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Article

Non-Thermal Plasma Treatment Improves Properties of Dormant Seeds of Black Locust (*Robinia pseudoacacia* L.)

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Abstract: This article deals with the breaking down of seed dormancy in black locust (*Robinia pseudoacacia* L.) seeds. These seeds have a hard testa that is impermeable to water and air. In this investigation, seeds of black locust were treated with non-thermal plasma (NTP) for 0, 5, 10, and 20 min. The testa of the treated seeds had an eroded surface (SEM analysis) and showed an increased oxygen content (EDS) and increased hydrophilicity (contact-angle measurement). The exposed seeds also had a higher water absorption (seed water uptake) than the untreated ones. In seeds treated for 20 min, a significant difference ($p < 0.05$) was recorded in seed germination (eight times greater than the control set) and in other parameters of initial seedling development, such as the fresh weight of both seedling root and shoot and all three indexes of seedling vitality. The detected changes in the seed surface and changes in germination proved that NTP can partly break dormancy in black-locust seeds.

Keywords: cold plasma; hard seed; physical dormancy; seed germination; seedling

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1. Introduction

In recent years, there has been increased interest in non-thermal plasma (NTP) in agriculture in general, and plant cultivation in particular.

Seeds and dried fruits are frequently treated by NTP to improve their ability to germinate and grow, but the whole plant can also be treated for a study of an adaptive response to stress (factors such as heat, cold, and pathogens). Recent research has also focused on the application of NTP on seeds [1–4] as a new, and possibly eco-friendly method—it is free of aggressive chemicals, e.g., in pre-sowing methods, and has very low energy consumption in comparison to, e.g., scarification or hot-water soaking. NTP can be a solution for seed-stimulation and surface-decontamination, increased seed-testa hydrophilicity, stimulation of gene expression related to plant growth, and resistance to pathogens; several representative works are mentioned. The seed treatment of maize (*Zea mays*), wheat (*Triticum aestivum*), and lupine (*Lupinus angustifolius*) [5,6] contribute to the enhancement of seed germination, and improve their phytosanitary conditions. Holubová et al. [7] advocated NTP treatment of seeds as a priming tool for application of mild stress to prime a plant for stronger stress. Decontamination of the seed surface from bacteria, fungi, and insect pests, causing minimal damage and growth enhancement, are the most reported positive effects of NTP on plants, e.g., for wheat grains, as reviewed in Scholtz et al. [8]. Han et al. [9] reviewed mechanisms of plasma action on various endogenous enzymes, including peroxidase, polyphenol oxidase, lysozyme, α-chymotrypsin, alkaline phosphatase, and pectin methylesterase and on the activation of others such as superoxide dismutase, catalase, and lipase. The transformation of conformational structures including primary and spatial structures induced by chemical reactive species during NTP treatment has also been reported.

In addition to the direct action of NTP, the use of disinfectant solutions generated by the action of NTP on various liquids may also be useful. The principles and use of plasma-activated water (PAW) as a green and sustainable technology in food processing and plant growth in agriculture were reviewed [10,11]. Its use for seed treatment and improving plant growth is also widely discussed there. Liquids with properties and effects similar to PAW may be prepared not only by NTP treatment, but also by artificially mixing the active ingredients, namely nitric acid and hydrogen peroxide [12].

In our previous review [13] we summarized the effect of NTP on some species of the *Fabaceae* family, for example, the following strategic crops: peanut (*Arachis hypogaea*), carob (*Ceratonia siliqua*), soybean (*Glycine max*), lentil (*Lens culinaris*), alfalfa (*Medicago sativa*), common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and clover (*Trifolium* sp.).. Our other studies [14,15] were devoted to the plasma disinfection and germination improvement of pine seeds. In general, affecting of initial seedling growth is closely related to the above effects on germination.

