https://doi.org/10.22364/eeb.22.05

UNIVERSITY OF LATVIA

ABSTRACTS
OF THE 82nd SCIENTIFIC CONFERENCE OF THE UNIVERSITY OF LATVIA

January – February 2024

Diversity of bacteria in drinking water samples from apartment buildings and hotels due to the incidence of *Legionella* spp. and free-living protozoa

Artjoms Mališevs*, Svetlana Makarova, Genadijs Konvisers, Juris Ķibilds, Linda Labecka, Olga Valciņa, Lelde Grantiņa-Ieviņa

Institute of Food Safety, Animal Health and Environment "BIOR", Latvia
*Corresponding author, E-mail: artjoms.malisevs@bior.lv

Key words: biofilms, *Legionella*, protozoa, water.

Biofilms in water supply system pipes are created by free-living protozoa (FLP). They can protect different bacteria from high temperatures, disinfectants. These bacteria can be pathogenic. For example, *Legionella pneumophila* that is a causative agent of Legionnaires' disease. The interaction of bacteria and protozoa inside biofilms can increase the resistance to antibiotics and virulence (Muchesa et al. 2018).

The aim of this study was to describe bacteria diversity in the drinking water supply systems of Riga apartment houses and hotels in Riga and Jurmala, identifying the main bacteria phylum and occurrence depending on water temperature, water taking place (kitchen or bathroom), address location (right or left bank of the river Daugava). Other aim of this study was to investigate the coexistence of FLP and Legionella spp. in these water samples.

Free-living protozoa were cultivated on Page's Amoeba saline with peptone yeast extract glucose (Vaerewijck et al. 2010). Hot and cold drinking water samples from apartments were tested for the presence of FLP using three methods: microscopy, PCR methods and 16S 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. 16S rRNA amplicon sequencing libraries were prepared according to Illumina's 16S Metagenomic Sequencing Library Preparation guide (document part #15044223 Rev. B), which uses primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 from Klindworth et al. (2013) to amplify a 464 bp long fragment of the V3-V4 regions of the bacterial 16S rRNA gene. Obtained sequences were analyzed using two resources: https://www.bv-brc.org/ for taxonomic analyzes, https://biit.cs.ut.ee/clustvis/ to make bacterial diversity clusters.

With microscopy FLP were detected in 55.6% of apartment samples and in 68% of samples from hotels. The most common genera were *Hartmanella, Vahlkampfia and Acanthamoeba*. From samples, which were detected as FLA positive with microscopy, using PCR methods it was found that 70% of samples from apartments and 74% from hotels were FLP positive.

From 16S results it can be see that there were more *Legionella*-positive samples that in same time were FLA positive (Fig. 1). The same situation was evident for apartment and hotel water samples.

Using 16S sequencing several bacterial phylum were detected in water samples. Most common were *Planctomycetota, Bacillota, Acidobacteriota, Candidatus Omnitrophota* in all samples. The most common phylum was Pseudomonadota with the sequence abundance in

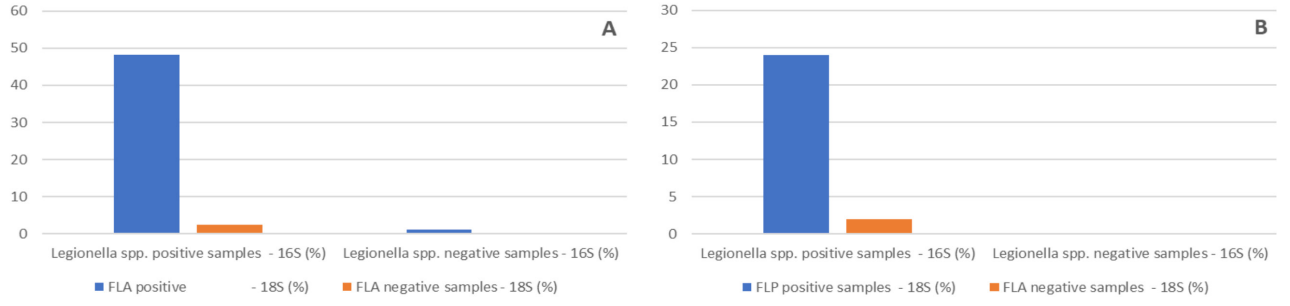

Fig. 1. Free-living amoeba and *Legionella* coexistence in apartment (A) and hotel (B) water samples detected with 18S and 16S rRNA sequencing.

average 32.13% from all bacterial sequences in cold water samples, 27.47% in hot and 32.92% in hotel water samples. *Deinococcota* were common in hotel water samples (average 7%).

Bacterial diversity clusters showed that there were no connection between sample taking place (kitchen or bathroom, left or right Daugava bank) and bacterial diversity, but hotel water samples were grouping based on hotel building. So it can be said that each building had its own and similar bacterial diversity. Shannon's diversity index was higher in cold water samples (3.19) vs. in hot water samples (2.72), and in hotel samples (2.32).

In water samples several genera of potentially pathogenic bacteria were detected, for example, *Coxiella, Leptospira, Listeria, Corynebacterium, Mycobacterium*, and *Pseudomonas*.

Using 16S sequencing several parasites of free-living amoeba were detected. *Candidatus Berkiella* is a parasite of *A. polyphaga* amoeba (Mehari et al. 2016). *Candidatus Amoebophilus* from phylum Zygomycota (fungi) can infect *Mayorella vespertilioides* amoeba (Mrva 2011). So there is a connection between bacterial diversity and FLP in water supply systems.

In conclusion, all methods should be used to analyze water samples. It is not possible to detect all protozoa with microscopy, but it helps to find positive samples and then analyze them with PCR and detect bacteria with 16S rRNA sequencing.

References

Calvez T., Trouilhe M.C, Humeau O. 2012. Detection of free-living amoebae by using multiplex quantitative PCR. *Mol. Cell. Probes* 26: 116–120.

Klindworth A., Pruesse E., Schweer T., Peplies J., Quast C., Horn M. 2013. Evaluation of general 16s ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41: e1.

Mehari Y.T., Hayes J., Redding K.S., Mariappan P.V.G., Gunderson J.H., Farone A.L., Farone M.B. 2016. Description of '*Candidatus Berkiella aquae*' and '*Candidatus Berkiella cookevillensis*', two intranuclear bacteria of freshwater amoebae. *Int. J. Syst. Evol. Microbiol.* 66: 536–541.

Mrva M. 2011. *Mayorella vespertilioides* Page, 1983 (Amoebozoa) – new host for the ectoparasitic fungus *Amoebophilus simplex* (Zygomycota). *Biologia* 66: 645–647.

Muchesa P., Leifels M., Jurzik L., Barnard T.G., Bartie C. 2018. Detection of amoeba-associated *Legionella pneumophila* in hospital water networks of Johannesburg. *South. African J. Infect. Dis.* 33: 72–75.

