Romanian Reports in Physics **71**, 701 (2019)

DISINFECTION FROM PINE SEEDS CONTAMINATED WITH *FUSARIUM CIRCINATUM* NIRENBERG & O'DONNELL USING NON-THERMAL PLASMA TREATMENT

B. ŠERÁ[1], A. ZAHORANOVÁ[2], H. BUJDÁKOVÁ[3], M. ŠERÝ[4]

[1] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Landscape Ecology, Ilkovičova 6, Bratislava 842 48, Slovak Republic
E-mail: *bozena.sera@uniba.sk*
[2] Comenius University in Bratislava, Faculty of Mathematics, Physics and Informatics, Department of Experimental Physics, Mlynská dolina, Bratislava 842 48, Slovak Republic
E-mail: *zahoranova@fmph.uniba.sk*
[3] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Microbiology and Virology, Ilkovičova 6, Bratislava 842 48, Slovak Republic
E-mail: *helena.bujdakova@uniba.sk*
[4] University of South Bohemia, Faculty of Education, Department of Physics, Jeronýmova 10, České Budějovice, Czech Republic
E-mail: *kyklop@pf.jcu.cz*

Received June 27, 2018

Abstract. *Fusarium circinatum* is a quarantine pest of trees that causes pitch canker disease in many pine species. The aim of this study was to test non-thermal, environmentally friendly plasma treatment for the disinfection of seed surfaces infected by *F. circinatum.* Inoculated seeds were plasma treated using a Diffuse Coplanar Surface Barrier Discharge apparatus working at atmospheric pressure and room temperature. The exposure times of the plasma treatment were: 0 s, 5 s, 10 s, 60 s, 180 s, and 300 s. Data analysis was performed with ANOVA test. A reduction of seedborne pathogens (14–100%) and seed germination (0–6.67%) was documented at the end of seed cultivation. Inoculated seeds remained free of mold infection for 12 days of cultivation on an agar surface in Petri dishes already after a short plasma treatment time of 60 seconds. Inoculated seeds treated for 5 s and 10 s had smaller seed germination (5.33% and 6.67% respectively) in comparison to samples without inoculation and plasma treatment (16%). Inoculated seeds treated for 60 or more seconds did not germinate. This work demonstrated the possibility of using plasma treatment against the dangerous *F. circinatum* fungus as a type of physical disinfectant method. The following research strategy will deal with the methodology elaboration of seed disinfection which would keep the seeds viable.

Key words: biotechnology, *Fusarium circinatum*, fungal inactivation, non-thermal plasma, *Pinus radiate.*

1. INTRODUCTION

The phytopathology fungus *Fusarium circinatum* Nirenberg & O'Donnell is registered as a quarantine pest (European and Mediterranean Plant Protection

Organization: A2 action list no. 306; [1]) with occurrence outside Europe. *F. circinatum* causes pitch canker, which affects many pine species (*Pinus* L.) [2] and Douglas fir (*Pseudotsuga menziensii* (Mirbel) Franco) [3]. Emergency measures to prevent its introduction and spreading within Europe are priorities in the current pine-pathogen research.

This pathogen produces airborne spores that can be spread by the wind and carried by native insects [4, 5]. Flying beetles can spread the disease to new areas. Long-distance spreading to new areas is more likely when people transport pathogen-infested wood, grass, logs, nursery stock, seeds, or sawdust [6, 7]. Other spreading vectors are: soil, forestry tools and equipment, planting containers, and forest nursery workers' clothing [8]. Hygiene measures are an important component of the control of this disease in containerized forest nurseries [9]. The seeds of pine species imported from countries where *F. circinatum* is present should be free of the pathogen [8].

Plasma consists of many active particles: radicals, electrons, ions, metastables, reactive species and radiation. Non-thermal plasma is characterized by low energy of its heavy particles (ions, molecules), which means it does not damage thermally sensitive material, but its electrons reach sufficient energy to participate in plasma-chemical reactions. Non-thermal atmospheric pressure plasma treatment is a modern biotechnological method that may be used for the decontamination of various surfaces of living tissue [10]. Thanks to its highly reactive composition, it can treat surfaces without the need for invasive chemicals [11]. The elimination of microbial infection from the seed coat by plasma treatment has been studied before [12–15].

There has been experience with using non-thermal atmospheric pressure plasma against microbial pathogens, such as the bacteria *Clavibacter michiganensis, Erwinia amylovora* and *Escherichia coli* [16] and the fungi *Microdochium nivale, F. culmorum, Trichothecium roseum, Aspergillus flavus, A. clavatus* [15]. The study of surface disinfection has been conducted using both Dielectric Barrier Discharge (DBD) and Diffuse Coplanar Surface Barrier Discharge (DCSBD), as the sources of non-thermal atmospheric pressure plasma (various electrode configurations) with satisfactory results [15, 17–21].