However, only a small number of experiments have focused on the breaking of seed dormancy by NTP [16–24]. The oldest information on the possibility of breaking dormancy in seeds was after direct NTP treatment of *Chenopodium album* seeds, where the growth curve was influenced by the speed and number of germinating seeds [16]. Direct seed treatment with NTP was specifically tested on the species *Erythrina velutina* [17], *Mimosa caesalpiniafolia* [18], *Leucaena leucocephala* [21], *Medicago sativa* [22], and *Pityrocarpa moniliformis* [24], and the results reported an increase in the hydrophobicity of the seed surface and greater seed germination. Changes in ABA/GA phytohormones as a function of *Raphanus sativus* seed growth shed light on the physiological processes during dormancy-breaking [20]. The direct exposure to NTP [19] and PAW treatment [23] of *Arabidopsis thaliana* provided a physiological contribution to the nature of the dormancy-breaking process. It is very likely that NTP can probably be used for the physical or physiological dormancy of seeds [13,25].

In this investigation, we tried to extend our earlier results to seeds of black locust (*Robinia pseudoacacia* L.). The black locust belongs to the *Fabaceae* family (Leguminosae, legumes), a large taxonomic group of dicots with a worldwide distribution. This species is native to the east coast of North America [26], where it grows mainly in river alluvium. The first black locust trees were introduced to Europe in the early 17th century [27]. Their attractive flowers and exotic appearance have made them a popular tree in parks and gardens [28]. Later, the tree began to be used to reinforce steep banks around rivers and also on sandy slopes, where it serves as a reinforcement against landslides. This light-loving tree is used to colonize poor and dry soils, which it tolerates very well [29,30]. The black locust is cultivated as a commercial tree in many European countries, including Germany, Hungary, Poland, Austria, Czech Republic, Slovakia, Switzerland, and Slovenia [27]. Compared to native European tree species, it grows quickly, is resistant to diseases and pests, and its roots fix airborne nitrogen in the soil [31,32]. Its hard wood is of good quality, dense, strong, and calorific [33]. Neither the tree growth nor the wood is significantly affected by poor habitat conditions, so it can be grown even in sandy soils [31,33,34]. It is also used as a honey tree [35] and as a medicinal tree (flowers and bark) and is a popular tree in urban greenery [28].

The black locust can be propagated both generatively and vegetatively. Seed production is very high and seeds retain their ability to germinate for a period of up to 30 years, so they are part of a permanent seed bank [32,36]. The black locust usually starts to fruit around the sixth year and produces seeds every year or every two years. For the generative breeding of black locust in nurseries, the seed is obtained by hand by picking the pods or mechanically by sieving the top layer of overlying soil [29,33]. The seeds of black locust are dormant and hard and do not germinate even under suitable conditions. Their dormancy is due solely to the impermeability of the seed testa to water and gases, so it is a physical dormancy [37,38]. Overcoming dormancy is possible by mechanical or chemical disturbance of the seed testa, allowing water to be absorbed. There are three main methods of pre-sowing treatment: seed scarification, maceration in sulfuric acid, and soaking the seed in hot water [39–42]. Mechanical or chemical damage of the seed testa causes water absorption and triggers physiological processes leading to seed germination.

Based on previous results, we attempted to improve the limited germination of black-locust seeds, caused by physical dormancy, using eco-friendly NTP technology. We aimed to test the assumptions leading to the influence of seed germination and initial growth and to determine whether NTP causes: (1) physicochemical erosion of the testa surface and changes in O/C, detectable by SEM and EDS; (2) increase in the hydrophilic seed surface; (3) improvement in seed germination; and (4) improvement in seedling growth.

Although black locust is an invasive species in Europe, it is still an important forest tree in many countries. Breaking the dormant seeds of black locust by NTP technology will speed up and simplify generative propagation of tree. Improving the germination of these dormant seeds with NTP has the potential for practical applications in forestry. Pre-prepared seeds of black locust could improve germination and initial growth parameters.

2. Materials and Methods

2.1. Non-Thermal Plasma Apparatus

The seeds were exposed to NTP produced by the DC electric discharge generated by a point-to-ring electrode system. This electric discharge was based on the cometary discharge described by [43]. The scheme of the apparatus used is depicted in Figure 1. The working point electrode was made of a medical stainless-steel needle Medoject (0.6 × 25 mm), connected through a resistor R = 3 MΩ to the positive terminal of the high-voltage DC power supply. The ring electrode, realized by a 1 mm diameter nickel wire forming a 10 mm diameter circle, was connected through an ammeter to the negative terminal of the DC high-voltage power supply. The distance between the working electrode and the ring electrode was 3 mm. The discharge voltage was set to 9.65 kV, the discharge current to 150 μA, and the source voltage to 10.1 kV.

