

Plants in Changing Environment

International conference of the Slovenian Society of Plant Biology

Biotechnical Faculty, University of Ljubljana

September 15 - 16, 2022, Ljubljana, Slovenia



Slovensko društvo za biologijo rastlin
Slovenian Society of Plant Biology

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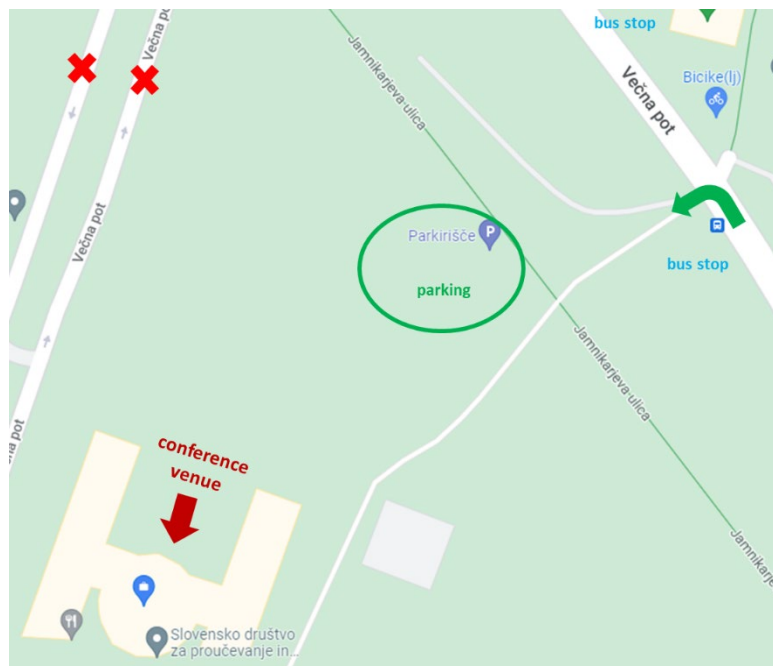
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GENERAL INFORMATION

The conference will start on Thursday, September 15th 2022 at 9.00 a.m. and will close on Friday, September 16th 2022 in the afternoon.

Venue



The conference will be held in lecture hall B1 of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana (see the map, QR code leads to parking site).

The conference reception desk will be open every day from 8.30 to 09.00.

Parking is possible on-site, but please use the one indicated on the map (green arrow and circle). Pedestrian or car access is not possible on the driveways on the west (red cross), as there is a construction site).

Coffee breaks and lunches will be served at the venue. The conference dinner will be held in Gostilna Čad (Cesta na Rožnik 18), 15 min walk from the venue.

Scientific program

Contributions will be presented as opening end closing lectures (OL, CL), invited lectures (IL), short lectures (SL) or posters (P).

The speakers are kindly requested to deliver their presentation material in Microsoft PowerPoint (PPT) or PDF format on USB at least 15-20 minutes before the beginning of the session. We recommend using the Standard (3:4) slide size. The length of short oral presentations is 10-12 minutes, allowing 3-5 minutes for discussion.

Posters will be displayed in the main corridor outside the lecture hall. Authors are kindly requested to mount their posters at the beginning of the conference and to dismount them after the lunch break on the second day in order to present their work throughout the period of the conference. At least one of the authors is requested to be available for discussion during the poster matchmaking session on Friday during the morning coffee break.

During the conference, we will also celebrate the 40th anniversary of the Slovenian Society of Plant Biology, with an Academy and lectures of the Society awardees.

Organizing committee

Špela BAEBLER (National Institute of Biology) – Chair

Tine GREBENC (Slovenian Forestry Institute)

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Jaka RAZINGER (Agricultural Institute of Slovenia)

Eva PRAPROTNIK (Agricultural Institute of Slovenia)

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CONFERENCE PROGRAM

Thursday 15 September

08:30 - 09:00 Registration

Opening session

- 9:00 Welcome address (Maja Ravnikar, director of National Institute of Biology, Špela Baebler, president of the organizing committee)
- 9:15 **Opening lecture:** Maria J. Pozo: Beneficial microbes enhance plant phenotypic and metabolic plasticity to cope with stress

Session 1: Applied plant biology research and agronomy applications (chairs: Alessandra di Francesco & Saša Širca)

- 9:50 Alessandra di Francesco: *Aureobasidium pullulans* as an alternative and sustainable strategy in agriculture (IL1)
- 10:20 Nina Devrnja: Characterization of β -cyclodextrin - fennel and basil essential oil inclusion complexes and effect on Colorado potato beetle growth (SL1)

10:35 - 11:05 Coffee break & posters

- 11:05 Saša Širca: Belatedly - climate changes affects the spread of tropical pests in agriculture (IL2)
- 11:35 Denis Kutnjak: Virome of tomato, weed plants and water in agroecosystems in Slovenia (SL2)
- 11:50 Ana Vučurović: Tomato mottle mosaic virus: newly discovered enemy affecting tomatoes and peppers (SL3)
- 12:05 Ales Lebeda: Wild and crop plant pathosystem interactions and variation: Case study *Lactuca* spp. – *Bremia lactucae* (SL4)
- 12:20 Mojca Juteršek: Growth penalty in *Nicotiana benthamiana* insect sex pheromone biofactories involves transcriptional deregulation of stress-growth balance (SL5)

12:35 – 13:45 Lunch & posters

Session 2: Plant metabolism, structure and function (chairs: Katarina Vogel-Mikuš & Boris Lazarević)

13:45 Katarina Vogel-Mikuš: Application of X-ray-based techniques in plant biology (IL3)

14:15 Boris Lazarević: Plant phenotyping using chlorophyll fluorescence and multispectral imaging (IL4)

14:45 Maria Müller: Glutathione-mediated plant response to different light intensities and spectra (SL6)

15:00 Anna Gasperl: Glutathione concentrations differ in maize bundle sheath cell compartments, depending whether sulfur is supplied via hydrogen sulfide (H₂S) or sulfate (SL7)

15:15 – 15:45 Coffee break & posters

Session 3: Plant interactions with other organisms (chair: Kristina Gruden)

15:45 Sarah Lebeis: Identifying chemicals that drive interactions between plants and microbes during microbiome assembly (IL5 - **online**)

16:15 Marko Petek: Assembly of potato endophytes using Nanopore and Illumina sequencing (SL8)

16:30 Zala Kogej: Epidemiology and diversity of 16SrV phytoplasma group infecting grapevine and hazelnut (SL9)

17:00 Academy of the Slovenian Society of Plant Biology with lectures of the Society Awardees

Marina Dermastia: Years with my society

Dominik Vodnik: TBA

18:00 Welcome reception

19:00 Conference Dinner (close to the venue)

Friday 16 September

Session 3: Plant interactions with other organisms (chair: Maria J. Pozo)

9:00 Kristina Gruden: Study of spatial responses in combination with network analysis reveals mechanisms of potato stress signaling (IL6)

9:30 Maja Križnik: The role of small RNA regulatory networks in tolerance and resistance responses of potato to potato virus Y infection (SL10)

9:45 Anna Coll: The ethylene response factor StERF49 negatively regulates potato immunity (SL11)

10:00 Andrea Schubert: Leaf sucrose controls grapevine susceptibility to Flavescence Dorée (SL12)

10:15 Katarina Šoln: Allelopathy of invasive *Fallopia* plants induced oxidative stress in radish seedlings (SL13)

10:30 - 11:30 Coffee break & poster matchmaking

Session 4: Natural ecosystems in changing environment (chair: Marjana Westergren & Silvio Schueler)

- 11:30 Marjana Westergren: Evolutionary potential in natural populations of forest trees (IL7)
- 12:00 Silvio Schueler: Phenotypic plasticity and genetic variation in forests trees: understanding drivers and developing applications for forest adaptation (IL8)
- 12:30 Tanja Mrak: Response of *Quercus pubescens* ectomycorrhizal fungi to abiotic changes in the Slovenian Sub-Mediterranean (SL14)
- 12:45 Angela Balzano: Plasticity of wood and phloem formation in tree species from drought-prone environments (SL15)
- 13:00 Špela Pungaršek: The differentiation and distribution of alpine *Luzula* sect. *Luzula* species in the Eastern Alps (SL16)

13:15 – 14:30 Lunch & posters

Session 5: Plant interactions with environment (chair: Edith Stabentheiner & Mario Pezzotti)

- 14:30 Edith Stabentheiner: Stressphysiology – the use of stress markers to evaluate oxidative stress (IL9)
- 15:00 Bekim Gashi: Response of onion plants to oxidative stress induced by heavy metals pollution of soils from industrial areas in Kosovo (SL17)
- 15:15 Maruša Pompe Novak: Grapevine in changing environment (SL18)

15:30 - 16:00 Coffee break

Closing session

- 16:00 **Closing lecture:** Mario Pezzotti: Grapevine biology in the post-genomics era
- 16:35 Concluding remarks

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Opening and closing lectures

Beneficial microbes enhance plant phenotypic and metabolic plasticity to cope with stress

Maria J. Pozo^{1*}, Javier Rivero¹, Javier Lidoy¹, Laura Dejana¹, Zhivko Minchev¹, Kristina Gruden², Salva Herrero², Victor Flors³, Ainhoa Martinez-Medina³

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Plant associated beneficial microbes can confer multiple benefits to the host plant, ranging from improved nutrient acquisition to enhanced resistance-tolerance to diverse stress conditions. This enhanced tolerance is commonly associated with an improved ability to activate defences upon challenges, leading to an enhanced metabolic and phenotypic plasticity. Using tomato and mycorrhizal fungi as a model system, pharmacological and genetic approaches, and omics analyses, we present examples of how mycorrhizal plants cope with abiotic and biotic stresses through priming of plant defense mechanisms, and how phytohormone signalling pathways are key regulators of such improved responses. We are particularly interested in the impact of mycorrhization on plant resistance to herbivore insects, finetuning plant primary and secondary metabolism to reduce their performance, and how the effects can even cascade up to other trophic levels. The results are discussed in the frame of our current knowledge on induced resistance by beneficial fungi, and the opportunities and challenges of their application as bioprotectors in sustainable agriculture.

Gruden, K., Lidoy, J., Petek, M Podpečan, V., Flors, V., Papadopoulou, K., Pappas M.L., Martinez-Medina A., Bejarano, E Biere, Pozo M.J. (2020). Ménage à Trois: Unraveling the Mechanisms Regulating Plant–Microbe–Arthropod Interactions, *Trends in Plant Science*, 25, 1215-1226, doi: 10.1016/j.tplants.2020.07.008

Pozo, M.J., Zabalgogeaçoa I., Vazquez de Aldana B.R., Martinez-Medina, A., (2021) Untapping the potential of plant mycobiomes for applications in agriculture, *Current Opinion in Plant Biology*, 60, 102034, doi: 10.1016/j.pbi.2021.102034

Rivero, J., Álvarez, D., Flors, V., Azcón-Aguilar, C. and Pozo, M.J. (2018), Root metabolic plasticity underlies functional diversity in mycorrhiza-enhanced stress tolerance in tomato. *New Phytol*, 220: 1322-1336. <https://doi.org/10.1111/nph.15295>

Rivero J., Lidoy J., Llopis-Giménez A., Herrero S., Flors, V., Pozo M.J. (2021) Mycorrhizal symbiosis primes the accumulation of antiherbivore compounds and enhances herbivore mortality in tomato. *J Exp Bot.* 2021 Jun 22;72(13):5038-5050. doi: 10.1093/jxb/erab171

Grapevine biology in the post-genomics era

Chiara Fattorini¹, Edoardo Bertini¹, Chiara Foresti¹, Erica D'inca¹, Marianna Fasoli¹, Giovanni Battista Tornielli¹, Sara Zenoni¹, Mario Pezzotti^{1,2*}

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Grapevine is a woody temperate perennial plant and one of the most important fruit crops with global relevance in both the fresh fruit and winemaking industries. Unfortunately, global warming is affecting viticulture by altering developmental transitions and fruit maturation processes. In this context, uncovering the molecular mechanisms controlling the onset and progression of ripening could prove essential to maintain high-quality grapes and wines. The availability of the grapevine genome sequence and of abundant transcriptomic data are the pre-requisite for identifying genes controlling specific developmental processes. Moreover to finally establish the biological role of the isolated genes, a platform for gene editing in grapevine will be also fundamental. The passage from omics to fundamental biology in grapevine is the objective of this presentation, through several examples.

Applied plant biology research and agronomy applications

***Aureobasidium pullulans* as an alternative and sustainable strategy in agriculture**

Alessandra Di Francesco*

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Aureobasidium pullulans is a ubiquitous yeast found in a variety of environments, including temperate, cold, warm, and arid areas (Bozoudi and Tsaltas, 2018). Furthermore, in a context of climate change, it is interesting to verify the evolution and spread of more extremotolerant species in more conventional environments. The yeast shows different morphological characteristics depending on growth conditions, which encompass various nutrient-rich and -poor media. In their later growth stages, regardless of the type of media, the colonies always turn dark olivaceous to blackish due to the production of chlamydospores, a characteristic feature of *A. pullulans* (Ramos and Garcia-Acha, 1975). The yeast has been regarded as both effective and safe for various applications in biotechnology (environmental remediation, agriculture, food technologies) and cosmetics (Prasongsuk et al., 2017). For agriculture, in particular, *A. pullulans* is useful against postharvest fruit and vegetable pathogens. In fact, fruits and vegetables are products subjected to fungal diseases which can cause losses up to 50%, according to the fruit and the country. An alternative to synthetic chemicals, it is considered a Biological Control Agent (BCA) with non-toxic antimicrobial activity against pathogenic bacteria and fungi. The yeast is able to produce a wide range of natural products, which have been extensively studied for their numerous applications in the above-mentioned sectors. Among the most common active compounds produced by this yeast are polysaccharides such as pullulan, β -glucan, aureobasidin, siderophores, and a “biosurfactant” composed of glycerol-liamocin. Despite this, they also find wide application in open fields. The interaction between fungal pathogen, host, and antagonist is influenced by several parameters as temperature, oxygen, and water activity that could determine the success of biocontrol activity. The BCAs are organisms that act through different modes of action (antibiosis, competition for space and nutrients, parasitism, resistance induction) (Sharma et al. 2009). The knowledge of these modes of action could enhance their viability and their potential in controlling plant pathogens and also could be useful in optimizing their commercial formulation long-term, stable, easily handled, and consistently effective against target diseases.

Bozoudi D., Tsaltas D., 2018. The multiple and versatile roles of *Aureobasidium pullulans* in the Vitivinicultural Sector. *Fermentation* 4, 85

Prasongsuk P., Pongtharin L. Imran A., Wichanee B., Hunsa P., 2018. The current status of *Aureobasidium pullulans* in biotechnology. *Folia Microbiologica* 63, 129–140

Ramos S., Garcia-Acha. I., 1975. A vegetative cycle of *Pullularia pullulans*. *Transactions of the British Mycological Society* 64, 129-135

Sharma R. R., Singh D., Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control* 50, 205–221

Belatedly - climate changes affects the spread of tropical pests in agriculture

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Climate change affects the frequency, intensity, and geographic distribution of pathogens. These changes can impact the occurrence and severity of pest infestations in crops by increasing the range of distribution, colonization of new hosts, and altering pest development cycles. Understanding the various impacts of climate change on agricultural ecosystems requires consideration of the specific interactions between an individual pathogen and its host(s) and the resulting effects on the interaction between the host and other species in the community. However, milder winter conditions and warmer summers are already affecting the distribution patterns of thermophilic pest species. Such group of pests includes the tropical group of root-knot nematodes (RKN, *Meloidogyne* spp.), which are considered the most damaging plant-parasitic nematodes. Among the plant parasites, only RKN cause crop losses of about 15% in subtropical countries. In addition, yield losses of 50-80% in vegetable crops caused by these nematodes are not uncommon. Damage and yield losses caused by this group are also expected to be greater in temperate regions, where environmental conditions are more favourable for pest colonisation, development, reproduction, and spread. In addition, intensive global trade, trends such as environmentally friendly crop production methods, and the lack of appropriate management strategies pose a serious risk of these pests to agricultural production in Europe. Two species, *M. incognita* and *M. javanica*, from the tropical RKN group have been recognised as the most rapidly spreading crop pests worldwide, as measured by country saturation rate among 424 crop pests and diseases (Bebber et al., 2014). Several species of the tropical group are able to survive winter in the field, especially in Mediterranean countries (Širca et al., 2021). Occurrence in the field poses an additional risk, especially in light of climate change and the fact that infestations are much more difficult to control in larger areas.

