicate that the production of positively charged fragments with the same mass and chemical composition in water required more appearance energy than that in vacuum. It was determined that the structures of fragments with the same mass and chemical composition depended on the environment. Additionally, L-glutamic acid and L-glutamine, with a similar mass but different chemical compositions, were found to undergo fragmentation in a similar manner. The obtained results also showed that L-leucine and L-isoleucine, with the same mass and chemical composition but different geometrical structures, were also found to undergo fragmentation in a similar manner.

EFFECT OF ACUTE AND CHRONIC ALCOHOL CONSUMPTION AND WITHDRAWAL ON RAT VISUAL EVOKED POTENTIALS: FOCUS ON ON/OFF RESPONSES (Biophysics)

Redas Dulinskas

Scientific supervisor:
Prof. Dr Osvaldas Rukšėnas, Vilnius University

Dissertation defended:
13 June 2020

Perception of stimulus onset and offset or stimulus duration is one of the fundamental functions in temporal information processing. Studies show that processing of stimulus onset and offset could be based on different neuronal mechanisms and, correspondingly, could be differently modulated by biologically active compounds. Alcohol is one of the most used and abusive drugs in the world. Alcohol consumption is related to various disorders and modulation of visual information processing at different levels in the visual system. Changes in the information processing chain of responses to stimulus onset and offset might cause the changes in the perception of stimulus parameters and alter the reaction time. Our studies demonstrated that at the level of neuronal population, the activation of the visual system during the visual stimulus onset is qualitatively different from that during the offset. The differences between these responses are noticeable at the cortical level in anesthetized and awake animals. Long-term alcohol consumption and abstinence had a strong long-term and, in some cases, irreversible impact on the visual information processing. Both of these conditions modulated only the last stage of stimulus onset processing at the level of visual cortex, but not at the level of lateral geniculate nucleus. Response to the stimulus offset was more susceptible to the effect of alcohol consumption and/or abstinence and was modulated at both visual cortex and lateral geniculate nucleus levels.

EFFECT OF AMINO ACIDS AND NMDA ON ELECTRICAL SIGNALLING PARAMETERS OF CHAROPHYTE *NITELLOPSIS OBTUSA* (Biophysics)

Indrė Lapeikaitė

Scientific supervisor:
Prof. Dr Osvaldas Rukšėnas, Vilnius University

Dissertation defended:
26 August 2020

In Plantae, information about environmental conditions can be transmitted through electrical signalling. Action potentials of plants possess the main characteristics of mammalian action potentials: they are all-or-none responses with inherent excitation threshold, duration, and shape. Despite the inherent characteristics, the parameters of plant action potentials are altered in response to environmental cues and chemical compounds. It is known that environmental amino acids can modulate or elicit action potential generation in plants by binding to glutamate receptor-like channels. The effect of amino acids on the electrical signalling parameters of

a separate intact plant cell is yet to be elucidated. The dissertation describes the effect pattern of amino acids and a synthetic ionotropic glutamate receptor channel agonist NMDA on the action potential parameters of a single Charophyta cell via microelectrode technique in current- and voltage-clamp modes; it also suggests standardised evaluation methods for action potentials in plants. Reported dose-dependent modulations, described inhibition of the effect by ionotropic glutamate receptors antagonists supports the functioning of glutamate receptor-like channels in Characeaen, confirms the modulation of action potentials as a possible response to environmental amino acids, and stresses the importance of multi-parametrical analysis of plant electrical signals.

EVALUATION OF ELECTRICAL BRAIN RESPONSES TO LINEAR CHIRP-MODULATED TONES: EFFECT OF TASK AND CHANGES IN NEUROPSYCHIATRIC DISORDERS (Biophysics)

Evaldas Pipinis

Scientific supervisor:
Prof. Dr Inga Griškova-Bulanova,
Vilnius University

Dissertation defended:
23 October 2020

There is a great need for objective neurophysiological biomarkers that could be used in diagnostics of neuropsychiatric disorders. Electroencephalogram, which represents rhythmic neural activation, has been widely utilized for this purpose. By using the basic property of the nervous system to "follow" periodic stimulation, the ability of the brain to generate particular frequencies can be tested. However, testing broad frequency range requires long recording times and is challenging to the subjects. To overcome the long duration of the assessment procedure, the stimulation with changing sound modulation frequencies – chirps– was proposed. This dissertation includes three separate studies: (1) evaluation of attentional effects on chirp-evoked responses; (2) study of changes in chirp-evoked responses in schizophrenia, and (3) study of changes in chirp-evoked responses in disorder of consciousness. Results show that brief (500 ms) low-carrier tones (440 Hz) amplitude modulated by chirp stimuli elicit clear responses that did not depend on the subject's level of attention. In a clinical sample of schizophrenia patients, chirp-evoked responses reveal impaired brain ability to synchronize at theta-beta and high gamma ranges and highlight association between response in 32–43 Hz range and hallucination symptoms. Chirp-evoked responses at the low gamma range differ between vegetative and minimally conscious state patients and are associated with the degree of consciousness impairment.

FACTORS DETERMINING CELL DEATH CAUSED BY ANTICANCER [I.E. ANTICANCER] THERAPIES IN LIVER CARCINOMA MODEL *IN VITRO* (Biophysics)

Mantas Šilkūnas

Scientific supervisor:
Prof. Dr Gintautas Saulis,
Vytautas Magnus University

Dissertation defended:
23 October 2020

The aim of this study was to investigate the anticancer potential of vitamins C (VC) and K3 (VK3) as well as their mixture, electrochemotherapy with bisphosphonates and bleomycin in an *in vitro* model of liver carcinoma. For the first time, the cytotoxicity of the combination of bisphosphonates and electroporation was evaluated in liver carcinoma model *in vitro*. Plasma membrane electro-permeabilization altered the cytotoxicity of pamidronate but not ibandronate. Also, cell media acclimatization to hypoxia condition was evaluated and used to develop hypoxic liver carcinoma model *in vitro*. It was also found that cell media supplementation with VC, VK3, and VC+VK3 consumes dissolved oxygen, forming H_2O_2 and thus creating temporary hypoxic conditions. It was found that VC, VK3, and VC+VK3 are cytotoxic to MH-22A liver carcinoma cells under hypoxia and normoxia conditions. This effect depends on the concentration of active substance/s and the time of exposure in a direct manner. It was also found that if a sufficient concentration of VC+VK3 is not reached, the opposite result is possible: stimulation of cancer cell proliferation. Assessing the cytotoxicity of the combination of bleomycin and electroporation was found that the efficacy of ECT depended on the amount of dissolved oxygen in the system and the lower oxygen content reduces the effectiveness of the applied treatment. The obtained results were linked to the clinical treatment of ECT, and the reasons for it are ineffective.