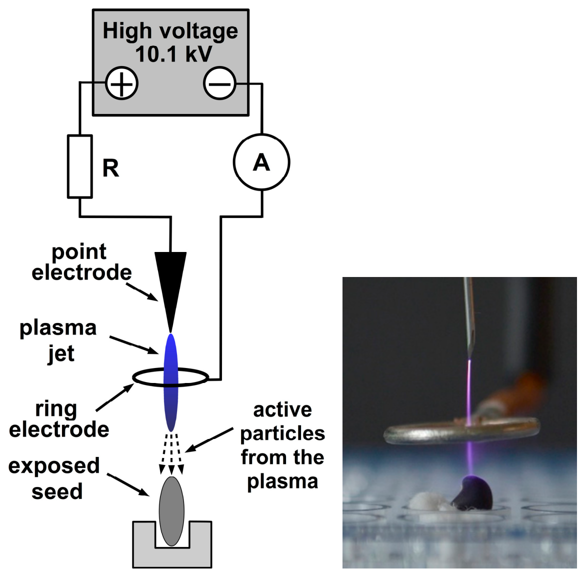


Figure 1. Scheme of non-thermal plasma apparatus (**left**) and photograph of electrical discharge (**right**).

The working electrode was oriented perpendicularly to the plane of the circular ring electrode so that its axis passed through the center of the plane of the ring electrode. A positive streamer was generated from the point electrode passing through the center of the ring, where a negative glow corona burned from the ring perpendicularly to the streamer.

As a result, the positive streamer extended further below the ring electrode and formed a plasma jet that treated the seed (Figure 1).

2.2. Seeds and Non-Thermal Plasma Treatment

The fruits of black locust (*Robinia pseudoacacia* L.) were plucked directly from the trees in the Royal Game Reserve in September 2020. The Royal Game Reserve (known as Stromovka) is one of the most important natural landscape parks in Prague. The park is located in the central part of the Prague Basin at an altitude of 185–220 m above sea level. (Warm climate area), with an average rainfall of 498 mm/year and a high groundwater level [44].

The fruits were obtained from a compact stand of black locust trees at the forest edge of the park. The seeds were manually peeled from the dried fruits (35 °C, 48 h) and stored in the dark at 23 °C until the experiment. The thousand seed weight and seed germination were determined to be 16.7 g and 5.56%, respectively.

A set of seeds was scarified with a small file in the micropyle and hilum area. The micropyle is the small opening between the envelopes of the plant egg, where the germ root grows from the seed during germination. The hilum is a scar lying near the micropyle from where the seed was attached to the plant placenta; it is produced by the separation of the seed from the placenta. The hilum and micropyle area are the weakest part of the seed testa. The seed germination of scarified black-locust seeds was 93.33%.

Only healthy dark seeds without obvious defects were selected for the experiment. The seeds were placed about 1.5 mm below the ring of the non-thermal apparatus (Figure 1) and exposed for 5, 10, and 20 min. The placement of the seeds tested was individual so that the hilum and micropyle area was directly treated. The untreated seeds (0 min) served as a control.

2.3. SEM and EDS Analyses

Scanning electron microscopy (SEM) images were obtained with a TESCAN MIRA 3 LMH (TESCAN, Brno, Czech Republic) equipped with a Shottky cathode. The elemental composition of the seeds was determined by energy-dispersive spectrometry (EDS) as an analytical method based on the detection of X-rays excited by the impact of an electron beam on the surface of the sample. Here, the EDS spectrometer (Bruker Quantax 200, Bruker, Brno, Czech Republic) at 10 kV of accelerating voltage as the part of electron microscope was used. Seed samples were mounted on aluminum specimen stubs using a double-sided adhesive carbon tape. To prevent surface charging, the seeds were coated with a 6 nm layer of gold.

2.4. Contact-Angle Measurement

The contact angles were determined using the Surface Energy Evaluation System (See System, Advex Instruments, Brno, Czech Republic). Measurements were performed by pouring 5 µL of distilled water onto the seed surface and recording the image of the drop with a CCD camera. The images were then processed by See System software to determine the contact angles.

