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Short Communication

The Effect of Low-Temperature Plasma on Fungus Colonization of Winter Wheat Grain and Seed Quality

Leszek Kordas[1], Wojciech Pusz[2]*, Tomasz Czapka[3], Ryszard Kacprzyk[3]

[1]Department of Agroecosystems and Green Area Management,
Wrocław University of Environmental and Life Sciences, pl. Grunwaldzki 24a, 50-363 Wrocław, Poland
[2]Department of Plant Protection, Wrocław University of Environmental and Life Sciences,
pl. Grunwaldzki 24a, 50-363 Wrocław, Poland
[3]Institute of Electrical Engineering Fundamentals, Wrocław University of Technology,
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

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Abstract

The aim of this research was to determine the potential effect of plasma on fungi colonizing winter wheat grain and the effect of this process on seed quality. The subject of our study was the winter wheat grain. The process of seed disinfection was conducted in a reactor with a packed-bed (wheat grain). The assessment of both the effectiveness of spore destruction and of seed quality were conducted under 3-, 10-, and 30-second exposures. The voltage was set at 8 kV. For the mycological tests, 200 seeds were selected from each variant of the experiment, including control treatment that did not undergo plasma processing. Half of the seeds, i.e. 100, were subjected on their surface to 10-minute disinfection with 0.5% solution of sodium hypochlorite. The other half were put on Petri dishes filled with glucose-potato medium acidified with citric acid (PDA). A detailed study of seed quality (germination energy and ability as well as leaf and root length and the dry matter of the plant), was conducted under laboratory conditions, in a Sanyo climatic chamber on Petri dishes in two independent series of 10 repeats for each duration of exposure. The experiment demonstrated that the exposure of winter wheat grain to low-temperature plasma resulted in the reduction of the number of colonies of fungi forming on grain in the optimum time of 10 seconds. The results also showed a positive effect of the use of cold plasma on the basic values determining seed lot quality as well as on the development of winter wheat in the initial growth stage.

Keywords: germination ability, germination energy, plasma reactor, fungal colonies

Introduction

In recent years attention has been paid increasingly not only to high crop quantity but also to the protection of crop quality.

Seeds associated with fungi are marked by lower vigor and germination ability, [1] and they also pose a threat to the health of humans and livestock feeding on infested seeds [2]. During the growing season and during storage crop seeds are highly prone to be infected and colonized by a number of fungi species, both pathogenic and saprophytic. These fungi reduce, to a large extent, seed quality as well as the level of consumption [1].

The occurrence of seeds infested with the fungi of the genus *Fusarium* is particularly important from the consumer's point of view. These fungi decrease the content of

*e-mail: wojciech.pusz@up.wroc.pl

gluten and amylasic activity, and also they have the ability to produce carcinogenic mycotoxins [3, 4].

One of the most popular methods to protect seeds against pathogens is the chemical method [1]. Its critical flaw, however, is high cost as well as a potential harmfulness to humans, animals, and the environment [5]. Nowadays, alternative methods of seed dressing are being pioneered, and they are expected to be marked by increased effectiveness, reduced harmfulness, and lower costs. One such inventive method seems to be a technology using the properties of low-temperature plasma [6]. Yet the use of plasma as a seed dressing raises serious doubts concerning not only the method's effectiveness but also its impact on seed quality. That problem was examined by a number of authors [7-9], whose studies focused on germination ability, increases in leaves and roots and dry matter, as well as changes in enzyme content. The results obtained by researchers are ambiguous and, regrettably, fail to provide clear insight into winter wheat. It is understandably imperative that further studies are carried out in this area.

Plasma is an ionized gas comprised of electrons, ions, atoms, and molecules. The term "plasma" was first introduced by an American researcher, Irving Langmuire, to determine the collection of charged particles emitted during electrical discharge in gases [6]. Cold plasma has been successfully used in electro-surgury, tissue engineering [10], modification of the surface of biologically consistent materials (e.g. transplants) [11], and also during the sterilization of various materials and instruments.

The objective of the studies was to determine the potential effect of plasma on fungi colonizing winter wheat grain and seed quality.

Experimental Procedures

The mycological studies were done in the Department of Plant Protection and the analysis of seed ability in the Department of Agroecosystems and Green Areas Management at the University of Environmental and Life Sciences in Wrocław, while grain exposure was provided in the Institute of Electrical Engineering Fundamentals at the Wrocław University of Technology.