MAGNESIUM-REGULATED TRPM7 CHANNELS IN HUMAN ATRIAL MYOCYTES (Biophysics)

Mantė Almanaitytė

Scientific supervisor:
Dr Regina Mačianskienė,
Lithuanian University of Health Sciences

Dissertation defended:
29 October 2020

The aim of this work was to characterize the role of Mg^{2+}-sensitive TRPM7 channels in the human heart. In particular, we wanted to determine the molecular identity and function of TRPM7-like channels in atrial cardiomyocytes under physiological and pathological conditions. The tasks: (1) to identify and characterize the biophysical properties of TRPM7-like currents in human atrial cardiomyocytes, (2) to examine/determine the pharmacological modulation of TRPM7 in the heart, (3) to validate the molecular identity

and distribution of native TRPM7 channels in human cardiomyocytes, (4) to determine the regulation of TRPM7 currents by protons in human atrial cardiomyocytes, and (5) to determine the modulation of TRPM7 function in human hearts with the cardiomyopathy. Scientific novelty: interest in TRPM7 channels has increased recently following the discovery that these channels participate in a variety of physiological/patho-physiological processes. We and others have demonstrated previously that in human atrial cardiomyocytes the TRPM7-like currents could be measured by gradual activation during dialysis with Mg^{2+}-free pipette solution.

THE INFLUENCE OF ORAL CONTRACEPTIVES ON FEMALE COGNITIVE FUNCTIONS AND AFFECTIVE PROCESSING (Biophysics)

Rasa Monˇciunskaitė

Scientific supervisors:
Prof. Dr Kastytis Dapšys,
Prof. Dr Ramunė Grikšienė, Vilnius University

Dissertation defended:
29 October 2020

Oral contraception (OC) is used by more than 100 million women globally. Therefore, it is important to consider the unintended, potentially far-reaching effects associated with OC use on women's neurobiology, psychology, and behaviour. Previous research shows that sex steroids are closely linked to women's emotional well-being and cognitive functioning. Studies reveal that OC use may be associated with structural and functional changes in the areas of brain related to cognitive functions (e.g. verbal memory, face recognition, verbal fluency, and visuospatial abilities) and affective processing. However, evidence of OC effect on the nervous system is often contradictory and inconclusive. The aim of the work was to evaluate the influence of OCs on female cognitive functions and emotional processing in a cohort of healthy females of reproductive age. We compared the performance in the tasks assessing working memory and spatial abilities, and the electrophysiological reactivity to affective images between naturally cycling women and OC users. To the best of our knowledge, this is the first study demonstrating that OC users and nonusers may employ different strategies to perform the mental rotation task. The performance did not differ between the groups in the working memory task. The results suggest altered affective picture processing among OC users with significantly blunted brain activity to the highly unpleasant images.

IDENTIFICATION OF THE ALLERGENIC TREE SPECIES AND THEIR IMPACT ON THE RESPIRATORY ALLERGIC DISEASES MORBIDITY IN CHILDREN (Ecology and environmental)

Irma Česnakaitė

Scientific supervisor:
Prof. Dr Audrius Dėdelė,
Vytautas Magnus University

Dissertation defended:
26 June 2020

Components of the green infrastructure are an important factor for improving people's well-being. However, tree pollen is related to the risk of allergic respiratory diseases and can exacerbate underlying symptoms. The aim of the dissertation was to identify the major allergenic tree species and to assess their impact on the respiratory allergic diseases morbidity in children. For identification of variety of species, the allergenic trees were selected based on the inventory of the green spaces of the city of Kaunas and using GIS. For the first time, while using an accurate and objective greenery measure in different types of environment of participants in the research (home, kindergarten), association was established between the abundance of the allergenic trees and the risk of respiratory allergies in preschool children. The results show that the most prevalent allergenic tree species are maple (44%) and birch (28%). Alder and hazel are the rarest species. The highest distribution of allergenic tree species and the highest number of children suffering from respiratory allergies was recorded in Dainava residential area, which is characterized by the highest population and the biggest number and density of trees producing allergenic pollen. Children living in the highest abundance zone of the allergenic trees have a statistically significant increased risk of allergy symptoms. The research participants allergic to anemophilous tree pollen had the most symptoms of respiratory allergies in April and May, when the trees, which are recognized as the main cause of polinosis, are in blossom.

IMPACT OF CLIMATE CHANGE ON CHANGES IN ORGANIC CARBON CONCENTRATION AND CARBON ACCUMULATION IN THE BIOMASS OF AGRICULTURAL PLANTS AND SOIL (Ecology and environmental)

Gintarė Juozapaitienė

Scientific supervisor:
Prof. Habil. Dr Romualdas Juknys,
Vytautas Magnus University

Dissertation defended:
25 September 2020

Global warming is currently the biggest global ecological problem that affects the ecosystems of the whole Earth. More and more studies show that carbon concentrations differ significantly when comparing individual plant parts, species, and growth conditions. Therefore, in order to increase organic soil carbon when assessing the potential carbon sequestration of selected plant varieties with better carbon sequestration conditions in current and warming climates, accurate carbon

concentration not only in their entire biomass but also in individual parts of the plant is essential. In this dissertation, the concentration and accumulation of total organic carbon was determined not only in the whole biomass of the studied species, but also in different parts of plants. Organic carbon concentration was found in the biomass of spring barley (*Hordeum vulgare* L.), field pea (*Pisum sativum* L.), and spring oilseed rape (*Brassica napus* L.) as well as in different parts of plants at single elevated CO_2 concentration (21°C/800 ppm), the combined effects of elevated CO_2 concentration and air temperature (25°C/800 ppm) and extreme climatic conditions (heat waves and droughts) was well below the commonly used value of 500 g kg^{-1}. The investigated carbon transfer through the plant-soil system also revealed that, compared to rapeseed, barley carries out carbon transfer to the soil more slowly.

