

The International Conference
of Young Scientists

**YOUNG SCIENTISTS
FOR ADVANCE
OF AGRICULTURE**

abstracts

2016



FOREWORD

The Division of Agricultural and Forestry Sciences of the Lithuanian Academy of Sciences is organizing the 5th Conference 'Young Scientists for Advance of Agriculture'. The aim of this traditional conference is to rally young researchers working in the fields of agronomy, forestry science, animal science and veterinary medicine, agricultural engineering, food safety and quality, agrarian economics and rural sociology, and to encourage their cooperation and the dispersion of the results of their research. This year it is an international conference the objective of which is to promote international collaboration of young scientists and exchange of up-to-date research results achieved by Lithuanian and foreign scientists.

Participants of the conference are junior research fellows, doctoral students, lecturers, and master's students engaged in scientific investigation at research and educational institutions of Lithuania, Latvia, Estonia, and Poland. This publication contains abstracts of the forty papers delivered at the conference. They are grouped by the sections of the conference and by the order of their presentation.

We hope that the conference will not only facilitate informing colleagues and the general public on the latest fundamental and applied research but will consolidate relations between research and educational institutions of different countries and spark interest of specialists in agriculture and business entities that have expressed considerable interest in the conference and supported its organization. We hope that the conference will play at least some role in generating more recommendations of better quality for the increase of competitiveness in agricultural production and business, and for the enhancement of environmental protection.

We are grateful to the conference participants, partners, and sponsors, and to everybody who has contributed to the organization of the conference and preparation of this publication.

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THE EFFECTS OF DIFFERENT FERTILIZATION ON SOIL WATER STABLE AGGREGATE STABILITY

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Agriculture is going through a modernization process where more and more emphasis is placed on soil as a valuable plant growth medium which can be either improved or deteriorate based on the land management. Nowadays farmers are not only focusing on short-term high yields but also interested in soil sustainability for a high profit in the long-term perspective. Therefore, in order to receive a better overview of the soil condition, regular soil analyses are carried out to determine soil acidity and nutrient contents. These are chemical analyses and by solely depending on them the expected yield might not meet expectations, because in many cases physical properties of the soil are not considered. One important physical parameter of soil that determines water retention and aeration is soil structure which consists of different size aggregates. Studies have shown that soil water stable aggregate stability (WAS) is significantly influenced by the use of farmyard manure (FYM) and the sole use of mineral fertilizers (MF) is not enough to maintain a decent WAS, especially if plant residues are removed. However, for many various reasons farmers – especially those in precision farming – are using continually less FYM and increasingly more MF. To get a better understanding of how FYM and MF with different rates affect the WAS in the climatic conditions of the Baltic countries, this study on barley and potato was carried out in Estonia, near Tartu, on a long-term (established 1989) nitrogen fertilizer (IOSDV) experiment in 2014–2015. The WAS of 0–2 mm soil fractions was determined by Eijkelkamp's wet sieving apparatus. This study found that: (1) the use of FYM increased significantly the WAS and had a positive impact on soil organic matter and pH; (2) barley is a more soil-friendly culture than potato; (3) the WAS of permanent grassland was constant, but in barley and potato varied considerably on both years, and (4) higher rates of MF caused a decrease in the WAS.

ALUMINIUM FRACTIONATION IN ACID AND LIMED RETISOL PROFILES

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Acid soils have a high proportion of Al in the exchange complex and are usually amended with lime to reduce the toxic effect of exchangeable Al. We studied how liming materials affect chemical parameters in Retisol (WRB, 2014), mainly focusing on the aluminium forms in solid fractions in the various horizons of acid soil profiles in Western Lithuania.

The profiles were conducted on plots of cultivated naturally acid and long-term limed (by 2 rates every 3–4 years) soils. Aluminium was extracted from the solid phase with the following solutions: ammonium oxalate (Al_o – non-crystalline Al), sodium pyrophosphate (Al_p – organically bound Al), copper chloride (Al_{cu} – organo-Al complexes of low and medium stability), lanthanum chloride (Al_{la} – the most labile organo-Al complexes), and ammonium chloride (Al_{NH_4} – exchangeable Al). Aluminium in the extracts was determined by atomic absorption spectroscopy.

It was revealed that in various horizons of acid and limed soil profiles, organo-aluminium complexes predominated over inorganic compounds of low crystallinity (Al_p always represented more than 55% of the Al_o). The largest ($239.80 \text{ mg kg}^{-1}$) concentration of toxic exchangeable Al was found in the acid soil profile of Ahp horizon, meanwhile in the limed soil profile it makes only 1.95 mg kg^{-1} . Organically bound Al concentration decreased in the limed soil profile. Liming replaces qualitative Al complexes with organic matter composition: it increases the organo-Al complexes of low and medium stability in down layers, but also reduces organo-Al complexes of high stability and labile organo-Al complexes in upper layers. It has been found that liming changed aluminium chemistry (compounds) to the whole profile of acid soil.

THE EFFECT OF A MYCORRHIZAL INOCULUM ON THE LEAF GREENNESS INDEX AND THE YIELD OF TOMATO PLANTS (*LYCOPERSICON ESCULENTUM* MILL.) GROWN IN A HEATED PLASTIC TUNNEL

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In an experiment conducted in 2012–2013, tomatoes were grown in coco coir inoculated with mycorrhizal fungi, in a heated plastic tunnel in the garden of the Research and Experimental Station of the University of Warmia and Mazury in Olsztyn. The experimental materials comprised seedlings of three tomato cultivars, 'Torero F₁', 'Growdena F₁' and 'Listell F₁', supplied by a horticultural farm. The second experimental factor was inoculation of the plant growth medium with endomycorrhizal fungi of the genus *Glomus*. The aim of this study was to determine the chlorophyll content of leaves of three tomato cultivars grown in coco coir inoculated with mycorrhizal fungi. Each treatment consisted of seven coco coir mats (100 x 20 x 7.5 cm) with four tomato plants grown in each mat, pruned to produce 23 clusters. The experiment was carried out in triplicate. Seedlings planted in rock wool cubes were initially placed next to openings made in coir mats. Immediately before planting, each seedling was supplied with 10 ml of a standard working solution containing mycorrhizal fungi. The mycorrhizal inoculum was applied directly to the coco coir. Mycorrhizal fungi were not used in the control treatment. The results showed that in most treatments, SPAD readings recorded in the fall were lower than in the summer, and the noted values were similar to those determined at the beginning of the growing season. The mycorrhizal inoculum improved the nutritional status of tomato plants that was determined on the basis of the leaf greenness index. The mycorrhizal inoculum increased the yields of all analyzed tomato cultivars.

LATVIAN MICRO AND SMALL ENTERPRISES IN RURAL AREAS: POSSIBILITIES FOR FUTURE DEVELOPMENT

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A vast majority of enterprises in Latvia are micro and small enterprises shaping the backbone of Latvian economy, providing jobs, social value and contribution to the state budget. However, as the tendencies of urbanisation strengthen and technological progress allows employing less workforce in agriculture, forestry and mining, and as data show that one person is needed for every 100 ha, what is the future of Latvian countryside? Even more, as Latvia is a state with a low population density and only Riga and the Riga region can ensure enough human knowledge capital to provide export of services both internationally and within the state, how can a rural area be developed and adjusted to the needs of a knowledge society? Research shows that optimal development of a rural area is ensured by its proximity to 'successful' cities and towns, such as Riga, Valmiera, Saldus, Broceni, Smiltene, and Dobele. Closeness to the city alone does not guarantee the development of a rural area as is shown by the example of 'non-successful' towns: Ludza, Balvi, Aluksne, Gulbene, and Kuldīga. Another key issue of development is diversity: the Riga region with diverse occupation areas provides a higher income level than the Vidzeme region with mainly agricultural enterprises. In the case of micro and small enterprises, one fact must be taken into consideration: some of them have growth potential and can transform into high-added-value enterprises, but others can maintain their equilibrium by providing livelihood and social stability in the region. These groups of enterprises must be dealt with differently and their development strategies differ greatly.





IDENTIFICATION OF GENETIC MARKERS FOR FREEZING TOLERANCE IN PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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Perennial ryegrass (*Lolium perenne* L.) is a key species for modern pasture agriculture. Despite its many superior properties, perennial ryegrass is sensitive to abiotic stresses and exhibits poor winter survival under harsh winters. Freezing tolerance is recognized as a major component of winter hardiness, although until now only a few quantitative trait loci for freezing tolerance have been detected in perennial ryegrass. Thus the genetic mechanism underlying this trait is still to be fully revealed.

We have conducted marker-trait association studies for freezing tolerance trait in perennial ryegrass using two different approaches: the candidate gene *LpIR1* allele sequencing in individual plants, and genome-wide association study (GWAS) in perennial ryegrass populations. In addition, a high-throughput screening method for freezing tolerance at the population level had to be developed.