Studies into Low-Temperature Plasma

The grain of winter wheat was the subject of our research. Seed disinfection was facilitated in a reactor with a packed-bed [12], where the so-called "packed-bed" was processed grain. The use of grain, whose permittivity is much higher than that of air, was expected to increase the field strength in gas gaps leading to discharges. Plasma, as the result of the discharges, would then provide the surface sterilization of grain.

The features of the reactor included: power supply and voltage of 100 Hz AC, plane-cylindrical geometry, aluminum electrodes, 100 ml volume, 80 mm diameter of the electrodes, and 20 mm distance between electrodes.

The discharges were executed in the bed with gaps filled with air under atmospheric pressure. The assessments of the effectiveness of spore destruction, and of seed quality were performed under exposures of 3, 10, and 30 seconds in duration, and power supply frequency 100 Hz (lower frequency) and 83 kHz (higher frequency). The voltage was set at 8 kV.

Mycological Analysis

For the mycological tests 200 seeds were selected from each variant of the experiment, including control treatment, where no plasma processing was involved. Half of the seeds, that is 100, underwent the process of surface disinfection, of ten minutes' duration, with 0.5% solution of sodium hypochlorite. The other half of the batch, 100 seeds, was put on Petri dishes filled with glucose-potato medium acidified with citric acid (PDA). After an incubation of between two and seven days' duration, at 22ºC, in darkness, the fungal colonies, grown on each one of the Petri dishes of 90 mm in diameter, were counted and identified. The specific identification of the sampled fungi was performed using macro- and microscopic observations, namely the morphology of hyphae, conidia and sporangia, of the colonies that had grown on culture media, applying the commonly accepted methods used in mycological laboratories. The fungi were identified by means of diagnostic keys [15-17].

Assessment of Seed Quality

A study into seed quality, germination energy, and ability in particular was undertaken under laboratory conditions in the climatic chamber

Sanyo (Incubator type MLR-352H – SANYO Electric Biomedical Co., Ltd., Osaka, Japan), on Petri dishes, 110 mm in diameter, in two independent series of 10 repetitions for each duration of exposure. The observations were performed at 20ºC (±1ºC) in natural light, 15,000 lx, with the light-darkness alternating pattern 16 to 8 hours. A paper filter was used as growth medium and was kept pemanently damp during germination. Germination energy and ability were assessed on days four and eight of the experiment. The measurements of the length of leaves and roots were obtained from all the plants on each dish, while dry mass was established employing the oven-drying method.

Statistical Analysis

All the data were analyzed employing an analysis of variance. Means were compared using Student's t-test (least significant difference – LSD) at $\acute{\alpha}$=0.05.

Results

In the mycological analysis of the non-disinfected and disinfected seeds a total of nine species of fungi were isolated (Tables 1 and 2). In both experiments, the highest

Table 1. The number and frequency of fungal colonies isolated from non-disinfected grain of winter wheat.

Genus	Duration of exposure (seconds)							
	0		3		10		30	
	total	%	total	%	total	%	total	%
Alternaria alternata (Fr.) Keissl.	106	42.4	10	15.9	11	40.7	21	60.0
Alternaria botrytis (Preuss) Woudenberg & Crous					1	3.7		
Aspergillus brasiliensis Varga. Frisvad & Samson	11	4.4	1	1.6	4	14.8		
Epicoccum nigrum Link	11	4.4					1	2.9
Fusarium culmorum (W.G. Sm.) Sacc	14	5.6	1	1.6	1	3.7		
Fusarium poae (Peck) Wollenw.			1	1.6				
Gibberella zeae (Schwein.) Petch					1	3.7		
Mucor hiemalis Wehmer	7	2.8						
Penicillium spp.			17	27.0	5	18.6	6	17.1
Rhizopus stolonifer (Ehrenb.) Vuill	100	40.0	24	38.1	4	14.8	7	20.0
Trichoderma spp.			4	6.3				
Non-spore-forming fungi	1	0.4	5	7.9				
Total	250a		63b		27d		35c	

Means followed by the same letter do not differ significantly. Student's t-test least significant difference (LSD) test, $\alpha \leq 0.05$.

share was claimed by the fungus *Alternaria alternata*, which, in the case of the non-disinfected seeds, and depending on the duration of exposure to low-temperature plasma, varied from 42% in the control group to 60% when the seeds were irradiated for 30 seconds (Table 1). The *A. alternata* fungus on the disinfected seeds accounted for 76% of all the fungi in the control group and for nearly 26% in the variant with 30 seconds of plasma duration (Table 2). Apart from *A. alternata*, the sample was characterized by a high proportion of fungi of the genus *Fusarium* and *Gibberella*, particularly in the case of seeds, and the disinfected species *Rhizopus stolonifer*, which in some combinations accounted for up to 40% of all obtained isolates.

