https://doi.org/10.22364/eeb.20.09

UNIVERSITY OF LATVIA

ABSTRACTS
OF THE 80th SCIENTIFIC CONFERENCE OF THE UNIVERSITY OF LATVIA

January – February 2022

Effects of different polysorbates on the growth of *Trichoderma asperellum*

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Key words: polysorbates, submerged cultivation, *Trichoderma*.

The work involves the development of an appropriate biotechnological method to obtain products with high biomass content and antifungal activity against phytopathogenic fungi. Economically viable production of soil microbiological fertilizers and plant protection agents have not yet been fully explored (Markets and Markets 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. Applying of microbial fertilizers and plant protection agents reduces the use of chemical fertilizers and pesticides (de Rezende et al. 2020). The addition of polysorbate surfactants (Tween) to the culture medium is a widely used method to stimulate the release of various substances produced by microorganisms, especially enzymes, as well as to accelerate the growth of mycelium and to protect extracellular enzymes from denaturation. The fatty acid moiety in this substance can be used by microorganisms as a carbon source (Zhang et al. 2012).

The aim of the study was to determine the morphological structure and dry biomass changes of *Trichoderma asperellum* MSCL 309 by cultivation in sugar-yeast extract medium with different concentrations of Tween 20, 40 and 80, as well as the antifungal effect of the cultivation liquid against the phytopathogenic fungus *Cladosporium herbarum* MSCL 267.

During the study, six Erlenmeyer flasks were filled with a defined liquid medium with additives. A suspension of *T. asperellum* was introduced into each flask. Inoculated media were placed on a shaker, cultivated for 72 h. To detect changes in the amount of *T. asperellum* biomass, cultivation medium with biomass was obtained from each flask every 24 h (for 3 days). The liquid was centrifuged and the supernatant was discarded, but the biomass in the sediment was weighed. In addition, the supernatant of the liquid was tested for antifungal activity against *C. herbarum* and *T. asperellum* biomass microscopy was performed.

The highest yield of *T. asperellum* dry biomass was achieved at 1.5 and 2% Tween 20 concentrations 72 h after the start of cultivation (36.9 and 37.3 g L^{-1}, respectively). The dry biomass yield of *T. asperellum* did not differ significantly at 0.2% Tween 20 concentrations and the control sample (13.1 and 14.5 g L^{-1}) after 72 h of cultivation. In all samples with different concentrations of Tween 20, a significant increase in the dry biomass of *T. asperellum* after 72 h of submerged cultivation was observed compared to the 0.2% Tween 20 concentration (Fig. 1A). The dry biomass yield of *T. asperellum* at the various concentrations of Tween 40 increased most rapidly after 48 h from the start of cultivation in samples in which an additional 1.5% and 2% Tween 40 concentrations (up to approximately 20 g L^{-1}) were introduced. No such increase was observed in samples with lower Tween concentrations, and the highest dry matter yield of *T. asperellum* was observed in these two samples after 72 h (38.5 and 42.5 g L^{-1}, Fig. 1B). The yield of dry biomass of *T. asperellum* after 72 h of cultivation was significantly increased in all samples compared to the control sample (20.0 g L^{-1}). In all samples with different concentrations of Tween 80, a significant yield of *T. asperellum* dry biomass was observed after 72 h of culture compared to the control (19.0 g L^{-1}). The highest yields of T. asperellum dry biomass were achieved at 1.5, 2, 1 and 0.2% Tween 80 concentrations 72 h after the start of cultivation (35.3, 35.0, 38.6 and 31.7 g L^{-1}, respectively). The amount of biomass obtained from the respective samples differed significantly. In the control sample, the yield of *T. asperellum* after 72 h reached 19.0 g L^{-1}, which is significantly less than in the samples with different concentrations of Tween 80 added (Fig. 1C).

The formation of sterile zones against *C. herbarum* phytopathogenic fungi could not be detected in any of the samples with different concentrations of Tween 20, 40 and 80. Inhibition of *C. herbarum* growth was observed with decreases in Tween 20, 40 and 80 concentrations.

No morphological differences were observed between samples with different concentrations of Tween 20. In all samples, regardless of Tween 40 concentration, an increase

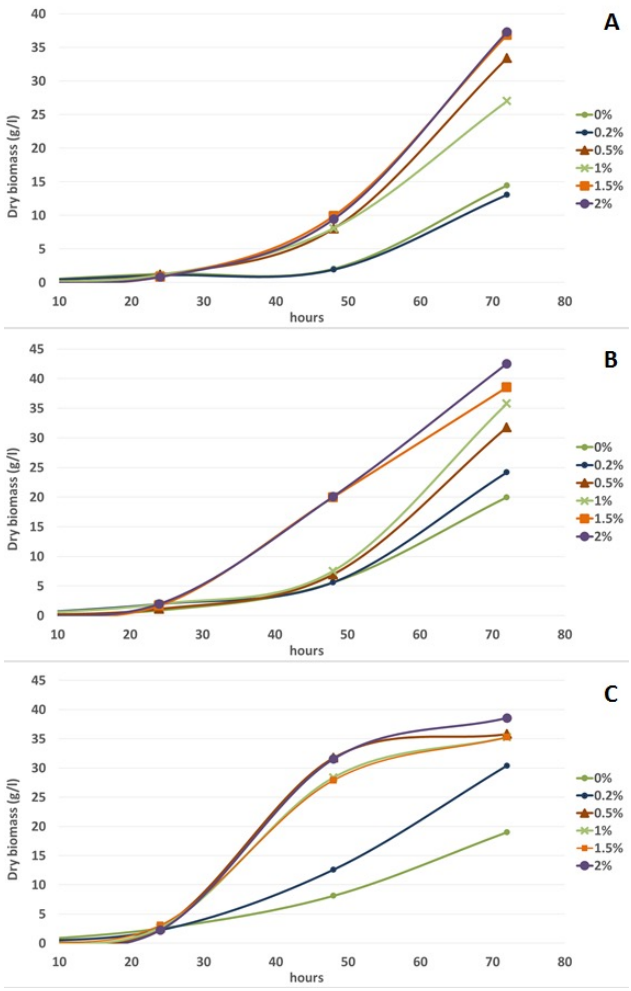

Fig. 1. Yield of dry biomass of *T. asperellum* at different concentrations of Tween 20 (A), Tween 40 (B) and Tween 80 (B).

in chlamydospores formation and hyphae accumulation was observed 48 hours after inoculation. As early as 24 h after the start of culture, an increase in hyphae accumulation was observed, which remained unchanged for up to 72 h in all samples with Tween 80. Chlamydospores began to form 48 h after the start of the experiment. No conidiophores and conidia were observed in the samples.

The highest yields of *T. asperellum* dry biomass were achieved at 1.5 and 2% Tween 20, 40 and 80 concentrations 72 h after the start of cultivation. None of the samples formed zones of inhibition against phytopathogenic fungi. It was observed that 72 h of *T. asperellum* supernatant inhibited the growth of phytopathogens at low concentrations of Tween 20, 40 and 80. Chlamydospores formed 48 h after inoculation, but no morphological differences were observed between samples with different concentrations of Tween 40 and 80, but no chlamydospores were observed in any of the samples with different concentrations of Tween 20.

Acknowledgements

This work was supported by the project "Scale-up research of the microbiological soil fertilizer and biocontrol agent obtainment in submerged and surface cultivation processes", ERDF Project No. 1.1.1.1/19/A/150.

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Resources of crop wild relative *Trifolium fragiferum* in Latvia: characterization of sites and comparative genetic diversity in the context of the Baltic Sea

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Key words: crop wild relatives, genetic diversity, mineral nutrition, soil properties, strawberry clover.

Trifolium fragiferum, a crop wild relative species, is distributed in northern Europe within the protected habitat "Boreal Baltic coastal meadows" (1630*). There is no comparative information available on requirements of *T. fragiferum* to edaphic conditions and soil salinity in native habitats, and genetic diversity of the species in the Baltic Sea region has not been characterized. Therefore, the aim of the present study was to compare soil chemical composition and aspects of mineral nutrition of accessions from eight geographically isolated sites of *T. fragiferum* in Latvia, as well as to characterize genetic diversity of these plants in comparison to populations from Denmark and Estonia, and cv. 'Palestine' (Fig. 1).

All identified geographically isolated *T. fragiferum* micropopulations in Latvia were located in the immediate vicinity of water reservoirs (sea, river, lake), except for TF4 in the degraded area of the city of Riga. Only TF1 grew in highly saline soil and TF5 and TF6 in moderately saline soil. The sites with *T. fragiferum* were mostly relatively small, ranging from a few hundred m^2 (TF2, TF2b, TF4) to several thousand m^2 (TF1). The number of individuals in the sites ranged from about 10 (TF2, TF3, TF7) to several thousand (TF1).

