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ABSTRACTS
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Investigations of macrozoobenthoss communities in lakes of Smiltene area

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Key words: Latvia, macrozoobenthoss, small lakes.

A study of zoobenthoss communities was performed in five small lakes situated in vicinity of Smiltene area, Latvia: Lake Blomes, Lake Bilskas, Lake Tiltlejas, Lake Vidusezers, and Lake Taperu. Investigations were implemented according to the project of management plans of Smiltene municipality. Lakes were small (1.0 to 10.0 ha), with a medium depth 1.5 m, but the deepest lake was Lake Taperu (2.0 m). Medium water transparency measured by Secchi disc was 1.5 m and more (3.5 m in Lake Vidusezers). Bottom ground consisted of sludge and detritus with admixture of sand. Littoral zone was made by *Phragmites australis, Typha latifolia, Acorus calamus, Equisetum fluviatile*, and *Carex* spp. Pelagic zone was formed by *Potamogeton natans, Potamogeton crispus, Nuphar lutea, Nymphaea alba, Hydrocharis morsus ranae* and *Myriophyllum* sp. Protected plant species, *Nuphar pumila*, was found in lake Bilskas (habitat 3150; Urtāns 2017). Bottom ground of Lake Vidusezers was practically fully covered with *Chara contraria*. Zoobenthoss indices showed high number of medium biomass (5.60 to 12.44 g m^{-2}).

Dominating macrozoobenthoss groups were Mollusca, Chironomidae, Trichoptera, Ephemeroptera, and Varia. Group Mollusca were dominated by *Limnaea ovata, Limnaea pereger, Physa fontilalis, Valvata piscinalis*, and *Bithynia tentaculata*. Mussels were dominated by *Pisidium amnicum* and *Sphaerium corneum*. Individuals of Chironomidae were detected in high numbers, however biomass of this group was low (0.50 to 1.95 g m^{-2}), because this population consisted mainly of young individuals (Figs. 1 and 2).

Group Ephemeroptera was formed exclusively by young individuals of two species, *Cloeon dipterum* and *Caenis horaria*. Group Varia was formed mainly by *Chaoborus flavicans, Acari* spp., *Corixa* sp. and Culicoideae. It can be concluded that investigated lakes of Smiltene city area have a rich biological diversity of macrozoobenthoss communities as well as form good food resource for fish. Rare and protected zoobenthoss species referred to in regulations of the Cabinet of Ministers of Latvia were not found.

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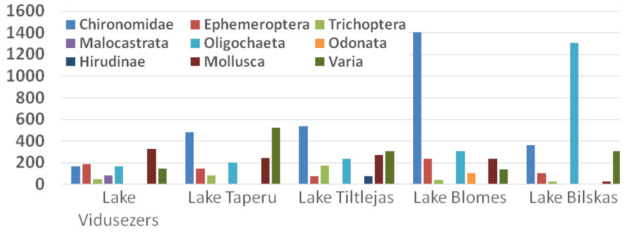

Fig. 1. Number of macrozoobenthoss individuals (individuals per m^2) in lakes of Smiltene area.

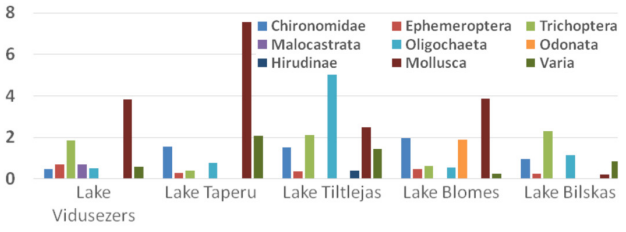

Fig. 2. Biomass of macrozoobenthoss (mg per m^2) in lakes of Smiltene area.

Insect eggs trigger inter-plant systemic acquired resistance and enhanced insect performance

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Key words: elicitors, defence signals, inter-plant communication, plant-herbivore interactions, systemic acquired resistance.

Recognition of molecular patterns from plant pathogens or herbivores trigger not only responses in local plant tissue but also activate a broad-spectrum plant defence priming in distal leaves against potential future attacks, leading to systemic acquired resistance (SAR). Additionally, attacked plants encode a signal that triggers SAR response in neighbouring plants lacking initial exposure to pathogen elicitors. However, the discrimination between molecular mechanisms involved in the SAR signal generation in local tissue and decoding in distal tissues or neighbouring plants are not fully understood. Here, we demonstrate that insect eggs induce not only intra-plant but also inter-plant SAR against *Pseudomonas syringae* via a root-mediated signal in *Arabidopsis thaliana*. Furthermore, egg-induced activation of SAR in neighbouring plants is coupled with increased insect larval performance on these plants. Thus, these results suggest that insects have evolved strategies to favour optimal development of future larvae by providing a niche of host plants with reduced infection and reduced anti-insect defences (Fig. 1).

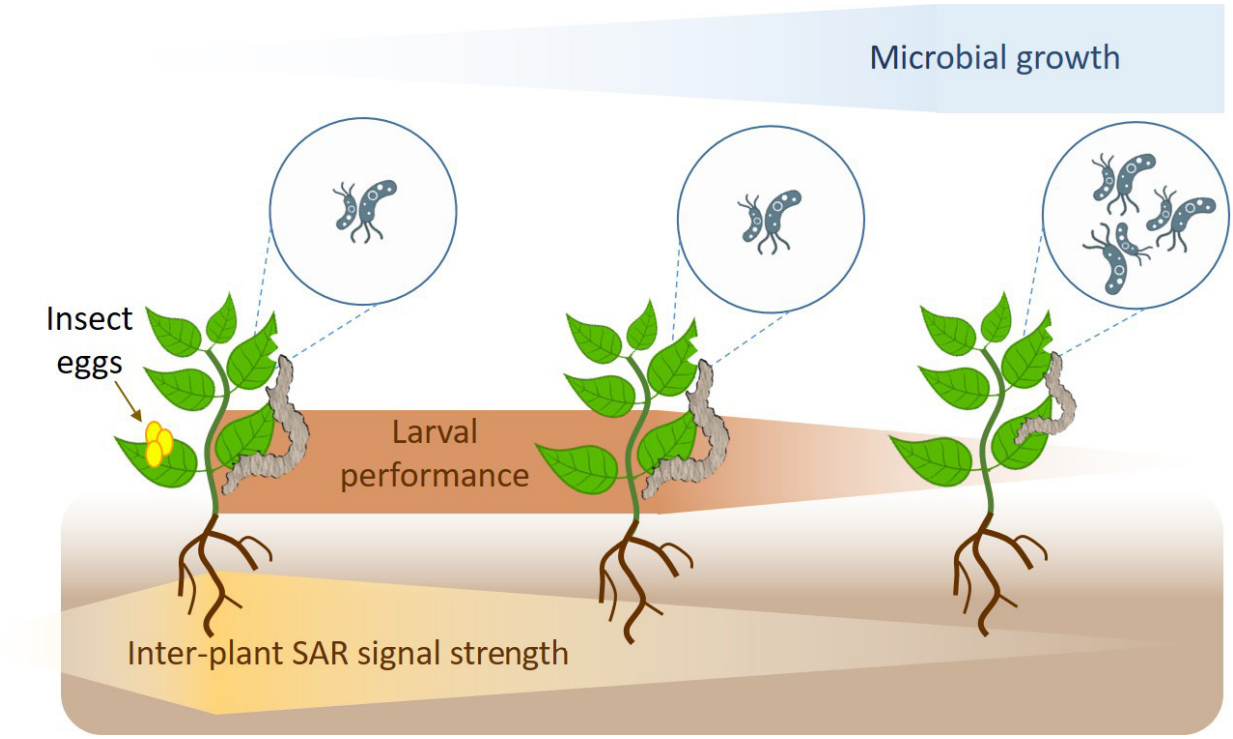

Fig. 1. Insects have evolved strategies to favour optimal development of future larvae by providing a niche of host plants with reduced infection and reduced anti-insect defences

Ranking of rivers of the Kurzeme Region, Latvia in accordance to the reproduction potential of river lamprey upstream from the migration barriers

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Key words: anadromous species, *Lampetra fluviatilis*, migration barriers, river lamprey, river connectivity.

With annual landings of 50 to 100 t, river lamprey *Lampetra fluviatilis* L. is one of the most important species in Latvian inland fishery. At the same time, river lamprey is also endangered species protected by Council Directive 92/43/EEK and other national and international acts of nature protection. One of the most important threats to this species is migration barriers that imped access to spawning grounds, and to reduce this impact several mitigation measures are implemented across Europe (Clemens et al. 2020). The benefit of different mitigation measures greatly depends on existing reproduction potential upstream from each barrier.

In this paper we describe results of our attempt to rank rivers in Kurzeme Region in accordance to the river lamprey reproduction potential upstream from the migration barriers. This research was conducted within Interreg project LI-310 "Cross-boundary evaluation and management of lamprey stocks in Lithuania and Latvia" (LAMPREY). Due to the project-related boundaries this research was confined to rivers located in Kurzeme Region and was conducted in relatively short period of time without any additional field surveys.

In the beginning a special method was developed for comparison of river lamprey reproduction potential in different rivers. This method consisted of two steps where the first step was a simple decision tree and the second step was calculation of "index of importance" for each barrier. Decision tree consisted of two questions: "Do migrating lampreys reach the barrier?" and "Is catchment upstream from the obstacle suitable for reproduction of lampreys?", and three answers to each question: "yes", "no" and "most probably no, but we are not sure". Different data sources were used to answer these questions, including the results of lamprey larvae monitoring, electrofishing surveys, catch statistics, available information on parameters of the river, and in some cases also photos or other indirect data. Only rivers with two positive answers qualified for the next step.

The second step included "index of importance" calculation for each barrier. This index was calculated using a specially developed formula:

$$IIbarrier = [(ALriver \times CSriver) + (ALtrib \times CStrib)] \times IFbarrier \times \sqrt{CA} \times Indhab\% + IFbarrier \times IInextbarrier \times (1 - IFnextbarrier)$$

where:

IIbarrier represents "index of importance" for the particular barrier; ALriver is available length of the river upstream from the barrier (km); CSriver is coefficient of suitability of the river upstream from the obstacle, from 0 (unsuitable) to 1 (perfect); ALtrib is available length of tributaries upstream from the barrier (km); CStrib is coefficient of suitability of tributaries upstream from the obstacle, from 0 (unsuitable) to 1 (perfect); IFbarrier is impact factor of the barrier, from 0 (no impact) to 1 (unpassable); CA is catchment area upstream from the obstacle (km^2); ndHab% is index of share of habitats upstream from the barrier (calculated by dividing the multiplication of square root of catchment area upstream from the particular barrier and total length of river and its tributaries upstream from the same barrier with multiplication of square root of total catchment area of this river and of sum of total length of the same river and its tributaries); IInextbarrier is calculated "index of importance" for the barrier located upstream from the particular barrier; IFnextbarrier is impact factor for the barrier located upstream from the particular parrier, from 0 (no impact) to 1 (unpassable).

Individual "indexes of importance" for each barrier were later summed up and the result used for ranking the rivers in priority order.

The greatest unexploited river lamprey reproduction potential (index value exceeds 11 000) in Kurzeme Region is located upstream from Ventas Rumba, a natural waterfall located 85 km from rivermouth of Venta River, which is the largest stream in Kurzeme Region. Other rivers can be grouped into four classes: (1) rivers with high lamprey reproduction potential upstream from barriers (index value > 100): Rīva, Roja, Riežupe and Alokste; (2) rivers with good lamprey reproduction potential (index value from 40 to 65): Grīva, Vārtāja, Virga, Pāce, Durbe, Tebra; (3) rivers

with moderate lamprey reproduction potential (index value from 20 to 31): Kauliņa, Dzirnavupe, Dakterišķe, Rudupe, Padure, Īvande; (4) rivers with low lamprey reproduction potential (index value < 20): Svente, Vanka and Alekšupīte. We believe that this rank illustrates the actual situation in Kurzeme region and can therefore be used as a tool for planning of various mitigation measures.

Indisputably, the most convenient method for assessing reproduction potential upstream from the migration barriers is a detailed field survey supported by GIS analysis. However, successful application of the previously described method for ranking rivers in Kurzeme Region in accordance to river lamprey reproduction potential suggests that this method can be useful in similar situations when swift ranking of impact of the barriers is needed but additional field surveys cannot be performed. Originally this method was focused on lampreys, but we believe that it can also be applied to other anadromous species.

Acknowledgements

We are grateful to our colleague Robertas Staponkus from Klaipeda University for providing useful comments in development of the method that we used for river ranking. Additionally, we would like to extend our gratitude for the support to all our colleagues who were involved in the LAMPREY project.

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Seeds as explant source for tissue culture inititation of seven rare coastal plant species of the Baltic sea

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Key words: Baltic sea, costal plants, *in vitro*, seed germination, tissue culture.

The efficient and sustainable use of natural resources is an essential component of the circular economy that can create products with a high added value. The plant tissue culture approach is a critical step in the introduction of such uses, as it enables the acquisition and use of plant resources for practical economic purposes and scientific research without harming nature, while benefiting both nature protection (conservation and reintroduction of rare plant species) and environmental conservation (recultivation of degraded territories).