Bebber, D. P., Holmes, T., Gurr, S. J. (2014). The global spread of crop pests and pathogens. *Global Ecology and Biogeography*, 23, 1398– 1407. <https://doi.org/10.1111/geb.12214>

Širca S., Gerič Stare B., Strajnar P., Knapič M., Žibrat U., Folcher L., Ollivier F., Buisson A., Chappé AM., Lurdes Inácio M., Rusinque L., Nóbrega F., De Andrade E., van Hesse E., Karssen G., Warbroek T., Bačić J., Abrantes I., Conceição I., Maleita C. (2021). Global warming and distribution of root-knot nematode species of the tropical group (MeloTrop): Euphresco: final report. Geneva: Zenodo, doi: 10.5281/zenodo.5171594

Characterization of β -cyclodextrin - fennel and basil essential oil inclusion complexes and effect on Colorado potato beetle growth

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Essential oils (EOs) obtained from plants can frequently exhibit toxicity and repellency/deterrence against insect pests. Fennel (*Foeniculum vulgare* Mill.) and basil (*Ocimum basilicum* L.) EOs are used in pest management applications, but the most-discussed constraint regards restraining the usage of these natural insecticides in common agricultural practices is related to the volatility and limited persistence of EOs under field conditions. Due to high volatility, the efficacy of these substances falls short after EO application and requires frequent reapplication. There is a great interest in developing formulations that improve the handling of EOs, prevent the photodegradation of their active ingredients, and control the release of volatiles through micro/nanospheres or capsules in solid or liquid form. The polymer-based EO nanoformulations provide the stability and optimal effectiveness of EOs with an anti-insecticidal effect. One of the main advantages of solid formulations is that they can have a longer shelf life and be applied in solid form or even mixed with water and sprayed. We investigated the formation and characterization of inclusion complexes (ICs) of beta-cyclodextrin (β -CD) with two selected EOs, using the co-precipitation method. The main constituents of EOs determined by GC-MS analyses were trans-anethole (37.77%) and α -pinene (23.51%) in fennel EO, and linalool (32.6%) and methyl chavicol (23.2%) in basil EO. The interaction between β -CD and EO molecules was assessed through Fourier-transform infrared spectroscopy (FT-IR) and it was used to characterize the ICs by evaluating the changes recorded in the ICs compared to the free β -CD and individual EO. FT-IR spectra of inclusion complexes displayed peak shifts and variations of intensity when it compared to FT-IR spectra of EOs and free β -CD. β -cyclodextrin resulted as an effective host in encapsulating selected EOs. The potato plants sprayed with ICs were examined by scanning electron microscopy (SEM) and showed ICs with lamellate or rhomboid morphology. Bioassay with Colorado potato beetle fed with potato plants covered with ICs showed that complexation with β -CD reduced insects' growth (body mass of IV instar larvae where 119.84 ± 1.48 mg and 120.79 ± 7.42 mg for IC with fennel and basil EO respectively in comparison to body mass of control larvae 138.97 ± 2.01 mg) and affected digestive proteolytic activity.

Acknowledgment: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007

Virome of tomato, weed plants and water in agroecosystems in Slovenia

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Viromics approaches, based on high-throughput sequencing, can provide untargeted overview of the viruses associated with different parts of an ecosystem. In the span of 2 years we have visited 14 tomato farms in Slovenia and systematically investigated the virome of tomatoes, surrounding volunteer and weed plants from different botanical families, and irrigation and surface waters in these agroecosystems. Using Illumina sequencing approach and subsequent bioinformatics, we analysed 436 plant samples pooled in 69 composite samples (by location and sample type), and 24 samples of tap, underground and surface water. We discovered 37 known and at least 56 new viruses across plant samples, belonging to at least 25 viral families, with rhabdoviruses, potyviruses and tombusviruses being the most abundant ones. Majority of new viruses were discovered in weed plants. Some viruses were detected both in tomato and weed samples, indicating a possibility of viral exchange between those two compartments. Nucleic acids of diverse plant viruses were detected in waters samples; here, viruses with known stable virions (tobamoviruses, tombusviruses) predominated. Complete viral genomes of new viruses were reconstructed from water samples (e.g., new tobamoviruses). Several viruses were found to be present both in water and plant samples, for example, a new tobamovirus, detected in *Plantago* sp. at one location, was further detected in water samples from different locations. This cross-environment viromics study highlights the usefulness of combining analysis of different sample types to better understand diversity and distribution of viruses in an ecosystem. It represents a baseline for establishing the epidemiological links between plants and environmental waters, and will help to detect and better understand possible future emergences of viral diseases in plants.

Tomato mottle mosaic virus: newly discovered enemy affecting tomatoes and peppers

Ana Vučurovič^{1*}, Jakob Brodarič¹, Irena Bajde¹, Antonio Tiberini², Marta Luigi², Ariana Manglli², Francesco Faggioli², Nataša Mehle^{1,3}

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Tomato mottle mosaic virus (ToMMV) was firstly described in tomatoes in 2013 in Mexico as a new member of the genus *Tobamovirus*, family *Virgaviridae* (1). After its first finding, ToMMV was detected in tomato and pepper crops in North and South America, and Asia causing significant losses. It was also detected in some European countries (2, 3, 4). ToMMV may cause severe symptoms in affected plants including leaf deformations, mottle, mosaic, and necrosis. Even though its epidemiology still needs to be clarified, we know that like other tobamoviruses, ToMMV can be transmitted mechanically by contact between plants and by doing common agricultural practices (such as pruning or harvesting). Probably, like the other emergent tobamovirus tomato brown rugose fruit virus (ToBRFV), it can also be spread by bumblebees. The economic importance of tomatoes and peppers, emerging nature of ToMMV and its similarities with emerging ToBRFV initiated the inclusion of ToMMV on EPPO Alert List. Since the most important potential pathway for introduction to new countries/regions are seed and planting material, rapid and accurate detection of ToMMV is a cornerstone of successful control. Therefore, we improved ToMMV diagnosis by developing a new high sensitivity diagnostic test to detect the virus in seeds and leaf material. Our newly developed test can simultaneously detect two emerging tobamoviruses, ToMMV and ToBRFV. The performance characteristics of the test are available in our recently published article (5). In addition, in the ARRS applicative project (<http://projects.nib.si/tobamo/>) we will try to fill the knowledge gaps related to the epidemiology of the virus, by studying the survival of the virus in a water environment and the possibility of transmission with irrigation water.

(1) Li R. et al. (2013) Genome announcements 1: e00794-13.

(2) Ambrós S. et al. (2017) European Journal of Plant Pathology 149: 261–268.

(3) EPPO Reporting Service (2020) Available online: <https://gd.eppo.int/reporting/article-6930> (accessed on 20 June 2022)

(4) Fowkes A. R. et al. (2022) New Disease Reports 45: e12067.

(5) Tiberini A. et al. (2022) Plants 11: 489.

Wild and crop plant pathosystem interactions and variation: Case study *Lactuca* spp. – *Bremia lactucae*

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Wild and crop plant pathosystems are exciting models for complex study of host-pathogen interactions (Lebeda and Burdon, 2022). Extensive research on the wild and crop plant pathosystem, wild *Lactuca* spp. and *L. sativa* - *Bremia lactucae* (lettuce downy mildew), has been conducted in the Czech Republic since the end of the 1970s (Lebeda et al., 2002, 2008). Recent phylogenetic studies suggest that *B. lactucae* might be restricted only to cultivated *L. sativa* and wild growing *L. serriola* (Runge et al., 2021). European populations of *L. serriola* were generally highly susceptible to *B. lactucae* under *in-vitro* tests. However, large variation in *L. serriola* race-specific resistance was found both among and within individual populations (Lebeda et al., 2008). Temporal changes in virulence frequencies over the period were recorded in *B. lactucae* populations on *L. serriola*. In many v-factors (v11, v14, v16, and v25–28), fluctuations were observed, some (v14 and v17) shifting to higher frequencies, and others (v5/8 and v23) decreasing (Lebeda et al., 2008). Many of the *Dm* resistance genes in cultivated lettuce originate from *L. serriola*, but a rapid surmounting of that resistance in the crop by new downy mildew races is often observed. Resistance in *L. saligna*, which is considered as a nonhost resistance, seems to be most efficient and durable (Lebeda et al., 2014). In addition, the pathogenicity structure of two *B. lactucae* populations from *L. serriola* and *L. sativa*, respectively, have been investigated. While the pathogen populations from both hosts were characterised by the fast dispersal of v-factors, those from *L. serriola* showed a balanced selection pattern while those from *L. sativa* showed a directional selection pattern. This indicates founder effects, most likely caused by host jumps, that result in a fast radiation and colonization of susceptible lettuce cultivars (Runge et al., 2021).

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Lebeda A. and Burdon JJ (2022). *Phytopathology* (in press)

Lebeda A., Petrželová I. and Maryška Z. (2008). *Eur J Plant Pathol* 122, 127-146.

Lebeda A., Pink D.A.C. and Astley D. (2002). *Advances in Downy Mildew Research*. In: Spencer-Phillips, P.T.N., Gisi U. and Lebeda A, (Eds.) *Advances in Downy Mildew Research*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 85-117.

Lebeda A., Křístková E., Kitner M., Mieslerová B., Jemelková M. and Pink D. A. C. (2014). *Eur J Plant Pathol* 138, 597–640.

Runge F., Gärber U., Lebeda A., and Thines, M. (2021). *Eur J Plant Pathol* 161, 411-426.

Growth penalty in *Nicotiana benthamiana* insect sex pheromone biofactories involves transcriptional deregulation of stress-growth balance

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Plant biofactories are a sustainable platform for production of high value compounds, among which are insect sex pheromones, used in integrated pest management as a species-specific control of insect pests, reducing application of conventional insecticides. Recently, we have constructed transgenic *Nicotiana benthamiana* plants (“Sexy Plants”, SxP) that successfully produce and emit a blend of moth (Lepidoptera) sex pheromone compounds (*Z*)-11-hexadecen-1-ol and (*Z*)-11-hexadecenyl acetate. However, high production yields cause severely stunted growth, which presents a hindrance for bigger-scale commercial production of pheromones in plants. To characterize the molecular perturbation leading to growth arrest and find key bottlenecks or triggered signalling pathways that could be addressed and fine-tuned in the next generation of pheromone producing plants, we have analysed the whole-genome transcriptome and evaluated it in relation to growth and pheromone production in lines with low and high production yields. We have also generated a comprehensive functional *N. benthamiana* genome annotation as well as gene translations to *Arabidopsis thaliana*, enabling functional information transfer by using Arabidopsis knowledge networks. Analysis of the transcriptional reprogramming revealed a strong, stress-like response with downregulation of genes related to photosynthesis, DNA synthesis, protein synthesis, lipid synthesis and cell wall metabolism, and upregulation of genes related to secondary metabolism, hormone metabolism, stress, protein degradation and lipid degradation. Interestingly, we observed activation of jasmonic acid and downregulation of gibberellic acid signalling. This illuminated the possibility that the growth penalty was not solely a consequence of a higher metabolic burden imposed upon constitutive expression of a heterologous biosynthetic pathway, but rather an orchestrated growth inhibition resulting from activation of specific stress-related signalling pathways, namely the stress-growth balance regulated by the interplay between jasmonic and gibberellic acid signalling.

Mateos-Fernández R., Moreno-Giménez E., Gianoglio, S., Quijano-Rubio A., Gavaldá-García J., Estellés L., Rubert A., Rambla J. L., Vazquez-Vilar M., Huet E., Fernández-del-Carmen A., Espinoza-Ruiz A., Juteršek M., Vacas S., Navarro I., Navarro-Llopis V., Primo J., Orzáez D. (2021). Production of volatile moth sex pheromones in transgenic *Nicotiana benthamiana* plants. *BioDesign Research*, doi: 10.34133/2021/9891082.

Corn-cockle (*Agrostemma githago*) preparation exhibit plant growth regulator activity

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Corn-cockle (*Agrostemma githago*) is the main ingredient of Agrostemin®, a natural plant preparation used as a biostimulant in agriculture, which elicits many positive responses. The objective of the present study was to determine and quantify whether *A. githago* has auxin and cytokinin-like growth regulator (PGRs) activities at different stages of its life cycle from seed to young plant. The *Cucumis* and *Triticum* bioassays were used to determine cytokinin-like activities, and the *Cucumis* and Mung bean bioassays were used to determine auxin-like activities. The water extract of seeds, fresh and dry plant material at different stages of development showed auxin-like activity in the cucumber cotyledon root and Mung bean bioassays. The activity in the cucumber bioassay was equivalent to about 0.3 mg L⁻¹ Indole-3-butyric acid (IBA) and in the mung bean bioassay it was equivalent to about 0.5 to 1 mg L⁻¹ IBA. Both the *Cucumis* cotyledon expansion bioassay and the *Triticum* bioassay showed weak cytokinin-like activity.

Ambrožič-Dolinšek, J., Ornik, D., Branda, R., Molnar, Z., Ciringer, T. (2022) Does biostimulant Agrostemin® exhibit plant growth regulator activities?. *Phyton: annales rei botanicae*. doi: 10.12905/0380.phyton61-2022-0109.

Cold plasma for effective decontamination of buckwheat grains artificially infected with *Fusarium graminearum*

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Fungi from the genus *Fusarium* are one of the most economically important plant pathogens that commonly infect various crops. Besides causing the decay of plants, many of them are also capable of producing mycotoxins, which are harmful to both humans and animals. Their consumption can induce acute intoxication or lead to chronic effects, such as some types of cancer or even death. Genus *Fusarium* has been traditionally associated with temperate crops as these fungi require somewhat lower temperatures for growth and mycotoxin production. One of the most problematic diseases caused by *Fusarium* species worldwide is the “Fusarium head blight” of small-grain cereals, which is associated with many different species of this genus, but *F. graminearum* is still the primary and most aggressive causal agent (Ferrigo et al., 2016). As for most cereals and pseudocereals, fungi present the leading cause of buckwheat diseases at all stages, from germination to harvest and post-harvest stages. Nowadays, seeds and grains are mainly treated with fungicides to suppress fungal growth, but these chemical substances leave residues and negatively impact the environment and our health. Therefore, new environmentally friendly technologies are sought for in the light of reducing the usage of pesticides. The emerging use of cold plasma technology seems to offer promising solutions for decontaminating fungi and their toxins from seeds (Mravlje et al., 2021). This study aimed to test the effectiveness of cold plasma (CP) treatment for decontamination of buckwheat grains artificially contaminated with spores of *F. graminearum*. We used a large-scale custom-made inductively coupled radio frequency plasma system operating under vacuum conditions. We tested two exposure times, 45 and 90 s, and found a 20% and more than 90% reduction in grain contamination compared to the control. The longer CP treatment time also significantly suppressed the fungal growth rate to less than 10% of the control. However, both CP treatments also decreased the germination of buckwheat grains (to 73% after 45 s and 27% after 90 s of CP treatment), making this kind of CP treatment applicable in post-harvest (food and feed storage) but not for further sowing.

Ferrigo, D., Raiola, A., & Causin, R. (2016). Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules*, 21(5). <https://doi.org/10.3390/molecules21050627>

Mravlje, J., Regvar, M., & Vogel-Mikuš, K. (2021). Development of cold plasma technologies for surface decontamination of seed fungal pathogens: Present status and perspectives. *Journal of Fungi*, 7(8). <https://doi.org/10.3390/jof7080650>

Morphological seed characterization of composite genetic resources of *Phaseolus* sp.

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The ability of *Phaseolus* sp. to adapt to both different environmental factors and cultivation methods has resulted in a great deal of genetic diversity between different populations and also between beans from the same genetic source. In this study, we used 56 *Phaseolus* sp. composite genetic resources; including 54 common bean (*Phaseolus vulgaris*) and 4 runner bean (*Phaseolus coccineus*), to evaluate seed-related differences among them. A composite had an average of 2 to 3 components that differed in seed coat pattern/colour. In total, we evaluated 141 different components of bean genetic resources using 16 descriptors developed by International Plant Genetic Resource Institute (IPGRI), Community Plant Variety Office (CPVO), and Improvement of sustainable *Phaseolus* production in Europe for human consumption (Phaselieu). Seeds were evaluated using six quantitative traits (seed length, seed width, seed thickness, seed length/width ratio, seed width/thickness ratio, and 100 seed weight) and ten qualitative traits (seed size, number of seed colours, seed colour, primary seed colour, predominant secondary seed colour, distribution of secondary seed colour, apparent seed veining, seed veining, seed shape, and seed brilliance). The results of principal component analysis showed that the first six components explained 76.84 % of the variability within the *Phaseolus* sp. collection used in this study. The average values of traits that most effectively distinguished the beans from each other were 12.98 mm seed length (8.77 – 22.46 mm), 45.00 g 100 seed weight (18.37 – 75.95 g), 7.74 mm seed width (5.33 – 14.55 mm), 6.07 mm seed thickness (4.38 – 9.89 mm), medium seed size (78.01 %), for seed colour, a colour mixture (50.35 %), and seeds with one colour (50.35 %). We will continue our research with the analysis of composites at the genetic and genomic level. The results obtained show that there is a morphological variability that can be exploited to create new bean varieties more suitable for breeders, farmers and consumers.

Nadeem M. A., Karaköy T., Yeken M. Z., Habyarimana E., Hatipoglu R., Çiftçi V., Nawaz M. A., Sönmez F., Shahid M. Q., Yang S. H., Chung G., and Baloch F. S. (2020). Phenotypic characterization of 183 Turkish common bean accessions for agronomic, trading, and consumer-preferred plant characteristics for breeding purposes. *Agronomy*, 10(2). doi:10.3390/agronomy10020272.

Sinkovič L., Pipan B., Sinkovič E., & Meglič V. (2019). Morphological seed characterization of common (*Phaseolus vulgaris* L.) and runner (*Phaseolus coccineus* L.) bean germplasm: A slovenian gene bank example. *BioMed Research International*, 2019, doi:10.1155/2019/6376948.