IMPACT OF THE COLONIES OF GREAT CORMORANTS (*PHALACROCORAX CARBO SINENSIS*) ON MAMMALS (Ecology and environmental)

Marius Jasiulionis

Scientific supervisor:
Dr Linas Balčiauskas, Nature Research Centre

Dissertation defended:
2 October 2020

Small mammals are indicators of forest ecosystem sustainability and play an important role in many other ecosystems worldwide. Diversity, abundance, dominance, sex and age structure, and other indicators of the small mammal community are influenced by environmental conditions. Changes in the structure of vegetation, undergrowth, composition of tree crown cover or forest litter affect communities of small mammals. Colonies of great cormorants are probably the fastest and most strong environmental factor in our climatic zone. Complex environmental effects of great cormorants lead to enormous ecosystem transformation. In this work, the effects of cormorants on mammals were scientifically evaluated for the first time. We found that the initial positive effects of cormorant colonies on the ecological and biological parameters of small mammals are changed to negative when the colony size reaches a critical level. Influence of cormorants on the accumulation of heavy metals and other chemical elements in mammalian tissues was evaluated, and a stable isotope ratio analysis of carbon and nitrogen was performed. Novelty of our investigation consisted in evaluating the immediacy of the impact of the cormorant colony on small mammals. The results showed for the first time how fast biogenic pollution were transferred and what its consequences were to small mammal ecology. We also observed changes in seasonal and daily activity patterns of mammals in the colonies of cormorants during bird breeding time.

JUVENILE-STAGE RESPONSE AND PLASTICITY OF DIFFERENT TREE SPECIES AND POPULATIONS UNDER THE IMPACT OF SIMULATED CLIMATE CHANGE AND OTHER ENVIRONMENTAL STRESSORS (Ecology and environmental)

Gintarė Bajerkevičienė

Scientific supervisor:
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Dissertation defended:
18 December 2020

The aim of this study was to evaluate the response of juvenile progeny of seven forest tree species and their populations to different combinations of climate change-related stressors, simulated in a phytotron under elevated CO_2 concentration: (i) heat + elevated humidity, (ii) heat + frost + drought, (iii) heat + elevated humidity + increased UV-B radiation doses + elevated ozone concentration, and (iv) heat + frost + drought + increased UV-B radiation doses + elevated ozone concentration. The significant effects of the complex treatments on sapling growth, physiological and biochemical traits were found. Species effect and species-by-treatment interaction were highly significant in most of the traits studied, indicating species-specific reactions to the applied treatments. For deciduous trees, the height increment was much higher under heat and elevated humidity treatment than in ambient conditions indicating a positive effect of elevated temperature and better water availability. Rates of photosynthesis in most of the tree species were greatest under heat and drought conditions. This indicates that heat waves can cause stress on certain tree species even at good availability of water and may negatively affect physiology, although this does not necessarily reduce tree growth. Concentrations of malondialdehyde and hydrogen peroxide varied a lot across treatments showing variable responses of tree species to stress, but hydrogen peroxide concentrations in all deciduous species were substantially lower than in ambient conditions and were low and not affected by treatments in coniferous species.

THE HABITAT PREFERENCES OF COMMON BUZZARD (*BUTEO BUTEO*) AND INTERACTION WITH OTHER BIRDS OF PREY (Zoology)

Aušra Kamarauskaitė

Scientific supervisors:
Dr Rimgaudas Treinys,
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Dissertation defended:
6 March 2020

For forest-dwelling raptors, nest sites are the most important recourse for reproduction, but they may be destroyed during forests felling. The Common Buzzard and the Lesser Spotted Eagle (*Clanga pomarina*) (LSE) breed in similar habitats. Return of the superpredator White-tailed Eagle (*Haliaeetus albicilla*) (WTE) to the ecosystem may induce brood defence behaviour in the mesopredator. The aim of the study was to investigate the habitat preference of the buzzard, spatio-temporal changes in habitat use, and interactions with larger raptors. The field experiment was performed to determine buzzard brood defence behaviour to WTE. Buzzard habitats between landscapes did not differ only in age of the stands. The plasticity of nest trees and nest site use were identified within changing landscapes. The buzzard does not avoid the LSE when choosing nest sites. Habitats of these species differed in the soil humidity of nest stand and in location within forest. The brood defence behaviour towards the WTE is mostly explained by the individual differences of buzzards. Mature stands are the most important component in buzzards' breeding habitats. Competition for nest sites between the buzzard and LSE is most likely to happen at the forest edge. Buzzards' brood defence behaviour was not related to the proximity to the nest sites of WTE.

BIOLOGICAL MECHANISMS OF STABLE APPLE TREE FRUITING (Agronomy)

Aurelijus Starkus

Scientific supervisor:
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Dissertation defended:
24 April 2020

The study was aimed at evaluating biological and agronomic characteristics of apple varieties of different origin and identifying genotypes with different mechanisms of yield self-regulation. We identified research criteria that allow objective characterization of apple varieties according to their yield self-regulation. It was found that yield habit of an apple variety could be objectively characterized by a complex of two features: blooming abundancy and fruitlet self-elimination. Twenty-one (21) unique alleles were identified. They are typical of the varieties with specific yield self-regulation habit or typical of the group of apple varieties with same yield self-regulation habit. It was shown that at least two yield self-regulation mechanisms exist: genetic and stress-induced. It was established that normal development of seed is a crucial factor for

apple tree varieties that eliminate a large number of fruitlets, while for varieties that eliminate a sparse amount of fruitlets, the number of seed is not so important. The relationship between apple fruit elimination and its photosynthetic parameters was determined. Importance of phytohormones on the regulation of yield has been shown. The impact of abscisic acid on yield self-regulation under environmental stress conditions was determined.