From our phenotypic data analysis it is obvious that tetraploid perennial ryegrass populations are inferior in freezing tolerance as compared to the diploid ones. Furthermore, there is an indication that the natural genetic diversity for freezing tolerance might be almost totally exploited in some of the existing cultivars. Candidate gene association analysis revealed two non-synonymous *LpIR1* gene SNPs being associated with a decreased cell membrane stability under the freezing conditions. A GWAS for a freezing tolerance trait was conducted in 122 diploid perennial ryegrass populations and yielded eight markers where seven of them were located within the exons of the genes, scaffold-mapped ESTs, or in close proximity to the predicted genes.

IDENTIFICATION OF NEW ALLELIC VARIANTS IN WINTER WHEAT HEAT SHOCK PROTEINS

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Common wheat (*Triticum aestivum* L.) is a worldwide agricultural crop and plays an important role in the human diet. However, wheat grain production is being challenged by environmental stress conditions such as pests, diseases, drought, or extreme temperatures, for example, sub-zero temperatures in the northern latitudes. A better understanding of the mechanisms involved in cold acclimation may lead to genetic germplasm enhancement for freezing tolerance. The aim of this study was to identify mutations in genes associated with cold acclimation.

Mutagenized population of two winter wheat cultivars ('Kena DS' and 'Gaja DS') were developed using ethyl methane sulfonate (EMS) mutagen. Targeting Induced Local Lesions in Genomes (TILLING) method was used to create point mutations in target genes. Two heat shock protein genes (*HSP90A*; *HSP90B*) were chosen for new allelic variants detection by High Resolution Melting (HRM) analysis with DNA specific primers in winter wheat TILLING population of generation M₂. A total of 306.2 kb and 227.7 kb of genomic DNA was screened for *HSP90A* and *HSP90B* gene, respectively. One missense mutation (P3212L) of *HSP90A* gene in exon 3 was identified and three missense mutations (T135M; L127F; R126C) and one silent mutation (L158L) of *HSP90B* gene in exon 2 were detected.

Plants with new allelic variants were propagated to generation M₄ in order to validate mutation phenotypes. We found that one missense mutation (P321L) in *HSP90A* gene and all three missense mutations (T135M; L127F; R126C) of *HSP90B* gene were present in generation M₄. Plants with identified mutations will be chosen for further study to verify the effect of these mutations on freezing tolerance in winter wheat.

TETRAPLOID INDUCTION IN ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* SSP. *MULTIFLORUM*)

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Annual ryegrass (*Lolium multiflorum* ssp. *multiflorum*) is a leafy, highly tillering grass of high palatability and digestibility, which also sets seeds in the year of sowing. The creation of novel traits in existing plants can be achieved by genetic manipulations and modifications, making plant variability and the advantages associated with these variability harnessed for more agronomical purposes.

Plant polyploidization is one of the trends utilized in plant breeding to phenotypically produce plants with distinct traits such as broader leaves and increased yields, which in turn increase their agronomic and economic value.

An efficient method for inducing tetraploids from diploid *Lolium multiflorum* ssp. *multiflorum* cultivars ($2n = 2x = 14$) *in vitro* can be achieved using colchicine and amyprophos methyl (APM). Both compounds inhibit the polymerization of microtubule thus arresting mitosis at the metaphase stage, thereby preventing the duplicated chromosomes from separating into the daughter cells.

The embryos excised from diploid seeds were germinated and subsequently treated with different concentrations of colchicine and amyprophos methyl for different time intervals. The treated plants were cultured in test-tubes and transferred to soil for plant development. Flow cytometry and chromosome counting at the root-tip confirmed the induction of tetraploids ($2n = 4x = 28$) from the diploid cultivars

Results showed that both colchicine and amyprophos methyl were capable of inducing tetraploids. However, the optimal tetraploid induction from diploid cultivars was achieved by using colchicine with a treatment concentration of $10 \times 10^3 \mu\text{M}$ and $8 \times 10^3 \mu\text{M}$ for 3 hours and cultured at 24°C . Amyprophos methyl (APM) had a high induction efficiency; however there is a relatively low survival rate of the treated plants.

EXOGENOUS PROLINE AS A TOOL FOR WINTER RAPESEED (*BRASSICA NAPUS* L.) FROST RESISTANCE

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Nature Research Centre

Under stressful conditions plants accumulate metabolites, particularly amino acids such as proline. The accumulation of these plastic and preventive substances changes the structure and characteristics of protoplasm.

The aim of the work was to test exogenous proline as a tool for the optimization of winter rapeseed frost resistance. The influence of exogenous proline for adaptation to cold of this plant was investigated under controlled laboratory conditions.

The seedlings of the winter rapeseed cultivar 'Hornet' were grown under photoperiod 16/8 h (day/night) and temperature of 18°C . At the three-leaf stage, plants were treated with L-proline 1 mM water solution. After this pretreatment seedlings were held at -1°C , -3°C , -5°C and -7°C for 24 h. Then seedlings were transferred into a plant growing chamber at the temperature of 18°C for 7 days. Survived plants were estimated. Free proline content was determined in leaves of seedlings after all freezing temperatures using the method described by Bates et al.

Evaluation of the effect of exogenous proline on rape freezing resistance at the temperature of -1°C revealed that all experimental seedlings – those treated with tested regulator and control seedlings – are resistant to cold. At a lower temperature (-3°C and -5°C) under the impact of L-proline, seedlings survival compared with control increased by 28% and 11%, respectively. The freezing temperature -7°C is deadly for rapeseed. Our study showed that L-proline produced a positive effect on free proline content. Pretreatment with proline at intensified freezing temperature (from -1°C to -5°C) influenced free proline accumulation and increased plant freezing resistance.

Thus free proline is involved in the response to cold stress and its level may be an indicator of cold-hardening and freezing tolerance.



EFFECT OF DIFFERENT TYPES OF BIODEGRADABLE WASTE COMPOST ON THE YIELD AND QUALITY OF WINTER WHEAT AND SPRING BARLEY

Ieva Narutytė

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Many countries (including Lithuania) practice composting of biodegradable waste. Composts have been used for fertilisation in agriculture for many years, yet there is still a need for more research on relationships between the different quality characteristics, quality characteristics as totality, and especially contamination of different types of biodegradable waste compost.

The experiment is carried out at the Elmininkai Experimental Station in the Anykščiai district, Lithuania. Design of the experiment: (1) not treated; (2) treated with green waste compost (leaves, grass, plant parts); (3) treated with green waste compost and mineral fertilisers; (4) treated with farm manure; (5) treated with farmyard manure and mineral fertilisers; (6) treated with biogas production waste compost; (7) treated with biogas production waste compost and mineral fertilisers; (8) treated with mineral fertilisers (winter wheat – $N_{90}P_{60}K_{90}$, spring barley – $N_{60}P_{40}K_{60}$); (9) treated with sewage sludge compost, and (10) treated with sewage sludge compost (matured using straw or leaves) and mineral fertilisers. Winter wheat and spring barley grain yield ($t\ ha^{-1}$) was re-calculated and expressed as the yield of 15% moisture content absolutely clean grain.

During the first year after compost incorporation, winter wheat was cultivated. Statistically, grain and straw yield did not increase significantly in the applications not fertilized with mineral fertilizers; however, during the second year all composts applied increased spring barley yield. As a result of the compost effect, barley grain yield increased as follows: affected by biogas production waste – 72.9%, green waste – 68.6%, farmyard animal manure – 58.9%, and sewage sludge – 45.2%; straw yield increased by 39.9, 36.8, 36.3 and 52.3%, respectively. Mineral fertilizers increased the winter wheat yield by 29.5% and that of spring barley next year – by 47.8% compared with zero fertilizer application.

PERSPECTIVES OF GROWING SOY IN ORGANIC AGROECOSYSTEMS

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Since Lithuanian climate is expected to get better for plants from warm regions and new improved soy strains have been established, it became important to carry out new investigations of soy. Former research into soy were carried out from 1926 and some singular experiments were made from time to time. However, soy was not adapted widely to the Lithuanian agricultural system and comprehensive studies are still needed.

For this purpose, new experimental plots of land were allocated in Dotnuva in 2015. The main objectives of this study were (1) to investigate soy cultivation properties and (2) to explore its nitrogen resort in organic farming agroecosystems. This study presents two years' research data. Climatic conditions varied significantly during the last two years. Soybean crops were able to produce available yield both years. However, soy was less productive in 2015, when the lack of moisture during the summer was one of the main limiting factors. The accumulated aboveground biomass was twice bigger during the warm and comparable moist summer in 2016. Nitrogen generation to the agroecosystem was better in 2016, too. The success of inoculation depended on climatic conditions during the first period of soy growing. Nodulation efficiency was significantly higher when avoiding drought stress. Technological properties were less but still an important factor for harvest formation, compared with climate conditions. Some features that improved the amount of yield were soy inoculation, smaller row spacing (25 cm vs. 50 cm), and different sowing time.

To summarize, soybean crops could be successfully introduced to Lithuanian organic farming systems. Different cultivation technologies could increase soybean harvest and improve accumulated nitrogen supply to the agroecosystem. Nevertheless, yearly climatic conditions play the key role for appropriate yield generation. This proves that further studies are needed. Forthcoming research on this study will provide perennial data. Comprehensive studies in Dotnuva have already analysed different new soybean strains, the use of soy in different crop rotation systems, soy inoculation efficiency etc. Based on all studies, practical recommendations will be prepared in the future.