Fig. 1. Location of *Trifolium fragiferum* sites in the Baltic Sea region used in the present study.

Concentrations of plant-available soil mineral elements showed significant differences between different sites and respective coefficients of variation ranged from 59% N to 139% Ca. Concentrations of mineral elements in plant leaf petioles and blades varied less. The analysis of the principal components showed similarities in terms of soil properties and mineral nutrition between micropopulations in dry, salt-free habitats along the river and sea. Based on these results, *T. fragiferum* can be characterized as a species capable of maintaining mineral homeostasis in tissues even when growing in substrates with sharply different concentrations of available minerals, different salinity and pH values.

The location of the sites in the immediate vicinity of the water reservoirs indicates the possible dispersal of the species by water and its association with the deposition of organic sediments. Unlike other clover species, *T. fragiferum* is characterized by the transformation of an inflorescence (flower head) into a fruit head consisting of inflated fruit capsules formed from the structures of the calyx after flowering (Zohary, Heller 1982). Fruit heads can provide seed propagation through water, which is thought to coincide with the spread of phytodetrite (Wolters et al. 2017). It cannot be ruled out that plant stolon fragments may spread along with phytodetrite, which may act as vegetative propagules.

Using 10 microsatellite markers, identically low level of genetic diversity was found in all *T. fragiferum* pmicroopulations of Latvia, as well as in plants from sites in Denmark and Estonia, which could be explained by the geographical isolation of the micropopulations and the nature of clonal reproduction of the species.

Significantly larger diversity was observed in seed material of cv. 'Palestine'. Populations were genetically differentiated with mutual F_{st} values above 0.050, except for TF5 / TF6 and TF5 / TF7. Interestingly, the genetic differentiation between the TF2 (Lielupe estuary) and TF9 (Bornholm island in Denmark) populations was relatively small (F_{st} = 0.069), although there are more than 600 km of air distance between them. The analysis of genetic distances between populations divided them into three groups. The first group included all coastal populations except TF10 (Haapsalu in Estonia). The second group consisted of populations from the river and lake shores as well as TF10, but cv. 'Palestine' formed a separate group. These results support the hypothesis of the spread of *T. fragiferum* in the northern Europe by two routes since the last ice age, inland and sea.

Acknowledgements

The study was supported by the Latvian Science Council project lzp-2020/2-0349 "Molecular, physiological and ecological evaluation of Latvian genetic resources of valuable wild legume species, *Trifolium fragiferum*, in a context of sustainable agriculture".

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Characteristics of phytoplankton of Lake Lubāns, summer 2021, Latvia

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Key words: phytoplankton, chlorophyll *a*, eutrophication.

Aim of this study was to obtain information of ecological quality and phytoplankton biological diversity of Lake Lubāns. Phytoplankton and chlorophyl *a* samples were collected in eight sampling stations at 0.5 m depths in July and August 2021. Phytoplankton samples were fixed by Lugol's solution. Phytoplankton was analysed according to Utermohl (1958) method by use of inverted microscope Leica DMIL. Chlorophyll *a* was detected with the help of multiparameter water quality sonde YSI 6600 V2. For this study it was important to know presence of potentially toxic algae, which would cause low ecological status of the lake. Ecological status of the lake were characterised by phytoplankton compound quotient (PCQ) index (Ott, Laugaste 1996) and quantity of Cyanobacteria biomass and species composition of phytoplankton.

Lake Lubāns is the largest lake in Latvia which is situated in the centre of the Eastern Latvian Lowland. It is highly transformed water body with clear water and shallow drainage basin. Lake Lubāns is surrounded by wetlands.

In July 2021 high water temperatures (27.5 to 28.7 °C) were observed, whereas in August were stated decrease of water temperature was evident (20.3 to 21.5 °C). Visibility according Sechi was low (0.6 to 0.8 m). All inspected sampling sites were characterised by intensive development of phytoplankton. The highest biomass was detected in pelagic zone: 10.85 mg L^{-1} in July and 13.56 mg L^{-1} in August. The lowest biomass was detected in littoral zone: 0.79 mg L^{-1} in July and 1.42 mg L^{-1} in August (Fig. 1). Development of cyanobacteria (blue-green algae) was detected in all sampling sites in July and August, where cyanobacteria formed 42 to 87% from total phytoplankton biomass.

The highest amount of chlorophyll *a* was detected in pelagic zone: 12.1 µg L^{-1} in July and 27.0 µg L^{-1} in August. The lowest amount of chlorophyll a was detected in littoral zone: 3.3 µg L^{-1} in July and 11.4 µg L^{-1} in August. Phytoplankton communities were dominated by potentially harmful Cyanobacteria colonies of *Microcystis* spp. and potentially harmful filamentous cyanobacteria *Planktolyngbya limnetica*. PCQ index (PCQ > 9) showed ecological quality of pelagic zone of lake as bad. Ecological quality of littoral zone was characterised as good till medium (PCQ = 3.5 – 6.1).

In the plankton community algae from seven algal divisions were detected: Cyanophyta (Cyanobacteria), Dinophyta, Cryptophyta, Chrysophyta, Euglenophyta, Bacillariophyta, Chlorophyta. Dominant algal taxa were *Anabaena* sp., *Aphanizomenon flos-aquae, Aphanocapsa* sp., *Chroococcus limneticus, Chroococcus turgidus, Coelosphaerium kuetzingianum, Coelosphaerium* sp., *Gloeocapsa* sp., *Gomphoshaeria naegeliana, Gomphoshaeria aponina, Gomphoshaeria lacustris, Merismopedia major, Merismopedia* sp., *Microcystis aeruginosa, Microcystis botrys, Microcystis ichthyoblabe, Microcystis wessenbergii, Oscillatoria* sp., *Oscillatoria tenuis, Planktolyngbya contorta, Planktolyngbya limnetica, Ceratium hirundinella, Glenodinium* sp., *Gymnodinium* sp., *Peridinium bipes, Peridinium cinctum, Cryptomonas* sp., *Rhodomonas* sp., *Dinobryon sertularia, Euglena* sp., *Phacus* sp., *Amphora ovalis, Aulacoseira granulata, Aulacoseira italica* var. *tenuissima, Cocconeis pediculus, Cyclotella* sp., *Cymatopleura solea, Fragilaria capucina, Fragilaria construens, Fragilaria crotonensis, Gyrosigma* sp., *Navicula gracilis, Navicula* sp., *Nitzschia acicularis, Nitzschia* sp., *Pinnularia* sp., *Surirella biseriata, Synedra acus, Synedra* sp., *Tabellaria fenestrata, Actinastrum hantzschii, Ankistrodesmus falcatus, Ankistrodesmus* sp., *Botryococcus braunii,*

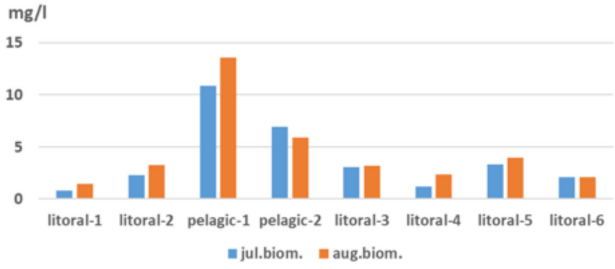

Fig. 1. Phytoplankton biomass observed in littoral zones (1, 2, 3, 4, 5, 6) and pelagic zones (1, 2) of Lake Lubāns in July and August 2021.

Coelastrum microporum, Cosmarium sp., *Cruccigenia* sp. *Desmodesmus* sp., *Eudorina elegans, Oocystis lacustris, Pediastrum boryanum, Pediastrum duplex, Pediastrum simplex, Pediastrum tetras, Selenastrum* sp., *Staurastrum* sp., *Tetraedron minimum*.

High biomass of harmful Cyanobacteria and high amount of chlorophyll a in the pelagial part of lake present evidence about processes of eutrophication just wide macrophyte and reed belt in littoral zone.

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Resources of crop wild relative *Trifolium fragiferum* in Latvia: dependence on nitrogen and rhizobial symbiosis

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Key words: crop wild relatives, mineral nutrition, nitrogen fertilizer, rhizobial symbiosis, strawberry clover.

