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research in Biology /Inovativie  
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1

## Plant communities with *Rhynchospora fusca* (L.) W. Aiton in Latvia

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*Rhynchospora fusca* is found in central Europe, the southern part of Scandinavia, the Baltic States, and the northeastern part of America. It is a suboceanic boreo-temperate species. Not surprisingly, in Latvia, the species is restricted to the Coastal Lowland, more precisely to the north-western part of it. Four sites are known and there are no historical records from other sites.

The study aims to describe and classify plant communities with *R. fusca* and compare them with plant communities in other countries. Three sites were visited, and vegetation was described in ten sample plots. Formal definitions of fen alliances were used in classification at the alliance level and cluster analysis was used to classify vegetation at the association level.

Similarly, as in other parts of its distribution range *R. fusca* grows in a shallow nutrient-poor lake, fen, and periodically flooded humid dune slacks in Latvia. It forms monodominant stands in 10-15 cm water accompanied by *Myrica gale*, *Carex lasiocarpa*, and other sedges bordering *Eleocharis multicaulis* community. In the humid dune slacks, acidophilous species such as *Andromeda polifolia*, *Vaccinium oxycoccos*, *Calluna vulgaris*, *Eriophorum vaginatum*, and *Eriophorum angustifolium* are present. *Rhynchospora fusca* stands were less dense than in the lake, and they occupy the central part of dune slacks. Moss layer was absent or weakly developed. *Sphagnum palustre* and *Sphagnum subsecundum* were the most common bryophytes there. The dune slacks are overgrowing with *Myrica gale*.

In fen, *R. fusca* does not dominate. It is found in *Rhynchospora alba* community overgrowing with *Myrica gale*. Frequent associates were *Carex lasiocarpa*, *Menyanthes trifoliata*, *Vaccinium oxycoccos*, and *Utricularia intermedia*. *Sphagnum teres*, *Sphagnum contortum*, *Campylium stellatum*, and *Scorpidium scorpioides* are abundant in the moss layer.

The first results of classification and similarities with plant communities from other parts of Europe will be discussed.

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## Development of an innovative approach to identify biological determinants involved in the between-animal variation in feed efficiency in sheep farming

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This cooperation project (lzp-2021/1-0489) is interdisciplinary research related to economic activity in agriculture and biotechnology, implemented by the University of Latvia in collaboration with the Latvian University of Life Sciences and Technologies. The purpose of this study is to determine genetic and molecular markers to identify individual animals in sheep herds with the maximum predisposition to feed digestibility or efficiency (FE) and weight gain, with the aim of their introduction into breeding. Feed accounts for 65–70% of the cost in the sheep industry, and thus, improving feed efficiency is important for the economy and the environment. FE is a major indicator of the efficiency of feed utilization. A low FE has a negative impact on the environment and increases production costs. The proposed project aims to better understand the mechanisms that affect animal-to-animal variation around the average feed efficiency observed in lambs reared in similar conditions, to gain knowledge to develop putative biomarkers, could be used to analyse live lambs, as a cost-effective and rapid tool for genetic selection or management decisions. To achieve the project goal, selection

of lambs from meat-type Latvian breeds and from Latvian dark head breed, feeding trial, ranking animals by groups based on feed efficiency will be conducted. Pre-grouped animals will be assessed for phenotypic parameters, potential genetic markers (using next-generation sequencing), molecular markers for metabolic efficiency, and product quality (body weight, height, fat content). Chemical and biochemical analysis of biological samples (blood, serum, stool samples) will be performed. Based on the obtained data, biostatistical methods will be used to develop algorithms for predictive models of various parameters, which will be tested in secondary lamb collection to ensure accuracy. We are planning to develop a cheap, simple and easily reproducible methodology of genetic and molecular FA markers in blood of live lambs for selection and/or for breeders.

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## Effects of Low Frequency Electromagnetic Radiation on *L. minor* growth parameters and generation of point mutations at GPx, CAT and APx genes

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Development of new technologies distributing electric power from power stations to our homes through a network of cables and wires, including numerous electric devices at working places and home environment become a source of electromagnetic radiation (EMR) much stronger than EMR of natural origin. To provide a better understanding of the impact of the EMR of anthropogenic origin on living organisms, we investigated the long-term effects of EMR on *Lemna minor*. In this study, plants of the *L. minor* laboratory clone were exposed to LF EMR (50 Hz) growing clones in Petri dishes placed on the coils generating magnetic flux ranging from 1 – 300  $\mu$ T. We examined the response of the plants on molecular (variability of DNA sequences of ascorbate peroxidase (APx), glutathione peroxidase (GPx), and catalase (Cat) genes) and physiological (plant growth, frond area, and number) level.

Comparison of growth parameters of *L. minor* clones exposed to 1  $\mu$ T, 2  $\mu$ T, and 300  $\mu$ T magnetic flux revealed positive effect stimulating growth of experimentally affected plants at 2  $\mu$ T. After the first 14 weeks of treatment, the growth parameters were lower in the directly exposed by LF EMR group than in the group grown distantly from the source of EMR. However, after 18 weeks from the beginning of the experiment no significant difference was observed between two groups of *L. minor* including directly and indirectly affected by LF EMR plants. Moreover, the signals of the impact of LF EMR on the plants rising point mutations were detected. The significantly enhanced number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones were revealed at the introns of APx ( $P=0.011$ ), GPx ( $P=0.009$ ), and Cat ( $P=0.044$ ) genes starting from the 10th week of the experiment.