Schroeder J.M, Booton G.C., Hay J. 2001. Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoebae* from humans with keratitis and from sewage sludge. *J. Clin. Microbiol.* 39: 1903–1911.

Solgi R., Niyyati M., Haghihgi A., Mojarad N. E. 2012 Occurrence of thermotolerant *Hartmanella vermiformis* and *Naegleria* spp. in hot springs of Ardebil Province, northwest Iran. *Iranian J. Parasitol.* 7: 47–52 .

Vaerewijck M.J.M., Sabbe K., Hende J., Bare J., Houf K. 2010. Sampling strategy, occurrence and diversity of free-living protozoa in domestic refrigerators. *J. Appl. Microbiol.* 109: 1556–1578.

First steps in *in situ* conservation of crop wild relatives in Latvia

Agnese Gailīte*, Dainis Edgars Ruņģis

Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Rigas 111, Salaspils LV–2169, Latvia
*Corresponding author, E-mail: agnese.gailite@silava.lv

Key words: genetic diversity, gene bank, wild plants.

Plant genetic resources (PGR) for food or agriculture are seeds and planting material with current or potential agricultural, economic and social value. In Latvia, genetic resources are conserved in the Latvian gene bank (LGB), field collections, and in vitro. LGB stores seed-propagated PGRs of Latvian origin (65 species-level taxa representing 43 genera). Most accessions are cereals and fodder crops. 45% of fodder crop accessions have been collected from natural populations. Wild species could be a valuable source of genetic diversity for the improvement of food and forage cultivars and varieties and are recognized as a critical resource with a vital role in food security and economic stability for the 21st century (Maxted, Kell 2009). Crop wild relatives (CWR) are wild plant taxa that have an indirect utility that results from their relatively close genetic relationship with crops (Maxted et al. 2013). They may belong to the same species as the crop or to a related species with which interbreeding is possible. The most important CWR species found in natural populations in Latvia are those related to fodder crops, medicinal and aromatic plants, wild berries, and fruits.

Several European countries have developed action plans and strategies for CWR conservation. According to Maxted et al. (2013), steps for the development of a CWR conservation strategy include: the creation of a national CWR list, prioritization of national CWRs, analysis of the ecogeographical diversity of priority CWRs, analysis of genetic diversity of priority CWRs, risk assessment of priority CWRs, gap analysis of in situ and ex situ conservation, creation of a national management plan (determination and implementation of *in situ* and *ex situ* conservation goals and activities), monitoring of conservation efforts, promotion of the use of CWR. The main tasks to ensure in situ conservation of Latvian CWR are currently being implemented or planned:

1) Creation of the national CWR list and prioritization of species,

2) Analysis of information sources to identify potential populations for *in situ* and *ex situ* conservation,

3) Initial surveys and research of potential conservation sites (existence and size of populations, threats, determination of land ownership),

4) Ecogeographical and genetic analysis of priority CWR populations,

5) Development of cooperation between PGR managers, interested CWR holders, national legislators and other stakeholders with an aim to officially nominate appropriate sites as CWR *in situ* conservation areas (genetic reserves),

6) Creation of a duplicate *ex situ* collection of materials collected from CWR reserves, ensuring their safe and sustainable preservation, as well as simplifying the task of fulfilling international obligations by issuing samples with a Standard Material Transfer Agreement,

7) Mapping of the responsibilities of each participating institution for the in situ and ex situ conservation of CWR populations and sites,

8) Nomination of the established national genetic reserves for inclusion into the European in situ conservation network.

The checklist of Latvian CWR species was created by harmonizing species found in the Flora of Latvian vascular plants, List of taxa 2005 (G. Gavrilova, V. Sulcs 2005, unpublished data) with species listed in the PGR forum CWR information system (CWRIS) (Kell et al. 2005), and non-agricultural and non-food species were excluded from the list. The result is a checklist with 440 CWR taxa.

A list of priority species has been created with specialists from scientific institutes and the University of Latvia. It also includes medicinal plant species whose genotypes are maintained in the Latvian University of Life Sciences and Technology collection. The list consists of 94 taxa from 18 families: 50% of prioritized taxa are forages, 29% are wild fruit and berries, 13% are medicinal plants, and 8% are edible plants/spices (Fig. 1). From these, 33 species from the priority list are included in the European priority CWR list, 26 are International Treaty Annex 1 species, and 17 are Red Data Book of Latvia species.

Further actions planned are:

1) Selection of priority *in situ* CWR conservation sites: selection of sites with the richest biological diversity, selection of sites with individual rare genotypes,

2) Creation of an *ex situ* CWR duplicate collection,

3) Nomination of selected sites as CWR *in situ* conservation sites (genetic reserves).

Acknowledgements

This work was supported by the Rural Support Service Republic of Latvia research aid Nr. 23-00-S0INZ03-000001 "Latvijā *in situ* audzēto pārtikā un lauksaimniecībā izmantojamo augu un to savvaļas radinieku ģenētisko resursu potenciāla apzināšana, ievākšana un izpēte (2023)".

References

Kell S.P., Knüpffer H., Jury S.L., Maxted N., Ford-Lloyd B.W. 2005. Catalogue of Crop Wild Relatives for Europe and the Mediterranean. University of Birmingham, Birmingham, UK.

Maxted N., Kell S. 2009. Establishment of a global network for the *in situ* conservation of crop wild relatives: status and needs. Background study paper No. 39.

Maxted N., Magos Brehm J., Kell S. 2013. Resource book for preparation of national conservation plans for crop wild relatives and landraces. University of Birmingham, United Kingdom.

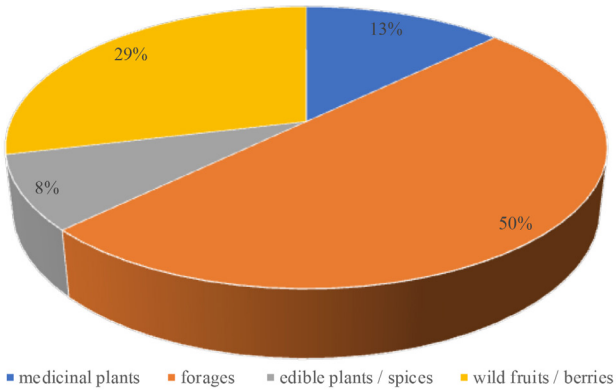

Fig. 1. Groups of prioritized CWRs.

Monitoring of ruderal rapeseed populations in areas potentially contaminated with genetically modified plants

Lelde Grantiņa-Ieviņa*[1], Juris Ķibilds[1], Lilija Kovaļčuka[1], Guntis Boikmanis[1], Karīna Ortlova[1], Artjoms Mališevs[1], Evija Bebre[1], Gederts Ievinsh[2]

[1]Scientific Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes 3, Rīga LV–1076, Latvia
2Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia
*Corresponding author, E-mail: valdis.pilats@daba.gov.lv

Key words: contamination, genetically modified organisms, ruderal rapeseed populations.

