IWOPA 2021

3rd INTERNATIONAL WORKSHOP ON PLASMA AGRICULTURE

GREIFSWALD, GERMANY

MARCH 01 – 03, 2021

BOOK OF ABSTRACTS

IWOPA 2021

3rd INTERNATIONAL WORKSHOP ON PLASMA AGRICULTURE
GREIFSWALD, GERMANY, MARCH 01 – 03, 2021

HOSTED BY

Leibniz Institute for Plasma Science and Technology
Felix-Hausdorff-Straße 2
17489 Greifswald
Germany

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FOREWORD

Dear colleagues and participants of the IWOPA 2021(formerly 2020),

Last year, the International Workshop Series on Plasma Agriculture should take place for the first time in Europe, in the Hanseatic City of Greifswald, in Germany.

Due to the Corona Pandemic and the accompanying travel restrictions, the conference had to be postponed to 2021 and we are still struggling with the challenges of the Corona Pandemic worldwide. We regret, that we can't welcome you in person in Greifswald very much.

By February 2020, everything was ready for your visit – the conference bags packed, field trips planned, talks and posters prepared and also the abstract book had been already printed.

Furthermore, we are highly appreciative of the submission of more than 65 abstracts in contribution to the success of this workshop and thank all participants for their commitment.

This publication comprises the abstracts of the invited speakers, as well as the abstracts selected for the contributed talks and poster presentations. The order of the abstracts corresponds to the chronological order of the presentations during the postponed conference at the time of print. Posters are ordered according to the topic. The responsibility for the content rests entirely with the authors.

Globalisation confronts society and research with new challenges not only with regard to pandemic situations. The application of plasma technology in agriculture and the food industry is highly relevant, considering the public and scientific discourse regarding

the replacement of chemicals and the need of increasing the global agricultural productivity, to guarantee the feeding of the world's continuously growing population.

The International Workshop on Plasma Agriculture is a key element in this process, focusing on aspects like food safety, seed germination, plant growth and development, on systems and methods for seed treatment, biological processes like stress reactions or resistances, as well as on technologies and methods for the treatment of seeds and plants.

Plasma research has a long tradition in Greifswald. Experiments with ionised gases have been conducted for more than 100 years in the Hanseatic City. Nowadays, Greifswald has become a worldwide known centre for plasma research, with professorships at the local university and two non-university research institutes. We are proud to hold such an important event on plasma applications in the areas of agriculture and food sciences. We hope that a digital symposium could provide the great opportunity to exchange and discuss the latest research results and new scientific ideas as well as state-of-the-art technologies, new approaches and challenges for the future to support the progress in this field - despite all the circumstances, or rather because of them.

Finally, we look forward to a profound conference, with inspiring presentations and an interesting exchange on the virtual venue. The LOC is grateful for the support of the IOC and greatly acknowledges the sponsorship of the Volksbank.

The Local Organizing Committee (LOC)

IWOPA 2021

CONTENT

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CONTRIBUTED LECTURES

POSTER

FOOD SAFETY, PRESERVATION, STORAGE & TRANSPORTATION

SEED GERMINATION, PLANT GROWTH & DEVELOPMENT

BIOLOGICAL PROCESSES, E.G. STRESS RESPONSES, RESISTANCES

SYSTEMS & METHODS FOR TREATMENTS OF SEEDS, PLANTS…

METHODS & PROCEDURES, SIMULATIONS

ENVIRONMENTAL ASPECTS

INVITED LECTURES

PHYSICS AND PLASMA CHEMISTRY OF PRODUCE WASHING, MISTING, AND PLANT GROWTH STIMULATION

Alexander Fridman
Nyheim Plasma Institut, Drexel University, Philadelphia, PA, USA
email: fridman@drexel.edu

The presentation is focused on physical and plasma-chemical fundamentals, mechanisms, physical and chemical kinetics of plasma-stimulated processes of fresh produce washing, misting, and plant growth. Major plasma sources under consideration include:

- Nano-second and micro-second pulsed dielectric barrier discharges DBD in air, and water/water-solution mist
- Atmospheric and reduced pressure microwave discharges in air
- Non-equilibrium gliding arc discharges stabilized in reverse vortex Tornado flow
- Gliding arc discharges submerged in water and water solutions

Major non-equilibrium plasma stimulated food processing and agricultural processes under consideration include:

- Sanitization of different types of fresh produce by plasma treated mist in different configuration of containers. Washing of different types of fresh produce by plasma activated water and water solutions, non-oxidative disinfection processes
- Simulation of plant growth by plasma activated water, optimization of nitrogen compound production, challenge of energy efficiency of the process.

Presentation is based on novel experimental and modeling results obtained in Nyheim Plasma Institute of Drexel University.

INFLUENCES OF PLASMA TREATMENT OF SEEDS ON THEIR MOLECULAR RESPONSES

Masaharu Shiratani[1] and Kazunori Koga[1, 2]
[1] *Faculty of Information Science and Electrical Engineering, Kyushu University, Japan*
[2] *Center for Novel Science Initiatives, National Institutes of Natural Sciences, Japan*
email: siratani@ed.kyushu-u.ac.jp

High-yield farming without antagonistic effects helps feed the growing world population. As one of potential high-yield farming method, plasma assisted farming has attracted attention for its significant contribution to improve crop and biomass yield with less environmental impact. Plasma induced stress responses are observed for plasma treated seeds probably due to reactive oxygen-nitrogen species (RONS) irradiated during plasma treatment [1, 2].

So far, we have developed a scalable dielectric barrier discharge plasma device to treat seeds [3]. For the treated seeds, we observed growth enhancement in the seedling stage, reduction of the harvest period and an increase of the harvest amount [4-6]. To reveal the mechanisms of the plasma induced effects, we have studied molecular mechanisms of the plasma treated seeds. We have carried out electron spin resonance measurements of seeds before and after plasma treatment. The concentration of radicals related to biochemical compounds in seeds was increased by plasma treatment. After the inhibition of seeds, the radical concentration decreased rapidly within 10 min and then it decreased slowly. The decay time constant of radical concentration after 10 min for the plasma treated seeds was shorter than that for the control (seeds without plasma treatment). This is probably due to enhanced water uptake characteristics of the plasma treated seeds. Furthermore, the concentration of plasma induced radicals corresponding to biochemical compounds seeds depends on the seed color. Plasma treatment induces changes in seed phytohormone balance [7, 8]. The phytohormone regulation correlates with the morphometric parameters and photosynthetic characteristics. In short, plasma treatment of seeds clearly modifies the molecular balance in the seeds.

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PLASMA ENHANCED BIOREFINERY

Masafumi Ito[1,2]
[1] Research Center for Plasma-Bio Science and Technology, *Meijo University, Japan*
[2] Faculty of Science and Technology, *Meijo University, Japan*

email: ito@meijo-u.ac.jp

Recently, the applications to biorefinery processes have attracted much attention owing to the wide-spread concerning of global warming and fuel depletion. In the biorefinery processes, the improvements of biomass-decomposition and ethanol fermentation efficiencies are required.

In our researches, we have investigated the effects of atomic oxygen radical treatment on the fungal-spore activation by monitoring amylase production of *Aspergillus oryzae* (*A. oryzae*), which is very beneficial microorganism and employed for decomposing starch to glucose in the process to produce ethanol. We exposed *A. oryzae* spores to oxygen radicals generated by a commercially available atmospheric-pressure radical source (FUJI CO., LTD. FPA-10). As a result, the germination rate was increased by 13 % and amylase activity secreted from the irradiated *A. oryzae* spores were increased by 40 %.[1]

Moreover, we have successfully promoted the growth of the budding yeast cells by 20 % through controlling the dose of oxygen or nitric-oxide radicals using atmospheric-pressure oxygen-radical source. [2]

A highly promising way of production of low-cost ethanol is utilization of lignocellulosic materials such as crop residues, grasses, sawdust, wood chips, oil palm empty fruit bunches, trunks, and fronds. These materials contain sugars polymerized in the form of cellulose and hemicellulose, which can be liberated and subsequently fermented to ethanol by microorganisms. However, there are some common problems related to decomposition of cellulose, lignin and vanillin in the biomass.

The efficiency of cellulolytic enzymes is important in industrial biorefinery processes, including biofuel production. We confirmed that the production of reducing sugar from oxygen-radical-pretreated carboxymethyl cellulose (CMC) was 1.7-fold higher than those from non-pretreated and oxygen-gas-pretreated CMC, and found that oxygen-radical pretreatment of cellulose enhances cellulolytic activity.[3]

Moreover, we treated YPD medium containg 5.0 mM vanillin with oxgen radicals for 20 min. The yeast growth and ethanol production rates were improved 4 and 7 times compared to those without oxygen-radical treatment.

This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1511021), Grant-in-Aid for Special Purposes (19H05462) and Plasma-bio consortium project (01221907).

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PLASMA ACTIVATED WATER AND AMMONIA SOLUTIONS TESTED AS FERTILIZERS

Z. Machala[1], G. B. Ndiffo Yemeli[1], S. Kyzek[2], R. Švubová[3], K. Kučerová[1], K. Hensel[1]

[1] *Division of Environmental Physics, Faculty of Mathematics, Physics and Informatics,*
[2] *Dept. of Genetics,* [3]*Dept. of Plant Physiology, Faculty of Natural Sciences,*
Comenius University in Bratislava, Slovakia
email: machala@fmph.uniba.sk

As envisioned by Graves et al. [1], improved utilization of organic waste for fertilizer has significant worldwide economic and ecological potential, which can be unlocked using plasma. Most animal types of organic fertilizer (manure, urine) contain ammonia, which is released into the environment and significantly contributes to the global warming. Atmospheric air plasmas enable very efficient nitrogen fixation into nitrogen oxides or into water [2-3]. Plasma activation of organic fertilizer offers the possibility to increase the nitrogen content and reduce the loss of greenhouse ammonia by converting it into less volatile but more fertilizing ammonium nitrate. In addition, plasma activation reduces odour and controls the bacterial population of the organic fertilizer mixture [1].

Plasma activated water (PAW) is formed by exposure of water to the cold plasma resulting in the production of reactive oxygen and nitrogen species (RONS, e.g. H_2O_2, NO_2^-, NO_3^-, $ONOO^-$, OH radicals) [4-5]. PAWs produced by different plasma sources and with various chemical compositions have been demonstrated to have effects on the improved seed germination and bacterial/fungal decontamination, and as nitrogen fertilizer they can enhance the plant growth [5-6].

We test various types of PAWs and ammonia solutions (up to 4 mM), activated by atmospheric air plasmas of the transient spark discharge with water electrospray or circulating water, and DC glow discharge with water cathode. The chemical composition of each PAW or plasma activated ammonia solution (PAA) is characterized by measuring pH and long-lived RONS concentrations (H_2O_2, NO_2^-, NO_3^-) by UV/VIS absorption spectroscopy. PAW only or PAW/PAA effects on wheat (*Triticum aestivum L.*), barley (*Hordeum vulgare L.*), and radish (*Raphanus sativus*) plant growth enhancement were investigated. After 4-12 weeks of plant growth, the effects of PAWs/PAAs watering on seedlings or adult plants were analysed by measuring growth parameters (above ground plant length, fresh and dry weight) and several physiological parameters, e.g. content of photosynthetic pigments, rate of photosynthesis and antioxidant enzymes activity.

The results showed that PAWs generated by various atmospheric air plasma discharges have the potential to improve the plant growth and increase the photosynthetic pigments concentration and other physiological plant parameters, without causing any genetic modification. The preliminary results with ammonia solutions suggest that ammonia as a source of nitrogen slightly improves the plant growth. PAAs farther enhance the plant growth, depending on the type of plasma activation (i.e. H_2O_2, NO_2^-, NO_3^- concentrations). The encouraging results with PAAs, i.e. chemically simplified experimental model of the plasma activated organic fertilizer represent the initial laboratory study as input data for future testing and up-scaling of plasma activation of real organic fertilizers.

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PLASMA-TREATED SUBSTRATES USING ATMOSPHERIC JET AND MULTI-JETS FOR AGRICULTURAL PURPOSE

Eric ROBERT[1], Audoin HAMON[1], Augusto STANCAMPIANO[1], Claire DOUAT[1], Sébastien DOZIAS[1], Kamal HAJISHARIFI[1,2] and Jean Michel POUVESLE[1]

[1] *GREMI, UMR 7344, CNRS/Université d'Orléans, Orléans FRANCE*

[2] Department of Physics and Institute for Plasma Research, Kharazmi University, Tehran, IRAN

email: eric.robert@univ-orleans.fr

We target and report on the use of either single or multi so-called cold atmospheric pressure plasma jets, based on Plasma Gun technology [1], to expose liquid solutions eventually containing seeds or immerged seedlings for agricultural applications. Besides, the upscaling capability of multi jet setups in comparison with single jet devices for the generation of attractive chemicals in solutions, the consideration of the diffusion of plasma produced reactive species in liquid samples is the focus of our investigations.

With the single jet configuration, two mode of exposures of liquid samples are considered: a "conventional" protocol where plasma jet plume impinges on the top of the solution and a more rarely investigated and documented method where the plasma jet is "transferred" into gas bubbles exiting from an immerged nozzle [2].

The talk will report on in-bubble plasma generation, water solution treatment using bubble plasma gun device, and the diffusion of reactive species in the liquid container. Using KI coloration as a broad range oxidation reporter, it is measured that while conventional plasma jet exposure leads to very slow and very inhomogeneous reactive species diffusion in the solution, generation of plasma in bubbles induces a sudden, very homogeneous and surprising oxidation of the whole volume of a few mL. Work is in progress to try and simulate the plasma generation in bubble and the consecutive reactive species delivery in the solution.

With the conventional protocol, Schlieren visualization indicates that air and helium mixing and flow properties above the liquid surface correlates with the oxidation patterns revealed in the solution. Air entrainment along and inside the plasma plume with plasma jet in free jet mode or impinging over various targets was recently reported with a dielectric barrier discharge based helium plasma jet [3].

Dealing with plasma solution generation using plasma jet and/or treatment of solution containing either attached or suspended cells or samples of interest for agriculture, it is suspected that neither slow diffusion using conventional plasma jet exposure nor opportunity with bubble arrangement were so far considered with sufficient care.

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ON THE PLASMA DECONTAMINATION EFFICIENCY OF NATURALLY OCCURRING TOXINS IN AGRICULTURE

N. Hojnik[1,2], M. Modic[1], J. L. Walsh[3], D. Žigon[1], U. Javornik, J. Plavec[4], B. Žegura[5], M. Filipič [2,5], U. Cvelbar[1,2*]

[1] *Jozef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia, EU.*

[2] *Jozef Stefan International Postgraduate School, Jamova cesta 39, SI-1000 Ljubljana, Slovenia, EU.*

[3] *Department of Electrical Engineering and Electronics, University of Liverpool, Liverpool, United Kingdom.*

[4] *Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia, EU*

[5] *Department of Genetic Toxicology and Cancer Biology National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia, EU.*

email: uros.cvelbar@ijs.si

Naturally occurring mycotoxins are stable toxic metabolites produced by various filamentous fungi species, which are frequently found on food crops such as cereals and nuts [1]. Currently, more than 400 structurally different mycotoxins have been identified, which can produce an assortment of toxic effects when consumed by vertebrates. Amongst these, aflatoxins are considered the greatest threat to human health, especially aflatoxin B_1 (AFB_1) to which more than half of the world's population is chronically exposed. AFB_1 is one of the most potent natural group 1 carcinogens, responsible for up to 155,000 (28 %) annual cases of human hepatocellular carcinoma cancer [2,3]. For this reason, mycotoxins have become one of the most critical dietary risk factors, with the situation being further exasperated by the alarming increase in their occurrence on widely consumed food stuffs [4]. Mycotoxin contamination is considered an unavoidable and unpredictable problem as they are readily found on crops where good agricultural, storage and processing practices have been implemented [5-8]. To combat this growing issue, food producers are urgently seeking novel non-thermal decontamination methods that are able to rapidly eliminate mycotoxin contamination with negligible impact on the treated food product and environment. Current non-thermal approaches include UV irradiation, pulsed light treatment, gamma irradiation and ozonation, which induce the breakdown of the mycotoxin molecule by the absorption of high energies or interaction with highly reactive chemical species [9,10]. Here we present an easily implemented approach using non-equilibrium plasma for targeted degradation of aflatoxin B_1. We identify rapid scission of the vinyl bond between 8- and 9-position on the terminal furan ring of aflatoxin B_1 as being of paramount importance for the suppression of toxic potential. The detailed plasma reactive species mediated degradation pathways are elucidated, and it is demonstrated that the approach not only renders aflatoxin B_1 harmless but does so in an order of magnitude less time than other methods currently under investigation.

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CAN PHYSICAL AND BIOLOGICAL TREATMENTS ENHANCE THE YIELD OF CROPS? PILOT STUDY ON RAPESEED.

Vladislav Curn[1], Eva Jozova[1], Petr Spatenka[2]

[1] *University of South Bohemia, Faculty of Agriculture, Ceske Budejoveice, Czech Republic*

[2] *SurfaceTreat, a.s., Turnov, Czech Republic*

email: curn@zf.jcu.cz

High quality seeds together with optimal exploitation of genetic potential of modern intensive cultivars of agricultural crops are main predisposition of high yield. One of key feature of farming technology is seeds and seed quality. To improve seed quality, to prevent transfer of pathogens and to protect germinating seeds and seedlings against attack of diseases and pest, seeds are often treated by pesticides. So seeds could be an important source of input of pesticides into environment. Decreasing of amount of pesticides, including seed dressing, is also one of goals of systems of Integrated Pest Management. The use of environmentally friendly methods of crop protection against pest and diseases represents one of the priorities for all subjects involved in the production, storage, trade and processing of agricultural products. New technologies, that would be able to eliminate (or reduce) undesirable microorganisms and their toxic products, and are not themselves toxic or don't represent any risk of additional intoxication are highly demanded.

Low temperature plasma (LTP) is well known technology used for many years especially in chemical and pharmaceutical industry for modifying physical and physiochemical properties of treated substances, surfaces, microparticles [1]. Recently this technology also has found application in plant biology and agriculture. Plasma treatment of seeds positively influenced seed germination of many agricultural crops including oilseed rape [2]. In general authors reported positively affected seed germination rate after plasma treatment of seeds, and in some cases positive effects were recorded also on plant growth in field conditions: growth rate, plant height, root length or fresh weight or yield. Also utilization of appropriate fungi in biological control and seed treatment was reported many times. Seed treatment with biopreparations should enhance plant growth and also have positive effect on pathogens and can suppress plant disease and activate plant defence mechanisms [3].

The main topic of presented study was application of dual seed treatment based on physical (low temperature plasma) and biological treatment on oil seed rape seeds. Treated seeds were assessed in small-scale field experiments in 2015-2017 as well as in large field experiments in 2017-2018. Our results showed positive effect of seed treatment on elimination of spores of fungal pathogens from seed surface, positive effect on early growth of seedlings, seedling emergence in field conditions, better canopy development and yield. In plasma or plasma together with *Trichoderma virens* treated variants were positively affected earlier flowering and ripeness of plants, length of roots, number of branches, length of siliguae and number of seeds as well as yield (12-32% higher yield in small scale experiments and 13-26% higher yield in large field experiments).

This study was supported by grants of Ministry of Agriculture of the Czech Republic QK1910070 and University of South Bohemia GAJU 027/2019/Z.

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PLASMA-DERIVED NITROGEN-SPECIES: FERTILIZERS OR SIGNALING FACTORS IN PLANT GROWTH AND DEVELOPMENT?