CHANGES IN SPRING BARLEY HARVEST WITH DIFFERENT ORGANIC FERTILIZERS

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The most prevalent soil in South-East Lithuania is low productive with low level of humus (organic carbon amount). In intensive farming, these factors create low agronomic value. Recommended for such farms are sustainable agricultural measures that help to create conditions that are closer to high-performance soils and a choice of fertilizers that help to stop processes of soil erosion. There has already been much research into organic fertilizers (manure, sapropel) in assessing the effectiveness of soil and plant agronomic condition. Organic fertilizers gradually release nutritious substances that help to reduce the quantity of fertilizer insertion during the growing period and reduce the amount of biogenic elements in the leaching losses into the deeper layers of the soil. The naturalization process is currently at the peak and is supported by the state. Growing numbers of large intensive farms consciously choose sustainable soil farming. The experiment was set up at the Vokė Branch of the Lithuanian Research Centre for Agriculture and Forestry (LRCAF) in 2016. Lysimeters were placed in loam and in sandy loam *Haplic Luvisol* soil. Each soil was represented by three replications, which monitored and evaluated leaching tendencies of biogenic elements in four-option plots with one control (no fertilizer). The main aim was to examine and evaluate the effects of organic fertilizers (Provita, phosphorite powder, potassium magnesia (NPC sources) ($N_{60}P_{60}K_{60}$), sapropel, and manure) in the plots and the quality of agricultural harvest. Sandy loam: 0.2 m thick, pH – 6.3, humus – 1.45–2.05%, mobile phosphorus (P_2O_5) and potassium (K_2O) – 208–244 mg kg^{-1} and 90–140 mg kg^{-1} , calcium (Ca) 1321–1874 mg kg^{-1} , magnesium (Mg) 212–261 mg kg^{-1} . Light-loamy: 0.2 m thick, pH 5.0–5.2, humus 1.81–1.98%, mobile phosphorus (P_2O_5) and potassium (K_2O) 203–214 mg kg^{-1} and 152–171 mg kg^{-1} , calcium (Ca) 837–913 mg kg^{-1} , magnesium (Mg) 127–141 mg kg^{-1} .

The spring barley grain harvest was mainly determined by organic fertilizers. Significant and visually observed increase in crops was in light loam plots (heavier plants, more productive stems). The yield of barley increased four times in sandy loam plots with NPK fertilizers (0.678–2.425 t ha^{-1}) and 3.5 times in the plot with manure (0.678–2.034 t ha^{-1}). In the light loam, grain yield basically did not differ (1.446–2.02 t ha^{-1}). A more significant increase in

barley yield was observed in the plot with manure – it increased by 0.5 times. Indicators of secondary products of spring barley (straw yield, the number of productive stems, 1000 cereal weight, the number of grains per ear, the length of the barley ear) in sandy loam and light loam soils were affected by manure. In two types of soils fertilized with manure its benefits were obvious. Spring barley produced more: (1) productive stems (from 436 to 456 units in 1 m^2 sandy loam and from 480 to 568 units in 1 m^2 light loam); (2) weight of 1000 grains (33.3–37.8 g sandy loam and light loam 40.2–44.2 g); (3) straw (from 1.927 to 2.892 t ha^{-1} in sandy loam, and from 1.397 to 2.35 t ha^{-1} in light loam); (4) a longer ear (3.2–3.6 cm in sandy loam, and 3.5–4.8 cm in light loam); (5) the number of grains per ear (from 9.4 to 11 in sandy loam, and from 12.1 to 16.1 in light loam).



THE INCIDENCE AND SEVERITY OF VERTICILLIUM WILT (*VERTICILLIUM* SPP.) ON WINTER AND SPRING OILSEED RAPE

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In Lithuania, winter and spring oilseed rape (*Brassica napus* L.) are the second most widely grown crops after winter wheat. The demand for oilseed rape products is increasing constantly, yet the productivity of rapeseeds is limited by various fungal diseases. Phoma stem cancer, alternaria black spot, sclerotinia stem rot, and Verticillium wilt are common and challenging diseases of oilseed rape. The control of those diseases is based on application of fungicides, except for Verticillium wilt. Verticillium wilt is a relatively new disease in Lithuania. Since 2008, when the first severe outbreak of the disease was recorded, Verticillium wilt has become a threat for oilseed rape production. The objective of the present work was to document the incidence and severity of Verticillium wilt on oilseed rape. The experiment design was a split plot in 4 replication with 2 plots (artificially infected soil with *Verticillium* spp. versus naturally occurring). All field experiments were carried out over one growing season (2015/2016) at the experimental fields of the Department of Plant Pathology and Protection, Institute of Agriculture, Lithuanian Research Center for Agriculture and Forestry.

BACTERIAL DIVERSITY IN SOIL UNDER DIFFERENT TILLAGE SYSTEMS

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Microbial diversity is one of the main factors that affects soil viability. Tillage also affects soil properties. Although the number of microbial species in soil is still being debated, the metagenomic approach to estimate microbial diversity predicted about 10^3 – 10^7 bacterial genomes in 1 g of soil. Despite the key role of bacteria in soil processes, there is still lack of information about bacterial diversity of soils as affected by different tillage systems. This study focused on metagenomic analysis of bacterial diversity in long-term experimental plots of *Dystric Epihypogleyic Albeluvisols* in the western part of Lithuania.

The experiment had a split-plot design where the whole-plot treatments were laid out in a randomized design with three replicates. The whole-plot treatments consisted of two tillage methods – deep ploughing (22–25 cm) and ploughless tillage (7–10 cm). The experiment field was fertilized with farmyard manure (40 t ha⁻¹). Three subsamples (0–20 cm) were collected on 22 October 2015. Genomic DNA from soil sample was extracted from approximately 200 mg field-moist soil by using the D6005 Fungal/Bacterial Miniprep set (Zymo Research®) following the manufacturer's instructions. To determine bacterial diversity and community composition, we employed a culture-independent approach of high-throughput pyrosequencing of the 16S rRNA gene. Metagenomic sequencing was made with Illumina MiSeq platform in Base Clear Company.

Our study was a preliminary attempt at observing bacterial diversity in soil under two common but contrasting tillage practices. The number of sequenced reads obtained for deep ploughing (154 904) was higher than in ploughless tillage (131 650). But the indices of microbial biological diversity were opposite: the values were higher in the ploughless tillage than in deep ploughing. These results lead to the conclusion that in deep ploughing we have few main dominant species, meanwhile in ploughless tillage there is major microbial diversity.



ENVIRONMENTAL RISK ASSESSMENT OF COMPOST PREPARED FROM DIFFERENT KIND OF BIODEGRADABLE WASTE

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Compost is an organic matter that has been decomposed and recycled as a fertilizer and soil improver. Composting is the most popular way of treatment of biodegradable waste. The result of the composting process is a new product which has micro and macro elements, organic matter, and other nutrients for plants and soil. On the other hand, depending on the origin, the compost produced may contain not only nutrients but also pollutants. Contamination of compost could be divided into two groups: inorganic (heavy metals) and organic (PAHs, PCBs and other) contamination. The aim of this research is to investigate contamination of composts with green waste (GWC), sewage sludge (SSC), mixed municipal waste (MMWC), and mixed municipal waste after mechanical biological separation (MMWAMBSC) and to evaluate the risk of using polluted composts. In my research, concentrations of seven heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) were determined and concentration of organic contaminants (PAHs, PCBs) were investigated. Depending on the materials used, the compost obtained contains higher or lower levels of contaminants. A comparison of two years' results shows that determined amounts of heavy metals were lower in the second year. It is expected that the quality of composts becomes better over time. On the other hand, two years is a very short time to make conclusions and next year we will continue our investigations. The total amount of PAH in these composts varied from 0.77 ± 0.008 mg/kg in SSC 2016 sample to 14.87 ± 0.115 mg/kg in MMWC 2015 sample. The total amount of PCBs in investigated composts varied from 3.2 ± 0.14 µg/kg in GWC 2015 sample to 505.1 ± 45.10 µg/kg in MMWC 2015 sample. According to the literature data, PAH concentration in composts should be less than 4 mg/kg (d.m.) and PCBs should be less than 400 µg/kg (d.m.). This study is important from the ecological point of view and is the basis for further investigations of persistent organic pollutants in Lithuanian-produced composts. Every year the amount of recycling and reuse of biodegradable waste is increasing, which is relevant to the topic.

POSSIBILITIES OF CULTIVATION OF PERENNIAL GRASS FOR ENERGY IN LIGHT-TEXTURED SOILS USING SEWAGE SLUDGE COMPOST FOR FERTILIZATION

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The European Union stresses the importance of biomass use and a variety of opportunities for development; it also promotes the use of plant biomass as an energy source as intensively and as widely as possible. Sewage sludge disposal is an urgent issue at the moment.