In this study seeds from seven rare coastal species were tested for their ability to germinate in *in vitro* conditions in order to establish tissue culture (Table 1). Whenever it was possible various genotypes for the same species were used. For each genotype 30 seeds were used (exception was *Calystegia soldanella* with five seeds) for culture establishment. Seeds were surface sterilized with a commercial bleach ACE (33 to 50%) for 10 to 12 min followed by three washes with sterile deionized water. Surface sterilized seeds were germinated in 100 mL plastic jars on half- strength Murashige and Skoog medium supplemented with sucrose (30 g L^{-1}) and agar (6 g L^{-1}) (Kļaviņa et al. 2006). Prior autoclaving pH was adjusted to 5.8. Seeds were germinated under 16-h photoperiod provided by a fluorescent light with photon flux density 50 µmol m^{-2} s^{-1} at 25 °C. Seeds of *Trifolium fragiferum, Linaria loselii* and *Calystegia soldanella* (Ko et al. 2004) needed additional treatment in order to germinate (Table 1).

Germination rate for the same species varied depending on the genotype and treatment. Generally, our data supported data found in literature (Klavina et al. 2006) that seeds of *Phleum arenarium* and *Plantago maritima*

Table 3. Species and seed sources of accessions used to initiate *in vitro* culture

Species	Seed origin (year of collection)	Days of germination	Germination (%)	Special treatment
Armeria maritima	National Botanical Garden, Latvia (2020)	46	37	–
	Vecdaugava, Latvia (2020)	46	43	–
	Nybro, Sweden (2017)	46	0	–
	Vakarbuļļi, Latvia (2020)	46	40	–
Calystegia soldanella	Plant World Seeds (2020)	7	100	Soaked for 3 h in concentrated H_2SO_4 before surface sterilization
Linaria loeselii	Ovīši, Latvia (2016)	41	23	Medium additionally supplemented with 100 mg L^{-1} gibberellic acid
	Ovīši, Latvia (2016)	60	0	–
Phleum arenarium	Ovīši, Latvia (2016)	15	100	–
Plantago maritima	Ohesaare, Estonia (2018)	28	70	–
	Bornholm, Denmark (2017)	28	100	–
Rumex sanguineus	Jelitto Seeds (2020)	15	90	–
Trifolium fragiferum	Liepāja, Latvia (2016)	35	0	scarification
	Lielupe, Latvia (2016)	35	3	scarification
	Ainaži, Latvia 2020	35	50	scarification
	Bornholm, Denmark 2017	35	13	scarification
	Rīga, Latvia (2019)	35	33	scarification
	Liepāja, Latvia (2015)	35	50	scarification

germinated excellently, but seeds of *Trifolium fragiferum* required scarification.

It has been reported earlier (Necajeva, Probert 2011) and was confirmed by our data that *Linaria loselii* seeds may stay dormant without additional treatment. Adding 100 mg L^{-1} gibberellic acid to the growth medium was successful to break the dormancy. Previously this treatment has been described for *Gentiana cerina* (Morgan et al. 1997). Further research about optimal gibberellic acid concentrations for *Linaria loselii in vitro* seed germination is still required.

Once Baltic see costal plant tissue culture potential will be evaluated, *in vitro* plantlets could be used as model systems for wide range of experiments.

Acknowledgements

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Production of *Trichoderma asperellum* biomass under different submerged cultivation regimes

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Key words: antifungal activity, biomass, submerged cultivation, *Trichoderma*.

The work involves the development of an appropriate biotechnological method to obtain products with low water content and high concentration of active microorganisms. Economically viable production of soil microbiological fertilizers and plant protection agents have not yet been fully explored (MarketsandMarkets 2019). During cultivation, *Trichoderma* spp. produces various valuable secondary metabolites (peptaibols, polyketides, pyrones, terpenes, etc.), so the liquid produced could have antifungal activity against phytopathogens. Production of microbial fertilizers and plant protection agents reduces the use of chemical fertilizers and pesticides (de Rezende et al. 2020).

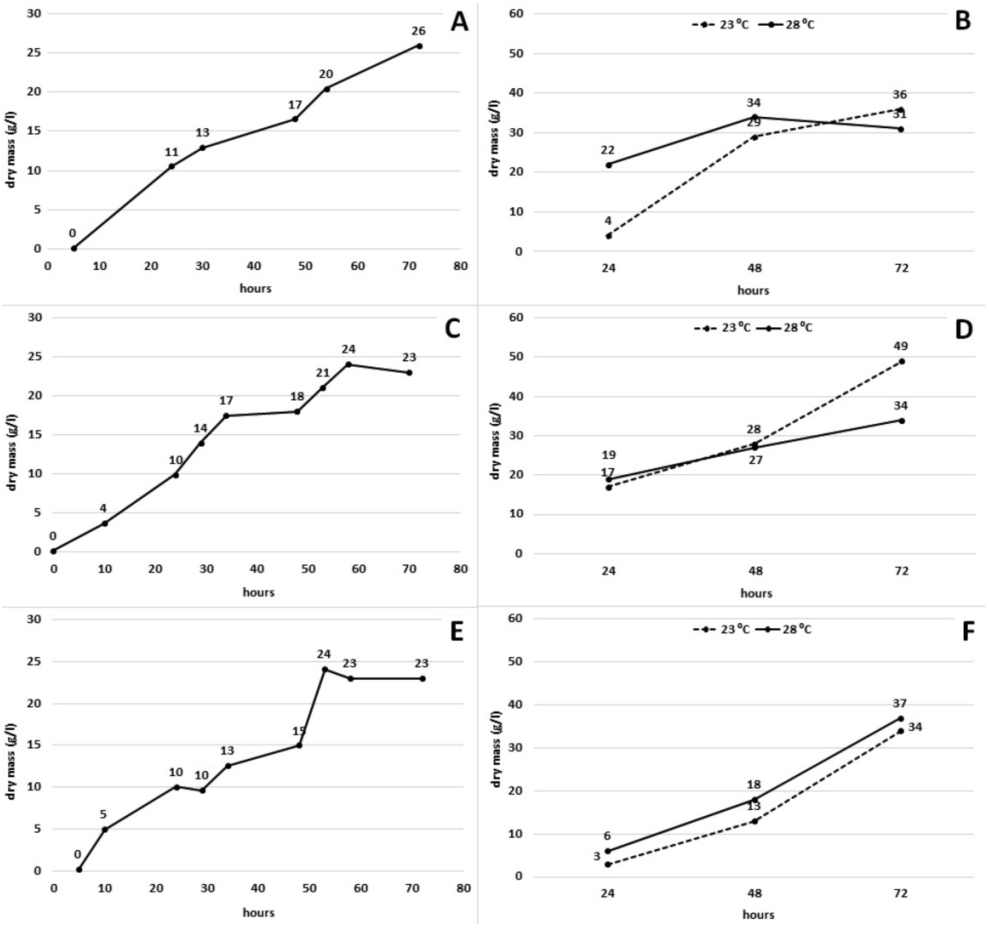

Fig. 1. Submerged cultivation of *Trichoderma asperellum* in different media. A, bioreactor with sucrose-yeast extract medium; B, Erlenmeyer flasks with sucrose-yeast extract medium; C, bioreactor with sucrose-yeast extract-peptone medium; D, Erlenmeyer flasks with sucrose-yeast extract-peptone medium; E, bioreactor with sucrose-yeast extract-peptone-$MgSO_4$-$CaCl_2$ medium; F, Erlenmeyer flasks with sucrose-yeast extract-peptone-$MgSO_4$-$CaCl_2$ medium.

The aim of the study was to investigate the growth of *Trichoderma asperellum* MSCL 309 at various regimes of the submerged cultivation and to determine the activity of its released metabolites against phytopathogenic fungi.

During the study, two experiments took place in parallel. The first experiment was set up in Erlenmeyer flasks, each flask was filled with a certain liquid medium with additives. A suspension of *T. asperellum* was introduced into each flask. Inoculated media were placed on a shaker, cultured for 72 h. To detect changes in the amount of *T. asperellum* biomass, culture medium with *T. asperellum* biomass was obtained from each flask every 24 h (3 days). The liquid was centrifuged. The supernatant was discarded, but the biomass in the sediment was weighed.

In the second experiment, a five liter bioreactor was used for the submerged cultivation process. A constant stirring speed was maintained, revolutions of 200 rpm, aeration of 2 L min^{-1} and a temperature of 28 to 30 °C.

The biomass obtained in the bioreactor was decanted and centrifuged. The liquid supernatant was discarded, but the biomass in the sediment was weighed to determine the percentage of dry biomass. In both experiments, the antifungal activity of *T. asperellum* supernatant against phytopathogens of *Fusarium oxysporum* MSCL 259, *Verticillium dahliae* MSCL 863 and *Cladosporium herbarum* MSCL 267 was determined.

The highest amount of *T. asperellum* dry biomass in the reactor with sucrose-yeast extract medium was detected after 72 h (26 g L^{-1}). The fastest biomass increase was observed between 5 and 24 h (Fig. 1A). The amount of dry biomass in submerged cultivation in flasks reached its maximum 48 hours after the beginning of the experiment (29 to 34 g L^{-1}). The amount of *T. asperellum* biomass did not change significantly between different temperatures (Fig. 1B).

The amount of dry biomass of *T. asperellum* reached its maximum amount in the bioreactor with sucrose-yeast extract-peptone medium after 58 h (24 g L^{-1}). The same amount of dry mass was also found 70 h after the start of the experiment (Fig. 1C). The highest amount of dry biomass in submerged cultivation in flasks was obtained after 72 h, at 23 °C 49 g L^{-1}, but at 28 °C 34 g L^{-1} (Fig. 1D).

The highest values of *T. asperellum* dry biomass in the reactor with sucrose-yeast extract-peptone-$MgSO_4$-$CaCl_2$ medium were found after 53 h (24 g L^{-1}), the indicator remained after 72 h from the beginning of the experiment. The fastest increase in biomass was observed between 48 and 53 h (Fig. 1E).

The amount of dry biomass in submerged cultivation in flasks reached its maximum after 72 h from the beginning of the experiment (34 to 37 g L^{-1}). The amount of *T. asperellum* biomass did not change significantly between different temperatures (Fig. 1F).

The highest yield of *T. asperellum* dry mass in the reactor was achieved in sucrose-yeast extract medium (26 g L^{-1} after 72 h), but in submerged cultivation in flasks – in sucrose-yeast extract-peptone medium (49 g L^{-1} after 72 h). No antifungal activity against phytopathogenic fungi was observed in any of the samples. In some samples, area of influence of phytopathogenic growth could be observed, without complete inhibition. Chlamydospores were observed in *T. asperellum* biomass, but no conidia were formed.

Acknowledgements

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Armeria maritima from a dry coastal meadow: Na and K tolerance and ion accumulation

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Key words: *Armeria maritima*, coastal plant species, electrical conductivity, growth, ion accumulation, salinity.

Rosette-forming perennial species *Armeria maritima* (Mill.) Willd. (Plumbaginaceae) is characteristically found on temperate open habitats with dry, saline, sandy or metal-rich soil. Existence of possible species-wide metal tolerance of *A. maritim*a is still under scientific exploration. Previously it was shown that *A. maritima* subsp. *elongata* accession from a dry coastal meadow in Southern Sweden has extremely high tolerance against heavy metals (biogenous Mn and Zn, as well as nonbiogenous Cd and Pb), acting as a metal hyperaccumulator (Andersone-Ozola et al. 2020). Comparative assessment of salt tolerance and accumulation potential of electrolytically active ions of diferent populations of *A. maritima* has been only seldom performed (Köhl 1997). *A. maritima* from salt marsh acumulated 3.8 g kg^{-1} Na in control soil and 21.2 g kg Na in saline soil (Cooper 1982). In natural conditions, *A. maritima* accession from a salt-affected relatively dry meadow accumulated 12 g Na kg^{-1} dry mass in leaves (Ievinsh et al. 2021). The aim of the present study was to analyze salinity tolerance of *A. maritima* subsp. *elongata* accession from a dry coastal meadow in controlled conditions. A special attention was paid to possible different growth responses and ion accumulation potential to separate treatment with NaCl or KCl, or simultaneous treatment with both salts.

Plants were established from seeds collected in a natural population (Nybrostrand, Sweden) and grown in a conditions of an automated greenhouse as described previously (Andersone-Ozola et al. 2020). Fully developed 2-month-old plants in a vegetative stage were asigned to one of 12 treatments (Table 1), five individual plants per treatment. Salt treatment was performed gradually, by not more than 44 mmol L^{-1} increments during five weeks, using NaCl and KCl solution. During treatments, plants started to develop generative structures. Plants were cultivated for eight more weeks after reaching full treatment. At the end of the experiment, plants were separated in roots, dry leaves, living leaves, flower stalks and inflorescences. All parts were carefully washed with deionized water, blotted dry and fresh mass and dry mass (after drying at 60 °C for 72 h) were measured. Plant tissues were homogenized by crushing and a sample (0.2 g) was taken for analysis of electrical conductivity, Na^+ concentration and K^+ concentration in water extract by respective LAQUAtwin compact meters (Horiba Scientific, Japan) and analysis of osmotic activity by a freezing point osmometer (Osmomat 3000, Gonotec, Germany).