Non-thermal plasma treatment (atmospheric hybrid cold-discharge plasma based on microcorona discharge on a single dielectric barrier) was used by Khamsen *et al.* [22] for the study of surface modification of rice seeds (*Oryza sativa* var. Indica cv. KDML105). According to their results, non-thermal plasma treatment not only modified the surface from a hydrophobic to a hydrophilic state, but also completely inhibited the fungi and substantiated a higher growth rate and germination percentage. Ideally, seed sterilization with non-thermal plasma treatment should be harmless to the plant seeds, generate no toxic residue, and provide efficient processing times at nearly room temperature.

The aim of this work was to confirm a disinfectant application of DCSBD in seed-surface infection by *F. circinatum*. We selected the seeds of Monterey pine to be the testing pine species based on the study of Martinez-Alvarez *et al.* [23]. Monterey pine trees are sensitive to *F. circinatum* and their seeds can be infected by the fungus.

2. MATERIALS AND METHODS

2.1. SEED INOCULATION

Before inoculation, Monterey pine seeds (*Pinus radiata* D. Don) were disinfested superficially. Seeds were immersed in a solution of 10% commercial bleach with Tween20 (1 drop/100 ml of solution) for 15 min, followed by 2 rinses with sterile distilled water for 5 min each. After that, they were immersed in hydrogen peroxide (33%) for 10 min and rinsed three times with sterile distilled water for 10 min each. Later, seeds were stirred in water for 24 h and then, after discarding floating seeds, they were air-dried in a laminar flow cabin for at least 2 h. Spores of *F. circinatum* were obtained by scraping a 7-day-old culture of a fungus colony growing on PDA at 25°C, adding 0.5% KCl + 1 drop of Tween20, and filtered through two layers of cheese cloth. The concentration was adjusted to give a spore suspension of 10^3 spores/µl. Seeds were immersed and stirred in this spore suspension for 30 s and then air dried.

The used methods are modified according to Evira-Recuenco *et al.* [24]. Inoculated seeds were divided into 6 samples; each sample contained at least 100 seeds. One of the samples was used as a control sample with infection and without plasma treatment (1^{st} control). Another sample of 100 seeds (not infected with fungi) was immersed in a solution of 0.5% KCl with Tween20 and was included in the experiment as a control (2^{nd} control).

2.2. SEED PLASMA TREATMENT

In our experiments, plasma treatment of seeds was carried out using a plasma source based on DCSBD at atmospheric pressure in ambient air (Fig. 1a). The used DCSBD plasma panel (Roplass, Brno, CZ) has an electrode system consisting of 16 pairs of parallel strip-line silver electrodes embedded 0.5 mm below the surface of 96% Al_2O_3 ceramic. DCSBD generates a thin uniform layer of macroscopically homogeneous plasma on the alumina plate (Fig. 1b). The DCSBD has already been described in greater detail by Cernak *et al.* [25].

The discharge was powered by 14 kHz sinusoidal high voltage with an amplitude of approximately 10 kV, supplied by HV Plasma Power Supply Lifetech VF 700 (Lifetech, Brno, CZ). The electrical parameters of the discharge were monitored by Pearson current probe Model 4100 (Pearson Electronics, Palo Alto, CA, USA) and two high voltage probes Tektronix P6015A (Tektronix, Beaverson, OR, USA). The signals from all three electrical probes were recorded by the Tektronix TDS 2014B digital oscilloscope (Tektronix, Beaverson, OR, USA). The total power consumed by DCSBD plasma source was calculated from measured time development of current/voltage waveforms (Fig. 1c) using the formula

$$P = \frac{1}{T}\int_0^T u(t)i(t)\mathrm{d}t\,.$$

The efficiency of energy transfer to the discharge is approximately 95%. The corresponding volume power density of plasma was determined to be approximately 80 W cm^{-3} taking into account the working input power of 400 W, the plasma layer thickness 0.3 mm and the active plasma area 200 × 80 mm.

