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EFFECT OF RF PLASMA TREATMENT ON THE GERMINATION AND PHYTOSANITARY STATE OF SEEDS**

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Optical spectroscopic techniques are used to study the spectral and energy characteristics and to determine the gas temperature of rf discharges for treating cereal and legume seeds. It is shown that active plasma particles produce a change in the morphology of seed coats. The optimal conditions for plasma processing prior to planting, such that its biological effectiveness is greatest, are realized for a specific discharge power of ~0.35 W/cm^3 and an exposure time of 5–7 min.

Keywords: *low-temperature plasma, rf discharge, seeds, germination, fungicide and bactericide effects, optical emission spectroscopy, scanning electron microscope, gas temperature.*

Introduction. The processing of materials with low-temperature nonequilibrium plasmas is one of the most efficient ways of modifying their properties. In recent years the application of plasma technologies in agriculture has been found to have a positive effect when used to treat seed and plant material prior to planting [1–10]. Plasma interactions enable faster growth and increase the germination of seeds [1–10], suppress the development of aggressive phytopathogens that cause diseases in growing plants [8,11–13], and improve the consumer quality of grains by reducing the amounts of toxics and nitrates in them [10]. It has been noted that the major active factors in plasma processing are plasma radiation, surface bombardment of seeds by active particles, and the formation of small bioactive molecules on the surface [4, 5].

Despite the available positive results on the use of plasma technologies for processing plant material, the effect of the various plasma interactions on the processes leading to improvement of the sowing properties of seeds has not been studied adequately. In particular, there are no data from studies of the parameters of plasmas in the vicinity of the zone where they come into contact with seed surfaces (needed to evaluate the role of excited particles and plasma radiation in activating intracellular processes in seeds). The mechanisms for the fungicidal and bactericidal effect of plasma processing have hardly been studied. The data obtained thus far indicate that plasma can have both stimulate and suppress the germination of seeds [13–15]. Thus, a detailed choice of operating modes as a function of the type and physiological state of seed material is required. The conditions for the processing of seeds must be selected in a way such that surface heating by the plasma is minimal.

In this paper we study the spectral energy characteristics and determine the gas temperature T_g of capacitively coupled rf discharges in the vicinity of the interaction zone for the seeds of agricultural crops. Optimum operating conditions are determined for stimulating germination of the tested seed material.

Experimental Apparatus, Material, and Techniques. Seed cultures that play a major role in the structure of agricultural commerce were chosen for testing: spring wheat *Triticum aestivum* L. ("Darya" variant, 2012), narrow-leaf lupine *Lupinus angustifolius* ("Pershatsvet" variant, 2012), and corn *Zea mays* L. (hybrid Nemo 216 SV, 2012) provided by the Institute of Plant Protection.

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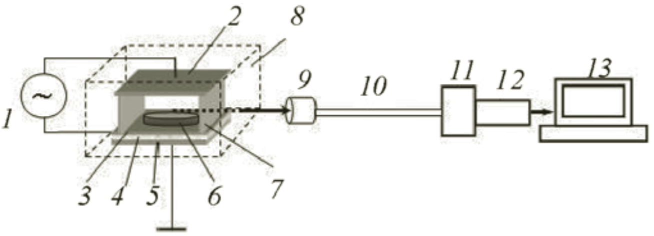


Fig. 1. A sketch of the experimental apparatus and the optical and spectroscopy measurement system: (1) rf oscillator, (2, 3) rf and grounded electrodes, (4) dielectric lining, (5) grounded metal base, (6) Petri dish, (7) quartz window in discharge vessel, (8) vacuum chamber, (9) collecting lens, (10) light guide, (11) spectrometer, (12) detection circuit based on a TCD1304 CCD array, (13) notebook computer.