Henrike Brust, Nicola Wannicke, Thalita Nishime, Robert Wagner, Stefan Horn, Jürgen Kolb, Thomas von Woedtke, Klaus-Dieter Weltmann
Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str. 2, 17489 Greifswald Germany
Email: henrike.brust@inp-greifswald.de

Reactive nitrogen species (RNS) comprise radicals (e.g. NO, NO_2•, NO_3•) and non-radicals ($ONOO^-$, HNO_2, N_2O_4) that have, depending on the specific chemical and physical environment, different half-life times and chemical conversion characteristics. Significant amounts of RNS can be found in cold atmospheric pressure plasma (CAPP). The type of excitation source (e.g. low frequency electrical energy, microwave radiation), plasma parameters (e.g. frequency, duty cycle) and the feeding gas composition (air, noble gases with gas mixtures containing nitrogen) are important parameters for quality and quantity of RNS occurrence within CAPP. Treatment of fluids (e.g. water) with CAPP containing RNS leads to a time-dependent deposition of nitrogen compounds into the liquid phase and a conversion to energy-stable nitrogen ions such as nitrite (NO_2^-) and/or nitrate (NO_3^-). The interaction of plasma-derived RNS with biological targets, especially with microorganisms and tissues from animals and human, have been studied and evaluated since many years. In the last decade, the positive effects of CAPP and plasma treated water (PTW) generated by CAPP on plant germination as well as on plant growth and developmental processes have been reported for different kind of plant species ranging from crops, herbs, vegetables to ornamental plants.

Gaseous and aqueous plasma-generated nitrogen-radicals can react with biomolecules located at the tissue surface layers of the target or can partially diffuse in sub-epidermal tissue layers and even enter cells. Low and high molecular weight molecules such as amino acids, nucleic acids, lipids or proteins undergo nitration and/or nitrosylation. Such chemical modification alters stability, activity and physiological function of the respective biomolecule and can provoke cellular responses resulting in physiological changes affecting stress adaptations and developmental processes of the whole plant.

Moreover, nitrate and nitrite have dual function in plants. Most plants can utilize nitrate and nitrite as a further nitrogen source, besides ammonia, for biomass production. In plants, nitrate and nitrite act as signaling components, too, for sensing the internal nitrogen status to adjust the C/N balance of primary metabolism and during stress adaptations. Thus, treatment of plants with CAPP containing nitrogen-species can have two functions: (a) fertilizing agents and (b) signaling component for plant growth and development.

SCALE-UP OF NON-EQUILIBRIUM PULSED DISCHARGE FOR SANITATION OF WHOLE AND CUT FRESH FRUITS AND VEGETABLES

Gregory Fridman[1*], Charles Bailey[1], Alexandr Blackbay[1], Jinjie He[2], Sidney Ortiz[2], Alexander Rabinovich[2], Alexander Fridman[2], Jasreen Sekhon[2], Christopher Sales[2]

[1] AAPlasma, LLC, Philadelphia, PA, USA

[2] Drexel University, Philadelphia, PA, USA

email: greg@aaplasma.com

Food safety is the ever-expanding global need. An important concern is the presence of bacteria and other pathogens on the surface of fresh produce. Plasmas, of course, are well-known for their strong antimicrobial properties. In the field of plasma medicine, a number of discharges have already been developed where plasma can successfully come in contact with living tissue, without damaging it, and achieve the desired rate of pathogen inactivation—usually within a few seconds of treatment. However, delivering plasma treatment to a 3-dimentional complex surface of foods, specifically of fresh produce, can be quite challenging: produce surface is complex and frequently multi-layered (e.g. a bag of spinach leaves), and the industrial processing rates are very high. For this reason, we have developed a plasma system to address this issue: plasma jet-like system where an air stream containing small droplets of water is passed through the discharge and onto the surface of produce. The key challenges, addressed in this talk, are the control of temperature of air and water passing through the discharge, and the resulting chemistry generated in the liquid and on the surface of produce. Mixing of plasma-treated water microdroplets with untreated air in the storage chamber is also a major issue with the scale-up and will be addressed in the talk.

For our validation experiments we utilize *E.coli*, *Salmonella*, and *Listeria* with Rifampicin-induced resistance. We coat Petri dishes with Rifampicin and thus are able to repeatably control and observe the rate of initial contamination of each fresh produce sample. As validation samples, packaged or open spinach, kale, lettuce, and strawberries are inoculated with up to 10^8 cfu (per sample). Samples are then placed into the misting chamber, as shown in Figure 1. Treatment times vary from 1 to 30 minutes in an 18” cubic chamber, simulating non-refrigerated storage. Following the treatment, the samples are placed in stomacher bags with 1 ml of PBS, stomached for 120 seconds, and 100 µl of this solution is plated on rifampicin-coated Petri dishes for overnight growth.

Figure 1. Photograph of 18”x18”x18” fresh produce fogging chamber with open and packaged whole strawberries and spinach being treated (the lasers are used to visualize the flow of plasma-treated water microdroplets).

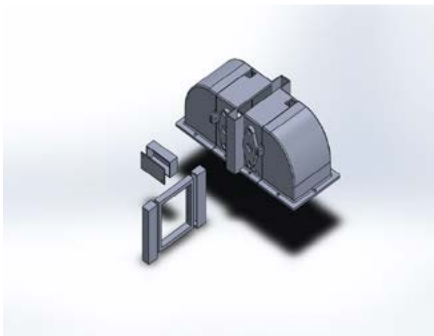

Figure 2. 3D rendering of the modular pulsed dielectric barrier discharge grid, nebulizers, and air recirculation fan.

Figure 2 shows a 3D rendering of the 10 Watt modular unit, developed for this project. Larger units with detailed schematics will be presented during the talk.

PLASMA ACTIVATED WATER AS NOVEL RESISTANCE INDUCER FOR PLANTS IN GREENHOUSE AND OPEN FIELD

Assunta Bertaccini[1], Enrico Biondi[1], Alessandro Canel[1], Vittorio Colombo[2,3,4], Nicoletta Contaldo[1], Matteo Gherardi[2,3], Romolo Laurita[2], Set Perez[1], Yuri Zambon[1]

Alma Mater Studiorum-*Università di Bologna, Bologna, Italy*
[1]Department of Agricultural Sciences, Plant Pathology, [2]Department of Industrial Engineering, [3]Industrial Research Centre for Advanced Mechanics and Materials.
[4]Interdepartmental Center for Agri-food Industrial Research.
E-mail: vittorio.colombo@unibo.it

In this work, an atmospheric pressure dielectric barrier discharge was used to produce plasma activated water (PAW). In order to evaluate the effectiveness of PAW as an alternative means to control plant diseases, three different phyto-pathosystems (i.e. plants infected by phytopathogens) were treated. Phytopatogens are involved in severe diseases affecting agronomic relevant crops. Management of these diseases has mainly focused on the use of copper compounds or antibiotics, on the control of insect vectors and on roguing infected plants. Since all these methods present some drawbacks, many efforts are devoted to novel control strategies. Plasma activated water (PAW) is presented as innovative and alternative possible management tool to control plant diseases due to phytopathogens, such as bacteria and phytoplasmas (insect-transmitted cell wall lacking bacteria), both involved in plant diseases. Sterile deionized water (SDW) was exposed to a nanosecond pulsed dielectric barrier discharge, inducing the production of nitrites (22±0,24 mg/l) and hydrogen peroxide and the reduction of pH (2,78±0,47). Three different phyto-pathosystem were investigated: tomato plants infected by *Xanthomonas vesicatoria* (Xv) [1], phytoplasma infected periwinkles (both in greenhouse), phytoplasma infected grapewines (in vineyards). Tomato plants ‘Moneymaker’ and ‘VF 10’ were individually grown in pots. The plants at 3°-4° leaf stage were uprooted, and the root apparatus was soaked for 10 min into PAW. 6 days after soaking, the tomato plants were experimentally inoculated with Xv and the disease severity was assessed 21 days after the inoculation. To evaluate the increase of phenylalanine ammonia-lyase (*pal*) gene (defense-related gene) transcripts by using RT-PCR. Phytoplasma infected and healthy periwinkles micropropagated shoots were exposed to PAW for about 25 minutes and a study of the expression of genes involved in plant defense response (phytoalexin metabolism) was performed. Parallel experiments were carried out treating grapevine plants in vineyards in which the phytoplasma presence was previously verified. Treatments were performed during three years by injecting into the vascular tissues of each selected plant (60 plants in total) 10-20 ml of PAW or SDW (as control). Quantitative RT-PCR analyses were carried out to determine the expression level of genes involved in the plant defense response.

Tomato plants treated with PAW and experimentally inoculated with Xv presented a significantly reduced disease severity, evaluated counting the number of leaf spots caused by the pathogen. Focusing on periwinkles micropropagated shoots, PAW treatment resulted in an overexpression of genes specifically involved in the phytoalexin metabolism. In the field trials, PAW-treated plants showed a reduction of symptoms. No phytotoxicity was observed in the PAW treated grapevine plants. The PAW ability to enhance some of the plant defense mechanisms improving the health status of the treated plants was experimentally demonstrated on three different phyto-pathosystem, both in greenhouse and open field conditions.

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LONG-TERM OBSERVATIONS AS A TOOL FOR ESTIMATION OF PLANT RESPONSE TO SEED TREATMENT WITH COLD PLASMA AND ELECTROMAGNETIC FIELD

Vida Mildaziene[1], Zita Nauciene[1], Rasa Zukiene[1], Laima Degutyte-Fomins[1], Asta Malakauskiene[1], Giedre Pauzaite[1], Anatolii Ivankov[1], Irina Filatova[3], Veronika Lyushkevich[3]

[1]Vytautas Magnus University, Lithuania;
[3]B. I. Stepanov Institute of Physics, National Academy of Sciences, Belarus

The effectiveness of pre-sowing seed treatment with low cold plasma (CP) and electromagnetic field (EMF) was reported for numerous plant species. In the majority of studies, these effects are estimated by changes induced in seed germination and early seedling growth. We performed long time observations of treatment effects on plant growth in the field that lasted from 2 to 5 years for perennials (Norway spruce, *Picea abies*; purple coneflower, *Echinacea purpurea* (L.) Moench; red clover, *Trifolium pratense* L.; Smirnov's rhododendron, *Rhododendron smirnowii* Trautv.) or 4-5 months for annual plants (common buckwheat, *Fagopyrum esculentum* and hemp, *Cannabis sativa L.)*.

The effects on plant height and number of branches for the wooden plant species Norway spruce and Smirnov's rhododendron persisted at least for 5 years. The best growth in the period of the first 3 years both for Norway spruce and Smirnov's rhododendron was observed in the treatment groups with the inhibited germination. However, in the 4th-5th year of growth situation have changed, so that the best germinated group (growing from seeds treated with vacuum and EMF) of Smirnov's rhododendron exhibited the most positive growth response. Five years after sowing Norway spruce seedlings from the vacuum treated group had the largest height (29% larger in comparison to control) although this treatment had no significant effect on germination; seedlings from CP treated groups (germination was inhibited) had were slightly (8%) higher; seedlings growing from EMF treated seeds (germination was stimulated) were 20% smaller in height in comparison to the control counterparts. Positive effects of CP and EMF treatments on the growth of the purple coneflower seedlings observed in the first vegetation season persisted in the second vegetation season, however, an increase in the amount of the phenolic acids was observed only in the first year. The effects of treatments with CP and EMF on morphometric parameters were dependent on the cultivar of red clover and were much stronger (from 24% to 49%) after growing in the field for 5 months in comparison to the effects observed on germination or in the early growth stage. However, in the second vegetation season morphometric parameters of seedlings growing from the treated seeds were not different from the control. Seed treatment with CP and EMF slightly (10-20%) suppressed germination of common buckwheat under field conditions, however the results of morphometric analysis performed after 4 months growth in the field have revealed substantial strong positive and cultivar-dependent effect on biomass (from 17 to 97%) and on the seed yield (from 17 to 85%). Both CP and EMF treatments strongly stimulated germination (by 25-32%) of hemp seeds, but morphometric analysis of plants grown in the field for 4 months have revealed that only EMF treatment induced positive changes in the height (+14%), weight of the above ground part (+66%) and number of influorescences (+70%) in the female plants, whereas CP treatments had tendency to slow down plant growth.

In summary, the obtained results show that effects of seed treatments with CP and EMF on germination and early seedling growth do not provide sufficient information to predict the impact on plant performance on a longer time scale. The effects of seed treatments on plant growth persist for the long time but their extent depends on the plant species and poorly correlates with the effects observed on germination kinetics and early seedling growth.

PLASMA APPLICATIONS FOR AGRICULTURE FROM SEEDS TO FIELD

Hiroshi Hashizume[1], Hiroko Mizuno[1], Akiko Abe[1], Kenki Tsubota[1], Genki Yuasa[2], Satoe Tohno[2], Mikiko Kojima[3], Yumiko Takebayashi[3], Hiromasa Tanaka[1], Kenji Ishikawa[1], Masafumi Ito[4], Hidemi Kitano[1], Shogo Matsumoto[1], Hitoshi Sakakibara[1], Takayuki Okuma[5], Susumu Nikawa[2], Masayoshi Maeshima[1], Masaaki Mizuno[1], and Masaru Hori[1]

[1]*Nagoya University, Japan*
[2]*Fujitsu Client Computing Co., Ltd., Japan*
[3]*RIKEN, Japan*
[4]*Meijo University, Japan*
[5]*Kota Town, Japan*
e-mail:hashizume@plasma.engg.nagoya-u.ac.jp

Recently, non-equilibrium atmospheric-pressure plasmas (NEAPPs) have much attention of the application in biological fields such as medicine and agriculture. We previously developed the ultrahigh electron-density plasma source, and showed that ovarian cancer cells were killed with the plasma treatment.[1,2] Besides, we prepared the plasma-activated Ringer's lactate solution (PAL) by irradiating the plasma to the solution. We showed that brain tumor cells were selectively killed by PAL treatment.[3] Those results showed that the both of direct and indirect treatments were effective for cancer therapy. On the basis of the results, we have further applied the plasma treatment to plants to the agricultural technology. In this study, we focus on the cultivation of rice and strawberry, and performed the plasma treatment on the different growth stages from seeds to plants in the field during the cultivation.

Firstly, we directly irradiated to the rice seeds by the ultrahigh electron-density plasma source. The early growth after germination was promoted with the physiological activation. And then, the growth-promotion effects were detected through later stage in the plants from the plasma-treated seeds, which were cultivated in the growth chamber. Next, we investigated the effects of NEAPP on the rice plants in the paddy field during the cultivation. After the transplant of seedlings in the field, we periodically treated the plants with the direct irradiation or indirect treatment with PAL solution. Under the optimum treatment, the traits of the plasma-treated plants were not only the growth promotion but also the increase and improvement of yield such as brown rice.

In addition, we periodically treated the strawberry plants with cold plasma in the greenhouse during the cultivation periods in two ways; the direct irradiation and the PAL solution. Both treatments produced various beneficial results from the plasma-treated area; the yield increase, and the increase of the antioxidant content in fruits. Our health can be improved by taking the antioxidants. Those results suggested that the plasma treatment with different approaches on the various plants according to the growth stages were effective, such as the yield increase and the quality improvement, to be expected as a new technology for the agricultural application.

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CHANGING THE PLANT TISSUE MORPHOLOGY AND PHYSIOLOGY BY PLASMA TREATMENT

Nevena Puač[1], Nikola Škoro[1], Suzana Živković[2], Milica Milutinović[2], Slađana Jevremović[2] and Zoran Lj. Petrović[1,3]

[1] *Institute of Physics-National Institute of Republic of Serbia, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia*

[2] *Institute for Biological Research "Siniša Stanković"-National Institute of Republic of Serbia, University of Belgrade, Bul. despota Stefana 142, 11060 Belgrade, Serbia*

[3] *Serbian Academy of Science and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia,*

email: nevena@ipb.ac.rs

In parallel with the plethora of biomedical applications that employ atmospheric pressure plasma systems, another field of plasma applications is growing – plasma agriculture [1]. Chemical species in plasma, with ample amounts of Reactive Oxygen and Nitrogen Species (RONS), are responsible for triggering various mechanisms and effects in plant cells. For example in treatment of seeds, the rich plasma chemistry changes the coat of the treated seed resulting in changes of wettability, better water uptake, an increased percentage and speed of germination [1-3]. Another application of atmospheric pressure plasmas is for treatments of plant calli. In biological research and biotechnology the plant callus (pl. calli) is induced from plant tissue and it forms growing mass of plant meristematic cells. Plant callus is widely used in plant biology both for basic research and industrial production: in plant biotechnology as a tool for genetic manipulation of plants, for micropropagation, for studies of plant metabolism and cellular development, commercial production of natural products that cannot be chemically synthesized etc.. Puač et al. have studied the influence of RONS on *Daucus carota* calli showing their long-term influence [4].

We have used plasma needle type of the atmospheric pressure plasma device for direct plasmas treatments of plant calli. The operational frequency of the device was 13.56 MHz and working gas was helium. The flow of helium was kept constant at 1 slm. The detailed characterization of the discharge was performed by optical emission spectroscopy, mass spectroscopy and electrical measurements. The direct plasma treatment of the plant calli was used to investigate the plasma-cell interactions and to follow the response of the plant tissue several hours and days after the treatment. We have used calli of model plant (*Daucus carota*) and of plants with specific issues, like small bearded irise (*Iris reichenbachii*) who mainly produce non regenerative calli that do not enter the process of somatic embryogenesis (SE). Firstly, in treatments of *D.carota* calli we found that the formation of SE can be initiated even under non- permissive conditions. Similar effects were obtained with *I. reichenbachii* calli where the atmospheric plasma treatment induced significant morphological and physiological changes in non-embryonic calli toward SE formation that were followed also with the enhanced production of arabinogalactan proteins.

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OVERVIEW AND FUTURE PROSPECTS OF PLASMA FARMING

Seong Bong Kim[1], Seungmin Ryu[1], Jaesung Oh[1]

[1]*Plasma Technology Research Center, National Fusion Research Institute*

email: sbkim@nfri.re.kr

'Plasma Farming' is the comprehensive plasma application to the entire agricultural phases from farm to table. It includes cultivation like plant farm and animal farm, postharvest, food safety, and even waste treatment. Since the plasma farming project began in 2014, it is now in the 2nd season. In the 1st season from 2014 to 2017, we mainly carried out case studies such as feasibility test of various plasma sources, seed treatments, microorganism inactivation, food storage, etc. Now we are investigating the core element technologies of plasma technology based systems like plant farm system, animal farm system, food storage system, and food safety system. There are many engineering and technical issues to develop the core element technologies. Furthermore, we have faced commercialization issues regarding as regulations, evaluation and standardization, which are necessary to provide technology based devices and systems to markets.

Here we would like to introduce the plasma farming project and show highlight results. The issues and challenges to be solved will be also discussed.

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DEGRADATION OF RESIDUAL AGROCHEMICALS BY NON-THERMAL PLASMA

Juergen F. Kolb[1], Katja Zocher[1], Peter Gros[2], Raphael Rataj[1], Veronika Hahn[1], Tilo Schulz[1], Michael Schmidt[1], Bruno Honnorat[1], Volker Brüser[1], Peter Leinweber[2] and Klaus-Dieter Weltmann[1]

[1]*Leibniz Institute for Plasma Science and Technology,*
Felix-Hausdorff-Strasse 2, 17489 Greifswald, Germany
[2]*Faculty of Agricultural and Environmental Science,*
University of Rostock, Justus-von-Liebig-Weg 6, 18051 Rostock, Germany
email: juergen.kolb@inp-greifswald.de

The introduction and rise of agrochemicals was essential for the necessary increase of farming yields. Fertilizers, herbicides and pesticides are in the meantime indispensable to supply ever growing populations and for an efficient exploitation of limited resources, in particular cultivable land. Concurrently, extraction methods and processing steps that often involve chemicals, e.g. solvents, contribute significantly to the production of affordable food and feed products. However, the reliance on agrochemicals for several decades now, has resulted in environmental problems and potential threads for consumers, which are today becoming more pressing and to the attention of the general public. Therefore, a paradigm shift is advocated that includes alternative methods for farming and food processing and in addition requires alleviating the environmental impact of agrochemicals [1]. Non-thermal plasma offer a potential solution for many of the respective problems. The strength of the different available methods is in particular the possibility to degrade and decompose also rather resilient chemical compounds that are often difficult or not sufficiently degradable by other means. Accordingly, plasma processes provide novel ways for environmental remediation and protection, including effluents from agricultural operations [2]. Especially the contamination of water poses an immediate challenge but also opportunity for plasma treatments. Based on our experience for the decomposition of pharmaceutical residues in water, we have investigated different plasma concepts and sources for the degradation of substances that are relevant for agricultural production [3]. These include geosmin, which due to its odor is detrimental to food quality, glyphosate (Roundup®), which is currently discussed rather controversial for its potential carcinogenicity, and perfluorocarbons (PFCs), which are sometimes found as a contaminant of fertilizers and in general responsible for rather problematic contaminations of soil. Depending on the pollutant, different strategies and approaches were developed for the treatment of water, such as submerged corona-, spark- or microwave generated plasma, or the application of pulsed dielectric barrier discharges and transient arc discharges at or along the water surface. The different methods will be presented and their respective advantages as well as the possibilities for a comprehensive water treatment for agricultural needs will be discussed. This includes in particular reaction mechanisms with different compounds. While oxidative processes, especially with hydroxyl radicals, are effective for the successive disintegration of many anthropogenic pollutants and their intermediates, PFCs are more efficiently destroyed by reduction reactions. However, both pathways can be provided by non-thermal plasma, depending on discharge configuration and operating parameters.