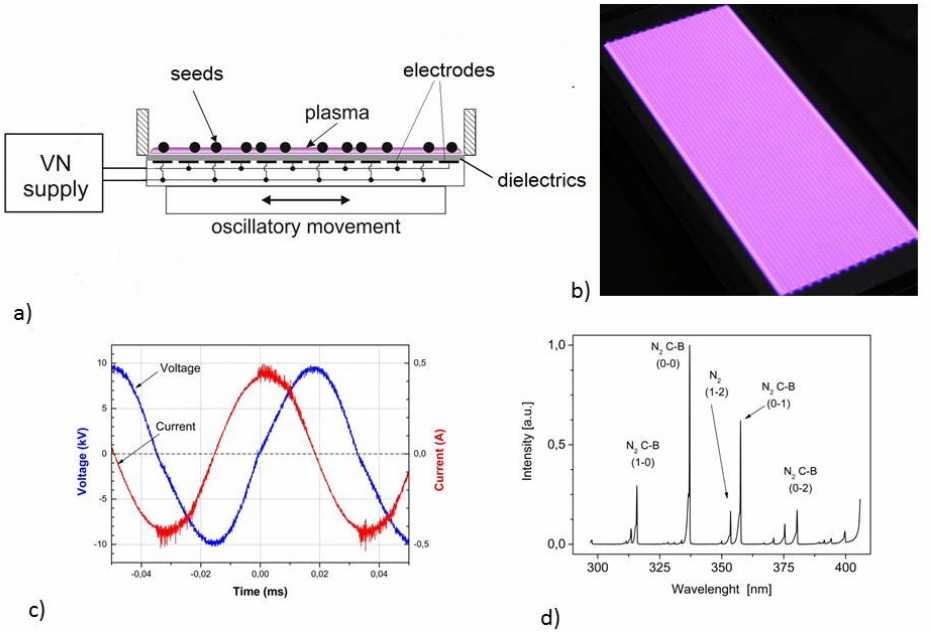

Fig. 1 – Diffuse Coplanar Surface Barrier Discharge (DCSBD) plasma source. Experimental setup (1a.), photo of the thin plasma layer (1b.), time development of voltage and current waveforms (1c.), typical emission spectrum of DCSBD plasma generated in ambient air at atmospheric pressure at input power 400 W (1d.).

AvaSpec–2048 Thermo-Electric Cooled Spectrometer (Avantes, Apeldoorn, Nederland) in a spectral range of 300–400 nm and resolution 20px/nm was used for the optical emission spectroscopy of DCSBD plasma. Spectrum was integrated for 1000 ms. A typical emission spectrum of the DCSBD plasma generated in ambient air at input power 400 W is shown in Fig. 1d, with dominant 2nd positive system of molecular nitrogen $N_2(C^3\Pi_u - B^3\ \Pi_g)$, used to determine temperatures in nitrogen-containing discharges at atmospheric pressure, particularly in non-isothermal plasma. Peaks were identified using Spectrum Analyser [26] and simulated in Specair 2.2 [27]. The vibrational temperature of 3300 K (±100 K) and the rotational temperature of 370 K (±30 K) were determined from the comparison of measured and simulated emission spectra of 2nd positive system of molecular nitrogen. These results confirm the non-equilibrium character of DCSBD plasma, where the electron temperature, represented by vibrational temperature, is high enough for electrons to participate in chemical reactions. The temperature of neutral molecules and ion, represented by rotational temperature, is about room temperature. The fact that DCSBD air

plasma can be touched by hand is a simple illustration that this kind of plasma is non-isothermal and safe to use.

Plasma treatment of seeds was realised at an input power of 400 W. DCSBD was fixed to the vortex to ensure uniform rotation of 330 rpm of the seeds on a ceramic surface and homogeneous plasma processing. As depicted in Fig. 1a, the treated seeds were placed in the plasma layer on ceramic. The plasma treatment times were: 0 s (1st control, see above), 5 s, 10 s, 60 s, 180 s, and 300 s. One set of samples was without fungal infection and without plasma treatment (2nd control, see above).

2.3. SEED CULTIVATION

Seeds were submitted for cultivation following the sample set splitting as it has already described. Twenty seeds per one sterile plastic Petri dish (90 mm diameter, Sarstedt, Numbrecht, Germany) were placed onto a surface of Sabouraud agar, a solid medium suitable for the cultivation of fungi (prepared according to the instructions of its manufacturer Biolife, Milano, Italy). All handling of seeds was carried out in a sterile flow box. Five parallel Petri dishes were used for one seed sample (100 seeds were used for one set).

Petri dishes were cultivated at 28°C for 12 days. During the experiment, two characteristics were observed (1) mold presence: appearance of a mold colony forming around seeds presented in percentage (maximum of 20 colonies per one Petri dish is 100%) and (2) seed germination: percentage of germinated seeds (seed germinated if 1 mm radicle was visible). Percentage of germinated seeds was calculated according to the method described by Sera *et al*. [28].