In conclusion, the data on molecular and physiological levels provide evidence that *L. minor* clones experimentally affected by LF EMR respond to the impact depending on the time and the magnetic flux density.

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## NEMORALISATION OF CONIFEROUS STANDS IN LATVIA

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Establishment of native and alien deciduous broadleaved species (*Acer platanoides*, *Tilia cordata*, *Quercus robur* and *Ulmus laevis*, *Acer pseudoplatanus* and *Tilia platyphyllos*) has been stated in extensively managed mature and over-mature conifer - *Pinus sylvestris* and *Picea abies* - stands in Latvia during the last decades. The process which involves establishment of deciduous broad-leaved species in coniferous stands is called as nemoralisation of coniferous stands. It is characterised by a large number of seedlings of broad-leaved species, young trees and the formation of a subcanopy layer in stands. The research objective was to identify changes in deciduous broad-leaved forest stands, which are formed by the transformation of coniferous stands in Latvia, based on investigation on the age of individuals and the peculiarities of stand structure and the composition of the shrub and tree layer of pine and spruce stands. Thus eight forest stands were selected to characterize the transformation process of coniferous forest stands with various broad-leaved species located in different ecological regions of Latvia. Our research revealed that the establishment of some deciduous broadleaved individuals into the studied coniferous stands occurred 40–50 years ago or during the seventies and eighties of the 20th century. The results showed that the predominant age of alien broad-leaved tree species in the research stands was 15–25 years, which indicates that the introduction of large numbers of seedlings of alien broad-leaved species in the coniferous stands occurred at the end of the 20th century. As a result, stable and mainly shade tolerant and mixed stands consisting of three to four tree species have formed in the final phase of the conifer replacement process or nemoralization, often together with *Picea abies*.

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## Preliminary results of *Sarcocystis* spp. infection in birds of prey in Lithuania

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Preliminary results of *Sarcocystis* spp. infection in birds of prey in Lithuania

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*Sarcocystis* spp. are cyst-forming protozoa parasites. They maintain prey-predator two-host life cycle. *Sarcocysts*, as an asexual vital form, are formed in the muscles and CNS of the intermediate host, and oocysts/sporocysts, as a result of sexual multiplication, develop in the small intestine of the definitive host. Birds of prey are usually studied as definitive hosts while their role as intermediate hosts are poorly described.

In the period of 2014-2020 various birds of prey within Accipitriformes, Falconiformes, Strigiformes orders were collected. Legs and/or chest muscles were methylene-blue stained, when prepared by squash method and analysed under a light microscope (LM). Microscopic sarcocysts were detected in *Accipiter gentilis*, *Buteo buteo*, *Strix aluco* and *Asio otus* (4/27 14,8%). Based on ITS1 region sequence analysis sarcocyst isolated from *Buteo buteo* muscles was identified as *S. halioti*. Intestinal scrapings were analysed by direct light microscopy. Oocysts/sporocysts were detected in *Buteo buteo* (5/7, 71.4%), *Accipiter gentilis* (11/11), *Buteo lagopus* (2/2), *Milvus migrans* (1/1), *Strix aluco* (1/3 33.3%). Further molecular examinations are needed for *Sarcocystis* spp. found in intestinal samples.

As pathogenic *Sarcocystis* species, *S. halioti* was isolated from muscle tissue other internal organs such as brain, lungs and liver must be investigated. Studies should be performed to determine which species of *Sarcocystis* spp. are excreted with faeces to the environment, as well to identify vital organ infestation.

**Keywords:** *Sarcocystis*, Accipitriformes, Falconiformes, Strigiformes, molecular identification

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## Impact of light source on distribution of greenhouse whiteflies *Trialeurodes* spp. (Hemiptera: Aleyrodidae) on tomatoes in greenhouses

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Nowadays, vegetable growing in greenhouses during the cold and dark winter seasons is an integral part of agriculture. To ensure better plant growth and production various artificial light sources are used. Lately, beside the other light sources light emitting diodes are introduced in greenhouse systems, as they are safer, easier to control digitally and are cooler than high-pressure sodium vapor lamps.

In greenhouses not only plants can thrive on favourable conditions, various pests also flourish. Light is one of the most important factors in the insect life. Light can promote or disturb insect development and life cycles. Whiteflies *Trialeurodes* spp. (Hemiptera: Aleyrodidae) are known to feed on many plant species, including agriculturally important ones. In favourable conditions whiteflies can rapidly reproduce and cause vast plant damage. Whiteflies feed on plant fluids, and infested leaves may lose vigor, become yellow and may drop prematurely. In case of severe infestation plants can be destroyed. Whiteflies must eat large quantities of dilute sap in order to obtain the necessary nutrients. The liquid and excess sugar ends up being excreted as shiny, sticky honeydew on plant surface that may lead to black sooty mold that grows on the foliage. Still what harms plants the most is a whitefly's ability to transfer plant viruses, as they can transmit more than 100 viruses.