GENETIC BACKGROUND OF PLANT RESISTANCE TO GALL MITE AND FRUIT ANTHOCYANIN QUALIY IN *RIBES* SPP. (Agronomy)

Ingrida Mažeikienė

Scientific supervisor:
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for Agriculture and Forestry

Dissertation defended:
24 April 2020

The aim of thesis was to investigate the genetic potential of *Ribes* spp. to enhance the plant resistance to the blackcurrant gall mite and improve the quality of anthocyanin in fruits; to identify and validity of molecular markers for selection of plants with inherited resistance to blackcurrant gall mite and blackcurrant reversion virus. Results of this study allowed constructing a new linkage map of molecular markers of *R. nigrum*; to identify newly AFLP molecular marker for *P* gene; to establish the genetic nature of resistance to gall mite and BRV in *Ribes* spp.; to implement a set of molecular markers for identification of plants with pyramidal resistance to gall mite and BRV; to create new *R. nigrum* cultivars with pyramidal resistance; to determine the composition of anthocyanins in fruits of *Ribes* species and interspecific hybrids; to create genetic unique inter-specific hybrids with new composition of phenolic compounds for breeding process in the future; to evaluate the prospects of target breeding of blackcurrants and new varieties;to develop new varieties of blackcurrant that meet ecological and economic standards with pest and pathogen resistance locus. Published results are valuable for the optimization and implementation of blackcurrant breeding and new cultivar selection by MAS.

INFLUENCE OF SEED AND FERTILIZER RATES ON THE BIOLOGICAL POTENTIAL OF HEMP, DEVELOPMENT AND EVALUATION OF PROSPECTIVE SELECTION LINES OF HEMP (Agronomy)

Ernestas Maumevičius

Scientific supervisor:
Prof. Dr Natalija Burbulis,
Vytautas Magnus University

Dissertation defended:
12 Novermber 2020

The experimental objective aims to determine the effect of seed and fertilizer rates on the biological potential of hemp varieties cultivated for fibre and to evaluate hemp breeding lines developed in Lithuania *in vitro* and *in vivo*. Preparing the thesis the following research tasks were implemented: the effect of seed and fertilizer rates on the quantitative and qualitative parameters of hemp fibre yield, weed contamination and quantitative and qualitative parameters of fibre were estimated; the factors determining the process of dedifferentiation of somatic cells of hemp in *in vitro* culture were determined; the breeding plant numbers of hemp created by the selection method of family groups were evaluated, and a productive hemp selection line intended for seed and adapted to the Lithuanian climatic conditions was selected. The conducted research is relevant in providing practical recommendations to Lithuanian growers of sowing hemp for fibre. The performed *in vitro* research on hemp breeder's reference numbers is important for the development of genetic diversity of hemp by biotechnological methods. The performed *in vivo* research on breeder's reference numbers is relevant for the development of Lithuanian hemp varieties. The developed hemp selection line "KAN-21" was transferred to the State Plant Service Plant Variety Division for the registration of the new variety. Economic value tests are carried out in Lithuania; distinctives, uniformity and stability tests are conducted in Hungary.

QUALITY OF BIOFUEL ASH BASED FERTILIZER PRODUCTS AND THEIR EFFECT ON SOIL AND PLANT (Agronomy)

Kristina Bunevičienė

Scientific supervisor:
Dr Romas Mažeika, Lithuanian Research Centre for Agriculture and Forestry

Dissertation defended:
27 Novermber 2020

Biofuel ash is a complex and heterogeneous mixture of inorganic crystalline and amorphous minerals with organic matter. It is an alkaline raw material suitable for fertilizing acid soils. Currently, a large proportion of biofuel ash is still disposed of in landfills. The aim of the dissertation was to produce fertilizer products from biofuel ash, i.e. ash and lime kiln dust granules and ash/green waste compost mixtures and to evaluate their quality and effect on soil pH, changes in concentrations of mobile phosphorus, potassium, calcium,

magnesium, and heavy metals (Cd, Cu, Cr, Ni, Zn, Pb) in soil and their effect on plant chemical composition and yield improvement. The analysis of chemical and physicochemical quality indicators of fertilizer products revealed that these substances consist mainly of chemical compounds containing Ca^{2+} ions. The strength of the granules correlated with the amount of ash added to the mixture, i.e. the strength increases with increasing ash content in the granules, which weakens the release of nutrients from the granules. The granules obtained with the lowest concentration (30%) in the ash mixture were found to be the most suitable for fertilization. Such granules were also more effective in increasing soil pHKCl, as nutrient content in soil and plants and did not pose a risk of contamination. The results of the dissertation confirm that the processing and use of biofuel ash in agricultural soils can reduce the need for landfills, return valuable nutrients to the ecosystem, and neutralize soil acidification, thus making energy production from wood-burning more sustainable.

REGULATION OF STEVIA (*STEVIA REBAUDIANA* BERTONI) MORPHOGENESIS *IN VITRO* (Agronomy)

Neringa Juškevičiūtė

Scientific supervisor:
Prof. Dr Aušra Blinstrubienė,
Vytautas Magnus University

Dissertation defended:
4 December 2020

Stevia is a medicinal plant known for its self-accumulating natural low-calorie sweeteners, steviol glycosides, the seeds of this plant have a low germination (from 10% to 36.3%), moreover, propagation by seeds does not ensure the homogeneity of the population according to the desirable traits. For this reason, research investigations with the aim to develop alternative methods of successful stevia propagation are performed. Stevioside and rebaudioside A. are the main steviol glycosides the latter has sweet taste, while the former has undesirable bitter aftertaste. In order to change the ratio of these glycosides, an *in vitro* micropropagation scheme, in which the ratio of these biologically active compounds can be regulated using various exogenous factors, is required. Research objective was to develop an efficient *in vitro* stevia micropropagation scheme which would help to optimize the synthesis of secondary metabolites. The most suitable explant for stevia callus induction was identified. The optimal combination of growth regulators, which stimulated the highest callus mass augmentation, was selected. In MS medium callus genesis was more intensive compared to callus induction in B5 and White media. The most effective

cytokinin and its optimal concentration for direct organogenesis were selected. The regularities of auxins influence on rhizogenesis and proline and sucrose – on phenols, flavonoids and antioxidant activity has been determined. Positive effect of exogenous proline on ratio of steviol glycosides was established.