2.4. DATA ANALYSIS

The data sets were arcsin transformed (y = arcsinSQRT(x/n)) to obtain a normal distribution and subjected to a one-way analysis of variance (ANOVA). The dependent variables were mold presence and seed germination and the classification variables were treatment and control samples. A series of Tukey HSD post-hoc tests were run to answer the pair comparisons question. The analysis was performed using STATISTICA software (Statistika Cz 6, StatSoft CR, CR) at the significance level of 0.05.

3. RESULTS

3.1. SEED SURFACE DISINFECTION

After 3 days of cultivation, fungal filaments appeared on/around the individual seeds, which had been treated for 10 s or less. Total growth inhibition was found in samples after 60 s of plasma treatment, where no contamination was detected

during or at the end of the experiment (12 days, Fig. 2). A significant difference in mold spreading was found in samples with different treatment times (ANOVA, $P < 4.10^{-3}$). Statistically significant differences were found among samples in the 1st control, 5 s and 10 s treatments (Tukey HSD, $P < 4.10^{-3}$). Examples of representative Petri dishes from the experiment are shown in Fig. 3.

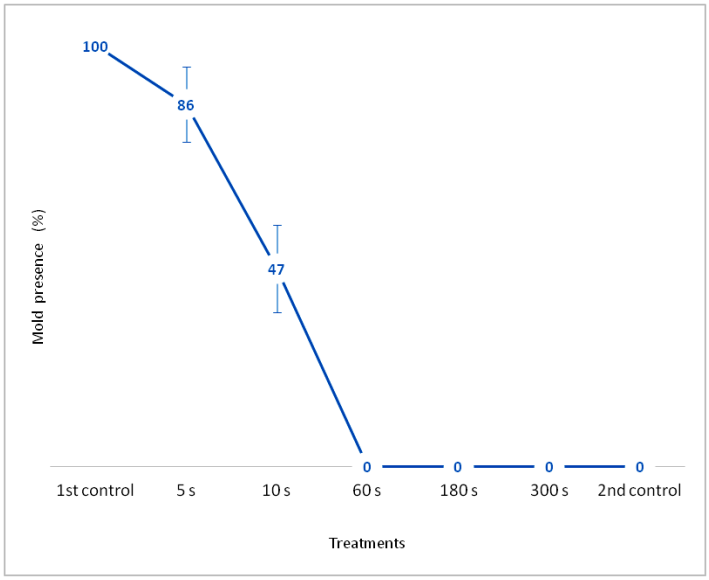

Fig. 2 – Mold presence of *Pinus radiata* seed surface inoculated with *Fusarium circinatum* after DCSBD plasma treatment on the 12th day of experiment. Seeds treated for 60 s or more had no fungal infections. Significant differences were found among samples in the 1st control, 5 s and 10 s treatments (Tukey HSD, $P < 4.10^{-3}$). 1st control was inoculated and without plasma treatment, 2nd control was neither inoculated nor plasma treated. For details see Materials and Methods.

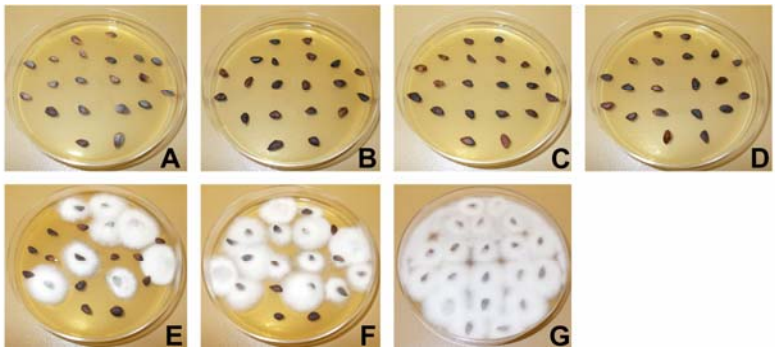

Fig. 3 – Cultivation of *Pinus radicata* seeds inoculated with *Fusarium circinatum*. Petri dishes represent control and experimental samples on the 12th day of experiment. Seeds treated with DCSBD plasma for 0 s (A, 2nd control), 300 s (B), 180 s (C), 60 s (D), 10 s (E), 5 s (F), 0 s (G, 1st control). 1st control was inoculated and without plasma treatment, 2nd control was neither inoculated nor plasma treated.