Trial was performed at the Vokė Branch of the Lithuanian Research Centre for Agriculture and Forestry in sandy loam Haplic Luvisol from 2012 to 2015. Mugwort (*Artemisia dubia* Wall.), Virginia fanpetals (*Sida hermaphrodita* (L.) Rusby) and Giant miscanthus (*Miscanthus x giganteus*) were planted from seedlings in the spring of 2012. Fertilization rate: control; $N_{90}P_{60}K_{90}$; 20, 40 and 80 t ha⁻¹ sewage sludge compost DM. Mineral fertilizers were applied every year, and sewage sludge was used once, in 2012, for 3 years.

Tests of herbaceous energy plants showed that the highest total DM biomass yield was produced by giant miscanthus and mugwort. Fertilization with 20 and 80 t ha⁻¹ of SSC significantly increased the mugwort biomass DM yield only on the third year (from 4.9 to 7.0 and 6.8 t ha⁻¹ respectively). Fertilization with SSC had no effect on biomass DM yields of Virginia fanpetals and giant miscanthus.

The biomass of Giant miscanthus was the most suitable for solid biofuel as it was characterized by the significantly lowest ash (1.96–3.01%), sulfur (0.082–0.098%) and total nitrogen (0.24–0.41%) contents.

However, the calorific value of these plants was only 18.4–18.7 MJ kg⁻¹, i.e. significantly lower than of Virginia fanpetals (18.5–19.0 MJ kg⁻¹) and mugwort (19.3–19.5 MJ kg⁻¹). Mugwort biomass had the highest calorific value, but it contained a lot of S (0.096–0.117%) and N_{total} (0.30–0.57%). Besides, its ash content was more than 1.5 times higher than that of other herbaceous plants (4.35–5.21%).

Application of SSC did not significantly change the calorific value of plant biomass. However, application of 20 and 40 t ha⁻¹ of compost reduced the ash content in Giant miscanthus and Virginia fanpetals biomass, S content in Virginia fanpetals biomass and N_{total} concentration in Giant miscanthus biomass ($p < 0.05$).

It was revealed that cultivation of energy plants, using sewage sludge compost for fertilization, does not exhaust the soil. Concentrations of total nitrogen (N_{total}) and mobile phosphorus (P_2O_5) in the soil substantially increased.

During the three-year trial the soil pH decreased. Fertilization of soil under herbaceous energy plants with sewage sludge compost weakened the soil acidification or even increased its pH ($p < 0.05$).

AGRICULTURE AND ENVIRONMENTAL AUDITING IN THE BALTIC STATES AFTER THEIR ACCESSION TO THE EUROPEAN UNION

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The Supreme Audit Institutions (SAIs) are the highest audit authorities which have the authority to pursue independent audits of national policies including the field of agriculture and environment and to evaluate their corresponding implementation in a particular state. Public audit has the capability to shed light on the sustainability of public policies and thus to bring about certain improvements to state activities in the concerned areas.

Each EU member state has a national supreme audit institution that offers expertise and submits recommendations to the involved public institutions. Since 2004 and following the EU enlargement, the SAIs of the Baltic states have been part of the pan-EU network of SAIs – the Contact Committee* which has had exclusive interest in the areas of audit of both agriculture and environmental auditing in the form of dedicated networks of experts and working groups. The concerned area has drawn attention due to its high implication on both the national budgets in the form of a support for local agriculture production and the common EU budget via funding from the European Agriculture Fund for Rural Development. From the point of view of environmental auditing, the area is of a great concern due to the serious implications which could be potentially caused in case of mismanagement planning and implementation of nature protection related policies. Both areas are of significant importance to all institutions since in the audit work by the SAI of Latvia it has been covered in 17 performance audits (agriculture – 10, environmental – 7), the SAI of Lithuania has conducted 31 performance audits (agriculture – 13, environmental – 18), and the SAI of Estonia reached the staggering 40 performance audits (agriculture – 12, environmental – 28).

Since the accession to the EU, the SAIs of the Baltic countries have covered a wide variety of different performance audit topics reflecting both the topicalities partly arising from the European decisions, opening of the EU funds (EDF, EAFRD), local issues, and other tendencies of national importance.





IS THERE A CONNECTION BETWEEN SEED WING SHAPE AND SEED DISPERSAL PATTERNS IN SCOTS PINE?

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Genetic stability of forests is an important factor under the stresses induced by the changing climate. Genetic diversity and a sound genetic structure are the main components of genetic stability. A sound genetic structure implies that related trees are intermixed in the stand and get less chance for mating. Seed dispersal distance and pattern markedly affects location of the related individual trees in a new natural regeneration. This factor depends on seed wing shape and wing size.

The wings of Scots pine seeds from a seed orchard were assessed according to the following classes: (1) elongated: the seed wing is elongated, narrow; (2) regularly rounded: the wing is oval, widest near the seed, the wing gradually peaks; (3) irregularly rounded: the seed wing is wide, the place of widening is stretched, the wing peaks sharply. In total, 800 seeds were investigated (20 seeds from each of the 40 clones). Elongated seed wings have the biggest wing area (80.02 mm²), perimeter (46.48 mm) and length (19.21 mm), but the smallest width (5.57 mm). Regularly rounded (class 2) seed wings dominated among the clones (16 clones or 40%). For nine of the 40 clones, the dominant seed wing class was found on more than 60% of the seeds of these particular clones.

The relationship between the seed wing size and colour was weak. There was a weak association between seed wing colour and wing length and perimeter. The length and the width of the cone do not have a significant effect on the seed wing colour. The seed colour varied markedly between the clones. It means that this feature is under strong genetic control.

There was no significant difference in the flying distance between the seed wing classes. On the other hand, there were significant differences in the direction of seed flight between the different seed wing shapes. This implies that when Scots pine stand naturally regenerated, the siblings from a mother tree may be located at one spot within the site, especially if there is no wind or it is weak.

RESEARCH ON GENE COPY NUMBER VARIATION IN SCOTS PINE

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Gene copy number variation (CNV) has been acknowledged to be a major source of genetic polymorphism among humans. Increased or decreased copy number of specific genes is also responsible for some diseases. CNVs have been shown to influence plant phenotypes in wheat, maize, potato, and others. The phenotype is influenced by increasing or decreasing summary gene expression due to the variation in the gene copy number. Despite the demonstrated effects of CNVs, research in this area of forest tree genetics is in its infancy.

We have focused on research of CNVs of resistance-linked genes of Scots pine which is a very important forest tree species in Latvia, both economically and ecologically. The hypothesis is that increased copy number of a resistance-linked gene would improve the resistance of Scots pine against fungal pathogens, thus decreasing economic losses. We have analysed CNV of the genes which encode products with proven efficiency against plant pathogens using several different genetic methods. We have found strong evidence for the existence of CNVs of several resistance-linked genes – *Pinus sylvestris* thaumatin-like protein gene, *Pinus sylvestris* defensin 2 gene, pinosylvin synthase gene, and others. Our findings still have to be supported by gene expression data but we are moving forward to develop CNV-based criteria for the selection of trees for breeding programmes aimed at increasing the resistance of *Scots pine* against various diseases.



PHYTOPHTHORA ALNI S.L. AND PHYTOPHTHORA PLURIVORA SPECIES COMPLEX VIRULENCE TEST ON ALNUS GLUTINOSA SEEDLINGS

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Black alder (*Alnus glutinosa*) is economically and ecologically important forest tree species growing on moist, temporarily waterlogged forest or riparian sites, where it forms unique ecosystems. Since the early 1980s, when a new *Phytophthora* disease of black alder has been spreading across Europe, *Phytophthora alni* complex (*P. x alni*, *P. x multiformis*, *P. uniformis*) have traditionally been identified as the main causal agent of it. Recently more and more studies show that not only *P. alni* s.l. can be responsible for alder decline. The knowledge of the driving forces and pathological processes of alder decline is limited in Lithuania. Understanding the main roles of the pathogens that cause alder decline could provide the basis for a management strategy against it in riparian ecosystems not only in Lithuania but also in Europe.

The experiment was performed in the Laboratory of Phytopathogenic Microorganisms and it was done in the Field Experimental Station of the Institute of Botany (Nature Research Centre). Inoculation tests were conducted on 1000 2-year-old seedlings of black alder with *P. x alni*, *P. uniformis* and *P. plurivora* zoospore suspensions to compare if alder seedlings are more sensitive to a single pathogen or to the zoospore suspensions of combined pathogens. The expectation of this study is to confirm the hypothesis that black alder seedlings are more susceptible and damaged by the combined *P. alni* s.l. and *P. plurivora* pathogens, compared to the seedlings inoculations with single species pathogen.