In recent years, one case of unintentional release of genetically modified plants (GMP) in the environment has been documented in Latvia. In 2021, as part of the monitoring of seed and plant propagating material, rape seed contamination with GT73 rape seeds was detected. The total area of sowing was 843.33 ha. All fields were destroyed (https://www.la.lv/gmo-del-iznicina-sejumus). Earlier, in 2017, in the framework of the project "Assessment of possible risks of genetically modified seeds and propagating material in the territory of Latvia and development of risk management recommendations in accordance with Latvian agro-economic conditions", two seed samples containing GM seed impurities, as well as genetically modified petunia seedlings and seeds were found. Therefore, the need to evaluate the spread of unintentional GMOs in the Latvian environment is relevant and justified. Similar studies have also been conducted in Lithuania (Paulauskas 2020), Germany (Franzaring et al. 2016), Switzerland (Hecht et al. 2014). No genetically modified plants were found in Lithuania. In Germany wild rapeseed was found in all study areas, both in the territory of processing plants and in publicly accessible places in their surroundings. The wild cruciferous species (mainly *Sinapis arvensis*) were found only in some places. Most canola plants were observed and sampled along roads and railway tracks. Only one plant was GM positive (Roundup Ready™) – in the port of Neuss (Franzaring et al., 2016). In Switzerland GT73 rapeseed was found at 3 locations. The GM canola plants found contained gox and CP4 epsps genetic elements and were resistant to herbicides. None of the collected plants of potential hybridization partner species were GM (Hecht et al. 2014).

The aim of the present study was to monitor ruderal rapeseed populations in areas potentially contaminated with genetically modified plants in Latvia. This study was done within the project Nr.23-00-S0INZ03-000038 "Monitoring of unintentional release of genetically modified plants in the environment and evaluation of environmental monitoring programs available in Latvia in connection with general monitoring of GMOs" (2023 – 2024). The following tasks were determined: 1) to carry out monitoring and collect samples of ruderal rapeseed plants in surroundings of ports, railway cargo handling areas, rapeseed processing factories, etc.; 2) to determine the presence of GMO screening elements in the collected samples and, if necessary, to identify the detected GMO events in individual samples. A methodology developed in Germany was chosen for the study (Sukopp, Schmitz, 2013). This floristic mapping method includes a 2 km zone around processing plants, ports, transshipment areas. If the company is located by the river, then this zone includes a stretch of the river 2 km upstream and 3 km downstream. Access roads, railway lines and relevant river sections are surveyed within a radius of 2 m around the respective company. Mapping of found rapeseed plants is carried out with notes, photos and GPS coordinates.

The monitoring was carried out in the period from 27.05.23. until 30.09.23. The surroundings of 54 objects were surveyed (Fig. 1). The number of combined samples taken was 139 (10 leaves each), of which 132 were rapeseed leaf samples, four were seed samples, but three samples were wheat, barley, and mustard. The pooled samples were from a total of 1357 plants and represented a population

Fig. 1. Location of 54 monitoring objects on the map of Latvia - surroundings of ports, railway cargo handling areas, and rapeseed processing factories.

from ca. 8900 plants. Out of 54 objects, 46 objects (85%) were found to have rapeseed plants in their surroundings. Increased attention was paid to objects located near protected natural areas, but these territories had few or no rapeseed plants. During the monitoring, four places in Riga were found, where the existing rapeseed populations could be described as perennial populations, but the situation must be monitored also in the next year. In total, 130 out of 139 monitored samples showed no amplification for any of the six screening elements (CaMV 35S promoter, T-nos, *pat*, pFMV, tE9, *bar*), as well as the rapeseed event DP-073496-4. Nine pooled rapeseed leaf samples showed late amplification ($C_t > 39$) for one or more screening elements, but such C_t values were considered negative. In the second year of the study, it is planned to survey additional 10 objects, as well as to carry out repeated monitoring in those objects in the vicinity of which large (> 100 plants) ruderal rapeseed populations have been detected.

References

Franzaring J., Wedlich K., Fangmeier A., Eckert S., Zipperle J., Krah-Jentgens I., Hünig C., Züghart W. 2016. Exploratory study on the presence of GM oilseed rape near German oil mills. *Environ. Sci. Pollut. Res.* 23: 23300-23307.

Hecht M., Oehen B., Schulze J., Brodmann P., Bagutti C. 2014. Detection of feral GT73 transgenic oilseed rape (*Brassica napus*) along railway lines on entry routes to oilseed factories in Switzerland. *Environ. Sci. Pollut. Res.* 21: 1455–1465.

Paulauskas A. 2020. GMO rizikos poveikio aplinkai vertinimas. 2014-2020 metų Europos Sąjungos fondų investicijų veiksmų programos 5 prioriteto „Aplinkosauga, gamtos išteklių darnus naudojimas ir prisitaikymas prie klimato kaitos“ 05.5.1–APVA-V-018 priemonė „Biologinės įvairovės apsauga“.

Sukopp U., Schmitz U. 2013. How to track genetically modified (GM) plants in the field? The VDI standard method of floristic mapping of GM plants as an efficient tool. BioRisk 8: 89–110.

Comparison of salt tolerance of *Tripolium pannonicum* in tissue culture using agar-solidified and liquid medium with a temporary immersion system

Liva Purmale*, Gederts Ievinsh

Department of Plant Physiology, Faculty of Biology, University of Latvia, 1 Jelgavas Str., LV–1004 Rīga
*Corresponding author, E-mail: liva.purmale@lu.lv

Key words: halophyte, *in vitro*, NaCl, sea aster, temporary immersion system.

Salinity is one of the major threats in agriculture. Plant species that grow in salt affected habitats could be great model organisms to understand physiological characteristics for life in saline environments (Meng et al. 2018). Sea aster [*Tripolium pannonicum* (Jacq.) Dobrocz.] is edible halophytic plant species belonging to Asteraceae family growing in salt marshes and coastal beach area of temperate region (Ramani et al. 2006). Plant tissue culture approach for sea aster was chosen as seed germination rate is low leading to very limited amount of available plant material.

