

2021

Cold Plasma For Insect Pest Control: *Tribolium Castaneum* Mortality and Defense Mechanisms in Response to Treatment

Dana Ziuzina

Technological University Dublin, dana.ziuzina@tudublin.ie

Robin Van Cleynenbreugel

Katholieke University Leuven, Ghent, Belgium

Paula Bourke

Technological University Dublin, paula.bourke@tudublin.ie

Follow this and additional works at: <https://arrow.tudublin.ie/schfsehart>



Part of the [Public Health Commons](#)

Recommended Citation

Ziuzina, Dana; Van Cleynenbreugel, Robin; and Bourke, Paula, "Cold Plasma For Insect Pest Control: *Tribolium Castaneum* Mortality and Defense Mechanisms in Response to Treatment" (2021). *Articles*. 500.

<https://arrow.tudublin.ie/schfsehart/500>

This Article is brought to you for free and open access by the School of Food Science and Environmental Health at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact arrow.admin@tudublin.ie, aisling.coyne@tudublin.ie, gerard.connolly@tudublin.ie.



This work is licensed under a [Creative Commons Attribution-NonCommercial-Share Alike 4.0 License](#)

FULL PAPER

Cold plasma for insect pest control: *Tribolium castaneum* mortality and defense mechanisms in response to treatment

Dana Ziuzina^{1,2} | Robin van Cleynenbreugel³ | Claudio Tersaruolo² |
Paula Bourke^{1,2} 

¹Plasma Research Group, School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland

²ESHL, School of Food Science and Environmental Health, Technological University Dublin, Dublin, Ireland

³Faculty of Engineering Technology, Katholieke University Leuven, Ghent, Belgium

Correspondence

Paula Bourke, Plasma Research Group, School of Biosystems and Food Engineering, University College Dublin, Dublin 4, Ireland.

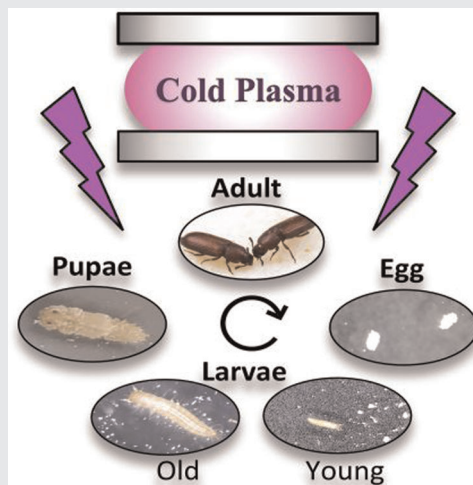
Email: paula.bourke@ucd.ie

Funding information

Science Foundation Ireland, Grant/Award Number: 14/IA/2626; Erasmus+, Grant/Award Number: R van Cleynenbreugel study exchange

Abstract

The insecticidal properties and mechanisms of high-voltage air-based atmospheric cold plasma using a contained dielectric barrier discharge reactor were investigated against *Tribolium castaneum* as an important bio-contaminant in stored grains spoilage. The mortality of 95.0%–100% for preadult stages can be achieved within seconds of treatment, but longer plasma exposure (5 min) is required to kill adult insects. Cold plasma treatment reduces both the respiration rate and the weight of insects and affects the levels of oxidative stress markers in adult populations. Sufficient toxicity is achievable through plasma process control in air to address the range of insect lifecycle stages that are disease vectors and pose risks for grain stability in storage. Balancing insecticidal activity with grains' quality retention can provide a route to sustainable integrated pest management.



KEYWORDS

cold plasma, insecticidal effect, mortality, oxidative stress, *Tribolium castaneum*

Abbreviations: ACP, atmospheric cold plasma; CAT, catalase; DBD, dielectric barrier discharge; GST, glutathione S-transferase; MDA, malondialdehyde; PTRT, post-treatment retention time; ROS, reactive oxygen species; SOD, superoxide dismutase.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Plasma Processes and Polymers* published by Wiley-VCH GmbH.