The seeds were processed on an experimental system based on a VChI-62-5-IG-101 rf current generator. A conceptual diagram and a detailed description of the system design are given in [8]. An α-type capacitively coupled rf discharge was excited at a frequency $f = 5.28$ MHz in a discharge vessel formed by two copper electrodes with diameters of 120 mm cooled with flowing water separated by a distance of 20 mm in a vacuum chamber with a volume of 0.053 m^3. Seeds (at least 100) were placed in a Petri dish mounted on the grounded electrode (item 3 in Fig. 1). Processing was done in an air atmosphere at a pressure $P = 40–80$ Pa. The specific power W applied to the discharge was varied from 0.34–0.65 W/cm^3. Depending on the type of seeds, the interaction time was 2–10 min. Three series of experiments were carried out in each operating mode to provide statistical data.

The efficiency of plasma processing of the seeds was determined from the change in their sowing properties (laboratory germination and biometric indicators of seedlings) and phytosanitary state relative to control (unprocessed) samples. Laboratory germination and infection by pathogenic microorganisms of the spring wheat were checked seven days after planting and of the corn and lupine, ten days later. All the experiments for testing the seeds were done in the phytopathology laboratory and laboratory for the protection of food and technical seed cultures at the Institute of Plant Protection in accordance with methods established in the standards documents GOST 12038-84 and GOST 12039-82 [16].

Since all the test seeds were exposed to vacuum during initial pumpdown of the discharge vessel to the working pressure before the discharge was turned on, the effect of the vacuum on the sowing properties of all these seeds was investigated. For this, some of the seeds (at least 100) were held in the vacuum chamber at a pressure of ~100 Pa for 7–15 min without subsequent plasma processing.

In order to clarify the role of the active plasma particles in etching of the seed coats, a study was made of the morphology of the surface of control and processed samples using an LEO 1455 VP scanning electron microscope (accelerating voltage 20 kV, probe current 10^{-7} A). The plasma parameters that could be used to estimate the local temperatures near the processed samples were monitored by optical emission spectroscopy. The spectral energy characteristics of the plasma were studied using an S100 high-throughput spectrometer in the range $\Delta\lambda = 200–1100$ nm [17]. The plasma emission from the part of the discharge above the seed surface was collected with a short-focal length quartz lens (9) attached to a quartz light guide (10) and fed to the input slit of the spectrometer (11) (Fig. 1). The light $I(\lambda \in \Delta\lambda)$ was detected with a CCD strip array (12) and the data were collected and stored for later processing by a computer (13).

The gas temperature T_g of the plasma was determined from the radiative intensity distribution $I(\lambda)$ in the electronically excited bands of the second positive (2+) system of N_2 without resolution of the rotational structure. Agreement between the measured rotational temperature T_{rot} and gas kinetic temperature T_g under these conditions follows from the relationship of the lifetime τ_c of the electronically excited $C^3\Pi_u$ state of the N_2 molecule including its quenching through intermolecular collisions and its rotational-translational relaxation time τ_{RT} ($\tau_c/\tau_{RT} >> 5$) [18]. The algorithm for determining T_g involves comparing the experimental profiles $I^{exp}(\lambda)$ with the distributions $I^T(\lambda)$ calculated for different rotational temperatures T_{rot} using the SPECAIR (version 2.1) program [19] with the real shape of the instrument function and amplitude characteristic of the detection system of the S100 spectrometer taken into account. The desired T_g is taken to be the value of T_{rot} for which the agreement between the distributions $I^{exp}(\lambda)$ and $I^T(\lambda)$ is best (Fig. 2). The estimated error $\Delta T_g/T_g$ including the methodological and instrumental error components does not exceed 15%.

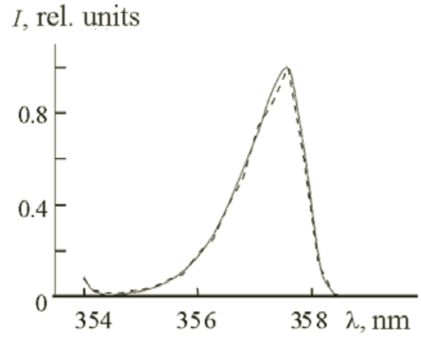


Fig. 2. Intensity distribution in the 0–1 (2+) system of N_2 (3579 Å): the dashed curve is experimental data and the smooth curve, a calculation using the SPECAIR program for $T_g = 330$ K.

