

Cold atmospheric pressure He-plasma jet and plasma ball interactions with the Venus flytrap: Electrophysiology and side effects

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ABSTRACT

Cold atmospheric pressure radio frequency plasma (CAPP) can play an important role in agriculture, medicine, biophysical and bioelectrochemical applications, disinfection and sterilization, synthesis of different compounds, nitrogen fixation, and treatment of surfaces. Here we found that reactive oxygen and nitrogen species, UV-Vis photons, and high-frequency strong electromagnetic fields with an amplitude of a few kV produced by a cold plasma jet can interact with bio-tissue and damage it if the plasma treatment is long enough. The electrophysiological effects of CAPP treatment of bio-tissue and electrical signals transmission were measured in the Venus flytrap. The plasma ball does not produce any visible side effects on the Venus flytrap, but induces electrical signals in bio-tissue with very high amplitude. Plasma (Kirlian) photography shows the existence of a blue aura around the plasma ball due to a corona discharge. Understanding the mechanisms of interactions between CAPP and bio-tissue and preventing side effects can contribute to the application of plasma technology in medicine and agriculture. The use of cold plasma in medicine and agriculture should be monitored for side effects from strong high-frequency electro-magnetic fields, UV photons, and reactive oxygen and nitrogen species to protect against undesirable consequences.

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1. Introduction

On Earth, plasma is generated naturally in lightning and the aurora borealis (Northern Lights) and aurora australis (Southern Lights). Since the beginning of the 20th century, the observed effects of aurora borealis on plants and trees have been associated with ions generated by atmospheric electricity near the Earth's surface.

Cold plasma is a widely used method in medicine, agriculture, and for the disinfection of water and food. Cold atmospheric pressure radiofrequency plasma (CAPP) can induce electroporation and morphological changes in the surface of biological tissues [1–3], modifications the wettability of plant seeds [4], gene expression [5], disease resistance and inactivation of bacteria [6–9], affect ion transport and electrochemical characteristics of biological objects. CAPP accelerates the water absorption and germination of seeds, plant growth, nutrient absorption, activates enzymatic

reactions [10] and the activity of ion channels [1–3,11]. However, little is known about the hazardous side effects of plasma interaction with bio-tissue. Reactive oxygen and nitrogen species (RONS) can initiate bio-tissue oxidation and peroxidation. Ultraviolet radiation can create genotoxic effects and DNA damage [5,12]; strong radiofrequency electromagnetic radiation can interact with a bio-tissue [13,14]. The electrophysiological effects in bio-tissue induced by CAPP have not been practically investigated, although the powerful high-frequency electromagnetic radiation produced by CAPP is used in dermatology, cancer therapies [15], dentistry, treatment of seeds, plants, and fruits in agriculture [1–3,11,15]. The effects of electric fields on vegetation have been the subject of research since the eighteenth century [16–21].

Plasma lamps can be used in agriculture to accelerate the germination of seeds, the growth of plant seedlings, and the corrugation of the surfaces of bio-tissue without the side effects of reactive oxygen and nitrogen forms generated by plasma jets [1–3,11,14]. A plasma lamp (ball) is a transparent ball of borosilicate glass filled with a combination of noble gases at atmospheric pressure with an electrode in the center of the sphere. The plasma inside the ball and bio-tissue outside act as capacitor plates, and the glass wall is the dielectric between them. Plasma balls were developed by Tesla [22], and their physical properties have been studied recently

Abbreviations: CAPP, cold atmospheric pressure radio frequency plasma; CAPPJ, cold atmospheric pressure radio frequency He-plasma jet; EMI, electromagnetic interference; RF, radio frequency; RNS, reactive nitrogen species; RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species; V, voltage.

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[13,23,24]. A commercial plasma ball was used for electrostimulation of seeds and plants [1,14].

Of particular interest for the study of electrophysiological processes induced by cold plasma in bio-tissue is the Venus flytrap (*Dionaea muscipula* Ellis) – the most famous plant among carnivorous plants that can quickly attack, capture, and digest insects. The leaves of the Venus flycatcher have miniature antennae or sensitive hairs that can receive, process, and transmit information about the insect's stimuli. Touching the trigger hairs that protrude from the upper epidermis of the leaves of the Venus flycatcher activates mechanosensitive ion channels and generates receptor potentials that can induce action potentials. The Venus flytrap has short electrical memory and memristors [25–30] the hydro-elastic curvature model [31], the upper leaf of the Venus flytrap is visualized as a thin, weakly curved elastic shell with the main natural curvatures depending on the hydrostatic state of the two surface layers of the cell, where different hydrostatic pressures are maintained. The uneven expansion of the individual layers leads to bending of the sheet, which is described in terms of flexural elasticity. External triggers, whether mechanical, electrical [25,27,30], magnetic [32], or CAPPJ [3,11], cause the pores connecting these layers to open; then the water flows from one layer to another layer inside the petal, and the petal quickly changes its curvature from convex to concave, and the trap closes [3,11,30,31]. The equations describing this motion were derived [31] and verified by experimental data [25,27–29].