Biological nitrogen fixation by legume-rhizobacterial symbiosis in temperate grasslands is an important source of soil nitrogen. On the other hand, in a context of coastal habitats, experimental evidence based on studies in dune grassland models containing legume species shows that in addition to plant productivity and nitrogen nutrition, legume-rhizobia interaction also determines plant community structure (van der Heijden et al. 2006). *Trifolium fragiferum* is a rare crop wild relative legume species, which in northern Europe is associated with coastal meadows. Legumes can obtain necessary N either in an inorganic form from soil or as organic N through symbiotic nitrogen fixation. An actual contribution of each type of N acquisition seems to be both genotype- as well as environment-dependent characteristic. So far, aspects of symbiotic nitrogen fixation in wild legume species is only seldom assessed, and there is no information on characteristics of rhizobia-plant interactions in the case of *T. fragiferum*. As a first step to fill this knowledge gap, the aim of the presnet study was to characterize dependence of different accessions of *T. fragiferum* in Latvia from their native rhizobia as well as additional nitrogen fertilization.

Dependence of all eight Latvian accessions of *T. fragiferum* and cv. 'Palestine' on added nitrogen as well as on symbiosis with native rhizobial bacteria was compared in controlled conditions. Asimbiotically cultivated, mineral-fertilized *T. fragiferum* plants gradually showed signs of nitrogen deficiency, appearing as decrease in leaf chlorophyll concentration, leaf senescence, decrease of growth rate (Fig. 1). This effect was partially genotype-dependent. Addition of nitrogen, inoculation with native rhizobia or both treatments significantly prevented the onset of these symptoms (Fig. 2), leading to both increase in plant shoot biomass as well as increase in tissue concentration of N. The actual degree of each type of response was genotype-specific. Accessions showed relatively similar degree of dependence on nitrogen (from 70 to 95% increase in shoot dry mass), but increase in shoot dry mass by inoculation with native rhizobia ranged from 27 to 85%. For several accessions (TF1, TF5, TF8) addition of nitrogen to already symbiotic plants resulted in additional biomass increase, but only TF5 positively responded to inoculation of nitrogen-fertilized plants. Nitrogen addition and plant symbiotic status significantly and in a genotype-dependent manner affected biomas partitioning. Most importantly, generative development was highly stimulated, especially, by combination treatment for TF1, TF2b, TF5 and TF6, but this characteristic was not affected in TF8.

In general, there were no correlation between growth stimulation and increase in tissue N concentration by the treatments. Both leaf chlorophyll concentration and chlorophyll *a* fluorescence parameter Performance Index were good indicators of changes in physiological status due to treatment with nitrogen or inoculation with rhizobia. Addition of N or rhizobial inoculant affected mineral nutrition at the level of both macronutrient and micronutrient concentration in different plant parts. As a result, each host plant-rhizobia combination resulted in appearance of a unique mineral nutrient profile within a plant, possibly reflecting differences in adaptation of

Fig. 1. Changes in morphology of *Trifolium fragiferum* plants from different accessions during cultivation in asymbiotic conditions. A, February 22; B, March 16.

mineral nutrition to changes in N metabolism among different accessions.

Usually, plant genotype dependence of symbiotic performance is evaluated for plants grown in N-free medium, allowing to estimate amount of fixed N directly from N analysis in shoots (Drew, Ballard 2010). In the present study, for practical reasons, control plants received near-optimal level of full mineral fertilizer, and it was revealed that addition of surplus nitrogen could completely replace rhizobial inoculation. On the other hand, strain genotype effects cannot be excluded.

It is well-known that high N supply reduces root nodule formation and/or nitrogen fixation efficiency in legumes (Streeter 1988; Ohyama et al. 2011). In field conditions, additional N fertilizer to dairy pastures had negative effects on morphology of *T. repens* plants and significantly decreased N_2-fixation activity (Harris, Clark 1996). In addition, even symbiotic legume plants favor uptake of mineral N from soil, as it is energetically less demanding process (Moustafa et al. 1969).

Additional studies aimed at revealing of genetic and functional diversity of rhizobial symbionts are necessary in order to fully understand differences found in different *T. fragiferum* accessions in respect to their native bacterial symbionts, as one of most important factors shaping their characteristic growth responses in highly heterogeneous conditions. Extremely important ecophysiological aspect of rhizobial symbiosis of *T. fragiferum* is related to interspecies competition in saline habitats, as shown by previous studies (Dūmiņš et al. 2021).

Acknowledgements

The study was supported by the Latvian Science Council project lzp-2020/2-0349 "Molecular, physiological and ecological evaluation of Latvian genetic resources of valuable wild legume species, *Trifolium fragiferum*, in a context of sustainable agriculture".

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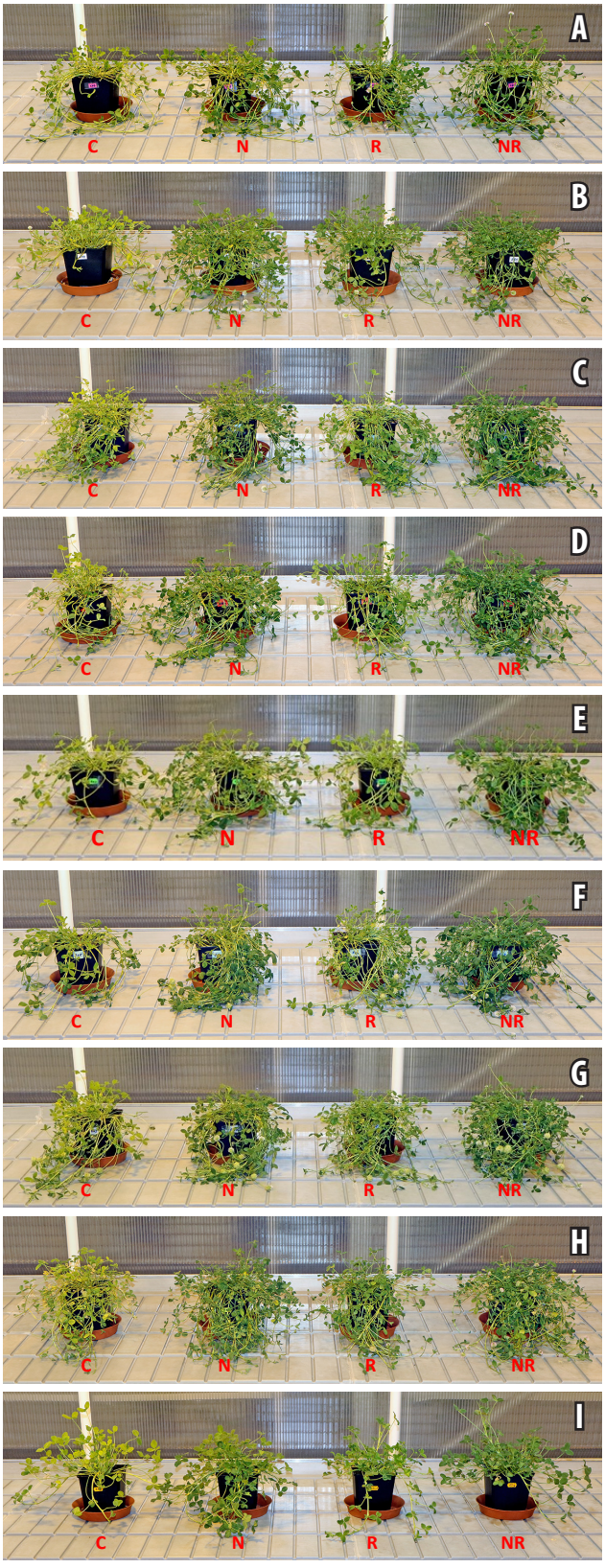

Fig. 2. Differences of *Trifolium fragiferum* morphology between control (C), N-treated (N), rhizobia-treated (R) and N + rhizobia-treated (NR) plants of different accessions. A, TF1; B, TF2; C, TF2b; D, TF3; E, TF4; F, TF5; G, TF6; H, TF7; I, TF8.

Resources of crop wild relative *Trifolium fragiferum* in Latvia: comparison of salinity tolerance

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Key words: crop wild relatives, ion accumulation, mineral nutrition, salinity tolerance, strawberry clover.

Soil salinization is one of most important problems in agriculture, and negative effects of salinity on crop productivity are becoming more severe on a background of global climate changes. Because of symbiosis with nitrogen-fixing rhizobacteria, salt tolerant forage legume species are especially important for saline marginal grasslands with characteristically low response to nitrogen fertilizers (Manchanda, Garg 2008). In this context, wild legume species from coastal habitats represent a valuable resource for developing salt resistant forage crops. *Trifolium fragiferum* is a perennial stoloniferous legume species, in nothern Europe associated with an endangered habitat "Baltic coastal meadow" (Janssen, Rodwell 2016). As salinity tolerance of *T. fragiferum* accessions in the Baltic Sea region has not been assessed, it was the aim of the present study.