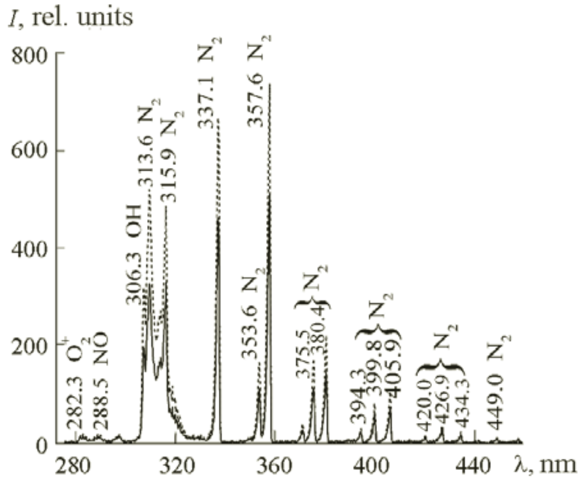


Fig. 3. Emission spectrum of an rf discharge in air ($W = 0.45$ W/cm^3): the dotted curve corresponds to the absence of seeds in the discharge vessel and the smooth curve was taken during processing of seeds.

Results and Discussion. The air plasma emission spectra for low specific powers ($W \leq 0.5$ W/cm^3) consist of molecular bands of N_2 (second (2+) and first (1+) positive systems), N_2^+ (first negative (1–) system), NO (β system), and hydroxyl OH (Fig. 3). The components of the plasma spectra do not vary during processing of seeds at $W \leq 0.5$ W/cm^3, but there is a drop in the intensity of the detected radiation, apparently because it is partially shielded by the seeds. No visible changes were observed in the texture of the coats of the processed seeds. When the specific power was increased to $W > 0.5$ W/cm^3, atomic lines of hydrogen (H_β, H_α), oxygen O I (7771.9, 8446.7 Å) and nitrogen N I (7468.3, 8629, 8680.2 Å), and, with seeds present, bands of carbon monoxide CO (Ångström system) (Fig. 4) were detected in the spectra along with the above mentioned components. The appearance of atomic hydrogen and nitrogen lines in the spectra is indicative of a change in the plasma electron energy distribution function and an increase in the fraction of fast electrons owing to dissociation of molecular species in the plasma with excitation of atomic levels with energies >10 eV. The CO bands appear to be related to etching of the seed coats and the entry of carbon atoms into the discharge, where they participate in plasma chemical reactions to form carbon containing molecules. Sees processed at higher powers W have darker coats than the control samples.

The variations in the gas kinetic temperature of the plasma with specific power during processing of seeds and when there are no seeds in the discharge vessel are shown in Fig. 5. The gas temperature clearly rises with increasing W. When seeds are introduced into the discharge vessel, T_g essentially remains the same. For optimal processing, the radial temperature distribution near the lower electrode is uniform to within the error in measuring T_g and this ensures maintenance of the same temperature conditions in different spatial regions above the surface of the samples as they are processed (Fig. 6).