In this article, we attempted to measure the electrical signaling and side effects caused by the plasma ball and the cold atmospheric pressure radio frequency He-plasma jet in the Venus flytrap.

2. Materials and methods

2.1. Plants

One hundred bulbs of *Dionaea muscipula* (Venus flytrap) were purchased from *Fly-Trap Farm* (Supply, North Carolina) and grown in well-drained peat moss in 250 mL plastic pots. **All plants were cultivated from seeds.** The soil was treated with distilled water.

Irradiance was 900–1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ PAR at the plant level. All experiments were performed on healthy specimens. The humidity in the laboratory was maintained at 45 – 50%. The temperature was 20 °C.

2.2. Chemicals

Ultra-high purity bottled helium was purchased from *Sexton Welding Supply* (Huntsville, AL, USA). Hydrogen peroxide solution and HNO_3 was purchased from *Sigma-Aldrich* (USA). The ozone test strips were purchased from *Macherey-Nagel Company* (Duren, Germany). They were used to determine the concentration of ozone in the air near the surface of a plasma ball and under the plasma jet.

2.3. Shielded extracellular electrodes

All measurements were carried in a laboratory at a temperature of 20 °C inside a Faraday cage mounted on a vibration-stabilized table. Teflon-coated silver wires (*A-M Systems, Inc.*, Sequim, WA, USA) with a diameter of 0.2 mm were used for preparation of non-polarizable electrodes. Reversible Ag/AgCl electrodes were prepared in the dark by electrodeposition of AgCl on 5 mm long silver wire tips without Teflon coating in a 0.1 M aqueous KCl solution. The anode was a high-purity silver wire and the cathode was a platinum plate. The electric current in the electrolytic cell was limited to 1 mA/cm² of the anode surface. Identical shielded

electrodes (Ag/AgCl or Cu) were used as working and reference electrodes for measurements of the potential differences in the plants. We allowed the plants to rest after the electrode insertion.

2.4. Data acquisition

Setup for experiments with a plasma ball is shown in Fig. 1. High-speed data acquisition was performed using NI-PXI-1042Q microcomputers with simultaneous multifunction I/O plug-in data acquisition board NI-PXI-6115 (*National Instruments*, Austin, TX, USA) interfaced through a NI-SCB-68 shielded connector block to shielded electrodes (Fig. 1). Data acquisition board NI-PXI-6115 (*National Instruments*, Austin, TX, USA) has a maximum sampling rate of 4,000,000 samples/s. Data acquisition board NI-6270 (*National Instruments*, Austin, TX, USA) was connected to shielded electrodes. Both systems integrate standard low-pass anti-aliasing filters at one-half of the sampling frequency.

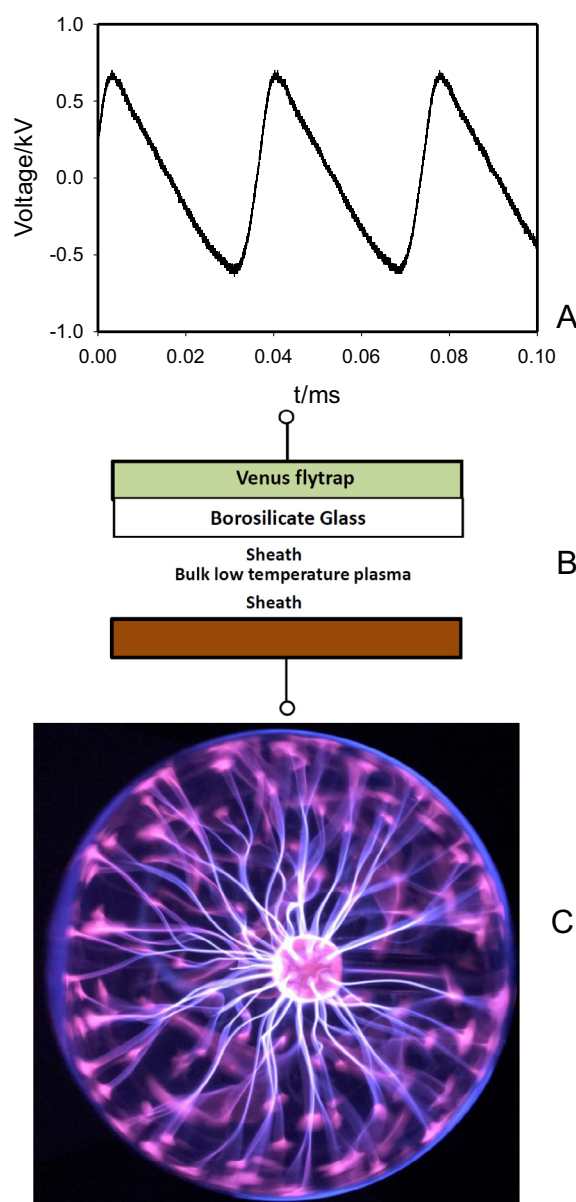


Fig. 1. (A) The electrical voltage on the surface of the plasma ball. (B) Plasma capacitor. (C) Plasma (Kirlian) photo of a plasma ball in the darkness with a time exposure of 15 s. The plasma photo shows a blue aura of 2–3 mm thick around the plasma ball. The temperature was 20 °C.