To clear up possible impacts of light source on development of one of the most harmful greenhouse pests – greenhouse whiteflies current experiments in the frames of the project “Investigation of innovative solutions and new method development for efficiency and quality increase in Latvian greenhouse sector [IRIS]” were undertaken. The study was performed in the polycarbonate greenhouse of Faculty of Agriculture of the University of Life Sciences and Technologies of Latvia. Three additional light sources were used: LED COB Helle Top LED 280 luminary, induction lamps and high-pressure sodium vapor lamps Helle Magna. Four varieties of tomatoes were grown under 16 hour photoperiod. Adult whiteflies were recorded by means of yellow sticky traps placed near tomatoes. Traps were monitored every 2 weeks. Whitefly's nymphs were recorded on tomato leaves in the same time. Differences in distribution of whiteflies among three light sources were observed. Acknowledgements. Investigation was supported by Latvian Rural Development Program 2014-2020 “Cooperation”, call 16.1 project Nr. 19-00-A01612-000010.

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## Microalgae originated biostimulants as a sustainable alternative for agriculture

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Currently maintaining agricultural sustainability is a major problem due to growing global population, climate change and rapid increase of food demand. General problems in agriculture are loss of harvest, plant diseases, abiotic stress which may result in loss of 20 – 40 % of the crop. Use of conventional methods, for instance - pesticides results in decreased sustainability, also many organic biostimulants are not sufficiently effective. As a sustainable solution microalgal biostimulants (MBS) and biofertilisires (MBF) can be used to enhance plant stress resistance and increase production yield. Microalgae are microscopic, unicellular, or filamentous photosynthetic organisms with small size (1 – 900 µm) that can growth in marine and freshwater environments. Research show that MBS and MBF can potentially be used to increase plant growth and productivity and enhance pathogen and stress factors resistance. Microalgal biomass can be used as a source of slow macro- (K, P, N) and micro-nutrient (Mn, Cu, Zn, Fe) release. Additionally, microalgal ability to produce phytohormones (gibberellin, auxin, cytokinin et al.) can stimulate plants innate defence mechanisms. In northern regions, also in Latvia agricultural production is quite complicated due to relatively cold climate. Use of cheap industrial growth substrates (wastewater, agricultural by products et al) can be used for microalgal biomass production for further application in agriculture. Moreover, selection of strains suitable for certain growth environments may increase production of microalgal biomass.

This study was performed within the framework of the project no. 19-04-A01620-000072 “Development and testing of microalgae originated prototype of plant growth stimulant and antimicrobial agent for autumn raspberries”, co-financed by European agricultural fund for rural development (EAFRD) and supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

**Keywords:** microalgae, biostimulants, crop nutrition, crop yield, sustainability.

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## Bacterial cellulose production on apple juice

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Biomaterials and biopolymers, such as bacterial cellulose (BC), are gaining increased importance as perspective eco-friendly materials with wide range of potential applications. BC is an exopolysaccharide produced mainly by certain acetic acid bacteria strains. BC exhibits unique physical and mechanical properties and has many potential applications starting from biomedicine to food industry. However, BC industrial production still is associated with several difficulties, one of which is to achieve high BC production yield. Costs of growth media for BC production can reach up to 30 % of the total production costs and, therefore searches for alternative growth substrates are challenging. For instance, as it has been showed in several studies, juice from low quality apples can be used instead of standard growth medium. At present food loss and refuse is one of the great global problems. Annually, about one third of all foods produced (1.3 billion tons/year) is lost during harvest, processing and retail. Approximately 30 – 40 % of annual apple harvest remains unsold. Apples can be used to produce apple juice which is reach in such sugars as glucose 9.3 – 32.2 g/L, fructose 66.1 – 96.0 g/L, sucrose 8.5 – 55.1 g/L and total sugars reaching 110.9 – 164.4 g/L. Moreover, apple juice is reach in macro- and micronutrients, including B group vitamins, that may also enhance bacterial growth and BC synthesis. In the current study BC synthesis on apple juice was evaluated. The optimal dilution factor of unmodified apple juice was 1:6 that corresponds to  $15.28 \pm 0.68$  g/L of total sugar concentration and resulted in  $0.89 \pm 0.01$  g/L of dry BC production, which is higher as compared with reported previously. Further research showed *Komagataeibacter hansenii* B-22 as the best BC producer strain on the unsupplemented apple juice ( $0.79 \pm 0.03$  g/L) as compared with two other strains *K. rhaeticus* P-1463 and *K. hansenii* DSM 5602, which produced  $0.67 \pm 0.03$  g/L and  $0.26 \pm 0.03$  g/L of BC, respectively. Currently the work is in progress for further apple juice media composition's optimisation including determination of optimal N source.