1 | INTRODUCTION

Cereal grains, including wheat, maize, rice, barley, and sorghum, are a principal component of the human diet, supplying approximately 50% of the world's caloric intake;^[1] however, up to 40% of the worldwide agricultural produce is lost due to plant diseases and pests.^[2] Stored grains are ravaged by a number of insect pests, resulting in qualitative as well as quantitative losses.^[3] Internally feeding insects, whether adult or larvae, feed on grain endosperm and/or the germ, resulting in grain weight loss, reduction in nutritive value, deterioration of quality, and reduction of seed viability and vigor. Externally feeding insects can damage grains by excrement contamination, empty eggs, larval molts, empty cocoons, and adult corpses.^[4] Moreover, insects are important vectors in disseminating fungal contaminants, which can increase mycotoxin contamination and decrease the quality of stored grains,^[5,6] making the preservation of grain quality during storage a continuous challenge.^[7]

Globally, around 3 million tons of pesticides are applied annually, with almost 500 active substances in use across the European Union as plant protection products.^[8] To control insect pests, chemically based insecticides, including both contact insecticide and fumigants, such as methyl bromide and phosphine, are applied throughout all developmental phases of plants and during crop storage.^[9] The common contact insecticides used in cereal grain storage are organochlorines, organophosphates, pyrethroids, and neonicotinoids.^[10,11] In light of the phasing out of methyl bromide, phosphine fumigation is commonly used; however, it also has limitations, such as long exposure times and ineffectiveness against different stored grain pests.^[12–14] Although insecticides play an important role in protecting crops, their extensive use, nontarget toxicity, and persistent nature have lifecycle impacts on soils, terrestrial and aquatic ecosystems, humans, and other animal life and contribute to the development of resistance in insects.^[8,11–14]

Among animal pests, insects cause by far the most damage to stored grains, as they are very flexible, adaptable to minimal nutritive environments, and are often regarded as permanent pests.^[14,15] The most harmful insect species belong to genera *Sitophilus*, *Rhyzopertha*, *Trogoderma*, *Sitotroga*, *Cryptolestes*, and *Tribolium*.^[15] *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), the red flour beetle, present worldwide, is a global pest of stored food products that belongs to a group of secondary grain insects infesting only damaged grains.^[16] Both the larvae and adults feed on a

variety of produce, including cereals, flour, starchy materials, cake mixes, spices, chocolate, powdered milk, and dry animal food, contributing to significant financial costs to food production industries.^[17] Apart from economic issues, the beetle secretes toxic quinones, carcinogens, thus posing a risk to the health of consumers.^[18] Therefore, novel control measures are urgently needed to address consumer risks, environmental impacts, and increasing resistance of the insects, to ensure long-term eco-safety and maximize production yields.

To date, multiple studies have investigated alternative methods to the use of chemical pesticides. These include natural plant-derived components,^[18,19] entomopathogenic bacteria and nematodes,^[20–22] insect growth regulators,^[23] physical control methods, such as heat, cold, and modified atmosphere technology,^[7,24,25] ozone,^[26,27] ionizing radiation,^[28] and combined treatment approaches.^[24,29] Atmospheric cold plasma (ACP) harnesses diverse and multiple mechanisms of action, and is increasingly being investigated to address bio-contamination and sustainability issues in the agri-food chains. Generated at atmospheric pressure, ACP consists of UV photons, neutral or excited atoms and molecules, negative and positive ions, free radicals, and free electrons, and has minimal or no thermal impacts on target foods.^[30] These characteristics provide a rich resource to develop alternative approaches to traditional fumigation methods for the preservation of agricultural commodities. Cold plasma can be modulated to provide a promising tool for effective cereal grain decontamination and to promote the functional properties of seeds^[31] and can be effectively applied for the elimination of pests in stored foods.^[32–37] However, it is still relatively underexplored and less understood as an insecticidal approach. The aim of this study was to investigate the insecticidal potential of cold plasma treatment using *T. castaneum*, red flour beetle, as an insect model. This study uses a well-characterized cold plasma processing device that uses air as the inducer gas, and takes cognizance of system and process findings to date to refine and understand how this scalable approach can be used for insect control to promote food security. The influence of plasma process parameters, such as treatment time, post-treatment retention time (PTRT), and mode of exposure, on insecticidal efficacy was evaluated in terms of the mortality rate of insects at various developmental stages, including egg, young, and late-stage larvae, pupae, and adult. The effects of plasma on the insects' respiration rate and weight and on enzymatic antioxidative defense systems, namely, superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), and concentration of malondialdehyde (MDA) as an oxidative stress marker, and protein content were measured in adult beetles to elucidate the process-associated mechanisms involved in cold plasma toxic effects.