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PLANT PATHOGEN CONTROL USING DIRECT SPRAY OF SOLUTION CONTACTING PLASMA OR PLASMA EFFLUENT GAS

Toshiro Kaneko[1], Keisuke Takashima[1], Shota Sasaki[1]
[1] *Graduate School of Engineering, Tohoku University, Japan*
email: kaneko@tohoku.ac.jp

Atmospheric-pressure plasmas in contact with liquid, which are defined as "gas-liquid interfacial plasmas", are widely used in medical and agricultural fields. In the gas-liquid interfacial plasmas, various kinds of stimuli such as electrical, chemical, pressure, temperature stimuli, are generated and delivered to biological cells and tissues, and affect the biological responses. Among these stimuli, reactive species as the chemical stimuli are particularly important factors. Therefore, we have experimentally investigated the generation and reaction of the reactive species [1,2], especially short-lived reactive species, using several lab-build gas-liquid interfacial plasma devices for agricultural applications.

First, we have developed an air plasma exposed solution (PES) spray device (Fig. 1) for plant pathogen control [3] and induction of pathogen resistance [4]. The direct contact with the plasma in the PES spray device is expected to yield high concentration of the short-lived reactive species, which is transported to target objects in a short time. Five seconds PES spray is found to significantly suppress germination of a fungal conidium of strawberry pathogen (*C. gloeosporioides*). In addition, the germination suppression efficacy is not monotonically modulated by the gas and solution flow rates for PES generation. It is suggested that several short-lived reactive species, generated with control of the solution flow rate, contribute to germination suppression.

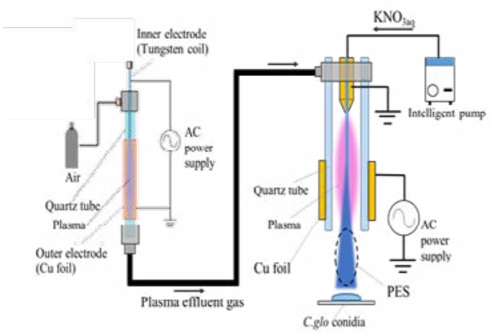

Fig. 1. Schematic of an air PES spray device.

In order to detect decay of the OH radical (one of important short-lived reactive species in PES), we try to introduce high-speed (~ 10 m/s) liquid flow in He plasma. This system gives the resolution of sub-milliseconds in the decay measurement of short-lived reactive species. Our preliminary result shows that the half-life of OH is estimated to be 55 **μ**s in our experimental system.

Second, an air plasma-effluent-gas dissolved solution (PEGDS) spray device (Fig. 2) has been developed. The PEGDS spray device relies on the dissolution of the plasma effluent gas into the distilled water for practical use in the farming field. Its performance is characterised by the conidium germination suppression effect on the plant pathogen and the reactive species generated in both gas and liquid phases. The results suggest that O_3 can be responsible for the germination suppression effect under the given operating condition, whose life-time is found to be shortened by the co-dissolved reactive species. This suggests that the O_3 dependent anti-bacterial effect can be tuned by the co-dissolved reactive species, which can balance the anti-bacterial effect, environmental release of the generated species, and plant responses.

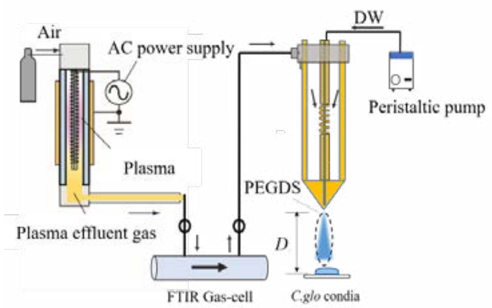

Fig. 2. Schematic of an air PEGDS spray device.

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SCALING PLASMA-LIQUID SYSTEMS – DIAGNOSTIC MEANS TO MEET THE NEEDS

S. Reuter[1,2*], B.M. Goldberg[3,2*], L. Hansen[4,2*], H. Kersten[4], S. Watson[1], B. Nisol[1], M.R. Wertheimer[1], A. Dogariu[2], and R.B. Miles[2,5]

[1]*Polytechnique Montréal, Engineering Physics Department, Montréal, Canada*
[2]*Princeton University, MAE Department, Princeton, USA*
[3]*Sandia National Laboratories, Combustion Research Facility, Livermore, USA*
[4]*Christian-Albrechts-University Kiel, Germany*
[5]*Texas A & M University, Department of Aerospace Engineering, College Station, USA*
E-mail: Stephan.Reuter@polymtl.ca

Non-equilibrium plasmas generate highly reactive species at low temperatures [1]. These plasmas have been widely studied for their application in medical therapy [2]. Plasmas for agriculture – other than plasmas in medicine – must provide high throughput to compete with existing agricultural techniques. They need to be efficient and cost effective. Scaling of existing plasma processes requires a fundamental insight into plasma liquid interaction [3]. Diagnostics of cold plasmas interacting with liquids are limited by high gradients of species' concentrations, multiphase transport processes, and sometimes turbulent interfaces of plasma, gas, and liquid phases [4]. Most conventional plasma diagnostics fail for non-equilibrium plasmas in these situations.

The talk will give an overview of scaling approaches for plasmas developed for agricultural application. It focuses on stochastic and interfacial processes that require dedicated diagnostic approaches to gain insight into real time events. For plasma at atmospheric pressure, most processes are influenced by either the flow [5], transporting reactive species, or by the electric field [6], initiating plasma chemical processes. Three approaches to measure low intensity stochastic events in flow and electric field on short time scales will be presented:

1) Time binning sorts measurements after recording, according to their jitter delay to increase the time resolution of fs-laser electric field measurements.
2) Advanced averaging results in an increased signal to noise ratio from procedures that average electric field measurements selected by an intensity threshold.
3) Space slicing allows to perform discretized 3D-measurements with multiple laser sheets guided into the volume that is measured.

Recording multiple signals for each event is paramount to get real time data and analyze stochastic processes in plasmas, building the basis to understand non-equilibrium energy transfer processes at atmospheric pressure and thus be able to scale plasma sources to the high throughput requirements of agricultural applications.

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CONTRIBUTED LECTURES

INVESTIGATION OF THE EFFICIENCY OF A DUAL MICROWAVE-RADIOFREQUENCY LOW-PRESSURE AIR PLASMA SOURCE ON FUNGAL AND MYCOTOXIN PROLIFERATION CONTROL

M. Soulier[1], T. Maho[1], C. Muja[1], Ph. Belenguer[2], J. Lo[1] and Ph. Guillot[1]
[1]*Laboratoire Diagnostics des Plasmas Hors Equilibre (DPHE), Université de Toulouse, INU Champollion, Albi, France*
[2]*Laplace, Université de Toulouse, UPS, INP, CNRS, Toulouse, France*
email: manon.soulier@univ-jfc.fr

Wheat represents one of the most cultivated crops in the world, widely consumed thanks to their facility to be transformed in starch or flour. However, its harvesting, transport and storage are key points that expose wheat to mold contamination and spoiling. Fungal contaminations have negative effects on production yields and also on human health [1]. Molds, such as *Aspergillus spp*., may produce mycotoxins during their metabolism process. Aflatoxins are carcinogenic mycotoxins, resistant to thermal or drying treatments during transformation processes [2]. Several studies in the literature showed fungal and aflatoxin reductions following non-thermal plasma treatments [3,4]. This work aims to evaluate the ability of a low- pressure air plasma to decrease mold contamination and aflatoxin production on wheat seeds.

Two plasma sources working on different ranges of frequencies have been associated and characterized to optimize seed treatment parameters. The first source is a 2.45 GHz Electron Cyclotron Resonance microwave plasma source (MW-ECR) placed at the upper part of the reaction chamber and able to sustain plasmas in a low-pressure range (10^{-2} – 10 Pa). The second one is a Coupled Capacitive Plasma (RF-CCP) 13.56 MHz radiofrequency source placed at the bottom of the reactor was added to enhance the MW-ECR plasma effect. An 85 liters reaction chamber filled with air at 1 Pa was used for plasma treatments.

Species identification and their spatial distributions were determined from OES (Ocean Optics HR2000+) and ICCD camera (Princeton Instruments PI-MAX) measurements. A linearly driven double Langmuir probe (Impedans ALP System) was used to calculate electron temperatures and densities. Measurements show ne variation from 10^{10} to 10^{11} cm^{-3} and Te value around 1 eV depending on experimental conditions. Gas temperatures, lower than 250°C, were also estimated by a K-type thermocouple. *Aspergillus spp*. native contamination on wheat seeds was assessed using standard plating technique [5]. Firstly, MW-ECR plasma efficacy as a fungal decontamination process was evaluated. Secondly, the contribution of a RF-CCP addition in the fungal decontamination has been investigated. In both cases, total aflatoxin concentration has also been quantified in order to assess the compound source potential in mycotoxin proliferation control.

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APPLICATION OF PLASMA ACTIVATED WATER ON MUSHROOMS

Erik Esveld[1], Bert Dijkink[1], Masja Nierop Groot[1],
[1]*Wageningen Food & Biobased Research, Bornse Weilanden 9, Wageningen, Netherlands*
email: erik.esveld@wur.nl

Listeria monocytogenes has become a growing concern in fresh fruits and vegetables. Fresh commercially grown *Agaricus bisporus* mushrooms have not been linked to listeriosis outbreaks although presence *of L. monocytogenes* on (sliced) mushrooms and the production environment has been reported in various studies [1,2]. We have investigated the use of plasma activated water (PAW) as method to decrease the microbial load (total counts, *Enterobactericeae*) on mushrooms including the potential presence of *L. monocytogenes*. In the industrial setting, PAW treatment of mushrooms is most feasible in the crates, before cold storage and subsequent processing.
Plasma activated water was prepared with a laboratory unit that is described elsewhere [3,4]]. A batch of 500 ml demineralized water was exposed for 20min to diffuse swirling air plasma plume. RF pulse power was controlled at 120W and the water was maintained at room temperature via external cooling. The generated NO_x from the air plasma dissolve in the water phase and are oxidized to predominantly NO_2^- (262 mg/l) and NO_3^- (161 mg/l) [4]. The resulting pH is was 2.45±0.02 and the ORP 811±6 mV_H.
Submerging of whole mushrooms in PAW in the ratio 2 l/kg for 10 or 30min reduced both the total and entero microbial count by 2 and 3.5 log(CFU/g), respectively. Reduction of *Listeria* spp. naturally occurring on the mushroom was 1 log(CFU/g).
Next, rinsing of mushrooms in a crate was simulated with a 16 cm bed of mushroom, sprayed for max. 10 min with recirculating PAW in the reduced ratio 0.4 l/kg. PAW activity gradually diminishes, as the pH rises from 2.5 to 2.9 during recirculation The bacterial inactivation after 10 min varied from 2.5 log(CFU/g) at the top to 1 log(CFU/g) near the bottom, which is due to the channeling of the trickling fluid.
The mushrooms do absorb 5-7% of PAW depending on the treatment duration. Nitrite levels in the mushroom before and after PAW treatment were measured and the implications will be discussed in the presentation.

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RADICAL PRODUCATION AND TREATMENT OF NUTRIENT SOLUTION USING PULSED DISCHARGES CONTACT WITH WATER SURFACE

Katsuyuki Takahashi[1,2], Koichi Takaki[1,2]
[1] *Faculty of Science and engineering, Iwate University*
[2] *Agri-Innovation Center, Iwate University*

email: ktaka@iwate-u.ac.jp

A pulsed discharge plasma in water and in contact with water has been attracting significant attention as a promising technology in various fields such as agriculture and environmental remediation. The pulsed discharge plasma enables the instantaneous production of a non-thermal plasma with various chemical species such as hydroxyl radical. Since hydroxyl radicals are a powerful oxidizing agent, they play an important role in the decomposition of chemical compounds and the sterilization of bacteria in water [1], which contribute to improve the plant cultivation environment [2]. In this study, sterilization and decomposition of 2,4-dichlorobenzonic acid (DCBA) in hydroponic nutrient solution using discharge inside bubble is investigated. Air gas is injected into water through a vertically positioned glass tube, into which high voltage wire electrode is placed to generate plasmas at low applied voltage. A magnetic pulse compression circuit is used to generate high voltage pulses. The amount of radical production by discharges are evaluated using indigo carmine solution used as a chemical probe. The nutrient solution is contaminated with *Ralstonia solanacearum*, a plant pathogenic bacterium, or DCBA. The number of colony forming unit of the *Ralstonia solanacearum* and concentration of DCBA decreased after the treatment. After discharge treatment, tomato or cucumber plants are cultivated using the nutrient solution treated by the discharges, and The plant height is evaluated as the growth rate of the plants. The results show that the growth of almost plants is inhibited by the bacteria or DCBA without discharge treatment; in contrast, the plants with discharge treatment grow healthy.

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CHARACTERISTICS OF HYDROGEN PEROXIDE GENERATION IN PIN-TO-WATER DISCHARGE SYSTEM

Sung-Young Yoon[1], Yong-Seong Byeon[1], Eun Jeong Hong[1], Junghyun Lim[1], Sangheum Eom[1], Seongbong Kim[1] and Seungmin Ryu[1]

[1] *Plasma Technology Research Center, National Fusion Research Institute, 37, Dongjangsan-ro, Gunsan-si, Jeollabuk-do, Republic of Korea*

email: smryu@nfri.re.kr

We demonstrated the H_2O_2 contained plasma treated water(PTW) generation by using pin-to-water discharges in spoke-like electrodes. The mechanism of H_2O_2 generation is studied by measurements and simulations on the plasma and PTW properties. The measured H_2O_2 concentration in PTW shows non-linear, i.e. peaked density rather than monotonic increase and saturation. The decrease of H_2O_2 density during the plasma treatment implies the change of plasma generating chemical species rather than H_2O_2 decay, since the life time of H_2O_2 in the liquid is number of days[1].

The plasma generating chemical species largely changed by gas temperature, especially vibrationally excited temperature of N_2[2]. The gas temperature is the key parameter for H_2O_2 concentration control knob. The gas temperature can be controlled by using various factors: power density, PTW temperature, and discharge time[3]. One can obtain high H_2O_2 concentration while the T_{vib} maintained under 4900 K. When the discharge system shows non-saturating gas temperature before H_2O_2 density saturation, discharge should be stopped when the H_2O_2 concentration reached to the necessary value.

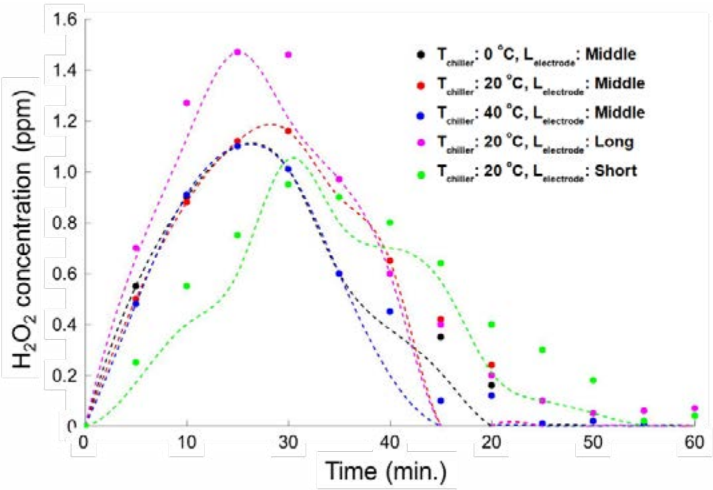

Figure 1. Measured (dot) and simulated (dashed line) H2O2 concentration in PTW for plasma treatment conditions. The short, middle, and long electrode lengths indicate the 34, 36, and 38 mm of electrode length, respectively

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EFFECTS OF COLD PLASMA TREATMENT ON SEED GERMINATION AND SEEDLING GROWTH OF TURKISH TEA

T. Çoruhlu[1], E. E. Akkaya[2], Kenan Şentürk[3], T. Şen[4]
[1] *İstanbul Yeditepe University, Turkey*
[2] *İstanbul Üsküdar University, Turkey*
[3] *İstanbul Gelişim University, Turkey*
[4] *İstanbul Beykent University, Turkey*

email: tcoruhlu@gmail.com

The effects of cold atmospheric-pressure plasma jet (CAPPJ) were investigated on germination and seedling growth of tea (*Camellia sinensis* L.) seeds. The experiments were carried out at the northeastern Black Sea region of Turkey during the year 2017-2018 under field conditions. The seeds were exposed to various durations of air plasma treatment using a home-made CAPPJ (18 kV, 12 kHz). Seeds were pre-treated for treatment durations of 2, 4, 6, 8, and 10 minutes. The preliminary results of ongoing study indicate that cold plasma treatment might promote the growth and modify the speed of germination. Plasma treated seeds (PTS) started to sprout 10 days earlier than the control group. Plasma treated seedlings were 3 times longer than the untreated controlled group. By using micro-Kjeldahl Method, it was found that nitrogen percentage of PTS increased by more than 75%. Wettability and surface properties were also examined by contact angle measurements, and Scanning Electron Microscopy (SEM).

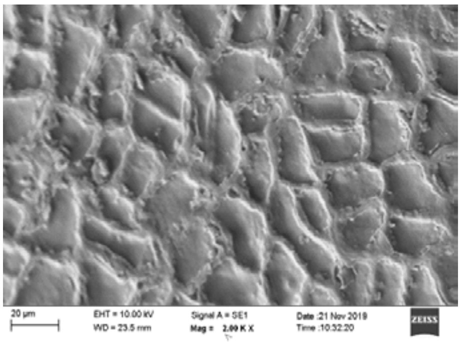

Figure: SEM image of the surface of the tea seed

This work is the first study on atmospheric cold plasma treatment of Turkish tea. Kuloba et al. [1] used DBD nitrogen plasma to study its effect on the polyphenol content in withered green tea leaves. Matan et al. [2] studied the combined effect of cold plasma and green tea extract on pathogens of fresh-cut dragon fruit. Amini and Ghoranneviss [3] found that plasma significantly reduced the population of different kind of microorganisms. Hamajima et al. [4] have studied the effect of plasma treatment on flavor components of the tea powder after plasma treatment.

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ELECTRON SPIN RESONANCE STUDY ON GERMINATION DYNAMICS OF PLASMA-ACTIVATED SEEDS OF RADISH SPROUTS

Kenji Ishikawa,[1,*] Ryo Arita,[1] Ryoya Sato[1], Hayate Tanaka[1], Masaya Hiromatsu[1], Tomoaki Yoshida[1], Pankaj Attri[1], Kunihiro Kamataki[1], Naho Itagaki[1], Daisuke Yamashita[1], Kayo Matsuo[1], Kazunori Koga[1,2], and Masaharu Shiratani[1]

[1] *Kyushu University, Japan*

[2] *National Institutes of Natural Science, Japan*

e-mail: ishikawa@plasma.kd.kyushu-u.ac.jp

Seed germination and growth enhancement of seeds, which were treated by nonthermal atmospheric pressure plasma, were reported by several research groups.[1-3] This is conceived a potential solution of declining world crop yields. In our previous study, we demonstrated that the germination and growth processes were enhanced 11% faster in the air plasma irradiation to seeds, as compared with that of the untreated seeds of *Arabidopsis thaliana*.[4] However, the mechanisms of these enhancements have not comprehensively clarified yet. Therefore, we investigated early germinating processes, such as water uptake, imbibitional swelling, metabolic activation, seedling germination and sprouting, using physicochemical analyses. In particular, electron paramagnetic centers in seeds of radish sprout *Raphanus sativus L.* were dynamically monitored during the germinating process of the plasma-activated seeds.