This study was performed within the project no. 19-00-A01612-000004 “Obtaining of bio-degradable polymers from renewable resources for production of protective coatings and packaging materials for fruits”, co-financed by European Agricultural Fund for Rural Development and supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

**Keywords:** bacterial cellulose, biopolymers, apple juice, *Komagataeibacter*, acetic acid bacteria.

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## Bacterial cellulose production on whey medium

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Bacterial cellulose (BC) is a biopolymer with a potentially high added value, produced by certain acetic acid bacteria (AAB) strains. Due to its great biodegradability and mechanical properties, BC has a wide range of potential applications in such fields as food industry, biomedicine, packaging, electronics et al. Up to date industrial-scale BC production still is mostly unsuccessful. In general, costs of growing media can reach up to third part of total production costs. Research shows that different kind of industrial and agricultural by-products can be used to decrease growth media costs. In milk industry only 10 – 20 % of total milk is converted into an end-product, other 80 – 90 % is the liquid fraction known as whey – a problematic by-product that may cause severe environmental pollution when utilised improperly. In the current study whey was evaluated as an alternative BC synthesis medium. It was shown that as compared with other strains of AAB, *Komagataeibacter rhaeticus* P-1463 showed highest production of BC ( $1.95 \pm 0.15$  dry BC weight) on unmodified whey. However, BC synthesis on whey was significantly lower than on the standard Hestrin & Schramm media ( $3.26 \pm 0.05$  g/L). Although it was shown that *K. rhaeticus* P-1463 can utilise both lactose ( $0.26 \pm 0.03$  g/L) and galactose ( $0.28 \pm 0.02$  g/L) as the main C source in modified HS medium, where glucose is replaced, respectively. It has been shown, that decreased whey concentration resulted in lower BC synthesis. Whey hydrolytic pre-treatment was evaluated and increased BC synthesis by enzymatic hydrolysis was ( $2.41 \pm 0.04$  g/L) was shown. Limiting factors were studied and no specific impact was found on BC synthesis, thereby showing that lactose is the carbon source being difficultly assimilable for AAB, and its consumption for BC synthesis is little or no affected by the presence of added growth factors. Therefore, further research for optimisation of whey medium for BC production is required.

This study was performed within the framework of the project no. 19-00-A01612-000004 “Obtaining of bio-degradable polymers from renewable resources for production of protective coatings and packaging materials for fruits”, co-financed by European Agricultural Fund for Rural Development and supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

**Keywords:** bacterial cellulose, whey, *Komagataeibacter*, biopolymers, wastewater treatment

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## Growth systems for microalgae biomass production

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Cultivating of microalgae has led to increasing commercial interest in their use for producing valuable substances for food, feed, cosmetics, pharmaceuticals, and biodiesel et al., as well as for mitigation of environmental pollution and CO<sub>2</sub> emissions. Some technical barriers exist on the commercialization of microalgal biomass and products thereof. These are mostly related to the cultivation of large quantities of microalgae biomass to meet energy demands, which is limited by the light-to-biomass conversion efficiency and harvesting systems as well.

Microalgae growth strongly requires light energy and CO<sub>2</sub> for photosynthetic production of biomass. Hence the design and use of effective cultivation system that allows light penetration and an efficient supply of CO<sub>2</sub> is an important requirement for the rapid cultivation of microalgae and their further commercial use. Microalgae can be cultivated generally in two different aquaculture systems, namely open pond and closed photobioreactors (PBR). The open ponds are the simplest systems, while closed (PBR) could be in form of plates or tubes. Microalgae receive sunlight directly through the transparent container walls or through light fibers or tubes that channel it from light collectors. As compared with open-air systems, there are distinct advantages to using closed systems, but technical challenges remain. Enclosed systems are advantageous for algae which are prone to contamination while open systems are suitable for robust microalgae, which can resist high pH or salinity when

cultivated e. g. in marine water. For most of these cultivation methods, the production processes are economically viable and technical improvements are still in progress. Recently there have been various approaches for cultivation and biomass harvesting developed. However, for effective and sustainable microalgae biomass production further development of more controllable, cost-efficient systems and manufacturing devices is required to maximize productivity, especially for large scale industrial use.

This study was performed within the EMFF funded project no. 20-00-F02201-000001 "Approbation of microalgae cultivation in closed aquaculture systems and evaluation of their efficiency in fish feed" supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

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## **Diatom-based assessment of the ecological status of the Venta River, Kuldīga (LV: Ventas upes ekoloģiskā stāvokļa novērtēšana Kuldīgas pilsētas teritorijā, izmantojot kramalģes kā bioindikatorus)**

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Changing land use, climate change, urbanization and population pressures on rivers is an important socio-ecological process that requires managements and biomonitoring. The Venta River at Kuldīga hosts the Ventas Rumba, the widest waterfall in Europe, a popular recreational site for tourists and local residents. The catchment includes residential areas with allotments, industrial areas and agricultural lands. Urbanization and intensive agricultural often have an observable impact on the river ecology, as increased pollution tends to interfere with physiological processes in living organisms, hence, causing pathologies which in turn can further reduce quality and expectancy of life at a societal cost. The monitoring of pollution in Venta is crucial for basing and timing of informed and appropriate municipal-level interventions for prevention and mitigation.