Effect of treatment with Na^+ alone (Fig. 1A), K^+ alone (Fig. 1B) or in combination (Fig. 1C) on plant growth was comparatively similar. Significant differences were found only for root biomass, where there was a more severe growth inhibition by K^+ at the two highest concentrations used in comparison to that by Na^+. Total biomass of *A. maritima* plants did not increase even at low salinity (Fig. 2), but dry mass of living leaves significantly increased by both Na^+ and K^+ treatments at 22 mmol L^{-1} concentration, while dry mass of roots significantly increased only in 22 mmol L^{-1} NaCl treatment.

Accumulation potential for Na^+ was the highest in dry leaves (up to 55 g kg^{-1}) followed by living leaves (up to 25 g kg^{-1}). Flower stalks accumulated only up to 14 g kg^{-1} Na^+, with 8 g kg^{-1} in inflorescences. Na^+ concentration in roots did not significantly increae with increasing substrate Na^+ concentration. Presence of equimolar concentration of K^+ in substrate diminished Na^+ accumulation by 37% only in dry leaves.

K^+ acumulation potentail was relatively higher than that for Na^+, with no significant differences between aerial parts reaching 110 g kg^{-1}, except lower concentration (60 g kg^{-1}) at 217 mol L^{-1} KCl in inflorescences. Presence of equimolar

Table 1. Experimental treatments used in the present study with *Armeria maritima* subsp. *elongata*. NaCl and KCl was used as a source of Na^+ and K^+, respectively

Molar concentration (mol L^{-1})	Ion concentration		
	Na^+ (g L^{-1})	K^+ (g L^{-1})	$Na^+ + K^+$ (g L^{-1})
0	0	0	0
22	0.5	0.85	0
44	1.0	1.7	0.5 + 0.85
87	2.0	3.4	1.0 + 1.7
217	5.0	8.5	2.0 + 3.4

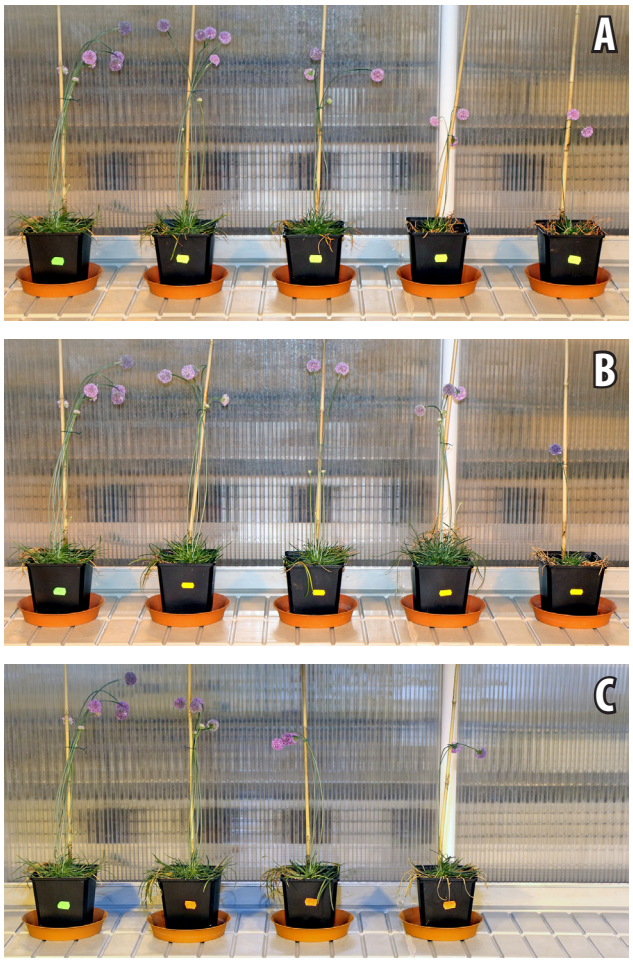

Fig. 1. Effect of increasing concentrations of NaCl (A), KCl (B), and equimolar concentrations of NaCl and KCl (C) on morphology of *Armeria maritima* plants. A & B, 0, 22, 44, 87, 217 mol L^{-1}; C, 0, 44, 87, 217 mol L^{-1} (from left to right).

cconcentration of Na^+ diminished K^+ accumulation in all plant parts by about 25%.

As a result, summed molar concentration of Na^+ + K^+, electrical conductivity, as well as osmotic activity in leaves were higher at 217 mol L^{-1} for K^+-treated plants, in comparison to Na^+-treated plants, with combination treatment resulting in average values. It is interesting that osmotic component not dependent on Na^+ and K^+ increased only in Na^+-treated *A. maritima* plants in a concentration-dependent manner, but it decreased in K^+-treated plants up to 87 mmol L^{-1}, followed by increase at 217 mol L^{-1}. This effect was characteristic only for living leaves and was not found in generative organs.

Thus, *A. maritima* plants from relatively salinity-unaffected dry coastal meadow showed high tolerance to salinity together with very high ion accumulation potential irrespective of catione (Na or K). Accumulation potential of Na^+ was clearly organ-specific, decreasing in order

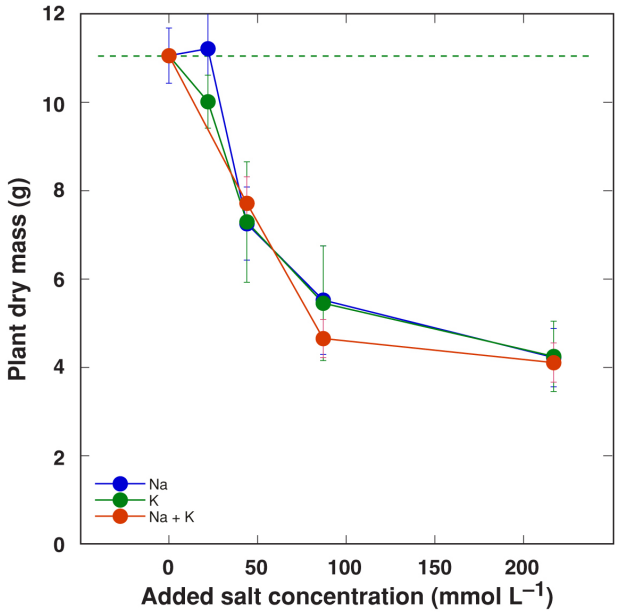

Fig. 2. Effect of different concentration of added salt in substrate on total dry mass of *Armeria maritima* plants. Na, as NaCl; K, as KCl; Na + K, as equimolar concentrations of NaCl and KCl.

dry leaves > living leaves > flower stalks > inflorescences > roots. In contrast, only *A. maritima* plants from a salt marsh population accumulated slightly more Na in leaves in comparison to that in roots, but plants from sandy soil as well as heavy metal populations accumulated significantly higher concentration of Na^+ in roots (Köhl 1997). Surprisingly, in that study, higher concentration of Na in roots (up to 30 g kg^{-1} dry mass) was reached in plants from sandy soil and heavy metal populations, with only up to 9 g kg^{-1} in plants from salt marsh population. According to the established threshold concentration values for Na^+ hyperaccumulation for coastal plant species of the Baltic Sea (Ievinsh et al. 2021), the particular accession of *A. maritima* can be classified as electrolytophytic Na^+ hyperaccumulator.

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Responses of two ecotypes of *Mentha aquatica* to salinity, heavy metals and mineral nutrient availability

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Key words: ecotypes, ion accumulation, *Mentha aquatica*, Pb, mineral nutrient availability, salinity.

Mentha aquatica L. (Lamiaceae) is a semi-aquatic plant species characteristic for European protected habitats EU 3260 "Water courses of plain to montane levels with the Ranunculion fluitantis and Callitricho-Batrachion vegetation" and EU 6430 "Hydrophilous tall herb fringe communities of plains and the montane to alpine levels". This perennial plant species has a high potential for clonal spread due to ability of procumbent shoots to produce adventitious roots at nodes as well as due to spread by underground rhizomes. An ecotype of *M. aquatica* found on coasts of the Baltic Sea in Finland and Sweden has been described as *M. aquatica* var. *litoralis*. Recently an accession of *M. aquatica* was found on a sea-affeted wet sandy beach in Ainaži, Latvia, possibly representing *M. aquatica* var. *litoralis*. In natural conditions, these plants accumulated 19.6 g kg^{-1} Na^+ in leaves (Ievinsh et al. 2021). The aim of the present study was to evaluate possible differences in physiological responses of the two accessions of *M. aquatica* to salinity, heavy metals (Pb), and mineral nutrient availability. Seeds of a standard type *M. aquatica* var. *aquatica* were obtained from a commercial source (Jelitto Seeds, Germany) and established plants were further propagated vegetatively. It was expected to find out that *M. aquatica* var. *litoralis* has higher salinity tolerance and ion accumulation potantial in comparison to these for *M. aquatica* var. *aquatica*.

Plants were cultivated in conditions of an automated greenhouse in a commercial garden soil with addition of 10% (v/v) quartz sand. In a salinity tolerance experiment, established *M. aquatica* plants were gradually treated with NaCl, reaching final concentration of 0.5, 1.0, 2.0, and 4.0 g Na^+ per L of substrate. In a heavy metal tolerance experiment, plants were treated with Pb acetate, with final concentration of Pb per L of substrate reaching 0.1, 0.2, 0.5, and 1.0 g. In a mineral nutrient availability experiment, plants were fertilized weekly with Kristalon Blue plus Calcinit fertilizers in a concentration 0.5, 1.0, or 2.0 g L^{-1}. For the last experiment, performed in summer season, only natural sunlight was used in contrast to the two first experiments performed in winter, where supplemented light with photosynthetic photon flux density of 350 μmol m^{-2} s^{-1} with 16 h photoperiod was used. Plants were cultivated for additional 8 to 10 weeks after reaching full treatment. After termination of experiments, plants were separated in different parts (vertical stems, leaves on vertical stems, procumbent stems, leaves on procumbent stems, underground stems, and roots) and number and length of stems, as well as fresh mass, dry mass and water content in different parts were measured.

Contrary to what was expected, salinity tolerance of var. *litoralis* was less pronounced than that in var. *aquatica*. Total dry mass of plants decreased by 47 and 60% for var. *litoralis* and by 13 and 27% for var. *aquatica*, at 2 and 4 g Na^+ L^{-1}, respectively. However, dry mass of underground shoots increased by 11% at 4.0 g Na^+ L^{-1} for var. *litoralis*, in comparison to control. In contrast, var. *aquatica* did not form any underground shoots. For var. *aquatica*, dry mass of vertically-oriented shoots and leaves on them increased by 33 and 16%, respectively, but mass of creeping shoots and leaves on them decreased by 53 and 72%, respectively, at 4.0 g Na^+ L^{-1}, in comparison to control plants.

However, pronounced differences in ion accumulation pattern and concentrations were found between the plants of the two genotypes. First, while accumulation potential for Na^+ in roots by the two genotypes was relatively similar, reaching 6 to 8 g kg^{-1}, plant leaves and shoots of var. *litoralis* accumulated significantly more Na^+ (15.5 g kg^{-1}) in comparison to that in var. *aquatica* (8.5 g kg^{-1}). Second, also all parts of var. *litoralis* accumulated significantly higher concentration of K^+ (up to 20 g kg^{-1}) in comparison to var. *aquatica* (up to 8 g kg^{-1}). In addition, increasing concentration of substrate Na^+ reduced K^+ accumulation for var. *litoralis* in above-ground parts, but increased it in leaves of var. *aquatica*.

Both ecotypes showed relatively high tolerance against Pb, with no changes in biomass for var. aquatica and decrease only by 18% for var. *litoralis* at 1.0 g Pb L^{-1} in substrate. For both ecotypes, majority of Pb accumulated in roots, reaching 1.1 and 1.5 g kg^{-1} for var. *litoralis* and var. *aquatica*, respectively. The respective concentration of Pb in above-ground parts was only 195 and 137 mg Pb kg^{-1}, at 1.0 g Pb L^{-1}.

Fig. 1. Effect of different levels of mineral nutrient availability (indicated as MIN in g L^{-1}) on morphology of *Mentha aquatica* var. *litoralis* (A) and *Mentha aquatica* var. *aquatica* (B) plants cultivated without or with 4 g L^{-1} Na (Na).

Increase in mineral nutrient availability from 0.5 to 2.0 g L^{-1} in natural light conditions resulted in relatively small increase in plant biomass (by 37% in var. *litoralis* and 35% in var. *aquatica*; Fig. 1). However, treatment with 4 g L^{-1} Na+ in these conditions did not result in significant changes in total biomass of var. *litoralis* plants, while biomass of var. *aquatica* plants decreased by 35%. Also, differences in Na^+ accumulation capacity between the two ecotypes were inverted, with roots of var. *aquatica* accumulating higher concentration of Na^+ in comparison to these of var. *litoralis*. Ion accumulation potential in above-ground parts was similar for the two exotypes. Consequently, it appears that higher ion accumulation capacity of *M. aquatica* var. *litoralis* in comparison to var. *aquatica* is evident only in high llight conditions.

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Calystegia sepium and *Calystegia soldanella* as model species in ecophysiological studies: propagation potential and opportunities

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Key words: *Calystegia sepium, Calystegia soldanella*, clonal species, ecotypes, propagation potential.

