Abstract. In this study, we investigated the effect of atmospheric plasma jet treatments on adults of the red flour beetle, Tribolium castaneum (Herbst) and the confused flour beetle, Tribolium confusum Jacquelin du Val in wheat grains. We evaluated the mortality of insects upon temperature and after plasma exposure by changing the gas (Argon, Argon/Oxygen and Argon/Nitrogen mixtures). Evidence for induced mortality was obtained upon Argon/Nitrogen plasma treatments, while the introduction of a survival effect was noted for Argon/Oxygen plasma. The results indicated that non-thermal plasma treatments may have a good potential to be developed as alternative methods of chemical insecticides for the control of storage pests.

Key words: atmospheric plasma jets, ecological methods, pests of stored products, pest control, plasma agriculture;

1. INTRODUCTION

One of the major problems of agricultural production is the presence of pests in stored products. These pests can contaminate food by spreading different diseases, as well as reduce the quality and yield of stored products, causing significant post-harvest losses from an economic point of view. The main strategy used in the preventing and repelling of pests in stored products is based on chemical methods which implies using insecticides with high toxicity levels. These methods have known efficiency, but they are not ecological ones. The persistence of vapours and residual by-products which can contaminate water and affect human health, as well as the pests’ resistance associated with insecticide
treatments, lead to the need for the development of non-toxic alternative pest control methods.

For example, a pesticide extensively used is bromomethane, also known as Methyl Bromide [1],[2] successfully applied as a fumigant in controlling pests due to its low cost and efficiency. After discovering its ozone-depleting properties, the use of Methyl Bromide as a pesticide was phased out [1]. Another fumigant, widely used in recent years to control pests in stored products, is ozone [3]. Because oxygen (O\textsubscript{3}) exists in the atmosphere, the plasmas generated at atmospheric pressure can be a good alternative for ozone generation. Other plasma species, atoms, metastable and gaseous radicals can also have a pesticidal effect. Nevertheless, these plasmas should be cold, therefore combining a low gas temperature close to the ambient, with a high electron temperature, the electrons with high energies being able to sustain the generation of active chemical species. Cold plasma applications include already biological applications [4], such as bacteria inactivation [5], blood coagulation [6], or cell proliferation [7].

The effect of plasma treatments on insects was discussed in a few papers only. An atmospheric pressure plasma jet operated in pulse mode using oxygen and water vapors was applied to Indian meal moth (Plodia interpunctella Hübner) [8]. In that study, the distance from plasma to the sample and the number of pulses varied. The experiment concluded on a proportional increase in the insect mortality rate to the number of pulses and, in contrast, the mortality rate increased with decrease of distance from the jet. Moreover, a dielectric barrier discharge treatment was evaluated on green peach aphids (Myzus persicae Sulzer), citrus mealy bugs (Planococcus citri Risso) and human body lice (Pediculus sp.) [9]. These insects were directly exposed to a helium plasma and even if a significant mortality rate of insects was observed after plasma treatment, this effect continued to increase over a period of time after treatment. Another study [2] whose goal was to investigate the plasma effect on insects was using a non-thermal plasma on the Mediterranean flour moth (Ephestia kuehniella Zeller) and Tribolium confusum (Coleoptera: Tenebrionidae), the same insect that was used in this study as well. That plasma source is also based on the dielectric barrier discharge configuration and to maintain the plasma discharge normal atmospheric air was used. That paper showed a positive correlation between the insect mortality rate and treatment time.

The other floor beetle Tribolium castaneum Herbst that was also used in this experiment was presented earlier in another paper study [10] in which the plasma stress on insects and the effect of plasma on maida flour was also investigated. In that paper, the Tribolium insect mortality rate increased when the electrode gap was decreased, the exposure time was increased and the applied voltage was increased. The plasma was generated in atmospheric air. However, these experiments were performed in a reactor chamber in which the pressure was lower than the atmospheric pressure.
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The flour beetles, *Tribolium castaneum* Herbst and *T. confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae) [2] are worldwide pests that feed on cereals and other stored products. These pests drastically decrease their quality and quantity [2]. The previously mentioned works prove that plasma can be considered a promising method to improve the seed’s quality and to increase the yield of various crops [11], [12], [13], [14], but also to reduce some insects attack in stored products [2].