The aim of this study was to assess the ecological status of the River Venta at Kuldīga using diatoms as bioindicators and to determine diatom diversity in the studied part of the river. A total of six sampling sites were selected in a 10 km long stretch of the river in the territory of Kuldīga town. The average distance between sampling sites was 1.5 km.

A total of 112 species were identified in all samples. The highest species diversity of 69 species was observed in sample 2 (individual rarefaction calculated if exactly 500 diatoms valves were counted in all samples). The lowest species diversity was observed in sample 6 (46 species). The most similar in terms of species are samples 1 and 5. This is probably because both sites have similar physical characteristics that include sandy beaches with a slow current.

The most abundant diatoms were *Amphora pediculus* (Kützing) Grunow, *Cocconeis placentula* Ehrenberg, *Amphora lybica* Ehrenberg, *Navicula capitatoradiata* Germain ex Gasse, *Ulnaria ulna* (Nitzsch) Compère and *Sellaphora nigri* (De Notaris) Wetzel & Ector, which are commonly found in meso- to eutrophic waters. Diatom analysis suggests that the Venta might be at risk of eutrophication. This could be reduced by controlling the use of fertilisers on the surrounding agricultural lands. The pollution might also enter in the river from neighbouring allotments. To reduce the risk of pollution, it should be checked whether all allotments with summer houses are connected to the urban sewerage network.

For the most abundant diatom taxa, the susceptibility to pollution was assessed by III (medium), but there were also diatoms with IV and V (very sensitive to pollution). This shows that the Venta River in Kuldīga overall has low levels of pollution. All study sites were rated as good on the IPS index.

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## Melnā mušķērāja ligzdošanas laika izmaiņas Latvijā kopš 20. gs. vidus

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The research paper analyses breeding time changes of the Pied Flycatcher since 1950s. Data about breeding of the Pied Flycatcher in nest box plots near Garkalne were collected from 2019 to 2021 and compared to the data from 1950s (the same region).

The aim of the scientific paper – to find out, what changes can be seen in breeding time of the Pied Flycatcher.

The results – during the time period from 1948 till 1959 the average laying date of the first egg varies within 10 days – from May 23 till June 1. In 2019 the average laying date was May 21 – it is significantly earlier than in 9 analysed years of 1950s out of 12. In 2020 the average laying date was May 20 – it is significantly earlier than in 12 analysed years of 1950s out of 12. In 2021 the average laying date was May 21 – it is significantly earlier than in 9 analysed years of 1950s out of 12.

The main conclusions of the research – in 2019 the average laying date of the first egg is significantly earlier than in the middle of 20th century. Reasons of these changes are not fully understood. It would be important to collect more data in the following years to avoid the impact of weather conditions of one particular year and to make conclusions about changes in the breeding time of the Pied Flycatcher and its reasons.

**Key words:** climate change, *Ficedula hypoleuca*, phenology

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## Genetic Diversity of Common Carp *Cyprinus carpio* L. Strains Maintained in Lithuania

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Modern investigations into genetic diversity of common carp, *Cyprinus carpio* L. strains maintained in Lithuania started only recently. In total 43 fin clips from alive old carp reproducers representing 5 different strains, as well as 4 hybrid lines, kept in Šilavotas carp farm were collected in 2020. Initially, genetic diversity was evaluated based on sequencing of mtDNA D-loop of selected 20 specimens and genotyping of all 43 carp individuals using 11 DNA microsatellite loci. Newly created and tested primer pair Carp\_F1/Carp\_R1 for carp mtDNA targeting D-loop enabled amplification of ~900 bp fragments. Obtained DNA sequences that represented all 5 carp strains (Bubiai, Šilavotas, Germanian, Hungarian and Ukrainian) maintained in Šilavotas carp farm had identical mtDNA D-loop haplotype. Based on microsatellite data analysis, representatives of carp strains and hybrid lines introduced into Šilavotas carp farm at different periods of the second half of the 20th century (originating from Germany, Hungary, Czechoslovakia, Ukraine) possessed 3-6 private alleles unique to each carp strain and hybrid line. Representatives of an old Lithuanian carp strain Bubiai had the higher allelic diversity (37 different alleles) in comparison to the carps attributed to more recently developed Šilavotas strain possessing the lower allelic diversity (28 different alleles). Lithuanian Bubiai carp strain possesses unique allelic and genotypic composition in comparison to all other studied carp strains and hybrid lines indicating Bubiai strain as the most important genetic resource for conservation, maintenance, breeding and applications in selection programs.

**Keywords:** *Cyprinus carpio*, carp strains, genetic research, Lithuania

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## **Influence of peat substrate composition on indicators of physiological vitality of cloudberry *Rubus chamaemorus* during the rooting period**

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The growing of cloudberry in Latvia and globally is still done in rather small areas and harvests are mainly produced in wild stands, thus making cloudberry cultivation a promising research field. Berry plantations in extracted peat bogs are known to protect the upper peat layer from wind erosion and water loss, and to reduce GHG emissions. Production of high-quality berry yields contribute to the local economic development.