Calystegia sepium (L.) R.Br. is a clonal climbing plant species found in a variety of habitats. It is an umbrella species of the European protected habitat EUH 6430 "Hydrophilous tall herb fringe communities of plains and the montane to alpine levels", but exhibits high diversity in respect to a range of habitats where it can be found. Thus, at one extreme, *C. sepium* is a component of nitrophilous mesophytic vegetation of river banks and other similar habitats; at the other, it is frequently located in relatively arid semi-ruderal habitats like road verges. Moreover, *C. sepium* is often found on coastal habitats, being an important species of salt-adapted vegatation complexes of the Southern Baltics (Hulisz et al. 2016). These facts raise the question on existence of different ecotypes of *C. sepium* as based on genetical variation in certain adaptive characteristics. Due to predominantly vegetative mode of propagation of the species (Klimeš, Klimešová 1994), it is highly possible that certain populations consist of a single locally well-adapted clone of *C. sepium*.

Taxonomically closely related species, *Calystegia soldanella* (L.) R.Br. ex Roem. & Schult., is a coastal-specific creeping clonal plant with a cosmopolitic distribution, characteristic for sand dunes (Arafeh, Kadereit 2006). The species has pronounced drought and, presumably, salinity tolerance, but the latter has not been assessed experimentally. The aim of the present study was to establish an experimental system for using *C. sepium* and *C. soldanella* as model species in ecophysiological studies in controlled conditions, using different accessions of *C. sepium* from various habitats as well as *C. soldanella* seeds from a commercial source as material for vegetative propagation.

Rhizome fragments from various accessions of *C. sepium* were collected in three different habitats in June and July 2020 (Table 1). The rhizome fragments were used to establish a laboratory stock culture of *C. sepium*. Seeds of *C. soldanella* were purchased from Plant World Seeds, UK. Before planting, seeds were treated with concentrated H_2SO_4 for 3 h to interrupt physical dormancy.

Successful development of established plants from rhizome fragments of *C. sepium* was achieved within two weeks (Fig. 1). After transfer to vegetation containers, plants

Fig. 1. Young established plants of *C. sepium* formed from mature rhizome fragments.

Table 1. Plant material used in the present study for establishment of laboratory stock culture of *Calystegia sepium* and *Calystegia soldanella*

Species, accession	Habitat	Establishment of stock culture	Characteristics	Locality (coordinates)
C. sepium 1	Coastal, sandy beach with perennial vegetation	Rhizome fragments	White flowers	Mērsrags, Latvia (N 57°21'57" E 23°7'21")
C. sepium 2	Inland, mesophytic vegetation on banks of pond	Rhizome fragments	White flowers	Salaspils, Latvia (N 56°51'32" E 24°30'38")
C. sepium 3	Inland, dry grassland vegetation on a steep river bank	Rhizome fragments	Pink flowers	Ogre, Latvia (N 56°48'59" E 24°36'59")
C. soldanella	Coastal, sand dunes	Seeds	NA	NA

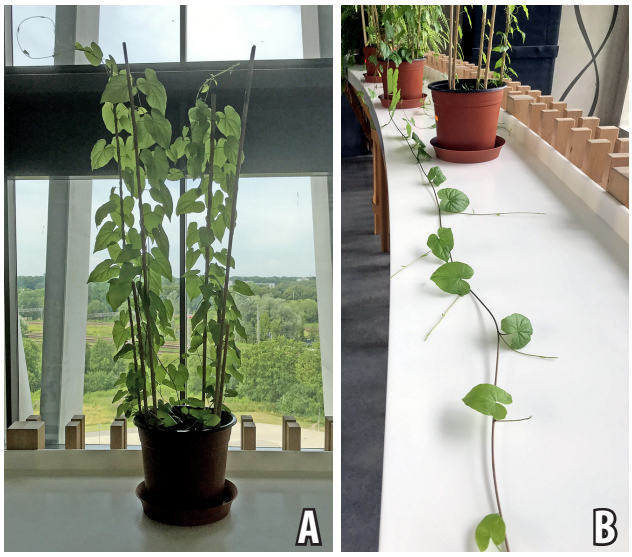

Fig. 2. Dvelopment of climbing (A) and creeping (B) stems of *Calystegia sepium*.

Fig. 3. Dvelopment of creeping stems of *Calystegia soldanella*.

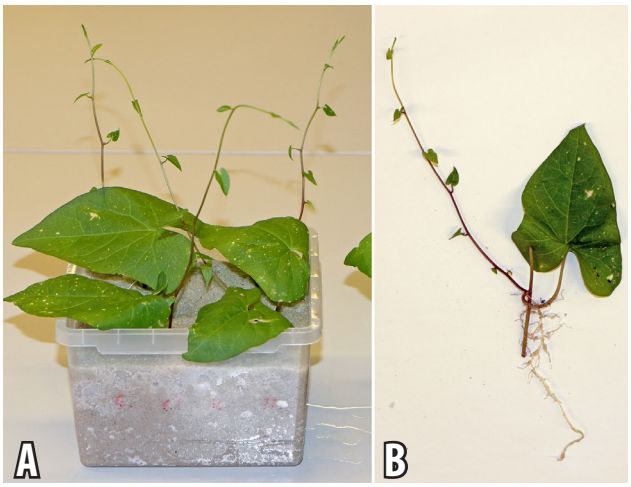

Fig. 4. Propagation of *Calystegia sepium* by fragments of creeping stem with single leaf (A) and rooted explant of *Calystegia sepium* (B).

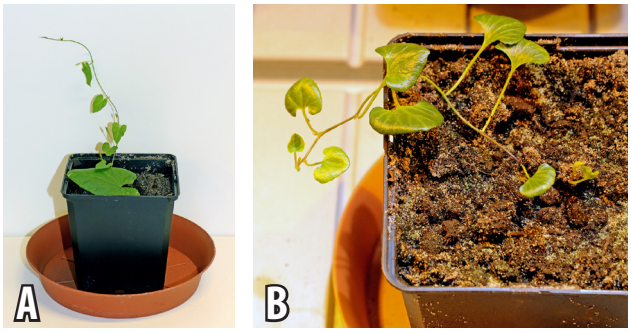

Fig. 5. Established plants of *Calystegia sepium* (A) and *Calystegia soldanella* (B) propagated by fragments of creeping stem with single leaf.

showed vigourous development of climbing shoots for the next four weeks (Fig. 2A), but after that, development of creeping stems started (Fig. 2B) in parallel to flowering. Plants of *C. soldanella* showed active development and growth of creeping stems during prolonged period of time (more than 10 weeks) without initiation of flowering (Fig. 3). Different plant parts were further used for clonal propagation of *C. sepium* and *C. soldanella*, including newly-formed rhizome fragments and creeping stem fragments with a single leaf.

The best results for all accessions of *C. sepium* and *C. soldanella* was achieved by using fragments of creeping stems with single leaf, placed in wet quartz sand. These explants showed 100% establishment with active development of previously dormant vegetative bud and formation of root at leaf nodes (Fig. 4). Immature rhizome fragments had no potential for propagataion. Rooted stem explants easy established in soil. However, in contrast to *C. soldanella*, which further developed well (Fig. 5), propagated plants of *C. sepium* showed only short phase of growth of climbing stems, followed by flowering and development of creeping stems within six weeks and further induction of senescence of above-ground parts.

In conclusion, both mature underground rhizomes and creeping stem fragments with a single leaf had high potential for use in vegetative propagation of *C. sepium,* but the plants established from stem fragments had only limited growth potential. In contrast, creeping stem fragments with a single leaf from *C. soldanella* had high propagation potential with ability for prolonged vegetative growth of established plants.

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Strawberry clover (*Trifolium fragiferum*) in the Baltic Sea region: scientifically alluring clonal legume species and undervalued economic resource

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Key words: abiotic stress tolerance, anthropogenic stress tolerance, crop wild relatives, *Trifolium fragiferum*.

In a view of climate changes and growing anthropogenic impact on ecosystems, increasing sustainability of agricultural production is of critical importance. In order to ensure productivity and increased added value in this complictaed situation, it is necessary to develop new more diverse crop cultivars better adapted to increasing environmental heterogeneity and being able to give high quality yields in conditions of changing climate. Crop wild relatives (CWR) are valuable as a genetic resource in this respect, representing a source of environmental tolerance-associated characteristics. Several international strategies, especially, at the European level, have been developed in recent decades aiming at conservation, study and sustainable exploitation of crop genetic diversity. One of the important aspects of these strategies is related to exploration of local CWR resources.

In Latvia CWRs are relatively poorly represented, with only pernennial forage grasses and legumes being more widely accessible. Legumes are especially important in a view of agricultural sustainability both as high quality protein crops as well as nitrogen fixing species leading to increased soil fertility withot a need to apply high doses of mineral nitrogen fertilizers. While scientific attention has been focused recently on wild genetic resources of several traditional clover (*Trifolium*) species in Latvia and other countries of the region for their potential use as forage crops, one extremely rare wild clover species, *Trifolium fragiferum*, has remained neglected. While not commercially used in Europe, *T. fragiferum* has been cultivated in other regions of the world showing exceptional agronomical qualities, including salinity and disease tolerance. In order to understand the diversity of *T. fragiferum* accessions in Latvia and their potential importance as a breeding material for forage crop development, it is necessary to perform thorough agrobiological evaluation of the available plant material.

We have previously hypothesized that in conditions of the Baltic region *T. fragiferum* can outcompete *Trifolium repens* only in saline coastal habitats due to significantly higher salinity tolerance of the former (Dūmiņš et al. 2017). Thus, it appears that wild accessions of *T. fragiferum* need to be explored as potential donors of genes for abiotic stress tolerance. However, there is no information available in the scientific literature on heavy metal or trampling tolerance of *T. fragiferum* genotypes. Most importantly, genetic diversity among *T. fragiferum* introductions has been reported to be sufficient to develop cultivars with high tolerance to abiotic stress factors, especially, soil salinity (Rumbaugh et al. 1993).

Symbiosis with nitrogen-fixing rhizobia has been described as an important constituent in mineral nutrition of *Trifolium* species. The outcome of the interaction between closely related species in conditions of high substrate salinity could be affected by efficiency of symbiotic nitrogen fixation. Resistance of rhizobial bacteria to soil salinity may differ from that of their host, moreover, the response of symbiotic nodule to salinity can be different from both individual responses (Kaushal, Wani 2016). In nutrient-limiting conditions, rhizobial inoculation usually has positive effect on growth of clover species and other legume plants, depending both on compatibility and efficiency of used bacterial strains. Therefore, dependence of particular accession of *T. fragiferum* from their native rhizobial symbionts can be evaluated in sterile cultures. In further, genes related to efficiency of symbiotic relationship for N-fixing can be another target for improvement of production of forage yield and quality in low N-input environment-protecting conditions.

The concept on the basis of the proposed study is that it is possible to perform preliminary evaluation of different accessions of potentially valuable CWRs in controlled conditions in relatively short time to reveal any functional differences related to stress tolerance, in parallel to molecular analysis of their genetic diversity and ensuring conservation *ex situ* by means of tissue culture. Therefore, in controlled conditions, tolerance of all available accessions of *T. fragiferum* to abiotic and anthropogenic factors, as well as effect of biotic factors will be evaluated.

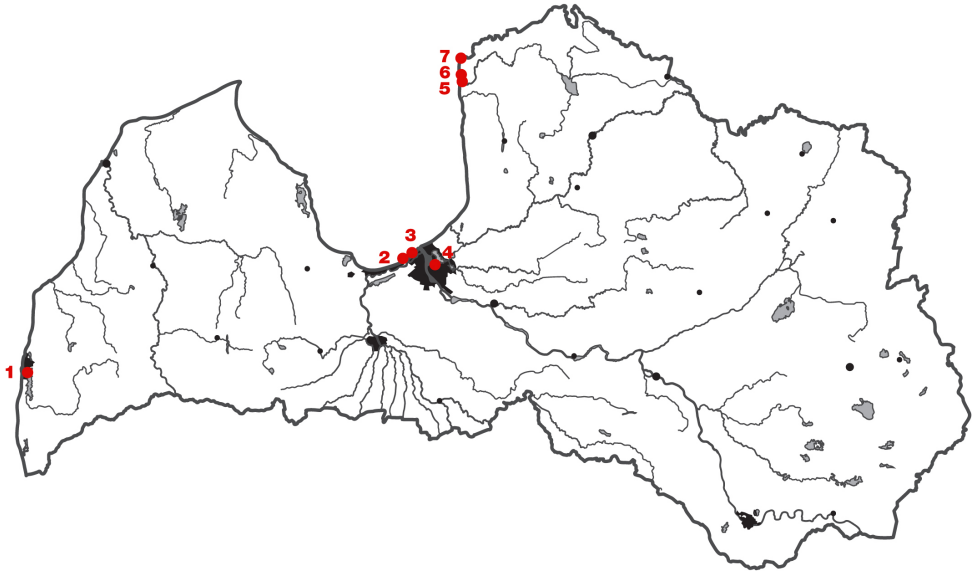

Fig. 1. Location of identified (micro)populations (accessions) of *Trifolium fragiferum* in Latvia.

We have identified seven spatially separated (micro) populations of *T. fragiferum* in the territory of Latvia (Fig. 1): wet meadow on shore of Lake Liepāja, city of Liepāja (1); bank of River Lielupe near estuary, city of Jūrmala (2); bank of River Buļļupe, city of Rīga (3); meadow in industrial territory in Skanstes region, city of Riga (4); wet coastal meadow in nature reserve Randu Pļavas, Salacgrīva region (5 & 6); coastal meadow, city of Ainaži (7). One of them, No. 4, has not been previously known and represents an unique genetic material. As a reference genotype, an elite commercial *T. fragiferum* cv. Palestine developed in Australia will be used.