3.2 SEED GERMINATION

Seeds started to germinate in samples of both controls and after 10 s plasma treatment on the 3rd day of cultivation. At the end of the experiment (12 days), most seed germination (16%) was found in seeds without fungal infection and without plasma treatment (2nd control). Seed germination in the 2nd control significantly differs from samples of the 1st control, 5 s and 10 s (Tukey HSD, $P < 2 \cdot 10^{-4}$). Inoculated seeds treated for 1st control, 5 s and 10 s had 2.67%, 5.33% and 6.67% germination respectively at the end of the experiment (no significant difference). Seed germination samples of 5 s and 10 s were between germination of 1st control (2.67%) and 2nd control (16%) (Fig. 4). Inoculated seeds treated for 60 or more seconds did not germinate.

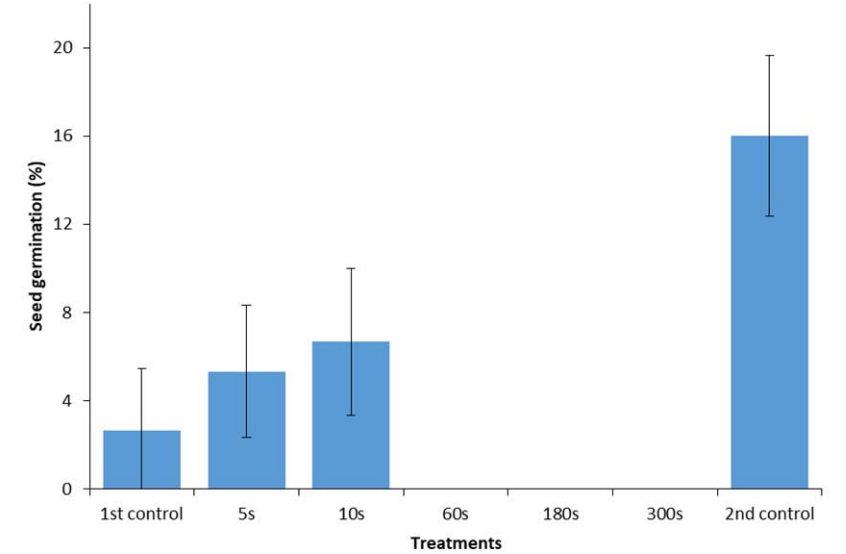

Fig. 4 – Percentage of seed germination of *Pinus radiata* seed (average and SD are given) inoculated with *Fusarium circinatum* and treated with DCSBD plasma on the 12th day of experiment. No seed germination was observed if the duration of DCSBD treatment was 60 s and longer. 1st control was inoculated and without plasma treatment, 2nd control was neither inoculated nor plasma treated.

4. DISCUSSION

4.1. SEED SURFACE DISINFECTION

It is well known that alcohol at a concentration between 50% and 80% and hot water are effective disinfectants. Alcohol is suitable as a combined cleaning and disinfection agent. The use of alcohol is limited, because it fixes proteins by a

process of protein denaturation. Plant and tree seeds are living organisms containing proteins, so they are not suitable for surface disinfection with alcohol.

Agusti-Brisach *et al.* [29] found that hot water of ±51°C for 30 min can be used to substantially reduce *F. circinatum* contamination of *P. radiata* seeds. This article indicates hot water as potentially useful for pine seed disinfection against *F. circinatum*. Non-thermal plasma generated using air DBD significantly reduced fungal inoculation of *Gibberella fujikuroi* from rice seed surface [30]. The *G. fujikuroi* fungus is related to the *F. circinatum* species. This study proved that a DBD device is harmless to the viability of rice seeds in addition to demonstrating strong antifungal activity.

The presented result (Fig. 2 and Fig. 3) corresponds with disinfection using the same DCSBD device, where the total growth inhibition of *Fusarium nivale* and *F. culmorum* was observed for 60 s of exposure [15]. Non-thermal atmospheric pressure plasma treatment was used to degrade mycotoxins produced by *Fusarium* spp., *Aspergillus* spp., and *Alternaria alternata*. It was found that all the investigated mycotoxins were completely degraded by plasma treatment for 60 s [31]. The question is which technology, whether hot water or plasma (or a combination), is more effective and environmentally friendly. Further studies on the efficiency should be provided.

4.2. SEED GERMINATION

The seeds of some plant species usually germinate well after non-thermal plasma treatment, *e.g.* some grains [18, 32]; *Chenopodium album* agg. and *Papaver somniferum* [33, 34]; *Zea mays* [35]; *Pisum sativum* [19]; *Brassica napus* [36]; *Morus nigra* [37]; *Raphanus sativus* [38]), but others do not (*e.g. Avena sativa* [28]; *Rhododendron smirnowii* [39]). The type of plasma apparatus/discharge and the parameters of the plasma treatment are crucial. Significant differences were found in the germination and early growth of *Fagopyrum esculentum* [39] and *Cannabis sativa* when different apparatus/parameters were used [40]. So, different pine species will probably respond to plasma treatment in different ways.