2.5. Low temperature plasma ball

A conventional commercial Nebula Plasma Ball (Model NX-PS-8, Theefun, China) was used as a source of cold atmospheric pressure radiofrequency plasma for electrostimulation of the Venus flytrap (Schematic 1, Fig. 1). Electromagnetic interference was measured with a CalTest CT2982B 10 kV high voltage divider probe (CalTest electronics, Yorba Linda, CA, USA) connected to a LeCroy wave runner LT322 oscilloscope (LeCroy, Chesnut Ridge, NY, USA).

2.6. Low-temperature atmospheric pressure radio frequency He-plasma jet (CAPPJ)

The CAPPJ method was described earlier [1–3]. The plasma was powered with a high voltage pulsed DC system consisting of a Matsusada AU-10P60 10 kV DC (Matsusada Precision Inc., Japan) power supply, an IXYS PVX-4110 pulse generator (Directed Energy, Inc., Fort Collins Colorado), and a Rigol DG1ZA203504066 arbitrary waveform generator (Rigol Technologies USA Inc., Beaverton, OR, USA). The system operated with a pulse amplitude of 8 kV, a pulse frequency of 6 kHz, 1 μ s pulse width, and a ~ 70 ns pulse rise and fall time. The entire system is placed in a metal enclosure to reduce electromagnetic interference.

2.7. Images

A photo camera Nikon D3x with an AF-S Micro Nikkor 105 mm 1:2.8 G ED VR lens (Nikon, Japan) was used to photograph the plants.

2.8. Plasma (Kirlian) photography

Plasma or Kirlian digital photography was conducted in a dark room [33–36]. Plasma photography creates the latent image using light emitted by cold atmospheric pressure radiofrequency plasma.

2.9. Temperature control

Digital laser temperature gun Etekcity lasergrasp 800 (Etekcity, Anaheim, CA, USA) was used to measure the temperature of the plasma jet, water, plants, and air.

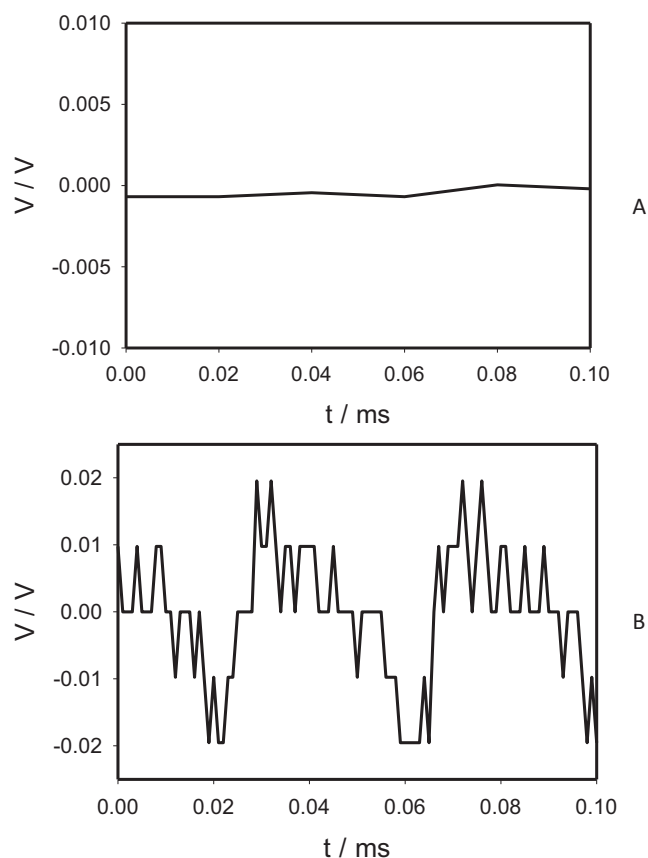
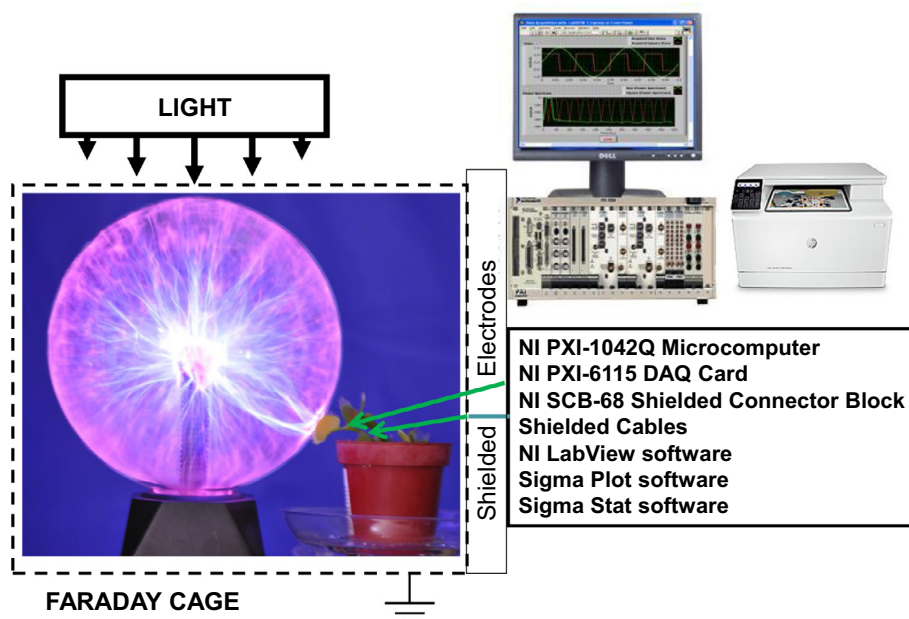