Effect of drought and belowground herbivory on maize morphological and physiological features

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Climate change is expected to have a major impact on European agriculture in the future as it can affect crops and their pests. Wireworms (Coleoptera: Elateridae) are already serious maize pests and global changes in precipitation patterns could trigger unexpected changes in host plant–pest interactions. In this study, we tested single and combined effects of drought and belowground wireworm herbivory on maize. For this three factorial pot experiment we selected two maize varieties with different genetic predispositions to drought tolerance, i.e., a drought tolerant variety FuturiXX and a drought intolerant variety ZP341. Water deficiency and/or herbivory stress treatments started in the 4th week of maize growth. Various physiological parameters, such as chlorophyll *a* fluorescence, effective quantum yield of photosystem II, photosynthetic rate, relative water content, leaf stomatal conductivity and transpiration rate were measured at three time points (on day 14, 21 and 28 after adding wireworms and changing watering regime). At the end of the experiment, morphological parameters such as average stem diameter, shoot length, shoot mass and root mass were measured, along with herbivory damage, which was determined as the number of tunnels made by wireworms. The greatest differences occurred on day 21, when water deficient FuturiXX plants with no wireworms had lower relative water content, lower leaf stomatal conductivity and lower effective quantum yield of photosystem II compared to other FuturiXX treatments. On day 21 also well-watered ZP341 plants infested with wireworms had higher leaf stomatal conductivity and higher transpiration rate compared to other ZP341 treatments. In addition, the number of tunnels made by wireworms was higher in ZP341 variety. Drought negatively affected shoot length in both varieties, and shoot mass, root mass, and average stem diameter in ZP341. Overall, herbivory and drought stress combined had a greater effect on shoot length and shoot mass than single stress in both varieties.

Chávez-Arias C. C., Ligarreto-Moreno G. A., Ramírez-Godoy A., and Restrepo-Díaz H. (2021). Maize Responses Challenged by Drought, Elevated Daytime Temperature and Arthropod Herbivory Stresses: A Physiological, Biochemical and Molecular View. *Frontiers in Plant Science*, 12, 702841. doi.org/10.3389/fpls.2021.702841

Kozina, A., Lemic, D., Bazok, R., Mikac, K. M., Mclean, C. M., Ivezić, M., et al. (2015). Climatic, edaphic factors and cropping history help predict click beetle (Coleoptera: Elateridae) (*Agriotes* spp.) abundance. *Journal of Insect Science*, 15(1), 1–8. doi.org/10.1093/jisesa/iev079

Effect of root inoculation of strawberry plants with entomopathogenic fungus *Metarhizium brunneum*: hyperspectral analyses and plant physiology

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Certain soil microbes can enhance plant nutrition and protect plants against biotic and abiotic stresses. For this reason they have a huge potential to help farmers reduce fertilizer and pesticide input toward a more sustainable agri-and horticulture, which is a main goal of the EU project Excalibur. A two-factor strawberry tunnel pot experiment was performed in 2020 on frigo strawberry plants (*Fragaria × ananassa* Duch.) cv. 'Clery'. We investigated the effect of soil inoculations (factor 1) of an entomopathogenic fungus *Metarhizium brunneum* 1868 and an aboveground pest (factor 2), the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) (Hemiptera, Aphididae) on strawberry plants, and their interactions. Several physiological, agronomical and entomological parameters were evaluated. Using hyperspectral imaging combined with Savitzky-Golay 2nd order derivatives, kernel principal component analysis and support vector machines, we were able to distinguish between the treatment groups with 100% accuracy. Inoculating strawberry plants with *M. brunneum* significantly affected the number of flowers and fruits per plant, fruit weight, rhizoplane colonization, potential photochemical efficacy of PSII (Fv/Fm) and cobalt leaf concentration. Potassium and chromium were borderline insignificantly increased in *M. brunneum* treated plants. Endophytism in roots and leaves was negligible. A significant correlation between Fv/Fm and rhizoplane colonization was observed ($r^2=19,7\%$). Root inoculation with *M. brunneum* didn't significantly affect any of the gas exchange photosynthesis parameters or leaf chlorophyll content. The addition of aphids *C. fragaefolii* significantly increased the incidence of *Sphaerotheca macularis* infection, but not physiological status of plants or leaf chlorophyll content. Due to the fact that *M. brunneum* positively affected some physiological and agronomic parameters it will be further tested in field experiments within project Excalibur.

Keywords: Biological control; integrated pest management; agricultural entomology; entomopathogenic fungi; hyperspectral imaging; remote sensing; plant-microbe-pest interaction; plant physiology; rhizosphere competence;

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Role of glutathione in potato response to French marigold essential oil

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Volatile organic compounds (VOCs) have a role in communication of plants with other organisms. The effect of VOCs from French marigold (*Tagetes patula* L.) essential oil (EO) on potato (*Solanum tuberosum* L.) plants was studied to examine the interaction between this ornamental plant species and potato as an important agricultural crop. According to comprehensive microarray analysis, EO affected the expression of numerous potato genes after 8 h of exposure. From 124 identified transcripts annotated to glutathione metabolism pathway in potato (sot00480), 43 were with significantly altered expression (35%), and 42 of them were up-regulated. Glutathione (GSH) is an essential low-molecular metabolite, which participates in the removal of reactive oxygen species (ROS), stress-generated signal molecules involved in immune responses. Transitions between glutathione reduced (GSH) and oxidized forms (GSSG) are finely controlled by complex enzymatic machinery. Thus, in order to examine in more details the role of glutathione in potato ROS balance after perception of VOCs, the expression of 10 selected genes involved in glutathione metabolism was further analysed by the qRT-PCR method. For this purpose potato plants were exposed to EO for 8 h during one or three consecutive days. Samples were collected immediately after EO removal, or after 5 and 10 days. Results showed that gene coding for γ -glutamylcysteine synthetase (γ -GCS), first in the line of GSH synthesis, had the highest level of expression after the first 8 h of exposure, indicating a fast response to VOCs perception. In other experimental groups synthesis of GSH, in which were involved GSH synthase (GSS) and glutamyltranspeptidase (GGT), was decreased. A similar pattern was observed for genes coding for enzymes involved in redox glutathione transduction (glutathione reductase - GR, isocitrate dehydrogenase - IDH, and dehydroascorbate reductase-like protein - DHAR). However, the highest alternation in expression between exposed potato and corresponding controls was observed for glutathione S-transferase (GST) genes, indicating that the glutathione-based response mechanism is an important component of ROS scavenging mechanisms in VOCs-induced responses of the potato. In addition, the potential of glutathione to sequester and detoxify exogenous VOCs by glutathione S-transferases, opens new research avenues.

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***In vitro* shoot propagation and regeneration of rocket (*Eruca sativa* Mill.) using organogenesis and somatic embryogenesis**

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Eruca sativa (rocket, *Brassicaceae*), is known in traditional medicine for its therapeutic, while young plants are used as salad, vegetable, or green foods. Genetic improvement of the rocket has been limited due to the appearance of biennial plants and the requirement for isolation barriers. Plant tissue culture offers a platform to overcome existing barriers to improving this species. The present study aimed to achieve a feasible protocol for shoot regeneration and propagation of rocket cv. Domaća rukola and to evaluate the use of AgNO₃ to improve regeneration efficiency. Proliferation of shoots from seedling epicotyl, shoot organogenesis from root, hypocotyl and cotyledon seedling explants, and somatic embryogenesis from immature zygotic embryos were studied. Murashige and Skoog (MS) basal medium in combination with a low KIN concentration of 0.1 mg l⁻¹ with frequent subculture over a period of 3 weeks was found to be optimal for shoot multiplication with a multiplication index of almost 3 and only 9.3% of vitrified shoots without necrosis. Different concentrations of 2,4-D, BA or TDZ in combination with NAA and with or without AgNO₃ were tested for shoot regeneration from seedling explants. As a result, the hypocotyl explants cultured on MS with a combination of TDZ 1.0+NAA 0.1+AgNO₃ 5.0 mg l⁻¹ provided healthy shoots with a satisfactory regeneration rate of 25.4% and 2.2 mean number of shoots per regenerating explant. Immature zygotic embryos cultured on MS medium containing 1.0 mg l⁻¹ 2,4-D showed the highest regeneration frequency (78.76%) as well as the number of regenerated somatic embryos per explant (5.13) with low incidence of callusing and necrosis. KIN at 0.5 mg l⁻¹ enabled the best conversion of somatic embryos into healthy plants (22.5%). The results provide an opportunity to use established regeneration protocols in rocket breeding improvement programs.

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Pavlović S., Vinterhalter B., Zdravković-Korać S., Vinterhalter D., et al. (2013). Recurrent somatic embryogenesis and plant regeneration from immature zygotic embryos of cabbage (*Brassica oleracea* var. *capitata*) and cauliflower (*Brassica oleracea* var. *botritis*). *Plant Cell Tissue Organ Cult (PCTOC)*, doi: 10.1007/s.11240-012-0279-6.

Banjac N., Vinterhalter B., Krstić-Milošević D., Milojević J., Tubić Lj., et al. (2019). Somatic embryogenesis and shoot organogenesis from the hypocotyl slices and free radical scavenging activity of regenerants of collard greens (*Brassica oleracea* L. var. *acephala*). *Plant Cell Tissue Organ Cult (PCTOC)*, doi:10.1007/s11240-019-01595-6

Antifungal activity of selected plant extracts obtained by supercritical fluid extraction using CO₂

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Fungi are an integral part of the natural environment, and although they have many beneficial functions, they also contribute significantly to food spoilage. Pathogenic fungi are responsible for up to 20% loss of global crop yields yearly, making the use of fungicides critical to consumer protection. Modern agricultural pest management relies heavily on the use of synthetic fungicides, and resistance to many of the effective fungicides has gradually spread in pathogen populations. Many plant extracts show antifungal activity and have the potential to replace the synthetic fungicides. The antifungal activity of plant extracts is attributed to the presence of secondary metabolites such as alkaloids, phenols like flavonoids, and terpenoids, and the choice of extraction method is extremely important to provide comparable final products that maintain the primary composition of the source material. New technological approaches to extract these compounds are gaining increased attention across a variety of research fields, such as the use of supercritical fluid extraction (SFE). Supercritical fluid extraction using CO₂ (SFE-CO₂) has gained attention as an environmentally well-accepted extraction method, as CO₂ is a non-toxic, inert, and available solvent, and the extracts obtained are chemically of greater or different complexity compared to those of conventional extracts. The SFE-CO₂ extracts of *Achillea millefolium*, *Calendula officinalis*, *Chamomilla recutita*, *Helichrysum arenarium*, *Humulus lupulus*, *Tarax-acum officinale*, *Juniperus communis*, *Hypericum perforatum*, *Nepeta cataria*, *Crataegus* sp. and *Sambucus nigra* were studied in terms of their compositions and antifungal activities against the wheat- and buckwheat-borne pathogenic fungi *Alternaria alternata*, *Epicoccum nigrum*, *Botrytis cinerea*, *Fusarium oxysporum* and *Fusarium poae*. Extracts of *C. recutita* and *H. arenarium* were the most effective, inhibiting the growth of most fungi by 80 to 100%. Among the fungal species, *B. cinerea* was the most susceptible to the treatments with the SFE-CO₂ extracts, while *Fusarium* spp. were the least. This study shows that some of these SFE-CO₂ extracts have promising potential for use as anti-fungal agents for selected crop-borne fungi.

Schoss K., Kočevar Glavač N., Dolenc Koce J. and Anžlovar S. (2022). Supercritical CO₂ plant extracts show antifungal activities against crop-borne fungi. *Molecules* 27, 1132. Doi:10.3390/molecules27031132.

***In vitro* bioavailability of phenolics from broccoli: Impact of high growing temperature**

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Plants challenged with high temperature (HT) undergo many adaptive mechanisms at molecular levels to keep normal physiology function. They can adapt to different temperatures by changing their transcriptome, proteome and metabolome, or even by activating cell death mechanisms (Bita and Gerats, 2013). Changes like these could have significant outcome on the plant nutritional value and present serious challenges for global food security. In scope of this work, we investigated the effect of high growing temperature on the bioavailability of total phenolics, flavonoids and flavonols from aqueous extract of broccoli (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.) seedlings subjected to *in vitro* model of human digestion. As a control, seedlings grown under regular temperature were used. In each phase, except oral, the bioavailability of total phenolics was higher from the HT-group. Total flavonols were also significantly better bioavailable from the HT-group in each of the phase. Flavonoids also showed the tendency of higher bioavailability from HT-group, however only in oral phase that was significant ($p \leq 0.05$). Based on this, we conclude that growing broccoli seedlings at elevated temperature ensures better bioavailability of its phenolic compounds.

Bita C.E., Gerats T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, doi:10.3389/fpls.2013.00273.

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Influence of temperature stress on the mass share of chlorophyll, lycopene, carotenoids and porphyrins in broccoli

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Climate change is a global problem that has changed both the physiological and biochemical parameters of plants. Considering that plants are sessile organisms, their survival depends on the effective activation of metabolic mechanisms of resistance to such stress. As part of this study, we investigated the impact of low and high temperature water on the content of chlorophyll, lycopene, carotenoids and porphyrins in broccoli (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.). For all pigments, except chlorophyll *a*, a statistically significant difference was observed between the group treated with water of high temperature and the remaining two groups (control and group treated with water of low temperature). In the group treated with water of high temperature, the highest proportion of chlorophyll *b* (5.35 ± 0.34 mg/g of dry weight (sm)), lycopene (0.28 ± 0.016 mg/g sm), and porphyrins (28.27 ± 1.09 mg/g sm) was recorded. The proportion of carotenoids, on the other hand, was the lowest in this group (0.40 ± 0.19 mg/g sm). Based on this, we conclude that chlorophyll *b*, lycopene, porphyrins and carotenoids of broccoli are significantly more sensitive to high temperature water stress than to low temperature water.

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Surveying and inventorying of on-farm conservation of plant genetic resources for food and agriculture in Slovenia

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Plant genetic resources for food and agriculture (PGRFA) are the foundation for food security and sustainable crop production. They are the building blocks for selecting and breeding crops that are more nutritious, productive, and resilient to pests, diseases, and environmental changes. Of special importance are landraces, old varieties and other heterogeneous material that are adapted to specific cultivation areas, but due to various environmental influences, population growth and the abandonment of the traditional way of farming they are in danger of disappearing. In Slovenia, the programme for conservation of PGRFA is conducted in the frame of Public Service for Plant Genetic Resources; however, a systematic survey, inventory, and monitoring of *in situ* and on-farm conservation have not been performed yet. With the aim to identify areas and/or farms where old varieties and landraces are still cultivated a two-year project was conducted. For apple and pears, the survey was made in the entire territory of Slovenia, whereas for fodder crops, cereals, vegetables, medicinal and aromatic plants, and grapevine inventorying was performed in three selected areas, i) Upper Posočje Region, ii) Haloze and iii) Slovenske gorice. The results indicated that there are less and less farmers who still cultivate old varieties. There is therefore an urgent need to prepare a programme for on farm conservation and management of PGRFA in order to prevent genetic erosion. This will also contribute to the realization of the objectives of the recently adopted Plant Genetic Strategy for Europe (ECPGR, 2021), where the first action includes ‘a significant expansion of *in situ* conservation of crop wild relatives, wild food plants, and on-farm varieties, through more systematic survey, inventory, and active conservation and management’.

ECPGR. (2021). Plant Genetic Resources Strategy for Europe. European Cooperative Programme for Plant Genetic Resources, Rome, Italy, 92 pp.

From glaciers to refrigerators: biocontrol potential of *Aureobasidium subglaciale* against pre- and postharvest plant pathogens

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Apples are infected by numerous fungi known as storage rots, which cause significant pre- and postharvest loss of produce. The rising public concerns about the use of chemical phytopharmaceuticals, bans of numerous fungicides in many countries, and increasing organic production motivates the search for safer and environmentally friendlier biocontrol agents such as the use of naturally occurring antagonists to prevent fruit rots. *Aureobasidium subglaciale* (Zalar, de Hoog & Gunde-Cimerman) Zalar, Gostinčar, Gunde-Cimerman, a close relative of commercially exploited *A. pullulans*, seems particularly interesting for postharvest application based on its preliminary biocontrol potential and adaptation to low temperatures. To investigate its potential to control postharvest decay we have sequenced the genomes of 15 available strains of *A. subglaciale*, investigated biocontrol relevant traits and tested antagonistic activity against the three relevant pathogenic fungi: *Botrytis cinerea*, *Penicillium expansum* and *Colletotrichum acutatum*. The genome analysis uncovered clear population structures of the strains and linkage disequilibrium indicated the clonality of the species, a desirable trait for commercial use of biocontrol agent. Several physiological traits of *A. subglaciale* relevant in biocontrol applications were identified, such as growth at low temperatures (even at 0 °C), tolerance to high temperature stress and elevated concentrations of solutes (sorbitol and CaCl₂). Also strong production of several extracellular enzymes (e.g. caseinases, β glucosidases, chitinases, and cellulases) and siderophores was noted – all acting directly on the phytopathogenic fungi either by mycoparasitism or by contributing to its successful out-competing strategy. Furthermore, the selected strains significantly reduced postharvest decay of apples at low temperatures caused by *B. cinerea*, *C. acutatum* (over 60% reduction) and *P. expansum* (approximately 40% reduction). Altogether, these data uncover substantial biotechnological and biocontrol potential of *A. subglaciale* leading towards alternative bio-based disease control of fresh fruits.