RETROTRANSPOSON COPY NUMBER VARIATION IN THE GENOMES OF CONIFERS

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With the availability of genomic re-sequencing data, it is increasingly recognised that the complete genome sequence of one individual vastly underestimates the variations existing in the wider population. The variable part of genomes includes transposable elements (TE), pseudogenes, as well as individual genes and gene copy number variation. Retrotransposons (RE) are a class of TE especially frequent in plant genomes and are also found within gene introns and untranslated regions displaying gene regulatory function. Frequencies of TE in the genome could vary due to their replicative mode of transposition, loss or removal of elements, selection and genetic drift. Genome size variation is associated with possible adaptation to environmental conditions, geographical distribution, growth characteristics and morphology, and can have an adaptive and evolutionary role. Woody plants are characterized by long generation times, a high level of gene flow, and high effective population sizes. Given the low level of sequence divergence and monophyly of gymnosperm genomes, we investigated the distribution of Loblolly pine (*P. taeda*) specific RE families in pine species and more evolutionarily distant gymnosperm genera. We also investigated the intra-species variation of these REs in eight Scots pine (*P. sylvestris*) genotypes. This information gives insight into the age, distribution, and activity of particular RE families, and indicates any possibilities of horizontal transfer. Various aspects of the RE families were compared among gymnosperm genomes, including their distribution, copy number and sequence divergence. Despite the monophyletic genome structure, RE copy number varies greatly between and within pine species. Our results reflect the rapid sequence divergence of RE families shared between species and the unequal distribution of REs among genera. RE activity has been shown to be induced by stress conditions. While the majority of transposition events are neutral, advantageous mutations may also be established in the presence of selective pressure.



EFFECTS OF DIFFERENT EXOGENOUS AUXINS ON ASPEN MORPHOGENESIS *IN VITRO* IN THE CONTEXT OF AUXIN TRANSPORT INHIBITION BY 2,3,5-TRIIODOBENZOIC ACID

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Plant organ development is targeted and regulated by auxin which is known to be synthesized in one place of a plant and transported to another. Therefore the auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) was used in this study for auxin analysis. Since, as part of the hormone cross-talk, auxin is known to modulate gibberellin (GA) responses, the effects of paclobutrazol (PBZ), a GA synthesis inhibitor, were also taken into consideration. Thus, the following plant growth regulators were investigated using aspen (*Populus tremula* L.) shoot culture: auxins indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), TIBA, and PBZ. These were added to the otherwise hormone-free nutrient medium in glass culture tubes (150 × 20 mm). IAA and IBA were tested at the concentrations of 1, 3, and 5 $\mu\text{mol}\cdot\text{l}^{-1}$, TIBA – at 1, 5, and 15 $\mu\text{mol}\cdot\text{l}^{-1}$, and PBZ – at 0.5 $\mu\text{mol}\cdot\text{l}^{-1}$. The morphometric characteristics of developing plants were evaluated. The most obvious effect of TIBA was on root development, as it decreased the root number and total root length. In respect of shoot development, the use of 5 $\mu\text{mol}\cdot\text{l}^{-1}$ TIBA led to the increase of shoot length and also to the increased number of adventitious shoots. In the next step, 5 $\mu\text{mol}\cdot\text{l}^{-1}$ TIBA concentration was used to analyse auxin ability to restore the TIBA impacts on aspen *in vitro* culture. Interestingly, the comparison of IAA and IBA effects revealed obvious differences between these two auxins: average root number per explant was increased by IBA and decreased by IAA, while the latter had a positive effect on shoot growth (also in direct contrast to IBA which decreased average shoot length). These differences between the two auxins were also confirmed by testing IAA and IBA in combination with TIBA. Thus, only IBA, and not IAA, was able to restore root number decreased by TIBA. Finally, when PBZ was tested, an interesting fact was observed that PBZ (whose main effect in all combinations was a significantly increased root number) was able to increase also the shoot length if added alone but not in the combination with TIBA. Meanwhile, IAA, when added to the combination of TIBA and PBZ, increased the shoot length significantly. In conclusion, IAA by itself seems to have a significant role in promotion of aspen shoot growth, while undisturbed auxin transport or exogenous IBA is required for root formation on aspen explants.

SUSCEPTIBILITY OF *PINUS CONTORTA* AND *PINUS SYLVESTRIS* TO *HETEROBASIDIUM* ROOT ROT

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The aim of this investigation was to compare the susceptibility of lodgepole pine and Scots pine. Long-term sample plots were established in 4 stands, in total 4686 trees and 244 stumps from previous generation were inspected to analyse the spread of *Heterobasidion* spp. Fungal isolates were obtained from wood discs gathered from 1223 decayed trees and wood samples taken from 244 previous generation Scots pine stumps.

To evaluate the susceptibility of both tree species to basidiospore infection, 193 standing trees were cut and 446 wood discs were examined in total. To identify *Heterobasidion* spp. infection *Heterobasidion* spp. conidiophores were used. Macro- and microscopic morphological features of the fungal mycelia were used to determine all pure cultures, which were isolated from wood. For identification of *Heterobasidion* spp. species intersterility tests were performed, and somatic incompatibility tests were made to analyse dynamic of infection.

In conclusion, both *Heterobasidion* species can infect *P. contorta*, but mainly root rot was caused by *H. annosum*. *H. parviporum* infection was negligible ($p < 0.05$). *P. contorta* were more frequently infected by *H. annosum* s.l. ($p < 0.05$) than *P. sylvestris*.



THE EFFECT OF SELECTED BIOSTIMULANTS ON SEED GERMINATION IN FOUR PLANT SPECIES

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In 2015, an experiment was conducted in the laboratory of the Department of Horticulture at the University of Warmia and Mazury in Olsztyn (NE Poland). Three replications of the experiment were performed in three independent series. The object of this study was to evaluate the germinative energy and germination capacity of seeds of four ornamental plant species treated with various biologically active substances. The first experimental factor constituted seeds of four ornamental plant species: China aster (*Callistephus chinensis* L. Nees), Scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), Common zinnia (*Zinnia elegant* L.), and French marigold (*Tagetes patula* L.). The second factor was the biostimulants used for seed pre-conditioning: Effective Microorganisms (EM) at a concentration of 20%, *Trichoderma* – 2.4 g dm⁻³, and Goëmar Goteo – 1%. Seeds soaked in distilled water were the control. Seeds were conditioned in flasks; 150 seeds of each plant species were soaked in solutions of biological compounds for 30 minutes. The seeds were dried on sterile filter paper under relatively aseptic conditions and were placed in PS 100 mm Petri dishes (50 seeds per dish) lined with two layers of filter paper soaked in conditioning substances. Germinative energy and germination capacity were evaluated after three days and eight days, respectively. The plant species compared in the study responded differently to the tested biostimulants. French marigold seeds were characterized by the highest average germinative energy and germination capacity in three experimental series (mean values) and in each of the series. Scarlet sage seeds had the lowest germinative energy, and Common zinnia seeds had the lowest germination capacity in experimental series 1 and 3.

QUANTITATIVE DIFFERENCES OF BIOACTIVE COMPOUNDS IN APPLES PICKED FROM DIFFERENT POSITIONS OF AN APPLE-TREE

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The aim of this research was to evaluate the differences of bioactive compound distribution in apples cv. 'Ligol' that were picked from various positions of an apple-tree: from bottom, top, east and west sides. Apples were grown in the orchards of the Institute of Horticulture, a branch of the Lithuanian Research Centre for Agriculture and Forestry. Results were formed from two years of data. Statistical differences were evaluated using Tukey's HSD test at $p = 0.95$. The amounts of all compounds are given in dry mass.

Evaluation of phenolic compounds. Amounts of hyperoside significantly varied from 0.028 µg/g (bottom) to 0.171 µg/g (top), rutin varied from 0.014 µg/g (bottom) to 0.023–0.025 µg/g (west side and top), reynoutrin varied from 0.004 µg/g (bottom) to 0.053 µg/g (west side), avicularin varied from 0.034 µg/g (bottom) to 0.069–0.099 µg/g (all other positions). However, there were no significant differences in the amounts of isoquercitrin, quercitrin, procyanidin B1, catechin, procyanidin B2, epicatechin, procyanidin C1, phloridzin, and chlorogenic acid. Fruits from the bottom position had a significantly smaller amount of quercetin glycosides (0.156 µg/g) in comparison to fruits from other positions (avg. 0.408 µg/g).

Evaluation of triterpenic compounds. The amount of corosolic acid was significantly lower in fruits picked from the top (0.755 µg/g) in comparison to other positions (avg. 1.172 µg/g), betulinic acid varied from 0.067–0.077 µg/g (east, west, and top) to 0.133 µg/g (bottom), oleonic acid varied from 1.586–1.765 µg/g (east, west, and top) to 2.174 µg/g (bottom), and ursolic acid varied from 6.846–8.120 µg/g (east, west, and top) to 10.140 µg/g (bottom).

Evaluation of carbohydrates. The ratio between monosaccharides and polysaccharides varied from 1.53 (bottom) to 1.16 (top); 507.9 µg/g (monosaccharides) to 351.6 µg/g (polysaccharides) for bottom, and 406.9 µg/g (monosaccharides) to 351.6 µg/g (polysaccharides) from top.

In conclusion, fruits picked from the bottom of the tree had the smallest amounts of phenolic compounds, but the highest amounts of triterpenic compounds and the highest number of monosaccharides. Fruits from the top had the largest amounts of phenolics, but the smallest quantity of triterpenes. In most cases, fruits from the east side had a similar amount as fruits from the bottom, and fruits from the top had similar amounts of bioactive compounds as fruits from the west side.