MYCOTOXINS IN SPRING CEREALS AND THEIR EFFECT ON THE QUALITY OF GRAIN PRODUCTS (Agronomy)

Yuliia Kochiieru

Scientific supervisor:
Dr Audronė Mankevičienė, Lithuanian Research Centre for Agriculture and Forestry

Dissertation defended:
10 December 2020

The aim of the study was to determine the distribution of mycotoxins in the chain of spring wheat products (grain – bran – flour – flour fractions – bread) and in the individual components of grain/flour (starch, gluten); to evaluate the relationship between mycotoxin contamination and technological properties of flour, and the risk of contamination of whole grain flour and grain products; to establish the influence of harvesting time on mycotoxin variation in the grain of different spring cereal species. Research results showed that the occurrence of *Fusarium* spp. fungi and mycotoxins produced by them in the grains of spring oats, triticale, and wheat was most influenced by the harvesting time, crop species, and meteorological conditions of the growing season. Due to the rainy growing season and harvesting period (2017), the concentrations of DON and ZEA in the spring triticale and wheat grains were several times higher than the permissible limits set forth in the EU regulation. The distribution of DON and its derivatives (3-ADON, 15-ADON) in the grain products (whole-meal flour-bran-white flour) depended on the weather conditions of the growing season and grain contamination level. The sifting was not effective in diminishing DON level, especially for highly contaminated wheat samples. It was detected that DON is stable at 170°C, and high DON levels in whole-meal flour and white flour could not be converted or decomposed during baking. The concentrations of mycotoxins in starch and gluten remained relatively high. In rainy years, a delay in harvesting resulted in diminished values of the main quality indicators of spring cereals, including mass per hectolitre and falling number. Negative correlation was found in highly contaminated spring cereals between mycotoxins (DON, 3-ADON, 15-ADON, ZEA and T-2) and protein content, flour viscosity, rheological properties of wheat dough and bread baking properties.

THE EFFECT OF CROP COVER AND SOIL WATER RETENTION ON PHYSICO-CHEMICAL AND BIOPHYSICAL QUALITY OF SOILS OF DIFFERENT ORIGIN (Agronomy)

Mykola Kochiieru

Scientific supervisor:
Dr Virginijus Feiza, Lithuanian Research Centre for Agriculture and Forestry

Dissertation defended:
10 December 2020

The study was aimed to investigate the influence of contrasting land use and tillage methods on soil carbon dioxide efflux, content of water-stable aggregates in different soil horizons and their relationship with physical and chemical properties and plant root parameters in the soils of different genesis (Cambisol and Retisol). Research results showed that topsoil CO_2 effluxes under contrasting vegetation cover, land use, and management conditions in Cambisol and Retisol were directly related to soil temperature, volumetric water content, macropore network and root volume. The response of soil water-stable aggregates (WSA) to freezing-thawing processes depended on many factors, including soil texture, soil organic carbon content, and water content during freezing process and agronomic practices. In Cambisol and Retisol, soil organic carbon content had a positive direct effect on the formation of WSA. The potential of Retisol to increase the content of water-stable aggregates within the whole 0–40 cm soil layer was found to be higher than that of Cambisol. The content of WSA, averaged across soil types, land uses and soil layers, tended to decrease in the following order: WSAAD – air-dry soil > WSAFC – soil with water content at field capacity > WSANS – soil near full saturation.

EFFECT OF DIFFERENT CHEMICAL COMPOSITION AND STRUCTURE OF LIMING MATERIALS ON ACID SOIL NEUTRALIZING (Agronomy)

Donata Drapanauskaitė

Scientific supervisor:
Dr Romas Mažeika, Lithuanian Research Centre for Agriculture and Forestry

Dissertation defended:
17 December 2020

The study was aimed to determine the efficiency of liming materials and the relationship between their chemical composition, pellet strength, size, and the speed of the soil neutralization process, and to determine the influence of different liming materials on the yield and harvest quality of crop rotation: spring barley, spring wheat, pea. Research results showed that K, Na, Si, Fe, and Al reduce the neutralizing value and reactivity of liming materials. Also, the analysis of physicochemical indicators of liming materials showed that the pellet strength correlates with the size of the pellet, i.e. the strength of the pellet increases with increasing

pellet size, which slows down the neutralization efficiency of the liming materials. The physical state of liming materials does not affect the chemical composition, but it affects the physicochemical parameters. It has also been found that under excess soil moisture conditions, soil neutralization efficiency increases for all liming materials, but the soil moisture content has the greatest effect on the performance of pelletized liming materials.

THE PHENOTYPIC ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* STRAINS AND WHOLE GENOME SEQUENCE-BASED PREDICTION OF RESISTANCE DETERMINANTS (Veterinary)

Jurgita Aksomaitienė

Scientific supervisor:
Prof. Dr Mindaugas Malakauskas,
Lithuanian University of Health Sciences

Dissertation defended:
3 July 2020

The aim of the study was to determine antimicrobial resistance phenotypes of *Campylobacter jejuni* isolated from different sources and to investigate the genetic mechanisms of resistance and population structure using a single nucleotide polymorphisms-based phylogenomic analysis. In this study, the antimicrobial resistance of the 341 strains of *C. jejuni* was identified using phenotypic assay based on standardized agar dilution method considered as a gold standard of AMR detection. Whole genome sequencing (WGS) based characterization of resistant *C. jejuni* strains provided more useful information about the presence of known/unknown AMR genes or mutations, deletions, intergenic regions and other AMR genetic determinants. Comparative genomic analysis of *C. jejuni* strains using whole genome sequencing has provided new insights into genetic antimicrobial resistance defined by the ability to correctly identify AMR determinants associated with an antimicrobial resistance phenotype. A DNA sequence-based study makes it possible to define preferable multidrug-resistance for detection of AMR determinants and to get important knowledge to combat the increasing threat of AMR of *C. jejuni*.