ZAJC, Janja, ČERNOŠA, Anja, SUN, Xiaohuan, FANG, Chao, GUNDE-CIMERMAN, Nina, SONG, Zewei, GOSTINČAR, Cene, 2022. From glaciers to refrigerators: the population genomics and biocontrol potential of the black yeast *Aureobasidium subglaciale*. *Microbiology spectrum*. <https://pubmed.ncbi.nlm.nih.gov/35880866/>, DOI: 10.1128/spectrum.01455-22.

Plant metabolism, structure and function

Application of X-ray-based techniques in plant biology

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Trace elements are essential components of living systems, but at the same time, they can be toxic at concentrations beyond those necessary for their biological functions. In addition, the toxicity can be extended to other non-essential elements of very similar atomic characteristics that can mimic the properties of a trace element. Trace element malnutrition affects more than half of the world's population, while on the other hand industrialization, traffic and extensive use of fertilizers have resulted in exceedingly high concentrations of non-essential elements in food crops, posing risks to human health. To be able to develop and improve phyto-technologies that enhance the production of safe and quality food, knowledge of the basic mechanisms involved in trace and non-essential element uptake, transport, accumulation and ligand environment in plants is needed. Such studies are nowadays supported by highly sophisticated X-ray-based techniques, such as synchrotron-based X-ray fluorescence spectrometry, proton-induced X-ray emission and X-ray absorption spectroscopy, enabling imaging of element distribution and determination of speciation and ligand environment of trace elements in biological tissues and cells with high spatial resolution and sensitivity. Selected case studies of metal distribution and speciation in selected model and crop plants, achieved by interdisciplinary work, will be presented.

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Plant phenotyping using chlorophyll fluorescence and multispectral imaging

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High-throughput phenotyping methodologies such as chlorophyll fluorescence and multispectral imaging represent tools for non-invasive quantification of plant performance in different environments. These techniques are widely used from basic ecophysiological studies to applied research in crop breeding programs. Plant phenotyping enables quantifying relevant traits reliably and automatically, saving time and human labour. This discipline combines new sensing technologies with automated image analysis and advanced statistical methods such as machine learning. The lecture will cover a background and most important traits obtained by chlorophyll fluorescence and multispectral analysis, and the results of several studies (nutrient deficiency stress, drought stress, and virus infections) using plant phenotyping methods will be presented.

Glutathione-mediated plant response to different light intensities und spectra

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Light intensity and spectrum composition affect photosynthesis and thus growth, development and yield. Prolonged excess light may lead to an overload of electron transport chains (photosynthesis, respiration), due to insufficient availability of the electron acceptor NADP⁺. Excess electrons react with molecular oxygen to form reactive oxygen species (ROS) such as singlet oxygen or H₂O₂, which can act as signaling molecules at low levels or damage nucleic acids, lipids and proteins when antioxidant capacity is exceeded. Among the antioxidant mechanisms in plants, components of the ascorbate-glutathione cycle play a central role in controlling H₂O₂ levels and the NADP⁺/NADPH ratio [1]. Since ascorbate and glutathione metabolism is cell compartment specific and cell compartments are affected differently by stress, it is important to monitor ascorbate and glutathione content at the subcellular level. We aimed at linking light-mediated effects on photosynthesis, growth, and development to the regulation of glutathione metabolism at whole leaf and subcellular level. Plants were exposed to 50, 250 or 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light or light of different spectral composition. Adaptations to light treatments were monitored by analyzing photosynthesis and growth parameters, gene expression, free amino acids in leaf extracts and glutathione in leaf extracts and in cell compartments. In a previous study, we found that excess light can damage plants [2], but below a certain threshold, wheat can recover from drought stress. This is due to increased amino acid and glutathione levels resulting from high or increased expression of genes encoding antioxidant enzymes [3]. At the subcellular level, we confirmed the results for glutathione from whole leaf extracts by finding that excess light and far-red light increased glutathione in organelles of wheat and *Arabidopsis*, respectively.

[1] Kocsy G., Tari I., Vanková R., Zechmann B., Gulyás Z., Poór P., Galiba, G, 2013. Plant Science 211, 77-91.

[2] Heyneke E., Luschin-Ebengreuth N., Krajcer I., Wolkinger V., Müller M., Zechmann B. 2013, BMC Plant Biology 13, 104.

[3] Gyugos M., Ahres M., Gulyás Z., Szalai G., Darkó E., Végh B., Boldizsár A., Mednyánsky Z., Kar R.K., Dey N, Simon-Sarkadi L., Galiba, G, Kocsy G. 2019. J Agro Crop Sci 16, 1-9.

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Glutathione concentrations differ in maize bundle sheath cell compartments, depending whether sulfur is supplied via hydrogen sulfide (H₂S) or sulfate

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We aimed to determine how leaf glutathione homeostasis is regulated upon variation in sulfur supply in maize, a model C₄ plant. In C₄ plants, sulfate metabolism is only located in the chloroplasts of bundle sheath cells (not in mesophyll cells). Glutathione may perhaps regulate the flux through sulfate metabolism. However, the latter is disputed by some studies, in which plants were fumigated with hydrogen sulfide (H₂S). Also, glutathione may function as a storage and transport molecule of sulfur in the plant. To get insights in such presumed functions of glutathione in C₄ plants, we assessed how leaf glutathione contents respond to a variation in sulfur supply, especially H₂S supply. Therefore, we detect glutathione contents in different subcellular compartments via immunogold-labeling and transmission electron microscopy and quantify glutathione concentrations with software-assisted imaging. We focus on chloroplasts, as sulfur metabolism is mainly located inside this compartment and because glutathione is synthesized in chloroplasts (and the cytosol). Our preliminary data showed that thiol (glutathione + cysteine) contents in maize bundle sheath tissue were significantly increased, when plants were supplied with H₂S, even when H₂S was the sole source for sulfur. We found the subcellular glutathione distribution in bundle sheath cells to be compartment-specific, in response to sulfur supplied via H₂S or sulfate. We propose that subtoxic H₂S treatment may accelerate the flux of glutathione from chloroplasts to the cytosol. This hypothesis is in agreement with the uptake and use of H₂S and sulfate for the synthesis of thiols and other organic compounds in plants.

Zechmann B., Mauch F., Sticher L., Müller, M. (2008). Subcellular immunocytochemical analysis detects the highest concentrations of glutathione in mitochondria and not in plastids. *Journal of Experimental Botany*. doi: 10.1093/jxb/ern243

Kopriva S. (2011). Nitrogen and Sulfur in C₄ plants. doi: 10.1007/978-90-481-9407-0_7.

Ausma T. and De Kok L.J. (2019). Atmospheric H₂S. Impact on plant functioning. *Frontiers in Plant Science* 2019, doi: 10.3389/fpls.2019.00743.

Hawkesford M. and De Kok L.J. (2006). Managing sulphur metabolism in plants. *Plant Cell and Environment*, doi: 10.1111/j.1365-3040.2005.01470.x.

Expression of recombinant CEP2 protease from the green alga *Chlamydomonas reinhardtii* and its activation in bacteria *Escherichia coli*

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Cysteine endopeptidase CEP2 from the green alga *Chlamydomonas reinhardtii* belongs to papain-like cysteine proteases (PLCPs). In plants, PLCPs are associated with regulated cell death, also called programmed cell death (PCD) under physiological conditions (such as plant development). A subfamily of PLCPs from the model plant *Arabidopsis thaliana*, RD21, are particularly important as enzymes involved in PCD, as they initiate PCD and mediate the plant-pathogen immune response. Cysteine endopeptidase CEP2 from *C. reinhardtii* shares many similarities with members of the RD21B group of enzymes, which leads us to believe it may have a similar role in plant cell death as RD21B enzymes. Firstly, in order to more easily predict biochemical properties of CEP2, we performed a bioinformatic analysis of the protease. Based on its amino acid sequence and the use of an internet tool we prepared a model tertiary structure of the protein. We also compared the sequences of CEP2 and RD21B, respectively, and used that knowledge to predict the role and position of specific parts of the sequence and the domains in the polypeptide chain. Our laboratory work began with the preparation of the recombinant protein CEP2 in *Escherichia coli* strain Rosetta-gami 2. This was followed by protein isolation and subsequent analysis of the protein's activation in the presence of a mildly acidic buffer. We noticed the protein gets processed during expression and isolation and that it is similarly active with and without incubation in a buffer with a lower pH value. Subsequently, we determined the protein's activity pH profile which helped us establish the pH value for the protein's optimal activity which is slightly acidic, at 5.0. Lastly, based on results from an experiment during which the protein's autocatalytic activity was inhibited, we predict that the activation happens by means of another protease rather than by autoprocesing. The identity of said protease, which is present in the *E. coli* lysate, remains to be elucidated.

Diversity and Content of Carotenoids and Other Pigments in the Transition from the Green to the Red Stage of *Haematococcus pluvialis* Microalgae Identified by HPLC-DAD and LC-QTOF

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Haematococcus pluvialis is a unicellular freshwater alga containing many bioactive compounds, especially carotenoids, which are the strongest antioxidants among the pigments. This study evaluates the composition and content of carotenoids and other pigments in both stages of algae life cycle, especially in the green vegetative stage, less studied in comparison to the red stage. To determine the composition and content of carotenoids, a combination of HPLC-DAD and LC-QTOF-MS was used. The content of carotenoids in the green vegetative stage was significantly lower than in the red stage. In the green vegetative stage, 16 different carotenoids and other pigments were identified. Among the total 8.86 mg g⁻¹ DW of pigments, 5.24 mg g⁻¹ DW or 59% of them were chlorophyll a with its derivatives, and 3.62 mg g⁻¹ DW or 41% of them were free carotenoids. After the transition from the green to the red stage, the carotenoid composition was replaced by secondary carotenoids, astaxanthin and its esters, which predominated in the whole carotenoid composition. In addition to free astaxanthin, 12 astaxanthin monoesters, 6 diesters and 13 other carotenoids were determined. The majority of 37.86 mg g⁻¹ DW pigments were monoesters. They represented 82% of all pigments, and their content was about 5 times higher than both, diesters (5.91 mg g⁻¹ DW or 12% of all) and free carotenoids (2.4 mg g⁻¹ DW or 6% of all). The results of the study contribute to the data on the overall pigment composition and content of *H. pluvialis* algae and provide the basis for further improvement of cultivation of the *H. pluvialis* alga.

Key words: photobioreactor, microalgal culture, *Haematococcus pluvialis*, carotenoids, astaxanthin

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***Gentiana clusii* Perr.&Song. cultured in vitro - secondary metabolite profile and evaluation of antioxidant activity**

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Gentiana clusii Perr. & Song. (Gentianaceae), is a rare plant species found in the Alps, the Pyrenees, the Black Forest Mountains and the Carpathians. *G. clusii* is endemic for Europe which implies the need for its conservation. *G. clusii* plants were collected at fruitful stage in June 2015 at Velika Planina Mountain, Republic of Slovenia. Seeds of *G. clusii* were germinated on MS basal medium. Shoots cultured on WPM + 0.5 mg/L KIN were used as starting material in propagation and rooting experiments. Medium with IBA at 0.5 mg/L was chosen as optimal for rooting. HPLC screening of secondary metabolites of wild plants collected in nature and in vitro plants revealed the presence of two main classes of compounds typical for gentians – secoiridoids and xanthones. Secoiridoids gentiopicrin and sweroside were identified in both aerial parts of wild plants and shoots of *in vitro* plants, while root extracts contained amarogentin in addition. Aerial parts of wild plants contained gentiacaulein-1-*O*-primeveroside as dominant xanthone glycoside. HPLC also revealed the presence of gentiakochianin-1-*O*-primeveroside, norswertianin-1-*O*-primeveroside, 1,8- dihydroxy-3-methoxy-7-*O*-primeverosyl xanthone, and xanthone aglycons-norswertianin, gentiacaulein, and gentiakochianin. HPLC analysis shown that *in vitro* culture considerably promoted the production of secondary metabolites in *G. clusii*. The selected protocol for shoot propagation (WPM + 0.5 mg/L KIN) increased the content of sweroside, gentiopicrin and norswertianin-1-*O*-primeveroside for more than 2-fold compared with the wild plants. IBA promoted norswertianin-1-*O*-primeveroside and norswertianin production in root cultures; their contents were enhanced 6.4- and 18.6-fold, respectively, compared with the wild plants. The extract of these roots displayed high antioxidant capacity. The established shoot and root propagation protocols facilitate *in vitro* conservation of *G. clusii*, and provides a promising tool for the large scale production of valuable secoiridoids and xanthones.

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Krstić D., Janković T., Aljančić I., Šavikin-Fodulović K., Menković N., Milosavljević S. (2004) Phytochemical investigation of *Gentiana dinarica*. *Biochemical Systematics and Ecology*, doi: 10.1016/j.bse.2004.03.

Molecular characterization of *Chlamydomonas metacaspases* and their detection by activity-based protein profiling

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Regulated cell death (RCD) is an important mechanism that enables the careful elimination of unwanted cells in various organisms. Metacaspases (MCAs), the structural homologs of animal caspases, were one of the first protease families to be associated with RCD in non-metazoan organisms. Despite their structural similarity to caspases, metacaspases have been found to have distinct catalytic properties and functions. In plants, MCAs are associated with various responses to biotic and abiotic stresses, but understanding of their precise roles in these processes is still limited. To shed light on the role and molecular mechanisms of these proteases, we studied MCAs from the green alga *Chlamydomonas reinhardtii*. It is a representative of the early-diverged Viridiplantae, but unlike higher plants, it encodes only one member of each of the two metacaspase types found in algae (CrMCA-I and CrMCA-II), which facilitates their molecular and functional characterization. Using recombinantly expressed CrMCAs, we were able to biochemically characterize the two metacaspases and investigate their regulation with calcium. By removing a highly hydrophobic loop within the p10 domain of CrMCA-I, we recently became the first to recombinantly produce and characterize a type I metacaspase of the green lineage. We also validated metacaspase-specific activity-based probes (ABPs) that allowed us to label and identify active isoforms of both metacaspases *in vitro*. To investigate the role of metacaspases in oxidative stress, we exposed both wild-type and mutant strains lacking the genes for CrMCA-I or CrMCA-II to hydrogen peroxide and performed activity-based protein profiling of cell extracts. Using this approach, we were able to gain insight into the involvement of cysteine proteases in oxidative stress in *C. reinhardtii*.

van Midden K.P., Peric T., Klemenčič M. (2021). Plant type I metacaspases are proteolytically active proteases despite their hydrophobic nature. FEBS Letters. doi: 10.1002/1873-3468.14165.

Crown reduction alleviates drought stress in sessile oak and Norway spruce seedlings

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Seedling stage represents the most vulnerable phase in a life of a tree. When planting a forest, the newly planted tree seedlings are exposed to sudden changes in conditions, often including a lack of shading and drought stress. Drought stress frequency and intensity are, however, increasing due to climate change and are expected to further rise in the future. As a consequence, we can expect higher tree seedling mortality shortly after the planting. To explore the possibilities to improve seedling performance under drought stress, we compared the effect of two drought stress-protective treatments on seedlings of two tree species. Potted seedlings of sessile oak and Norway spruce were treated with hydrogel amended in the substrate, crown reduction, or their combination. Half of the seedlings were subsequently exposed to long-term drought stress maintained by reduced irrigation. The isohydric Norway spruce seedlings showed a higher stomatal conductance (g_s) and transpiration rate (E) in the wet treatment than anisohydric sessile oak. The drought-treated spruce seedlings experienced a reduction both in g_s and E while only minor differences caused by drought were recorded for oak. Similarly, the photosynthetic rate of spruce seedlings was more affected by drought treatment than in oak seedlings. While hydrogel amendment did not prove to be a sufficiently effective action against drought stress, crown reduction led to increased soil humidity and thus postponed the drought impacts. The drought-treated seedlings with reduced crown had higher both g_s and photosynthetic rate than the corresponding treatment without crown reduction. This also indicates that seedlings with reduced crown are less prone to carbon starvation and should have a better survival rate under the drought stress. Crown reduction therefore might be an effective tool to alleviate the drought stress after seedling planting by postponing the soil drying and thus providing more time for plant establishment in a new habitat.

Imaging of element distribution in whole leaves of hyperaccumulating *Noccaea* sp. from Slovenia using table-top micro-X-ray fluorescence

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Micro-X-ray fluorescence (μ XRF) imaging provides 2D distribution of several biologically relevant and potentially toxic elements in hydrated or fresh organs and tissues of plants, including hyperaccumulating species (Donner et al., 2013). These are plants that can accumulate extraordinarily high concentrations of one or more metal(loid)s in their above-ground biomass without apparent toxicity symptoms (Brooks et al., 1977). In spring 2022, two different hyperaccumulating species, namely *Noccaea caerulescens* and *Noccaea praecox*, were sampled at two sites in Slovenia: a non-polluted site (Lokovec) and a polluted site (Žerjav), respectively. The distribution of phosphorus, sulfur, chlorine, potassium, calcium, manganese, iron, zinc, and, when detectable, lead was determined using a 15 μ m beam in cryo-fixed and freeze-dried mature leaves using table-top μ XRF (Horiba XGT-9000, Japan). Distribution maps revealed preferential allocation of zinc to vascular bundles in both species, while in *N. caerulescens*, zinc was also allocated to the upper part of leaves and leaf edges. In the leaf of *N. praecox*, lead co-localized with zinc and calcium in the vascular bundles, with more lead found at the bottom part than in the upper part of the leaf, in line with reports for *Arabidopsis halleri*, another hyperaccumulating species (Höreth et al., 2020). Other elements were more evenly distributed across the whole leaf in both species.