The objective of this paper is to elucidate if the exposure of wheat grains infested with adults of *T. castaneum* and *T. confusum* to non-thermal plasma influences the insect mortality. The study begins with the presentation of experimental setup and procedures. First, the natural mortality of insects and mortality caused by the temperature, in plasma off mode, were observed. We have found that even if we used a small amount of oxygen and nitrogen in argon plasma, their effect is determinant for plasma induced insect mortality.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL SETUP

The experimental setup used to investigate the cold plasma effect on insects and wheat kernels is shown schematically in (Fig. 1). It is composed from a plasma jet source, a glass treatment vessel and the sample which contains wheat grains and insects placed inside the vessel. To maintain a controlled but yet not air-tight environment, the tube was closed with PTFE (polytetrafluoroethylene) bonnets on the top and bottom.
For performing the experiments, we used an atmospheric a stable, stationary pressure plasma jet, based on a DBD (Dielectric Barrier Discharge) configuration, described in more detail elsewhere [15]. The plasma is generated inside a thin glass tube with a 6.5 mm outer diameter, and 3.5 mm inner diameter. The discharge is powered via an annular aluminium RF electrode wrapped around the tube. The glass tube is inserted in the treatment vessel through the upper bonnet and plasma expands out of tube as a stationary filament of about 6 cm length, oriented perpendicularly to the sample which is placed at 8 cm far from the tube exit.

The treatment vessel was made by connecting two glass tubes of identical 75 mm diameter, and lengths of 90 and 60 mm. A sieve was used between the two glass tubes. This sieve had multiple functions: firstly, it is the holder of the wheat sample and, secondly, it forces the gas (carrying the plasma generated species) to circulate among the grains. The gas is afterwards evacuated at the bottom of the tube. An image of the treatment setup is presented in Fig. 2.
2.2 SAMPLE PREPARATION

Test insects used in the experiments were adults of *T. castaneum* and *T. confusum* (about 30 days old) from laboratory populations reared on artificial diet (wheat and corn flour 50:50, powder milk and 7% yeast powder) in controlled conditions (25°C, 70% RH, 16 hours light and 8 hours dark) at the R&D Plant Protection Institute Bucharest. Each sample consisted of 30 grams of wheat and 25 insects.

2.3 WORK PROTOCOL

The experiments were performed using Argon (Ar), Oxygen/Argon (O₂/Ar) and Nitrogen/Argon (N₂/Ar) mixtures as discharge gases. The following treatment parameters were used: argon gas flow rate at 3000 sccm (Standard Cubic Centimeters per Minute - flow rate gas unit), the oxygen gas flow was set in the range of 1-8 sccm (Oxygen/Argon gas ratios 0-0.27%) and the nitrogen gas flow was set in the range of 0-80 sccm (Nitrogen/Argon ratios from 0 to 2.67 %) and the RF (13.56 MHz) power was set in the range of 90-130 W. In this study, the plasma exposure time varied between 10 and 15 minutes.

The temperature reached by the samples during treatments was evaluated using a thermocouple (type THERMOMETER, TM-936) inserted inside the grain samples. The thermocouple reading was performed at the end of each treatment, in first seconds after switching off the RF power. In this way the temperature evaluation errors produced by RF induced currents in the thermocouple wires are avoided.

In order to evaluate the insect mortality, we counted the total number of dead insects, at various periods of time (days) after the treatment date. To compare the treatment effects, we have defined a specific parameter as follows (1):

\[
\text{insect mortality (measured at day } n, \%) = \frac{\text{number of dead insects until day } n}{\text{initial number of insects}} \times 100 \quad (1)
\]

The mortality is a day-dependent parameter, which increases when new death events are counted during the time elapsed from the previous day.

The mortality percentage inherently includes the contributions of natural mortality and those caused by the gas atmosphere, as well as the temperature reached during treatment. Therefore, this study began with an evaluation of natural insect mortality and of mortality induced by the argon atmosphere in plasma off mode, for the two species. Furthermore, we accomplished an experiment in which the effect of temperature on the samples was evaluated as follows: the samples were heated and the insect mortality was counted, in plasma off mode.
3. RESULTS AND DISCUSSIONS

3.1. NATURAL INSECT MORTALITY

In general, control samples are an important part in every biological experimental study. In the present study, we separately determined the natural insect mortality in order to account for its contribution to the overall insect mortality measured at various times after plasma treatment and these values were used as control samples. The results are presented in Fig. 3 and Fig. 4, for *T. castaneum* and *T. confusum*, respectively. We have found that natural insect mortality has a negligible influence on the results, because at the time scale of measurements (1-15 days) it is very low. However, in order to exclude any error introduced by the possible variability in natural mortality, each set of triplicated plasma-treated samples was accompanied by a set of three control samples, stored in the same experimental conditions. The mortality was analysed comparatively for both insect species used in this study.

![Graph showing T. castaneum natural insect mortality and Argon atmosphere - plasma off](image1)

![Graph showing T. confusum natural insect mortality and Argon atmosphere - plasma off](image2)

Fig. 3 - Time dependence of mortality due to natural causes and due to exposure to Argon atmosphere on *T. castaneum*.