The implementation of the project will lead to new scientific knowledge in the fields of agricultural biology and nature conservation. The obtained knowledge will have both fundamental and practical importance. From the fundamental point of view, new information will be acquired on population structure and possible functional differences of extremely rare coastal plant species. From the practical point of view, agronomically important characteristics of *T. fragiferum* accessions will be derived allowing to evaluate possible use of these genetic resources for improvement of forage legume cultivars, appropriate for use in heterogenous environmental conditions.

Acknowledgements

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NaCl tolerance and ion accumulation in *Rumex sanguineus* plants

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Key words: electrical conductivity, ion accumulation, *Rumex sanguineus*, salinity, water content.

Rumex sanguineus L., commonly found in Europe, is a perennial dock species characteristic for moist marginal woodland habitats, roadsides and wastelands. Presence of bright green leaves with dark red veins makes it attractive as an ornamental plant or as an element used in food decoration (Fig. 1). However, there is no information available on ecophysiological characteristics of *R. sanguineus*. According to ecological indicator values, the species has zero salinity tolerance, but at the north-eastern border of the distribution range it is associated with coastal habitats. As several wetland-adapted *Rumex* species from coastal habitats have shown relatively high salinity tolerance and electrolytically active ion accumulation capacity (Ievinsh et al. 2020), the aim of the present study was to evaluate tolerance of *R. sanguineus* to NaCl treatment in controlled conditions.

Seeds of *R. sanguineus* were purchased from Jelitto Seeds (Germany), germinated, and established plants were grown in commercial garden soil with addition of 20% (v/v) quartz sand in an automated greenhouse. Plants were gradually treated with NaCl, reaching final concentration of 1, 2, 3, and 5 g Na^+ per L of substrate. Five individual plants per treatment was used, control plants were watered with deionized water. Plants were fertilized each week using soluble fertilizer Kristalon Green plus Calcinit (Yara International, Norway). Plants were grown for additional five weeks after the full treatment. At the termination of the experiment, roots were separated from substrate and washed, but leaves were separately harvested according to their age group (old, middle, young leaves). Number of leaves in each group, fresh mass, dry mass and water content of all parts of each individual plant were measured. Electrical conductivity, Na^+ concentration and K^+ concentration in tissue extracts of leaf blades, leaf petioles, storage roots and small roots were measured separately using LAQUAtwin compact meters (Horiba Scientific, Japan).

Fig. 1. Characteristic appearance of leaves of *Rumex sanguineus*.

NaCl treatment even at 1 g L^{-1} resulted in plant growth inhibition (Fig. 2). Number of leaves decreased linearly with increasing substrate Na^+ concentration, but leaf fresh and dry mass were even more sensitive to salinity (Fig. 3). The most sensitive parameter was root growth, showing more than 50% decrease in biomass already at 1 g L^{-1} (Fig. 2). Water content significantly increased in all plant parts at 1 to 3 g L^{-1} Na^+, with tendency to decrease with further increase in substrate Na^+ concentration up to 5 g L^{-1}.

Control plants growing in a substrate without added NaCl accumulated relatively high concentration of Na^+ in their tissues, reaching 8, 14 and 16 g kg^{-1} in young, middle and old leaves, respectively. Increasing substrate Na^+ concentration resulted in linear increase of tissue Na^+ concentration in leaf blades, with significantly lower increase in leaf petioles. As a result, maximum Na+ concentration was in older and middle leaf blades (> 45 g kg^{-1}), with lower value in young leaf blades (30 g kg^{-1}). Na^+ concentration in both storage roots and small roots was low and did not significantly increase with increasing substrate Na^+ concentration (1 to 4 g L^{-1}). Accumulation potential for K^+ was relatively higher as that for Na^+, especially, in leaf

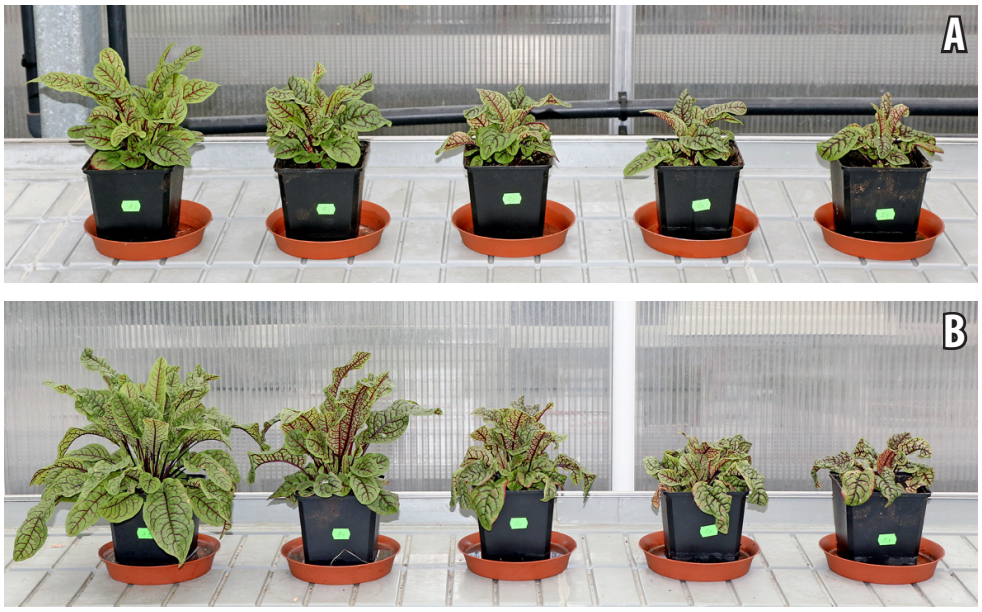

Fig. 2. Morphology of *Rumex sanguineus* plants as affected by different concentrations of Na^+ after the final treatment (A) and three weeks later (B). From left to right: control plant, plants treated with 1, 2, 3, 5 g L^{-1} Na^+.

petioles, but K^+ concentration in both leaf blade and leaf petiole tissues decreased with increasing Na^+ concentration in substrate. Due to salinity-induced increase in tissue water content, electrolytical activity in leaf tissue water did not increase with severity of treatment up to 3 g L^{-1}, and only at the highest treatment (5 g L^{-1}), plant tissues tended to have higher electrical conductivity.

It can be concluded that *R. sanguineus* plants have relatively high salinity tolerance and pronounced ion accumulation potential in vegetative stage, as well as tendency to accumulate water in leaf blades and leaf petioles in saline conditions, similarly to these characteristic for coastal accessions of other moisture-adapted *Rumex* species, such as *Rumex hydrolapathum, Rumex longifolius,* and *Rumex maritimus*.

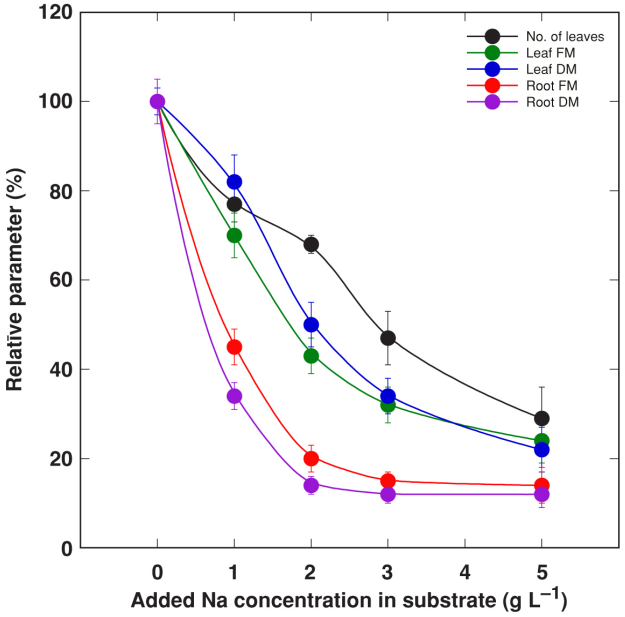

Fig. 3. Relative effect of increasing concentration of Na^+ on morphological parameters of *Rumex sanguineus* plants.

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Plantago coronopus and *Plantago maritima*: comparison of salinity tolerance and ion accumulation of the two coastal species

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Key words: coastal species, electrical conductivity, ion accumulation, *Plantago coronopus, Plantago maritima*, salinity

Studies aiming at understanding abiotic stress tolerance mechanisms of taxonomically closely related species is of great importance. Genus *Plantago* has gained a special attention in this respect, as there are several species with a coastal-specific distribution and putative salinity tolerance, as *Plantago maritima* and *Plantago coronopus*, in addition to wide-spread species with no supposed salt tolerance, as *Plantago major*. Differences in salinity tolerance between *P. coronopus* and *P. major* were shown to be related to differences in accumulation pattern of Na^+ and K^+ (Al Hasan et al. 2016). Recently, *P. maritima* has been identified as a typical K^+-excluding species, regulating tissue electrical conductivity by changes in Na^+ concentration (Ievinsh et al. 2021). However, direct comparison for salinity tolerance of the two coastal *Plantago* species has been performed only seldom. Therefore, the aim of the present study was to compare salinity tolerance and accumulation potential of electrolytically active ions, Na^+ and K^+, in different parts of soil-grown *P. coronopus* and *P. maritima* in controlled conditions.

Plants were grown from seeds collected in a natural population (*P. maritima*, Ohesaare, Estonia) or purchased from Jelitto Seeds (*P. coronopus*). Plants were cultivated in conditions of an automated greenhouse in individual 1.2 L containers in mixture of commercial garden soil with addition of 20% (v/v) quartz sand. Plants were gradually treated with NaCl, reaching final concentration of 0.5, 1, 2, 5, and 10 g Na^+ per L of substrate and grown for additional seven weeks after the full treatment. At termination, plants were separated in different parts (storage root, fine roots, senescent leaves, old leaves, middle leaves, young leaves, flower stalks, inflorescences), and fresh mass, dry mass and water content was determined.

For *P. coronopus*, plants treated with 0.5 to 2 g Na^+ L^{-1} had significantly higher total dry mass in comparison to control, with the largest increase by 43% at 0.5 g L^{-1}. At 10 g Na^+ L^{-1}, total plant mass decreased only by 23%. Moreover, dry mass of living leaves was significantly higher in plants at all NaCl treatments in comparison to control. Water content significantly increased in all living leaves by all treatemnts. Growth of flower stalks was significantly stimulated at the lowest Na^+ concentration (Fig. 1A). However, number of inflorescences did not differ significantly. Number of leaves was significantly higher for plants treated with 0.5 to 5.0 g Na^+ L^{-1}, with maximum increase by 79% at the lowest Na^+ concentration.

On dry mass basis, maximum Na^+ concentration was found in senescent leaves of *P. coronopus* plants treated with 5 g Na^+ L^{-1}, reaching 130 g kg^{-1}. For plants, treated with 10 g Na^+ L^{-1}, living leaves accumulated 115, 87, and 55 g Na^+ kg^{-1}, for old, middle, and young leaves, respectively. Flower stalks had the same concentration of Na^+ as young leaves, but Na^+ concentration in storage root, fine roots and inflorescences was identical and reached only 20 g kg^{-1}. In contrast, Na^+ concentration on tissue water basis was identical in all living leaves and fine roots, reaching 0.5 mol Na^+ L^{-1} in plants treated with 10 g Na^+ L^{-1}.

Total dry mass of Na^+-treated *P. maritima* plants significantly increased at 0.5 and 1.0 g L^{-1} by 32%, tended to decrease by 18% at 5 g L^{-1}, and significantly decreased by 51% at 10 g L^{-1}. Only plants treated with 0.5 and 1 g Na^+ L^{-1} had higher dry mass of living leaves than that of control plants. Water content significantly increased in all living leaves by all treatments. Number of inflorescences significantly increased at 0.5 to 2.0 g Na^+ L^{-1}, by maximum 43% at 2.0 g L^{-1} (Fig. 1B). However, there was no significant increase in height of flower stalks. Number of leaves significantly increased for plants treated with 0.5 to 5.0 g Na^+ L^{-1}, with the highest increase by 32% at 0.5 to 1.0 g Na^+ L^{-1}.