The aim of the study was to determine the most suitable substrate composition for successful rooting of cloudberry. A hermaphroditic cloudberry variety Nyby was vegetatively propagated by dividing rhizomes and then planted in 3 variations of peat substrate with different levels of acidity and nutrient supply. Variations were formed by mixing bare peat (K) with limed and fertilized peat substrate (M1) in the following proportions: 1) K, 2) 1K:1M1, 3) M1.

The survival and vitality of plants were recorded by measuring chlorophyll fluorescence activity (Fv/Fm), a complex fluorescence parameter (Performance Index), and the concentration of total chlorophyll in SPAD units, which indicate the overall physiological status of plants. Morphological parameters as number of young shoots, leaf amount and size were recorded.

First year results marked higher young shoot vitality and photosynthetic productivity of variant (K), which held the highest values of all three chlorophyll-related parameters ( $p < 0,05$ ). The lowest values for these indicators were recorded in substrate M1. The highest survival rates (number of shoots and leaves) were also observed in variant K, the lowest – in variant M1.

Although rooting of cloudberry in M1 was the least successful, in the second year of development the highest number of shoots per pot (on average 4.1), which described cloudberry productivity, corresponded to M1. There were no significant differences between the variants regarding the size of leaf blades in the first year. However, in the second year, the largest leaves also corresponded to M1 - on average 5.23 cm, as for the K1 variant - 4.59 cm.

Successful cloudberry rooting is essential for high survival rates of rhizomes and their ability to form young shoots and leaves. Results indicate that unfertilized peat (K) is most suitable for cloudberry rooting, as it ensured the highest survival and vitality of plants. However, in the second year, higher green mass production in M1 indicates the positive effect of fertilization on the rooted cloudberry in further development.

**Key words:** cloudberry, rooting, peat, shoot physiological status, chlorophyll fluorescence.

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## **Sugar-free fractionated red beetroot juice biological activity evaluation**

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In the case of type II diabetes, the most important preventive and therapeutic effect gives a diet with a minimal amount of easily digestible carbohydrates. Vegetable juices positioned as healthy food products, because of high content of phenolic and other biologically active compounds. However, due to the high glycemic index, juices contraindicated in diabetes, while juices with a reduced glycemic index are not available on the market.

We have developed a technology for the fractionation of red beetroot juice based on molecular weight using ultrafiltration. The resulting fraction can stimulate duodenal absorption of iron, increase blood hemoglobin level in iron deficiency anemia and enhance capillary blood flow more effectively than native juice do. Both effects are extremely important for patients with diabetes because the impaired blood supply to tissues and organs is an important pathogenetic factor in the development of diabetic renal failure, blindness, gangrene. The sugar content in fractionated beet root juice is 5-7%, which makes its use in diabetes problematic.

The purpose of the study was to develop a technology for removing sugar from fractionated red beetroot juice and assessing the safety of its functional properties.

The native red beet juice fractionated and fermented fractionated juices were studied. Fermentation was carried out using pre-activated yeast *Saccharomyces cerevisiae*. In experiment in vivo chickens were ingested once per os 0,17-0,35-0,70 mg of iron separately, as well as in combination with 1 ml of the studied juice. After 100 minutes, the iron content in blood, duodenal mucosa, liver, and spleen was determined. The balance of trace elements in chickens' body was evaluated by the iron quantity obtained with diet and excreted with droppings for 3 days. According to the same scheme, a similar experiment was conducted on laboratory rats with experimental iron deficiency anemia, in which the exposure time was 3 days.

It was found that after 5-day fermentation, the sugar content in the native and fractionated juice fell to 0.5-0.7%. Fermentation did not affect the ability of both types of juice to stimulate intestinal absorption of iron and its concentration in blood. However, hemoglobin level and the total retention of trace elements in chickens body administered fermented juice have decreased due to reduction of accumulation in liver. In rats with alimentary iron deficiency, the dynamics of indicators of trace element metabolism did not depend on the fact of juice fermentation. The described phenomenon requires a deeper study.

Key words: beetroot juice, sugar, iron, diabetes

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## The role of natural dietary antioxidants in animals under oxidative stress

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The harmful effect of the most dangerous environmental heavy metal cadmium (Cd) is accompanied by an antioxidant-prooxidant imbalance in animal organ and tissues. Cd catalyses the formation of reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide in cell membranes following the oxidative stress and the risk of developing metabolic disorders and diseases. The protective action of nutrients with antioxidative activities in chickens exposed to Cd was investigated. As antioxidant dietary supplements the salts of zinc (ZnCl<sub>2</sub>) and selenium (Na<sub>2</sub>SeO<sub>2</sub>), and vitamin C (ascorbic acid) were used in the experiments with chickens administered orally 100 mcg of Cd (water solution of CdCl<sub>2</sub>). The antioxidative effect of developed new natural innovative product from red beetroot (fractionated juice) was studied in additional experiment, when chickens exposed to Cd with diet (50 mg /kg). At the end of the experiments the analyses of minerals (trace elements), oxidative stress indices in blood or organs, and parameters of humoral immunity (serum lysozyme and nonspecific circulating complexes) have been undertaken. The results of analyses in Cd-exposed chickens demonstrated the prominent increase of Cd concentration in blood serum, followed by rise of oxidative processes activity as evidenced the increase of malondialdehyde (MDA) and the decrease of antioxidant enzyme glutathione peroxidase (GSH-Px) levels. Suppressive Cd effect also reflected in the change of investigated humoral immune indices. All the experimental antioxidative nutrients demonstrated preventive effect against harmful Cd action. In the first experiment the most pronounced protective action manifested Zn supplement. This effect may be