2.5. Seed Water Uptake

The dry seeds were first weighed (m_0), then soaked in distilled water for 1, 2, 4, 6, and 24 h and weighed again (m). The following modified equation for water uptake, defined as the relative increase of mass [21], was used:

$$\text{water uptake} = \frac{m - m_0}{m_0}$$

2.6. Seed Germination and Early Growth Seedling

The tests were performed on three layers of filter paper in a 9 cm diameter Petri dish kept at 23 °C. Thirty seeds per dish were placed on filter paper moistened with 3 mL of distilled water. All experiments were repeated five times in parallel; thus, each treatment and control variant contained 150 seeds.

Data on number of germinating seeds, the shoot length (LS), root length (LR), fresh weight of shoot (FWS), and fresh weight of root (FWR) were measured on the 14th day of cultivation. Then, shoots and roots were dried separately at 60 °C for 24 h and the weight of the dried shoots (DWS) and roots (DWR) was determined.

The seedling length (L) was obtained as sum of length of root and length of shoot for each seedling; the fresh (FW) and dried weight of seedling (DW) were obtained in the obvious way. Root/shoot ratios were calculated from length (R/S_L), fresh weight (R/S_FW), and dried weight (R/S_DW) of root and shoot.

Seed germination (SG) was calculated as the percentage proportion between the number of germinating seeds and the number of seeds used in the germination test. Three seedling-vitality indexes were determined as the product of seed germination and seedling length (SVI_I), seed germination and seedling fresh weight (SVI_II), and seed germination and seedling dry weight (SVI_III).

The approach to the parameters of seed germination and early growth seedlings is described in more detail in [14,45].

2.7. Data Analysis

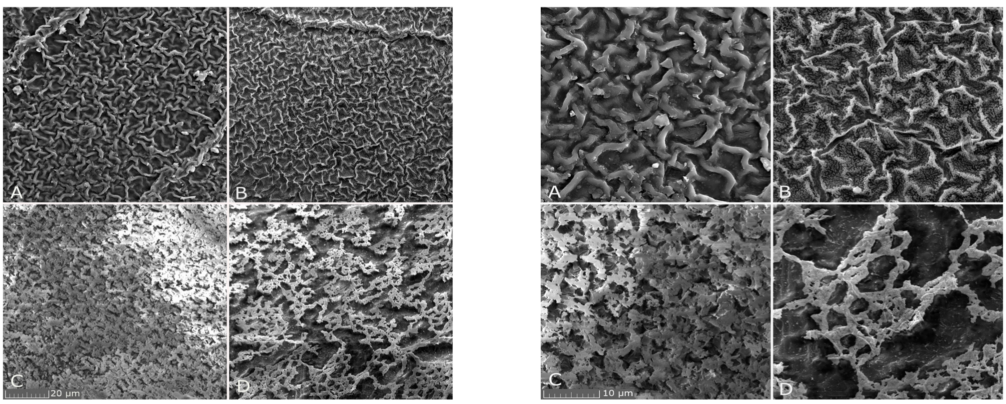
Experimental measurements were repeated five times. The dataset was first logarithmically transformed (y = log(x)) to normalize the data. This dataset was analyzed with a statistical package STATISTICA software (Statistica 13, StatSoft Inc., Tulsa, OK, USA). All statistical tests were performed at the significance level of 0.05.

One-way analysis of variance (ANOVA) was used to evaluate the influence of the NTP treatments on the measured characteristics. The detailed testing of the experimental variances was done using the Tukey HSD test for multiple comparisons. The data from the Tukey HSD test are presented in detail; significant differences at $p < 0.05$ are indicated by different letters.

3. Results

3.1. SEM and EDS Analyses

The SEM analyses found that gradual deterioration of the seed surface layer was apparent (Figure 2). Degradation of the seed surface was evident in seeds treated with NTP for 10 and 20 min at a magnification of 2000×. At higher magnification (5000×), surface degradation was visible even after a 5 min treatment. Peeling of the upper layers of the seed surface treated with NTP was observed after 20 min of exposure, at both magnifications used (Figure 2).