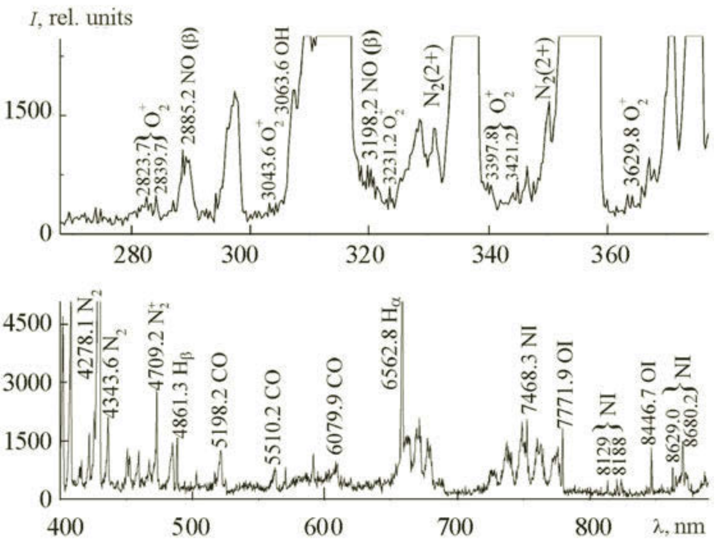
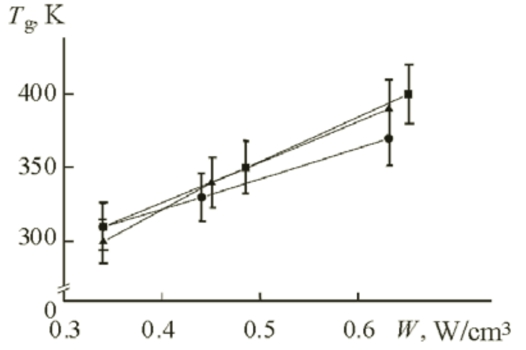


Fig. 4. Emission spectrum of a plasma during processing of wheat seeds (P = 66.5 Pa, W = 0.6 W/cm^3).



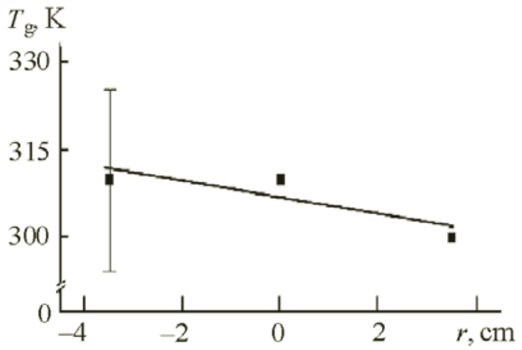


Fig. 5. Gas temperature of the plasma as a function of specific power applied to the discharge without seeds (■) and during processing of lupine *Lupinus angustifolius* (●) and wheat *Triticum aestivum* L.(▲); the processing time is 5 min and the temperature was measured using the v'–v'' = 0–1 (2+) system of N_2.

Fig. 6. The radial distribution of T_g in the plasma near the grounded electrode.

A study of the growth properties of the tested seeds as a function of the exposure time to the plasma (Fig. 7) indicates that plasma processing for 2.5–5.0 min has no negative effect on the growth and biometric characteristics of the seeds with a high viability (lupine) and stimulates germination in seeds with lower viability (spring wheat and corn). The most suitable conditions for processing the seeds, with the best germination properties, occurred at $W \approx 0.35$ W/cm^3 when $T_g \leq 310$ K. Spectral analysis of the plasma shows that etching of the seed coat is negligible under these conditions and causes minimal damage. When $W \geq 0.6$ W/cm^3 and for treatment times >7 min, the germination of the seeds is reduced owing to substantial destruction of the seed coat, which suppresses the growth functions.

Plasma processing also has a fungicidal action which suppresses the most dangerous phytopathogens for the seed cultures tested here: the fungi *Fusarium* spp. and *Alternaria* spp. (Fig. 8). The best result among all the tested seeds was obtained for exposure times of 5 min. In particular, the biological efficiency of processing lupine seeds against the agents of bacterial and some fungal diseases (*Colletotrichum gloeosporioides*, *Kabatiella caulivora*) is 100%, and the overall rate of infection was reduced by 77%. The rate of infection for seeds of spring wheat by *Fusarium* fungi was reduced by 16% compared to the control.