due to synergistic and antagonistic interactions between Cd and Zn at the molecular level. Se and ascorbic acid supplemented in the diet also caused an improvement in the parameters indicating the harmful effects of Cd. It resulted in the decrease of Cd accumulation, balancing the indicators of oxidative processes and immunomodulating effect. The results of the additional experiment showed that administration of fractionated red beetroot juice to Cd-exposed chickens prevented prooxidative impact of this heavy metal in chickens. This effect provided due to the presence of betalain pigments with antioxidative capacities in fractionated beetroot juice.

Experimental results demonstrated the prospect of preventive role of Zn, Se, ascorbic acid and beetroot fractionated juice in the improvement of Cd-induced disorders in the body in case of environmental pollution with heavy metals.

Key words: cadmium, oxidative stress, dietary antioxidants

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## Molecular Typing of *Paenibacillus* larvae Isolates from Lithuanian Apiaries by using VNTR Allele Sizing Method

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*Paenibacillus* larvae is a Gram-positive bacterium which is known as the causative agent of the American foulbrood (AFB), a highly contagious and fatal, widespread disease of honeybees. There are known four main bacteria genotypes classified as ERIC I - ERIC IV and recently detected ERIC V genotype. ERIC (enterobacterial repetitive intergenic consensus) genotypes differ in their virulence and prevalence in honeybee colonies. In this study we used 108 independent *P. larvae* isolates collected from different geographical regions of Lithuania in the last decade. Bacterial samples were investigated for genetic diversity using multiple locus variable number of tandem repeat analysis (MLVA). For MLVA five primers pairs representing different gene loci were used in multiplex PCR, and analysed by capillary electrophoresis (QIAXcel system). The aim of the study was by using MLVA method detect which ERIC genotypes are most common in Lithuania apiaries, identify and differentiate subtypes of defined genotype, and evaluate the ability of the QIAXcel Advanced System, using capillary gel electrophoresis, to be used as an automated improvement to the gel electrophoresis technology for sizing of *P. larvae* VNTR products. Molecular analysis data showed that 100 % of *P. larvae* bacterial isolates from Lithuania represent the ERIC I genotype, and capillary electrophoresis results did let us to differ *P. larvae* strains into 9 subtypes according to different length of VNTR.

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## Molecular identification of protozoan *Sarcocystis* species in sheep from Lithuania

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Representatives of the genus *Sarcocystis* (Apicomplexa: Sarcocystidae) are parasitic protozoa broadly distributed in mammals, birds, and reptiles. They are distinguished by a two-host life cycle based on the prey-predator relationships. Sarcocysts are mainly formed in muscles or CNS of the intermediate host, and sporocysts develop in small intestine of definitive host. The intense *Sarcocystis* infection can result in reduced wool and meat production of sheep. *Sarcocystis* species differs in their pathogenicity, therefore it is essential to accurately identify species of these parasites. Usually,

Sarcocystis species diagnosis is based on sarcocysts isolation and genetic characterization. However, such approach is not effective in epidemiological studies. The aim of the study was to develop a rapid and efficient molecular methodology for the identification of Sarcocystis species in sheep meat. Diaphragm and oesophageal samples from 40 sheep raised in different Lithuanian regions were examined for Sarcocystis spp. About 25 g of muscle samples were digested with pepsin. Sarcocystis species were identified using species-specific nested PCR targeting cox1 and sequencing. The prevalence of Sarcocystis infection was very high (40/40 diaphragm and 39/40 oesophageal samples were positive). Out of five Sarcocystis species tested, in analysed samples *S. tenella* and *S. arieticanis* were confirmed. This is the first study to identify Sarcocystis species in sheep from Lithuania. *Sarcocystis tenella* (n=39 in the diaphragm; n=34 in the oesophagus) was detected slightly more frequently than *S. arieticanis* (n=36 in the diaphragm; n=30 in the oesophagus). The species found in Lithuania are distributed via canids which are common in the studied area. It should be noted that detected species are more pathogenic compared to those transmitted via felids and opportunistic birds. The molecular diagnostics technique suggested in this work can be applied examining other economically important animals.

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## NEW TOOL SUITABLE FOR GENETIC STUDIES – AXENIC LEMNA MINOR LINE

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Common duckweed (*Lemna minor* L.) is widely used for phytoremediation and environment monitoring of wastewater in vivo as well plants could be efficiently grown in Petri dishes and quickly multiplied in laboratory conditions. In this work we used in vivo collected *L. minor* clones, being inhabited with bacteria and algae naturally co-existing in tissues of fronds and roots, to establish laboratory axenic line free from the symbionts and having 2n chromosome number as these features of model system are essential for getting reproducible results of the experiments.