Fig. 2. Electrical signals between shielded electrodes in an aqueous solution of 1 M NaCl without a plasma ball (A) and in the presence of a plasma ball (B) at a distance of 1 cm from an electrochemical cell with an electrolyte solution. The measurements were performed at 500,000 scans/s with a low-pass filter at 250,000 scans/s. The temperature was 20 °C.

2.10. Statistics

All experimental results were reproduced at least 16 times using different plants. Software SigmaPlot 12 (Systat Software,



Schematic 1. Diagram of an experimental setup for detecting the responses of the Venus flytrap to a plasma ball.

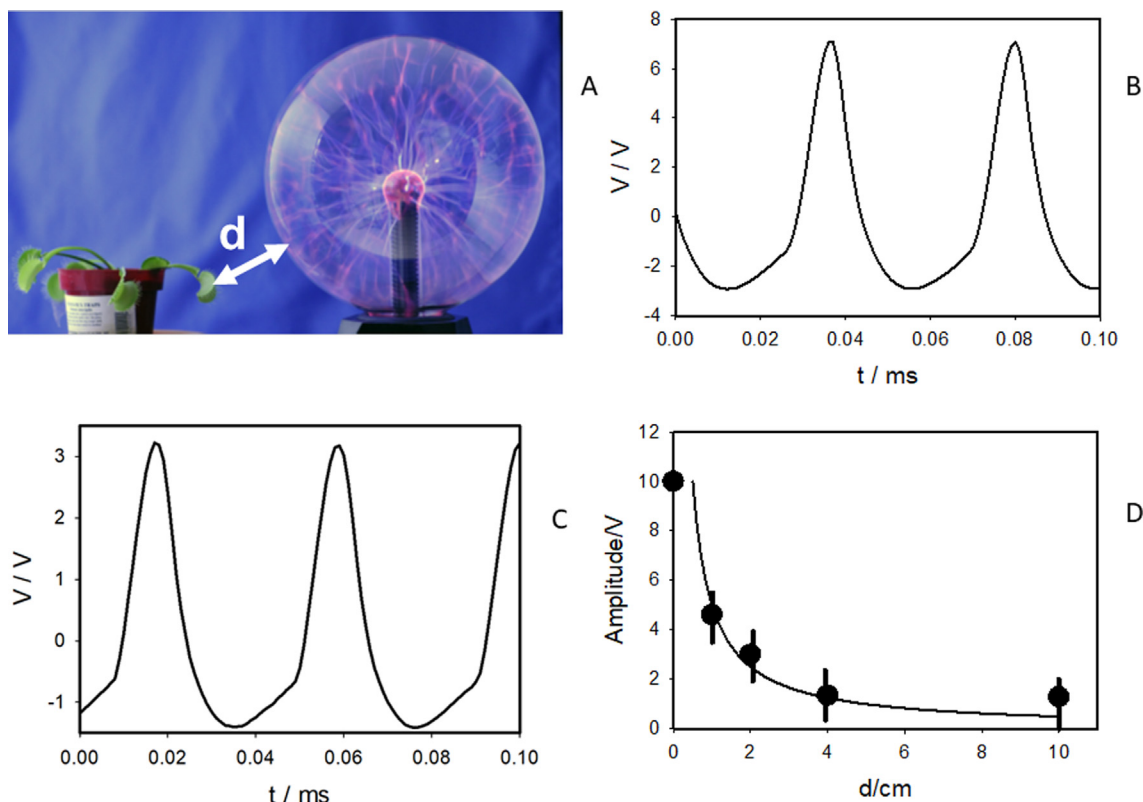


Fig. 3. Electrical signals in the Venus flytrap. The shortest distance between the trap and the plasma ball d was 0 cm (B), 1 cm (C). (D): Dependence of the amplitude of electrical signals oscillations in the Venus flytrap on distance between the trap and the surface of the plasma ball. The solid line was plotted according to Eq. (1). Shielded electrodes were inserted into the lobe and petiole. The distance between the electrodes was 3 cm. The measurements were performed at 1,000,000 scans/s with a low-pass filter at 500,000 scans/s. The temperature was 20 °C.