Brooks R.R., Lee J., Reeves R.D., and Jaffre T. (1977). Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of geochemical exploration*, doi: 10.1016/0375-6742(77)90074-7.

Donner E., de Jonge M.D., Kopittke P.M., and Lombi E. (2013). Mapping element distributions in plant tissues using synchrotron X-ray fluorescence techniques. In: Maathuis F. (eds) *Plant Mineral Nutrients. Methods in Molecular Biology*, vol 953. Humana Press, Totowa, NJ, doi: 10.1007/978-1-62703-152-3_9.

Höreth S., Pongrac P., van Elteren J.T., Debeljak M., Vogel-Mikuš K., Weber M., Braun M., Pietzenuk B., Pečovnik M., Vavpetič P., Pelicon P., Arčon I., Krämer U., and Clemens S. (2020). *Arabidopsis halleri* shows hyperbioindicator behaviour for Pb and leaf Pb accumulation spatially separated from Zn. *New Phytologist*, doi: 10.1111/nph.16373.

Metabolomic evaluation of five *Nepeta* species (fam. Lamiaceae) using liquid chromatography with multistage mass spectrometry

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The aim of this study was to determine qualitative composition of methanol-soluble metabolites in leaves from *in vitro*-grown plants of phylogenetically distinct *Nepeta* species producing iridoids (*N. sibirica* L., *N. spicata* Benth., *N. argolica* Bory & Chaub., and *N. rtanjensis* Diklić & Milojević) and iridoid-lacking *N. nervosa* Royle ex Benth., adopting UHPLC-LTQ OrbiTrap MS technique. Totally, 52 compounds were identified based on their monoisotopic masses, MSⁿ fragmentation, and previously reported MS data and they were clustered into four major groups: phenolic acid derivatives (23 compounds), flavonoids (10 compounds), iridoids (13 compounds), and 6 compounds belonging to other classes. Both phenolic and iridoid profiles of the tested species were found to be very diverse regarding both qualitative content of compounds and their relative representation in extracts. Rosmarinic, 5-*O*-cafeoylquinic, and caffeic acids were found to be the dominant phenolics in all analyzed species, especially in *N. argolica*, *N. rtanjensis*, and *N. nervosa*. Iridoids were present in the form of glucosides and the most abundant compounds from this group were epideoxyloganic acid, geniposide, and aucubin. Although iridoids were not detected in *N. nervosa* extracts, this species was revealed as a rich source of phenolic acids and flavonoids. Conversely, *N. spicata* was found to produce iridoids but negligible amounts of phenolic compounds. Extracts of two closely related endemic species originating from the Balkan Peninsula, *N. argolica* and *N. rtanjensis*, were very similar in both phenolic and iridoid profiles. The UHPLC-LTQ OrbiTrap MS method, in combination with chemometrics data processing, was proved to be simple, rapid, and accurate strategy for unambiguous taxonomic determination and chemical differentiation of *Nepeta* species.

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Biosensor of jasmonic acid signaling pathway reveals spatiotemporal patterns of plant's immune response in potato (*Solanum tuberosum* L.)

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The spatiotemporal dimensions of plant's response to stress determine plant's ability to withstand it. The main three hormones that are involved in the spread of the immune response are salicylic acid, ethylene and jasmonic acid (JA). We could follow them with biosensors such as transcriptional reporters, which are based on a promoter sequence that contains analyte-responding cis-regulatory elements and drives transcription of the reporter gene. We developed a transcriptional reporter of JA signaling pathway in potato. Transcriptomic analysis led to the selection of JA-responsive gene. Its promoter region was amplified from genomic DNA of potato genotypes Rywal, Désirée and PW363 according to homologue gene promoter in the genome of doubled monoploid potato clone of *S. tuberosum* Group Phureja. Interestingly, all of the amplified promoter sequences from Rywal and Désirée were identical. This and one more variant amplified from PW363 were fused with yellow fluorescent protein coding sequence, transiently expressed in *Nicotiana benthamiana* and treated with volatile derivative of JA (MeJA). The first variant showed higher activity and was therefore selected for the production of biosensoric stable transformants of potato. Stable transformants were confirmed to be MeJA-responsive. Further on, we followed the onset of the response to wounding and Colorado potato beetle (CPB) attack on the levels of cells and tissue. The sensor revealed that JA-dependent response at the site of the wounds occurred in a spatially restricted pattern. Besides, sensor's response begun at different times depending on the distance from the wound. Moreover, the sensor disclosed how CPB's two different feeding behaviours, probing and biting, evoked different spatiotemporal responses in potato leaf tissue. Response to probing resembled wounding while biting caused considerably weaker and delayed response. Our work now continues with ascertainment of the expression of other stress-responsive genes at the identified interesting regions and times.

Structural diversity and bioactivity of biflavonoids from ginkgo (*Ginkgo biloba* L.), a "living fossil" plant with exceptional adaptability to environmental changes

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The ginkgo (*Ginkgo biloba* L.) is the only living dioecious species in the Ginkgoaceae family. It is characterized by a large genome and high stress tolerance, including abiotic and biotic stresses and dioecious reproduction. Ginkgo trees, often more than 200 years old, are found as ornamental plants in many European cities. The leaves and fruits have been used in medicine for several centuries and the best known beneficial effect is on cognitive ailments. Its good stress tolerance and medicinal use may be related to the presence of various phytochemicals, as biosynthesis and diversity of specific metabolites are crucial for survival in a stressful environment. The biologically active compounds in ginkgo are from the group of flavonoids, terpenoids, alkylphenols and alkylphenolic acids, carboxylic acids, lignans, proanthocyanidins, polyphenols, and polysaccharides. To date, more than one hundred flavonoids with unique structures have been identified. In a GinkoBiFlav project (HRZZ-UIP -2019-04-1018), we focus on biflavonoids in ginkgo. Biflavonoids are flavonoid dimers consisting of two identical or non-identical flavonoid units linked in a symmetrical or asymmetrical manner. Although they have biological activity, their role in plants is unknown. Recently, we have summarized literature data on the diversity, presence, and biological activity of biflavonoids in ginkgo, which we will present. We will also discuss other research directions that could elucidate the role of biflavonoids in ginkgo, possibly related to adaptation to a changing environment.

Šamec D., Karalija E., Dahija S., Hassan S.T.S. (2022). Biflavonoids: Important Contributions to the Health Benefits of Ginkgo (*Ginkgo biloba* L.). *Plants*, 11(10):1381. <https://doi.org/10.3390/plants11101381>

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TGACG-binding (TGA) transcription factors in potato (*Solanum tuberosum* L.)

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Plants respond to pathogen infections with a complex network of signaling cascades, which are orchestrated by phytohormones and ultimately affect the expression of defense-related genes. One of the most important hormonal pathways in plant immunity is the salicylic acid pathway, which has been extensively studied in *Arabidopsis thaliana*, where it regulates the expression of *PR-1* defense gene via NPR transcription cofactors and TGACG-binding (TGA) transcription factors. Although this pathway has also been studied in some economically important crops, little is known about TGA-mediated transcriptional regulation in potato (*Solanum tuberosum* L.), one of the most widely produced crops around the world. Through phylogenetic analysis of potato TGAs (StTGAs), we selected candidates likely involved in the potato defense response and analyzed their protein sequences *in silico*. We identified a TGA protein (StTGA2.1) with an unusually compact molecular architecture that lacks the complete DNA-binding domain. We show that overexpression of *StTGA2.1* can compensate for salicylic acid deficiency in potato immune response to potato virus Y infection. Furthermore, we show that StTGA2.1 associates with a full-length TGA, StTGA2.3, to modulate the expression of a class III peroxidase. Using structural analyses and molecular dynamics simulations, we provide insights into the structural characteristics of StTGA2.1-StTGA2.3 heterodimer binding to target DNA. Overall, we provide new insights into the function of TGA transcription factors in potato.

Role of aquaporins for water and nutrients balance in cork oak and grapevine plants

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Aquaporins are water channels, belonging to Major Intrinsic Protein (MIP) family. They play crucial roles under various stresses and can transport many other small solutes besides water. Plant aquaporins are divided in 4 subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin26-like intrinsic proteins (NIPs) and small intrinsic proteins (SIPs). Cork oak and grapevine are two highly important economic plants of Portugal by providing source for cork and wine. However, climatic factors such as drought stress significantly affect the sustainability of these ecosystems. Therefore, it is essential to understand their molecular mechanism of water and nutrient transport. Full genomic sequences of both species identified the presence of AQP, however their putative roles are difficult to understand due to their varied temporal and spatial expressions. Our aim to characterize PIPs, TIPs and NIPs of cork oak and grapevine was achieved by their expression in *aqy-null S. cerevisiae*. Water and glycerol transport in the yeast strains were assayed through stopped-flow-spectroscopy. Results showed that PIP2s and TIP2s of both plants are functional for water transport, while NIP6;1 and NIP1s of both plants enhanced the glycerol transport. pH regulation of these aquaporins was also determined. Putative transport of atypical substrates was also assessed by the yeast growth assays.

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Tracking ecosystem carbon phenology of a karst secondary forest using permanently mounted phenocam

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Remote sensing offers a valuable resource for monitoring plant phenology. It also enables us to observe how changes in spectral reflectance oscillate with plant photosynthesis and more holistically, ecosystem carbon exchange (i.e., carbon phenology). There are increasing efforts to link vegetation reflectance and carbon dynamics at the ecosystem-scale across EC study sites and couple this information with local, regional or global terrestrial carbon models. Apart from the satellite-derived estimates of C exchange phenology, which are prone to atmospheric effects and often lack temporal consistency, proximal sensing in high temporal resolution (<1 h) using RGB or RGB+NIR cameras (i.e. phenocams) mounted above or sideways from the investigated ecosystem can be used.

Here, three years of phenocam data were compared with eddy covariance-measured C exchange of a drought-prone secondary forest from the Karst region in SW Slovenia. The aim was to evaluate phenodata sensitivity to certain phenological thresholds such as vegetation greenup and browning or maximum yearly performance. Several vegetation indices (VIs) were calculated from phenocam dataset, either simple RGB VIs or VIs that include NIR channel.

On general, indices performed similarly in explaining the timings of critical phenological stages. We observed several days of overestimation of vegetation season length using phenocam indices with greater discrepancy for the timing of autumn browning compared to spring greenup. Leaves seemed to end their assimilation period prior to observe this on their spectral signature. Less asynchrony was found for maximal yearly ecosystem performance particularly when using NIR-included VIs. Nevertheless, the results showed that phenocams offer improvements for studying carbon phenology at the ecosystem scale despite certain challenges.

Plant interactions with other organisms

Identifying chemicals that drive interactions between plants and microbes during microbiome assembly

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While plant rhizospheres are among the most diverse microbial communities on Earth, only a subset of these microorganisms successfully colonize internal root tissue. Although many plant and microbial factors contribute to this filtering effect, a mechanistic understanding of root microbiome establishment remains largely enigmatic. We use a range of experimental approaches across multiple plant hosts and bacterial isolates to identify broadly conserved mechanisms mediating interactions in plant roots. This approach led us to explore a role for plant-derived myo-inositol, which can be a sole carbon source by bacterial isolates with high root colonization. While plant myo-inositol synthesis and signaling was previously implicated in pathogen susceptibility studies, our findings suggest a more general role that host inositol stores play in impacting non-pathogenic microbial root colonization. We also explore how the phytohormone salicylic acid directly modulates internal root microbiomes through a serial passaging experiment over four generations of six *Arabidopsis thaliana* genotypes that vary in salicylic acid production and signaling ability. Diversity of bacterial communities decreased over the four passages, presumably enriching for specific taxa that are either sensitive or resistant to salicylic acid and/or its downstream plant responses. Together, these results highlight two selective mechanisms that contribute to internal root microbiome assembly.

Study of spatial responses in combination with network analysis reveals mechanisms of potato stress signalling

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Interaction of a plant with another organism initiates complex changes in the signalling network, which result in gene activity changes and reprogramming of the cell metabolism. A systems biology approach was adopted to understand the mechanisms and dynamics involved in potato stress response. We collected multi-layered omics datasets with spatiotemporal resolution. A qualitative model of potato plant immune signalling network was constructed describing the biosynthesis and signal transduction pathways for all major phytohormones and linked to tuberisation signalling. The prior knowledge from literature was expanded with information on the viral and plant component interactions, protein-protein interactions and protein-DNA interactions in plant *Arabidopsis* and translated to potato. Additionally, potato smallRNA regulatory network and gene regulatory networks were constructed from potato experimental data and overlaid with prior knowledge network. The resulting robust qualitative model offers new insights into the plant-virus interaction by expanding the knowledge on critical properties of plant defence signalling, thus producing novel hypotheses to be tested in the wet lab. Examples of two tested and confirmed hypothesis will be given.

Assembly of potato endophytes using Nanopore and Illumina sequencing

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Endophytes encompass microbes, which for all or part of their lifetime colonize the internal of plant tissues. They can be commensals with no apparent effect on their host, beneficial plant growth-promoting microbes or also latent pathogens. Beneficial endophytes can directly promote plant growth through nitrogen fixation, synthesis of enzymes or peptides that provide nutrients, or by the production of phytohormones. On the other hand, they can protect the plant either directly, by antibiosis or competition for nutrients with the pathogens, or indirectly, by priming the plant's immune response.

We isolated three microbes, two bacteria and one fungus, from tissue culture cultivated potato plants propagated in a sterile environment for several years without any visible signs of infection. Thus, it is reasonable to assume that they are endophytes tightly associated with potato plants. To precisely determine their phylogeny and get insight into their potential functions, we sequenced their genomes by a combination of Nanopore and Illumina sequencing. For bacteria, most contiguous genomes were obtained using tricycler (Wick et al., 2021), by combining the output of several long-read assemblers followed by Illumina reads polishing. For the fungus, a hybrid assembly using WENGAN (Di Genova et al., 2021) produced the highest quality genome. Taxonomic analysis suggests that one of the isolates belongs to a novel species. We will present its gene repertoire, comparison to phylogenetically most similar bacteria and biochemical determination of some metabolic functions. Our assembled genomes facilitate hypothesis-driven functional characterisation of the isolated endophytes and the understanding of their interactions with the host plant.

Wick R.R., Judd L.M., Cerdeira L.T. et al. Tricycler: consensus long-read assemblies for bacterial genomes. (2021). *Genome Biology*, doi: [10.1186/s13059-021-02483-z](https://doi.org/10.1186/s13059-021-02483-z).

Di Genova A., Buena-Atienza E., Ossowski S. et al. (2021). Efficient hybrid de novo assembly of human genomes with WENGAN. *Nature Biotechnology*, doi: [10.1038/s41587-020-00747-w](https://doi.org/10.1038/s41587-020-00747-w).

Epidemiology and diversity of 16SrV phytoplasma group infecting grapevine and hazelnut

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Phytoplasmas are plant pathogenic bacteria that cause severe economic losses of crops worldwide. In the EU, the quarantine Grapevine flavescence dorée phytoplasma (FD) of the 16SrV group causes many problems in vineyards. In Slovenia, numerous new outbreaks occurred in 2021, and several vineyards had to be destroyed. The American leafhopper (*Scaphoideus titanus*) efficiently transmits epidemic strains of FD in vineyards (1). However, grapevines can also be infected with non-epidemic isolates of 16SrV phytoplasma transmitted from alder plant hosts by other sap-sucking insects (2). In addition to grapevine, 16SrV phytoplasma was confirmed to cause disease in hazelnut orchards in Slovenia (3). Large populations of the leafhopper *Orientus ishidae* were observed in infected hazelnut orchards. Infestation with 16SrV phytoplasma was detected in 17% of the 64 insect samples examined, suggesting that the leafhopper could be involved as a vector of the phytoplasma. To characterize the genetic diversity of 16SrV phytoplasma in grapevine, hazelnut, and insect vectors, we performed nucleotide sequence analysis of the *map* gene. The most common genotype present in grapevine in all Slovenian wine-growing regions was M54 (84% of 176 tested), which is known to cause epidemics in France and Italy (2). Other determined genotypes that can be transmitted by *S. titanus* and cause outbreaks were M38 (6%), M51 (3%), M50 (2%), and M122 (1%). Three of these epidemic genotypes also occurred in hazelnut (31 analysed shrubs): M38 (42%), M50 (26%), and M122 (6%), which were also detected in *O. ishidae*. Non-epidemic strains of grapevine were identified in only 6% of 16SrV-positive hazelnut samples. In a few samples of grapevine and hazelnut, we detected *map* genotypes that had not been described previously. They are currently being further genetically characterized in order to determine their possible origin and their epidemic potential on hazelnut and grapevine. Knowledge of genetic diversity and epidemiological routes of spread in hazelnut orchards will help us efficiently plan further crop protection strategies.

(1) Martini, M. *et al.*, (2002). Mol. Cell. Probes. 16/3: 197–208.