Fig. 4. Time dependence of mortality due to natural causes and due to exposure to Argon atmosphere on *T. confusum*;

3.2 EFFECT OF ARGON ATMOSPHERE ON INSECT MORTALITY

To observe the effect of the Argon atmosphere on insects, we performed an experiment in which the samples were exposed 15 min to the flowing gas. The setup configuration was the same used for plasma treatments, but the discharge was
off. The changes in insect mortality, as compared to the control sample, were not significant (Fig. 3, Fig. 4). We concluded that, in such exposures of short duration and not air-tight treatment enclosure, Argon atmosphere practically did not affect insect mortality.

3.3 EFFECT OF SAMPLE HEATING ON INSECT MORTALITY – PLASMA OFF

Even in the case of cold plasmas, a part of the electrical power fed to the discharge is inherently spent for gas heating, which in turn can lead to the heating of the treated samples. In the case of filamentary plasma source and the power conditions used here, temperatures up to 70°C have been determined sometimes on the samples. Therefore, during treatments the insects were exposed simultaneously to the influence of temperature (thermal effect) and influence of chemical species formed in plasma (plasma effect). In order to infer the contribution to mortality of the thermal effect, we performed additional experiments consisting in the exposure of insects to the flowing gas in absence of plasma (“plasma off”), but with the samples heated at temperature values similar to those measured during plasma treatments. The results, presented in Fig. 5, show an important influence of temperature on insect mortality which should be considered in the interpretation of data describing the “plasma on” experiments. For high temperatures (more than 60°C) almost total mortality was noted from the first day, even in absence of plasma. In contrast, the data show that in these short time experiments, the thermal effect is less important if the temperature values are lower than 50°C. In addition, an increase of the late mortality was not observed in such conditions.

![Fig. 5 - Mortality dependence upon temperature as measured in the first day after the thermal treatment](image-url)
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3.4 THE EFFECT OF PLASMA TREATMENTS ON INSECT MORTALITY

Treatments with plasma generated in pure Argon at 3000 sccm led to sample temperatures of about 70°C, regardless of the used RF power. At these high temperatures, the thermal effect dominates the mortality, therefore it would be difficult to distinguish the plasma effect from the thermal one. In plasma experiments performed with Argon/Oxygen and Argon/Nitrogen mixtures, lower temperatures in the range of 45-55°C were measured in particular conditions. This behaviour was expected: compared to Argon, part of the injected electric power is spent for excitation of vibrational and electronic states of molecules, a smaller part remaining for gas heating. Therefore, further experiments were performed in gas mixtures, as described below.

3.4.1 Argon/Oxygen plasma treatments

In a previous paper [1] devoted to the study of Blattella germanica (L.), Frankliniella occidentalis (Pergande), and Planococcus citri (Risso) species, it was shown that Helium/Oxygen plasma can influence the mortality differently, depending on the species: for P. citri, the mortality increased with the addition of oxygen, but in contrast, for the other two species, oxygen did not significantly influence mortality.

In a similar reasoning, the parameter that was modified here was the oxygen concentration in the Argon main gas (gas mixture ratio). The RF power was set to 90 W and the plasma exposure time was 15 minutes for every sample. The mortality dependence upon the oxygen concentration in the mixture, at various times after treatment, is presented in Fig. 6, for T. castaneum, and in Fig. 7 for T. confusum. With respect to time behaviour (day after treatment), one can note that the late mortality did not change much from the first day value, indicating that the early days dominated the overall mortality. Regarding the oxygen concentration effect, mortality decreases at higher oxygen concentrations: as measured in the first day after treatment, for T. castaneum the mortality decreased from 88% for the Argon case to 65% when 2.8 sccm oxygen was added to 3000 sccm Argon and to 1.3% at 8.4 sccm addition. In a similar way, the mortality of the T. confusum decreased from 96% to 51%, and to 0 %, respectively. This trend was confirmed by an experiment in which only insects (T. castaneum, without grains) were exposed to plasma in the same conditions as above mentioned: at 4.2 sccm oxygen in 3000 sccm argon, all insects were found dead in the first day (100% mortality), while only 7 insects of 25 (28%) were found dead in 8.4/3000 (sccm) Argon/Oxygen mixture. These mortality values are higher compared to those measured on the samples consisting of insects mixed with grains, pointing out that the wheat offered a protective environment for the insects.
On the same Figures 6 and 7 the temperature reached by the samples is inserted (scale on the right axis). At each oxygen concentration, the sample temperature was different, with the lowest value at 8.4 sccm. Higher temperatures characterize the samples treated at low oxygen flows, raising the suspicion that the high mortality noted at low oxygen concentration is related to sample temperature, aspect which will be discussed later in the paper.