Maximum concentration of Na^+ on dry mass basis for *P. maritima* was also found in senescent leaves, reaching

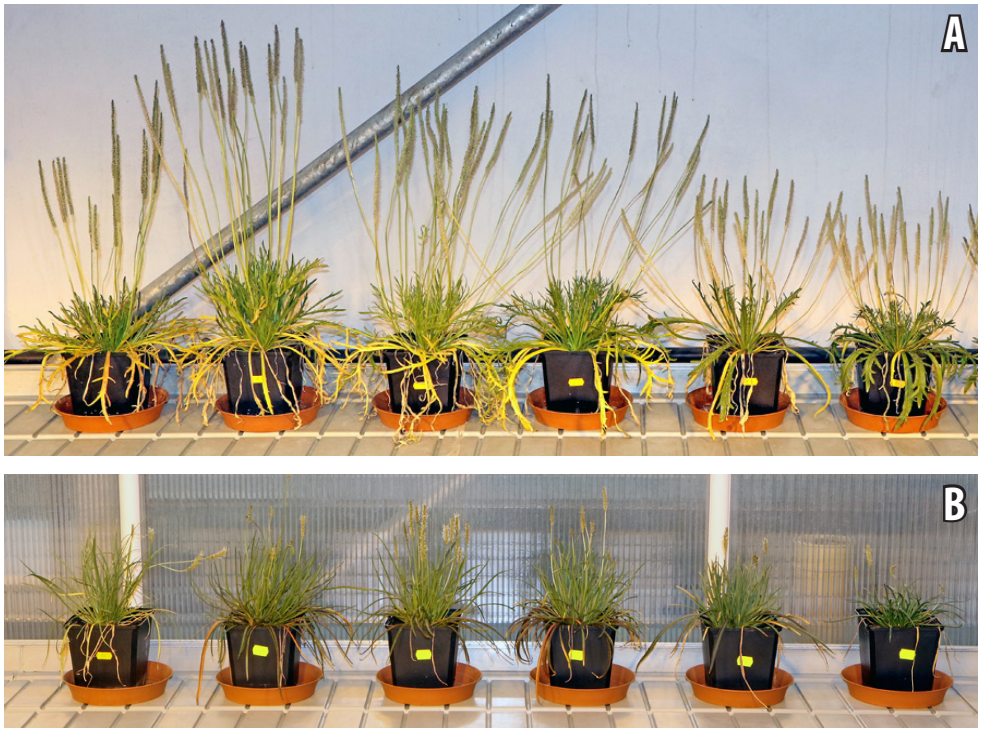

Fig. 1. Morphology of *Plantago coronopus* (A) and *Plantago maritima* (B) plants as affected by different concentrations of Na^+. From left to right: control plant, plants treated with 0.5, 1, 2, 5, 10 g L^{-1} Na^+.

120 g kg^{-1}. Maximum accumulation of Na^+ in living leaves, reached in *P. maritima* plants treated with 10 g Na^+ L^{-1}, was 80, 60, and 45 g kg^{-1}, for old, middle and young leaves, respectively. However, flower stalks had only 30 g Na^+ kg^{-1}, and inflorescences only 10 g kg^{-1}. On tissue water basis, only older leaves reached Na^+ concentration 0.5 mol L^{-1} for plants treated with 10 g Na^+ L^{-1}, but due to relatively higher water content in middle and young leaves, this value was only 0.36 and 0.32 mol L^{-1}, respectively.

It appears that both *Plantago* species had extremely high salinity tolerance and exhibited significant growth stimulation at moderate salinities, but *P. cornopous* appeared to be realtively more tolerant in comparison to *P. maritima*. Both species can be characterized as highly electrolytophytic, excluding K^+ and accumulating Na^+ at extremely high concentration, and using mostly Na^+ for osmotic adjustment.

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A preliminary study of weed soil seedbank in faba beans and winter wheat

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Key words: arable weeds, germination method, soil seedbank, species diversity.

Weed soil seedbank is one of the important factors regulating the dynamic of weed communities in arable fields. The soil seedbank buffers the effects of aboveground weed control and serves as a reservoir of new plants, as well as of genetic diversity of the weed species (Haring, Flessner 2018). At the same time, the species diversity of weed soil seedbank has been proposed as an index of sustainable intensification of agriculture (Storkey, Neve 2018). Therefore, assessment of the size and diversity of the weed soil seedbank should be an important tool to evaluate both the short-term efficacy and long-term sustainability of weed control. This study aimed to develop and test a suitable method of assessing the soil seedbank, and to compare the results from two different fields in Latvia.

Seed samples were collected in two fields in Zalenieku parish where faba beans (*Vicia faba* L.) and winter wheat (*Triticum aestivum* L.) were cultivated in 2020. In each field, 30 soil cores were collected in a 66 × 310 m plot, following a W-shaped transect. The soil cores were collected using an Edelman auger for combination soils (diameter 7 cm) at 20 cm depth. The sub-samples from 0 to 10 and 10 to 20 cm were processed separately. The samples were stored at –20 °C. To concentrate the samples they were washed using two sieves (eye diameter 3 and 2.5 mm) with organza cloth between the sieves where the seed-containing fraction of the soil was collected. After concentration, the samples were dried for 24 h at room temperature and then stored at 4 °C. The weed species were identified using the germination method. Concentrated samples were spread in a 1 to 1.5 mm layer on a moist peat substrate in 1 L plastic pots. The pots were kept in a greenhouse from May to October and watered as needed. Germinated seedlings were scored once a week and the total number of seedlings per sample was used to calculate the average number of seeds m^{-2} per 10 cm of soil depth. The inverse Simpson index and Shannon diversity index were calculated to compare species diversity in each field and at each depth. In the faba bean field, the above-ground weed vegetation was scored five times within the period from the end of April to the middle of June in 48 plots (0.25 m^2).

The total number of weed seeds per m^2 per 10 cm depth detected in faba bean field was 511 at 0 to 10 cm and 390 at 10 to 20 cm depth. In the winter wheat field the mean number was 312 seeds m^{-2} and did not differ between the depths. In total, 12 weed species were found in the soil samples collected in the faba bean field and 11 species in the samples collected in the winter wheat field (some plants were identified at the genus level; Fig. 1). Most of the species were typical arable weeds and were also identified in the above-ground vegetation: *Apera spica-venti* L., *Brassica napus* L., *Capsella bursa-pastoris* L., *Chenopodium album* L., *Papaver* spp., *Euphorbia helioscopia* L., *Sonchus arvensis* L., *Stellaria media* (L.) Vill., *Thlaspi arvense* L. and *Viola arvensis* Murr. However, soil samples also contained seeds of *Betula* spp., *Epilobium hirsutum* L., *Plantago* spp., *Chaenorrhinum minus* (L.) Lange and *Juncus* spp. An interesting finding was the large presence of viable *B. napus* seeds in the winter wheat field, where it had not been sown since 2017. The seedbank accumulation of *B. napus* can result due to seed loss at harvest (Rasmussen et al. 2003). *B. napus* seeds persist in soil due to secondary dormancy and clay soils are more favourable for the survival of these seeds (Gruber et al. 2014). Species diversity was higher in the 0 to 10 cm layer in the faba bean field (Simpson 1/D = 7.1, Shannon = 2.14), compared to the 10 to 20 cm layer (Simpson 1/D = 4.7, Shannon = 1.82), but there was no difference between the two depths in the winter wheat field (Simpson 1/D = 6.0 and 6.1, Shannon = 2.06 and 1.97, respectively).

The total number of identified seedlings in the samples was low, compared to the quoted accepted median value: 500 to 5000 seeds m^{-2} (Borgy et al. 2015). In a study conducted in different fields located in a wide geographical area, values between 33 and 33322 seeds m^{-2} are reported (Schwartz et al. 2015). However, 13 species identified in the above-ground vegetation in the faba bean field were not found in the soil samples, so further improvement of soil seedbank analysis method is required. More intensive sampling can be achieved by collecting several cores per 1 m^2 at each sampling point that are further combined in

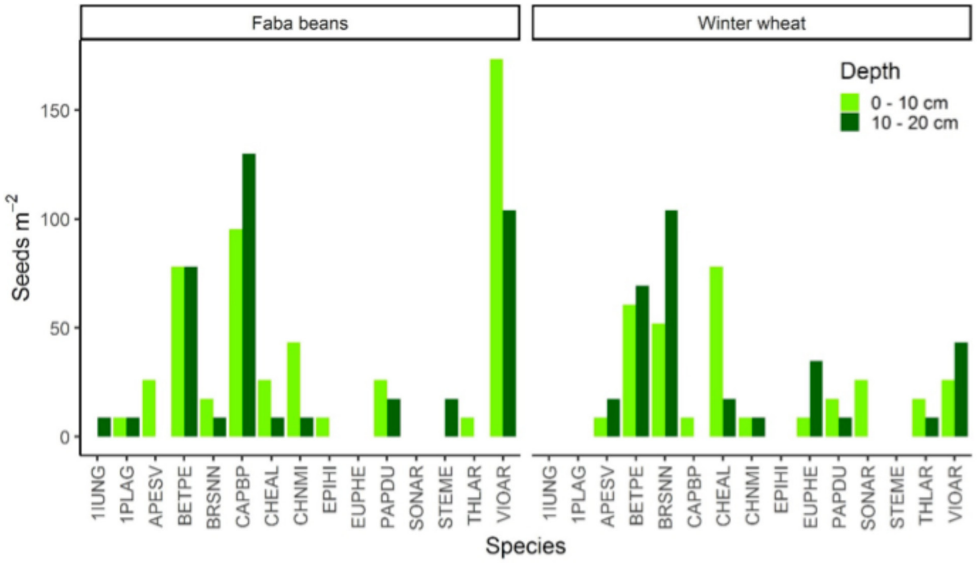

Fig. 1. Weed species identified in the soil samples from two fields using germination method and the average number of seeds m^{-2} per 10 cm depth. The species and genera are identified by the EPPO code.

a single sample. Species identification can be enhanced by cold stratification, germination-promoting chemicals (nitrate or gibberellic acid), as well as more frequent mixing of the sample layer on the peat substrate. In conclusion, weed soil seedbank research needs to be further developed as a tool to understand both the immediate and the long-term impact of different weed management strategies in Latvia.

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Fungal endophyte diversity in fruits of black elder (*Sambucus nigra*) revealed by culture-dependent approach

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Key words: elderberry, endophyte diversity, endophyte isolation, fruit stage.

Content of medicinally relevant compounds in plants can be affected by fungal endophytes (Jia et al. 2016) suggesting that plant-endophyte interaction is an interesting target to manipulate for enhancing pharmacologically active compound biosynthesis (Schmidt et al. 2014; Maggini et al. 2017). However, plant-endophyte interaction is a spatially and temporally dynamic complex process (Turner et al. 2013). Thus, knowledge of temporal and spatial shifts in fungal endophyte communities is essential, before a deeper exploration of the biochemical effects of fungal endophytes on medicinal plants can be undertaken. In the present study, fungal endophytes of fruits of the medicinal tree *Sambucus nigra* were explored using culture-dependent approach. Effects of isolation technique, fruit developmental stage, and sampling sites on the alpha diversity of assessed endophytic communities were investigated using a culture-dependent approach.

Four wild *S. nigra* populations located in Latvia (site coordinates: Lat 56.58694444 Long 21.34666667; Lat 56.611193 Long 24.189293; Lat 56.68583333 Long 22.39472222; Lat 56.65305556 Long 22.47027778) were included in the study. *S. nigra* fruits at three developmental stages, immature fruits reaching 50 to 70% of mature fruit size; immature fruits of deep green colour reaching 90% of mature fruit size; mature fully colored ripe fruits, were collected in 2019 in July, August and September, respectively. Sampled fruits were surface sterilized with household bleach and 70% (w/w) ethanol. Endophytic fungi were isolated from sterilized fruits applying two isolation methods for each sample: dilution to extinction method and fragment plating method essentially as described by Unterseher and Schnittler (2009). Isolation medium YMA at 10% concentration (w/v) was used for fungal isolation. Plates were incubated at 4 °C in the dark for up to two months with regular (once within two weeks) inspections. Observed fungal colonies were transferred to fresh 100% YMA medium for final morphotype characterisation. Based on the morphotype data, alpha diversity indices Chao 1 index and Shannon index were estimated using the packages *fossil* (Vavrek 2011) and *vegan* (Oksanen et al. 2020) in R software version 4.0.2.

Dilution to extinction method allowed for recovering of 126 fungal endophyte morphotypes in total (on average three morphotypes per plant per season). Significantly higher number (one-tailed *t* test, $t = 16.02$, $df = 39$, $p < 0.001$) of fungal morphotypes, 363 morphotypes, was isolated using fragment plating, on average 13 morphotypes per plant per season. Interestingly, only 6% of all isolated fungal morphotypes were overlapping between both methods, suggesting that the application of distinct isolation methods can yield complementary rather than repetitive results. Fragment plating also recovered significantly higher fungal diversity in comparison to the dilution-to-extinction method. An average Chao1 index for sampling site reached 156 ± 126 and 40 ± 28 for fragment plating and dilution-to-extinction, respectively (one-way ANOVA, $F_{1,16} = 10.07$, $p = 0.0059$) and Shannon diversity index 3 ± 0.7 and 2 ± 0.7 for fragment plating and dilution-to-extinction (one-way ANOVA, $F_{1,15} = 17.97$, $p = 0.0007$).

Fruit ripening stage had a pronounced effect on fungal endophyte morphotype richness (ANOVA, $F_{(1.961;\ 76.46)} = 74.51$, $p < 0.0001$). Chao1 diversity index showed that berries at later developmental stages (shortly before developing color and fully ripe berries) hosted significantly more diverse fungal endophyte communities compared to immature berries (immature berries vs fully ripe berries: *t* test, $df = 18$, $p = 0.0059$; immature berries vs mature uncolored berries: *t* test, $df = 18$, $p = 0.036$).

Morphotype richness, Chao1 index, and Shannon diversity index did not differ significantly between sampling sites. However, multidimensional scaling of morphotype data (principal coordinate analysis) showed site-specific grouping of sampled trees based on fungal endophytes isolated from fully ripe berries. These results suggest that the fungal endophyte communities of *S. nigra* are partially shaped by the growth sites and this effect becomes more pronounced later in season. However, the site effect does not affect the overall alpha diversity of *S. nigra* fungal

endophtyes.