(**a**) (**b**)

Figure 2. Scanning electron microscopy of the black-locust seed surface after NTP treatment with these expositions: A, 0 min; B, 5 min; C, 10 min; and D, 20 min. Figure (**a**) magnification 2000×; Figure (**b**) magnification 5000×.

The EDS determined the contents of carbon, nitrogen, oxygen, magnesium, silicon, sulphur, chlorine, sodium, potassium, and calcium on the surface of the treated seeds' testa. For the purposes of this study, only the variable oxygen content of the seeds is relevant. The ratio of oxygen to carbon content (O/C) increased from 0.28 ± 0.03 to 1.4 ± 0.3 (Figure 3).

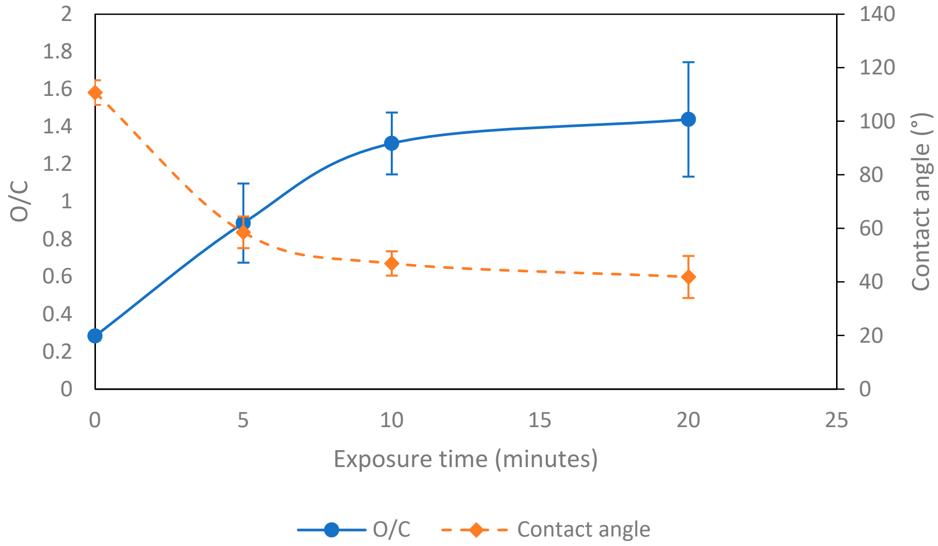


Figure 3. Oxygen-to-carbon (O/C) ratio and contact angle in exposed black-locust seeds as a function of non-thermal plasma exposure time.

3.2. Contact-Angle Measurement

The contact angle describes the hydrophilicity or hydrophobicity of the surface, which is related to the ability of the seeds to germinate. Figure 3 shows that the contact angle decreased with increasing exposure time from (111 ± 5) ° to (42 ± 8) °. An exposure time of 10 min was the turning point of this treatment, and the increase in the O/C ratio was slow.

3.3. Seed Water Uptake

The effect of NTP treatment duration on the water uptake of the seeds is shown in Figure 4. The water uptake increased with increasing treatment duration. The highest imbibition values were recorded for seeds treated with NTP for 20 min. A breakpoint of 400 min (x-axis) was evident for all treatment times used.

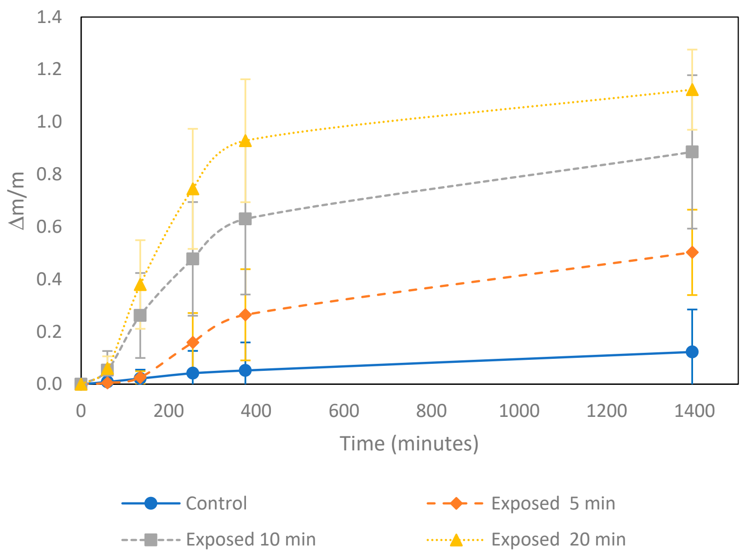


Figure 4. Dynamics of water uptake by black-locust seeds after non-thermal plasma treatment.