Inc., San Jose, CA, USA) was used for statistical analysis of experimental data.

3. Results

3.1. Electrical signals in the Venus flytrap induced by a plasma ball

The common commercial Nebula plasma ball (Fig. 1C) is a transparent sphere of borosilicate glass with a diameter of 20.32 cm, filled with rare low-pressure gases. The plasma (Kirlian) photo shows a blue aura (corona) around the plasma ball (Fig. 1C). The mechanism of aurora generation is known as a corona discharge observed in gases which occurs on the charged surface carrying a high frequency high voltage and low electrical current [36]. The photo-camera should have a long exposure time to allow the faint light from the cold plasma to accumulate. Turning off the lights is essential to capturing the faint light given off by corona around the plasma ball. The plasma photo shows a blue aura of 2–3 mm thick around the plasma ball (Fig. 1C).

The plasma inside the ball and the plant tissue outside the glass sphere can be considered as the plates of a RF capacitor (Fig. 1B). The plasma is generated inside the ball by a high radio frequency voltage from the central internal electrode. If the Venus flytrap is placed on the outer surface of the ball or near the ball (Fig. 1), the capacitive coupling can energize a high voltage load up to several kV with a frequency of 26.81 kHz (Fig. 1A). The electrical signal generated by the plasma ball is not confined by the glass sphere and propagates into the surrounding air in the form of electromagnetic interference (EMI). The EMI was measured using a CalTest CT2982B 10 kV high-voltage divider probe connected to a LeCroy

wave runner LT322 oscilloscope. The amplitude of the electrical signal on the surface of the plasma ball was 2 kV.

UVA-Vis radiation (380 – 700 nm) penetrates through the glass surface of the plasma ball. While the plasma inside a ball is surrounded by a glass wall and does not produce significant amount of RONS outside the ball, the high frequency electromagnetic radiation does propagate outside. The detection of ozone at the surface of the plasma balls by commercial ozone test strips for 10 min did not show the formation of ozone in the air near the glass surface.

The plasma ball can interact with the bio-tissue and induce electrical signaling in plants. In control experiments, electrical signals between shielded electrodes in an aqueous electrolyte solution of 1 M NaCl without a plasma ball (Fig. 2A) and with the plasma ball switched on (Fig. 2B) with distance of 1 cm from the electrolyte solution were measured at a very high speed of data acquisition of 500,000 scans/s with a low-pass filter of 250,000 scans/s. The potential difference between the shielded electrodes in experiments without powering the plasma ball is about 0 mV (Fig. 2A). When the plasma ball is switched on, the high-frequency electrical signals between the electrodes in an aqueous electrolyte solution have amplitude of ± 20 mV (Fig. 2B).

If a pair of shielded electrodes is inserted into a lobe and a petiole in the Venus flytrap, the plasma ball induces electrical signaling between the electrodes in the plant with a very high amplitude (Fig. 3). The Venus flytrap has a natural antenna that can detect electromagnetic radiation. The electrical signals in the Venus flytrap are asymmetric and have the same oscillation frequency as the plasma ball. Prolonged treatment of the Venus flytrap with a plasma ball for up to 3 h does not close the trap and does not cause visible side effects.