Effect of increasing substrate salinity was evaluated using four Latvian accessions of *T. fragiferum* from sites with different substrate salinity, in comparison to the accession from highly saline habitat on the island of Bornholm (Denmark) and cv. 'Palestine'. All accessions tolerated presence of 5 g L^{-1} Na in substrate, but significant differences were evident in respect to negative effect on morphological parameters and biomass accumulation between different accessions. The major differences in salinity responses between various *T. fragiferum* genotypes were at the level of dry biomass accumulation as well as water accumulation in plant tissues, resulting in relatively more similar effect on fresh mass. Na^+ and Cl^- accumulation capacity was organ-specific, with leaf petioles accumulating more, followed by leaf blades and stolons.

It was expected that the accessions from more saline habitats (as TF1 and TF9) would show higher salinity tolerance in identical conditions of the controlled experiment in comparison to the accessions from habitats with low salinity (as TF2, TF4, TF7). This indeed was the case when relative effect of salinity was compared, as plants from accession TF9 were the most tolerant to 2 g L^{-1} Na treatment, but TF1 plants were the most tolerant to 5 g L^{-1} Na. However, in absolute terms, when looking for the accession producing the highest biomass at high salinity, *T. fragiferum* accessions TF1, TF2, TF7 and TF8 produced identically high amount of biomass at 5 g L^{-1} Na, with values for TF4 (Fig. 1) and TF9 (Fig. 2) being significantly lower. Consequently, from a practical point of forage production, relatively intolerant cv. 'Palestine' (TF8) still would have

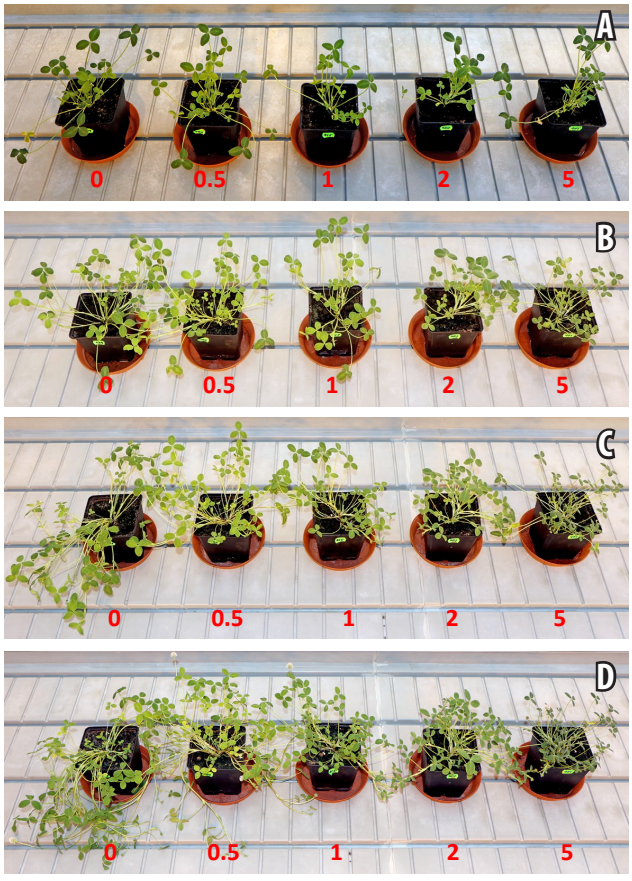

Fig. 1. Changes of morphology of *Trifolium fragiferum* plants (accession TF4) under effect of increasing doses of NaCl. A, February 23; B, March 2; C, March 9; D, March 16. Numbers indicate added Na concentration in substrate (g L^{-1}).

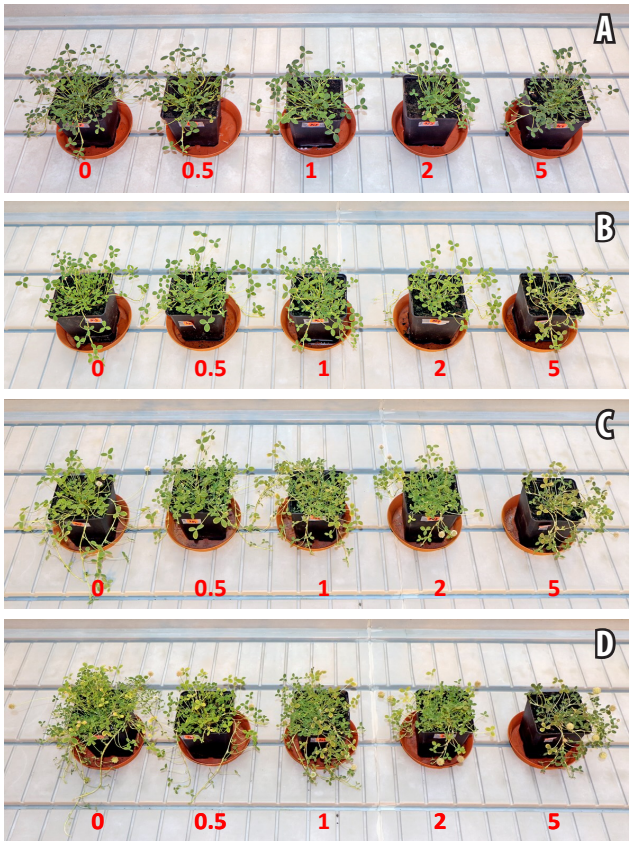

Fig. 2. Changes of morphology of *Trifolium fragiferum* plants (accession TF9) under effect of increasing doses of NaCl. A, February 23; B, March 2; C, March 9; D, March 16. Numbers indicate added Na concentration in substrate (g L^{-1}).

higher yield when cultivated in saline soil because of extremely pronounced biomass production ability of control plants, when compared to relatively most tolerant accession TF9 from saline coastal habitat in Bornholm (Fig. 2). In this respect, the most promising Latvian accession of *T. fragiferum* were TF1 from a saline wet shore meadow of Lake Liepājas: the accession showed the highest relative tolerance to 5 g L^{-1} Na and also were among the accessions with the highest absolute biomass production capacity at this salinity level. Physiological differences of accessions to increasing salinity was supported also by differences in mineral nutrient concentrations in plant tissues under the effect of salinity, resulting in unique, accession-specific mineral profiles. High intraspecies morphological and physiological variability in responses of *T. fragiferum* accessions to salinity allow to describe them as ecotypes.

Acknowledgements

The study was supported by the Latvian Science Council project lzp-2020/2-0349 "Molecular, physiological and ecological evaluation of Latvian genetic resources of valuable wild legume species, *Trifolium fragiferum*, in a context of sustainable agriculture".

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Air humidity-induced leaf petiole elongation in *Ranunculus sceleratus* and salinity tolerance: possible relationship with ethylene

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Key words: ethylene, humidity-induced responses, ion accumulation, leaf petiole elongation, *Ranunculus sceleratus*, salinity.

Aquatic and several wetland plants have an ability to respond to flooding by elongation of shoot parts as an adaptive mechanism. Thus, elongation of leaf petioles of *Ranunculus sceleratus* plants after flooding allow leaf blades to reach water surface. The response depends on action of endogenously produced ethylene, and it has been suggested that entrapment of ethylene in submerged plant tissues is responsible for petiole elongation (Park et al. 2011). Many plant species from coastal habitats are frequently and concomitantly subjected to fluctuations in both water level as well as substrate salinity. It is generally accepted that plant growth inhibition by salinity is due to deleterious ionic, osmotic or reactive oxygen species-related effects of NaCl, but participation of hormonal signals in salinity response seems to be logical (Beklheiri, Mulas 2013).

Preliminary experiments have shown that *R. sceleratus* accession from a sea-water affected coastal wetland habitat is extremely tolerant to NaCl and heavy metals in substrate, with high accumulation capacity in aboveground parts for Na^+ (Landorfa-Svalbe et al. 2020) and Cd, Mn, Pb and Zn (Andersone-Ozola et al. 2020). Therefore, the aim of the present study was to establish a model system for assessing possible ethylene-dependent responses related to salinity tolerance, using *R. sceleratus*.