4.3. SEED SURFACE DISINFECTION IN RELATION TO SEED GERMINATION

The infection with *G. circinata* caused a reduction of seed germination and DCSBD plasma treatment increased germination of the infected seeds. The difference in seed germination between un-inoculated and inoculated seeds was noticeable (1st and 2nd control samples, Fig. 4). The seeds with a deactivated surface with DCSBD treatment did not germinate. These results correspond to the conclusion of Mitra *et al.* [13], the optimal treatment time for killing microbes damaged the seed vitality. They found that a significant reduction of the seed-borne

microbial contamination was observed after 120 s and 300 s of non-thermal plasma treatment in chickpea seeds (*Cicer arietinum*). On the other hand, exposure to 60 s of non-thermal plasma treatment showed an improved characteristic of chickpea germination. Compared with them, our experiment did not use natural contamination, but a targeted inoculation of *F. circinatum,* and the contaminated seeds were part of the germination test. It is very likely that a type of microbial infection can affect both seed germination and growth. The parameters and designs in these experiments [13, 30] are partly similar to our experiment.

Our described experiment is the first presented relation between pine seeds and fungi infection after non-thermal plasma treatment generated from a DCSBD device. Although this plasma treatment did not result in both the conservation of seed germination and seed-surface decontamination, we believe that new experiments using different and more precisely optimized parameters of plasma treatment will bring mold inactivation on vital seeds [see 13, 22, 30]. Further experiments will be focused on the duration of plasma treatment between 10 s and 60 s (Fig. 2. and Fig. 4).

Surface disinfection by plasma treatment has its place in modern technology, its use is being thoroughly investigated in many research fields [10, 41]. According to these investigations, non-thermal plasma treatment is likely to inactivate *F. circinatum* from soil, forestry tools and equipment, planting containers, and forest nursery workers' clothing [8, 20, 41]. Above all, non-thermal atmospheric pressure plasma treatment is completely free of chemicals and thus may be considered to be an environmentally acceptable technology in green biotechnology.

5. SUMMARY

The results presented herein demonstrate the ability to inactivate seed surface inoculated with *F. circinatum* by non-thermal atmospheric pressure plasma (used Diffuse Coplanar Surface Barrier Discharge device). This was the first time the phytopathology fungus of trees was found to be sensitive to plasma treatment. This biotechnology is a promising step in seed technology.

Acknowledgements. This work has been running as part of COST programme solution (Action FP1406) and was partly supported by the Slovak Grant Agency for Science VEGA No. 1/0628/15, by the Slovak Research and Development Agency under the contract No. APVV-16-0216, and by the ERDF SPECTRA+ No. 20240120002 „Centre of Excellence for the Development of Settlement Infrastructure for the Knowledge Economy".