(2) Malembic-Maher, S. *et al.*, (2020). PLOS Pathog. 16/3: 1–28.

(3) Mehle, N. *et al.*, (2019). Eur. J. Plant. Pathol. 155/1117–1132

The role of small RNA regulatory networks in tolerance and resistance responses of potato to potato virus Y infection

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In recent decades, small RNAs (sRNAs) in plants have been shown to play an important role in fine-tuning defense responses against various pathogens, including viruses. The objective of our study was to identify the key sRNA components that control the establishment of potato resistance to potato virus Y (PVY) in tolerance and hypersensitivity response (HR). Using high-throughput sequencing technology, we compared the expression of sRNAs among tolerant, resistant, and susceptible potato genotypes. This information was linked to expression profiles of their target transcripts and used to construct sRNA regulatory networks. We discovered an interesting new sRNA-gibberellin regulatory circuit that is activated only in tolerant plants. We also discovered that sRNA regulation in tolerant interactions resembles that required for the establishment of mutualistic symbiosis. We used a spatially resolved approach to gain detailed insights into the spatial distribution of sRNAs in HR. More than 30 sRNAs were found to exhibit different gradients around the site of virus entry. Few sRNAs showed opposite expression patterns in response to PVY infection in tolerant and resistant plants, suggesting that some common modules of the sRNA regulatory network are activated but the behavior of its components is different. Selected sRNAs identified in this study are now being further functionally characterized to confirm their role in the HR response of potato to PVY.

The ethylene response factor StERF49 negatively regulates potato immunity

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Potato is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogens causing severe crop losses worldwide. It is thus important to improve our understanding of potato-PVY interaction for more efficient crop breeding strategies. The analysis of previously published transcriptomics data (Baebler *et al.*, 2014) suggested that the ethylene response factor StERF49 is an important player in Ny-mediated hypersensitive response to PVY, therefore we further investigated the role of this transcription factor (TF) in potato immune response. The use of stable transgenic potato plants with *StERF49* silenced revealed that the gene has a positive role in local viral accumulation and symptom development. We additionally confirmed this is not a specific response to the virus and we showed that StERF49 increases potato susceptibility to *Ralstonia solanacearum* as well. We performed RNAseq experiments on PVY infected and mock inoculated leaves of *StERF49*-knockdown potato plants. Several genes involved in the protein degradation pathway seem to be regulated by *StERF49* after PVY infection. On the other hand, the StERF49 is strongly accumulated only in nuclei and cytoplasm of PVY infected cells. Considering these results we hypothesize that PVY might interfere with the host mechanisms for its own benefit by preventing degradation of StERF49. We are further studying the complex network of plant signalling pathways involved in this response using Y1H and transactivation assays. We identified two ethylene responsive TFs that activate the expression of *StERF49* gene. Our results show the importance of StERF49 and its negative role in potato defence response. This work will contribute to a better understanding of the complex network of plant defence signalling pathways.

Baebler Š, Witek K, Petek M, Stare K, Tušek-Znidaric M, Pompe-Novak M, Renaut J, Szajko K, Strzelczyk-Zyta D, Marczewski W, *et al.* 2014. Salicylic acid is an indispensable component of the Ny-1 resistance-gene-mediated response against Potato virus Y infection in potato. *Journal of Experimental Botany* 65: 1095–1109.

Leaf sucrose controls grapevine susceptibility to Flavescence Dorée

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Flavescence dorée (FD) is an insect-borne phytoplasma disease severely affecting vineyards of most European grape-growing areas (1). Affected plants cannot be cured and must be uprooted in order to limit spread of the pathogen. FD infection affects carbohydrate status and metabolism in grapevine leaves (2). Occasionally natural recovery of FD symptoms and disappearance of phytoplasma is observed, and most susceptible genotypes are more likely to undergo recovery. Understanding the biological bases of this process could provide a curative approach to management of the disease (3). We compared leaf soluble sugar content in grapevine genotypes with different susceptibility to FD and found a positive relationship between sucrose levels and tolerance to disease. Sucrose concentration was higher in FD-affected than healthy plants, and the activity of sucrose-cleaving enzymes was repressed at high infection levels. In order to provide a causal evidence of the role of sugars in tolerance, we attempted to increase sucrose content in infected plants to prove a role in recovery from FD. Shoot girdling successfully increased leaf sucrose content, and we correspondently observed a more sustained recovery rate in plants where sucrose leaf content was higher. Girdling also activated expression of trehalose-6P biosynthetic genes. Taken together, these results show that a signal originated by elevated sucrose and possibly transduced through trehalose-6P contributes to inducing recovery from FD in grapevine.

- (1) Roggia C, Caciagli, L, Galetto, D., Pacifico F, Veratti F, Bosco D, Marzachi C (2013) Flavescence dorée phytoplasma titre in field-infected Barbera and Nebbiolo grapevines. *Plant Pathol.* 63:31-41 <https://doi.org/10.1111/ppa.12068>
- (2) Prezelj, N., Covington, E., Roitsch, T., Gruden, K., Fragner, L., Weckwerth, W., et al. (2016). Metabolic consequences of infection of grapevine (*Vitis vinifera* L.) cv."Modra frankinja" with Flavescence Dorée phytoplasma. *Front. Plant Sci.* 7, 711 <https://doi.org/10.3389/fpls.2016.00711>
- (3) Pagliarani C, Gambino G, Ferrandino A, Chitarra W, Vrhovsek U, Cantù D, Palmano S, Marzachi C, Schubert A (2020) Molecular memory of Flavescence dorée phytoplasma in recovering grapevines - *Horticultural Research* 7:126. <https://doi.org/10.1038/s41438-020-00348-3>

Allelopathy of invasive *Fallopia* plants induced oxidative stress in radish seedlings

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Allelopathy is a process in which the allelopathic plant releases secondary metabolites into the soil, that results in the growth inhibition of neighbouring plants. This allelopathic mechanism is also present in invasive plants, such as Japanese knotweed (*Fallopia japonica*) and Bohemian knotweed (*F. ×bohemica*). The aim of our study was to characterise the mechanism behind the allelopathic interactions, which includes molecular, cellular, biochemical, and morphological changes in the target plants. Radish (*Raphanus sativus*) was selected as the test plant. After 3 days of exposure to the aqueous extracts of knotweed rhizomes, the roots of radish seedlings were prepared for transmission and scanning electron microscopy, spectrophotometric analysis, high pressure liquid chromatography, and gene expression (RNA-Seq) analysis.

Our results showed that knotweed extracts affected the root tip surface, especially the cells of the root cap, which exhibited swollen cell walls. At the ultrastructural level, it was evident that the plasma membrane detached from the cell wall, the amount of endoplasmic reticulum bodies increased, and numerous electron-dense inclusions were observed in the cytoplasm of root cap cells. Damage to mitochondria was also observed. The progression of oxidative stress was evident from the increase in proline and the change in several non-enzymatic antioxidants: the concentration of total glutathione and total cysteine increased, while total ascorbate decreased in the treated radish roots. Knotweed extracts also induced upregulation of several genes associated with the regulation of the glutathione-ascorbate cycle, including glutathione peroxidase (*GPX2*, *GPX6*, *GPX7*, *GPX8*), ascorbate peroxidase (*APX3*) and dehydroascorbate reductase (*DHAR*). All of these changes contributed to the root length of the treated radish being reduced by more than 60% and may also lead to programmed cell death, as several genes related to protein degradation were upregulated in radish seedlings treated with knotweed extracts.

Šoln K., Žnidaršič N., Dolenc Koce J. (2021). Root growth inhibition and ultrastructural changes in radish root tips after treatment with aqueous extracts of *Fallopia japonica* and *F. ×bohemica* rhizomes. *Protoplasma*, doi: 10.1007/s00709-021-01668-4.

Šoln K., Likar M., Dolenc Koce J. (2021). Effects of rhizome extracts from invasive knotweed species *Fallopia japonica* and *F. ×bohemica* on radish seed germination and root growth of seedlings. *Allelopathy Journal* 52, 1: 103–117.

Influence of grapevine leafroll-associated virus 3 on physiological indicators in grapevine

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Grapevine leafroll disease (GLD) is one of the most significant grapevine viral diseases, and grapevine leafroll-associated virus 3 (GLRaV-3) is one of the main causes of this disease. The aim of this work was to investigate the effect of GLRaV-3 strains (genetic variants) on physiological parameters on three standard red cultivars Merlot, Cabernet Franc and Pinot Noir and an indigenous Croatian wine grape variety Tribidrag. Total soluble proteins, superoxide dismutase (SOD) activity, hydrogen peroxide, photosynthetic pigments and plant growth were evaluated following 6 and 18 months of inoculation with individual GLRaV-3 strains. The results showed different degree of sensitivity of the tested cultivars to the infection caused by GLRaV-3 strains. After 6 months, we determined that Tribidrag was the most susceptible to infection with GLRaV-3 compared to other cultivars (especially compared to Cabernet Franc and Pinot Noir). Longer infection (18 months) with GLRaV-3 strains singled out Merlot as the most sensitive grape variety as evidenced by decreased growth and photosynthetic pigments. Considering the relative scarcity of this type of research, findings obtained in this study can be used as a guideline for future studies of the impact of virus infections on grapevine.

Hančević K., Čarija M., Radić T., Černi S., Radić Brkanac S., Gaši E., Mucalo A., Likar M., Zdunić G., Regvar M., Saldarelli P. (2022). Coexistence of grapevine plants and grapevine leafroll-associated virus 3 in Croatia. Proceedings of 57th Croatian & 17th International Symposium on Agriculture, 19 – 24 June 2022, Vodice, Croatia, (plenary, international peer review, full paper), in press.

Song, Y., Hanner RH., Meng B. (2021). Probing into the Effects of Grapevine Leafroll-Associated Viruses on the Physiology, Fruit Quality and Gene Expression of Grapes. *Viruses*, doi: 10.3390/v13040593.

Nutritional Effects of old Slovenian and Hungarian Mulberry Varieties on Silkworm's growth and silk quality

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Former sericultural regions of Europe retained a number of centuries-old white mulberry (*Morus alba* L.) trees, which represent both, a valuable natural heritage and outstanding monuments of the very early attempts of sericultural activities. The aim of the presented research was to collect data on the eco-geographical locations of historical mulberry trees in Slovenia and Hungary and to screen the content of the main metabolites in the leaves in order to determine genotypes of local origin that are superior in terms of individual compounds defined as feed markers for silkworm larvae (*Bombyx mori* L.). Local mulberry genotypes maintained in the mulberry gene bank were chosen based on previous determinations of primary and secondary metabolites in leaves sampled at the tree's place of origin (Urbanek Krajnc et al., 2019; Šelih et al., 2020). Mulberry leaves are rich in proteins and phenolics. The main phenolic compounds in mulberry leaves were identified as caffeoylquinic acid derivatives with predominant chlorogenic acid. The flavonol fraction contained rutin, quercetin malonyl-hexoside, isoquercetin, quercetin acetyl-hexoside and quercetin dirhamnosyl-hexoside, while the predominant kaempferol glycoside was kaempferol acetyl-hexoside. The Slovenian and Hungarian mulberry genotypes had significantly higher total protein contents, lower total phenolic contents and differed significantly in some individual phenolics compared to the reference sericultural and fruit varieties. Significant differences were found in the contents of the macro- and microelements, namely S, Mn, Fe and Sr. Based on correlative statistics and multivariate analysis, we can conclude that proteins, some specific phenolics and elements have a positive influence on larval growth, cocoon size and silk thread parameters. The results of the study indicate that the local mulberry varieties are suitable for the production of high-quality silk cocoons and raw silk.

Šelih M., Mikulič Petkovšek M., Krajnc D., Berčič R. B., Urbanek Krajnc A. (2020). Screening of leaf metabolites in historical mulberry trees (*Morus alba* L.) from different eco-geographical regions of Slovenia. *Trees*, <https://doi.org/10.1007/s00468-020-01974-z>

Urbanek Krajnc A., Ugulin T., Paušič A., Rabensteiner J., Bukovac V., Mikulič Petkovšek M., Janžekovič F., Bakonyi T., Berčič R. L., Felicijan M. (2019). Morphometric and Biochemical Screening of Old Mulberry Trees (*Morus alba* L.) in the Former Sericulture Region of Slovenia. *Acta Societatis Botanicorum Poloniae*, DOI: 10.5586/asbp.3614.

Localisation studies of the multifunctional protein HCPro from potato virus Y in epidermal leaf cells of *Nicotiana clevelandii* using confocal microscopy

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Potato virus Y (PVY) is the most devastating potato pathogen worldwide (Kreuze et al., 2020). The virus belongs to the genus *Potyvirus* and form flexuous filamentous particles containing a single-stranded RNA. The PVY genome encodes for a polyprotein that is cut into 10 mature proteins. In addition, another protein is produced from an overlapping coding sequence (Chung et al., 2008). Most of the viral proteins are multifunctional; they are involved in different stages of the viral cycle, establishing a complex and dynamic interaction network between viral and plant proteins (Rodrigo et al., 2017). In order to develop sustainable and efficient plant protection strategies it is important to understand the role of different viral proteins in the PVY infection cycle. To that end, here we focus on localisation of the HCPro protein using a PVY clone. We prepared the clones with the viral protein fluorescently-tagged and followed its localisation over time in bombarded epidermal leaf cells of *Nicotiana clevelandii* using confocal microscopy. We observed an even distribution of fluorescence throughout the cytoplasm and also in endoplasmic reticulum (ER) and/or cytoskeleton associated lines. The localisation of HCPro seemed to change over time. To confirm the subcellular distribution, we transiently transformed *N. clevelandii* infected with the fluorescently-tagged clone with different organelle markers. We confirmed HCPro localisation within the cytoplasm and as dot-like inclusions seemingly associated to the ER. These preliminary results will help, to not only decipher the role of HCPro in the viral cycle, but also to better understand the complex mechanism of the PVY infection cycle.

Chung B.Y-W., Miller W.A., Atkins J.F., Firth A.E. (2008) An overlapping essential gene in the Potyviridae. Proc. Natl. Acad. Sci. U.S.A. 105, 5897–5902

Kreuze J.F., Jeevalatha A., Figueira A.R. (2020) Viral Diseases in Potato. Potato Crop Its Agric Nutr Soc Contrib to Humankind doi: 10.1007/978-3-030-28683-5

Rodrigo G., Daròs J.A., Elena S.F. (2017) Virus-host interactome: Putting the accent on how it changes. J Proteomics 156: 1–4

Natural ecosystems in changing environment

Evolutionary potential in natural populations of forest trees

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Long-term local survival of natural populations of forest trees relies on standing genetic variation and their capacity to adapt to changing environments. Selection gradients were studied in a natural population of European beech (*Fagus sylvatica*), which is a subject of genetic monitoring. Adaptive traits related to growth, reproduction and phenology were assessed for all adult trees on a 1 ha plot. Effective reproductive success, a fitness proxy, was estimated via parentage analysis using the seedling extension of MEMM. Log-linear models were used to compute selection gradients correlating adaptive traits with fitness. Heritability was estimated using mixed models based on pedigrees reconstructed using nuSSRs. The study revealed skewed effective reproductive success, significant selection gradients and low to moderate, albeit significant, heritability for all traits. However, low phenotypic variance for phenological traits in combination with moderate selection gradients resulted in low responses to selection, which brings concerns on the general capacity of forest tree populations to adapt in-situ to fast environmental changes.

Phenotypic plasticity and genetic variation in forests trees: understanding drivers and developing applications for forest adaptation

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Human-driven climate change is expected to reverse a global multimillion year cooling trend and drive our climate back to the conditions of the Pliocene or even Eocene. This tremendous change affects forest ecosystems and trees as its major foundation by increasing both tree growth and tree mortality as well by facilitating large scale forest disturbances. Notably, climate change strongly disrupts the link between climate and the local adaptation of forests trees and thus challenges the future of forest tree populations and traditional forest management practices. Adaptive genetic variation within and among forest tree populations as well as phenotypic plasticity have been suggested as solutions to facilitate adaptation to the changing climate. Here, I provide an overview about present meta-analysis of provenance trials across Europe, which may provide the basis for assisted migration schemes across Europe. I demonstrate that guided seed transfer may help to increase forest resilience and sustain forest productivity and the forests role as carbon sink. Moreover, I aim to quantify the drivers of phenotypic plasticity in tree seedlings by testing for effects of weather conditions during pollination and seed maturation on the performance of seedlings from open-pollinated seed orchards and seed stands. The latter shows that climate conditions of the maturation year have significant effects on seedling growth, vitality and phenotypic plasticity. Finally, I discuss the necessity for a better understanding of the genetic and environmental drivers of phenotypic variation as precondition to improve forest adaptation to climate change.