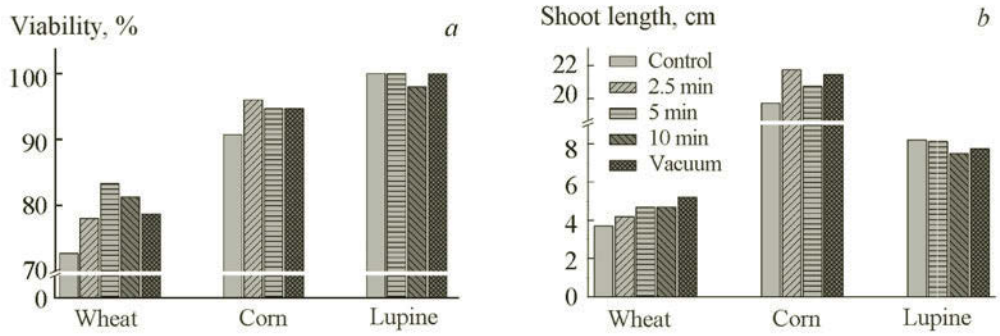


Fig. 7. Effect of plasma processing on the viability of seeds and the biometric characteristics of seedlings.

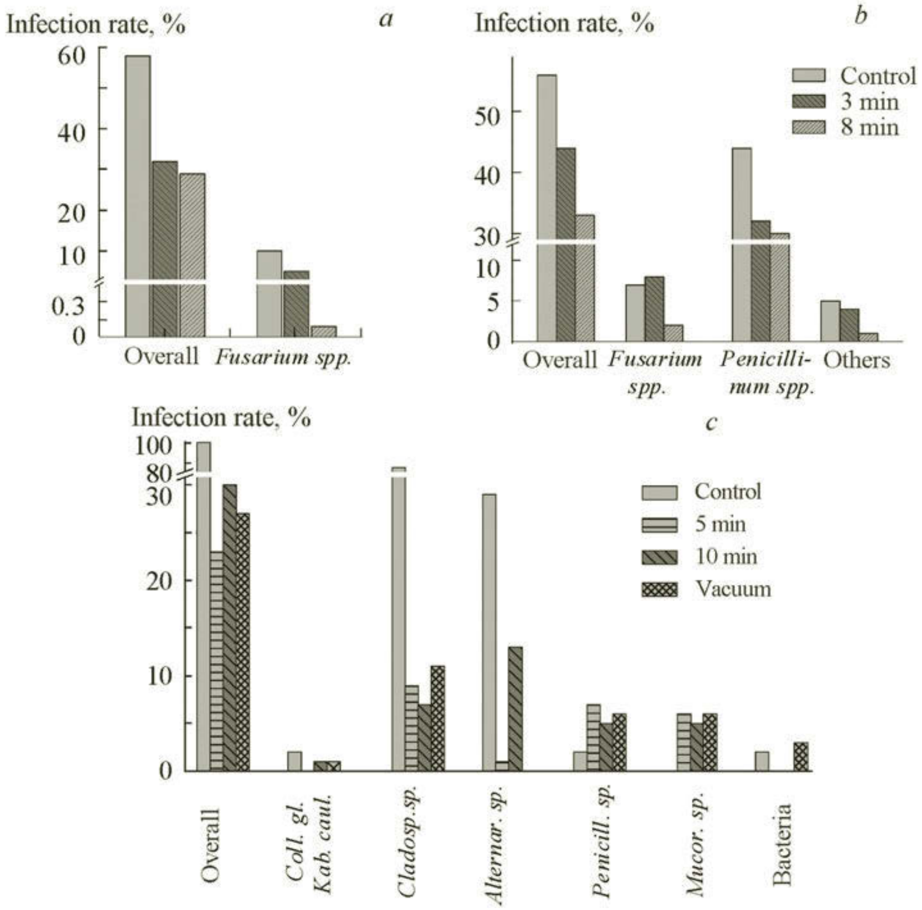


Fig. 8. Rate of infection of seeds as a function of exposure time to plasma: (a) Spring wheat, (b) corn, (c) lupine (P = 66.5 Pa, W = 0.34 W/cm^3).