Plant tissue culture for *T. pannonicum* was initiated from seeds, collected on island of Hiiumaa, Estonia and island of Bornholm, Denmark. A batch of 20 seeds from each group was surface sterilized by immersion for 15 min in 15% (v/v) commercial bleach solution Domestos (Unilever, London, Great Britain) followed by three washes of deionized autoclaved water (5 min each). Seed germination rate on Murashige and Skoog (MS; Murashige, Skoog 1962) agar-solidified medium (30 g L^{-1} sucrose, pH 5.8) was low (5 to 10%). After 40 days, plantlets were placed on multiplication medium consisting of MS supplemented with 30 g L^{-1} sucrose, 0.5 mg L^{-1} 6-benzylaminopurine, 6.5 g L^{-1} agar (pH 5.8). For salt tolerance experiment, multiplication medium was supplemented with Na (0, 1, 2.5, 4 and 5 g L^{-1}) in form of NaCl. Ten explants per treatment for each genotype were placed in jars with agar-solidified medium and in temporary immersion system (TIS) bioreactors (Plantform, Hjärup, Sweden) with liquid medium (multiplication medium without agar, immersion

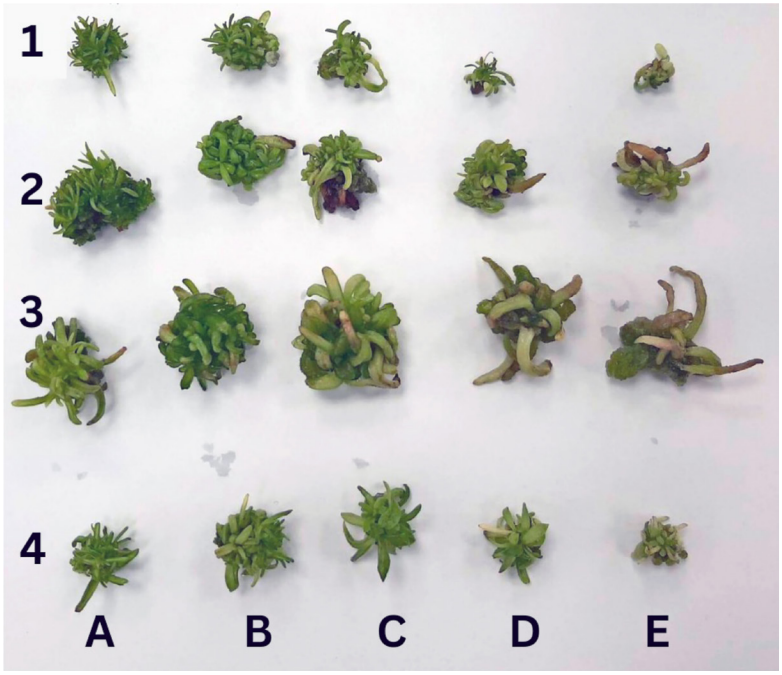

Fig. 1. *Tripollium pannonicum* plantlets of two genotypes, grown for 4 weeks with different Na concentrations and growth conditions: 1, Hiiumaa, agar-solidified medium; 2, Hiiumaa, liquid medium in Plantform biorector; 3, Bornholm, liquid medium in Plantform biorector; 4, Bornholm, agar-solidiefied medium. A, control; B, Na 1 g L^{-1}; C, Na 2.5 g L^{-1}; D, Na 4 g L^{-1}; E, Na 5 g L^{-1}.

in the medium twice daily for 10 min, aeration every 2 h for 10 min.). After 4 weeks the experiment was terminated (Fig. 1). Explant biomass was measured before and after drying for 4 days at 60 °C. The dried tissues were used for determination of concentration of soluble ions (Purmale et al. 2022).

In all treatments plantlets grown in liquid medium in TIS bioreactors were heavier than grown in agar-solidified medium (both fresh weight and dry weight). Increased Na concentration corelated with increased Na accumulation in tissues, and increased values of electrical conductivity. Increased Na concentration in the medium had no effect on K concentration in *T. pannonicum* explant tissues.

References

Meng X., Zhou J., Sui N. 2018. Mechanisms of salt tolerance in halophytes: Current understanding and recent advances. *Open Life Sci.* 13: 149–154.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 15: 473–497.

Purmale L., Jēkabsone A., Andersone-Ozola U., Ievinsh G. 2022. Salinity tolerance, ion accumulation potential and osmotic adjustment in vitro and in planta of different *Armeria maritima* accessions from a dry coastal meadow. *Plants* 11: 2570.

Ramani B., Reeck T., Debez A., Stelzer R., Huchzermeyer, B., Schmidt A., Papenbrock J. 2006. *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. *Plant Physiol. Biochem.* 44: 395–408.

Incidence of *Coxiella burnetii* DNA in milk samples of dairy cows in the years 2022 – 2023

Guntis Boikmanis*, Žanete Šteingolde, Juris Ķibilds, Lelde Grantiņa-Ieviņa, Aivars Bērziņš

Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes 3, Riga LV–1076, Latvia
*Corresponding author, E-mail: guntis.boikmanis@bior.lv

Key words: milk, *Coxiella burnetii*, Q fever, prevalence.

This study investigates the incidence of *Coxiella burnetii* DNA in milk samples from dairy cows in Latvia during the period from January 2022 to September 2023. *C. burnetii*, the causative agent of Q fever, is a pleomorphic Gram-negative obligate intracellular bacterium known for its highly infectious nature (Anderson et al., 2013) and its high resistance to external environmental stress (Coleman et al. 2005). Q fever is a zoonotic disease that can be asymptomatic or manifest as acute or chronic conditions in humans, posing significant risks, especially to individuals in direct contact with infected livestock (Angelakis et al. 2013).

The aim of the study was to determine the presence of *C. burnetii* DNA in dairy cows of combined milk and bulk milk samples from dairy cows to identify infected dairy sheds and to make comparative analysis with previous studies.

The research utilized molecular biology techniques to analyze milk samples collected from dairy herds across Latvia as part of the annual National Animal Infectious Disease Surveillance Plans for compulsory brucellosis and leucosis testing. DNA was extracted from the samples using QIAmp DNA mini kit and Dneasy mericon Food Kit. Real-time PCR amplification of the *C. burnetii* target gene DNA IS1111 using the ADIAVET COX REAL TIME reagent kit. Information on the number of herds and animals in the districts is obtained from the free-access statistics of the Agricultural Data Centre.