Response of *Quercus pubescens* ectomycorrhizal fungi to abiotic changes in the Slovenian Sub-Mediterranean

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Abiotic stresses are the major environmental factors that adversely impact tree growth, forest productivity and determine the geographic distribution of tree species. Trees in highly variable climates, such as Sub-Mediterranean, have evolved several mechanisms for coping with abiotic stresses that may arise throughout the year. Ectomycorrhizal (ECM) fungi as root-associated symbionts which support tree nutrition and water supply are inevitable part of the tree response to abiotic stresses, but temporal studies that would offer insight into resilience of ectomycorrhizal ECM communities in stress prone climates are scarce. Our study investigated vitality, community composition, diversity, and function of *Quercus pubescens* Willd. ECM fungi in relation to environmental parameters at two limestone and one flysch plot in Sub-Mediterranean Podgorski Kras (Slovenia) for two years (June 2016-May 2018). This period was characterized by drier than average summers 2016 and 2017 and a summer wildfire in 2016. The ratio of vital to non-vital ECM root tips dropped below one in August 2016 and remained below one until January 2018 which could reflect shortage of carbohydrates for ECM fungi due to tree repair mechanisms following drought and wildfire. The most abundant ECM taxa on root tips were those belonging to *Tomentella* and other Thelephoraceae, *Sebacina* and *Cenococcum*. High intra-site variability was observed, with ECM communities varying significantly among the plots. On the other hand, community composition was stable over time. Species richness varied with mean air and soil temperature, relative air humidity, and solar radiation. Dominance was related to mean soil temperature. The most abundant exploration type observed at the site was short distance, which was associated with precipitation.

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Mrak T., Šibanc N., Brailey-Jones P., Štraus I., Gričar J., Kraigher H. (2021) Extramatrical Mycelium and Ectomycorrhizal Community Composition of *Quercus pubescens* in a Sub-Mediterranean Stress-Prone Environment. *Frontiers in Forests and Global Change*, doi: 10.3389/ffgc.2021.599946

Plasticity of wood and phloem formation in tree species from drought-prone environments

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Natural disturbances such as droughts and heat waves due to climate change affect wood and phloem formation and forest structure, especially in semi-arid environments where tree survival is threatened by increases in harsh conditions. Therefore, better knowledge of the growth responses to climatic factors of tree species growing in such environments is needed to develop appropriate strategies for sustainable management. Monitoring xylogenesis (i.e., wood formation) and phloem formation provides information on tree growth and physiological responses to variations in intra-annual climatic parameters. We have analysed wood and phloem characteristics of tree species growing in semi-arid environments to address questions related to tree performance and plasticity under changing environmental conditions. Here we would like to provide an overview of our recent research and the results obtained in several case studies. In the first case, we studied the cambial activity of mature trees of *Pinus pinea* from southern Italy and *Pinus halepensis* from southern Spain, focusing on phloem formation. The observed phloem structure was a consequence of the ability of the cambium to produce new phloem cells throughout the entire year. Production of new sieve cells and collapse of older ones was balanced to maintain a relatively constant width of uncollapsed phloem as a critical tissue for translocation of photosynthetic products. Variation in radial dimensions of sieve cells along radial rows provided useful information for tracking the dynamics of phloem formation in Mediterranean trees. Intra-annual density fluctuations (IADFs) occurred on the xylem side, indicating high plasticity and prompt response of wood production by the cambium to climatic fluctuations. In the second case study, we followed the cambial activity of *Quercus ilex* trees growing at a site in southern Italy during a very dry year to assess how xylem and phloem production is affected by strong seasonal climatic fluctuations. The lowest production of xylem cells was observed in the dry late spring and summer, while it was highest in autumn. We detected cambial production of phloem cells throughout the entire year, even during the periods when no xylem was produced. Our results suggest that phloem production under stressful conditions is either favoured or less influenced by environmental factors than xylem production. We are currently monitoring xylogenesis in *Vitis vinifera* to assess the adaptability of its xylem and phloem to increasing drought, and intend to use the information to optimize decisions related to viticultural management.

The differentiation and distribution of alpine *Luzula* sect. *Luzula* species in the Eastern Alps

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Luzula sect. *Luzula* (Juncaceae) is one of the taxonomically most intricate groups of angiosperms, where diversification is driven by true polyploidy and agmatoploidy (fission of chromosomes), leading to a number of different karyotypes: the largest, full-size chromosomes are designated as AL type, the intermediate, half-size chromosomes as BL-type, and the smallest, quarter-size chromosomes as CL-type. Due to hybridization, combinations between these basic karyotypes are possible. For the Eastern Alps eight species with six karyotypes were reported. Four of them can be found above the timberline in the subalpine and the alpine belt (karyotypes in parentheses): tetraploid *L. alpina* (12 AL + 24 BL) is most common in the siliceous Central Alps, tetra- and hexaploid *L. multiflora* (24 AL or 48 AL) is widespread across all Eastern Alps, diploid *L. sudetica* (48 CL) is widespread on mostly siliceous bedrock and diploid *L. expectata* (24 BL) is widespread on mostly calcareous bedrock. However, morphologically, the taxa are often difficult to distinguish and genome size data coupled with karyological investigations are currently the most reliable way for their identification. Especially the distinction between tetraploid *L. alpina* and *L. multiflora* is highly problematic in absence of chromosome counts and the distribution of both taxa as well as their ecological preferences remain unknown. Our extensive field sampling across the Eastern Alps, coupled with relative genome size estimations, revealed that multiple ploidy-levels often co-occur at the same localities, which could point to multiple independent polyploidisation events. To unambiguously classify all investigated *Luzula* individuals sharing the same ploidy level, we counted their chromosomes from germinated seeds collected at half of the sites. In addition, we used amplified fragment length polymorphism (AFLP) fingerprinting to disentangle the relationships among all the sampled populations. Our preliminary results show that the diploid *L. expectata* and *L. sudetica* are clearly divergent and well differentiated from tetraploids. On the other hand, we could not observe any clear divergence between the tetraploid *L. alpina* and *L. multiflora*. Finally, on each locality we listed the accompanying vascular plant species of all collected *Luzula* individuals and via their Landolt indicator values we will examine the ecological differentiation among different ploidy levels as well as among different species.

Conservation of priority grassland habitats in Slovenia through the establishment of seed bank and *in situ* restoration – project presentation

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Semi-natural grasslands are remnants of traditional agricultural landscape in Europe. However, their surface in Europe and Slovenia is decreasing due to agricultural intensification on one hand and land abandonment on the other, which leads to overgrowth with shrubs and forest. In 2021, we started a five-year-long project called 'LIFE for Seeds' with the leading partner DOPPS – Birdlife Slovenia and partners from three Slovenian protected areas (Triglav National Park, Notranjska Regional Park and Goričko Nature Park Public Institute), aiming to preserve the most valuable grassland habitats in Slovenia. The project focuses on establishing a seed bank of 300 characteristic, indicator taxa found on three EU priority habitats that are included in the Natura 2000 network: 6210(*) - semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) – important orchid sites, 6230* - species-rich *Nardus* grasslands and 3180* - turloughs. According to the latest national report of the Habitats Directive 2013-2018, all the three priority habitat types have either unfavourable status or bad status. In this project, we will restore 74,1 ha of high nature value grasslands at seven Natura 2000 sites (Notranjski trikotnik, Goričko, Julijske Alpe, Drava, Krimsko hribovje – Menišija, Ljubljansko barje, Škocjanski zatok) using green hay and brush-harvested seed mixtures. Three conservation measures will be designed for the production of native seed material and grassland restoration, which will be included in the national agricultural policy after 2027. In order to increase genetic diversity of seed material of wild grassland angiosperms stored in Slovenia, we will collect and store 12.000 accessions of seeds of 300 characteristic plant species of HT 3180*, 6210(*) and 6230*. This will contribute to conservation of biodiversity at all levels: genetic, species and ecosystem, which are all equally important for maintenance and preservation of ecosystem services – the natural benefits, on which we are all dependent.

Jogan N., Kotarac M., Lešnik A. (2004): Opredelitev območij evropsko pomembnih negozdnih habitatnih tipov s pomočjo razširjenosti značilnih rastlinskih vrst. Končno poročilo. Center za kartografijo favne in flore, Miklavž na Dravskem polju, 961 pp.

Poročanje po 17. členu Direktive o habitatih: <https://zrsvn-varstvonarave.si/informacije-za-uporabnike/katalog-informacijavnega-znacaja/porocanje-po-17-clenu-direktive-o-habitatih/> Downloaded: 20.06.2022

Phenotype of *Lactuca serriola* L. from Central Chile and Argentina

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Prickly lettuce (*Lactuca serriola* L., Asteraceae), an allochthonous species in South America, is recently widely distributed in the central Chile, and occurs also in Argentina. Mountains of Andes strongly influence differences in climate (Mediterranean vs. arid with continental characteristics) between both geographically close areas, and represents a natural barrier for dispersal of achenes. The set of 182 seed samples of prickly lettuce was acquired on 129 sites in Central Chile in 2016 and 2017, and 70 samples originated from 53 sites in Mendoza district, Argentina from 2020. Collecting sites in Chile cover area of 31°54,818'S to 35°48,742'S, 70°13,103'W to 71°59,666'W, with elevation 18 – 2 365 m a.s.l. In Argentina 31°20'47,3'' S to 33°55'21,5'' S, 67°32'04,5'' W to 69°54'37,8'' W, and elevation of 504 – 2 726 m a.s.l. Plants were cultivated in the greenhouse of Palacký University in Olomouc in 2021, and assessed for 18 morphological traits on leaves, stem and flowers, and for 2 developmental characteristics. Cauline leaves of all samples were divided, and their taxonomic status was precised to *Lactuca serriola* f. *serriola*. Presence of trichomes in the inflorescence, a trait developed typically in dry areas and/or in higher elevations, was recorded in 59 samples from Chile and 7 samples from Argentina. These samples can be classified as *L. serriola* var. *coriacea*. Eleven samples representing 2 sites in Chile and 6 sites in Argentina developed leaf rosette. Plants develop leaf rosette in locations with long vegetation season and comfortable access to ground water, and this trait is genetically fixed. Surprisingly, formation of leaf rosette was observed in plants originating from the elevation of 2 726 m a.s.l. in Argentina. The period between beginning of bolting and flowering was 12 – 98 days for samples from Chile, and 20 – 63 days for samples from Argentina. Populations of *Lactuca serriola* are considered young in South America, however our preliminary results indicate that they are well adapted to local environmental conditions, reaching high altitude and modifying length of life cycle to the short vegetation period in mountains and arid zones, so that they may survive in newly acquiring areas. Data are recently elaborated more precisely and compared to DNA profiles of samples and other biological characteristics (e.g. disease resistance), with respect to original ecological conditions in collecting sites.

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Feráková V., 1977. The genus *Lactuca* L. in Europe. Univerzita Komenského.

Lebeda A, Křístková E, Khoury CK, Carver D, Sosa CC, 2022. Distribution and ecology of wild lettuces *Lactuca serriola* L. and *Lactuca virosa* L. in central Chile. *Hacquetia*, 21(1): 173 – 186. DOI: 10.2478/hacq-2021-0019.

Chemical constituents and biological activities of two *Nepeta* species: *N. cataria* L. and *N. nuda* L.

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Many *Nepeta* species (fam. Lamiace, subfam. Nepetoideae, tribe Nepetae) are well known for their pharmacological properties and are widely used in folk medicine as diuretic, diaphoretic, anti-tussive, anti-spasmodic, anti-asthmatic, febrifuge and sedative agents, against gastrointestinal and respiratory hyperactive disorders (Formisano et al., 2011). They also show biopesticide potential and display behavioural effect on cats. Various biological activities of *Nepeta* species are closely related to their main secondary metabolites, among which terpenes and phenolics predominate. Two herbaceous perennial *Nepeta* species investigated within the present study are widely distributed in EuroAsia. *N. cataria* L. is spreaded from Central Europe to the Iranian plateaus and in Central Asia, and *N. nuda* L. grows throughout Europe and European part of Russia, the Crimea, in the Dnieper area and on Carpathians in Ukraine, as well as in Siberia (Boikova and Grishkina, 2019).

Untargeted metabolomics approach adopting UHPLC/LTQ Orbitrap–MSⁿ revealed iridoids and phenolic acids as the major groups of compounds in methanol extracts of both *N. cataria* and *N. nuda*. UHPLC/(±)HESI–MS² profiling of major compounds from these groups of metabolites revealed that rosmarinic acid was the major phenolic acid in both species, while from the group of iridoids, 1,5,9-epideoxyloganic acid predominated. Methanol extract of *N. cataria* was rich in iridoid aglycone *cis,trans*-nepetalactone. Based on the bioautography assays, methanol extracts of *N. cataria* and *N. nuda* show moderate α -amylase activity, and display significant antioxidative activity, which is primarily ascribed to high content of rosmarinic acid.

Formisano C., Rigano D., Senatore F. (2011). Chemical Constituents and Biological Activities of *Nepeta* Species. *Chemistry & Biodiversity* 8: 1783-1817.

Boikov, O. and Grishkina K. (2019). Content is of tannins of the *Nepeta pannonica* growing in Tula region, *Problems of Scientific Thought* 7(3): 12-14.

Stem CO₂ efflux, growth respiration and stem increment of beech and spruce trees

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Stem respiration is an important component of an ecosystem's carbon budget. It provides energy for living tissue maintenance and growth processes, and it returns a part of carbon allocated to the stem back to the atmosphere as CO₂. Besides environmental factors (such as temperature or water availability), stem respiration depends highly on tree energy demands for stem growth. Determining the relationship between stem growth and stem respiration would help to reveal the response of stem respiration to changing climate, which is expected to substantially affect tree growth. In this study, we measured stem CO₂ efflux and irreversible tree stem increment of beech and spruce trees during four growing seasons (May – October) at two different sites. Stem CO₂ efflux was measured using automated chamber systems and stem increment using automated dendrometers, both installed at the breast height. Then the respiration needed for stem growth was estimated using the mature tissue method. Seasonal stem CO₂ efflux amounted to between 690 and 1100 g CO₂ per m⁻² of the stem surface. The seasonal stem CO₂ efflux of spruce was higher compared to the beech as well as seasonal irreversible stem increment. The proportion of seasonal growth respiration to total stem CO₂ efflux ranged between 38-70% and, despite bigger increment, it tended to be slightly lower in spruce compared to beech. The highest growth respiration was observed in June and July in beech and in July and August in spruce, with maximum contribution to monthly stem CO₂ efflux of 92 and 73%, respectively. The growth respiration showed a time lag behind stem increment. The regression between growth respiration in the irreversible increment, therefore, differed during the period before and after the maximum stem increment rate. We consider this to be a result of the later onset of stem wall thickening which, we assume, is more energetically demanding than is cell elongation.

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Climatic regulation of leaf and cambial phenology in *Quercus pubescens*: their interlinkage and impact on xylem and phloem conduits

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Increased frequency and severity of stressful events affects the growth patterns and functioning of trees which adjust their phenology to given conditions. Here, we analysed environmental effects (temperature, precipitation, VPD and SWC) on the timing of leaf phenology, seasonal stem radial growth patterns, and xylem and phloem anatomy of *Quercus pubescens* in the sub-Mediterranean in the period 2014–2019, when various adverse weather events occurred, i.e. spring drought in 2015, summer fire in 2016 and summer drought in 2017. Results showed that the timings of leaf and cambium phenology do not occur simultaneously in *Q. pubescens*, reflecting different environmental and internal constraints. Although year-to-year variability in the timings of leaf and cambial phenology exists, their chronological sequence is fairly fixed. Different effects of weather conditions on different stages of leaf development in spring were observed. Common climatic drivers (i.e., negative effect of hot and dry summers and a positive effect of increasing moisture availability in winter and summer) were found to affect the widths of xylem and phloem increments with more pronounced effect on late formed parts. A legacy effect of the timing of leaf and cambial phenology of the previous growing season on the timing of phenology of the following spring was confirmed. Rarely available phloem data permitted a comprehensive insight into the interlinkage of the timing of cambium and leaf phenology and adjustment strategies of vascular tissues in Mediterranean pubescent oak to various environmental constraints, including frequent extreme events (drought, fire). Our results suggest that predicted changes in autumn/winter and spring climatic conditions for this area could affect the timings of leaf and stem cambial phenology of *Q. pubescens* in the coming years, which would affect stem xylem and phloem structure and hydraulic properties, and ultimately its performance.

Gričar J., Jevšenak J., Hafner P., Prislan P., Ferlan M., Lavrič M., Vodnik D., Eler K. (2022). Climatic regulation of leaf and cambial phenology in *Quercus pubescens* : their interlinkage and impact on xylem and phloem conduits. *Science of the Total Environment*, doi: 10.1016/j.scitotenv.2021.149968.

Physiological changes in pubescent oak (*Quercus pubescens*) under induced drought conditions

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Tree mortality in forests worldwide is increasing due to water stress caused by climate change. Water stress can be catastrophic for trees as it can cause stomatal closure and loss of hydraulic conductivity, which in turn decreases the rate of carbon assimilation and can subsequently increase pest susceptibility. This effect can be more pronounced in species that have shallower root systems and are more dependent on rainwater, an example being the pubescent oak (*Quercus pubescens*). We sought insight into this phenomenon by choosing groups of pubescent oak trees on Podgorski kras, Slovenia and arranging rainwater exclusion, in order to magnify water stress. We monitored soil and meteorological conditions alongside tree physiology parameters (stomatal conductivity, sap flow velocity) in the 2021 growing season in both rain exclusion plots and in control plots. Rainwater availability in the measuring period proved to be heterogeneous, with a dry first half of the season with little rain, followed by larger rainfall in September. Because of that, we sampled data from three 5-day windows, according to different soil water content (SWC) levels: Baseline (early season with baseline SWC levels), Drought (low SWC levels, resulting from a prolonged lack of rainfall) and Recovery (an increase in SWC levels because of rainfall, after drought). As expected, increasing water stress caused a noticeable decrease in the trees' physiological function, most notably in decreasing stomatal conductivity. Overall, this experiment has proven to be a useful basis for further research on the relationship between severe water stress and pubescent oak physiology. In the future, we intend to use these findings to predict the progress of tree mortality in the worsening climate change we face today.