ANALYSIS OF CHANGES IN THE COMPOSITION OF DAIRY FAT TO DEVELOP THE COMPUTER PROGRAM FOR SCREENING OF FATTY ACIDS IN RAW MILK (Veterinary)

Lina Laučienė

Scientific supervisor:
Prof. Dr Loreta Šernienė,
Lithuanian University of Health Sciences

Dissertation defended:
3 July 2020

This thesis aimed to evaluate the effect of season, processing, and storage on the composition of dairy fatty acids in processed milk to develop a computer program prototype for fatty acid screening in procured raw milk. To date, no research has been carried out in Lithuania to determine the profile of fatty acids in the technological dairy chain covering every major step of the technological process and to analyze the relationship between the raw material, end product, and by-product under commercial settings. Thus, this renders the present case study relevant and new. As a result of this study, a prototype computer program was developed and tailored for the specific needs of dairy processor. This tool enables the screening of the composition of fatty acids of procured cow milk data provided by the Lithuanian Central Milk Testing Laboratory (Lith., *Lietuvos centrinė pieno tyrimų laboratorija*), by respective period, region, or selected raw milk producers. This program can help dairy processors to produce export products of the desired (standard) fatty acid composition and become more competitive in both domestic and foreign markets.

PROTOZOA OF THE GENUS *SARCOCYSTIS* IN CATTLE: IDENTIFICATION, DISTRIBUTION AND IMPACT ON THE HOST ORGANISM (Veterinary)

Vytautas Januškevičius

Scientific supevisor:
Prof. Habil. Dr Saulius Petkevičius,
Lithuanian University of Health Sciences

Dissertation defended:
26 August 2020

The aim of the study was to determine the prevalence of sarcocystosis and species composition of *Sarcocystis* spp. infecting cattle and to evaluate the effect of infection on animal organism and meat quality. Thus far, the species composition of sarcocysts in cattle has not been investigated. In the present study, biochemical parameters of the blood from naturally infected cattle were analysed and compared for the first time. Variation of these parameters depending on the intensity of infection was determined. By the common morphological method, it was preliminary determined that the most pathogenic *S. cruzi* is dominant in cattle. The effect of sarcocyst infection on the quality of meat from animals slaughtered for food, nutritional value and sanitary quality as well as risks to human health were evaluated. By DNA analysis of sarcocysts found in muscles, three species of sarcosporidia were identified in cattle: *S. bovifelis*, *S. cruzi*,

and *S. hirsuta*. The analysed *cox1* region showed high variability of species, which differed in genetic diversity and structure of populations. An effective and relatively rapid method based on digestion of muscular tissues and PCR with species-specific primers was developed for the identification of sarcosporidia species in cattle muscle. In the cattle samples analysed by original molecular diagnostic method for *Sarcocystis* parasites, four *Sarcocystis* species were identified: human pathogen *S. hominis* (13.7%), *S. cruzi* (96.1%), *S. bovifelis* (71.6%), and *S. hirsuta* (30.4%).

PARAMETERS OF THE REPRODUCTIVE ACTIVITY AND REGULATION OF THE SEXUAL CYCLE IN BEEF HEIFERS AND COWS (Veterinary)

Audronė Rekešiūtė

Scientific supervisor:
Prof. Habil. Dr Henrikas Žilinskas,
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Dissertation defended:
28 August 2020

To investigate the dynamic changes of ovaries and their functional derivatives, progesterone concentration and oestrous cycle synchronization during different breeding periods in beef heifers and cows of different breeds. Our work allowed evaluating the latest synchronization technologies (presynchronization, synchronization, and timed artificial insemination) while using veterinary medications registered in Lithuania for heifers and cows of various beef breeds. The morphometric dynamic of ovaries and their derivatives and progesterone concentration in synchronized and nonsynchronized oestrous cycle in different groups of heifers and cows of beef cattle were performed and compared. Also, different levels of progesterone modules, having influence on pregnancy at different synchronization programs, were also evaluated. In order to assess the potential genetic significance and influence on reproductive efficiency, gene GNRHR sequencing and polymorphism of genes encoding enzymes involved in sex hormone synthesis (GNRHR, LHCGR, PGR) were performed. It aimed to identify association of specific gene variations with ovulation and fertilization levels in beef cattle breeds. In the case of high genetic diversity in beef cattle population, both within and between breeds, differences between breeds are observed, for assessment of the most important traits of reproduction. Therefore, our research allows making more efficient use of beef cattle reproduction, while aiming to increase conception rates.

ANALYSIS OF CHEMICAL COMPOSITION INDICATORS OF EXTRUDED LUPINES (*LUPINUS* L.), FABA BEANS (*VICIA FABA* L.) AND PEAS (*PISUM SATIVUM* L.) AND EFFICIENCY OF THEIR USE IN THE NUTRITION OF DAIRY COWS (Animal sciences)

Ieva Kudlinskienė

Scientific supervisors:
Prof. Dr Asta Racevičiūtė-Stupelienė,
Prof. Habil. Dr Romas Gružauskas,
Lithuanian University of Health Sciences

Dissertation defended:
26 August 2020

The aim of research was to analyse the chemical, amino acids and fatty acids composition of untreated and extruded diverse species of legume family and to assess the efficiency of their use in the diets of dairy cows. In this dissertation, the chemical and amino acid composition and the fatty acid composition (before and after the extrusion process) of legume family plants (*Fabaceae*) included in the national list of plant varieties in Lithuania were analysed, and the efficiency of these materials in the nutrition of dairy cows was evaluated. Moreover, for the first time, the effect of the extruded faba beans (*Vicia faba* L.) on the milk sensory and technological properties, composition of amino and fatty acids , and on the formation of volatile flavour compounds was evaluated. Furthermore, studies have been carried out to assess the impact of extruded plants of the legume family (*Fabaceae*) on fermentation processes in the rumen of dairy cowsand on biochemical blood parameters. Concerning the results of the conducted study, it is possible to establish compound feed recipes and diets for dairy cows supplemented with extruded plants of legume family (*Fabaceae*) without adversely affecting their performance, milk composition, and sensory properties.