The findings revealed that 164 samples, or 15.46% of the total, tested positive for the presence of *C. burnetii* DNA. Furthermore, the bacterium was identified in 66 out of 657 herds, spanning 21 counties, which accounts for 58% of the country's total counties. Comparative analysis with previous studies indicates a slight decrease in the prevalence of *C. burnetii* in combined milk and bulk milk samples. Despite this, the bacterium remains common in more than half of Latvia's counties (Fig. 1), underscoring the need for effective control measures. The prevalence of *C. burnetii* in

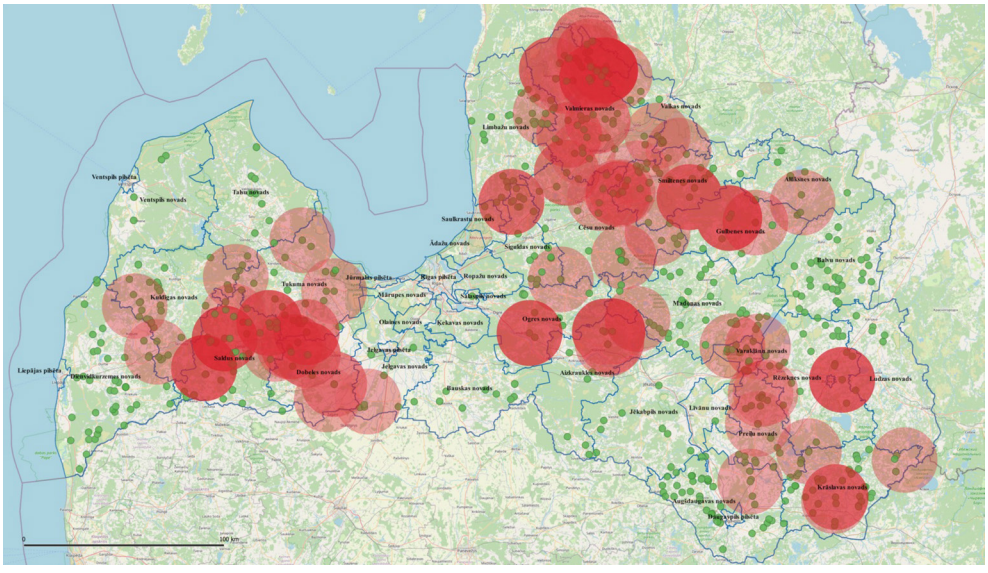

Fig. 1. The spatial distribution of dairy cow sheds analyzed during January 2022 to September 2023 for *Coxiella burnetii* DNA in milk samples. The circles in green indicate locations of sheds where all samples analysed were negative, with size corresponding to a 5 km radius around the farm. The red circles represent sheds where at least one positive sample has been found, with a radius of 30 km around the shed, thus marking the radius within which *Coxiella burnetii* is likely to occur.

dairy herds and its implications for public health have been subjects of concern not only in Latvia but across the globe, incorporating a comparative analysis with other countries provides a broader context for understanding the dynamics of Q fever transmission and control measures globally. In comparison to prevalence in Estonia (27.16%) (Neare et al. 2023), Poland (24.81%) (Szymańska-Czerwińska et al. 2022), Spain (60.1%) (Yáñez et al. 2024), Italy (14.3%) (Ferrara et al. 2022) and the global average (37.0%) of the Rabaz et al. (2021) study indicates a 15.46% prevalence rate of *C. burnetii* DNA in milk samples from dairy herds, falls within the lower to mid-range of the global prevalence rates reported. However, it's important to note that direct comparisons between studies can be challenging due to differences in sampling methods, diagnostic tests, and the specific populations studied.

The actual prevalence data of *C. burnetii* in Latvia's dairy herds, provides valuable insight into the situation within the country and contributes to the broader understanding of Q fever's global epidemiology. The persistence of the bacterium in a significant proportion of herds highlights the importance for ongoing surveillance, research, and need for customized control strategies.

References

Anderson A., Bijlmer H., Fournier P.E., Graves S., Hartzell J., Kersh G.J., Limonard G., Marrie T.J., Massung R.F., McQuiston J.H., Nicholson W.L., Paddock C.D., Sexton D.J. 2013. Diagnosis and management of Q fever. United States, 2013: recommendations from CDC and the Q fever working group. MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports. Centers for Disease Control, 62(RR-03): 1–30;

Angelakis E., Million M., D'Amato F., Rouli L., Richet H., Stein A., Rolain J.M., Raoult D. 2013. Q fever and pregnancy: disease, prevention, and strain specificity. *Eur. J. Clin. Microbiol. Infect. Dis.* 32: 361–368.

Coleman S.A., Fischer E.R., Howe D., Mead D.J., Heinzen R.A. 2005. Temporal analysis of *Coxiella burnetii* morphological differentiation. *J. Bacteriol.* 186: 7344–7352.

Ferrara G., Colitti B., Pagnini U., D'Angelo D., Iovane G., Rosati S., Montagnaro S. 2022. Serological evidence of Q fever among dairy cattle and buffalo populations in the Campania region, Italy. *Pathogens* 11: 901.

Rabaza A., Fraga M., Corbellini L.G., Turner K.M.E., Riet-Correa F., Eisler M.C. 2021. Molecular prevalence of *Coxiella burnetii* in bulk-tank milk from bovine dairy herds: Systematic review and meta-analysis. O*ne Health* 12: 100208.

Szymańska-Czerwińska M., Jodełko A., Osiński Z., Niemczuk K., 2022. Screening for *Coxiella burnetii* in dairy cattle herds in Poland. *J. Veterin. Res.* 66: 549-557.

Yáñez U., Álvarez J., Pisón C., Acción A., Becerra J.J., Jiménez A., Gisbert P., Herradón P.G., Peña A.I., Prieto A., Díaz-Cao J.M., Quintela L.A., 2024. Prevalence, risk factors, and relationship between reproductive performance and the presence of antibodies against coxiellosis in dairy farm milk tanks in the northwest of Spain. *Animals* 14: 367.

Characterisation of arbuscular mycorrhiza-mediated intra- and inter-plant defence pre-activation and priming responses in *Daucus carota*

Annija Kotova[1], Zigmunds Orlovskis*[1,2], Daniels Pugačevskis[1], Ēriks Voroņins[1], Soon Jae-Lee[3]

[1]Latvian Biomedical Research and Study Centre, Rātsupītes 1 k-1, Rīga, LV-1067
[2]Department of Plant Physiology, Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia
[3]Department of Ecology and Evolution, University of Lausanne, Switzerland
*Corresponding author, E-mail: zigmunds.orlovskis@biomed.lu.lv

Key words: *Daucus carota*, inter-plant signals, mycorrhiza, plant-fungi interactions, plant defence priming.

Organic agriculture and its relevance are growing significantly, along with it, a lot of attention is being paid to the development and improvement of biological plant protection. Worldwide, agricultural yields are limited due to pest and pathogen problems, therefore the necessity of developing alternative agricultural methods that can enchance crop natural defensive abilities is ever increasing (Gamage et al. 2023). Arbuscular mycorrhizal fungi (AMF) and other symbiotic soil microbes have clear potential towards this goal. AMF hyphae create a subterranean network of inter-connected root systems betwen plants, forming common mycelial network (CMN) that is proposed to function not only as a provider of essential mineral nutrients, but also as an information highway by transfering diverse chemical signals between plants (Barto et al. 2012). However, it is still not clear how AMF affects the host plant and the surrounding plant population in agroecosystems. It remains to be investigated how plants transmit signals to each other via AMF in nature and whether the receiver primes or preactivates defence against attacking pathogens.