REFERENCES

1. OEPP/EPPO, *Data sheets on quarantine pests Gibberella circinata*, OEPP/EPPO Bulletin **35**, 383 (2005).

2. T. Quesada, V. Gopal, W. P. Cumbie, A. J. Eckert, J. L. Wegrzyn, D. B. Neale, B. Goldfarb, D. A. Huber, G. Casella, and J. M. Davis, *Association mapping of quantitative disease resistance in a natural population of loblolly pine (Pinus taeda L.)*, Genetics **186,** 677 (2010).
3. T. R. Gordon, S. C. Kirkpatrick, B. J. Aegerter, D. L. Wood, and A. J. Storer, *Susceptibility of Douglas fir (Pseudotsuga menziesii) to pitch canker, caused by Gibberella circinata (anamorph = Fusarium circinatum)*, Plant Pathol. **55**, 231 (2006).
4. E. G. Brockerhoff, M. Dick, R. Ganley, A. Roques, and A. J. Storer, *Role of insect vectors in epidemiology and invasion risk of Fusarium circinatum, and risk assessment of biological control of invasive Pinus contorta*, Biol. Invasions **18**, 1177 (2016).
5. R. J. Ganley, M. S. Watt, L. Manning, and E. Iturritxa, *A global climatic risk assessment of pitch canker disease*, Can. J. Forest Res. **39**, 2246 (2009).
6. N. Lopez-Lopez, G. Segarra, O. Vergara, A. Lopez-Fabal, and M. I. Trillas, *Compost from forest cleaning green waste and Trichoderma asperellum strain T34 reduced incidence of Fusarium circinatum in Pinus radiata seedlings*, Biol. Control **95**, 31 (2016).
7. C. L. Swett, B. Porter, G. Fourie, E. T. Steenkamp, T. R. Gordon, and M. J. Wingfield, *Association of the pitch canker pathogen Fusarium circinatum with grass hosts in commercial pine production areas of South Africa*, Southern Forests **76**, 161 (2014).
8. T. R. Gordon, C. L. Swett, and M. J. Wingfield, *Management of Fusarium diseases affecting conifers*, Crop Prot. **73**, 28 (2015).
9. A. R. Morris, G. Fourie, I. Greyling, E. T. Steenkamp, and N. B. Jones, *Re-use of seedling containers and Fusarium circinatum association with asymptomatic Pinus patula planting stock*, J. Forest Sci. **76**, 177 (2014).
10. M. G. Brelles, *Biological and Environmental Application of Gas Discharge Plasmas*, California State Polytechnic University, CA, USA, 2012.
11. M. Baier, M. Gorgen, J. Ehlbeck, D. Knorr, W. B. Herppich, and O. Schlueter, *Non-thermal atmospheric pressure plasma: Screening for gentle process conditions and antibacterial efficiency on perishable fresh produce*, Innov. Food Sci. Emerg. Technol. **22**, 147 (2014).
12. P. Basaran, N. Basaran-Akgul, and L. Oksuz, *Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment*, Food Microbiol**. 25**, 626 (2008).
13. A. Mitra, Y. F. Li, T. G. Klämpfl, T. Shimizu, J. Jeon, G. E. Morfill, and J. L. Zimmermann, *Inactivation of surface-borne microorganisms and increased germination of seed specimen by cold atmospheric plasma*, Food Bioprocess. Technol. **7**, 645 (2014).
14. M. Selcuk, L. Oksuz, and P. Basaran, *Decontamination of grains and legumes infected with Aspergillus spp. and Penicillium spp. by cold plasma treatment*, Biores. Technol. **99**, 5104 (2008).
15. A. Zahoranova, M. Henselova, D. Hudecova, B. Kalinakova, D. Kovacik, V. Medvecka, and M. Cernak, *Effect of cold atmospheric pressure plasma on the wheat seedlings vigor and on the inactivation of microorganisms on the seeds surface*, Plasma Chem. Plasma Process. **36**, 397 (2016).
16. I. Mraz, P. Beran, B. Sera, B. Gavril, and E. Hnatiuc, *Effect of low-temperature plasma treatment on the growth and reproduction rate of some plant pathogenic bacteria*, J. Plant Pathol. **96**, 1 (2014).
17. K. Ebihara, M. Takayama, T. Ikegami, K. Ogata, H. D. Stryczewska, Y. Gyoutoku, and T. Sakai, *Development of agricultural soil sterilization using ozone generated by high frequency dielectric barrier discharge*, J. Adv. Oxid. Technol. **9**, 170 (2006).
18. C. Hertwig, A. Leslie, N. Meneses, K. Reineke, C. Rauh, and O. Schluter, *Inactivation of Salmonella enteritidis PT30 on the surface of unpeeled almonds by cold plasma*, Innov. Food Sci. Emerg. Technol. **44**, 242 (2017).
19. T. Stolarik, M. Henselova, M. Martinka, O. Novak, A. Zahoranova, and M. Cernak, *Effect of low-temperature plasma on the structure of seeds, growth and metabolism of endogenous phytohormones in Pea (Pisum sativum L.)*, Plasma Chem. Plasma Process. **35**, 659 (2015).