BOTRYTIS CINEREA: DIVERSITY, DISEASE FORECASTING AND CONTROL

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Diseases caused by *Botrytis* spp. are influenced by a complex of biological factors, such as the host, environment, chemical and physical interactions between the pathogen and the host. *Botrytis* spp. causes diseases on 170 families of cultivated plants. It is very important to understand pathogen biology, incidence, and control. *Botrytis cinerea* causes grey mould in strawberries, which may cause up to 50% losses of an annual yield. Diseases caused by *B. cinerea* are controlled by various methods, such as the use of fungicides, alternative plant protection products, or reduction of humidity. The aim of the research was to identify the composition of pathogens of *Botrytis* species causing strawberry and onion rots, to characterize their diversity, to evaluate efficiency of traditional and alternative plant protection measures. It was found that the dominant *Botrytis* spp. pathogen causing diseases in strawberry and onion was *B. cinerea*. Isolates grown on different agar media depended on the host, temperature, and light conditions. *B. cinerea* control with iMETOS@sm forecasting model showed positive effect on strawberry and onion plants. According to our results, treatment with alternative plant protection products can reduce the contamination of strawberries with grey mould and prolong the disease-free period of fruits.

This work was carried out within the framework of the long-term research programmes 'Horticulture: agro-biological basics and technologies' and 'Harmful organisms in agro and forest ecosystems' implemented by LAMMC.



BIOLOGICALLY ACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN PIGMENTED-FLESH POTATOES GROWN IN LITHUANIA

Nijolė Vaitkevičienė

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Over the past decade, potatoes with pigmented flesh (purple-fleshed and red-fleshed) have been receiving increased interest from researchers and consumers due to their richer nutritional value, antioxidant activity, appearance, and flavour. They contain biologically active compounds – natural antioxidants: phenolic compounds, phenolic acids, and anthocyanins. Most of these compounds are of nutritional and pharmacological interest and determine the quality of plant raw material.

The aim of the present study was to investigate total content of phenolic compounds, quantitative and qualitative composition of anthocyanins and phenolic acids, and antioxidant activity in the tubers of the pigmented potatoes cvs. 'Red Emmali', 'Blue Congo', 'Vittelotte'. The total content of phenolic compounds was estimated by the Folin-Ciocalteu colorimetric method. Anthocyanins, phenolic acids and their derivatives were measured using HPLC assay, and antioxidant activity was determined by the DPPH method.

The results showed that significantly the highest content of total phenolic compounds (350.40 mg 100 g⁻¹ d. m.), anthocyanins (266.58 mg 100 g⁻¹ d. m.), and phenolic acids (448.40 mg 100 g⁻¹ d. m.) were accumulated by the cv. 'Vitelotte'. Malvidin and petunidin compounds were identified as the main anthocyanins in the tubers of 'Blue Congo' and 'Vitelotte', and pelargonidin compounds in 'Red Emmalie'. The predominant phenolic acid in the tested tubers was chlorogenic acid which accounted for 65.99–69.33% of the total content of phenolic acids. The maximal antioxidant activity was determined in the tubers of 'Vitelotte': it was 1.59 times higher than that in the tubers of 'Blue Congo', and 2.5 times higher than in the tubers of 'Red Emmalie'.

The results of this study suggest that the content of biologically active compounds in potato tubers depends on the genetic properties of the cultivar. According to chemical composition, the tubers of cv. 'Vitelotte' were found to be the most valuable.

THE IMPACT OF THE SUBSTRATE TYPE ON NITRATE ASSIMILATION IN GREEN VEGETABLES

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In this study the impact of the substrate type (neutralized peat, coco peat, rock wool, zeolite, and vermiculite) on nitrate assimilation in green vegetables was evaluated. Red and green leaf lettuce plants (*Lactuca sativa* 'Red Cos' and 'Green Cos'), and tatsoi (*Brassica rapa* var. *rosularis* 'Rosetto' F₁) were cultivated in a greenhouse in spring (21/17°C), under high-pressure sodium (HPS; 18 h, 180 μmol m⁻²s⁻¹) light for 21 days from sowing. The plants were watered with mineral nutrient solution equally. The contents of nitrate and nitrite, nitrate reductase activity, total protein contents, chlorophyll index, and photosynthesis intensity were estimated. The obtained results show that the substrate type has a remarkable effect on growth patterns, nitrate assimilation indices, and nutritional value and safety of green vegetables. Most of the biomass was accumulated in all green vegetables, cultivated in vermiculite and rock wool; however, the nitrate contents, as well as photosynthesis indices were the highest in the red and green lettuce cultivated in peat. In tatsoi, on the contrary, the lowest nitrate content was determined in the plants cultivated in peat, and the highest – in vermiculite. In all cases, the increase in nitrate content in vegetable tissues coincides with the remarkable increase in nitrite content. Nitrate assimilation processes are closely interrelated with photosynthetic performance parameters.

This research was funded by a grant (No. MIP-60/2015) from the Research Council of Lithuania.





SMART CITY SPECIALISATION OPPORTUNITIES IN LATVIA

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Today, theoretical and policy documents in literature widely use two 'smart' terms: 'Smart City' and 'smart specialization', often as if they were synonyms. However, both the concept of the origin and the initial application are different, though they have common features. On the city level, a number of policy development initiatives have focused on the concept of 'smart cities', which was originally developed around the ICT (information and communication technology) infrastructure as a means of connecting the city, thus contributing to its growth opportunities.

More recently, the marking 'smart' was elevated to regional policy. Originally associated with industrial specialization, the smart specialization strategy is currently being actively discussed in regional studies, both in leading policy-making and in cognitive academic research.

Rapid development of the modern city requires a broader interpretation of both concepts, not only of the means of technological innovation. The empirical relationship has been found between the development of smart specialization and the appearance of Smart Cities (in the broad sense). However, this does not mean that all regions (especially with poorly developed production index) should be involved in the research, development and/or growth of technology (hi-tech)-based industrial development strategy. Smart development is a multidimensional concept that consists of sustainable economic growth and of sustainable urban/regional development based on sustainable competitive advantages. It is also viewed as a way to raise the quality of life. This means that social and environmental capital plays an equally important role in the next infrastructure/ICT development. Together, the concepts of 'smart cities' and smart specialization are able to use the concept of 'intelligent development' based on knowledge and innovation (smart cities) and the strategy-based competitive local capacity (smart specialization) selection.

Research supported from National Research programme EKOSOC-LV.

MLST GENOTYPES AND ANTIBIOTIC RESISTANCE OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM INFECTED CHILDREN AND FROM POULTRY PRODUCTS

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Background: *Campylobacter* are among the most frequently reported food-borne diseases worldwide. This thermo-tolerant pathogen is more common in poultry and is one of the leading causes of gastroenteritis in humans. However, most *Campylobacter* infections are self-limiting and antimicrobial treatment is necessary only in severe prolonged cases. Antimicrobial resistance of *Campylobacter* has increased dramatically in many countries, and such strains continue to exist with high-level resistance.

Objectives: The aim of this study was the association between Multilocus sequence typing (MLST) genotypes and antibiotic resistance of *C. jejuni* strains isolated from children clinical cases and from broiler meat.

Methods: In total, 199 *C. jejuni* isolates including isolates from children ($n = 101$) and from broiler products ($n = 98$) were genotyped by MLST and tested against phenotypic resistance to ciprofloxacin, tetracycline, gentamycin, ceftriaxone and erythromycin by the agar dilution method.

Results: MLST revealed 45 known and 23 novel sequence types (ST) out of 199 tested *C. jejuni* strains. ST-5 was dominant among children clinical cases. ST-464 and novel ST-6410 were dominant in broiler products. In total, 94% of *C. jejuni* strains were resistant to ciprofloxacin, 59.8% to ceftriaxone, 42.7% to tetracycline, and only two strains were resistant to erythromycin. All children and broiler strains were sensitive to gentamycin. Interestingly, multidrug resistance of the strains assigned to novel STs was found more often. Further, strains assigned to novel sequence types displayed higher minimum inhibitory concentrations to some antibiotics (MIC for 69.8% of strains in range of 32–256 $\mu\text{g/ml}$ to tetracycline).

Conclusions: The study revealed that 97% of *C. jejuni* strains were resistant to at least one of 5 tested antibiotics. *C. jejuni* strains belonging to novel MLST genotypes displayed multidrug resistance more often. Further, the higher MIC's of *Campylobacter* strains attributed to novel ST were revealed in comparison to already known ST.