Taken together the reported results suggest that elderberry fungal endophyte communities and their diversity are largely affected by fruit developmental stage and sampling sites. Results of different isolation techniques are complementary rather overlapping therefore the combined application of different isolation techniques can produce a more objective representation of assessed fungal endophyte communities.

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Influence of sample homogenization and DNA extraction methods on the quantitative and qualitative parameters of DNA in the detection of GMO impurities

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Key words: DNA extraction, feed, food, genetically modified organisms, GMO, homogenization, seeds.

Sample homogenization and DNA extraction methods are essential in the detection and quantification process of genetically modified organisms (GMO) in food, feed, and seeds. The homogenization depends from the type of the equipment available in the GMO testing laboratory. Depending on the size of the ground particles, it may be necessary to significantly change the test portion size of the sample in order to accurately quantify a specific level of GMO admixture. Therefore, it is important to grind the samples to the finest possible particles, from 0.5 to 0.75 mm in size (Joint Research Centre 2014). In several studies the impact of sample matrix and DNA extraction method on GMO testing have been evaluated (Cankar et al. 2006; Corbisier et al. 2007; Debode et al. 2012; Žel et al. 2015). Matrices whose DNA extraction and reference gene amplification results are reproducible have been considered as stable (soybean grains, flour, soybean meal, maize kernels, corn flakes) while more processed matrices (oil, chocolate, and sugar) can give various unexpected and problematic results (Klein et al. 1998; Debode et al. 2012; Žel et al. 2015). Standard ISO 24276:2006/A1:2013 states that the purpose of nucleic acid extraction methods is to provide further analysis with DNA of suitable quality. ISO 21571:2005/A1:2013 clarifies that the quality of DNA depends on the average length, chemical purity, and structural integrity of the extracted DNA. The choice of method depends on the user experience, considering the application scope of the method and the sample matrix. The quantity and quality of DNA can be evaluated by determining the absorbance at several wavelengths with a spectrophotometer. Measurements at OD_{260} must be greater than 0.05 (according to ISO 21571:2005 B 1.8). The ratio $OD_{260/230}$ should be in the range of 1.6 to 2.3 (Corbisier et al. 2007). For the $OD_{260/280}$ ratio, the optimal values are > 1.6 and < 2.2, but in the GMO detection successful amplification has been obtained even at values of 1.4 (Turkec et al. 2015). Ratio $Abs_{260/280}$ slightly below 1.8 indicates that there is an optimal amount of DNA, but there are also some impurities of proteins and aromatic compounds. Low $Abs_{260/230}$ indicates the presence of carbohydrates that may affect PCR (Corbisier et al. 2007).

The aim of the study was to evaluate the combination of sample homogenization devices and different DNA extraction kits on the quantitative and qualitative parameters of the extracted DNA, depending on the type of matrix. DNA was isolated in the period from June 2018 till June 2020. The total number of samples and certified reference materials (RM) was 219. DNA was isolated from seed samples in three replicates and from other samples in two replicates. The DNA concentration and absorption at 230, 260 and 280 nm were detected with NanoDrop ND-1000. Matrices from which DNA is relatively easy to obtain were: RM (number of extractions n = 25), cereals and seeds (125), plant-based animal feed (rapeseed cake, linseed cake, soybean sprouts, sunflower sprouts; 34), foods with relatively little technical processing (tofu, soy drinks, desserts, protein, sauces, corn groats, chips, flakes, baby food, fruit candies; 59), fresh vegetables (8), and meat products (68). Products with relatively high technical processing were chocolate (28), canned corn (22), oil (33), and sugar products (8). The following sample homogenization methods were evaluated: without homogenisation or grinding (for liquids and powders), knife mill Grindomix GM 200 (Retsch), laboratory blender (Waring), coffee mill Profi J Cook (ProfiCook), and a mortar and pestle. Following DNA extraction kits were used: Wizard® magnetic DNA purification system for food (Promega Inc.), NucleoSpin Food Mini kit (Macherey-Nagel), and DNeasy *mericon* Food kit (Qiagen). The DNA suitability for PCR was assessed by amplification of plant chloroplast intron gene *trnL*, and plant taxon-specific genes: high mobility group gene for maize, cruciferin A gene for oilseed rape, and lectin gene for soybeans.

Amplification of the gene *trnL* or a plant taxon-specific gene for powdered and liquid matrices was achieved in 100% of RM, 50 to 71% of oil samples, 78 to 85% of soy sauces, desserts, and other soy products. Another study has found that both virgin and fully refined oils were able to

Table 1. DNA concentrations (ng μL^{-1}) depending on the homogenisation equipment and DNA extraction method. W, Wizard® magnetic DNA purification system for food; NS, NucleoSpin Food Mini kit; *m*, DNeasy *mericon* Food kit

Type of homogenization	DNA extraction kit	Cereals and seeds	Processed cereals	Fresh vegetables	Meat products	Chocolate	Canned corn	Sugar products
Grindomix GM200	W	29.4 ± 10.7	53.4 ± 28.5	–	–	–	–	–
	NS	91.6 ± 93.7	–	27.9 ± 0.3	–	–	4.3 ± 1.9	–
	m	39.8 ± 22.2	–	–	129.1 ± 46.8	–	2.5 ± 0.6	–
Waring blender	W	22.8 ± 15.7	–	–	24.1 ± 1.2	–	–	–
	NS	288.7 ± 450	562.9 ± 788	90.5 ± 2.9	92.2 ± 107.9	31.3 ± 18.3	18.2 ± 8.0	0.7 ± 2.8
	m	32.5 ± 33.1	–	–	128.9 ± 5.3	–	–	–
Profi J Cook	W	35.7 ± 27.3	13.6 ± 5.6	–	–	–	2.8 ± 0.4	–
	NS	53.7 ± 36.7	–	–	48.1 ± 17.5	17.4 ± 1.3	40.3 ± 12.5	–
	m	17.3 ± 8.7	–	–	–	–	2.8 ± 0.1	–
Mortar and pestle	W	–	2.9 ± 1.5	6.47 ± 0.87	9.4 ± 6.2	3.0 ± 0.9	0.9 ± 0.5	–
	NS	–	–	–	54.3 ± 59.8	19.1 ± 8.6	–	–
	m	–	–	–	86.7 ± 69.2	2.6 ± 2.2	2.2 ± 0.2	–

give high copy number gene *5.8S* amplification, although the DNA concentration was lower (< 0.2 ng μL^{-1}, sample size 2 g) than in the present study (Debode et al. 2012). DNA concentrations depending from the homogenisation equipment and extraction method are given in Table 1. Amplification of the gene *trnL* or a plant taxon-specific gene for cereals and seeds, as well as fresh vegetables and canned corn, was successful in 100% of all combinations of mills and DNA extraction kits used. Three of the meat products from which DNA was extracted with the Wizard kit could not be amplified for the trnL or soy lectin gene because they may not contain plant-derived ingredients or the kit may not be suitable for meat products. Overall, PCR amplification was achieved in 91% of meat products, 50% of chocolate and 25% of sugar products. On average, higher DNA concentrations were obtained by isolating DNA with the NucleoSpin Food Mini kit. The lowest concentrations were obtained with Wizard® kit, probably because for this kit the DNA was isolated from 100 mg, while using other two kits from the 200 mg sample, and because Proteinase K is not used in this set of reagents. Furthermore, it is possible that DNA binds better to the column membrane than to the magnetic beads. When comparing the purity of the obtained eluates according to the $Abs_{260/280}$, on average both the Dneasy mericon Food kit and the NucleoSpin Food Mini kit eluates had an optimal degree of purity, except for oils. On average, lower DNA concentrations were obtained from samples for which homogenization was chosen by mortar and pestle, while higher DNA concentrations and the most appropriate absorption measurements were obtained from samples ground with a Grindomix GM200. From the selected sample pool, nine samples contained GMO impurities.

For the future it is recommended to implement the *5.8S* rlPCR protocol for testing oil samples due to its high sensitivity. Recommendations for the development of GMO monitoring plans are to avoid sampling of such products that are highly technically processed, instead it is recommended to analyse the raw materials used.

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Cs-133 ecotoxicity: approbation of acute and chronic tests

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Key words: caesium-133, ecotoxicity, *Daphnia magna, Thamnocephalus platyurus, Lemna minor*.

Disposal of radioactive waste is a global problem that can be significantly attributed to the situation in Latvia, with the liquidation process of the Salaspils nuclear reactor facility lasting more than ten years (Riekstina et al. 2016). Due to their relatively long stability, radioactive isotopes such as caesium (Cs), strontium and others are hazardous to the environmental ecosystem and human health, accumulating in the cooling water residues of nuclear reactors (Avery 1996).

The chemical effects of radioactive Cs on higher plants and other test-organisms often coincide with the properties of the stable Cs. To a large extent, Cs intoxication is related to its ability to interfere with the biochemical processes of potassium uptake. Cs is known to accumulate in aquatic organisms both from nutrient sources and from Cs dissolved in water and suspended in solid particles and sediments (Avery 1996; Burger, Lichtscheidl 2018).

Cs is an alkali metal that is naturally found in various ores and to a lesser extent in soil, water. The assimilation and entry of Cs into the cell is thought to be related to potassium transport systems. In plants, Cs is absorbed through the roots and leaves, affecting cell growth, size increase and signal transduction to cell organelles. It has been reported that Cs in plants can significantly affect stomata activity, thereby disrupting transpiration processes (Burger, Lichtscheidl 2018).

The aim of this study was to approbate acute and chronic Cs-133 ecotoxicological tests on various organisms representing different food chain levels. The ecotoxicity of the analysed samples was evaluated for both prokaryotic organisms, such as *Pseudomonas putida*, and eukaryotic ones, e.g., crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, macrophytes *Lemna minor* and some terrestrial higher plants. Evaluation of Cs-133 toxicity for *P. putida* was performed by inhibition of the enzymatic (fluorescein diacetate hydrolysis, FDA) activity, while for crustaceans a standard protocol according to Daphtoxkit F and Thamnotoxkit F (MicroBioTests, Belgium) was provided, respectively. Terrestrial plants were used for seed germination tests. Changes in plant biomass were determined for the aquatic plant *L. minor* after 8 days of incubation with CsCl.

The FDA hydrolysis activity of *P. putida* was examined after 48 h of incubation and indicated an inhibition of 1.0, 10.0 and 50.0 mg L^{-1} CsCl by 4.47, 7.20 and 28.20%, respectively. An intensity of FDA hydrolysis characterizes the activity of proteases, lipases, esterases and other enzymes of heterotrophic microorganisms (Guilbault et al. 1964).

For crustacean *D. magna*, the lethal concentrations

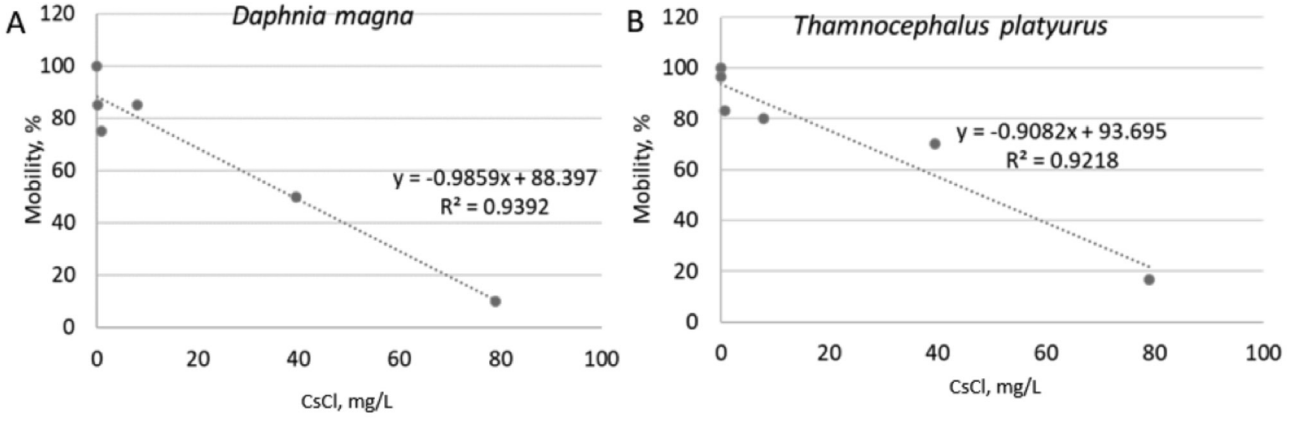

Fig. 1. Mobility of *Daphnia magna* (A) and *Thamnocephalus platyurus* (B) at different CsCl concentrations ranged from 0 to 100 mg L^{-1}.

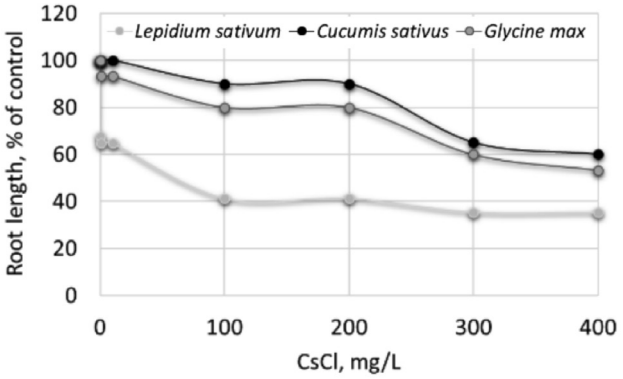

Fig. 2. Root length of cress *Lepidium sativum*, cucumber *Cucumis sativus* and soya *Glycine max* after four day germination in the presence of CsCl followed by four day growth in hydroponics.