20. H. D. Stryczewska, K. Ebihara, M. Takayama, Y. Gyoutoku, and M. Tachibana, *Non-thermal plasma-based technology for soil treatment*. Plasma Process. Polym. **2**, 238 (2005).
21. J. Vrajova, L. Chalupova, O. Novotny, J. Cech, F. Krcma, and P. Stahel, *Removal of paper microbial contamination by atmospheric pressure DBD discharge*, Eur. Phys. J. D **54**, 233 (2009).
22. N. Khamsen, D. Onwimol, N. Teerakawanich, S. Dechanupaprittha, W. Kanokbannakorn, K. Hongesombut, and S. Srisonphan, Rice (*Oryza sativa* L.) *Seed sterilization and germination enhancement via atmospheric hybrid nonthermal discharge plasma*, ACS Appl. Mater. Interfaces **8**, 19268 (2009).
23. P. Martinez-Alvarez, R. A. Fernandez-Gonzalez, A. V. Sanz-Ros, V. Pando, and J. J. Diez, *Two fungal endophytes reduce the severity of pitch canker disease in Pinus radiata seedlings*, Biol. Control **94**, 1 (2016).
24. M. Evira-Recuenco, E. Iturritxa, and R. Raposo, *Impact of seed transmission on the infection and development of Pitch Canker Disease in Pinus radiata*, Forests **6**, 3353 (2015).
25. M. Cernak, D. Kovacik, J. Rahel, P. Stahel, A. Zahoranova, J. Kubincova, A. Toth, and L. Cernakova, *Generation of a high-density highly non-equilibrium air plasma for high-speed large-area flat surface processing*, Plasma Phys. Control. Fusion **53**, 124031 (2011).
26. Z. Navratil, D. Trunec, R. Smid, and L. Lazar, *A software for optical emission spectroscopy – problem formulation and application to plasma diagnostics*, Czech J. Phys. **56**, 944 (2006).
27. C.O. Laux, *Radiation and nonequilibrium collisional-radiative models*, von Karman Institute for Fluid Dynamics, Lecture Series 2002-07. In: Fletcher D., Charbonnier J.-M., Sarma G.S.R., Magin T. (eds.), *Physico-chemical Modeling of High Enthalpy and Plasma Flows*, Rhode-Saint-Gense, Belgium, 2002.
28. B. Sera, P. Spatenka, M. Sery, N. Vrchotova, and I. Hruskova, *Influence of plasma treatment on wheat and oat germination and early growth*, IEEE Trans. Plasma Sci. **38**, 2963 (2010).
29. C. Agusti-Brisach, A. Perez-Siera, J. Armengol, J. Garcia-Jimenez, and M. Berbegal, *Efficacy of hot water treatment to reduce the incidence of Fusarium circinatum on Pinus radiata seeds*, Forestry, **85**, 629 (2012).
30. Y. K. Yo, J. Cho, T. C. Tsai, D. Staack, M. H. Kang, J. H. Roh, D. B. Shin, W. Cromwell, and D. Gross, *A non-thermal plasma seed treatment method for management of a seedborne fungal pathogen on rice seed*, Crop Sci. **54**, 796 (2014).
31. L. ten Bosch, K. Pfohl, G. Avramidis, S. Wieneke, W. Viöl, and P. Karlovsky, *Plasma-based degradation of mycotoxins produced by Fusarium, Aspergillus and Alternaria species*, Toxins, **9**, 97 (2017).
32. A. E. Dubinov, E. M. Lazarenko, and V. D. Selemir, *Effect of glow discharge air plasma on grain crops seed*, IEEE Trans. Plasma Sci. **28**, 180 (2000).
33. B. Sera, M. Sery, V. Stranak, P. Spatenka, and M. Tichy, *Does cold plasma change the seed dormancy? Study on seeds of Lambs Quarters (Chenopodium album agg.)*, Plasma Sci. Technol. **11**, 750 (2009).
34. B. Sera, I. Gajdova, M. Sery, and P. Spatenka, *New physicochemical treatment method of Poppy seeds for agriculture and food industries*, Plasma Sci. Technol. **15**, 935 (2013).
35. M. Henselova, L. Slovakova, M. Martinka, and A. Zahoranova, *Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma*, Biologia, **67**, 490 (2012).
36. P. Puligundla, J. W. Kim, and C. Mok, *Effect of corona discharge plasma jet treatment on decontamination and sprouting of rapeseed (Brassica napus L.) seeds*, Food Control, **71**, 376 (2017).
37. V. Mildaziene, G. Pauzaite, A. Malakauskiene, R. Zukiene, Z. Nauciene, I. Filatova, V. Azharonok, and V. Lyushkevich, *Response of perennial woody plants to seed treatment by electromagnetic field and low-temperature plasma*, Bioelectromagnetics **37**, 536 (2016).

38. A. L. Mihai, D. Dobrin, M. Magureanu, and M. E. Popa, *Positive effect of non-thermal plasma treatment on radish seeds*, Rom. Rep. Phys. **66**, 1110 (2014).
39. B. Sera, I. Gajdova, M. Cernak, B. Gavril, E. Hnatiuc, D. Kovacik, V. Kriha, J. Slama, M. Sery, and P. Spatenka, *How various plasma sources may affect seed germination and growth. Proceedings of the International Conference on Optimalisation of Electrical and Electronic Equipment 2012*, OPTIM Paper **1–5**, 1365 (2012).
40. B. Sera, M. Sery, B. Gavril, and I. Gajdova, *Seed germination and early growth responses to seed pre-treatment by non-thermal plasma in hemp cultivars (Cannabis sativa L.)*, Plasma Chem. Plasma Process. **37**, 207 (2017).
41. V. Scholtz, J. Pazlarova, H. Souskova, J. Khun, and J. Julak, *Nonthermal plasma – A tool for decontamination and disinfection*, Biotechnol. Advances **33**, 1108 (2015).