3.4. Seed Germination and Early Growth Seedling

The values of the seed germination (SG), fresh seedling weight (FW), fresh shoot weight (FWS), and seedling vitality indices (SVI_I, SVI_II, and SVI_III) were found to be significantly different in seeds treated with NTP for 20 min compared to those of the control set (see Table 1). The seed germination of seeds treated for 20 min increased to 45.67%, which was 803% compared to the control. The fresh length of seedling and the fresh length of the shoots for the seeds treated for 20 min were 84.95 mm and 57.71 mm (152% and 161%, respectively, compared to the controls). The values of seedling vitality indices calculated for seeds treated for 20 min were SVI_I = 3698.67 mm, SVI_II = 4.41 g, and SVI_III = 0.60 g, respectively, and this corresponded to 915%, 1026%, and 1000% compared to the control sets. No significant differences were found for the other evaluated parameters (Table 1).

Table 1. Parameters of seed germination and initial seedling growth in black locust after non-thermal plasma treatment.

Treatment	**SG (%)**			**SVI_I (mm)**			**SVI_II (g)**			**SVI_III (g)**		
	Mean	**SE**	**HSD**	**Mean**	**SE**	**HSD**	**Mean**	**SE**	**HSD**	**Mean**	**SE**	**HSD**
0 min	5.56	2.22	a	404.44	287.84	a	0.43	0.26	a	0.06	0.03	a
5 min	22	3.43	a	1499.33	263.46	ac	1.86	0.26	ac	0.31	0.06	ab
10 min	43.33	4.59	b	3058	564.37	bc	3.92	0.65	bc	0.65	0.12	b
20 min	44.67	6.72	b	3698.67	467.22	b	4.41	0.67	b	0.6	0.1	b
Treatment	L (mm)			LS (mm)			LR (mm)			R/S_L (mm)		
	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD
0 min	56	21.07	a	35.89	11.27	a	20.11	10.18	a	0.52	0.13	a
5 min	68.31	7.4	a	47.78	4.12	a	20.54	3.88	a	0.42	0.06	a
10 min	68.31	7.97	a	47.54	4.67	a	20.76	3.7	a	0.43	0.05	a
20 min	84.95	7.79	a	57.71	6.52	a	27.24	1.39	a	0.48	0.03	a
Treatment	FW (g)			FWS (g)			FWR (g)			R/S_FW (g)		
	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD
0 min	0.07	0.02	a	0.06	0.01	a	0.01	0	a	0.14	0.05	a
5 min	0.09	0	ab	0.08	0	ab	0.01	0	a	0.11	0.02	a
10 min	0.09	0.01	ab	0.08	0.01	ab	0.01	0	a	0.13	0.01	a
20 min	0.1	0.01	b	0.09	0.01	b	0.01	0	a	0.13	0.01	a
Treatment	DW (g)			DWS (g)			DWR (g)			R/S_DW (g)		
	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD	Mean	SE	HSD
0 min	0.011	0.002	a	0.01	0.002	a	0.001	0	a	0.061	0.035	a
5 min	0.014	0.001	a	0.013	0.001	a	0.001	0	a	0.086	0.015	a
10 min	0.014	0.001	a	0.013	0.001	a	0.001	0	a	0.098	0.005	a
20 min	0.013	0.001	a	0.012	0.001	a	0.001	0	a	0.104	0.007	a

Mean and standard error (SE) are given. Tukey HSD test was used: significant differences at $p < 0.05$ are indicated by different letters. See detail in Materials and Methods.