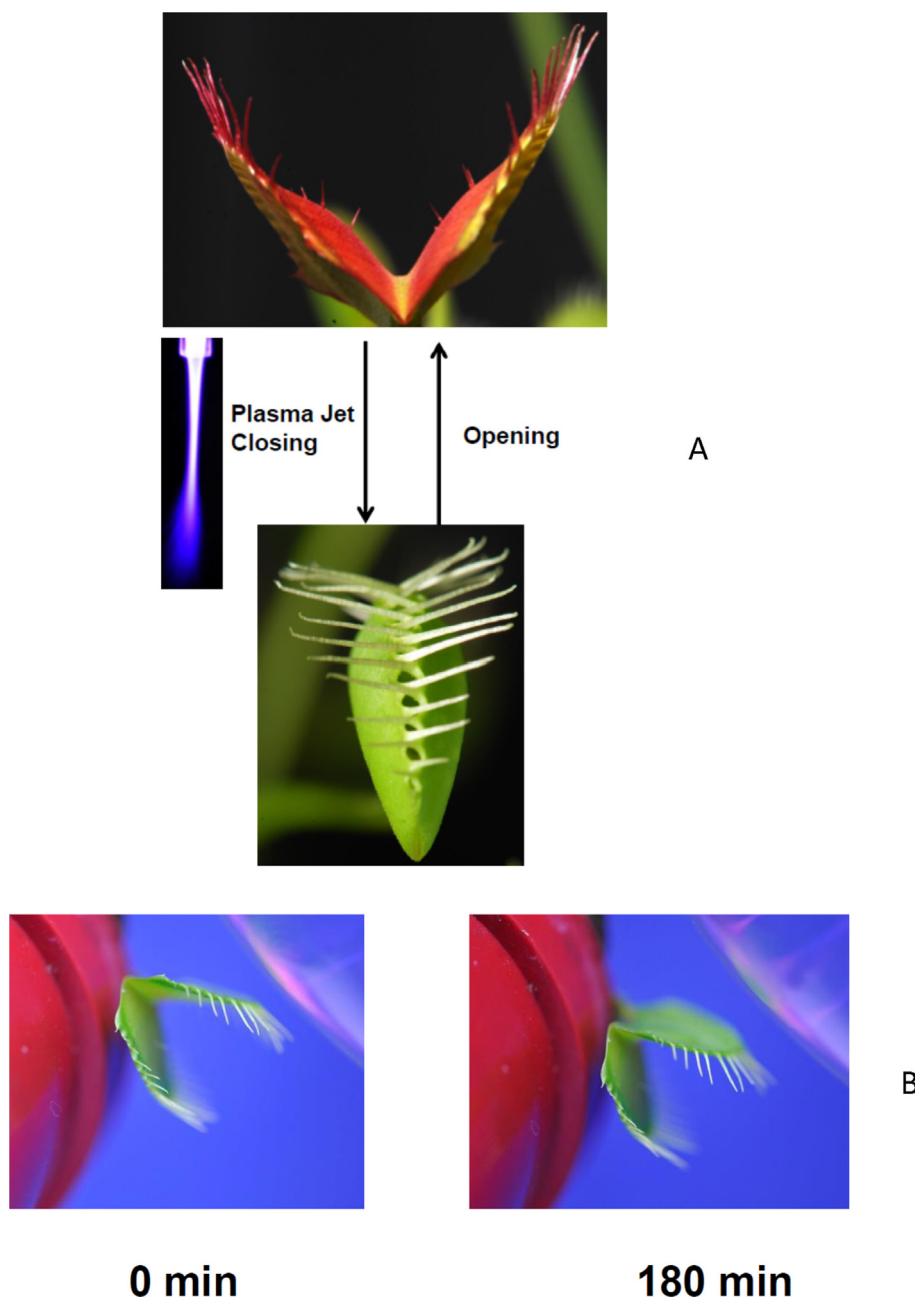


Fig. 4. (A) Closing the trap with a cold atmospheric pressure He-plasma jet in a fraction of a second; (B) Processing the Venus flytrap with a plasma ball treatment for 3 h does not cause the trap to close.

The amplitude of electrical signals in the Venus flytrap decreases with the distance between the trap and the plasma ball (Fig. 3) and can be described by the equation:

$$\text{Amplitude} = (0.05 \text{ Vm/Distance}) \quad (1)$$

These electrical signals are known in plant electrophysiology as electrotonic potentials [29]. Their amplitude decreases exponentially with the propagation distance along the vascular bundles. The amplitude of the passive electrotonic potentials depends on the magnitude of the stimulus. Electrical signals can propagate along the plasma membrane over short distances in plasmodesmata and long distances in vascular bundles.

Prolonged treatment of the Venus flytrap with a plasma ball for up to 3 h does not close the trap and does not cause visible side effects (Fig. 4B).

3.2. Interaction of a cold atmospheric pressure He-plasma jet with the Venus flytrap: Side effects

Short treatment of the Venus flytrap with a cold atmospheric pressure radiofrequency plasma jet can close the trap in a fraction of a second (Fig. 5A). It was shown that H_2O_2 and/or HNO_3 produced by CAPPJ are responsible for closing the trap [3,11,29].

Prolonged treatment of the trap with a cold He-plasma jet can cause side effects and damage to the Venus flytrap (Fig. 5). Direct measurements of the plant electrical signaling induced by the plasma jet proved to be more complicated due to a large amplitude of electrical signals from the plasma jet transmitted in plants. A cold atmospheric pressure plasma jet produces morphing effects with or without direct contact with the Venus flytrap (Fig. 5). Reactive oxygen and nitrogen species are identified as the main cause of