Samples were seeds of *Raphanus sativus L.*. The samples were first graded in sizes of 3.0-3.2 and 3.2-3.4 mm using a punched-hole mesh. Then, all the seeds were weighed and sorted according to their weights. The graded samples were divided into two groups and each was used to prepare for the untreated and treated seeds. The plasma treatments were typically conducted using the air dielectric barrier discharge (DBD) for 3 min.[1] The seeds planted each in one well of 96-well microtiter plates were incubated in a chamber at 22°C with both dark and light conditions. The germination was observed using a time-lapse camera. Seed's organic radicals were analyzed by electron spin resonance (ESR) technique, and measured at temperatures below 0°C, preventing dielectric losses for water imbibition in the seeds.

The air DBD treatments of the seeds enhanced to germinate. The result of kinetics of the seed germination is, as an example, shown in Figure. The untreated seeds had ESR signals for singlet (S=1/2) with an asymmetric shape around g-values of 2.03. This ESR signal intensity was changed by the water imbibition. The signal was monitor during the incubation for germination and sprouting. The ESR signals might be identified to organic radical such planar forms of $CO_2^{\,-}$ or phenol [5], and a further identification is required. Summarized all the results, we conclude that the air DBD treatment activates seeds of radish sprout, resulting through interactions between the absorbed water and the residual radicals.

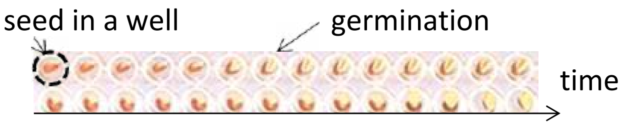

FIGURE 1. Kinetics of germination of plasma-activated seeds of radish sprout *Raphanus sativus L.*

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PLASMA FUNCTIONALIZED WATER AND AIR: FROM BECH TO PROTOTYPE FOR FRESH FOOD SAFETY

Jörg Ehlbeck[1], Mathias Andrasch[1], Jörg Stachowiak[1], Oliver Schlüter[2], Paula Bourke[3], Uta Schnabel[1, 3]

[1]*Leibniz Institute for Plasma Science and Technology, Greifswald, Germany*

[2]*Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany*

[3]*College of Sciences and Health, Technological University Dublin, Dublin, Ireland*

email: ehlbeck@inp-greifswald.de

The development of technical processes for food production have to face the challenge of processing natural raw materials characterized by a huge variety of their natural parameters. Therefore, only large sample numbers allow statistical significant results. To exacerbate the situation treatment conditions and procedures are often dependent on sample numbers. Thus, upscaling of experiments is an essential need to transfer new technologies to industry.

Over the past few years several procedures based on functionalized water and air have been scaled up successfully. To illuminate this process in more detail the example of the treatment of fresh-cut produce is selected. Further examples of scaling are only briefly outlined.

Fresh-cut produce like lettuce may contain a very high microbial load, including human pathogens. Therefore, the need for antimicrobial agents at post-harvest stages to prevent microbial growth is evident. Sanitation steps based on non-thermal plasma (NTP) opens up innovative food processing possibilities by application at different points along the food chain; for production, modification, and preservation, as well as in packaging of plant- and animal-originated food. Plasma differs from the gaseous state of matter by a certain amount of free charge carriers caused by ionization processes of the gas atoms and molecules due to the supply of energy.

This talk describes innovations resulting in a pilot scale fresh produce processing system based on cold plasma functionalized water [1]. The primary focus was on antimicrobial efficacy in line with the importance of food safety to the fresh produce processing sector. The treatment of natural products with changing parameters (size, surface, water content) is challenging for the design and optimization of cold plasma processes. To overcome these challenges, a specific plasma process based on microwave plasma operated with compressed air was established to deliver plasma processed air (PPA) as the antimicrobial agent to process water. This served to functionalize the water (PTW) with antimicrobial properties.

To successfully scale up the application, an understanding of the antimicrobial properties, the chemical composition of PTW and the food quality characteristics of color and texture was developed. The optimized PTW production and decontamination process was implemented into pilot-plant scale lettuce-washing thus demonstrating the industrial scalability and applicability.

Acknowledgment

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CONTROLLING *CAMPYLOBACTER SPP* AND *SALMONELLA SPP* CONTAMINATION IN POULTRY PROCESSING USING COLD PLASMA

Soukaina Barroug[1], Marta C. Carazo[2], Krishna S. Kumar[1], Dana Ziuzina[1], Bourke Paula[1, 3]

[1]School of Food Science and Environmental Health, Technological University Dublin, Ireland
[2]Department of Food and Agricultural Equipment Engineering, Institute of Plant Biotechnology, Polytechnic University of Cartagena (ETSA), Cartagena, Spain.
[3]School of Biological Sciences, IGFS, Queens University Belfast, Northern Ireland, UK
(12 pts, italic, centered)
email: Paula.Bourke@tudublin.ie, soukaina.barroug@mytudublin.ie

Campylobacter jejuni and *Salmonella spp.* are leading causes of outbreaks of foodborne disease globally, which is often attributed to the consumption of contaminated poultry products. Both pathogens employ biofilms as a survival mechanism under environmental stress, which facilitates persistence in food processing and cleaning in place practices. Cold plasma (CP) is an emerging non-thermal technology successfully demonstrated for decontamination and preservation of an increasing range of foods. Plasma functionalized liquid and gas plasma are two modes of delivery of atmospheric cold plasma (ACP) under investigation for application in food processing. The advantages of CP are operation at low temperatures, compatibility with heat sensitive materials, short processing times, energy efficiency, and demonstrated antimicrobial efficacy. This study investigated the antimicrobial effect of Plasma Functionalized Water (PFW) against planktonic cells of *Campylobacter jejuni* and *Salmonella enterica* Typhimurium. Deionized water was functionalized by treatment with different CP devices, namely the reactive species specificity (RSS) pin system comprising spark or glow discharge and the microwave discharge based MiniMip system. PFW was prepared by separate and/or sequential operation of the Spark or Glow discharge. Process parameters included PFW Generation Time (GT) ranging from 20-35 min, plasma discharge mode, and PFW Contact Time (CT) 0-20min with the target. Using the MiniMip system, process parameters explored used were liquid treatment volume, ranging from 100 ml to 600 ml and treatment times of 10 to 15 min. The volumes of liquid exposed to the plasma-processed air over different GT have analyzed to detect the impact on *S.* Typhimurium inactivation aligned with reactive oxygen and nitrogen species concentrations within the PFW. Results demonstrated that PFW generated by RSS was effective against both *C. jejuni* and *S.* Typhimurium*.* Inactivation efficacy was a function of GT, CT, and the type of challenge microorganisms. Combining spark and glow discharge treatment enhanced pathogen inactivation. Within short CT, reductions of up to 6 $\log_{10}$CFU/ml were achieved depending on the parameters applied.

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THE EFFECT OF PLASMA TREATMENT OF CROP SEEDS ON PLANT GROWTH AND DISEASE RESISTANCE DURING VEGETATION

Irina Filatova[1], Veronika Lyushkevich[1], Svetlana Goncharik[1], Alexander Zhukovsky[2], Natalia Krupenko[2], Joanna Kalatskaja[3], Vida Mildaziene[4], Giedre Pauzaite[4]

[1] *B.I. Stepanov Institute of Physics, National Academy of Sciences of Belarus,*
[2] *RUE «Institute of plant protection», Belarus*
[3] *V.F. Kuprevich Institute of Experimental Botany, National Academy of Sciences of Belarus*
[4] *Vytautas Magnus University, Lithuania*
email: filatova@presidium.bas-net.by

The effect of pre-sowing plasma seed treatment of maize (*Zea mays* L.), narrow-leaved lupine (*Lupinus angustifolius* L.) and winter wheat (*Triticum aestivum* L.) on seed health and germination, as well as plant resistance to common diseases during vegetation was studied in laboratory and field experiments.
Plasma treatment was *performed* in a planar geometry capacitively coupled 5.28 MHz plasma reactor in ambient air at a pressure of 200 Pa. The input power density was 0.025 W/cm^3. The treatment duration was 2, 4, 5 and 7 minutes. In the field tests, a plot area of 25 m^2 used for each investigated species. We evaluated pathogenic diseases at different plant stages assessing the phytosanitary state of plants according to the indices of disease development and spread of disease.
The results of laboratory tests showed that plasma seed treatment contributed to a decrease in contamination of maize seeds with *Penicillium* spp. by 20.7% also positively affected germination and the length of the sprout. Various pathogenic fungi have been detected on lupine seeds in control including *Cladosporium* sp., *Alternaria* as well as causing anthracnose fungi *C. gloeosporioides* and *K. caulivora*. No *C. gloeosporioides* and *K. caulivora* fungi were observed on seeds after plasma treatment. Plasma treatment was effective also in suppressing *Cladosporium* sp. and *Alternaria* sp. by 67% and 28% respectively. Plasma treatment reduced contamination of winter wheat seeds with *Alternaria* spp. and *Fusarium* spp.
It is shown that plasma treatment is an effective tool in suppression of a number of fungal crop diseases such as boil smut of maize, root rot of lupine and winter wheat at different growth stages. At the stage of V9 (9th leaf visible) the infection level in maize plants from treated seeds was 3 times less than that in control. Root rot disease development of lupine at the first stages (3rd-4th leafs emerged) of growth did not exceed 6.9% in plants from the treated seeds while reached to 47.8 % in control. Pre-sowing seed treatment led to suppress the anthracnose spreading on narrow-leaved lupine up to the flowering stage, and the infection level remained much lower than in control even at green bean stage. The anthracnose fungus is seed-borne and infected seed is the main source of inoculum dispersal and disease outbreaks. Thus, the observed absence of the anthracnose spread in lupine crops can be associated with a good antifungal effect of pre-sowing plasma treatment against anthracnose causing fungi *C. gloeosporioides* and *K. caulivora.*
It was revealed that due to a decrease in the level of seed infection, stimulation of field germination, early seedling growth and plant resistance to pathogens during the vegetation period, the grain yield of all investigated crop species increased compared to control plants. An increase in activity of non-enzymatic antioxidants (proline, anthocyanins as well as total phenolic content) in roots of maize seedling were observed which may indicate a significant role of plasma seed treatment in improving the plant resistance to biotic and abiotic stress during the vegetation.

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AIR PLASMA TREATMENT EFFECTS ON SEEDS PHYSIOLOGY, GERMINATION AND SEEDLING.

Paolo F. Ambrico[1], Milan Šimek[2], Marianna Ambrico[1], Massimo Morano[3], Václav Prukner[2], Angelantonio Minafra[2], Ignazio Allegretta[3], Carlo Porfido[3], Giorgio S. Senesi[3], Roberto Terzano[3]
[1] *C.N.R., Istituto per la Scienza e la Tecnologia dei Plasmi, Bari, Italy*
[2] *C.A.S., I.P.P. v.v.i., Department of Pulse Plasma Systems, Prague, Czech Republic*
[3] *Department of Soil, Plant and Food Sciences, University of Bari ALDO MORO, Bari, Italy*
[4] *C.N.R. , Istituto di Protezione Sostenibile delle Piante, Bari, Italy*
email: paolofrancesco.ambrico@cnr.it

The need of environment-friendly strategies for food production and plant protection makes the use of plasma-assisted technologies in agriculture as an innovative practice [1]. The non-equilibrium character of low temperature plasmas enables the production of reactive oxygen and nitrogen species (ROS and RNS) efficient in the decontamination, sterilization and chemical modification of surfaces. On seeds, the plasma treatment affects the basic physiological processes improving germinability and biometric parameters of plantlets [2].

We focused on the effect of plasma treatment on the morphology of seeds, germination and radicle development, either by using imaging techniques such as high-resolution computed X-ray microtomography (μXCT) or by following the development of seeds germination in vitro and in an artificial soil-like substrate. Since seeds germination relies mainly on the accumulation of mineral macronutrients (such as N, K, Ca, Mg, P, and S) and micronutrients (such as Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni, generally in the range of ppm) in certain seed tissues [3], X-ray micro-fluorescence (μ-XRF) was used to investigate the localization of mineral elements in seeds before and after plasma treatment [4].

Our results show that plasma treatment changed neither seed structure nor morphology. The increase of the germination rate was observed together with better developed root apparatus and improved biometric parameters. Moreover, we observed that macro- and micro-nutrients concentrated in the radicle, moving from the endosperm and the cotyledons. Redistribution of these elements could explain the faster germination of the plasma-treated seeds, being a similar process as generally observed in seed tissues during germination. The plasma treatment is therefore somehow anticipating and implementing the mobilization of key-nutrients towards the radicle, resulting in faster and higher germination of the seeds as well as improved characteristics of the plantlet, especially at the root level.

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STIMULATION OF NATURAL SWEETENERS BIOSYNTHESIS IN STEVIA REBAUDIANA (BERTONI) BY DIELECTRIC BARRIER DISCHARGE AND CAPACITIVELY COUPLED COLD PLASMA

Rasa Žūkienė[1], Veronika Lyuskevich [2], Irina Filatova[2], Kazunori Koga[3], Masaharu Shiratani[3], Vida Mildažienė[1]

[1] *Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania*

[2] *B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, Minsk, Belarus*

[3] *Kyushu University, Fukuoka, Japan*

email: rasa.zukiene@vdu.lt

Seed treatment with non-thermal or cold plasma (CP) improves agricultural performance of crops, leads to stimulation of germination, biomass production, and disease resistance. However, a detailed molecular mechanism of mentioned effects is still missing, preventing the possibility of controlled modulation of plant. Besides of improved adaptivity and higher yield of treated plants, the quality of plant production and the concentrations of valuable secondary metabolites can be increased by CP.

The aim of this study was to determine the effect of *Stevia rebaudiana* (Bertoni) seed treatment (2, 5, 7 min) with dielectric barrier discharge (DBD) and capacitively coupled (CC) CP on the amount and ratio of stevioside (Stev) and rebaudioside A (RebA) in the leaves of stevia. Stev and RebA are the most abundant steviol glycosides (SGs) responsible for the sweetness of stevia and nowadays widely used as natural sweeteners. As compared to Stev, RebA has an additional glucose monomer that gives it a higher sweetening potency and therefore is the most preferred component of the stevia leaf extracts. RebA also lacks the bitter aftertaste characteristic to Stev.

Both types of CP had strong stimulating effect on steviol glycosides (SGs) in stevia leaves 8 weeks after germination. CC CP increased the RebA concentration 1.5-fold and the concentration of Stev 7-11-fold depending on treatment duration. The optimal 2-min pre-sowing seed treatment with DBD CP increased the RebA concentration 2-fold, Stev - 14%, RebA+Stev - 37%, RebA/Stev ratio - 1.7-fold. The treatment of longer duration (5-7 min) had lesser effect than 2 min. However, the concentrations of other bioactive compounds as phenolics and flavonoids were drastically decreased by both types of CP resulting in lower antioxidant activity of stevia leaf extracts rich in SGs.

It can be concluded that a short time pre-sowing treatment of seeds with CP can be a powerful tool for the enhancement of biosynthesis/accumulation of secondary metabolites in plants and can have an economic benefit.

APPLICATIONS OF CHITOOLIGOSACCHARIDES PRODUCED IN BEAM-PLASMA REACTORS FOR PLANT GROWTH STIMULATION

Tatiana Vasilieva[1], Oscar Goñi[2], Patrick Quille[2], Shane O´Connell[2], Irina Naumova[3], Olga Galkina[3], Htet Ko Ko Zaw[1]

[1]*Moscow Institute of Physics and Technology*

[2]*Plant Biostimulant Group, Shannon Applied Biotechnology Centre, Institute of Technology Tralee*

[3]*Ivanovo State Agricultural Academy n.a. D.K. Belyaev*

email: tmvasilieva@gmail.com

Low molecular weight chitosan oligosaccharides produced by means of non-thermal Electron-Beam Plasma (EBP) were tested as plant growth stimulants and plant protectors. Beam-plasma reactors of new type (see also [1]) and controllable chitosan degradation in them are described. Water-soluble chitosan oligosaccharides of average molecular mass M_w ~ 1000 Da with polydispersity index 1.5 (hereinafter abbreviation COS designates these products) were obtained from crab shell chitosan of molecular mass 500 kDa in the EBP of oxygen. Plasma treatment only slightly increased the deacetylation degree from 90% of the original biopolymer up to 97%. Ten-minute chitosan treatment in the EBP under optimized conditions resulted in the yield (85% and even higher) of water-soluble COS, while the conventional chemical chitosan hydrolysis usually takes several days. Besides, no hazardous by-products and toxic exhausts or wastes are generated in beam-plasma reactors when the chitosan is processed.

The COS biostimulating activity was investigated on *Arabidopsis thaliana* (Col-0) that is conventionally used as a model organism in plant biology. The COS additives to the cultivation media Phytagel™ BioReagent (Sigma-Aldrich, Germany) in concentrations 0.25-1.0 % w/v significantly (up to 40% with respect to reference plants) intensified the root growth of *Arabidopsis thaliana* seedlings. Tomato plants (*Lycopersicon esculentum*, cv. Micro-Tom) and barley *Hordeum vulgare*, one of the most popular grain crop cultures, were selected for field tests. Foliar application of this formulation at 0.025% w/v on unstressed tomato resulted in up to 10% increase of a number of plant productivity indicators such as flower production or fruit yield compared to the untreated control.

Wetting the spring barley seeds sown into the soil by 1% water solution of the COS significantly enhanced the seeds germination. The germination increased from 79% (control seeds wetted by pure water) up to 91%. The germination of seeds treated by COS solution was observed 3 days earlier than that of the control seeds without COS application.

Application of the COS for wetting also increased the barley green mass. The surface leaf areas of control group of plants was 3.1 m^2 and 9.3 m^2 at the tillering and stem elongation phases respectively, while for grasses treated by COS these values were 4.7 m^2 and 11.6 m^2. Stimulation of dry biomass gain was observed during all barley vegetation period, however the most pronounced effect of the COS application (up to 45.8 g of biomass/100 g of plants) took place at the stem elongation stage.

Also, solutions of the COS in concentrations 500-1000 μg/mL inhibited the growth of various yeast-like and filamentous fungi (e.g. *Aspergillus flavus*, *Pemphigus betae*, and *Cladosporium herbarum*) responsible for damaging many agricultural crops.

Thus, the results of the study carried out demonstrate that plasma chemical technologies based on the EBP are promising for effective resources saving and environmentally friendly production of bioactive water-soluble COS for agricultural applications.

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PLASMA ACTIVATED WATER FOR AGRO

A.J.M. Pemen[1], T. Huiskamp[1], W.F.L.M. Hoeben[1], P.P. van Ooij[2], P.H.M. Leenders[2]
[1] *Eindhoven University of Technology, Electrical Energy Systems, The Netherlands*
[2]*VitalFluid, Nijmegen, The Netherlands*
email: a.j.m.pemen@tue.nl

Water can be activated by applying plasma in contact with the water. Plasma activated water (PAW) typically contains hydrogen peroxide, nitrates, nitrites, and peroxynitrite, and typically has a pH ranging from 2-4.The components of PAW and the low pH have proven synergistic antimicrobial effects against bacteria, biofilms, yeasts and other microorganisms.

In this contribution, we describe a system for the production of PAW at high yield and high production rate. A compact and solid-state dual resonant system has been realized and successfully demonstrated for industrial PAW production. The system generates up to 80 kV dual-resonant high-voltage pulses with an oscillation frequency of 1 MHz, pulse rep-rate up to 20 kHz and an average power of 300 W. The paper describes a detailed comparison of various topologies, a detailed design procedure, simulations and overview of the practical realization and verification of the power modulator. The system is able to activate around 100 L/hour.

In addition, we will give an overview of several pilot demonstrations of promising applications of PAW. In agro applications, such as nitrogen fixation for fertilizer production, and the prevention of plant disinfections in horticulture.