RESEARCH ON THE EFFECTS OF BREED, LACTATION, PRODUCTION, AND NUTRITION ON MILK PROGESTERONE CONCENTRATION AS BOVINE REPRODUCTION MARKER (Animal sciences)

Aurimas Gavelis

Scientific supervisor:
Prof. Dr Arūnas Juozaitis,
Lithuanian University of Health Sciences

Dissertation defended:
27 August 2020

The aim of this study was to determine the effects of cattle breed, lactation, production, and nutrition on the variation of milk progesterone concentrations by evaluating the performance of cattle. The significance of the P4 concentration as a real-time biological marker for the assessment of the ability of a cow to be impregnated has been studied. It has been identified that in the lactation period of 61 to 70 DIM, when the cows were undergoing insemination, the average P4 concentration could correlate with the ability of a cow to be impregnated and the interval between parturition. The development of the P4 concentration at the beginning of oestrus and 12 hours after the beginning of oestrus has been investigated

and the forms of effects of cattle breed, lactation, milk production performance, and milk composition on the abovementioned indicators identified. The effects of the investigated indicators on the P4 concentration are unambiguous. It is important for practitioners that the threshold on 61 to 70 DIM period when the cows were undergoing insemination was established. When estimating the P4 concentration during oestrus in line with the heat of cow, such factors as breed, lactation phase, milk production performance, and milk composition should be taken into account. In terms of the P4 concentration, reproductive function of cows both in conventional and organic farms are analogous, thus, farming method has no significant impact on the ability of cattle to be impregnated.

PHENOTYPIC AND GENETIC EVALUATION OF THE CALVING EASE AND RELATIONSHIP WITH CALF MORTALITY, COW PRODUCTIVITY, REPRODUCTION, EXTERIOR AND HEALTH IN THE LITHUANIAN BLACK AND WHITE CATTLE POPULATION (Animal sciences)

Arvydas Kardišauskas

Scientific supervisor:
Prof. Dr Vida Juozaitienė,
Lithuanian University of Health Sciences

Dissertation defended:
28 August 2020

The aim of the study was to investigate phenotypic and genetic parameters of calving ease in the population of Lithuanian Black-and-White cattle and to evaluate the genetic correlation among calf mortality, the indicators of productivity, exterior, health, and reproductive traits of cattle. The indicators of calving ease in the population of Lithuanian Black-and-White cattle have been investigated and their phenotypic and genetic correlations with the indicators of calf mortality rate, cow exterior, productivity, and milk somatic cells indices have been estimated. The relationship between the calving type evaluation in cows and electrical conductivity of milk, the occurrence of mastitis pathogens, and other reproductive traits has been assessed. The heritability coefficients of calving ease in cows of the population of Lithuanian Black-and-White cattle were established. Sustainable and efficient management of the genetic resources of dairy cattle is an integral part of disease control. The outcomes of the investigations and analyses carried out in this study have shown that selection on the basis of calving performance indicators will have a positive effect on bovine mastitis, metabolic disease, and reproductive indicators. This will lead to the improvement of animal welfare and longevity, increase in profitability of dairy farms, more expedient and rational use of livestock, and achievement of well-balanced agricultural growth and development.

THE COMPOSITION, STRUCTURE, SYNANTHROPIZATION AND IMPACT OF URBAN FORESTS ON THE QUALITY OF LIFE OF RESIDENTS (Forestry)

Ana Bernat

Scientific supervisor:
Prof. Dr Vitas Marozas,
Vytautas Magnus University

Dissertation defended:
6 March 2020

The work aims to investigate the composition, structure, stages of synanthropization of urban forests and ecotones and the impact of these forests on the residents' quality of life. Phytocenoses of urban forests affected by different stages of synanthropization and the influence of various forest edges on the forest vegetation of the city were assessed in a comprehensive manner using multivariate analysis methods. Phytocenoses of pine forests with different stages of synanthropization were compared to phytocenoses of relatively natural pine forests. The results are complementary to the totality of the knowledge gathered so far about urban forests and their vegetation, while allowing us to take a more objective and novel look at urban forests, assess them more thoroughly, and broaden our horizons and understanding of the complex processes taking place in urban forests. Research conducted during the doctoral studies found that almost all urban forests are more or less affected by synanthropic processes. The edges of the forest affect the phytocenoses of urban forests, but these effects are not very strongly expressed. Urban forests are an essential element of urban life of people, who tend to nurture, protect, and conserve them. There is a direct link between urban forests and other green areas in quantity/distribution/distance and well-being of the population. The results provide new knowledge about the composition and structure of urban forests, their functions, and their interaction with the well-being of the population.