Initial experimental system involved plastic containers (48 L) where intact *R. sceleratus* plants in a phase of vegetative rosette growth were placed on individual plates. Treatment with NaCl was performed by gradually increasing amount of NaCl in watering solution, reaching final concentration of Na in plant substrate 4 g L^{-1}. Gaseous environment inside the containers were modified by placing glass vials with ethylene-releasing chemical 2-chloroethylphosphonic acid (ethephon) or 1-methylcyclopropene-releasing chemical SmartFresh (MCP). MCP acts as a competitive inhibitor of ethylene action by irreversible binding to ethylene receptors, and is able to block plant responses to both endogenously-produced and exogenously-applied ethylene. Containers were closed with lids and ventilated once a day, also replacing all gas-releasing solutions. Treatment were performed for six days. Due to experimental conditions, high humidity inside containers induced fast elongation of leaf petioles (Fig. 1). This effect was ethylene-dependent,

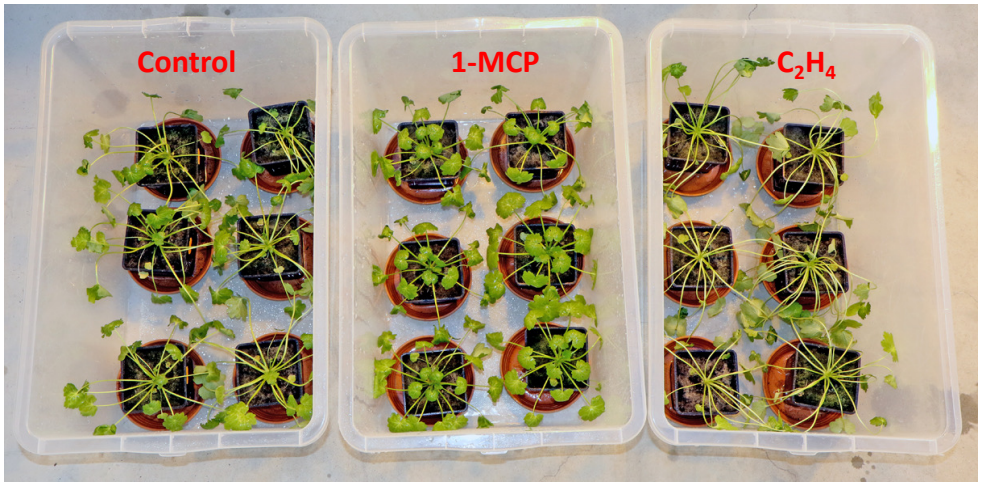

Fig. 1. Experimental system of asessing air humidity-induced ethylene-dependent elongation of leaf petioles in *Ranunculus sceleratus*. Container lids removed, five days after start of the treatment.

as presence of MCP completely blocked it. Moreover, exogenous ethylene further promoted petiole elongation. Within 6 days, treatment with exogenous ethylene resulted in elongation of petioles of all already formed leaves. After removal of ethylene, development of new leaves was evident within 24 h. In this system, NaCl had only minor inhibitory effect on leaf petiole growth. However, exogenous ethylene decreased but MCP increased Na^+ and K^+ accumulation in leaf blades of NaCl-treated plants, without significant effect in leaf petioles.

As high air humidity itself could have been affecting ion accumulation or other physiological responses, it was necessary to customize the experimental system allowing to exclude high humidity effects. This was achieved by adding silica xerogel pellets as desiccant. The experimental system was calibrated for five days with different amounts of silica xerogel per container against plant petiole elongation response, and it was found that 200 g of silica xerogel in a shallow dish was efficient enough to completely stop induced petiole elongation down to the value of control plants kept outside the container at ambient air humidity of a greenhouse (65 – 75%).

The experimental system with plants was also calibrated with different concentrations of ethylene in gaseous environment against elongation response of leaf petioles. It was found that 10 μL L^{-1} ethylene was the most efficient in promoting elongation, but at 100 μL L^{-1} ethylene several non-specific effects appeared, including desiccation of plant tissues.

Further, efficiency of the designed experimental system was tested in conditions of salinity treatment. Two subsystems (with high humidity or with desiccant) were used, each containing control plants or NaCl-treated plants in a gaseous environment containing (i) pure air, (ii) MCP, (iii) ethylene, (iv) MCP + ethylene. In high humidity conditions, NaCl inhibited petiole elongation by 25%, ethylene treatment fully reversed this inhibition and stimulated elongation in comparison to that in control plants. MCP treatment fully reversed this ethylene effect. In desiccated conditions, NaCl inhibited petiole elongation, which was reversed by ethylene without additional elongation stimulation. However, MCP only partially inhibited ethylene effect on petiole elongation.

Ethylene affected Na^+ and K^+ accumulation in leaves, but the most pronounced effect of ethylene on ion accumulation was evident in plant stems. In high humidity conditions, ethylene inhibited Na^+ accumulation in NaCl-treated plants, but MCP reversed this effect. At decreased humidity, Na^+ accumulation potential was less than at high humidity, and ethylene treatment led to further decrease, but MCP was only partially capable in reversing this effect. K^+ accumulation was inhibited in NaCl-treated plants at both humidity levels, and ethylene treatment led to further inhibition of accumulation, but MCP was not able to reverse this effect.

It appears that stimulation of endogenous ethylene production in *R. sceleratus* plants at high air humidity or in flooded conditions reverses inhibitory effect of salinity on plant growth and concomitantly inhibits accumulation of Na^+ in tissues. However, further improvement of the experimental system is necessary, to allow for more precise control of air humidity to obtain higher possible degree of generalization of the obtained results. Also, other model species less sensitive to increased air humidity need to be tested in the system.

Acknowledgements

We sinecerly thank Dr. biol. Una Andersone-Ozola for a kind help with plant establishment.

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Evaluation of the physiological and genetic diversity of the Latvian population of strawberry clover (*Trifolium fragiferum*), a crop wild relative species

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Key words: crop wild relatives, genetic diversity, mineral nutrition, strawberry clover, stress tolerance, symbiotic rhizobia.

Global climate change and increasing anthropogenic impacts are also increasing the heterogeneity of agro-ecosystems. In order to maintain stable and high-quality yields in such conditions, it is necessary to increase the diversity of crop varieties, paying particular attention to their resistance to abiotic stress. Crop wild relatives are a particularly valuable resource in achieving this goal (Dempewolf eet al. 2014). The objective of the study was to obtain a new knowledge on genetic and biological diversity of Latvian accessions of crop wild relative legume species, *Trifolium fragiferum*, in the context of sustainable agriculture. Several specific tasks were defined to achieve the goal: (i) to evaluate tolerance of accessions to abiotic and anthropogenic factors in controlled conditions; (ii) to characterize genetic diversity of accessions; (iii) to establish tissue culture of all accessions; (iv) to characterize edaphic conditions in natural habitats; (v) to evaluate symbiotic dependence of accessions in controlled conditions.

Trifolium fragiferum seed material from eight identified Latvian micropopulations (accessions) was used for the experiments. Biological and genetic comparisons were performed with the Australian commercial cultivar 'Palestine' (TF8). Samples of strawberry clover from Bornholm (Denmark, TF9) and Haapsalu (Estonia, TF10) were also used in the study of genetic diversity, but TF9 was also used in the study of salt resistance. Vegetation pot experiments were performed under controlled conditions in a greenhouse, comparing the growth and physiological

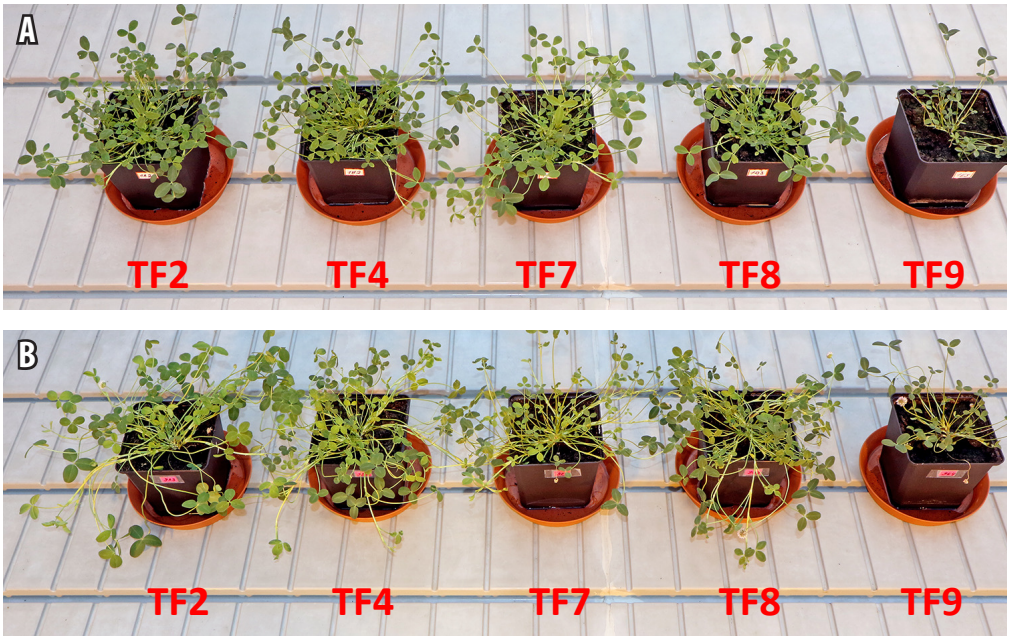

Fig. 1. Morphological differences of *Trifolium fragiferum* plants from different accessions. A, 3-week-old control plants; B, 4-week-old control plants.

response of selected genotypes to increasing soil salinity, increased moisture and soil flooding, repeated cutting, repeated trampling, increasing concentrations of heavy metals (Pb and Cd), nitrogen fertilizers and symbiosis. In addition, a field study of Latvian strawberry clover habitats was carried out to determine the chemical composition of the soil and the mineral nutrition of the plants.