INACTIVATION OF MICROORGANISMS IN MAPLE PEA (*PISUM SATIVUM* VAR. *ARVENSE* L.) SPREAD

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Plant-based spread made from maple peas is prone to spoilage by microorganisms after production if no additional preservation treatment is applied. Experiments were carried out at the Department of Food Technology, Latvia University of Agriculture, to investigate thermal inactivation of microorganisms during *sous vide* pasteurisation (60.0, 70.0, 80.0, 90.0 and 100.0°C / 5, 10, 15, 20, 25, 30, 35 and 40 min) of maple pea spread with herb seasoning. Pea spread (50 ± 1 g), made from ground re-hydrated cooked maple peas 'Bruno' together with salt, citric acid, oil and seasoning, was filled in transparent PA/PE film pouches, hermetically sealed under vacuum (20 mbar) and heat treated in a water bath with subsequent rapid cooling in ice-water. Total plate count was determined on Plate Count Agar; identification of the selected bacteria was completed with API 50 CHB kit. Survival curves were constructed as a function of the temperature applied. Several linear and non-linear models were fitted to the *sous vide* inactivation kinetics. Joinpoint regression analysis was used to identify breakpoints of non-linear survival curves where statistically significant change over time in linear slope occurred, in order to calculate D and z values. The linear model had the poorest fit to the experimental data while biphasic model produced the best fit to all the survival curves indicated by the lowest root-mean-square error and the highest R² values. Calculated linear D values were significantly lower than tail D values (p < 0.001). *Bacillus spp.* were found in all samples regardless of thermal treatment regime. Treatments at 60 and 70°C are the least suitable for *sous vide* processing.

This study was supported by the FP7 Research Project N° 61378 EUROLEGUME.

PLANTS FOR FOOD PRODUCTS WITH HIGHER NUTRITIONAL VALUE

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Public demands for food safety and health benefits have been rapidly rising in recent years. The assortment of food produce is quite wide in the Lithuanian market. However, the products on offer do not always have high nutritional value, and consumers increasingly prefer new, natural, more attractive products of better quality.

The aim was to determine and compare the biochemical content of Jerusalem artichoke (*Helianthus tuberosus* L.) cvs. 'Sauliai', 'Rubik', 'Albik', 'Anastas' tubers, white mulberry (*Morus alba* L.) cvs. 'Plodovaja 3', 'Turchanka' and 'Smuglianka' leaves and mints (*Mentha*) cvs. *Mentha spicata* 'Moroccan', *Mentha piperita* 'Glacialis', *Mentha piperita* 'Swiss', *Mentha suaveolens* 'Apple', *Mentha gentilis* 'Ginger', *Mentha suaveolens* 'Variegata' leaves.

The amount of dry matter, crude protein, fibre, and ash in Jerusalem artichoke tubers and mulberry leaves were determined by standard methods. The total phenolic compounds content (TPCC) mg GAE/1g in the dry matter was established by the Folin-Ciocalteu colorimetric method. The essential oil was extracted by the hydro-distillation method using a Clevenger-type apparatus.

The main indicator of the quality of aromatic plants is the amount of essential oils. The highest amounts of essential oil were found in the leaves of *Mentha spicata* 'Morocca' and in *Mentha piperita* 'Glacialis'. The statistically significant least amount of essential oil was found in the leaves of *Mentha suaveolens* 'Variegata' by 0.78% compared with *Mentha spicata* 'Moroccan'.

The results show that significantly highest content of dry matter was in 'Rubik' and 'Anasta' tubers – 25.9%, and 19.38% in 'Albik'. Analysis of crude protein showed that significantly highest content – 7.79% – was in the 'Albik' tuber. The content of crude fiber ranged from 3.49% to 4.76%. The highest amount of crude ash (7.26%) accumulated in 'Anastas' cv. tubers. The highest concentration of phenolic compounds in Jerusalem artichoke tubers was found in cv. 'Rubik'.

In mulberry leaves, depending on the cultivars, the content of dry matter was almost similar and varied from 92.64% to 93.06%. The investigation showed that the significantly highest (16.93%) crude protein content was in



COMPETITIVENESS OF LITHUANIAN DAIRY PRODUCE IN TRADE WITH RUSSIA AND IN ALTERNATIVE MARKETS

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In the free trade doctrine, there arise conflicts when the countries take protectionist measures without reason. Russia, which imposed an embargo on certain agricultural products, such as beef, pork, poultry meat, dairy products, fruit and vegetables against some countries of the world (on 7 August 2014) is an example of such a country. The problem of our research arises from the conflict between theory and practice (Imagawa, 2004; Bojnec, Fertő, 2014; Carraresi, Banterle, 2015).

Since the beginning of the embargo, the EU agri-food sector has managed to compensate the losses in export sales to Russia by increasing exports to alternative markets. The main goal of the present research is to recognize the level of competitiveness of the export of dairy products in trade between Lithuania and Russia, or alternative markets.

The object of research is the analysis of competitiveness of trade in dairy products using the International competitiveness index in a specific (local) region (LIIC). The index is an adoption of Balassa index. It is the ratio of sector k's export from exporting country to importing country relative to exporting country's total exports to the importing country and divided by the ratio of sector k's importing country's import from the world relative to the importing country's total imports.

The results of the research showed that Lithuanian dairy products were competitive in the 28 countries of the EU. The highest competitiveness was in trade with Italy, Poland took the second place, although the index had a significant decline.

Lithuanian dairy produce is also competitive in the countries outside the EU. The value of the index confirmed the decreasing competitiveness in Russia as it decreased almost two times. The highest competitiveness was in trade with Singapore, Malaysia, and Vietnam. The value of the index in Belarus has been steadily growing – from 0 to 26.5. It shows that Lithuania has potential to trade in dairy products with these economies.

'Smuglianka' cv. leaves. Substantially more (5.13%) crude fiber accumulated in 'Smuglianka' cv. leaves, and less – 10.16% – in 'Plodovaja 3'. The content of crude ash in mulberry leaves ranged from 8.98% to 11.67%. TPCC in the studied mulberry leaves species varied between 13.48 to 26.88 mg GAE 1g⁻¹ DM. A significant higher amount of phenolic compounds was found in mulberry leaves of cv. 'Smuglianka'.

Our research has shown that the examined plants have good prospects for the production of food products with additional nutritional value characterised by a higher amount of proteins, minerals, phenols, essential oils as described by permitted health claims made on the nutritional value of food according to the Commission regulation (EU) No 274/2014.

PHENOLIC AND ORGANIC ACID COMPOSITION OF STEAM-BLANCHED AND DRIED PARSLEY LEAVES

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Parsley leaves are a common *Apiaceae* species regularly used in food as additives. They contain various bioactive compounds, including phenolic compounds and organic acids with distinct antiradical activity. The aim of research was to analyse the effect of steam-blanching and different drying methods on total carotenoid content, organic acids and phenolic compounds, antiradical activity of phenolic compounds, and colour of parsley leaves. Gravimetric, spectrophotometric and high performance liquid chromatography methods were used. Moisture content in parsley was $78.98 \pm 0.79\%$ and decreased to under 9.00% during the drying process. The highest carotenoid content was observed in steam-blanching (SB) samples and using convective and microwave-vacuum (MV) drying, and varied in different concentrations. Total phenolic compound content in parsley increased with steam and drying processing; phenolic acid and flavonoids were detected in smaller amounts. The antiradical activity increased in a tendency similar to phenolic compounds. Ascorbic acid was thermo-unstable and decreased significantly. Principal component analysis was performed to estimate the relationship between analysed organic acids and phenolic compounds and processing methods. Parsley contains high amounts of phenolics, organic acids, and carotenoids. The thermal and drying process affects bioactive compounds significantly. Less pronounced organic acid phenolics were determined in fresh and steam-blanching parsley, while most of the compounds were observed in 3.0 min steam-blanching followed by MV drying. Changes in phenolic composition and organic acid content could be explained by chemical reactions during processing and analysis methods applied.

Presented research was supported by the national research programme AgroBioRes (2014–2017).

SAFETY EVALUATION OF NISIN-PRODUCING *L. LACTIS* STRAINS

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Inhibition of food spoilage and pathogenic bacteria in order to extend the shelf life of products is one of the main goals in food industry. Achieving this goal through biopreservation using natural antimicrobial compounds is of considerable interest and attracts special attention of consumers. One of the biggest considerations in biopreservation in recent years is dedicated to lactic acid bacteria (LAB) and bacteriocins produced by them. Safety of bacteriocin-producing LAB, intended to be used for food production, is an important aspect. From the enzymatic point of view, strains should not produce harmful enzymes like β -glucosidase or β -glucuronidase. The aim of this study was to select bacteriocin nisin-producing *Lactococcus lactis* strains with no activity of β -glucosidase or β -glucuronidase out of 12 nisin producing *L. lactis* strains, isolated from local cow and goat milk, fermented wheat and buckwheat samples. The enzymatic tests were carried out using the API ZYM kit. No activity of β -glucuronidase was detected, though four strains of plant origin were found to produce strong β -glucosidase activity. Other evaluation of enzymatic activities revealed none or weak activities determined for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α -galactosidase, α -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase. Weak phosphohydrolase activities were determined for all of the strains except one strain isolated from raw goat milk, which had strong activity of this enzyme. Eight nisin-producing *L. lactis* strains were found to be safe regarding the enzymatic activity, and could be applied for biopreservation after further testing.

This research was funded by a grant (No.MIP-63/2015) from the Research Council of Lithuania.