GENETIC DIFFERENTIATION OF SCOTS PINE MITOCHONDRIAL DNA HAPLOTYPES AND BOG POPULATIONS BASED ON MORPHOLOGY AND DNA MARKERS (Forestry)

Monika Sirgėdienė

Scientific supervisor:
Prof. Dr Darius Danusevičius,
Vytautas Magnus University

Dissertation defended:
8 May 2020

The aim of this study was to assess the genetic background for differentiation of the Scots pine population in raised bogs based on DNA markers and screen for morphological markers for the Scots pine mitochondrial DNA haplotypes in Lithuania. Results of this study showed that in raised bogs, the gene pool of the Scots pine is significantly differentiated from nearby dry sites populations even under high gene flow pressure from vast areas of surrounding Scots pine stands. The strength of this differentiation

depends on the bog size and distance between the pine trees in the bog and the edge of bog. Populations in large raised bogs are more genetically diverse than in small bogs and are sufficient genetic diversity for long term adaptive development as a distinct gene pool. The high frequency of specific the mtDNA haplotypes on the raised bogs strongly supports the distinct original founder populations on raised bogs. Morphotypes of the Scots pine in raised bogs are predominantly affected by environment conditions within the microsite in the bogs and to a lesser extent by genotype, but there certainly is a genetic background for the morphotypes. The mitochondrial haplotypes have not preserved distinct morphology types (based on tree stem bark, cone or seed morphology), however, the frequency variation of the mtDNA on the raised bog sites shows that there is strong support for original founder populations in the raised bogs preserving the type B haplotype.

IMPACT OF ENVIRONMENTAL FACTORS ON SUSTAINABILITY AND PRODUCTIVITY OF LARCH STANDS (Forestry)

Kšištof Godvod

Scientific supervisor:
Prof. Dr Gediminas Brazaitis,
Vytautas Magnus University

Dissertation defended:
25 June 2020

Studies have revealed that stands of European, Japanese, and Hybrid larch trees are the most productive stands in Lithuanian forests. A phytocenotic analysis of larch stands has shown the existence of 55 herbaceous plant species in larch stands. Three of them were invasive. Nine percent fewer species were found in mixed larch stands. The undergrowth of targeted tree species was found in 17.5% sample plots and only 6.8% of these plots had a perspective undergrowth. Stand density and habitat have a greater impact on the emergence of the undergrowth than the age of the stand. The evaluation of the sanitary condition of larch stands has shown that European larch trees of a good and average sanitary condition account for more than 85%. The condition of European and Hybrid larch trees was similar. Productivity analysis of larch trees has revealed that pure larch stands of age class III–VI in the most suitable sites (Nc) are on average 15.2–23.9% more productive than mixed larch stands, while pure stands of Hybrid larch trees are on average 10.8% more productive than European larch stands. The study has shown that precipitation has a greater impact on the radial increment of European larch trees than the temperature.

KEY FACTORS LIMITING SCOTS PINE STANDS SUSTAINABILITY UNDER THE PRESSES OF GLOBAL CHANGES (Forestry)

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Dissertation defended:
13 November 2020

The aim of this study was to determine changes in the condition and productivity of Scots pine populations and stands of pines of different genetic groups in different environmental conditions (meteorological, background and local pollution, and pest invasions). Different reactions of stem radial increment formation of individuals of different populations of the Scots pine to the influence of meteorological factors in Central Lithuania were determined. It has been shown that different groups of genetic trees have different responses of the Scots pine to natural environmental factors. There are also differences in the response of different groups of genetically related trees of Scots pine individuals to the needle-eating insects and air pollution. The importance of tree genetic diversity for the condition and growth of tree crowns has been assessed, when acting in combination with the tree's competition index, meteorological factors, and local or background contamination.

NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) AND SCOTS PINE (*PINUS SYLVESTRIS* L.) WOOD PROPERTIES DEPENDENCE ON GROWTH CONDITIONS AND STAND MANAGEMENT (Forestry)

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The global demand for wood products as natural raw materials is increasing every year, and wood is becoming an even more important renewable resource due to the development of the bioeconomy. However, the deteriorating forest biodiversity and the growing public expectations regarding the increase of the protected areas reduce the areas of commercial forests both in Lithuania and in Europe. One of the ways to grow higher amount of good quality wood could be the development of plantation forestry: appropriate selection of growing conditions, tree species composition, initial tree density, and, in later stages, tree thinning intensity. Such plantations could be successfully established by combining the needs of the timber industry, the forestry sector, the public interest, and taking into account environmental and ecosystem sustainability aspects. The aim of this study was to determine and compare the physical and mechanical wood properties of the Scots pine and the Norway spruce in the stands of different densities. Non-destructive

and destructive methods were used to determine wood quality indicators: dynamic and static modulus of elasticity, bending strength, wood density, properties of annual rings. Differences between the above indicators in stands with different initial stand densities were assessed. Wood quality indicators were also modelled based on environmental conditions data, and the significance of environmental conditions for the studied indicators was assessed. The obtained results allow to compare the influence of growing conditions on wood properties and to model the desired wood properties for future stands. This work provides new knowledge for plantation forestry and wood sciences.

ACCURACY OF LEAF AREA INDEX (LAI) ESTIMATED BY THE HEMIVIEW SYSTEM AND ITS APLLICATIONS IN RESEARCH OF SCOTS PINE STAND HEALTH AND PRODUCTIVITY (Forestry)

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The Leaf area index (LPI) is one of the most important structural indicators of stands, describing many biological and physiological processes related to crown vegetation. Leaf area index (LAI) quantifies the amount of leaf area bearing by a tree or whole stand normalized by the unit of crown projected or the whole stand ground area. The object of the study was the plant/leaf area index obtained by the method of hemispheric photographs. The identified applications have shown that the use of the hemispherical photography method is limited in mixed and perennial stands with abundant undergrowth, craze, or a perspective second grove. All indirect leaf/plant area determination methods obtained lower absolute numerical crown total surface area index values compared to the reference projected surface area: hemispherical photography method about 80%, mixed method – 11%. In a middle-aged, pure pine forest of natural origin, the PAI determined with the help of digital hemispherical photography best reflects the taxonomic characteristics of the stand determined in a circular plot with a radius of about 10 ± 1 m (about 314 m^2). At that time, the most informative part of the hemispherical photograph is that which covers the entire area of the study plot without going outside it and is calculated for each stand individually, depending on the average height of the stand. By complying with these requirements, the API can explain up to 70–80% of the variability in total stand tax rates. This is a norm that should be followed when choosing the size of the test plots.