While analyzing the potential impact of the length of seed exposure to plasma on the intensity of fungal colonization, it was found that the greatest decrease in fungal colonies was demonstrated in the case of the disinfected and non-disinfected seeds treated with plasma for 10 seconds. 27 colonies were formed on the non-disinfected seeds isolated in this variant, whereas 250 were identified in the control group. A similar correlation was observed in the case of disinfected seeds where the 10-second seed exposure to plasma led to the formation of 35 colonies, and the control combination yielded 146 (Tables 1 and 2). An extension of the seed-plasma interaction to 30 seconds resulted in the more mature fungal colonies on or in the seed.

The impact of the duration of exposure on the germination energy and ability is presented in Table 3. The table shows that the highest germination energy, on average by 5.5 percent higher than the control group, was observed for wheat under exposure of 10 seconds. When the time was extended to 30 seconds, a marked decrease in germination energy was observed, albeit this was not lower than that of germination without prior exposure.

The effect of cold plasma on the initial growth of winter wheat seedlings occurs in the first three seconds. The extention of time to 10 seconds triggered a slight reduction of the length of leaves even below that of the leaves, which were not treated with plasma. The reverse was true for seedling roots. Any cold plasma exposure of seeds resulted in shorter seedling roots, almost by 100%, but the values were not significantly different.

As far as an increase in dry matter is concerned, the highest positive effect of cold plasma was noticed for the three-second exposure. The subsequent seconds did not cause a significant reduction in the dry matter, even if it was below the control treatment

Discussion of Results

The study has demonstrated that with the prolongation of plasma treatment the number of fungal colonies on the seeds decreases. These findings seem to be in accord with those of other researchers [7, 18-20].

A similar study was conducted by Selcuk et al. [20]. The authors examined the effect of low-temperature plasma on the occurence of the fungi of the genus *Aspergillus* and *Penicillium* in several crop species seeds, including seeds of *Triticum durum*. They observed a positive correlation between the use of low-temperature plasma and the reduc-

Table 2. The number and frequency of fungal colonies isolated from disinfected grains of winter wheat.

Genus	Duration of exposure (seconds)							
	0		3		10		30	
	total	%	total	%	total	%	total	%
Alternaria alternata (Fr.) Keissl	111	76.0	13	28.9	2	5.7	10	25.6
Alternaria botrytis (Preuss) Woudenberg & Crous	3	2.1						
Aspergillus brasiliensis Varga. Frisvad & Samson	2	1.4			7	20.0	5	12.8
Fusarium culmorum (W.G. Sm.) Sacc	3	2.1	1	2.2	3	8.6	4	10.3
Fusarium oxysporum Schltdl.			1	2.2				
Gibberella avenacea R.J. Cook	7	4.8	1	2.2	8	22.9	2	5.1
Gibberella intricans Wollenw	7	4.8	7	15.6	1	2.9	2	5.1
Gibberella zeae (Schwein.) Petch			1	2.2				
Penicillium spp.			7	15.6	7	20.0	10	25.6
Rhizopus stolonifer (Ehrenb.) Vuill.	4	2.7	14	31.1	4	11.4		
Trichoderma spp.	3	2.1			1	2.9		
Non-spore-forming fungi	6	4.1			2	5.7	6	15.5
Total	146a		45b		35d		39c	

Means followed by the same letter do not differ significantly. Student's t-test least significant difference (LSD) test, $\alpha \leq 0.05$.

tion of fungi presence in the seed crops under study. It was furthermore established that the influence of plasma on fungal spermosphera depended on the surface of the seeds. Similar results were obtained by Morar et al. [7]. They examined the significance of highly intensive electric field to fungi occurrence on bean seeds. They showed a link between an increase of electric field and the decrease in the occurrence of pathogenic fungi on bean seeds. The effect was particularly noticeable in the fungi of the genus *Alternaria*, *Penicillium*, *Aspergillus*, and also *Fusarium*.