Daucus carota hairy root culture is widely used in scientific research and forms symbiosis with model AMF species Rhizophagus irregularis. The carrot has its whole genome sequence annotated (Xu et al. 2014), hence was chosen as model species to study both intra-plant and

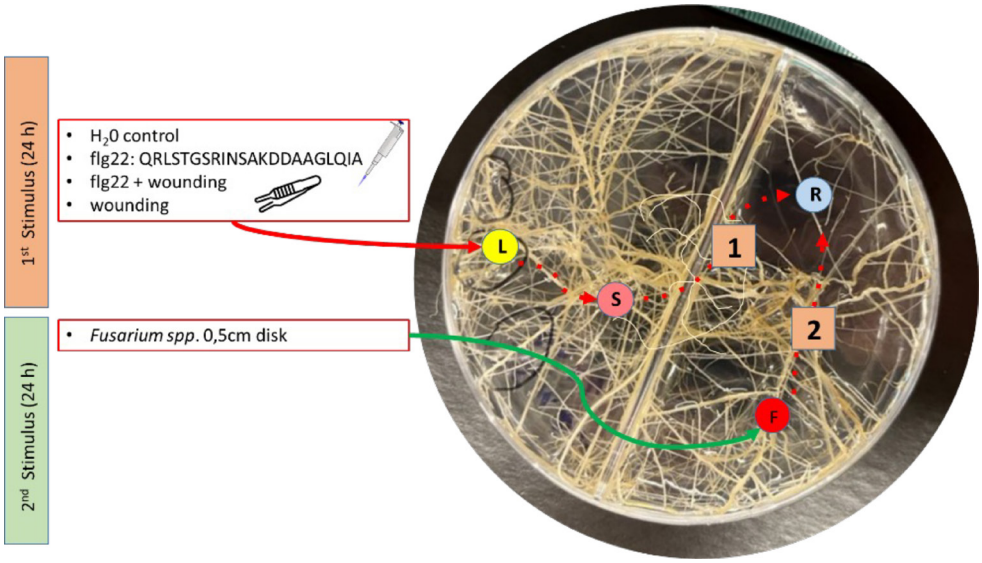

Fig. 1. Schematic experimental design. Two *D. carota* hairy root cultures are grown in a single petri dish with a barrier in the middle completeley seperating the agar substrate of both root systems. One side the root system (L - local tissue) was stimulated with bacterial flagellin and mechanical wounding with forceps (1st stimulus). Root samples from systemic (S) root tissues as well as the other side of the septated petridish with the receiver (R) roots will be samped for marker gene expression analysis 24 h after the application of local stimulus. These data will highlight the root responses that represent defence preactivation (1). In a seperate set of petri plates the 2nd stimulus (*Fusarium sporotrichoides*) is added to test receiver root system for 24h in order to characterise the potential defence priming (2).

inter-plant signals. The aim of the study is to examine the preactivation of systemic and inter-plant defense reactions of *D. carota* hairy roots by determining the activity of biotic stress response marker genes. We applied mechanical wounding in combination with plant immune elicitor peptide flg22 on 8 week old plants as primary signal to evaluate root response preactivation in AMF-connected receiver root system or additionally added 0.5 cm diameter *Fusarium* spp. disk 24 h after the primary signal to evaluate defence priming in the receiver roots (Fig. 1). Ongoing experimental work will generate novel data to better evaluate the potential role of AMF in transmitting plant immunogenic signals that aid in defence against fungal pathogens.

Acknowledgements

The study is supported by the Latvian Science Council project lzp-2021/1-0056 ''Investigate biotic stress induced inter-plant molecular signals and responses in mycorrhizal fungi-connected plants."

References

Barto E.K., Weidenhamer J.D., Cipollini D., Rillig M.C. 2012. Fungal superhighways: do common mycorrhizal networks enhance below ground communication? *Trends Plant Sci.* 17: 633–637.

Gamage A., Gangahagedara R., Gamage J., Jayasinghe N., Kodikara N., Suraweera P., Merah O. 2023. Role of organic farming for achieving sustainability in agriculture. *Farming Syst.* 1: 100005.

Xu Z.-S., Tan H.-W., Wang F., Hou X.-L., Xiong A.-S.. 2014. CarrotDB: a genomic and transcriptomic database for carrot. *Database* 2014: bau096.

Investigation of arbuscular mycorrhiza-mediated systemic and inter-plant defence responses in *Medicago trucatula*

Daniels Pugačevskis[1], Annija Kotova[1], Ēriks Voroņins[1], Soon Jae-Lee[3], Zigmunds Orlovskis*[1,2]

[1]Latvian Biomedical Research and Study Centre, Rātsupītes 1 k-1, Rīga LV–1067, Latvia
[2]Department of Plant Physiology, Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia
[3]Department of Ecology and Evolution, University of Lausanne, Switzerland
*Corresponding author, E-mail: zigmunds.orlovskis@biomed.lu.lv

Key words: inter-plant signals, *Medicago truncatula*. mycorrhiza, plant defence genes, *Rhizophagus irregularis*.

In response of growing demand for food all around the world (Ritchie, 2022), there is increasing necessity for novel agricultural plant defence technologies and tools (Statista Research Department 2023; Nicolopoulou-Stamati et al. 2016). Arbuscular mycorrhizal fungi (AMF) potentially can be used for enhancing plant defence mechanism, because of their ability to connect plants in a network which may transmit inter-plant signals. However, the molecular mechanisms of signal exchange between multiple plants using AMF and its effects on plant defence against pathogens remains to be investigated (Thirkell et al. 2017; Gavrin, Schornack 2020; Genre et al. 2020). This would potentially yield novel insights into future engineering of plant defence mechanisms and design of sustainable agroecosystems using beneficial microbes.

Arbuscular mycorrhizal fungus *Rhizophagus irregularis* and legume family (Fabaceae) plant *Medicago truncatula* were chosen as models for mycorrhiza mediated inter-plant signal research. Two experimental plants were grown in a single pot. After plant inoculation with AMF spores and