Keeping the seeds in vacuum simulates their germination capacity to some extent (Fig. 7), but in a number of cases an increase in bacterial and some fungal infections was observed (Fig. 8c). It was found that subsequent plasma processing in the optimal processing mode suppresses the development of pathogenic microflora and helps improve the initial growth properties (Figs. 7 and 8). Significant changes in the morphology of the seed surfaces as a result of exposure to the plasma were observed (Fig. 9). In particular, it was found that the surface of unprocessed seeds has a distinct texture (reticular for grain and papillose for lupine) that becomes less distinct and becomes more diffuse after exposure to the plasma (even for the optimum processing modes).

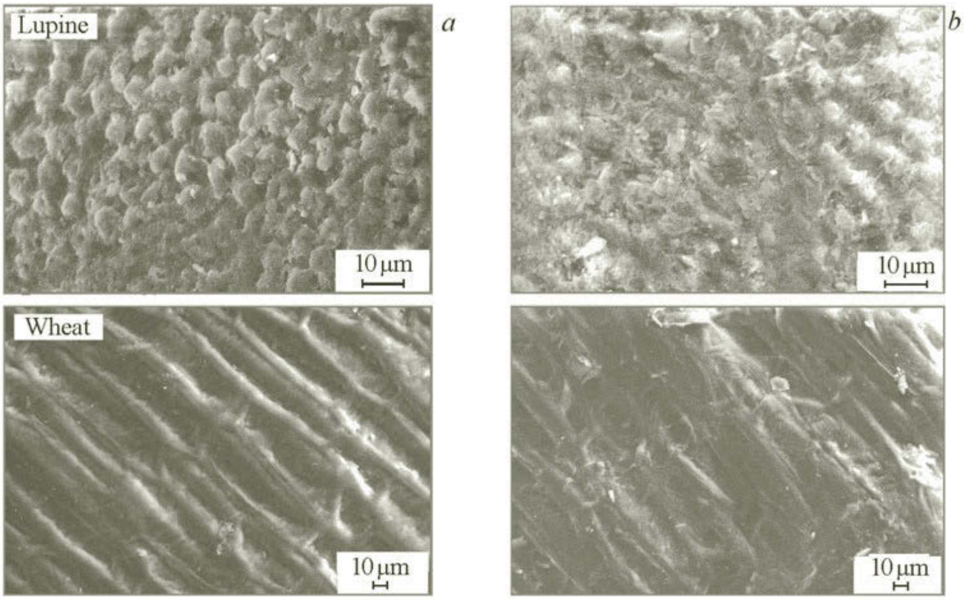


Fig. 9. Scanning electron microscope images of the surface of the seed coating of wheat (*Triticum aestivum* L.) and lupine (*Lupinus angustifolius*) before (a) and after (b) plasma processing (P = 66.5 Pa, W = 0.34 W/cm^3, t = 5 min).

We can, therefore, say that one of the most important factors affecting the stimulation of germination in seeds by plasma processing is etching of the seed coat. We assume that the active plasma particles (atomic oxygen O I, O_2^+ ions, and OH radicals) acting on the seeds produce the fungicidal and bactericidal effects of plasma processing. Etching of the surface of the seed coats makes them more permeable by water and gas and, thereby, increases the efficiency of metabolic processes that influence the rate of seed germination, as confirmed by the data of [2, 5, 20].

Conclusions. We have shown that processing seed material (*Triticum aestivum* L., *Lupinus angustifolius*, *Zea mays* L.) with low-temperature nonequilibrium rf plasmas can stimulate the growth and development of the plants in the first stages of ontogenesis by activating the metabolism of the seeds, increasing their resistance to stress factors, and strengthening their fungicidal and bactericidal resistance. The most favorable conditions for preplanting plasma processing of seeds are realized with a specific power of 0.34–0.4 W/cm^3 and an exposure time of 5–7 min. One of the most important factors in plasma processing responsible for stimulating seed germination and providing bactericidal and fungicidal effects appears to be exposure of the treated material to the active plasma particles (oxygen atoms O I, O_2^+ molecular ions, and OH radicals) which causes changes in the morphology of the seed coats.

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