Totally 29 lines of *L. minor* previously collected in different locations and river's basins in Latvia and Lithuania were examined by flow cytometry for further use in experiments devoted to obtaining measurements of the impact of different environmental factors. Line entitled Sta2 confirmed desired properties including 2n chromosome number (ploidy). Based on elimination of any attached symbionts the two-step purification procedure of the selected line was performed for establishing of experimental line suitable for genetic studies. Efficiency of purification was checked by confocal laser scanning microscopy.

As the result developed axenic line of *Lemna minor* L. containing 2n ploidy and free from any symbiotic bacteria or algae for studies of genetic diversity and variations caused by biotic or abiotic factors can be more precise in comparison with results obtained by using *L. minor* samples collected in vivo.

**Keywords:** common duckweed, flow cytometry, confocal laser scanning microscopy, axenic laboratory line

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## Evaluation of phytochemical composition and cytotoxic activity of wild grown and cultivated *Daphne mezereum* extracts: potential of cultivation of the species

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**Background.** *Daphne mezereum* is a perennial, deciduous shrub that belongs to the *Thymelaeaceae* family. Based on Latvian ethnobotanical knowledge as well as current practices, *D. mezereum* has been used for digestive and skin problems, especially for toothache and tooth extraction. The content of biologically active components in plants is significantly affected by growing conditions, year, and genotype. It is important to analyse plant phytochemicals to find the best cultivation conditions for commercial purposes.

**Aim.** The aim of this study was to investigate the phytochemical composition and cytotoxic activity of extracts of *D. mezereum* collected in Latvia to analyse the domestication potential of wild populations of *D. mezereum* in organic farming.

**Methods.** Plant parts for chemical analysis and plantlets for field trials were collected from three wild-grown *mezereum* populations DAF01 (Rāmuļi), DAF02 (Jaunalūksne), and DAF10 (Dzērbenes distr.). Plantlets in 2019 were planted in an organically certified experimental field. Due to the very slow development of *D. mezereum* under field conditions, it was possible to collect the sample for chemical analysis only from the one-field-grown population. The ethanol and methanol extracts were prepared from twigs, fruits, leaves, roots and bark of wild-grown and cultivated specimens. LC-MS methods were used to determine the phytochemical composition and terpenoid content in *D. mezereum* extracts. The cytotoxic activity of *D. mezereum* extract was determined in myoblast (C2C12), fibroblast (3T3), and neuroblast (Neuro-2a) cell lines using the MTT assay.

**Results.** The main compounds identified in *D. mezereum* extracts were terpenoids, coumarin and flavonoid derivatives, and a slight amount of flavones. Gniditrin, daphnetoxin, and exocariatoxin were the dominant terpenoids in *D. mezereum* extracts. The content of terpenoids in *D. mezereum* twigs and bark was significantly higher than that in fruits, leaves and roots. According to the cytotoxic activity results (IC<sub>50</sub>), *D. mezereum* extract was more effective in the Neuro-2a (68.7 ± 1.6 µg/mL) than in the C2C12 (139.3 ± 1.14 µg/mL) and 3T3 (140.8 ± 1.17 µg/mL) cell lines.

**Conclusion.** Although commercial cultivation of tested populations of *D. mezereum* is not promising due to the slow adaptation and development of plants, both wild-grown and cultivated *D. mezereum* are rich sources of diterpenoid constituents and possess selective toxicity to different cell types.

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## Study of the antioxidants and nutrients in cloudberry (*Rubus chamaemorus* L.) in Latvia

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Cloudberry (*Rubus chamaemorus* L.) is a species of the boreal zone with a distribution areal in the northern hemisphere and the Latvian cloudberry localities are close to the southern distribution area of the species in Europe. It is an economically important plant that is already cultivated in the Fennoscandia. Fresh cloudberry fruits and compounds derived from fruits and leaves contain several health-promoting substances: vitamins, flavonoids and phenolic acids with antioxidant properties (tannins, flavone, quercetin, naringenin) The most valuable components are those with antioxidant

properties, such as ascorbic acid, carotenoids, and polyphenolic compounds. The aim of the study is to create and analyse a collection of cloudberry samples representing Latvian population. Processed cloudberry seeds will be further used as breeding material for establishment of agricultural culture adapted to regional climatic and ground conditions.

Cloudberry samples from eight deposits in Latvia were analyzed. Presence of antioxidants including ascorbic acid,  $\beta$ -carotene, xanthophylls and total phenols were determined in the berries. Macroelements - N, P, K, Ca, Mg, S and microelements - Fe, Mn, Zn, Cu, Mo, B were determined in the leaves. Level of all detected antioxidants found in berries harvested in Baltais and Zaļezers bogs was higher in comparison to samples representing Nītaure, and Lielais un Pemme bogs. Highest content of all identified macro- and microelements was found in the leaves harvested in Lauga and Pelečāre mires.

The study was funded by the project "Evaluation of the cloudberry (*Rubus chamaemorus* L.) genetic resources of Latvia and Belarus as a background for the breeding program and conservation" (2019 – 2021).

**Key words:** cloudberry, antioxidants, phenolic compounds, macro- and microelements