- Allen C. G., Macalady A. K., Chenchouni H., et al. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, doi:10.1016/j.foreco.2009.09.001.
- Li X., Benye X., Xiuchen W., Choat B., Feng J., Jiang M., Tissue D. (2022). Unlocking Drought-Induced Tree Mortality: Physiological Mechanisms to Modeling. *Frontiers in Plant Science*, doi: 10.3389/fpls.2022.835921
- Nardini A., Casolo V., Borgo D. A., Savi T., Stenni B., Bertoncin P., Zini L., McDowell N. G. (2016). Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms differentially affected by an extreme summer drought. *Plant, Cell and Environment*, 39, 618–627.

Orchid diversity and conservation in Eastern Thailand

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Orchidaceae is the largest flowering plant family in Thailand, with ca. 1,200 species. Many species are demanded for ornamental purposes such as *Paphiopedilum* spp., *Vanda* spp., *Rhyncostylis* spp., *Dendrobium* spp., etc. However, many orchid species populations are declining due to habitat change and over-collection. Fortunately, Thailand seems to have succeeded in hotspot detection in conserved areas such as National Parks, Wildlife Sanctuaries, and conserved forests. In this study, we aimed to explore the wild orchid species in eastern Thailand, during September 2020-August 2021. At least once a season we explored Khao Yai National Park, Khao Kitchakut National Park, Khao Soi Dao Wildlife Sanctuary, and the conserved area of Rayong Botanical Garden. Photographs of the flowering orchid individuals were taken for identification. If they were uncommon species, their flowers were collected and preserved in 70% alcohol. Premature capsules of some orchids were bought for *in-vitro* propagation at Plant Genetic Resources Center, Suranaree University of Technology. We found 133 orchid species in 64 genera. They are epiphyte 90 spp., terrestrial orchid 42 spp. and holomycotrophic orchid 1 species. Among them, 13 vulnerable species, 2 endemic species and 4 rare species were recorded. There are 41 spp. in 30 genera, 68 spp. in 41 genera, 75 spp. in 43 genera, and 3 spp. in 3 genera were found in Khao Yai National Park, Khao Kitchakut National Park, Khao Soi Dao Wildlife Sanctuary, and Rayong Botanical Garden, respectively. Seeds of 31 spp. in 21 genera were collected for *in-vitro* propagation and 16 spp. (51.6%) germinated to seedlings. All information about orchid species in these conserved areas could be used for ecotourism with ecological activities. The seedlings could be also used for re-introduction to strengthen the diversity in a population.

Keywords: conservation, orchid seed propagation, species list, Thai orchids

Plant interactions with environment

Stressphysiology – the use of stress markers to evaluate oxidative stress

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Plants have to cope with tremendous challenges due to climate change and global warming. Heat, drought and soil salinization are just a few of them with increasing global relevance in the next years and decades [1]. Stress, especially when photosynthetic or respiratory electron chains are affected, results in an increased production of reactive oxygen species (ROS) followed by oxidative stress and finally oxidative damage with far-reaching consequences for plants [2]. However, ROS are Janus-faced molecules and redox regulation and thus production and scavenging of ROS is part of nearly every aspect in plant development and interaction with the environment [3, 4]. Oxidative stress is still a “hot” topic in stress physiology. More than 5.000 hits are listed in Web of Science for 2021 when searching for “oxidative stress and plants”. Many of these papers use components of the antioxidative defence system to identify and to qualitatively and quantitatively evaluate oxidative stress. This antioxidative defence system consists of low molecular weight or lipid soluble antioxidants (e.g., ascorbate, glutathione, proline, tocopherol) and several enzymes (e.g., peroxidase, catalase, superoxide-dismutase) [2]. Additionally, other stress markers - better labelled as “effect marker” - are used to evaluate (oxidative) damage (e.g., membrane leakage, photosynthetic pigments, photosynthesis, respiration, chlorophyll fluorescence). But there is a huge variation in reports on antioxidant activities and possible oxidative damage, thus tolerating a wide scope in the interpretation of stress scenarios. Reliable and informative stress markers are crucial for better understanding what’s going on in plants exposed to stressful situations. This is essential for managing the manifold challenges plants have to cope with in a changing environment.

- [1] Vicente-Serrano S.M., Quiring S.M., Pena-Gallardo M., Yuan S. and Dominguez-Castro F. (2020). A review of environmental droughts: Increased risk under global warming? *Earth Science Reviews*, doi:10.1016/j.earscirev.2019.102953.
- [2] Gill S.S. and Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, doi: 10.1016/j.plaphy.2010.08.016.
- [3] Noctor G., Reichheld JP. and Foyer C.H. (2018). ROS-related redox regulation and signaling in plants. *Seminars in Cell & Developmental Biology*, doi: 10.1016/j.semcdb.2017.07.013.
- [4] Nadarajah K.K. (2020) ROS Homeostasis in abiotic stress tolerance in plants. *International Journal of Molecular Sciences*, doi:10.3390/ijms21155208.

Response of onion plants to oxidative stress induced by heavy metals pollution of soils from industrial areas in Kosovo

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Heavy metals contamination of soils especially agricultural soils around industries or by other human activities is a major concern for the environment and living organisms, they can accumulate in crops affecting food quality, plant productivity and human health. In this study, the effects of Cd, Cr, Ni, Pb and Zn pollution in agricultural soil and their accumulation on onion (*Alium cepa* L.) were evaluated at the biochemical aspect with ALA-D enzyme response as a biomarker, δ -aminolevulinic acid (ALA) and total chlorophyll content in leaves of this plant. The aim of this study was to determinate the level of oxidative stress induced by heavy metals pollution in agricultural soil and their accumulation on onion cultivated in the two regions, Mitrovica and Obiliqi in Kosovo. Soil samples were randomly selected from the agricultural areas in the two regions, Mitrovica and Obiliqi, which are considered the most industrial polluted regions in Kosovo. Results show that Pb and Zn concentration in soil samples from Mitrovica (1953-2576 mg kg⁻¹) and Obiliqi regions (138-179 mg kg⁻¹) and their bioaccumulation level were significantly higher in comparison with the control group. Higher Pb and Zn concentration in leaves of onion from polluted regions resulted in the higher significant inhibition effect of ALA-D activity which caused an increased in ALA content and decrease the total chlorophyll content. Furthermore, Pb concentration in Mitrovica region exceeds more than 100 times the European average (EU) value for Pb and can be considered highly contaminated soil with Pb. Onion plants bioaccumulated more than twenty times higher Pb values compared with EU regulation for foodstuff. The obtained results strongly suggest that the activity of ALA-D is entirely dependent on the concentrations of Pb and Zn on onion leaves. Onion plants are very useful for evaluation of soil contamination with heavy metals. This study indicates that the ALA-D activity can be used as a very sensitive biomarker for the evaluation of heavy metal pollution and their risk for the food chain and public health of these regions.

Morkunas I., Woźniak A., Mai V., Rucińska-Sobkowiak R., Jeandet P. (2018). The role of heavy metals in plant response to biotic stress. *Molecules*, doi: 10.3390/molecules23092320.

Singh S., Parihar P., Singh R., Singh V.P., Prasad S.M. (2016). Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. *Frontiers in plant science*, doi: 10.3389/fpls.2015.01143.

Grapevine in changing environment

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Climate change that increased temperatures results in more precipitation falling as rain rather than snow, earlier snow melt, and increased evaporation and transpiration. Thus, the risk of drought increases, as summer droughts are longer and more frequent. Grape production is highly dependent on agroclimatic factors, water availability being one of the most important. However, the drought is not the only stress factor to which the grapevines are exposed to; in a vineyard, grapevines are simultaneously exposed to combinations of several abiotic (drought, extreme temperatures, salinity) and biotic stresses (phytoplasma, viruses, bacteria and fungi). Climate change is also significantly affecting the distribution, virulence and abundance of plant pathogens, increasing the likelihood of combined occurrence of abiotic and biotic stresses, which is already a very common event. Therefore, it is crucial to understand the processes that take place in plants in response to abiotic stress and disease and to implement the knowledge into agricultural practices. At the Department of Biotechnology and Systems Biology of National Institute of Biology, we investigate the response of grapevine to virus and phytoplasma infection and to drought and salinity stress, and to combination of them, as well as the resistance of newly bred fungal resistant lines and new rootstocks to the occurrence of drought and salinity stress.

Dermastia M., Škrlić B., Strah R., Anžič B., Tomaž Š., Križnik M., Schönhuber C., Riedle-Bauer M., Ramšak Ž., Petek M., Kladnik A., Lavrač N., Gruden K., Roitsch T., Brader G., Pompe Novak M. 2021. Differential response of grapevine to infection with 'Candidatus Phytoplasma solani' in early and late growing season through complex regulation of mRNA and small RNA transcriptomes. *International journal of molecular sciences* 22 (7): 3531-1-3531-28.

Škrlić B., Pompe Novak M., Brader G., Anžič B., Ramšak Ž., Gruden K., Kralj J., Kladnik A., Lavrač N., Roitsch T., Dermastia M. 2021. New cross-talks between pathways involved in grapevine infection with 'Candidatus Phytoplasma solani' revealed by temporal network modelling. *Plants* 10 (4): 646-1-646-18, DOI: 10.3390/plants10040646.

Buesa I., Pérez-Pérez J.G., Visconti F., Strah R., Intrigliolo D.S., Bonet L., Gruden K., Pompe Novak M., De Paz J.M. 2022. Physiological and transcriptional responses to saline irrigation of young 'Tempranillo' vines grafted onto different rootstocks. *Frontiers in plant science* 13: 1-17. DOI: 10.3389/fpls.2022.866053.

Jež Krebelj A., Rupnik Cigoj M., Stele M., Chersicola M., Pompe Novak M., Sivilotti P. 2022. The physiological impact of GFLV virus infection on grapevine water status: first observations. *Plants* 11(2): 1-15. DOI: 10.3390/plants11020161.

Modified BPM expression alters phenotypic traits of Arabidopsis in response to heat stress

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In the *Arabidopsis thaliana* (L.) Heynh. genome, six *BPM* genes encode BPM proteins - specific protein adaptors of the cullin E3 ligase complex that polyubiquitinate substrate proteins as part of the ubiquitin-proteasome pathway and direct them for degradation via the 26S proteasome. Research have shown that BPM proteins not only regulate various developmental processes, but also have an important role in phenotypic and physiological adaptability and the ability of plants to survive in a changing environment. The *Arabidopsis* line overexpressing *BPM1* gene (*oeBPM1*), line with downregulation of *BPM1*, 4, 5, and 6 (*amiR-bpm*), and wild type (wt) were exposed to 40 °C for six hours at two developmental stages - two (2L) and eight (8L) rosette leaves. The first group of plants was exposed at 2L stage, the second at 8L stage, while the third group was exposed at both stages (2L+8L). Plant growth and chlorophyll fluorescence parameters were determined using a high-throughput plant phenotyping platform (GrowscreenChamber, Jülich Plant Phenotyping Center). The *oeBPM1* showed a decrease in projected leaf area (A_{PT}) after exposure to heat stress at 2L. On the other hand, both *amiR-bpm* and wt showed significantly lower A_{PT} than their respective controls after exposure at 8L and 2L+8L. Immediately after exposure, all lines showed an increase in the maximum quantum yield of photosystem II (F_v/F_m) at 2L, with an average of 0.77 compared with 0.75 in control plants. However, in *oeBPM1* and wt stressed at 2L stage, F_v/F_m decreased to 0.76 and 0.74, respectively, over the following seven days. Heat stress applied at 8L stage increased F_v/F_m in *oeBPM1* and wt to an average of 0.80 compared with 0.76 in control plants, but not in *amiR-bpm*. Moreover, *amiR-bpm* exposed at 2L+8L showed a significant decrease in F_v/F_m (0.74) compared with control (0.77). Biomass measurement revealed lower dry weight of *oeBPM1* and *amiR-bpm* treated at 2L and 8L stages, respectively. These results suggest that overexpression of *BPM1* negatively affected the selected phenotypic traits of heat-treated Arabidopsis at the early developmental stage. Interestingly, a similar effect was observed in Arabidopsis plants with downregulated *BPMs* after exposure to heat stress at the adult stage.

UV-A stressing of basil (*Ocimum basilicum* L.)

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While short-wave UV light is known to be photodestructive and thus a strong stressor, long-wave UV, i.e. UV-A light (315 - 400 nm), has milder negative effects on plants. In addition, there are also reports of its beneficial effects. The nature and magnitude of UV-A effects depend on plant genotype, dose, wavelength, and factors interacting with the light regime. However, the available data do not allow us to determine the effect of the UV-A dose because there is insufficient information on the radiation doses used.

In our experiments, we exposed green basil (*Ocimum basilicum* L.) plants to UV-A of two UV wavelengths (365 and 385) at different doses (sum of 3.5, 7, 8, 16, 24, and 32 Wm⁻²) as supplementary light to basal red/blue (3/2) light of 260 μmol m⁻² s⁻¹. After two weeks, growth parameters were measured and plants were examined by fluorescence and multispectral imaging. In general, the effects of UV-A on basil were small. Even at higher doses, we did not detect significant negative effects on growth and function. Our results suggest that basil plants activate multiple mechanisms, such as UV-screening, photoprotective and antioxidant photosynthetic pigments, and adaptation of thylakoid processes to acclimate to the light environment. UV-A could be used to eustress green basil in indoor cultivation.

What happens with antioxidant potential of young broccoli under temperature stress?

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Due to climate change, sudden and intense changes in weather conditions are becoming more frequent. Sessile organisms such as plants cannot "escape" from high or low temperatures but adapt their metabolism to new conditions to survive. Oxidative stress is a common response of plants exposed to extreme temperatures. During temperature stress, the production of a large amount of ROS can be a major risk factor for plant cells and therefore stimulates the synthesis of antioxidants in them. As part of this paper, we investigated the impact of cold (ice) and hot water (80°C) on the antioxidant potential of broccoli (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.) in the developmental stage with 6-8 leaves. We used three methods of analysis, ABTS, FRAP and DPPH. The results showed that none of the treatments significantly changed the antioxidant potential measured by ABTS and FRAP method, however, both caused a decrease in potential as measured by the DPPH method. In particular, the control group accounted for 4.71 ± 0.40 % of radical inhibition compared to Trolox, the group treated with cold water for 4.13 ± 0.51 %, and the group treated with hot water for 3.69 ± 0.46 %. Such a difference in results between different methods is a consequence of different types of antioxidants present in samples that react differently with the radicals used. Based on these results, we conclude that using cold or hot water, we cannot improve the antioxidant potential of broccoli; moreover, according to the DPPH method, this potential was reduced.

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Content of bioactive substances and potentially toxic elements in wild edible plants in Celje basin

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Celje basin is one of the most degraded and contaminated areas in Slovenia, due to the industrial background. The demolition of the old zinc ore processing plant and use of contaminated waste material for embankment and driveways, resulted in wide dispersal of potentially toxic elements throughout the basin area. Because of the new trends of promoting wild plants in modern cuisine, we posed a question if in some areas in Celje basin, the use of these plants should be avoided due to contamination with toxic elements. The aim of this study was to determine the content of bioactive substances and the content of potentially toxic elements (Zn, Cd, Pb) in selected edible wild plants, as well as to study the effect of contamination with these elements on contents of bioactive compounds in selected plants. We sampled meadow soils (0-10 cm) and aboveground, green, edible parts of selected wild plants, in spring when they are usually collected for consumption. We took soil samples in different cadmium contamination zones, predicted from previous studies, ranging from uncontaminated to critically contaminated. Plant species surveyed were ground ivy (*Glechoma hederacea* L.), common daisy (*Bellis perennis* L.), common dandelion (*Taraxacum officinale* Wagg.), narrow-leaved plantain (*Plantago lanceolata* L.) and red clover (*Trifolium pratense* L.). Daisy was a greater accumulator of cadmium and lead than all other tested plant species. However, the analysis of zinc accumulation did not show significant differences between surveyed plant species. Bioactive compounds analysed were photosynthetic pigments (β -carotene, lutein, neoxantin, antheraxantin, violaxantin, chlorophylls a and b), and tocopherols (γ , δ , α). In the case of photosynthetic pigments, plant species in order from the highest to the lowest concentration were: dandelion, plantain, clover, ground ivy and daisy. Regarding chlorophylls, dandelion and plantain contained significantly higher concentrations, than daisy and ground ivy. Regarding carotenoids, plantain, dandelion and red clover contained significantly higher concentrations than daisy, while ground ivy contained significantly less carotenoids than plantain and dandelion. For most analysed bioactive substances, statistically significant differences were found between daisy and plantain and between daisy and dandelion. Concerning tocopherols (vitamin E), species from the highest to the lowest concentration were in order: plantain, dandelion, ground ivy, clover and daisy. In the case of tocopherols statistically significant differences were found between almost all plant species, excluding daisy and clover, as well as plantain and dandelion. Analysis of the effect of plant contamination with potentially toxic metals on bioactive compound concentration did not give statistically significant results in linear regression models.

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