COLD PLASMA FOR MICROBIOLOGICAL AND INSECT PEST CONTROL IN CEREAL GRAIN PRODUCTION

Agata Los[1], Dana Ziuzina[1], Daniela Boehm[1], Patrick J. Cullen[1,2], Paula Bourke[1,3]
[1] *School of Food Science and Environmental Health, Technological University Dublin, Ireland*
[2] *School of Chemical and Biomolecular Engineering, University of Sydney, Australia*
[3] *School of Biological Sciences, Institute for Global Food Security, Queens University Belfast, Northern Ireland, UK*
email: Paula.Bourke@TUDublin.ie; Dana.Ziuzina@TUDublin.ie

The potential of Atmospheric Cold Plasma (ACP) for controlling microbiological and insect pest contaminants of wheat grains was investigated. The evaluations were performed using dielectric barrier discharge (DBD) system and a contained reactor treatment. The samples were treated for 0 - 20 min using direct and indirect modes of plasma exposure. The antimicrobial effect of ACP was tested against a range of bacterial and fungal contaminants, including strains isolated from wheat grains (Fig. 1A). Direct plasma treatment for 20 min was required to significantly reduce the concentration of all pathogens. The efficacy of ACP treatment decreased in the following order (maximal reduction factor, $\log_{10}$ CFU/g): *B. atrophaeus (3.88) > A. candidus (2.77) > P. citrinum (2.57) > P. chrysogenum (2.13) > A. flavus (1.95) > P. verrucosum (1.74) > A. niger (1.56) > B. atrophaeus spores (0.94).* Importantly, repeated sublethal plasma treatment did not induce resistance to ACP in either *B. atrophaeus* or *A. flavus*. Effect of ACP treatment on sporulation of *B. atrophaeus* during grain storage was also investigated. Insecticidal efficacy studies employed *Tribolium castaneum* at various developmental stages (Fig. 1B). To achieve 100% mortality the following treatment times were required: egg and young larvae - 30 s, old larvae and pupae – 60 s, adult – 300 s. This study demonstrates the potential of ACP for microbiological and insect pest control in cereal grain production and storage. Co-contamination studies are recommended to investigate the complex interactions between mycotoxigenic spoilage fungi and insects to assess ACP potential to achieve long-term safe storage of cereal grains.

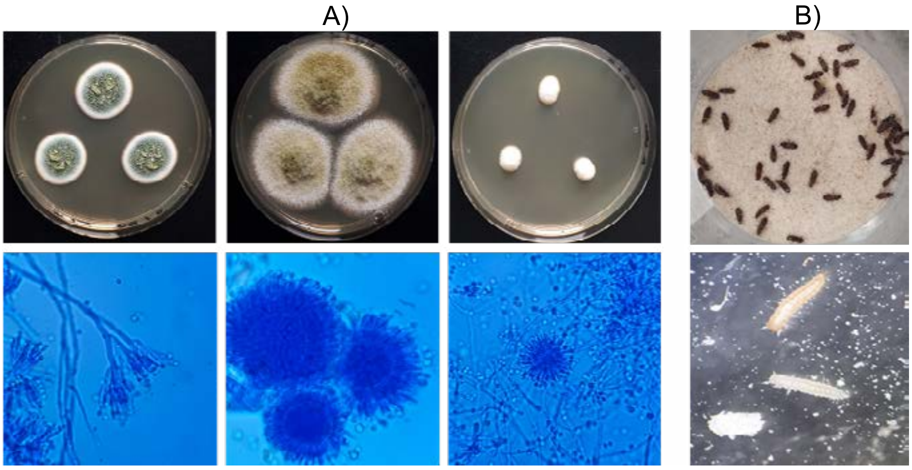

Figure 1. A) Wheat fungal isolates. Top panel – colonial morphology and bottom panel – optical microscopy images (magnification 1000 X); B) *Tribolium castaneum* . Top image – adult and bottom image – egg and larvae stages (magnification 4 X)

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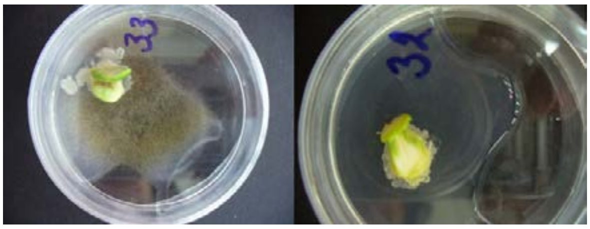
MICROWAVE ARGON PLASMA TORCH FOR AGRICULTURE APPLICATIONS

Plamena Marinova[1], Evgenia Benova[2], Violeta Bozhanova[3], Tzenko Vatchev[4], Ivo Yanashkov[4], Lilyana Nacheva[5], Snezhana Milusheva[5], Nataliya Dimitrova[5]

[1]Faculty of Forest Industry, University of Forestry, Sofia, Bulgaria
[2]DLTIS, Sofia University, Sofia, Bulgaria
[3]Field Crops Institute - Chirpan, Bulgaria
[4]N. Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection, Sofia, Bulgaria
[5]Fruitgrowing Institute, Plovdiv, Bulgaria
email: plamena_am@abv.bg

The microwave discharges of various types operating at atmospheric pressure usually produce non-equilibrium plasma that can be used for biological systems treatment. In many cases, the treatment should be carried out without thermal damage while providing a high active particles concentration and UV radiation to have for example efficient bactericidal effect. In the surface-wave-sustained plasma torch the treatment is in the active discharge zone. This results in high concentrations of short-lived active particles together with electromagnetic field and UV radiation at plasma-sample interface.

The plasma source for investigations presented in this paper is SWD operating at 2.45 GHz produced by surfatron type electromagnetic wave launcher in Argon at atmospheric pressure (plasma torch)[1]. In our case, we have produced Argon plasma torch with $T_g < 1000$ K and at some conditions even close to the room temperature [2,3]. The low gas temperature allows us to apply it for treatment of seeds, in vitro plants and for inactivation of pathogens in agriculture.



In vitro embryo culture of cherry treatment. Unsuccessful disinfection after 10 s treatment – left and successful disinfection after 10 s treatment – right

It has been observed that SWD Argon plasma is able to provide highest percentage of disinfection for very short treatment times. The main goal of the investigation is to gather experimental results about the effect of plasma treatment on wide range of biological systems. The cold plasma at atmospheric pressure treatment has been applied for decontamination of cherry embryos upon in-vitro culture or for seed decontamination prior to planting and to study the effect during the growth of the plants.

Acknowledgments:
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MICROWAVE DOWNSTREAM ATMOSPHERIC PLASMA FOR DISINFECTION OF LENTIL SEEDS

Saeedeh Taheri[1], Graham Brodie[2], Dorin Gupta[2], Mohan V. Jacob[3]
[1] *University of Melbourne, Parkville, Melbourne, VIC, Australia*
[2] *University of Melbourne, Dookie, VIC, Australia*
[3] *Electronics Material Lab, College of Science and Engineering, James Cook University, QLD*
email: saeideh.taheri@gmail.com

Non-thermal plasma has been proven to have great potential for seed treatments with the aim of seed germination enhancement or surface disinfection. Lentil is one of the important pulse crops in Australia, whose yield potential is threatened by some seed-borne diseases like Botrytis grey mould (BGM). These diseases cost an annual loss of $2.7 million in Australia [1]. Chemical seed treatment is often necessary to reduce seed to seedling transmission of this pathogen; however, there is a growing concern over the development of chemical resistance and issues with human health due to exposure to chemicals in the environment.

In a preliminary experiment, a downstream microwave plasma was used to treat BGM infected lentil seeds, using microwave powers of 100, 500 and 1000 W for exposure times of 0, 15 and 30 s. The plasma was created with Argon at 15 NL/min atmospheric pressure in a quartz tube with a diameter of 4 cm and a length of 40 cm. Two grams of lentil seeds were treated with non-thermal plasma by placed seeds 1 cm below the end of the tube at the exit of the plasma in a single-layer arrangement.

The result of the preliminary experiment showed that the BGM infected seeds percentage (IS%) reduced from 80% to 40% and germination of the seeds (G%) increased from 35% to 60% by applying microwave power of 500 W for 15 s. Germination of the lentil seeds reduced when applying microwave power of 1000 W for 30 s. Therefore, an experiment was designed based on a full factorial design to study the effect of factors including gas flow, gas type (argon and air), microwave power, time and lentil seed's moisture content on the seed pathogen and seed viability. This experiment is currently ongoing, and more results will be presented at the conference.

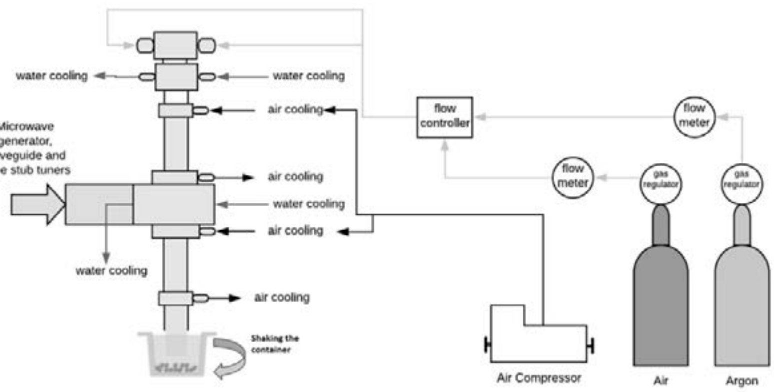

Figure 1 the schematic of the microwave driven plasma used for lentil seeds treatment

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POSTER

FUNGAL PLANT PATHOGENS: INHIBITORY EFFECT OF SURFACE DIELECTRIC BARRIER DISCHARGE PLASMA

Paolo. F. Ambrico[1] , Milan Šimek[2] , Caterina Rotolo[3] , Massimo Morano[3] , Angelantonio Minafra[4] , Marianna Ambrico[1] , Stefania Pollastro[3] , Francesco Faretra[3] , Rita M. De Miccolis Angelini [3]

[1] C*.N.R., Istituto per la Scienza e la Tecnologia dei Plasmi, Bari, Italy*

[2] *C.A.S., I.P.P. v.v.i., Department of Pulse Plasma Systems, Prague, Czech Republic*

[3] *Department of Soil, Plant and Food Sciences, University of Bari ALDO MORO, Bari, Italy*

[4] *C.N.R., Istituto di Protezione Sostenibile delle Piante, Bari, Italy*

email: paolofrancesco.ambrico@cnr.it

In the current global context, with an increasing number of undernourished people and worldwide food demand for the steady population growth[1], the correct and reasonable use of all the available plant protection measures is expected to play a key role in limiting agricultural yield losses caused by various plant diseases, during both the pre- and post-harvest production stages. Fungal diseases represent one of the main problems affecting the quality as well as quantity of agricultural production and food industry. Crop protection is usually achieved by the widespread use of synthetic fungicides. It is now recognized that an extensive use of such fungicides is associated with a range of environmental risks[2]. Innovative technologies, based on sustainability, human safety, and long-term eco-safety, are increasingly required. The atmospheric pressure low temperature plasma is becoming a suitable alternative/complementary tool[3]. In this contribution, we report on the effect of plasma treatment on important phytopathogenic fungi. Selected fungi cause quantitative and qualitative losses of agricultural production both in the field and postharvest. In detail, we focus our attention on the in vitro direct inhibitory effect of non-contact SDBD on spore germination of Botrytis cinerea, Monilinia fructicola, Aspergillus carbonarius and Alternaria alternata. Relatively short treatment time (few minutes) were required to completely inactivate the fungi on the growth medium. Morphological analysis of spores made by Scanning Electron Microscopy imaging suggests that the main mechanism is etching that could be attributed either to Reactive Oxygen Species or UV radiation, even though with a minor contribution, produced by plasma. Spectroscopic analysis of plasma generated in humid air provides an important evidence that the gas temperature of the active plasma layer should not play decisive role being very close to room temperature. In vivo experiments were also carried out on cherry fruits. The results demonstrate that inactivation of spores by direct inhibitory effect of surface DBD plasma is giving the fresh produce a shelf life longer that the artificially inoculated untreated one. Moreover, pre-treatment of fruit before inoculation improves the resistance to pathogen maybe activating the self-defense of the plant tissues.

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STUDY AND OPTIMIZATION OF COLD ATMOSPHERIC PLASMA TREATMENT FOR FOOD SAFETY AND QUALITY IMPROVEMENT (PLASMAFOOD)

Marco Dalla Rosa[1,2], Romolo Laurita[3], Vittorio Colombo[2,3], Pietro Rocculi[1,2], Carla Severini[4], Massimiliano Rinaldi[5], Sauro Vittori[6], Clemencia Chaves-Lopez[7], Massimo Mozzon[8], Dario De Medici[9]

Alma Mater Studiorum- University of Bologna, Italy

[1] Department of Agricultural and Food Science, [2] Interdepartmental Centre for Agri-Food Industrial Research, [3] Department of Industrial Engineering,

[4] Department of Sciences of Agriculture, Food and Environment – University of Foggia, Italy

[5] Department of Food and Drug, University of Parma, Italy; [6] School of Farmacy, University of Camerino, Italy; [7] Faculty of Bioscience and Agro-Food Environmental Technology, University of Teramo, Italy;[8] Department of Agricultural, Food and Environmental Sciences, Polytechnic University of the Marches, Italy; [9] Istituto Superiore della Sanità, Italy

email: romolo.laurita@unibo.it

Cold atmospheric plasma (CAP), which in the past has been mainly used in the medical field, has recently drawn considerable attention as a novel non-thermal technology for food product decontamination [1, 2]. Despite the high potentiality, there are still many aspects about CAP technology that are unknown or that need clarification, in particular regarding food quality, functional characteristics and potential toxicity of treated products [3]. A scientific assessment prior to authorisation to ensure the novel technologies safety is required by EFSA. The main objective of PLASMAFOOD project is to provide a deeper knowledge about aspects still scarcely investigated about CAP treatment of foods to fill the gaps identified in the literature and therefore promoting the application of this emerging novel technology. Five different categories of food products were chosen based on their different characteristics, different kinetics of quality degradation and different issues related to safety. In particular, food products are minimally process fruit and vegetables, semidried fruit, dry fruit, fish products and molluscs. The project is divided in 6 Work Packages, each one dealing with a specific aspect, such as processing, safety in relation to pathogenic microorganisms, moulds and mycotoxins and biogenic amines formation, quality in terms of colour, enzymatic activity, lipid oxidation and microstructure and nutritional issues related to bioactive components content, antioxidant activity and in-vitro effect on cell lines, that will be investigated through the collaboration of the five Research Units involved in different activities according to their specific expertise.

Acknowledgments: This work was in supported by Ministero dell'Università e della Ricerca (MIUR) PRIN 2017 project PLASMAFOOD

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PLASMA-ACTIVATED WATER - AN EMERGING AGENT FOR SPROUT PROCESSING

Mihai Mareş[1*], Andra-Cristina Bostănaru[1], Valentin Năstasă[1], Iuliana Motrescu[2], Eugen Hnatiuc[3]

[1] *Department of Public Health, Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine, Iași (Romania)*

[2] *Department of Biophysics, Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine, Iași (Romania)*

[3] *Department of Electrical Engineering, Gh. Asachi Technical University, Iași (Romania)*

email: mmares@uaiasi.ro

The sprouts are important sources of nutrients (proteins, vitamins, and minerals) and other health-maintaining compounds (polyphenols, glucosinolates, isoflavones, glutathione, sulforaphane etc.) [1]. They have been viewed as a functional food since many years and the sprout consumption increases constantly. Sometimes, including sprouts in the diet is not completely harmless as they have been associated with numerous outbreaks of foodborne illness mainly due to *Escherichia coli* O157:H7 and *Salmonella species*, and the challenge to obtain a safe product still persist [2].
Contaminated sprout seeds have been recognized as the most important source of pathogenic microorganisms, while the irrigation water is considered the second cause of contamination. Numerous practices have been proposed to control the sprout microbial contamination, from physical treatments to chemical interventions, but obtaining an important reduction of bacterial load and preserving the germination ability of the seeds is quite difficult to accomplish in the same time.
Taking into consideration our previous work on antimicrobial activity of plasma-activated water (PAW) [3], a new interventional method for sprout microbial quality assurance is proposed. This multi-target approach is based on both antimicrobial and germination enhancement properties exhibited by PAW. Both *Escherichia coli* O157:H7 and *Salmonella species* are highly susceptible to PAW with up to 6-7 log reduction after 5-10 minutes contact. Its effects depend on the concentration of oxygen and nitrogen reactive species - short-lived radicals like peroxynitrite ($ONOO^-$) and long-lived radicals like hydrogen peroxide (H_2O_2), nitrate (NO_3^-), nitrite (NO_2^-), and ozone (O_3). The most effective treatment is the decontamination of seeds before sprouting combined with another treatment before packaging. If the seeds are not treated, pre-existing bacteria can multiply during sprouting period and form biofilms that are more difficult to destroy using chemical interventions.
PAW represents a very promising agent for sprout processing, but in order to maximize its effects specific treatment conditions should be predetermined for each type of sprout seed.

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INVESTIGATION ON THE EFFECT OF NON-THERMAL DBD PLASMA ON RESIDUAL FLUDIOXONIL REDUCTION DEPENDING ON VARIOUS CONDITIONS

Sangheum Eom[1], Junghyun Lim[1], Hyeongwon Jeon[1], Jong-Seok Song[1], Jung Woo Yoon[1], Seong Bong Kim[1], Hyo Sub Lee[2] and Seungmin Ryu[1,*]

[1] *Plasma Technology Research Center, National Fusion Research Institute, Gunsan, Jeonbuk 54004, Republic of Korea*

[2] *Chemical Safety Division, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Jeonbuk 55365, Republic of Korea*

email: smryu@nfri.re.kr

In this study, effect of non-thermal atmospheric pressure (NTAP) dielectric barrier discharge (DBD) plasma on the reduction of residual fludioxonil was investigated. Fludioxonil is fungicide, which is inhibits the spore germination and stops mycelial growth []. Cylindrical DBD plasma source installed gas distribution system was adopted to produce the desired plasma activated chemical species (O_3). In order to evaluate the effect of plasma treatment on reduction of residual pesticide, experiment was performed according to three different conditions, such as treatment time and concentration of O_3, and surface roughness of microscope slide glass. Based on the results, plasma treatment is recommended as the conditions of 10 min treatment with O_3 concentration of 11.89 μL/L as shown in Figure 1.

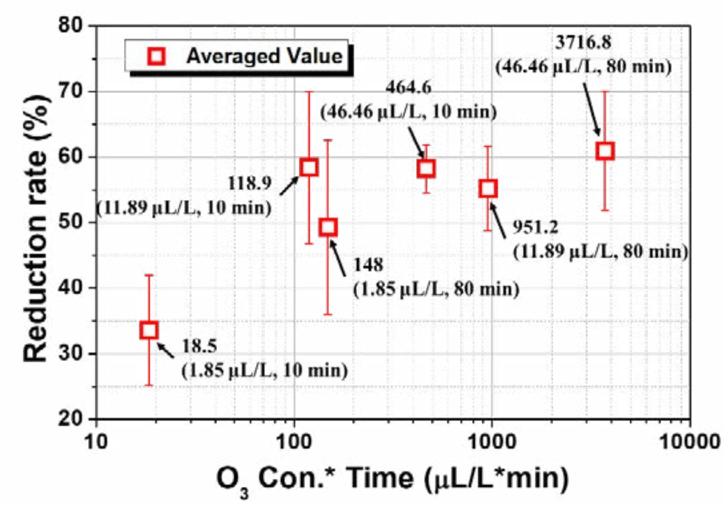

Figure 1 Averaged reduction rates of plasma treated fludioxonil according to the calculated CT value. Error bars mean standard deviation. ($P< 0.05$)

Acknowledgements

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INVESTIGATION OF THE CHEMICAL COMPOSITION OF PLASMA-TREATED WATER BY MIDIPLEXC AND ITS ANTIMICROBIAL EFFECT ON *L. MONOCYTOGENES* AND *P. FLUORESCENS* MONOSPECIES SUSPENSION CULTURES

Oliver Handorf[1], Harald Below[2], Uta Schnabel[1, 3], Jan-Wilm Lackmann[1], Katharina Riedel[4], Jörg Ehlbeck[1]

[1] *Leibniz Institute for Plasma Science and Technology (INP), Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany*

[2] *University of Greifswald, Institute for Hygiene and Environmental Medicine, FerdinandSauerbruch-Str., 17475 Greifswald, Germany*

[3] *Environmental Sustainability and Health Institute, Technological University Dublin, Greenway Hub, Grangegorman, Dublin 7, Ireland*

[4] *University of Greifswald, Institute for Microbial Physiology and Molecular Biology, Felix-Hausdorff-Str. 8, 17489 Greifswald, Germany*

email: oliver.handorf@inp-greifswald.de

Plasma generated products (PGC) conceived by microwave induced plasma (MidiPLexc) were physicochemical investigated in their molecular composition. Therefore, plasma treated water (PTW) was generated by treatment with the MidiPLexc within a 1 l glass bottle for a pre treatment time of 100 s, 300 s and 900 s. The PTW was further investigated in their composition of reactive oxygen/nitrogen species (RONS). For this purpose, the hydrogen peroxide concentration of the PTW was determined electrochemically using chronoamperometry, and the anionic components were determined using ion chromatography. As a result, hydrogen peroxide concentrations of up to 720 mg/l, nitrite concentrations of 1600 mg/l and nitrate concentrations of 72.3 mg/l could be detected after 900 s pre- treatment time of the water. The molecules obtained by ion chromatography were fractionated and subsequently confirmed by mass spectrometry. Once an overview of the chemical composition of PTW had been obtained, its effects on pathogens like *Pseudomonas fluorescens* and *Listeria monocytogenes*, which both are of particular importance in the food production industry, were tested. In that process, a maximum reduction in the colony forming units (CFU) of 4 log_{10} steps for *P. fluorescens* and 3 log_{10} steps for *L. monocytogenes* could be observed. The LIVE/DEAD assay showed a maximum reduction in the ratio G/R of 67 % for *P. fluorescens* and 38 % for *L. monocytogenes*. In addition, the XTT showed a maximum cell metabolism reduction of 96 % for *P. fluorescens* and 91 % for *L. monocytogenes*.