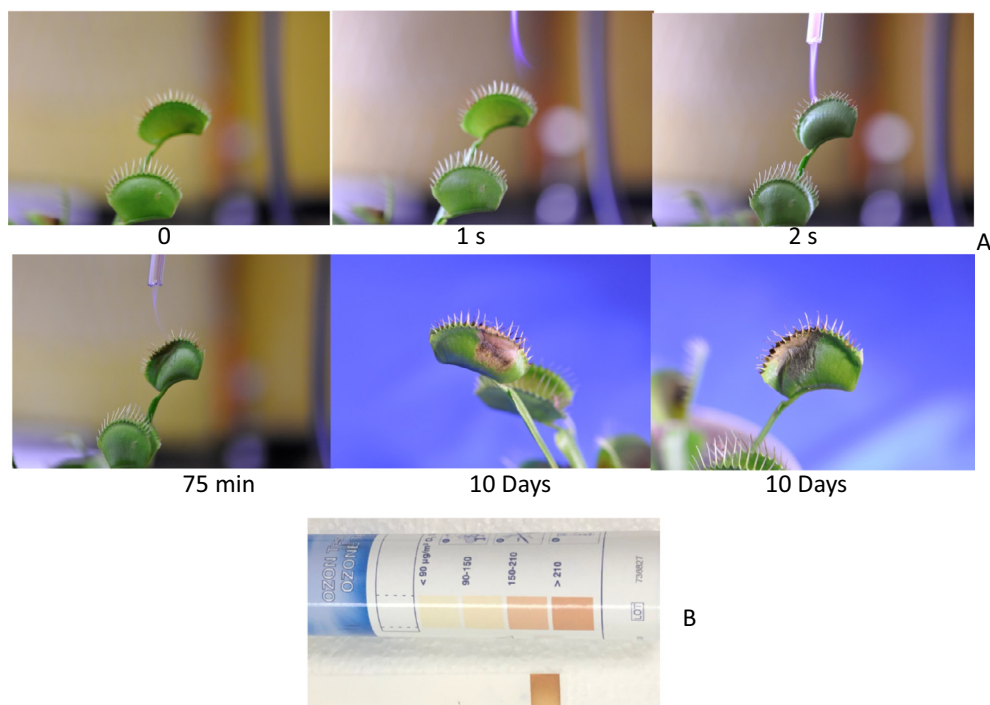
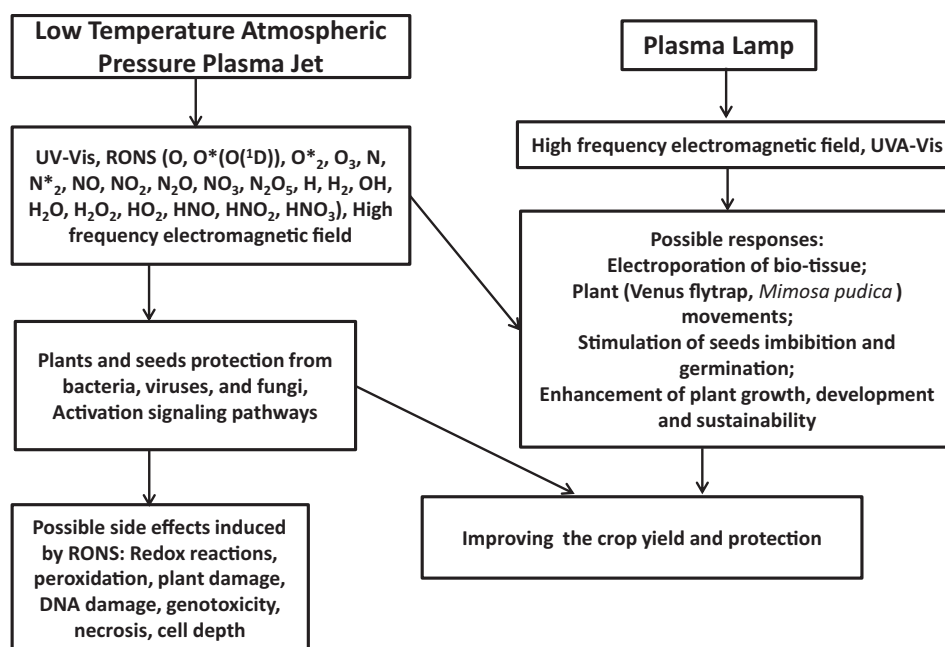


Fig. 5. (A) The closure and compression of the Venus flytrap induced by atmospheric pressure He-plasma jet and photos of the trap damage 10 days after CAPPJ treatment for 75 min. (B) Test for ozone concentration after 10 min exposition to CAPPJ. The ozone test strips when placed under the cold plasma jet at a distance of 1 cm for 10 min, show production of ozone by the He plasma jet in a concentration of 200 $\mu\text{g}/\text{m}^3$ or more. The temperature was 20 °C.



Schematic 2. Schematic diagram of the interaction of cold atmospheric plasma jet and plasma ball with a plant tissue.

the observed phenomenon [11]. ROS can regulate Ca^{2+} and K^+ permeable channels [37,38].

Short treatment of the Venus flytrap with CAPPJ for 3 min or less does not induce visible side effects. Prolonged treatment of the Venus flytrap with a cold atmospheric pressure He-plasma jet causes severe damage to the plants, which are visible even 10 days after treatment (Fig. 5A). Deposition of a 10 μL drop of 0.88 M H_2O_2

or 10 mM HNO_3 to the midrib of the Venus flytrap induces action potentials and closes the trap without visible side effects within 10 days of the trap opening. CAPPJ generates high concentration of ozone (Fig. 5B), which can produce significant damage on plants. Ozone and RONS can cause several types of symptoms including necrosis, which usually occurs first along the leaf margins, but can expand over time [39–41].