SEASONAL OCCURENCE OF INTESTINAL PARASITE INFECTION IN LITHUANIAN VILLAGE DOGS

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Intestinal helminth infections in dogs may cause very different clinical symptoms depending on the parasite species and abundance (Barutzki et al., 2003), or may be a source for human infection (Deplazes et al. 2011; Otranto et al. 2011). The aim of this study was to examine the occurrence of intestinal helminth infections in Lithuanian village dogs in different seasons. Randomly selected adult dogs for which it was possible to individually sample the feces from the ground were included into the study.

Dog fecal samples were examined every month in the period from May 2013 to June 2014 using flotation in zinc chloride solution and microscopy. Taeniidae-positive faecal samples were further processed by sequential sieving through nylon nets followed by Taeniidae egg isolation and identification using an inverted microscope (egg F/Si method) (Mathis et al., 1996). DNA extraction from isolated Taeniidae eggs was performed as described by Štefanić et al. (2004) followed by multiplex PCR according to Trachsel et al. (2007) for the simultaneous detection of *E. granulosus* (all strains), *E. multilocularis* and *Taenia* spp. *Toxocara* eggs were identified according to measurements (Fahrion et al., 2011). The average prevalence of *Toxocara canis* in dogs was 3.62% (95% CI 1.95–5.12), *Taeniidae* – 2.16% (95% CI 1.64–2.80), *Eucoleus aerophilus* – 2.97% (95% CI 2.35–3.70), *Capilaria putorii* – 0.35% (95% CI 0.16–0.66) and *Trichuris vulpis* – 0.69% (95% CI 0.41–1.09). The infection of dogs with *T. canis* and *C. putorii* was higher ($P < 0.05$) in winter (4.89%; 95% CI 3.27–6.99 and 0.70%; 95% CI 0.19–1.78) than in summer (2.60%; 95% CI 1.52–4.13 and 0.00%; 95% CI 0.00–0.56). The infection with *T. vulpis* was higher ($P < 0.05$) in spring (1.45%; 95% CI 0.63–2.85) than in summer (0.31%; 95% CI 0.04–1.10) and autumn (0.49%; 95% CI 0.10–1.42).

Out of eight sequenced Taeniidae-egg-positive fecal samples, four were confirmed as *T. pisiformis*, one as *E. granulosus* s.l., one as *E. multilocularis*, one as *T. hydatigena*, and from one sample no sequence could be determined.

DETERMINATION OF GENETIC DIVERSITY PARAMETERS OF *PERCA FLUVIATILIS* BY USING IPBS MOLECULAR MARKER

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Wild Eurasian perch (*Perca fluviatilis*) is a freshwater predatory species common to different geographical areas and is one of the most distributed fish species in Latvian and Lithuanian water reservoirs. The study of perch morphology and genetic divergence allows gaining valuable knowledge about genetics of fish populations and also regarding environmental changes of their living area.

The aim of this study was to collect samples of *P. fluviatilis* from different Latvian and Lithuanian reservoirs and to compare data of genetic diversity of all specimens by using molecular markers, namely, retrotransposons, a type of mobile genetic elements. The applied iPBS method is based on the ability of transposable elements to move themselves or their copies in different places of genome under the influence of stress factors.

Blood and muscle tissue samples were collected from five Latvian and four Lithuanian reservoirs. Totally, 180 high-quality DNA samples were amplified using iPBS-specific primers and then electrophoresed for 18 hour at 40 V on agarose gel. All collected data were processed and visualized using Popgene 3.0 and NTSYS software. Three of 26 selected primers showed good applicability with 128 loci, 95% of them were polymorphic. Genetic identity of all nine populations varies from 0.8689, between Lake Cīrišs in Latvia and the River Nemunas in Lithuania, to 0.9807 between lakes Kāla and Babītes in Latvia. Positive correlation between genetic and geographic distances of nine reservoirs is not observed.



DETECTION OF ZONOTIC HEPATITIS E VIRUS IN DIFFERENT ANIMAL SPECIES

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Hepatitis E virus (HEV) is recognized worldwide as an emerging human health pathogen that causes liver disease. Domestic pigs and wild boars could represent an important source of infection. Ingestion of raw or undercooked contaminated game or domestic pig meat and a direct contact with infected animals pose a significant risk for foodborne transmission. Studies on potential animals reservoirs of HEV, especially infection in wildlife, have never been conducted in Lithuania before.

Therefore, to investigate if HEV is commonly present, domestic pigs (n = 384), wild boar (n = 312), roe (n = 166), red deer (n = 108), moose (n = 34), and European bison (n = 3) sera samples from different geographic areas of Lithuania 2014–2015 were assayed by using a commercial HEV enzyme-linked immunosorbent assay kit (IDvet, France). HEV RNR was detected by reverse transcription-PCR (RT-PCR) and nested RT-PCR assays with primer pairs target the ORF1 and ORF2 parts of the HEV genome.

In the serological analysis, 43.75% (95% CI: 38.9–48.7) from domestic pigs, 57.05% (95% CI: 51.5–62.4) from wild boars, 11.76% (95% CI: 4.7–26.6) from moose, and 1.20% from roe deer (95% CI: 0.3–4.3) presented antibodies against HEV. None of red deer and European bison samples were positive for anti-HEV IgG. In pathological samples, 7.5% from domestic pigs and 13.3% from wild boars were positive for HEV RNA using ORF1 primers and 5.0% from domestic pigs and 8.3% from wild boars were positive using ORF2 primers in RT-PCR.

In conclusion, the present study revealed that wild boar and domestic pigs have to be considered as the main reservoir for HEV; however, low prevalence in moose and roe deer makes these species an unlikely reservoir in Lithuania. The proposed detection methods and sets of HEV-specific primers targeting the ORF1 and ORF2 regions allow screening for HEV in Lithuanian domestic pig and wildlife species samples.

PREVALENCE OF BLUETONGUE VIRUS INFECTION AMONG RUMINANTS IN LITHUANIA

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Bluetongue (BT) disease is a viral noncontagious infection of ruminants. The bluetongue virus (BTV) is transmitted by *Culicoides* spp. biting midges. The BTV is one of the genus *Orbivirus* species in the family of *Reoviridae*, and carries a double-stranded RNA genome. Sheep are considered the most vulnerable species for BT, but other ruminants are known to play a major role in BT epidemiology. There is no published data on BTV distribution in Lithuanian ruminants. The objective of this study was to describe the seroprevalence rate of the BTV in domestic and wild ruminant populations in Lithuania.

From 2012 to 2014, blood samples were randomly collected from ruminants in various districts of Lithuania. A total of 15,034 sera samples were collected and tested using an enzyme-linked immunosorbent assay (ID VET Innovative Diagnostics, France).

In 2012, serological evidence of the BTV infection was observed in 390 out of 5,278 animals accounting for a 7.5% (CI 6.8–8.2) prevalence rate among various species of ruminants. During 2013, the number of antibody positive samples increased to 26.9% (CI 25.2–28.6; $P < 0.01$). In 2014, the number of seropositive animals continued to increase to 34% (CI 31.6–36.4; $P < 0.01$).

Also, seropositive individuals were found in populations of sheep and goats, where seroprevalence in 2012–2014 was 1.9% (CI 1.3–2.5). Simultaneously, the study was held for wild ruminants farmed in captivity, such as deer (*Dama dama* and *Cervus elaphus*), mouflons (*Ovis orientalis*), and European bison (*Bison bonasus*). It was found that in this group of animals BTV seroprevalence was significantly lower than in the groups of domestic ruminants (0.64%, CI 0.4–0.9, $P < 0.0001$).

Our studies found that the BTV began a rapid spread in the Lithuanian bovine population. BTV seroprevalence among sheep, goats, and wild ruminants was significantly lower.

INNOVATIVE ADVERTISING CHANNEL FOR PROMOTION OF FOOD PRODUCTS IN LATVIA

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Sales are crucial for any business, including food products that are mass produced and face tough competition. Apart from exploiting sales channels that are close to end clients, food products require effective advertising campaigns to reach wide audience and influence the buying behaviour of potential clientele. Traditional promotion channels include TV commercials, banners in streets, flyers delivered to post boxes and distributed in streets, banners on specific websites and social networks, big video screens outdoors, printed media, small monitors indoors etc.

In 2015, an innovative advertising channel emerged in Latvia – the Illumine channel. It consists of an interconnected network of indoor and outdoor monitors that are placed in public places where people spend time waiting, chatting, or performing tasks. Such places include bus stops (for example, Riga International Bus Station), venues for events (for example, the Latvian Academy of Sciences), state offices, gyms etc.

While staying around these monitors, people normally pay attention to their content captured by dynamic change of images. A survey of food producers and distributors has shown several arguments for the choice of the Illumine channel instead of other advertising options:

- more cost-efficient than TV commercials while providing for the same dynamic way of presenting information;
- more eye-catching in comparison to static advertising channels such as banners, flyers, and printed media;
- more attention to content is paid while staying around small monitors rather than passing the big outdoor monitors;
- a wider (older) audience is targeted in comparison to online advertising that normally targets younger people.

To conclude, the Illumine channel is a unique advertising channel that provides for an efficient promotion of food products in Latvia.