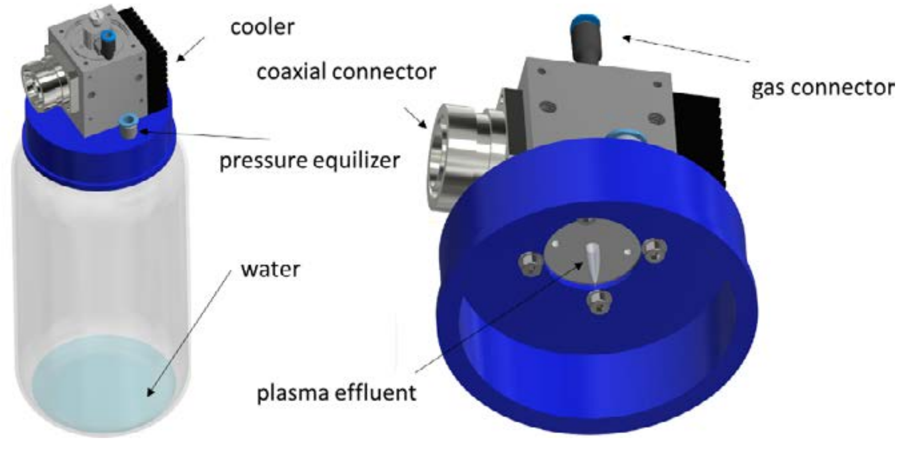

Figure 1: **Left: schematic illustration of the MidiPLexc with 1 l glas bottle attached to the bottle adapter of the plasma source. Right: schematic illustration of the MidiPLexc and the bottle adapter from below with illustration of the plasma effluent.**

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IMPORTANCE OF BENZENE RING STRUCTURE FOR BACTERICIDAL EFFICACY IN NEUTRAL pH OF RADICAL-ACTIVATED SOLUTIONS

Naoyuki Iwata[1], Hiroshi Hashizume[2], Jun-Seok Oh[3], Masaru Hori[2], Masafumi Ito[1]
[1] *Meijo University, Japan*
[2] *Nagoya University, Japan*
[3] *Osaka City University, Japan*
email: 183427003@ccmailg.meijo-u.ac.jp

Recently, biological applications of atmospheric-pressure plasmas are one of the most common plasma topics owing to their interesting characteristics. For example, Tanaka *et al*. reported the selective inactivation of cancer cells using a plasma-activated lactic solution. [1] L-phenylalanine (L-Phe), which is one of aromatic amino acids, was dissolved to a phosphate buffer (PB) solution (pH 6.3) and treated using an atmospheric-pressure radical source. The radical-activated L-Phe solutions killed *Escherichia coli* (*E. coli*) in a neutral pH and simultaneously promoted the growth of radish sprouts. [2] For this bactericidal method, an interaction between electrically neutral radicals and the benzene ring structure seemed essential.

To confirm the reliability of this speculation, L-alanine (L-Ala), which is an amino acid without the benzene ring sturcure, was treated using the radical source and the bactericidal efficacy was evaluated.

L-Phe and L-Ala were dissolved to the PB (pH 6.3) with the concentrations of 80 mmol/l and these solutions were treated using an atmospheric-pressure radical source (Tough Plasma, Fuji MFG Co., Ltd.). This radical source was designed for the selective supply of electrically-neutral species such as $O(^3P_j)$ without charged species or optical radiation. *E. coli* of 1×10^7 ml^{-1} was suspended to the treated samples and incubated at 30 °C and 250 rpm. The number of alive *E. coli* was estimated using a colony forming unit (CFU) assay at every 24 h of the incubation.

Figure 1 shows the death rate of *E. coli* in the radical- activated L-Phe and L-Ala. Cleary, the number of alive *E. coli* was reduced and reached the detection limit of the CFU assay by 24 h in the radical-activated L-Phe. On the other hand, *E. coli* in the radical-activated L-Ala was quite stable and not reduced at all in the experiment. This result strongly suggests that the benzene ring structure of L-Phe reacted with neutral radicals and produced new bactericidal species.

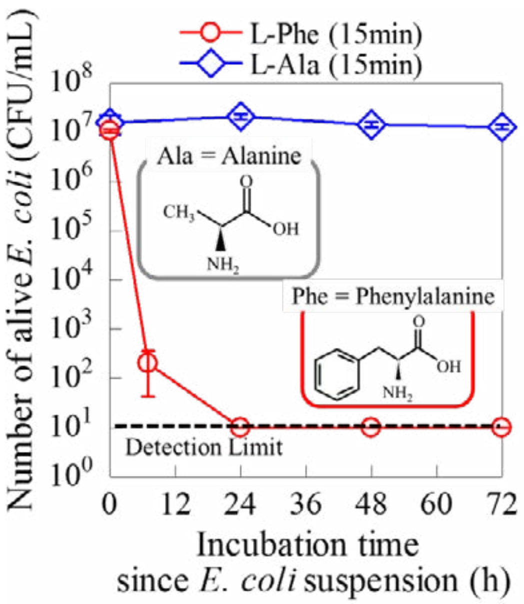

Fig.1 Bactericidal efficacy of radical-activated L-Phe and L-Ala.

This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1511021), Grant-in-Aid for Special Purposes (19H05462) and Plasma-bio consortium project (01221907).

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RELEASE OF ARABIDOPSIS SEED DORMANCY BY AIR DIELECTRIC BARRIER DISCHARGES

Jonas August[1,2], Thierry Dufour[1], Christophe Bailly[2]
[1] *Sorbonne Université, Laboratoire de Physique des Plasmas, UMR 7648, 75005 Paris, France*
[2] *Sorbonne Université, Laboratoire de Biologie du Développement, UMR 7622, 75005 Paris, France*
email: jonas.august@sorbonne-universite.fr
thierry.dufour@sorbonne-universite.fr
christophe.bailly@sorbonne-universite.fr

By its facility of culture and its small genome of approximately 135 Mbp, Arabidopsis is a model plant routinely investigated in seed biology. Although few studies in plasma agriculture are focused on seeds of this species, recent advances have shown how non-thermal plasma can affect their surface properties and metabolism which, in turn, enhance their germinative properties [1]. In our work, we investigate the influence of plasma treatments on physiological status of Arabidopsis seeds. We aim to determine whether the effect of plasma on dormancy release could be related to the water properties within the seed tissues.

Plasma is directly generated in air with a dielectric barrier discharge device (DBD). One of its electrodes is a metal mesh biased to the high voltage (5-10 kV) at low frequency (< 1 kHz). Several technical incrementations of the DBD have been achieved in particular the mesh parameter of the HV electrode. Combining electrical and optical diagnostic tools, we show how this parameter can be directly associated with the number and spatial distribution of the micro-discharges which, in turn can impact seed germinative properties.

Moisture content measurements of seeds equilibrated in a range of relative humidities have been performed to draw water sorption isotherms of Arabidopsis. Three distinct regions of water binding within seed tissues have been highlighted [2]. For each of these regions, seeds have been plasma-treated and compared with their respective controls to find out if a correlation can be found between water organization and plasma-triggered dormancy release. During this study, direct seed plasma treatments with different exposure times have been performed to assess plasma effects on dormancy breaking by evaluating germination rate in darkness at 25°C.

Plasma physico-chemical properties and plasma-seeds interface mechanisms are discussed to decipher the action of plasma on the early stages of germination.

Acknowledgements: SU IBPS funding

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EFFECT OF PLASMA ACTIVATED WATER ON EARLY DEVELOPMENT OF MAIZE PLANTS

K. Hensel[1], P. Ivanová[1], Z. Lukačová[2], R. Švubová[2]

[1]Faculty of Mathematics, Physic and Informatics and [2]Faculty of Natural Sciences, Comenius University, 842 48 Bratislava, Slovakia

email: hensel@fmph.uniba.sk

Atmospheric plasma has shown promising potential in various agricultural applications, where plasma is applied directly or indirectly (mediated by plasma activated gas or water) on seeds to stimulate germination or plants to modulate growth, and fruit yield [1, 2]. The plasma produces various gaseous nitrogen and oxygen reactive species that dissolve in water and produce plasma activated water (PAW).

The objective of the present study was to investigate the effects of the PAW generated by a transient spark discharge (TS) operated in ambient air in contact with tap water on the maize corn germination and growth, and root development of young maize plants (*Zea may*s L.). Guaiacol peroxidase (G-POX) activity of the seedlings, G-POX activity *in situ* and changes in the root cell walls lignification and suberisation was evaluated.

The PAW was generated by a positive DC-driven self-pulsing transient spark (TS) discharge at atmospheric pressure in the air. The discharge was generated in a reactor with water circulating through the discharge zone [3]. The exposure time of the plasma was set to 1 min/mL. Maize corns were imbibed in the PAW or the tap water for 6 hours and then cultivated in paper rolls for 3 days; the rolls were moistened either by PAW (treatment rolls) or tap water only (control). The priming treatment was designed using corns imbibed in PAW and then the cultivation continued with the tap water only. The *in situ* POX activity was analyzed after incubation of hand-made cross sections (0.5 mm thick) of maize roots with 4-metoxy-1-naphtol. For lignin and suberin visualization, hand-made cross section of maize roots (10, 50 and 70% from apex and base) were stained with fluoroglucinol-HCl and Fluorol Yellow 088 and deposits were observed by fluorescence microscope.

After 3 days of cultivation, roots from the priming treatment were significantly longer than control and alike the treatment rolls had modulated apoplasmic barriers development. G-POX activity was significantly higher due to PAW treatment already after imbibition in the corns and this effect was observed also after 3-days cultivation on the roots in a comparison with control. However, no positive effect on the G-POX activity was observed in the roots of treatment rolls. Probably the higher doses of reactive species given to the seedlings in this case caused a negative stress reaction. Staining of lignine and suberine confirmed acceleration in the lignification and suberisation of cell walls of exodermis, endodermis and also early and late metaxylems in the treatments with PAW.

The present study opens a new area of investigation of the effect of PAW on the maize plants which are one of the most important crops in the world. The enhanced apoplasmic barriers development or other modulations of the root tissues development is a promising way how to protect plants from various types of stresses like heavy metals/toxic metaloids or the drought which are being more evident in the last decades.

This work was supported by Slovak Research and Development Agency APVV-17-0382 and SK-PL-18- 0900, and by Scientific Grant Agency VEGA V-18-050-00.

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THE INFLUENCE OF THE COLOR OF THE SEED COAT ON THE GERMINATION AND AMOUNT OF PHYTOHORMONES IN THE SEEDS OF RED CLOVER (*TRIFOLIUM PRATENSE L.*) AFTER TREATING THE SEEDS WITH COLD PLASMA AND ELECTROMAGNETIC FIELD

Anatolii Ivankov[1], Rasa Žukienė[1], Zita Naučienė[1], Irina Filatova[2], Veronika Lyuskevich[2], Vida Mildažienė[1]*

[1] *Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania*

[2] *B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, Minsk*

email: anatolii.ivankov@vdu.lt

The objective of this study was to evaluate the effects of seed coat color on the germination of red clover (*Trifolium pratense* L.) seeds, seedling growth and the amount of phytohormones in seeds induce with electromagnetic field (EMF) and cold plasma (CP). Red clover seeds of the commercial variety 'Vyčiai' and 'Arimaičiai' were used in this study. Before the sowing, red clover seeds of different seed coat color were processed using two types of physical stressors, EMF and CP. The effects treatments on seed germination were estimated using an *in vitro* germination test. Kinetic parameters of germination were determined: Vi - the final germination percentage, Me - the median germination time, Qu – quartile deviation, uniformity germination in seed population. Morphometric analysis of seedlings was performed 7 days after sowing. Extracts for estimation of phytohormone content in seeds were prepared on the sowing day, phytohormone analysis was performed using high-performance liquid chromatography. The number of root nodules was measured for cultivar "Arimaičiai" that was growing in the substrate for 5 weeks.

It was established that germination kinetics *in vitro* of the red clover cultivars 'Arimaičiai' and 'Vyčiai' control seeds were independent of the seed coat color. The pre-sowing treatment of seeds with CP and EMF improved kinetic parameters of seed germination for both cultivars: the increase in germination rate (decreased Me) was largest for yellow seeds, smaller for black seeds and the smallest for brown seeds. The effect of seed treatment with stressors on the early growth of 'Arimaičiai' and 'Vyčiai' seedlings were also dependent on the color of the seed coat: the strongest positive effects for the 'Vyčiai' were obtained on the morphometric parameters of sprouts from the black seeds, whereas even negative effects were shown for the brown seed sprouts. Treatments increased length of sprouts growing from yellow and black seeds of 'Arimaičiai' cultivar, and weight of sprouts growing from brown seeds. The amounts of seed phytohormones involved in the control of germination were dependent on seed coat color in the seeds of both cultivars. The seed treatment with CP and EMF affects root nodulation, but the extent of this effect also depends on the seed coat color. The number of nodules significantly increased compared to the control, especially in seedlings from black seeds, where after 5 minutes of treatment with CP and 10 minutes of treatment with EMF the number of nodules increased by 27 and 13 times, respectively. For yellow seeds, the number of nodules increased by 36% after 5 minutes of treatment with CP and 98% after 10 minutes treatment with EMF, relative to the control.

PLASMA ACTIVATED WATER GENERATED BY TWO COLD PLASMA SOURCES: CHEMICAL PROPERTIES AND EFFECT ON PLANT GROWTH

Gervais Blondel Ndiffo Yemeli[1], Renata Svubova[2], Dominik Kostolani[2], Zdenko Machala[1]
[1] *Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, 84248 Bratislava, Slovakia*
[2] *Faculty of Natural Sciences, Comenius University in Bratislava, 84215 Bratislava Slovakia*
email: yemeli1@uniba.sk

Plasma activated water (PAW) is formed during an exposure of water to the cold plasma which causes chemical changes in water. Air plasmas produce reactive oxygen and nitrogen species (RONS) (e.g. hydrogen peroxide, nitrite, nitrate, peroxynitrite, hydroxyl radical, nitrogen monoxide radical, etc.). These RONS in PAW are responsible for the rapid germination of seeds, enhancement of the plant growth, destruction of bacteria and other germs harmful to the growth of the plants ([1], [2]). PAW has also a role as a fertilizing agent for plants ([2], [3]).

In this study various types of PAWs were generated by transient spark discharge with water electrospray and by glow discharge with water cathode at atmospheric pressure. After plasma treatment, each PAW was characterized by measuring long-lived RONS concentrations (H_2O_2, NO_2^-, NO_3^-) by UV/VIS absorption spectroscopy and its effects on barley (*Hordeum vulgare L.*) growth were investigated. After 4 weeks of plant growing, the effect of PAWs generated by the two plasma sources on barley seedlings were analyzed by measuring some growth parameters (plant height, fresh and dry weight) and some physiological parameters such as photosynthetic pigments, total soluble proteins content, antioxidant enzymes activity (superoxide dismutase (SOD), and guaiacol peroxidase (G-POX)), and photosynthetic rate.

The results suggest that PAWs generated by transient spark discharge with water electrospray and glow discharge at atmospheric pressure have the potential to improve the barley growth through the enhancement of the plant growth, the photosynthetic pigments concentration and total soluble protein content in roots and shoots. The effects of PAW on the plant growth depend on its chemical composition and we can identify the most suitable plasma parameters of PAW preparation to induce the highest effect on plant growth.

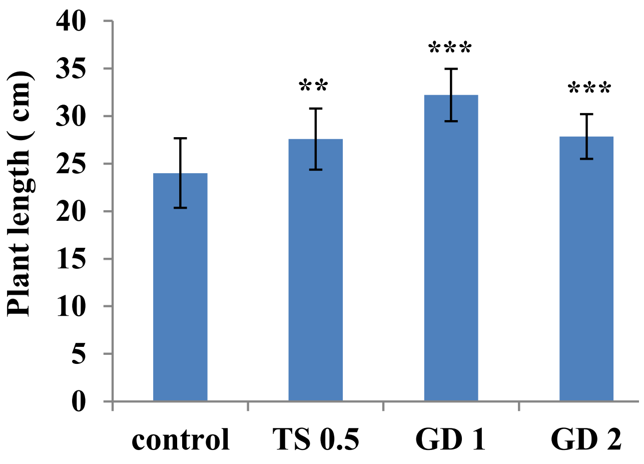

Fig 1: Plant height above ground of barley plants after 4 weeks of growing and watered with tap water (control), transient spark discharge flow rate 0.5 ml/min (TS 0.5) and glow discharge treatment time 1 and 2 min (GD 1 and GD 2). Values are expressed as a mean ± standard deviation (SD).

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A LOW-PRESSURE AIR PLASMA IMPROVING BELL PEPPER SEED GERMINATION AND VIGOR

M. Soulier[1], T. Maho[1], C. Muja[1], Ph. Belenguer[2], J. Lo[1] and Ph. Guillot[1]
[1]*Laboratoire Diagnostics des Plasmas Hors Equilibre (DPHE), Université de Toulouse, INU Champollion, Albi, France*
[2]*Laplace, Université de Toulouse, UPS, INP, CNRS, Toulouse, France*
email: manon.soulier@univ-jfc.fr

Since the 1950's, intensive agriculture and massive irrigation are means used in order to confront increasing population need in food production. However, these practices have shown adverse effects on biodiversity and population health [1,2]. Industrial development forced the separation between seed production and farmers. Seed selections performed by seed banks have led to a loss of more than 50 % seed varieties in 50 years. These measures restrain biodiversity and thus contradict the current mindsets concerning biological agriculture development. In recent years, French farmers, acting on agroecology, are cooperating on 'peasant seeds' production, *i.e.* seeds derived from a set of population, selected with non-transgressive methods of the plant cell and reproducible by the cultivator [3]. However, these varieties present in general lower germination yield. Several studies showed low-temperature plasma effects on germination yield improvement [4,5]. This work focuses on the ability of a low-pressure air plasma to increase germination yields and vigor of Cubo orange bell pepper seeds (providers: Agrosemens, France).

Two plasma sources working on different ranges of frequencies have been characterized and associated to optimize seeds treatment parameters. The first source is an Electron Cyclotron Resonance microwave plasma source (MW-ECR) fixed at the upper part of the reaction chamber and able to sustain plasmas in a low-pressure range (10^{-2} – 10 Pa). The second one is a Coupled Capacitive Plasma (RF- CCP) radiofrequency source placed at the bottom of the reactor to enhance the MW-ECR plasma effect. An 85 liters reaction chamber filled with synthetic air at 1 Pa is used for plasma treatments.