4. Discussion

The tested black locust had a natural seed germination rate under 6% and after the scarification of the hilum and micropyle area, the seed germination increased by over 90%. Therefore, disruption of seed testa significantly improved germination, so we can assume that the seeds dormancy is of physical origin [37,38]. Seed germination after NTP treatment was never at the same level as after scarification; however, the germination of the treated seeds was still significantly higher in comparison to the control sample (Table 1). Furthermore, a significant effect of NTP on initial seedling growth parameters was also recorded (Table 1). This was not related to the seed dormancy, but indicated the ability of NTP to improve seeds growth [25].

Despite the fact that the NTP treatment in other studies was usually carried out over the entire surface of the seed and for a larger number of seeds at once [1,4,6,14–16], in this study, the hilum and micropyle region of individual seeds (ca. 2 mm^2), as a sensitive part of the testa, were treated directly. SEM photographs showed visible modification of treated area, while the rest of the seed surface remained untouched (Figure 2), despite the boundary between the treated and untreated areas not being sharp. Moreover, in addition to mechanical surface modification, NTP treatment also caused the incorporation of oxygen-containing functional groups into the seed testa, increasing the O/C ratio until the pore layer is saturated (Figure 3). The surface of the seed became hydrophilic, leading to a reduction in the contact angle [46], see (Figure 3). This modification of both the O/C ratio and contact angle was at its maximum after approximately 10 min of exposure. However, the interesting fact remains that the water uptake was improved even after 20 min of exposure. As an explanation, it should be noted that while the O/C ration and contact angle were measured at the most exposed area, the water had soaked through the whole seed surface. Therefore, over a longer exposure time, a larger area of the seed is treated, and higher water uptake may be assumed.

Breaking physical dormancy consists of breaking through an impassable barrier, such as the seed testa, which leads to a subsequent increase in seed germination. Several targeted experiments that used the application of NTP on species that have physical seed dormancy have already been carried out (Table 2). The results obtained on *Erytrina velutina* [17], *Mimosa caesalpiniafolia* [18], and *Leucaena leucocephala* [21] provide very valuable and detailed information about the wetting and absorbance of the surface parts of the treated seed in relation to the seed dormancy (Table 2). Changes in seed surface and testa permeability and wettability were also demonstrated in black locust after the NTP application (Figures 2–4, Table 1). The topic of the wettability of seed surfaces treated with NTP was recently systematically reviewed [47].

Table 2. Plant species from *Fabaceae* family that have been tested for breaking dormancy using NTP.

Plant	NTP Treatment	Changes in Seed Testa	Seed Germination	Comments	References
Robinia pseudoacacia	Point-to-plane corona discharge in regime of transient spark; discharge voltage 9.65 kV, discharge current 150 µA, source voltage 10.1 kV; 3 mm distance; 5, 10, and 20 min	Eroded surface (SEM), increased content of oxygen (EDS), increase of hydrophilicity (contact angle), higher water absorption (seed water uptake)	Significant difference ($p < 0.05$) in seed germination (8×) in seeds treated for 20 min	Fresh weights of seedling root, fresh weights of seedling shoot, and all three indexes of seedling vitality were significantly better than in control samples.	Present manuscript
Pityrocarpa moniliformis	DBD; power of 10 kV and frequency of 400 kHz; duration 1.5, 2.0, 3.0, 4.0, and 5.0 min	Lowest contact angles of 64 and 61° after 5.0 and 4.0 min; electrical conductivity of soaking liquid and faster reaching phase III were higher in treated seeds	Highest cumulative germination after 5 min	NTP can be used in *P. moniliformis* seed-stimulation.	[24]
Medicago sativa (cultivars + wild plants)	Point-to-plane corona discharge in regime of transient spark; voltage of 4.6 kV; 3 mm distance; TS treatment: 5, 10, and 20 min; PAW treatment: pH≈ 3–4; c(H2O2) ≈ 100 mg/L; c(NO3−) ≈500 mg/L; c(NO2−)≈1 mg/L	Not tested	Seed germination increased (not significantly) from 21% (control) to 34% (TS + PAW treatment, 10 min) in wild plant	Significant increase in some initial growth parameters of seedlings in cultivars.	[22]
Leucaena leucocephala	DBD; pulses at 17.5 kV, frequency of 990 Hz; duration of 3, 9, and 15 min	Microcracks and contour in the hilar region and micropyle; electrical conductivity and pH of leached water correlated to imbibition time	Germination percentage changed from 4% (control) to 7% (treated)	Used NTP not sufficient to overcome dormancy. There are two resistance barriers to water penetration: integument surface and region of the macrosclereid cell wall in the seed.	[21]
Mimosa caesalpiniafolia	DBD; pulses at 17.5 kV, frequency of 990 Hz; duration of 3, 9, and 15 min	Saturation of the seed imbibition after 9 min	Germination rate was 8 times higher than control after 3 min; the best proportion of Richard curve after 30 min	Wettability and imbibition were directly related to the treatment duration.	[18]
Erythrina velutina	DBD; voltage of 10 kV, frequency of 750 Hz, power of 150 W; gas He, flux of 0.03 L/s; distance of 13 mm for 60 s	Contact angle decreased from 112° by 48% ($p < 0.05$); higher imbibition of 75% in micropyle and hilum	Germination rate significantly ($p < 0.05$) increase to 75 ± 3.8%; differences ($p < 0.05$) between Richards and sigmoidal curve parameters Vi, Qu, and Sk	Micropyle and hilum cooperate in the water absorption.	[17]