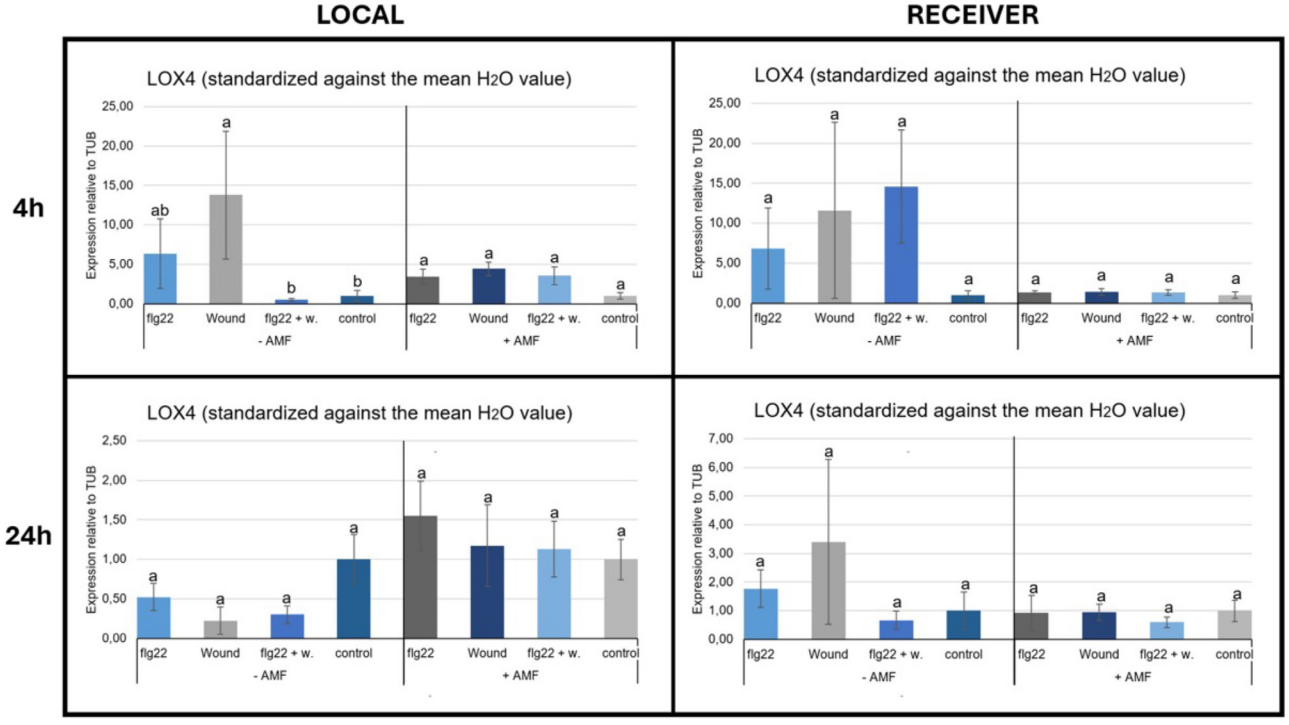

Fig. 1. *Medicago truncatula LIPOXYGENASE4* (*LOX4*) expression relative to *M. truncatula TUBULIN* (*TUB*) in local (L) and signal receiving (R) plant leaves, 4h and 24h after stimulation of local tissues in arbuscular mycorrhizal fungi colonized (AMF+) and mycorrhiza non-inoculated (AMF-) plants. All of the data from different stimuli – flagellin (flg22), mechanical damage (wound) and combination of both (flg22 + w.) – have been standardized to expression values in plants treated with deionized water H_20 (control). Bars represent Mean of 4 biological replicates. Statistical analysis performed with ANOVA and letters represent differences according to the Tukey post-hoc test.

8-week incubation to establish inter-plant connection, one of the plants was challenged with mechanical wounding and bacterial flagellin peptide (flg22). We collected the stimulated leaves (local tissue) as well as the distal (systemic) leaves of the stimulated plant. In addition, leaves from the inter-plant signal receiver were collected as well for the subsequent analysis of plant defence gene expression (*MtLOX2, MtLOX4, MtVSP1, MtPAD4, MtBGL, MtLYK5*).

AMF connection has affected both local plant defence gene expression as well as receiver gene induction against applied stimuli when compared to plants that were not inoculated or connected with *R. irregularis* (Fig. 1).

Our results suggest that AMF can change plant defence gene activity, mainly – decrease receiver plant defence gene expression. This could potentially lead to greater plant susceptibility to different type of pathogens which remains to be investigated in follow-up studies.

Acknowledgements

The study is supported by the Latvian Science Council project lzp-2021/1-0056 "Investigate biotic stress induced inter-plant molecular signals and responses in mycorrhizal fungi-connected plants".

References

Ritchie H. 2022. After millennia of agricultural expansion, the world has passed 'peak agricultural land.' Our World in Data. /https://ourworldindata.org/peak-agriculture-land visited February 26, 2024

Gavrin A., Schornack S. 2020. *Medicago truncatula* as a model organism to study conserved and contrasting aspects of symbiotic and pathogenic signaling pathways. In: de Bruijn F. (Ed.) *The Model Legume* Medicago truncatula. John Wiley & Sons, pp. 317–330

Genre A., Lanfranco L., Perotto S., Bonfante P. 2020. Unique and common traits in mycorrhizal symbioses. *Nature Rev. Microbiol.* 18: 11.

Statista Research Department. 2023. Global agricultural use of pesticides 2023-2027. Statista. /https://www.statista.com/statistics/1401556/global-agricultural-use-of-pesticides-forecast/ visited February 26, 2024/

Nicolopoulou-Stamati P., Maipas S., Kotampasi C., Stamatis P., Hens L. 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Front. Public Health* 4: 148.

Thirkell T.J., Charters M.D., Elliott A.J., Sait S.M., Field K.J. 2017. Are mycorrhizal fungi our sustainable saviours? Considerations for achieving food security. *J. Ecol.* 105: 921–929.

Role of arbuscular mycorrhizal mediated inter-plant signaling in *Medicago truncatula* resistance to fungal pathogens *Botrytis cinerea* and *Fusarium sporotrichoides*

Ēriks Voroņins[1], Annija Kotova[1], Daniels Pugačevskis[1], Zigmunds Orlovskis*[1,2], Soon Jae-Lee[3]

[1]Latvian Biomedical Research and Study Centre, Rātsupītes 1 k-1, Rīga LV–1067, Latvia
[2]Department of Plant Physiology, Faculty of Biology, University of Latvia, Jelgavas 1, Rīga LV–1004, Latvia
[3]Department of Ecology and Evolution, University of Lausanne, Switzerland
*Corresponding author, E-mail: zigmunds.orlovskis@biomed.lu.lv

Key words: inter-plant signals, mycorrhiza, plant-fungi interactions, plant defense.

Infections caused by necrotrophic and biotrophic fungi are an important problem in agriculture. For example, *Botrytis cinerea* causes annual economic losses of between 10 and 100 billion dollars worldwide (Watkinson et al., 2016). Biological alternatives of fungicides are actively researched to help reduce reliance on chemical fungicides. Plant colonization with arbuscular mycorrhizal fungi (AMF) is known to increase plant systemic resistance to *B. cinerea* (Pozo De La Hoz et al. 2021). Moreover, arbuscular mycorrhizae can form a common network of hyphae, connecting plants in a population (Barto et al. 2012). However, the specific molecular mechanisms for possible mycorrhiza induced inter-plant resistance remain to be investigated. This work aims to study the hyphal network of arbuscular mycorrhiza as a potential transmitter of danger and biotic stress signals between plants and its effect on plant resistance to pathogens.