Table 1
Cold atmospheric pressure plasma jet effects on seeds and plants.

Beneficial effects of low dose plasma applications	Possible side effects of high dose plasma applications
<ol style="list-style-type: none"> 1. Disinfection and sterilization of bio-surfaces (seeds, plants, fruits, etc.); 2. Morphological changes, corrugation, and hydrophilization of seed surfaces; 3. Acceleration of seed imbibition, germination and growth; 4. Enhancement of plant development, growth, and sustainability; 5. Electroporation of bio-tissue surfaces; 6. Control of plant signaling and behavior; 7. Catalysis of redox reactions; 8. Activation of ion channels; 9. Activation of specific signaling pathways; 10. Plasma control of plant morphing helps design adaptive structures and bioinspired intelligent materials; 11. Interaction with memristors and ion channel. 	<ol style="list-style-type: none"> 1. RONS and UV damaging effects; 2. Generation of strong electrical signals and oscillating fields in bio-tissue; 3. Change of electrical fields in bio-tissue; 4. Membrane breakdown; 5. Deactivation, oxidation or denaturation of enzymes; 6. Acidification of bio-tissue; 7. Peroxidation of lipids and bio-tissue; 8. Interaction with enzymatic and ion transport systems; 9. Chain reactions; 10. DNA modification; 11. Genotoxic effects; 12. Cell damage and death; 13. Necrosis.

4. Discussion

The trap of *Dionaea muscipula* can be closed by mechanical, electrical, chemical [7,25,29], and magnetic stimulation [32], or by an atmospheric pressure argon or helium plasma jet [3,11].

The present paper provides new insight into possible side effects of He-plasma jet interactions with plants. It is known that cold atmospheric pressure plasma in air produces RONS at room temperature. RONS serve as signaling molecules for most living organisms on Earth. Some reactive oxygen and nitrogen species are known to play important roles in plant physiology (Schematic 2). They can be very toxic to biological tissue such as mitochondria and can selectively kill bacteria, fungi, and viruses. At the same time, RONS are beneficial companions of plants' developmental processes and activation of ion channels [3,11]. Cold plasma can have an influence on the ion transport and electrochemical characteristics of a plant tissue. A cold atmospheric pressure He-plasma jet can cause side effects and damage to plants if the plasma exposure is long enough (Fig. 5, Schematic 2). The plasma ball does not produce any visible side effects on the Venus flytrap (Fig. 4B). Low-temperature atmospheric pressure plasma can play an important role in agriculture, medicine, biophysical, and biochemical applications (Schematic 2). Generated by the cold He-plasma jet reactive oxygen and nitrogen species, UV-Vis photons, and high-frequency strong electromagnetic fields with amplitude of a few kV can interact with seeds and plants (Schematic 2, Table 1).

Cold atmospheric pressure plasma jet affects the rate of seeds imbibition and germination, plant growth, development and sustainability, nutrient absorption, and enzymatic and ion channel activities. Pre-germination plasma treatment of seeds does not alter cotyledon DNA structure, nor phenotype and phenology of tomato and pepper plants [42]. There are models of plasma doses for evaluating various factors affecting the treatment of bio-tissues in biomedical applications [43]. Plasma treatment is a highly effective method of protecting seeds and plants from diseases and infection, improving crop yields and protection. Here we found that the cold atmospheric pressure plasma jet can also cause side effects and damage to plants if the plasma exposure is

long enough. The plasma lamp does not produce any visible side effects on the Venus flytrap. Table 1 summarizes the benefits and side effects of plasma applications to bio-tissue. Understanding the mechanisms of plasma interactions with plants could promote plasma-based technology in medicine and for plant developmental control, increasing yield, growth rates, and plant protection from pathogens.

5. Conclusions

This work provides new insight into the possible side effects of the interaction of cold plasma with plants. We have observed for the first time that cold plasma induces high frequency electrical waves with amplitudes of a few volts in a bio-tissue of the Venus flytrap.

It is well known that cold atmospheric pressure plasma produces RONS which can play an important role in plant physiology. RONS also can be very toxic to biological tissues and selectively kill bacteria, fungi, and viruses. At the same time, RONS are beneficial companions of plants' developmental processes and activation of ion channels [3,29]. Cold plasma can affect the ion transport and electrochemical characteristics of a bio-tissue. A cold atmospheric pressure He-plasma jet can cause side effects and damage to plants if the plasma exposure is prolonged enough (Fig. 5). The plasma ball does not produce any visible side effects on the Venus flytrap even if the exposition time is very long (Fig. 4).

Low-temperature atmospheric pressure plasma can play an important role in agriculture, medicine, biophysical and biochemical applications. Understanding the mechanisms of plasma interactions with bio-tissue can contribute to the development of new plasma technologies in medicine, agriculture, and material science.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Contributions

A.G.V. conceived the idea, analyzed the data, and participated in manuscript writing.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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