3.4.2 Argon/Nitrogen plasma treatments

In these experiments, the RF power was set to 130 W, which permits the discharge operation with a nitrogen gas flow up to 80 sccm, in reasonable heating conditions. When nitrogen was added to argon plasma, the discharge modified from a filamentary plasma, to a diffuse jet of a few cm length and the colour of the jet was also changed, according to presence of new plasma species. The plasma exposure time was 10 minutes for every experiment.

From Fig. 8 (T. castaneum) and Fig. 9 (T. confusum), it can be observed that the insect mortality decreased with the nitrogen concentration added in the discharge. As for the second day measurements, for T. castaneum, the insect mortality decreased from 90% corresponding to pure Argon, to 70% when 20 sccm nitrogen was added, and to 46% at 80 sccm nitrogen admixing. In similar conditions, the mortality of T. confusum decreased from 93% to 63% and to 57%, respectively. The temperature of the samples decreased only slightly when nitrogen was added in the plasma discharge, as it is shown in (Fig. 8) and (Fig. 9) on the right Oy axis.
3.5 EVIDENCE FOR PLASMA INFLUENCE ON INSECT MORTALITY

In Fig. 7, Fig. 8 and Fig. 9, a clear correlation between the mortality and temperature is observed. Therefore, a mean to discriminate the effect of plasma species from the heating effect had to be identified. With this aim, we plotted the mortality, in all plasma-studied cases, as a function of sample temperature. The plots are shown in Fig. 10 for T. castaneum and in Fig. 11 for T. confusum. The mortality induced by heating alone is also included in the graphs. In both Figures, the points corresponding to Argon/Nitrogen treatments indicate a higher mortality, compared with that caused by the thermal effect alone. The only exception, the point at 60 sccm, should be disregarded as it falls in the sum of error bars of temperature induced and plasma induced mortality. A contrary aspect is observed for Argon/Oxygen treatments were mortality points are always plotted lower than the thermal ones.

Fig. 8 The mortality dependence of Tribolium castaneum insect species with amount of nitrogen that was introduced in discharge. The figure includes also the temperature reached by the sample during treatment.
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Fig. 9 The mortality dependence of Tribolium confusum insect species with amount of nitrogen that was introduced in discharge. The figure includes also the temperature reached by the sample during treatment.

Fig. 10 Mortality values obtained for thermal, Argon/Oxygen and Argon/Nitrogen plasma treatments of T. castaneum infested samples. One note that the mortality induced by the process with Argon/Nitrogen is higher than the thermal one, in contrast to the process performed with Argon/Oxygen plasma.

Fig. 11 - Mortality values obtained for thermal, Argon/Oxygen and Argon/Nitrogen plasma treatments of T. confusum infested samples. One note that the mortality induced by the process with Argon/Nitrogen is higher than the thermal one, in contrast to the process performed with Argon/Oxygen plasma.

Therefore, it appears that the plasma process using Argon/Nitrogen mixture is more efficient regarding insect mortality, in comparison with the thermal treatment, thus adding a killing factor which can be assigned to the reactive plasma species. In contrast, the Figures highlight that plasma in Argon/Oxygen mixture helps in keeping the insects alive, the respective plasma chemical species adding a survival factor to the process.

4. CONCLUSION

Plasma agriculture is a recently proposed emerging field and one potential topic is pest control in stored products. This subject raises various questions, first one being to resolve if plasma treatments have any influence on the pests. In this context, wheat samples infested with adults of Tribolium castaneum and Tribolium confusum were submitted to atmospheric pressure plasma treatment and insect mortality was afterwards monitored.

In order to interpret the results, we have determined first the insects’ natural mortality and secondly the insect mortality caused by the gas used for plasma formation, as well as the insect mortality related to the temperature reached by the
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samples during the treatment. We have concluded that temperature is an important parameter: in the actual mortality caused by the plasma treatment, the contribution of the thermal effect may be strong enough to hide the presumptive plasma chemical effects. However, by performing systematic investigations using Argon/Oxygen and Argon/Nitrogen plasmas with various concentrations of argon and oxygen, we succeeded to conclude that plasma chemical effects are present, but they are dependent on the gas nature. Compared to the thermal treatment, at the same treatment temperature, the nitrogen-containing plasma enhanced mortality, while the oxygen-containing plasma enhanced the insects’ survivability. The identification of conditions leading to increasing insect mortality represents a solid ground for further testing plasmas as pest-controlling agents.

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