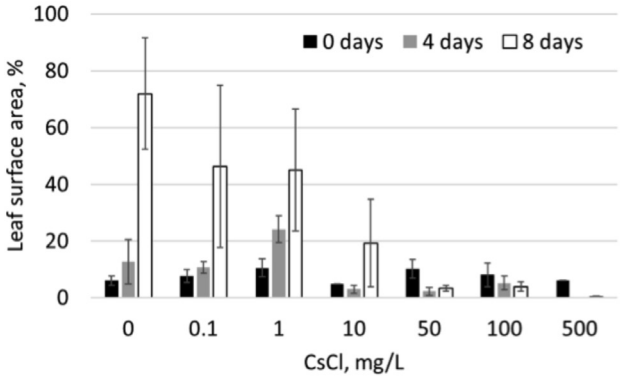

Fig. 3. Changes in *Lemna minor* plant biomass after 8 days of incubation at different CsCl concentrations and incubation periods (0, 4 and 8 days).

(LC50) for 24 and 48 h and size changes over a 6-day period were assessed. For *D. magna*, the LC50 (24 h) and LC50 (48 h) were determined to be 88.46 mg L^{-1} CsCl and 49.33 mg L^{-1} CsCl, respectively (Fig. 1A). In experiments with *T. platyurus*, the LC50 (24 h) was 60.95 mg L^{-1} CsCl (Fig. 1B).

Seed germination is one of the most widely used tests for eco- and phytotoxicity. From our experiments, it was determined that the presence of CsCl at the concentration range up to 500 mg L^{-1} did not affect seed germination after 4 days of incubation. However, when germinated seeds were transferred to a caesium-free liquid medium, an inhibition of the root growth was observed after 4 days of incubation in variants with seed germination occurred with 300 mg L^{-1} CsCl (*Glycine max* and *Cucumis sativus* were inhibited by 40 and 35%, respectively) and 100 mg L^{-1} CsCl (*Lepidium sativum* were inhibited by 62%, in comparison to control) (Fig. 2).

The aquatic plant *L. minor* is commonly used in ecotoxicological studies. According to our data, EC50 reached 0.1 mg L^{-1} CsCl, taking biomass growth as the evaluation criterion (Fig. 3).

The obtained data on the inhibitory effect of Cs-133 showed that the EC50 values under the tested conditions ranged from 0.1 mg L^{-1} CsCl (Lemna minor) to 88.5 mg L^{-1} CsCl (*D. magna* 24 h). In contrast, in further studies with Cs-137, caesium concentrations are expected to be 1000 to 10000 times lower. From this it can be concluded that the inhibitory effect of caesium as a metal can be ignored in experiments with Cs-137, but the mechanisms of Cs uptake will be similar.

Acknowledgements

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Valorisation of cellulose-containing wastes for production of biological pesticides

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Key words: cigarette butts, cellulose acetate, ecotoxicity, pesticides, slugs.

Biodiversity loss and ecosystem degradation are one of top threats facing humanity. One of important problems is the spread of invasive species in the soil leading to the annual loses in agricultural sectors. Activities such as global trade with ornamental plants, transports of soil, flowerpots, and pellets, dumping of soil and garden wastes may affect migration of slugs (Bergey et al. 2014; von Proschwitz 2020). Some of them spread very fast like *Krynickillus melanocephalus* (Dreijers et al. 2017). Species such as *Arion vulgaris* are considered as one of the worst pest slugs causing immense financial problems due to the damage of essential crop cultures grown in gardens and cultivations all over Europe (Jakubane et al. 2016).

World's ecosystem is notably affected by growing waste rates. Cigarette butts (CB) is the world's most prevalent source of waste litter causing contamination due to the presence of nicotine and hundreds of other toxic chemical substances (Kurmus 2020). High contents of CBs are commonly collected during clean-up of urban areas such as parks, beaches, and coastal environments (Araújoa, Costa, 2019). The Tobacco Atlas reported that during the year 2016, 5.7 trillion cigarettes were consumed worldwide. In 2019, the total consumption rate of cigarettes in Latvia reached 2.1 billion units. The aim of this study was to evaluate the potential inhibitory efficiency of CB extract as potential biological pesticide for the control of slug species, as well to evaluate their ecotoxicological properties.

Inhibitory tests of CB extract were performed as follows: the CBs were soaked in a mixture of isopropanol (70%) and glycerol (5 to 7%), extracted for 24 h at 50 °C followed by filtration through a paper filter. Isopropanol was used for the control tests. Tests were performed during single and double treatment of the slugs (slug *Krynickillus melanocephalus*) with CBs extract and isopropanol as a control (Fig. 1). It was determined that 100% of the slugs survived after a single treatment with the undiluted CB extract, whereas double spraying with the undiluted extract affected total death of the experimental slugs. Within the testing of double diluted CBs extract, 2/3 of the slugs survived after both single and double treatments. Slugs from the control group, which were treated with the isopropanol extract presented 100% survival after both single and double treatment (Fig. 1).

Ecotoxicological tests with CBs extract on crustaceans Daphnia magna were performed to determine the effective concentration (EC50) causing an adverse effect on the mobility of 50% of tested species within 48 h. Increase of the CB content in the extract affected gradual reduction of *Daphnia magna* mobility. The obtained data indicated that the EC50 (48 h) for CBs extract was 0.61 mL L^{-1} (Fig. 2).

The viability tests of *Planorbis* sp. were conducted by testing 24 and 48 h treatment of 0 to 0.6% CBs extract. Readings were taken after 24 h, then live subjects were transferred to CBs-free medium, and subsequent survivors were counted after 48 h period. Toxicity results indicated that the EC50 of CBs extract for *Planorbis* sp. after 24 h was 2.86 mL L^{-1}, but after 48 h it reached 3.05 mL L^{-1} (Fig. 3).

It was confirmed from the testing results that extracts of CB wastes can serve to control the infestation of terrestrial molluscs. Comparing *Daphnia magna* and *Planorbis* sp.

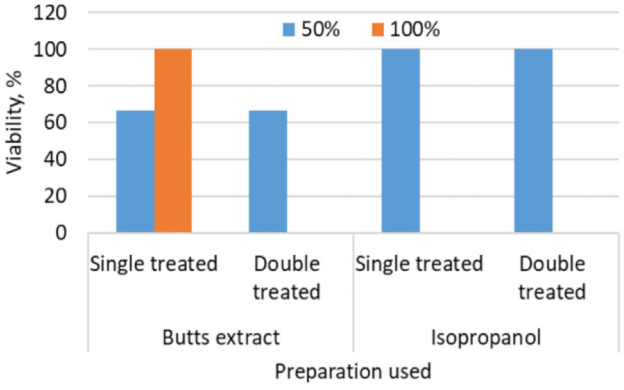

Fig. 1. Viability (%) of slugs after single and double spraying with CBs extract and isopropanol (control).

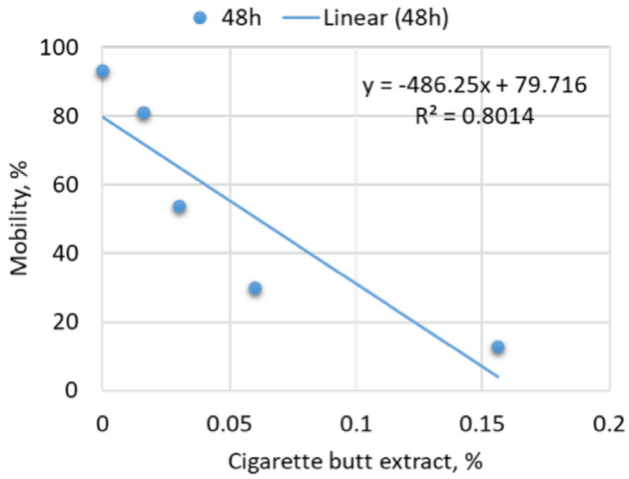

Fig. 2. Viability (%) of slugs after single and double spraying with CBs extract and isopropanol (control).

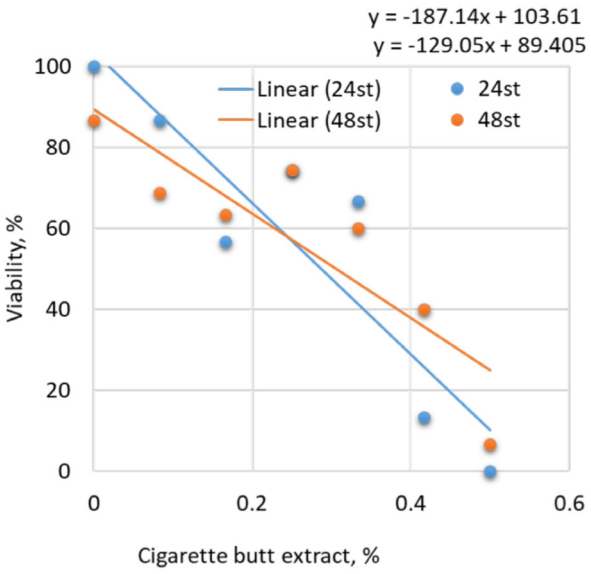

Fig. 3. Viability (%) of slugs after single and double spraying with CBs extract and isopropanol (control).

resistance to CBs extract, it was found that the LC50 (48 h) differed five-fold, with the higher sensitivity for *Daphnia magna* (LC50 48 h = 0.061%).

Obtaining an extract from CBs will provide an incentive to collect them, thereby reducing the spread of CBs, the harmful substances, and pathogens they contain in the environment. The biodegradable extract could be used to minimize the spread of slugs. Isopropanol as an extractant simultaneously acts as a disinfectant.

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Zymomonas mobilis genome scale metabolic model for validation of the genotype-phenotype relationship quality

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Key words: fluxomics, metabolic modeling, *Zymomonas mobilis*.

Zymomonas mobilis has a high specific productivity of ethanol, broad pH range for production (3.5 to 7.5), high alcohol tolerance, and it is classified as GRAS (generally regarded as safe) organism (Kalnenieks 2006; Rogers et al. 2007). *Z. mobilis*, in contrast to many popular bioconversion organisms, uses Entner-Doudoroff (ED) pathway instead of Embden-Meyerhof-Parnas (EMP) pathway. The ED pathway is energetically less efficient producing 50% less ATP than EMP (Kalnenieks et al. 1993). *Z. mobilis* consumes monosaccharides much faster than most other microorganisms and is able to convert up to 98% of carbohydrate substrates into catabolic end products (Goodman et al. 1982).

Genome scale metabolic models (GSM) are considered one of the best methods how to predict carbon distribution via biochemical pathways and to establish phenotype-genotype relationships. Several stoichiometric models of *Z. mobilis* have been developed previously with simulation-ready files (Pentjuss et al. 2013; Rutkis et al. 2013; Kalnenieks et al. 2014; Motamedian et al. 2016; Kalnenieks et al. 2019), while some others (Altintas et al. 2006; Lee et al. 2010; Widiastuti et al. 2011) are not presented in readable format and thus cannot be directly simulated. Only the latest published iHN446 (Nouri et al. 2020) has eliminated most of previously mentioned drawbacks and has been able to explain some publicly available transcriptome and proteome data on biochemical network topology scale.

To use iHN446 for purposes of biotechnological potential analysis, additional model validation steps were performed. We integrated the recently published ZM4 strain fluxomics data (Jacobson et al. 2019) into model, including glycolysis, TCA cycle, fermentation and the one carbon metabolism. Experimentally obtained ethanol, acetate, shikimate, and DHQ production rates were 77.2 ± 3.9, 0.27 ± 0.01, 0.21 ± 0.01, and 0.01 ± 0.002 mmol g^{-1} DW h^{-1}, respectively. Glucose uptake rate was 43.2 ± 2.2 mmol g^{-1} DW h^{-1}, and biomass specific growth was assumed 0.36 h^{-1}. All simulations were performed using CobraToolbox 3.0 (Heirendt et al. 2019) and Flux Balance Analysis (Orth et al. 2010).

iHN446 metabolic model showed very good results and was able to carry flux in accordance to previously mentioned integrated experimental data. But not all data was feasible for validation. After integration of experimental data on pentose phosphate pathway and all amino acid biosynthesis into iHN446, steady state could not be established. Also, glycine decarboxylase (Biocyc ID: GCVMULTI-RXN) was missing from the model.

iHN446 with integrated experimental data on pentose phosphate pathway lacked shikimate production, while the missing glycine decarboxylase was probably causing the discrepancy between the measured and simulated one carbon metabolism flux distribution. iHN446 published results correspond to growth on yeast extract medium. Other in silico growth mediums with less amino acids show that the model cannot simulate growth without simultaneous uptake of most amino acids.

After additional validation we conclude, that iHN446 primary metabolism in silico simulations correlates well with the available high-quality experimental data, but that secondary metabolism (amino acid biosynthesis and one carbon metabolism) is still lacking complete ZM4 metabolic information.

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