Species identification and their spatial distributions were determined from OES (OceanOptics HR2000+) and ICCD camera (Princeton Instruments PI-MAX) measurements. A linearly driven double Langmuir probe (Impedans ALP System) was used to calculate electron temperatures and densities. Measurements show n_e variation from 10^{10} to $10^{11} cm^{-3}$ and T_e value around 1 eV depending on experimental conditions. Gas temperatures, lower than 250°C, were estimated by a K-type thermocouple. Bell pepper peasant seeds initial characteristics were evaluated. Germination yield were determined respecting the AOSA rules. Moisture content, using a desiccator at 105°C, leachate conductivity and seed coat permeability with the tetrazolium penetration assay were evaluated as vigor characteristics. The influence of plasma effects on these different points was first investigated with only MW-ECR source and then with both MW-ECR and RF-CCP sources.

This study was supported by the Occitanie Region, France. The authors would like to acknowledge SAIREM SAS for their material and scientific supports.

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EFFECTS OF DIFFERENT MAGNETIC STRESSES ON RADISH SEED GERMINATION AND GROWTH

V. Nassisi[1,2], L. Velardi[1,2], L. Monteduro[1,2], E. Manno[2] and M. De Caroli[2]

[1] *Laboratorio di Elettronica Applicata e Strumentazione - LEAS, Department of Mathematics and Physics, University of Salento, Lecce – Italy*

[2] *INFN section of Lecce, Via Provinciale Lecce-Monteroni, Lecce - Italy*

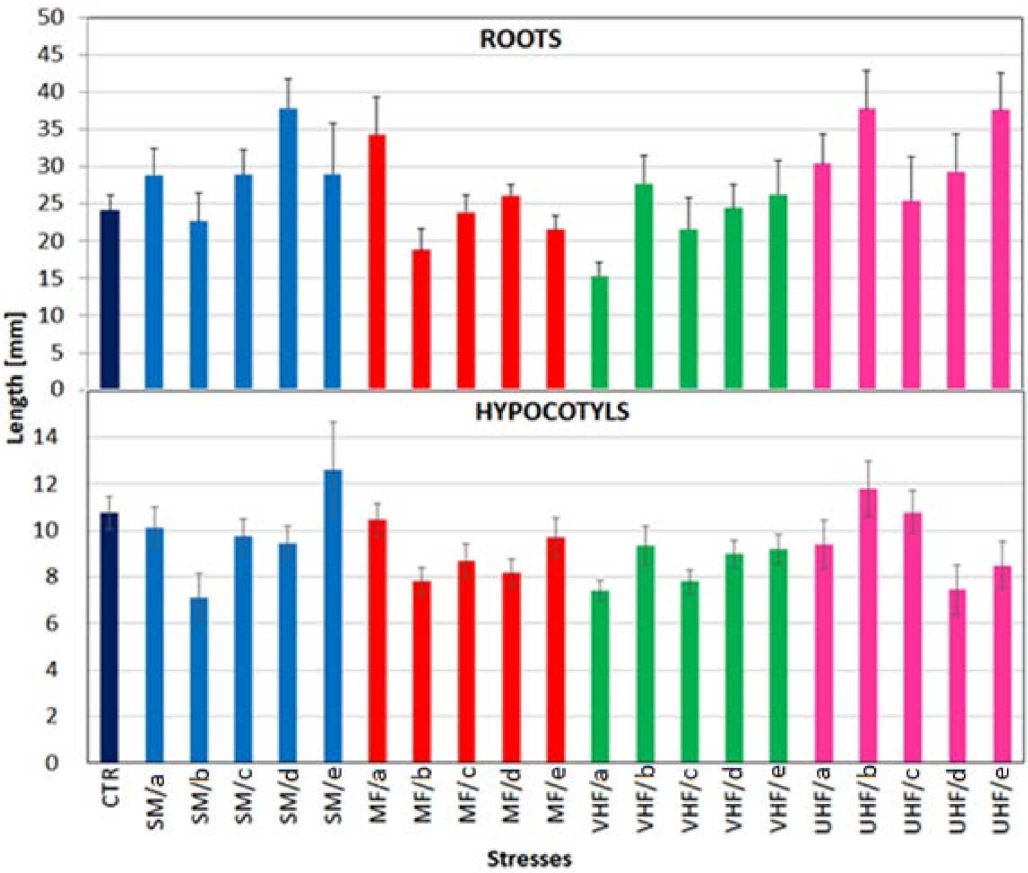
[2] *Di.S.Te.B.A., University of Salento, Lecce - Italy*

email: vincenzo.nassisi@unisalento.it

This work reports an interesting study on the behavior of radish seed (Raphanus sativum L.) germination and growth under electromagnetic stresses. Groups of uniform seeds were irradiated at five duration time values of 60 (a), 210 (b), 375 (c), 470 (d) and 830 (e) h at three radiofrequency values:

- medium frequency of 1 MHz: (MF/a), (MF/b), (MF/c), (MF/d) and (MF/e);
- very high frequency of 100 MHz: (VHF/a), (VHF/b), (VHF/c), (VHF/d) and (VHF/e);
- ultra high frequency of 900MHz, (UHF/a), (UHF/b), (UHF/c), (UHF/d) and (UHF/e).

The magnetic field was measured for the three frequencies and resulted of about 240 nT, and the associate electric field inside the samples was less than 71 V/m, owing to the electric permittivity exposed sample. Another group of uniform radish seeds was irradiated, as comparison, by a static magnetic (SM) field of 80 mT for the same time duration. Simultaneously untreated radish seeds were used as control (CTR). The results showed that all physical stresses induced by magnetic fields did not have strong effect on seed germination as well as on cell elongation growth of the hypocotyls. On the contrary, a stimulating effect was observed on the seedling root growth.



EFFECTS OF PLASMA ACTIVATED WATER ON GERMINATION OF BARLEY AND RADISH SEEDS

Tomislava Vukušić Pavičić[1], Jelena Vojvoda[1], Višnja Stulić[1], Dunja Šamec[2], Kinga Kutasi[3], Zoran Herceg[1]

[1] *Faculty of food technology and biotechnology, University of Zagreb, Croatia*

[2] *Ruđer Bošković Institute, Zagreb, Croatia*

[3]*Wigner Research Centre for Physics, Research Institute for Solid State Physics and Optics, Budapest, Hungary*

email: tvukusic@pbf.hr

Application of plasma activated water (PAW) is a novel method used in agriculture for germination enhancement. PAW differs in its chemical composition due to various plasma setups and working gases used for its generation [1]. The aim of this study was to compare the effect of PAW on germination of barley (*Hordeum vulgare* cv Golden Promise) and radish (*Raphanus sativus*). PAW used in this research was generated using high voltage electrical discharge plasma, plasma jet, and microwave plasma. In all plasma setups working gas was argon and treatment time was 10 minutes. After PAW production physic-chemical analysis was conducted to measure levels of generated radical species as well as change in temperature, pH and electrical conductivity. Germination test included 6-day incubation at 20 ^{o}C of seeds watered with PAW as well as control samples. Analysis of germination rate, lengths of roots and sprout, concentration of antioxidative molecules chlorophyll and proline were measured in sprout samples. A stressful environment results in the overproduction of proline in plants which indicates stress tolerance [2].
PAW showed enhanced germination of radish and barley seeds while plasma jet PAW caused the highest production of chlorophyll in radish samples. Radish seeds have shown better stress response over barley seeds due to high proline production.

Acknowledgment: This study has been funded by the European Regional Development Fund, through technical assistance of the Competitiveness and Cohesion Operational Programme "Equipping the semi-industrial practice for the development of new food technologies" (KK.01.1.1.02.0001).

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EFFICIENCY OF MICROWAVE DRIVEN DISCHARGE PROCESSED GAS FOR BIOLOGICAL DECONTAMINATION OF CROP SEEDS

N.Wannicke[1]*, R.Wagner[1], J.Stachowiak[1], J. Ehlbeck, T.M.C. Nishime[1], K-D. Weltmann[1] H. Brust[1]

[1] Leibniz Institute for Plasma Science and Technology, D-17489 Greifswald, Germany.

** Corresponding author email: nicola.wannicke@inp-greifswald.de*

Some of the world’s most common pesticides will be banned EU wide or withdrawn for health or environmental reasons in the next years, including fungicides used to treat cereal seeds. Alternatives have to be found, including physical methods, to protect seeds from pathogens as well as improved seedling development and ultimately secure yield. In the past, plasma-processed air (PPA) has shown promising properties for biological decontamination and sterilization of food products [1] food packaging [2] and crop seeds [3]. In this study, the antimicrobial effect of PPA generated by a microwave-induced non-atmospheric pressure plasma was investigated for cereal and legume seeds. For this purpose, *Bacillus atrophaeus* spores were inoculated onto seeds, subsequently treated with PPA at two different flow rates (73 slm and 63 slm), different filling regimes (single, repeated) and different gas exposure times (1 min to 20 min). Afterwards, recovery of viable spores on seed surface in relation to the control (no PPA treatment) was determined. Simultaneously, maximum germination, as well as germination speed were monitored. PPA treatment efficiency of artificially contaminated seeds was dependent on PPA flow rate, repetition of bottle filling and gas exposure time (Fig. 1). A maximum reduction of 3.39 log (CFU/ml) was achieved at 73 slm combined with single filling and 15 minutes of gas exposure, exemplarily for barley. To sum up, PPA proved effective for seed decontamination, while ensuring seed viability, if the optimal parameter are chosen. Considering a possible industrial application, energetic cost effectiveness has to be included as well.

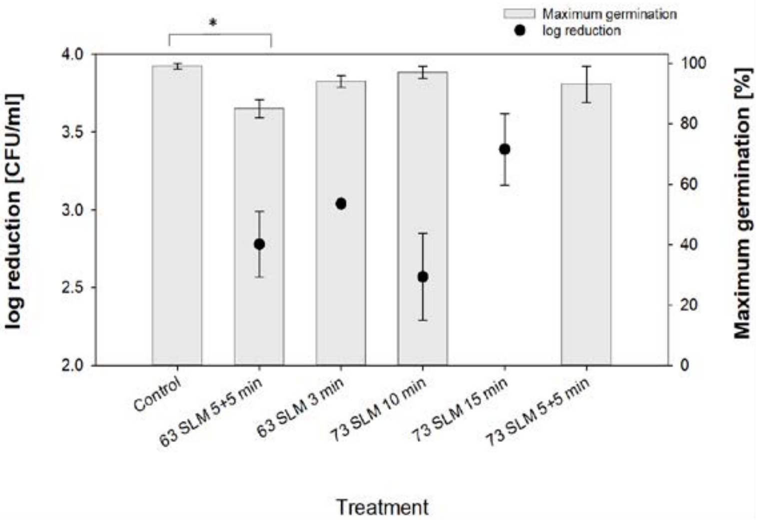

Fig.1 Mean reduction of viable spores relative to the control ± standard deviation (black circles, 1st y-axis) and mean maximum germination ± standard deviation (grey bars, 2nd y-axis) for the different treatments, exemplarily for barley seeds. Significant difference in mean maximum germination relative to the control is indicated by * with $p \leq 0.05$ (Student´s t-test, n=3). Maximum germination was determined after 62 h, except 63slm 3 min representing a time interval of 72h.

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LOW TEMPERATURE ATMOSPHERIC PLASMA EFFECTS ON SPROUTING AND PLANT GROWTH OF TURMERIC (*CURCUMA SPP.*) RHIZOMES IN THE GREENHOUSE AND THE FIELD.

S. R. Mentreddy[1], L. Duong[1], L. Nyochembeng[1]; E. Cebert[1]; S. Kumar[1], R. Gott[2], and K. G. Xu[2]

[1]Department of Biological and Environmental sciences, Alabama A& M University, Normal, AL 35762; [2]Department of Mechanical and Aerospace Engineering, University of Alabama in Huntsville, AL 35899

Abstract

Low temperature atmospheric plasma (LTP) technology is emerging as a tool for improving seed germination and enhancing plant growth. Several studies have shown that LTP enhanced seed germination, plant growth and grain and fruit yield of crops. However, LTP effects on enhancing sprouting and plant growth in root crop turmeric has not been reported to date. Turmeric (*Curcuma longa*), also known as the "Golden Spice" has proven anti-cancer, anti-inflammatory and anti-Alzheimer properties besides others benefits. However, lack of timely sprouting in some of the varieties often results in poor stand establishment. The objective of this research was to assess the effects of LTP on sprouting and plant growth of turmeric varieties known for poor stand establishment. Four turmeric varieties, VN12, VN18, VN23, and VN24 were each exposed to atmospheric pressure LTP for 0, 30, 60, 90, and 120 seconds, and the plasma treated and untreated control rhizomes assessed for sprouting and plant growth in the greenhouse and open field plots with four replications. All plasma treated rhizomes sprouted six to ten days earlier and achieved 100% sprouting nearly two weeks earlier than the untreated control. The plasma treated plants grew faster and were taller (37 – 39 cm) than untreated control (26-33cm) at 67 days after planting. The effects of LTP on plant growth are persistent in field conditions as well. The rhizomes exposed to 90 s sprouted faster and grew taller than other times of exposure in the greenhouse and the in the field. The LTP effects were consistent in both the greenhouse and in the open field plots. The study showed that cold or low temperature plasma offers potential for improving turmeric plant stand establishment and crop performance in open field production.

This research was supported by NSF EPSCoR RII Track 1 Grant OIA – 1655280.

Key words: Turmeric, *Curcuma longa*, Cold Plasma, rhizome, sprouting, growth, stand establishment

ROLES OF SEED PIGMENTS IN RESPONSES OF SEEDS TO PLASMA TREATMENT

Kazunori Koga[1, 2], Pankaj Attri[1], Ryo Arita[1], Ryoya Sato[1], Hayate Tanaka[1], Masaya Hiromatsu[1], Kayo Matsuo[1], Daisuke Yamashita[1], Kenji Ishikawa[1], Naho Itagaki[1], Kunihiro Kamataki[1], Masaharu Shiratani[1], and Vida Mildaziene[3]

[1] *Kyushu University, Japan*
[2] *National Institutes of Natural Science, Japan*
[3] *Vytautas Magnus University, Lithuania*
email: koga@ed.kyushu-u.ac.jp

Due to beneficial effects of cold atmospheric plasma treatment of seeds on seed germination and plant growth, the treatment has emerged as a potential method to increase agriculture production [1-5]. The mechanisms of the beneficial effects are currently under investigation. Here, to study roles of seed pigments in responses of seeds to plasma treatment, we treated gray and brown seeds of radish sprouts using our DBD plasma for 3 min. Stable organic radicals in seeds were detected using electron spin resonance (ESR) spectroscopy. ESR signal intensity of gray seeds was increased by plasma treatment, while that of brown seeds was not. For both gray and brown seeds, we studied chemical bonds and morphology of their seed coat before and after plasma treatment using XPS and SEM, and evaluated the antioxidant properties of these two kinds of seeds using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test. We will report the results at the conference.

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MODULATION OF PLANT CELL REDOX SIGNALING BY CAP – IS THERE A ROLE FOR PROTEIN AND LIPID OXIDATION PRODUCTS?

Kristian Wende[1], Sebastian Wenske[1], Johanna Striesow[1], Christine Stöhr[2], Klaus-Dieter Weltmann[3], Henrike Brust[3], Thomas von Woedtke[3]

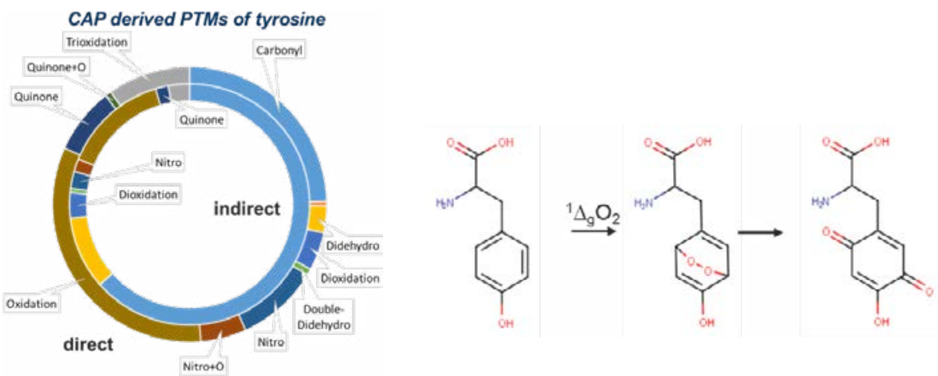
[1] *Leibniz Institute for Plasma Science and Technology, ZIK plasmatis, F.-Hausdorff-Str. 2, D-17489 Greifswald*

[2] *University of Greifswald, Institute for Botany, Soldtmannstr. 15, D-17489 Greifswald*

[3] *Leibniz Institute for Plasma Science and Technology, F.-Hausdorff-Str. 2, D-17489 Greifswald*

email: kristian.wende@inp-greifswald.de

Cold atmospheric plasmas (CAP) show a significant impact on both pre-harvest and post-harvest processes in agriculture. Beside the use of CAP as a microbiocide and/or fertilizer, it is applied for the stimulation of seed germination rate, sapling growth, and subsequent plant development. The stimulation of the germination and processes downstream of this event are under the control of phytohormones, especially ethylene, and reactive species (ROS, RNS) in combination with orthologs of the *Arabidopsis thaliana* proteins LSD1 (lesion stimulating disease 1), EDS1 (enhanced disease susceptibility 1), and PAD4 (phytoalexin deficient 4). H_2O_2 and singlet oxygen are assumed to be major ROS contributing to plant cell redox signaling events, and to target peptides, proteins, and cell wall components. While the details are not fully established, similarities with mammalian stress signaling regulation (including mitogen activated protein kinase pathway - MAPK, and nuclear factor erythroid 2-related factor 2 - NRF2) suggest specific and controllable effects of CAP in seed treatment. Singlet oxygen, a major and tunable component in the gaseous effluent of dielectric barrier jet plasmas, was recently shown to yield specific oxidative post translational modifications in model peptides thereby indicating its transport from the gaseous phase into a liquid or solid target (see figure). The observed modifications (including tyrosine quinones) contribute to redox signaling events and trigger changes in protein structure and function.



Oxidative and nitrosative post-translational modifications in the amino acid tyrosine after direct or indirect CAP treatment of model peptides (left). Tryosine quinone modifications indicate singlet oxygen ($^1O_2$) activity in the liquid (right).

In lipids, building blocks of cell membranes and cuticular waxes, CAP derived singlet oxygen leads to oxidation and chain cleavages, yielding in less oriented lipids exerting a reduced barrier functionality. In contrast, H_2O_2 does not show this potential in a solid supported lipid bilayer model, further supporting the significant role of short lived species for CAP effects in biological systems including plants.

XYLELLA FASTIDIOSA: A PLASMA APPROACH FOR THE RACE FOR THE CURE

Paolo F. Ambrico[1], Stefania Zicca[2], Pasquale Saldarelli[2], Maria Saponari[2], Marianna Ambrico[1], Donato Boscia[2]

[1] *CNR Istituto per la Scienza e la Tecnologia dei Plasmi sede di Bari, Italy*

[2] *CNR, Istituto per la Protezione Sostenibile delle Piante, sede di Bari, Italy*

email: paolofrancesco.ambrico@cnr.it

The quarantine bacterium *Xylella fastidiosa* occurred for the first time in the open field in Europe in 2013, in southern Apulia (Italy), posing a serious threat to the agriculture economy and the biodiversity of the whole area. Infections caused by the subspecies *pauca* strain "De Donno" of this bacterium turned to be extremely aggressive on olive, causing leaf scorching and desiccation symptoms, a disease named Olive Quick Decline Syndrome. Research efforts were devoted to gain basic knowledge of the genetic and epidemiology of the olive strain (e.g. host pathogenicity and range, insect vector(s), and fundamental drivers of its epidemics), in order to find strategies to control its spread or mitigate symptoms [1]. In this contest we explored the feasibility of using low temperature plasma to kill bacterial cells. Low temperature plasma is an environment rich of reactive oxygen and nitrogen species (ROS and RNS) efficient in the decontamination, sterilization, and chemical modification of surfaces. Moreover it presents numerous potential advantages over conventional methods, such as its nontoxic nature, low process operational costs, short treatment time at low temperatures.[2]

Preliminary experiments were conducted *in vitro* to test the biocidal effect of plasma produced by a surface dielectric barrier discharge on the bacteria. The results were quite encouraging showing that plasma reached high decontamination rate also on biofilm protected colonies of *Xylella* growing on solid media. Plasma application to trees will require developing protocols and tools able to reach the bacterium, which localizes and replicates in the xylem vessels. Thus, we explored the possibility to administer the plasma cure *via* water. The rationale was to use the plasma to activate water [3] as a biocidal agent that could freely move inside the xylem system. Preliminary results in liquid culture medium show a complete inactivation of *Xylella* cells, paving the way to test on model plants in confined conditions.