It was observed in the experiment that along with the extended period of plasma influence on the seeds there was a decrease in the number of fungal colonies of the genus *Fusarium*. This confirmed the results obtained earlier by Azharonok et al. [21]. The authors claimed that the extended time of plasma and radio waves exposure caused a decrease in the number of fungal colonies on the seeds of white lupine. Paradoxically, when the time was further extended, the disinfecting effect was no longer discernible.

The experiment demonstrates clearly that the optimum time to subject seeds to low-temperature plasma is 10 seconds. When extended to just under 30 seconds there is an increase in colony numbers. The results of other authors show that the use of low-temperature plasma over a thirty-second interval brings about a reduction in the number of pathogenic fungal colonies forming on rice grain by 100% [22]. The differences in the results seem to indicate that the low-temperature plasma effect is dependent on several varying factors, which has been confirmed by Selcuk et al. [20].

The figures fall into the so-called low rate (<1 J/cm^2) category, and this tallies with the reference data. The relatively short time of the process, for an approximate tenfold decrease of colony number, shows the possibility of a practical application during initial seed disinfection, e.g. prior to the purchase. Therefore it is important to assess the costs of such a treatment. These results show the potential appeal of seed disinfection with plasma, and the necessity of the optimization of sterilization process as well as better knowledge of the effect of electrical properties of seeds, conditions of the process, and the design of proper equipment.

The effect of the duration of exposure on germination energy and seed quality is illustrated in Table 3. The table shows that the highest germination energy, on average by 5.5 percentage points higher than that of the control group, was observed in the case of wheat under the exposure of 10 seconds. When the time was extended to 30 seconds, there was a pronounced decrease in germination energy, but not greater than when there was no exposure. A similar correlation was observed for germination ability, but the results are expressed in lower numerical intervals; nevertheless this relationship is analogical to germination energy. The use of cold plasma positively affects both features and improves the quality of seeds; notwithstanding, the differences were not statistically significant. Volin et al. [23] came up with similar results: the exposure of cereals to plasma led to a slight acceleration in the dynamics of germination, but for the bean it was slower. Also, recent studies have shown a slight positive effect of stimulation by cold plasma on wheat grain [8], buckwheat [9], and bean [7].

Table 3. The effect of low-temperature plasma on select plant properties at the early stages of their growth.

Duration of exposure [s]	Germination energy [%]	Germination ability [%]	Leaf length [cm]	Root length [cm]	Dry matter of plants [g]
0	93.5	95.5	14.6	20.8	23.5
3	96	97.5	14.8	11.6	24.2
10	98	98.5	14.5	11.3	24.1
30	97	97.5	14.7	11.2	22.6
(LSD) $\alpha \leq 0.05$.	n.s.	n.s.	n.s.	n.s.	n.s.

The effect of cold plasma on the initial growth of winter wheat seedlings occurs within the first three seconds. The time extended to 10 seconds was slightly instrumental in reducing the length of the leaves even below the length of the leaves that were not treated with plasma. The reverse was observed for seedling roots. Any exposure of the seeds to cold plasma resulted in shorter seedling roots (by almost 100%), but the values were not significantly different. Henselová et al. [24] and Šerá et.al. [9] claim that cold plasma stimulation leads to beneficial plant growth.

As far as an increase in dry matter is concerned, the highest positive effect of cold plasma was noticed for the three-second exposure. The subsequent seconds did not cause a significant reduction in dry matter; this reduction was not even below that of the control treatment. Similar results were obtained for corn [24] and for beans [7]. The study by Šerá et al. [9] shows the positive effect of cold plasma on the volume of buckwheat dry matter.

In summary, there seems to be incontrovertible evidence that the exposure of winter wheat grain to low-temperature plasma results in the reduction of the number of colonies of fungi occurring on grains.

The results also show the positive impact of cold plasma on seed quality as well as on the development of winter wheat in the initial growth stage. The use of cold plasma instead of chemical seed dressing is fully justified. It may also be used as a method of seed disinfection before storage in grain elevators or granaries.

Conclusion

The exposure of winter wheat grain to low-temperature plasma results in the reduction of the number of colonies of fungi occurring on grain: the optimum time of exposure is 10 seconds. The use of cold plasma instead of chemical seed dressing is fully justified. It may also be used as a method of seed disinfection before storage in grain elevators or granaries.

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