When plants from different accessions were grown in controlled conditions, characteristic morphological differences between them were evident (Fig. 1). Plants from all micropopulations were characterized by relatively high resistance to the tested adverse environmental factors, but there were significant differences between individual micropopulations in terms of biomass accumulation and changes in physiological parameters due to these factors. The analysis of genetic diversity showed a low degree of diversification within individual populations, but micropopulations were genetically differentiated from each other, and a significant difference emerged between "marine" and "river/lake" coastal habitats.

The observed genetic and physiological diversity shows that the geographically isolated populations of strawberry clover in Latvia represent different ecotypes and are considered to be a valuable biological resource of crop wild relatives. Of particular value in terms of adaptive potential is the genotype TF1 from the salt-affected wet meadow on the shores of Lake Liepāja.

Acknowledgements

The study was supported by the Latvian Science Council project lzp-2020/2-0349 "Molecular, physiological and ecological evaluation of Latvian genetic resources of valuable wild legume species, *Trifolium fragiferum*, in a context of sustainable agriculture". We sincerely acknowledge participation of Lāsma Neiceniece (Faculty of Biology, University of Latvia) and Magnolia Garbarino (Pawling High Scholl, NY, USA) in this study.

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Occurrence of protozoa in drinking water – a comparison of different methods

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Key words: biofilms, microbial diversity, drinking water, protozoa.

Free-living protozoa (FLP) are able to create biofilms in water pipes and can protect pathogenic bacteria from high temperatures, disinfectants, can serve as a reservoir for bacterial populations, for example *Legionella pneumophila* which is a causative agent of Legionnaires' disease. The interaction of bacteria and protozoa can increase the resistance of microorganisms to antibiotics and their virulence (Muchesa et al. 2018).

The aim of this study was to compare different methods of identification FLP in the drinking water samples from water supply systems of Riga apartment houses and hotels, identifying the main FLP genus and occurrence depending on sample incubation, water temperature, address location (right or left bank of the River Daugava). Last point is important because that part of Riga which is at right bank have water supply from underground sources, but the left bank is using purified water from Daugava river.

Free-living protozoa were cultivated on Page's Amoeba saline (PAS) with peptone yeast extract glucose (PYG; Vaerewijck et al. 2010). Hot and cold drinking water samples from apartments (n = 76) and hotels (n = 50) were tested for the presence of FLP using three methods: microscopy, PCR methods and 18S rRNA sequencing. PCR was implemented using *Acanthamoeba* (Schroeder et al. 2001), *Vahlkampfidae, Amoebaidae* (Calvez et al. 2012) and *Vermamoeba* (*Hartmanella*) (Solgi et al. 2012) specific primers. 18S rRNA sequencing was proceeded using TAReuk454FWD1 (CCAGCASCYGCGGTAATTCC) (Stoeck et al. 2010) and V4r (ACTTTCGTTCTTGAT) (Bradley et al. 2016) primers which amplify 18S rRNA gene V4 region. The rRNA amplicons were sequenced with Illumina MiSeq.

With microscopy FLP were detected in 61 (80%) apartment samples and 45 (90%) samples from hotels. The most common genera were *Hartmanella, Vahlkampfia* and *Acanthamoeba*.

Using PCR methods it was found that 70% of samples from apartments and 74% from hotels were FLP positive. It was observed that presence of *Vahlkampfia* was lower in water samples when PCR methods were used: with microscopy this genus was detected in 20 samples from apartments and 18 from hotels, but with PCR in two and three samples respectively. There were no differences in FLP diversity between samples from left and right bank of Daugava in Riga.

Third method was 18S rRNA sequencing, and it showed that in hot water samples the abundance of *Vermamoeba* was higher than in cold water: 60 and 20.3% respectively (Fig. 1B). Other main taxa were: *Chromulinales* (golden algae), *Paracercomonas* (genus *Rhizaria*), *Echinamoeba*, *Oligohymenophorea* (class of ciliates). *Paramicrosporidium* were also detected, which is interesting because they are known as parasites of amoebae, and they belong to the clade *Rozellomycota* (*Cryptomycota*) (Quandt et al. 2017).

It was noticed that sample cultivation in PAS and PYG media raised *Vermamoeba, Chromulinales* and

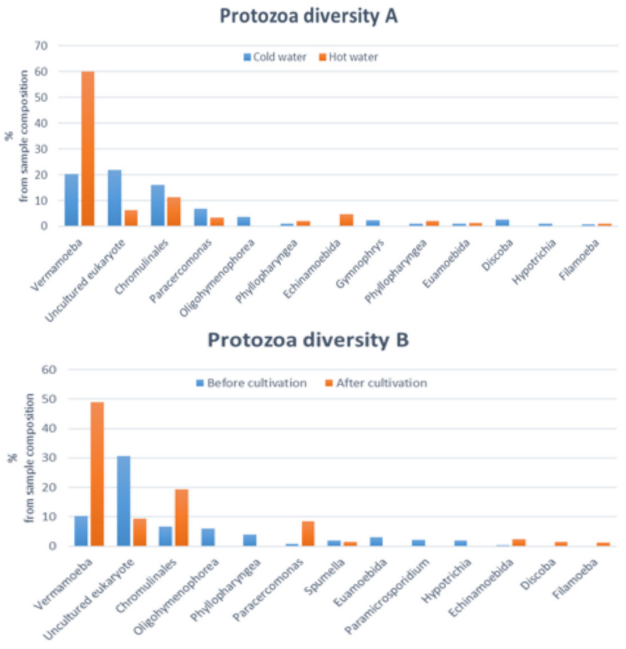

Fig. 1. Protozoa diversity in cold/hot water (A) and uncultivated/cultivated samples (B) (with 18S rRNA sequencing).

Paracercomonas proportion in particular: from 10.2 to 48.9% (Fig. 1A). Other main taxa were *Phyllopharyngea* (class of ciliates), *Euamoebida, Spumella* (golden algae).

Also it was noticed that in cold water samples and before cultivation there was bigger proportion of uncultured eukaryotes. In hotel samples it was noticed that 41.5% from eukaryotes were uncultured.

18S rRNA sequencing also did not show big difference between samples from right and left bank of Daugava, but at left bank was bigger uncultured eukaryote proportion.

After incubation of the samples diversity of protozoa decreases. This is because used media are suitable for growing amoebas, so *Amoebozoa* prevailed. It was noticed that water temperature affects diversity of amoeba – *Vermamoeba* inhabit water with higher temperature more.

As conclusion can be said also that at least two methods should be used to analyze water samples. With microscopy is not possible to detect all protozoa, but it helps to find positive samples and then analyze them with PCR or 18S rRNA sequencing. And from this study can be seen that *Vahlkampfia* are mostly detected only with microscopy, but not with PCR. The reason of that can be difficulties of DNA extraction.

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Temporal and spatial prevalence of Q-fever in dairy herds in the period from 2012 to 2021 and correlation with environmental factors

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Key words: *Coxiella burnetii*, epidemiology, q fever, spatial distribution.

Q-fever is widespread zoonotic disease caused by bacteria *Coxiella burnetii.* The goal of this study was to estimate the temporal and spatial prevalence of Q-fever in Latvian dairy herds in the period from 2012 to 2021 and correlation with environmental factors.

The spread of Q-fever is influenced by several factors, animal density, rainfall, temperature, terrain features, vegetation, wind speed (Nusinovici et al. 2015). Herd size as a risk factor identified in Ireland, Denmark (Paul et al. 2012), the Netherlands (Engelen et al. 2014), Portugal (Anastácio et al. 2016) and Italy (Barlozzari et al. 2020). Regional animal density – in Sweden (Nusinovici et al. 2015), France (Pandit et al. 2016) and Denmark (Agger et al. 2016). Strong winds and low precipitation have been identified as risk factors in Sweden (Nusinovici et al. 2015). According to the Van der Hoek study, a radius of 5 km is a risk area (Hoek et al. 2011). According to a study by Tissot Dupont, C. burnetii can spread up to 30 km in strong winds (Tissot-Dupont et al. 2004). Previous studies in Latvia have concluded that animal import and the territorial density of cattle and their herds, as well as their proximity to a positive farm, are important risk factors for the spread of Q-fever (Boroduske et al. 2017; Grantina-Ievina et al. 2022). The impact of woodland on the prevalence of Q-fever was considered in this work.