The initial stage of our study was to evaluate effective stimuli for inducing systemic responses in our plant model Medicago truncatula and provide visual differences in necrotic zone area in subsequent infection with fungal pathogens. To this end, we tested the effects of seven different stimuli (Fig. 1), applied to one local (L) plant leaf triplet. Each stimulus was applied to four plants (biological replicates). The experiment with all stimuli was repeated independently three times for each pathogen. We observed

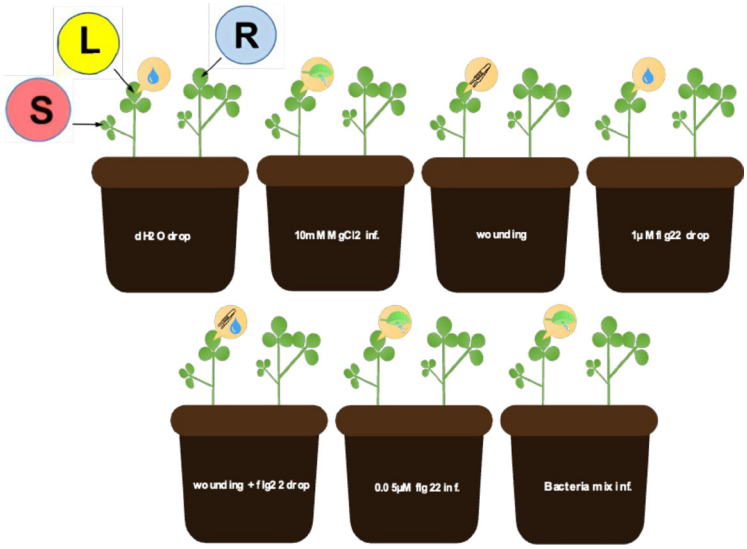

Fig. 1. Schematic representation of stimuli for *M. truncatula* systemic resistance to necrotrophic fungi. L, S, R icons reflect the local, systemic, and receiver plant regions. The drop volume is 5 µL. During syringe infiltration, we tried to cover the whole area of the leaf blade. For controls, we used water drop or $MgCl_2$ syringe infiltration. To simulate an insect attack we used mechanical wounding with forceps. For bacterial attack simulation, we used bacterial membrane peptide, flagellin (flg22), as well as a living bacteria mix containing *Pseudomonas agglomerans, Peudomonas syringae* pv. *tomato, Erwinia rhapontici, Dckeya chrysanthemi* at OD = 0.0005 each (measured at 625 nm). We also used a combination of wounding and flg22 to simulate simultaneous herbivore and bacterial attack that potentially could cause systemic acquired resistance against fungal pathogens.

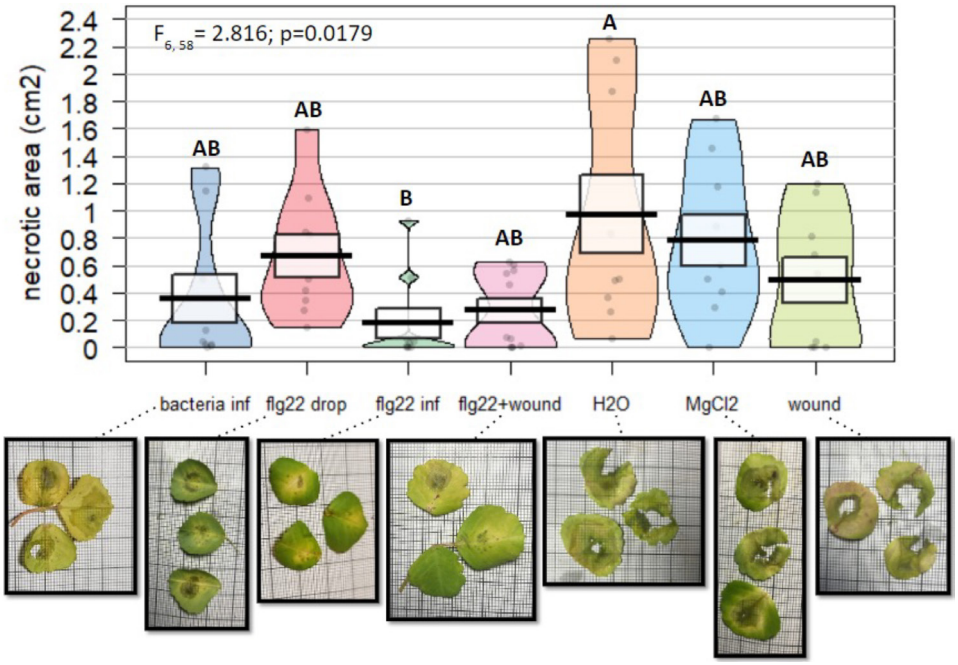

Fig. 1. Differences in areas of necrosis caused by *Botrytis cinerea* on *Medicago truncatula* systemic leaves. Stimuli on local leaves were applied as indicated in Fig. 1. 72 h after the initial stimuli we applied agar plugs of 14-day-old *B. cinerea* culture on the systemic leaves of the plant (S) that received the initial stimulus. Plants were placed in a high humidity sealed box, and 72 hpi necrotic areas were measured by the Image J visual tool. ANOVA and Tukey post-hoc tests were used to analyze the pooled data from all three experimental replicates.

that flg22 infiltration and flg22+wounding display the largest reduction in necrotic area of *B. cinerea* relative to the water control (Fig. 2). We conducted an identical experiment with another fungal pathogen, Fusarium sporotrichioides, and observed necrotic areas enlargement which corresponds to systemic acquired susceptibility caused by flg22 infiltration and flg22+wounding initial stimulation. ($F = 5.095$, $p = 4.34e\text{-}05$).

Our preliminary experiments provided the necessary data to reduce the number of stimuli to further investigate the pathogen resistance in receiver *Medicago* plants (Fig. 1.) that were inter-connected with signal senders via AMF. To this end, the first plant is stimulated by a primary stimulus (flg22 + wounding), while necrotic areas will be measured in the receiver plant region (R). This investigation will indicate possible pre-activation or priming of the receiver plant by a signal transmitted from the sender.

Acknowledgements

The study is supported by the Latvian Science Council project lzp-2021/1-0056 ''Investigate biotic stress induced inter-plant molecular signals and responses in mycorrhizal fungi-connected plants"

References

Barto E.K., Weidenhamer J.D., Cipollini D., Rillig M.C. 2012. Fungal superhighways: Do common mycorrhizal networks enhance below ground communication? *Trends Plant Sci.* 17: 633–637.

Pozo De La Hoz J., Rivero J., Azcón-Aguilar C., Urrestarazu M., Pozo M. J. 2021. Mycorrhiza-induced resistance against foliar pathogens is uncoupled of nutritional effects under different light intensities. *J. Fungi* 7: 402.

Watkinson S.C., Boddy L., Money N.P. 2016. *The Fungi*. 3rd Ed. Elsevier, Academic Press.