In wild alfalfa (*Medicago sativa*) species, and in other plant cultivars, a significant effect of NTP treatment on germination compared as to the untreated control was demonstrated [22]. The positive effect of NTP treatment on dormant seeds of *Pityrocarpa moniliformis* was also demonstrated [24]. Commercial cultivation of this Brazilian economically (timber) and socially (medicinal) important tree [48,49] can be facilitated by using of NTP.

NTP treatment for 10- and 20-min increased seed germination of black locust to 43.33 and 44.67%, respectively (Table 1). Seed scarification had a much greater impact on dormancy breaking than NTP treatment in the tested seeds. It should be noted that as in other reports, higher doses of NTP can inhibit, damage, or even kill the seeds [16,18,25,50]. For example in an experiment with Scots pine seeds [50], the following treatment times with NTP (produced by a diffuse coplanar surface barrier discharge apparatus) were used: 1 s, 3 s, 5 s, 10 s, 15 s, 20 s, 30 s, and 60 s. Significant inhibition of seed germination was recorded from times of 15 s and above. However, we did not observe this in our study.

A relatively complex study [42] that focused on the breaking the dormancy of black-locust seeds with different agents found that, e.g., the seed germination increased from 18% to 41% using sulfuric acid or extreme temperatures. The seed germination of 43–45% (Table 1) obtained in our study after NTP treatment for 10–20 min is probably a better result compared to the data of the mentioned experiment [42]. At this moment, we have a lack of information on specific steps required to remove dormancy in black locust effectively and in an environmentally friendly manner. Mechanical scarification is probably the most effective technology (see above), even it carries the risk of secondary infection. Nevertheless, NTP treatment may present an interesting alternative method. The question is whether the presented method, together with obtained seed germination would be sufficient to influence forestry practice. Further experiments will be directed in this promising direction. The presented NTP source is very suitable for base research on dormancy-breaking because it can be very precisely focused on a small space of a treated object. However, upscaled mechanisms for automatic seed treatment should be developed. Based on our results, two scenarios can be suggested: (1) the development of an NTP source such as a dielectric barrier discharge or low-pressure discharge that treats the whole seed and (2) some mechanisms of automatic seed-orientation during the NTP treatment.

5. Conclusions

In this experiment, we investigated the breaking of the physical dormancy of black-locust seeds using non-thermal plasma (NTP). Degradation of the seed surface was evident at 5000× magnification in seed treated for all treatment times used. The O/C ratio increased from 0.28 ± 0.03 (control set) to 1.4 ± 0.3 (20 min NTP treatment) and the highest water absorption was recorded at 20 min for seeds treated with NTP. The significant difference in seed germination was in seed treated for 20 min (44%) compared to non-treated seeds (6%), while the seed germination of mechanically scarified seeds was 93%. Despite the seed dormancy not being completely broken and the treatment not increasing the level of seed germination to that of scarified seeds, it may be concluded that NTP is an effective possible technology for stimulating germination in dormant black-locust seeds.

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