In the period from 2012 to 2015 Q-fever was more concentrated in Zemgale region and detected in Vidzeme region, also near city Preili (Fig. 1). In the period from 2016 to 2018 Q-fever has become more prevalent around nearby

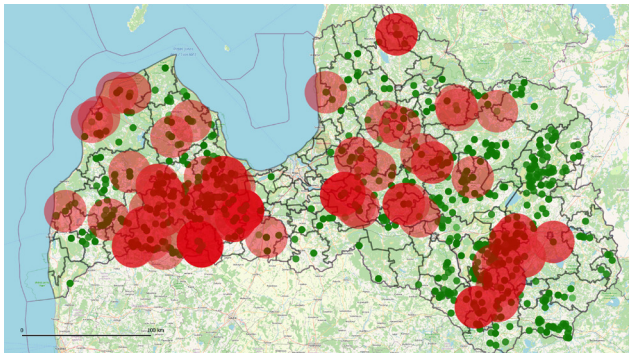
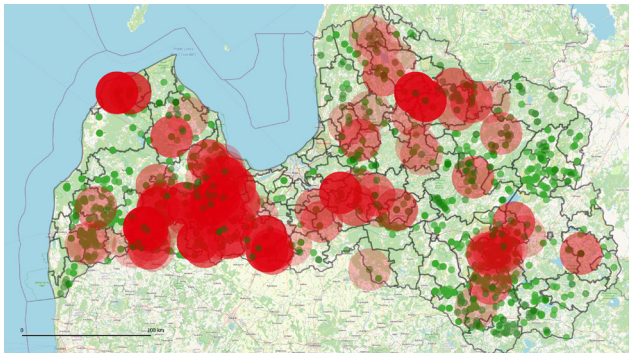


Fig. 2. Prevalence of Q fever in Latvia in 2016 – 2018. Negative samples are marked as green circles, with a risk area of 5 km around the farm. Positive samples are colored in red; the size of the circles is 30 km around the farm, thus marking the radius in which *Coxiella burnetii* is likely to spread. Scale 1 : 2 060 000.



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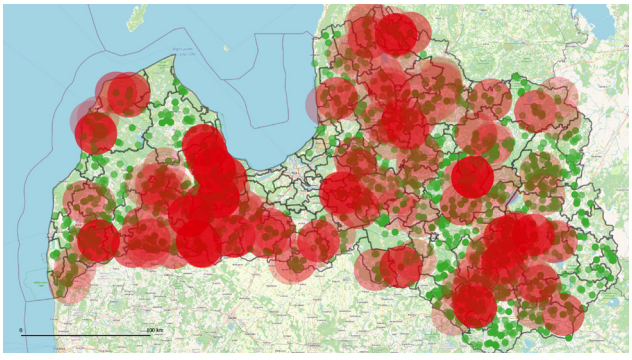

Fig. 3. Prevalence of Q fever in Latvia in 2019 – 2021. Negative samples are marked as green circles, with a risk area of 5 km around the farm. Positive samples are colored in red; the size of the circles is 30 km around the farm, thus marking the radius in which *Coxiella burnetii* is likely to spread. Scale 1 : 2 060 000.

farms as example can be seen around Daugavpils region (Fig. 2). In the period from 2019 to 2021 Q-fever was spread all over Latvia, for first time it was detected in Mazsalaca, Aglona, Ludza, Priekuļi, Tērvete un Kocenu counties (Fig. 3). Forests are one of the natural barriers that hold back the wind. Therefore, in theory, the prevalence in areas with more forests should be lower. Correlation analysis was performed using freely available statistical data of the State Forest Service. The correlation coefficient *r* obtained in the correlation analysis of the number of serologically positive herds in the region and forest cover on average over three periods of data was –0.183, which indicates a small negative correlation, but the value of the coefficient of determination R^2 was 0.0336. The correlation coefficient *r* obtained in the correlation analysis of the number of positive herds tested for the presence of *C. burnetii* DNA in milk samples in the region and woodlands was –0.05, which indicates an insignificant correlation, the value of the coefficient of determination R^2 was 0.0022.

In conclusion, woodlands correlate negative with the spread of Q-fever: regions with more forests have lower prevalence of Q-fever. Since correlation is low other affecting factors must be taken in account. No special preventive measures have been taken to control Q-fever at the national level, thus Q-fever has spread throughout the territory of Latvia.

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Evaluating the seed degradation potential of *Fusarium* epiphytic fungi isolated from seeds: methodology and initial results

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Key words: epiphytic fungi, *Fusarium*, soil seed bank, weeds.

Soil seed bank is of crucial importance for the survival and dynamic of annual plant populations, especially in arable lands. In turn, the fate of the seeds in the soil can be influenced by the fungi that colonize the seeds either before dispersal or later, when the seeds are buried. The effect of fungi on the seeds depends on the ability of a particular strain to infect and degrade the seeds, as well as on the defense capacity of the seeds (Davis et al. 2008). It is important to determine to what extent the intraspecific variability of the defense traits influences the persistence of the seeds in soil.

This study focused on two noxious weed species, *Avena fatua* L. and *Echinochloa crus-galli* (L.) Beauv. According to previous reports, different Fusarium species and isolates can degrade the seeds of *A. fatua* (de Luna et al. 2011) and decrease seedling emergence of *E. crus-galli* by either degrading the seeds or infecting the seedlings of (Mohler et al., 2012). The aim of this study was to test different methodological approaches allowing for assessment of fungal capacity to infect and degrade weed seeds at laboratory conditions and to determine the ability of *Fusarium* fungi isolated from the seeds of these weed species to infect and degrade the seeds in laboratory conditions.

Different approaches have been used to test the ability of fungi to infect seeds or seedlings. Usually the seeds are surface-sterilized to exclude the effect of other epiphytic microorganisms, although it is important to remember that in the field conditions interactions between the microorganisms determine the outcome of seed colonization and ultimately the seed fate. Seeds can be placed near to the growing mycelium or to the mycelial discs (de Luna et al. 2011; Links et al. 2014; Chen et al. 2018; Gianinetti et al. 2018), or inoculated with spores or spore suspension (Franke et al. 2014). Another important aspect is seed germination. Seed dormancy is important for the maintenance of the soil seed bank, while germination can allow seeds to escape fungal infection, if seedlings are not affected. If experiments are performed with non-dormant seeds, conditions that prevent seed germination, while not affecting fungal infection, such as low temperature or low water potential, can be used (Finch et al. 2013; Gianinetti et al. 2018).

Seeds of each species were collected at maturity in four (*A. fatua*) and three (*E. crus-galli*) constant locations in 2020 and in 2021. To isolate epiphytic fungi, seeds from 750 *A. fatua* plants and 350 *E. crus-galli* plants were collected in sterile containers and stored at 4 to 5 °C. Fungi were isolated within 48 hours from collection. Seeds were agitated in sterile 10 mM $MgSO_4$ and epiphytic fungi were isolated from rinsing solution on 10% yeast malt agar medium (YMA) using dilution to extinction method. Pure cultures of four *Fusarium* isolates isolated from the seeds of two *E. crus-galli* and two *A. fatua* populations collected in 2020 were used in further experiments (F1, F35, F55 and F76), as well as three *Fusarium* strains, *Fusarium graminearum, Fusarium oxysporum* and *Fusarium culmorum*, provided by the Microbial Strain Collection of Latvia.

Seeds from two *A. fatua* populations collected in 2020, from two *E. crus-galli* populations collected in 2020 and from three *E. crus-galli* populations collected in 2021. were used in three different experiments, using different seed infection methods. Three methods of seed infection were tested. (1) Seeds, either left intact or surface sterilized, were placed near mycelial discs on water agar medium; (2) non-sterilized intact or dehulled seeds were placed on water agar or full YMA near a growing mycelium; (3) surface-sterilized seeds were inoculated with a fungal spore suspension (250000 spores mL^{-1}) and placed on water agar. Non-inoculated seeds placed on the same medium were used as a control.