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ATMOSPHERIC PLASMA TECHNOLOGY WITH POTENTIAL FOR TREATMENT AND PROCESSING OF AGRICULTURAL PRODUCTS

Kerstin Horn[1], Sven Gerullis[1], Andreas Pfuch[1]
[1] *INNOVENT e.V Technologieentwicklung Jena, Jena, Germany*
email: kh1@innovent-jena.de

The production and processing of food are controversially discussed under the focus of increasing population numbers, environmental changes, hygienic requirements and varying needs. New technologies for the direct and indirect treatment of food offer potential for process optimization. One of these technologies are plasmas, which have various applications, especially under normal pressure conditions. INNOVENT e.V. offers a wide range of plasma systems for R&D.

Key aspects of our work with relation to agriculture are powder treatment, barrier layers, fine cleaning and adjusting wettability on technical surfaces. Corresponding activities in the field of Plasma Medicine ranges from surface cleaning and sterilization about projects for Plasma-Interaction with bacteria and fungi to pharmazeutical applications. [1, 2, 3]
For these purposes various systems from any manufacturers in a wide performance range are available.
Plasma-Single-Jets, Multi-Jets and DBD-Systems enable local, linear and planar plasma treatments.

Plasma applications for germ reduction on surfaces, antibacterial coatings and the treatment of powders and fine bulk materials have been successfully tested.
Special experience exists in the field of surface treatment of conveyor belts for use in the food industry. Atmospheric plasmas are successfully used for the treatment of the conveyor side and edges. This minimizes chemical cleaning processes and improves environmental compatibility. [4]
In a completed study, for a fast and easy treatment of powders, an atmospheric pressure plasma setup was build. Aim of this treatment was to increase the dispersability of hydrophobic powders (polymers such as PEEK and PI, ceramics such as hexagonal boron nitride) in polar liquids. A plasma treatment of powders materials lead to an attachment of oxygen-containing functional groups and to improved dispersibility of the modified powders in water. The improved surface characteristics further led to a better dispersability of the powders in aqueous chemical Ni electrolytes and hence to an incorporation in deposited metallic Ni coatings. This powder treatment plant can also be used for plasma treatment of plant materials such as seeds and other bulk materials.

INNOVENT e.V. is available as a partner for projects in the field of plasma agriculture.

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PLASMA-ASSISTED EXTRACTION OF MICROALGAL METABOLITES FOR DOWNSTREAMING

Katja Zocher, Raphael Rataj, Jan-Wilm Lackmann, Anna Steuer and Juergen F. Kolb
Leibniz Institute for Plasma Science and Technology (INP), Felix-Hausdorff-Straße 2, 17489 Greifswald, Germany
Email: katja.zocher@inp-greifswald.de

Microalgae are rich in metabolites, which have attracted industrial interest not only for biofuel production, but also as a source for the development of pharmaceuticals and nutraceuticals. Carotenoids and other pigments, as well as polysaccharides, and proteins, provide potential health benefits [1]. The main difficulty for extracting those compounds is a sturdy algal cell wall. Due to its strength, classical extraction methods have a high energy demand, accompanied by long treatment times, the use of environmentally harmful solvents, and/or an unwanted alteration of the extractives because of chemicals or heat development. Novel extraction techniques are therefore attractive. Accordingly, we have established a spark discharge plasma source, which proofed to be able to address aforementioned problems of other methods [2].

Green microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* and in addition, the red microalga *Cyanidium caldarium* were chosen for our study. Spark discharges, generated with 100-ns high-voltage pulses, were ignited directly in the algal suspension in a continuous flow system, and administered with a repetition rate of 4 Hz. The sparks delivered just enough energy to break the cell walls, but little to no heat-related side effects on the extractives were observed. Extraction yields were compared to yields achieved with microwave treatment. For Chlorella vulgaris, plasma effects on extracted proteins were evaluated by proteomic analysis. Schlieren imaging was conducted to quantify the generated shockwave pressure within the spark discharge.

Proteomic analysis revealed qualitative differences on extracted protein types for spark and microwave treatment. Furthermore, reactive species generated by the plasma had almost no effect on proteins [3]. With atomic force microscopy, the precise tensile strength of the microalga could be determined, which allowed a comparison with the shockwave pressure generated from the spark discharges. We could proof that the shockwave pressure in the system is high enough to exceed the tensile strength of the algae, which can thus be seen as the main reason for the successful cell wall rupture.

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A COAXIAL DIELECTRIC BARRIER DISCHARGE REACTOR FOR TREATMENT OF SEEDS IN DIRECT MODE

Nishime, T. M. C.[1], Wannicke, N.[1], Horn, S.[1], Weltmann, K.-D.[1], Brust, H.[1]

[1] *Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany*

email: thalita.nishime@inp-greifswald.de

The continuous increase in food demand caused by a worldwide population growth leads to urgent search for new alternative techniques for the different sectors of agriculture. Conventional technical solutions including the use of chemical treatments can efficiently protect the seeds. However, the excessive use of agrochemicals lead to substantial impacts on the environment [1]. Non-thermal atmospheric pressure plasmas have recently drawn much attention for their potential usage in agricultural processes. Many investigations show that plasma can be used in many steps of pre-harvest, such as seed treatment for germination and growth enhancement, decontamination of seeds with plasma, treatment of water and etc [2]. Thus, plasma applied to agriculture is an interesting alternative solution that can potentially increase food production with minor impact on the ecosystem.

In the present work, the development and characterization of a dielectric barrier discharge (DBD) reactor with coaxial geometry working with argon for direct treatment of seeds is presented. Direct plasma treatment provides a much richer and active environment that can quickly act on the target. The laboratory scale reactor allows homogeneous treatment of seeds due to the coupled shaking platform where seeds with different sizes (such as wheat, lupine and rape seeds) can be treated. To test the reactor efficiency for agricultural applications, seeds were treated with different exposure times and power values and it could be observed that plasma treatment accelerates the germination of wheat seeds, as shown in Figure 1.

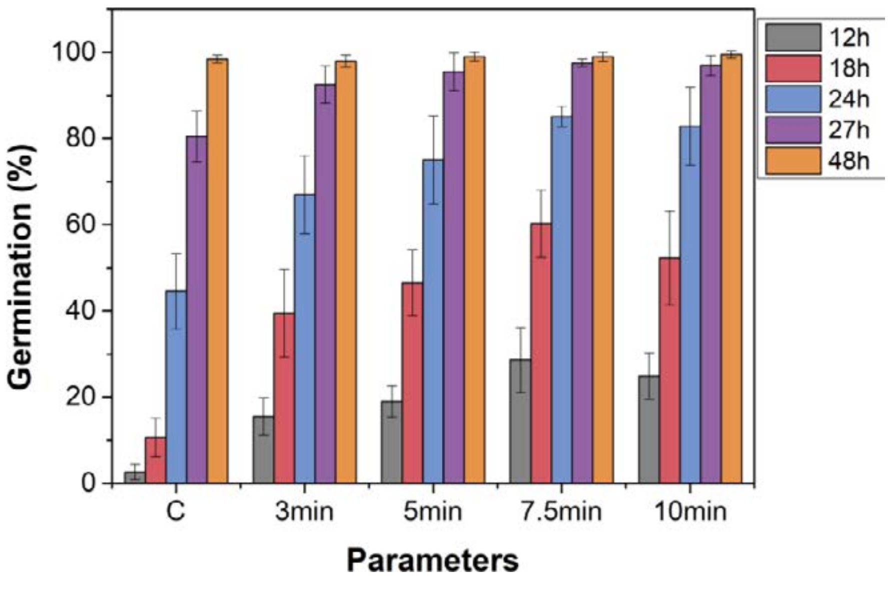

Figure 1: Germination acceleration of wheat seeds treated with different exposure times (Control, 3, 5, 7.5 and 10 min).

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MICROWAVE PLASMA TREATMENT OF BARLEY SEEDS

Petrukhina D.I., Karpenko E.I., Pomyasova M.G., Shishko V.I., Tkhorik O.V., Kharlamov V.A., Gorbatov S.A., Shesterikov A.Yu.

Russian Institute of Radiology and Agroecology. 109 km, Kievskoe shosse, Kaluga region, Obninsk, 249032 Russia

email: daria.petrukhina@outlook.com

The present work aims to study the ability of the non-thermal atmospheric pressure plasma (NTAPP) to improve the germination of spring barley seeds (*Hordeum vulgare* L.) and to protect from fungi. We used argon MW-plasma torch (2.43 MHz, Ar 15 L/min) and seeds harvested in 2018.

The duration of seed NTAPP treatment was 30-sec, 1, 5, 10, 15, 20, 25 and 30-min. The distance between the nozzle outlet and the glass dish with seeds (Ø28 mm) was 26 mm and 175 mm. The treatment was performed in air at atmospheric pressure and room temperature. Each sample contained 50 seeds. All experimental treatments were replicated three times. The seeds temperature was controlled using a thermographic camera and was 33–35 ºC in all experiments. The seeds were planted on a moist filter paper and kept in a thermostat at 20±0.5 °C under a light-dark regime. The laboratory germination study was performed after 7 days.

Results showed that NTAPP treatment did not statistically significantly influenced the length of root and sprout of barley when compared to the control sample. There was an increase in the wet-weight per-seedling after the 5- and 10-min treatment, and in dry-weight — after the 30-sec and 1-min. Furthermore, we observed a statistically significant increase in moisture content in barley seeds after 10-min NTAPP treatment. Thus, the treatment with MW-plasma torch was probably harsh (high power input, long treatment times) and considerably reduced the laboratory germination of barley seeds (germinated — when it had a radicle of 1 mm at least.).

Also, the barley seeds contamination with negative fungal microflora decreased. However, the decontamination from Helminthosporium disease turned out the be more challenging. The level of fungal contamination was evaluated after 7 days of incubation as the ratio between the number of infected plants and the total number of investigated samples. The disease severity was estimated according to [1]. NTAPP treatment did not significantly change the level of morbidity for Helminthosporium disease when compared to the control. However, we observed a decrease in the level of fungal infection and disease severity of fusariose after 30-min NTAPP treatment. Similarly, the disease severity and level of fungal infection such as genera Aspergillus (after 1-min) and Penicillium (after 30-sec or 25-min) decreased by half. These results may indicate that to control the effect of microwave plasma treatment it is necessary to find the optimal parameters for achievement of positive biostimulation in seeds [2].

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BUFFER CAPACITY OF A PLASMA-TREATED LIQUID DETERMINES SPOROCIDAL EFFICACY

Alexander Pogoda[1], Ansgar Schmidt-Bleker[1], Jörn Winter[1], Juergen Kolb[1]
[1]Leibniz Institute for Plasma Science and Technology (INP), Greifswald
Email: alexander.pogoda@inp-greifswald.de

The interaction of plasmas with liquids has a central role for many different applications of atmospheric pressure plasmas, such as they are in particular relevant in plasma medicine or plasma agriculture. Reactive species can either be transported from the gas phase into the liquid or be formed in the gas-liquid boundary layer itself [1]. The efficacy of plasma treatments, e.g. any microbial inactivation, depends on the respective differences of both approaches and on the subsequent fate of reactive species in the liquid. Accordingly, we studied the inactivation of bacterial spores by a plasma-treated buffer solution with respect to its buffer capacity. Different buffer capacities make it possible to bind and concentrate reactive species, whereby the timing of the reaction, resulting in the antimicrobial effect, can be controlled.

A discharge was ignited by means of a tungsten electrode on the surface of a K_2HPO_4 solution contained in a 1x1 cm^2 quartz cuvette. The goal of this configuration was the production of peroxynitrous acid (ONOOH) in the solution for its known antimicrobial properties [2].

By time-resolved in-situ UV spectroscopy, we observed and analyzed significant formation and degradation processes during plasma treatment. The method allowed the detection of ONOOH in plasma-treated liquids despite the short lifetime of ONOOH of 0.8s. The temporal resolution of the UV spectrometer was 50 ms.

The generation of ONOOH is promoted and depending on hydrogen peroxide (H_2O_2) and Nitrite (NO_2^-), which are provided by the exposure of the aqueous solution to plasma. Accordingly, in a buffer with a higher capacity, higher concentrations of H_2O_2 and NO_2^- can be accumulated and more ONOOH can be produced. We found that the achievable antimicrobicity scales with the capacity of the buffer solution. If the treatment exceeds a certain time and/or the buffer solution is below a certain threshold of the buffer capacity. H_2O_2 and NO_2^- that were accumulating during the plasma treatment is readily degraded and only NO_3^- remains in the solution as a stable species. According to the data of the UV measurement the generated concentrations of ONOOH were rather low.

Since the generation of reactive species and the dynamic of their interaction is also crucial for the decontamination of seeds or the stimulation of germination and growth, the method can potentially help to improve the design of respective treatment protocols.

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FTIR-MEASUREMENT IN HERMETIA ILLUCENS FARMING

Michael Schmidt[1], Gürbüz Daş[2], Manfred Mielenz[2], Jörn Winter[1], Ansgar Schmidt- Bleker[1], Jürgen F. Kolb[1], Cornelia C. Metges[2]

[1] Leibniz Institute for Plasma Science and Technology, Felix-Hausdorff-Straße 2, 17489 Greifswald, Germany

[2] Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

email: M_Schmidt@inp-greifswald.de

Farming of Hermetia Illucens (Black Soldier Fly, BSF) larvae is of interest, because of their high protein and fat content. With two kilogram of feed one kilogram of BSF larvae can be grown in less than 20 days. Thus, the usage of these species as feeding stuff in pig and poultry farming or aquaculture is getting into the focus. Also, the use as human food is under investigation.

To improve and optimize the farming of Hermetia Illucens it is of interest not only to control the feed quality, temperature, and humidity but also to monitor the gas composition in the farming vessel. The feeding stuff, the larvae, and the frass, which is the excretion of the larvae, are supposed to produce different gas compositions. These are of interest for the researchers in order to obtain information about the process phases and ways to optimize larvae feeding. In this contribution, the feasibility of Fourier-Transform InfraRed (FTIR) spectroscopy for the analysis of the gases in such a farming process is discussed. Therefore, different phases of this process were investigated by guiding gas from the farming vessel through an FTIR-spectrometer. In the spectra, carbon dioxide, ethanol, ammonia, and water could be identified and quantified with respect to the phase of the farming process. An example of the obtained spectra is given in Fig 1. The time resolution was less than 2 minutes, which should be sufficient for process observation and control. Thus, it was found that FTIR- spectroscopy delivers useful and reasonable results.

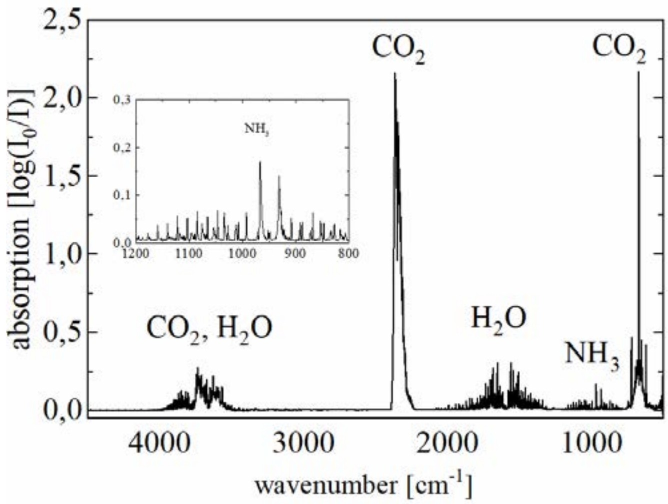

Fig 1: FTIR-spectrum of gas from a farming vessel filled with Hermetia Illucens larvae

DIELECTRIC CHARACTERIZATION FOR THE NON-INVASIVE EVALUATION OF BONE QUALITY FOR FARM ANIMALS

Michael Oster[1], Wenzuo Wei[2], Fukun Shi[2,3], Juergen F. Kolb[2,3] and Klaus Wimmers[1]
[1]*Leibniz Institute for Farm Animal Biology,*
Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[2]*Institute of Physics, University of Rostock,*
Albert-Einstein-Strasse 23-24, 18059 Rostock, Germany
[3]*Leibniz Institute for Plasma Science and Technology,*
Felix-Hausdorff-Strasse 2, 17489 Greifswald, Germany
email: oster@fbn-dummerstorf.de

Monogastric species such as pigs rely on adequate amounts of calcium and phosphorus in their feed to ensure for growth and performance as well as to account for physiological turnover rates. Both calcium and phosphorus form the bone mineral hydroxyapatite, which is deposited in skeletal and dental systems. Since the skeleton system represents the principle mineral storage for pigs, bone integrity require great attention to meet animal welfare aspects. However, the status of bone mineralization and bone structure in an individual is usually assessed by invasive methods, which require sedation of the animal (DEXA) or tissue sampling at slaughter (micro-CT). To this aim, the current project aims to develop a SOP to derive non-invasive proxies of bone mineral status, which would further allow repeated measurements *in vivo*.
This SOP will be based on the evaluation of bone quality by impedance spectroscopy. The transmission and reflection of an oscillating low voltage signal is determined by the permittivity and conductivity of the investigated material, i.e. bone tissue. Conversely, these dielectric parameters are related to composition and structure. We investigated long porcine femurs with respect to age, weight and gender of the respective animal. For a comparison with the electrical analysis, pertinent characteristics of the bone were determined from micro-CT images. A strong correlation of permittivity and conductivity in particular with bone volume fraction but also trabecular thickness was found. Both parameters are a direct measure of bone density and therefore indirectly for the formation of mineral hydroxyapatite. Moreover, specific measurement frequencies could be identified that even allowed distinguishing different sites along the bone. Accordingly, the method could be further developed for a comprehensive and non-invasive evaluation of tissue properties and the general status of the animal, e.g. by open-ended probes. In the long-term, proxies of bone health and welfare will be developed according to the individual nutritional mineral status in respective housing conditions of farmed animals.

INVESTIGATION OF REACTION BEHAVIOR BETWEEN VOLATILE AMMONIA AND AIR PLASMAS BY DIFFERENT TREATMENT METHODS

Yu Zhu, Zhenping Zou, Zilan Xiong
State Key Laboratory of Advanced Electromagnetic Engineering and Technology,
Huazhong University of Science and Technology, Wuhan, China

email: zilanxiong@hust.edu.cn

Volatile NH_3 pollution from agriculture is a main factor of smog formation in China, and NH_3 emissions have also exacerbated the global greenhouse effect. The problem of manure pollution in livestock and poultry farming not only emits a large amount of polluting NH_3 into the air, but also loses a large amount of N elements, which reduces the fertility of reuse of manure. As reported by D.B. Graves and R. Ingels, *et al*, using plasma to treat organic waste like animal waste could not only reduce volatile organic carbon (VOC) compounds, CH_4 and NH_3, but also improve the commercial value of fertilizer that can be made from organic waste, for example, enhance the fertility by forming NH_4NO_3 [1][2]. In this study, we investigated the interaction between gaseous ammonia and air plasma generated under three applied electrical conditions, as well as three types of treatment methods. Our primary results show that only in certain treatment method we could observe obvious NH_4NO_3 formation in gas phase. However, we did not find NH_4NO_3 formation in continuously discharge in air+NH_3 mixture driven by both pulse voltage and sinusoidal voltage. Meanwhile, the gaseous production varies with the discharge density and the treatment method.

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