Germination percentage of the seeds placed near mycelial discs was 0 – 12.5% in *A. fatua* and 25.0 – 62.5% in *E. crus-galli*. Moreover, 93.8 – 100% of the *A. fatua* seeds and 71.4 – 100% of the *E. crus-galli* seeds had signs of infection, i.e., hyphae from the mycelial discs reached

seed surface. However, only 0 – 10% *A. fatua* seeds proved decayed when tested with a cut-test, irrespective of the treatment. Only surface-sterilized *E. crus-galli* seeds were decayed (21.1 and 14.7% depending on the population). There were no decayed seeds in the experiment where seeds were placed near growing mycelia, possibly due to interference of the native seed microbiota or, in seeds placed on water agar, due to poor mycelial growth. In the experiment where seeds were inoculated with spore suspension, 41.7% *A. fatua* seeds decayed in the control treatment, indicating, that surface sterilization was not effective enough to protect the seeds. Inoculation with F55 isolate spore suspension resulted in a significant increase in the proportion of decayed seeds (91.7%). No *A. fatua* seeds germinated.

In *E. crus-galli* seeds, background contamination of non-inoculated control seeds with *Fusarium* was less pronounced than in *A. fatua*, only in one of the populations 7.7% seeds were infected. The proportion of infected *E. crus-galli* seeds varied between the populations. The proportion of decayed seeds did not differ significantly from control (3.8%), although decay was observed in seeds inoculated with F1, F35, F55 and *F. culmorum* spores (7.1 – 28.6%); and 0 – 40.0% of the seeds germinated. The proportion of infected seedlings was different between both the populations and the isolates. The proportion of infected seedlings in treatments with F35 (two populations), F1 (one population) and F55 (one populations) was significantly different compared to control.

In conclusion, assessment of fungal capacity to infect and degrade seeds was strongly affected by the method applied for seed infection. The results of this study show that some of the epiphytic *Fusarium* fungi isolated from seeds have potential to infect *E. crus-galli* seedlings and to degrade *A. fatua* seeds. This ability differs between the isolates and may depend on the seed characteristics in different populations.

Acknowledgements

This research is funded by the Latvian Council of Science project "Characterization and dynamics of seed-associated microbiome in weed soil seed-bank", project No. lzp-2020/1-0163.

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Presence of unintended genetically modified organism contamination in food, feed, their additives and food supplements obtained in retail and online shops

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Key words: food and feed, contamination, genetically modified organisms.

In the Rapid Alert System for Food and Feed there have been 679 notifications about genetically modified (GM) food or feed till 2018 (Rostoks et al. 2019). During 2008 – 2014 the largest numbers of border rejections by European Union (EU) due to unauthorized GM or novel food where from China, the United States, Hong Kong, Thailand, and India (Cuello et al., 2020). Among top 10 countries with the highest numbers of incidents recorded on the GM Contamination Register within the time period 1997 – 2013 were Germany, USA, France, Canada, The Netherlands, Australia, Austria, Italy, Sweden, and Japan (Price, Cotter 2014). A scientific study has gathered information from official reports (2000 – 2013) from EU Member States on the likelihood that food and feed samples that are non-compliant with GM organism (GMO) regulations can be detected in official controls. These are samples that contain authorized GMO events above the 0.9% threshold for food and feed, but are not labelled, or they contain unauthorized GMO events above the 0.1%. A higher probability of detection was found for feed samples compared to food samples. The authors conclude that the regulatory framework for food does not guarantee consumers' right to free choice of food and that consumers are likely to be misled as to whether food and feed contains GMOs (Areal, Riesgo 2021). Comparing various crops in terms of the possibility to be contaminated with GMO in the top there are rice, maize, oilseed rape/canola, soybean, flax and cotton (Price, Cotter 2014; Bohanec et al. 2017). The AquAdvantage® Atlantic salmon has been not so far detected as unauthorized GMO anywhere but laboratories have developed appropriate detection methods (Debode et al. 2018). In recent years the incidence of detection of GM microorganisms (GMM) carrying antimicrobial resistance genes in food and feed enzymes, additives and flavourings has increased (Fraiture et al. 2020).

In the present study a screening for GMO contamination in food, feed, their additives and food supplements obtained in retail and online shops in Latvia was performed. The total number of samples was 91. One sample was received from the Food and Veterinary Service Border Control Department. Four samples were purchased in stores of third countries goods in Riga, but the remaining samples were purchased in various stores or by ordering from these companies' websites. A total of 67 food and 10 feed samples were analyzed, as well as 10 food additives and food supplements and four enzyme mixtures and animal feed additives. Eighteen food and feed samples contained Atlantic salmon. Detection and quantification of GMO in food and feed samples was done according to the official methods approved in EU. Detection and identification of GMM was done according to the methods published in recent studies (Paracchini et al. 2017; Fraiture et al. 2020, Berbers et al. 2020 etc.). Bacterial isolates from several samples were obtained using standard microbiology techniques.

A higher proportion of GMO contaminated samples was found for samples from outside the EU (13.73%) compared to samples from the EU (7.5%), but the difference was not statistically significant. In total GMO were found in 10 samples. Comparing food samples with animal feed samples, it was detected that 10.45% of food samples and 30.00% of animal feed samples had GMO contamination. From these samples non-compliant to GMO regulations were three food and three feed samples (Table 1). Of the 14 samples of food and feed additives and food supplements, three samples were non-compliant, i.e., containing live bacteria with recombinant DNA. One of these samples was choline chloride with 60% corn. From this sample *Bacillus cereus* / *Bacillus subtilis* isolate was obtained with partial recombinant plasmids pGMBsub01, pGMBsub03, and pGMBsub04, that have been found in other studies as well (Paracchini et al. 2017; Berbers et al. 2020). From L-arginine sample *Bacillus velezensis* was isolated containing part of the cloning vector pCCR9 and plasmid pGMBsub01. The third sample was supplementary mineral feed for cows containing several vitamins. From this sample *Bacillus*

Table 1. List of food and feed samples containing GMO impurities. LOQ, limit of quantification; m/m, mass percent

No.	Product, country of origin	Target organism	Detected GMO events (% m/m ± SD)	Compliance with GMO labeling requirements
1-8	Silk tofu, USA	Soy	Soy: GTS40-3-2 (0.15 ± 0.11), A2704 (0.13 ± 0.01), MON89788 (0.03 ± 0.01)	Compliant, because GM soybean impurities are < 0.9 % m/m
1-15	Dough mix, South Korea	Soy, maize, wheat, rice	Soy: MON89788 (74.75 ± 20.94)	Non-compliant, as GM soybean impurities are > 0.9 % m/m and it is not indicated on the label that product contains GMOs
1-24	Canned cat food, Spain	Salmon, vegetables	Soy: MON89788 (78.74 ± 9.70)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-8	Silk tofu, USA	Soy	Soy: A2704 (< LOQ)	Compliant, because GM soybean impurities are < 0.9 % m/m
2-15	Granulated corn bait, Lithuania	Maize	Soy: A2704 (< LOQ), GTS40-3-2 (6.74 ± 2.41), MON87701 (0.25 ± 0.07), MON87708 (3.40 ± 0.11), MON89788 (< LOQ). Maize: MON863 (< LOQ)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-16	Granulated maize and soy mixture – fish feed, Lithuania	Maize, soy, canola	Soy: GTS40-3-2 (1.28 ± 0.05), MON87701 (101.41 ± 6.67), MON87708 (0.01 ± 0.00), MON89788 (1.02 ± 0.20). Maize: MON863 (< LOQ), 89034 (< LOQ)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-39	Chips with grilled corn taste, South Korea	Maize	Maize: TC1507 (0.01 ± 0.00)	Compliant, because GM soybean impurities are < 0.9 % m/m
2-40	Flavored cheese chips, USA	Maize	Maize: MON810 (15.72 ± 5.84), DAS59122 (0.63 ± 0.05), MIR604 (99.80 ± 9.30)	Non-compliant, as GM maize impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-41	Breakfast cereals, USA	Maize	Maize: MON810 (28.04 ± 2.64)	Compliant, because on the label it is stated that it contains bioengineered food ingredients
2-42	Blue cheese flavored chips, USA	Maize	Maize: DAS59122 (71.36 ± 13.27), MIR604 (7.70 ± 2.26)	Non-compliant, as GM maize impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs

paralicheniformis was isolated containing part of the plasmid pGMBsub01. One sample (mixture of L-leucine, L-isoleucine, L-valine and vitamin B6) was considered as suspicious, meaning that *Bacillus safensis* culture was isolated, and the marker gene *cat86* of chloramphenicol resistance in isolated culture was detected.

In conclusion, the study indicates presence of GMO contamination in food, feed, their additives and food supplement samples, which emphasizes the need for improved monitoring.

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