2 | EXPERIMENTAL SECTION

2.1 | Insect rearing conditions and sample preparation

T. castaneum was obtained from Blades Biological and reared in plastic containers on 95% organic wheat flour with 5% brewer's yeast mixture in a 34°C incubator in the dark.^[24] Fluon (Blades Biological) was used to prevent the escape of adult insects. *T. castaneum* non-sexed adult insects (4 weeks old), pupae (25–27 days from oviposition), old larvae (20 days from oviposition), young larvae (2–3 days old), second instar larvae (7 days from oviposition), and 48-h-old eggs were used to study the insecticidal effects of ACP. To collect eggs, non-sexed adult insects were incubated in pre-sieved (250 µm) wheat flour for 24 h at 34°C. After incubation, adult insects were removed and flour was sifted through a 300 µm sieve. Eggs were collected and further incubated for 24 h at 34°C.

2.2 | ACP system setup

The ACP system used in this study was a high-voltage (HV) dielectric barrier discharge (DBD) system with a maximum voltage output in the range 0–120 kV_{RMS} at 50 Hz, described in detail by Pankaj et al.^[38] and fully characterized by Moiseev et al.^[39] and Milosavljević and Cullen.^[40] The samples were subjected to contained ACP treatment at 80 kV under atmospheric pressure and atmospheric air used as the working gas. The total distance between the two aluminum disc electrodes (diameter: 15 mm) was approximately 50 mm, which was equal to the height (20 mm) of the polypropylene container (310 × 230 mm) utilized as a sample holder and the thickness of the top (10 mm) and the bottom (7 mm) dielectric barriers. Samples were placed inside the container and subjected to either direct (within the area of plasma discharge) or indirect (outside the area of plasma discharge) mode of plasma exposure as described previously.^[41] Both direct and indirect treatments were achieved simultaneously, that is, two samples per container: one for direct treatment and one for indirect treatment. Before treatment, each container was sealed with a high-barrier polypropylene bag (B2630 Cryovac) to retain plasma-generated reactive species inside the pack and therefore ensure that the reported activity is in relation to a closed processing system.

2.3 | ACP treatment

The schematic diagram of the experimental setup is shown in Figure 1. To examine the insecticidal effects of

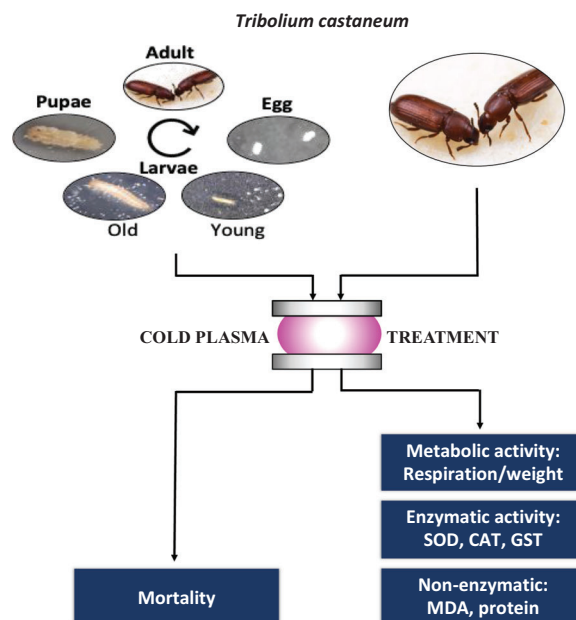


FIGURE 1 Experimental design. CAT, catalase; GST, glutathione *S*-transferase; MDA, malondialdehyde; SOD, superoxide dismutase

ACP, *T. castaneum* adult insects, old larvae, or pupae were transferred to Petri dishes (10 insects per dish) containing whole kernel organic wheat with 20% (by weight) wheat flour (total mass of food: 5 g). Insects' eggs or young larvae were fixed on a piece of black adhesive tape (10 eggs per tape), which was then fixed at the center of the Petri dish (two tapes per dish). The samples were exposed to 0–20 min of treatment and PTRTs of 0 and 24 h at 15°C. For eggs and young larvae control samples, the tape was exposed to the corresponding ACP treatment time and PTRT before insects' fixation. Similarly, for adult insects, pupae, or old larvae controls, plasma-processed food (whole grains and flour) was used. A separate set of controls consisted of eggs or young larvae fixed on an untreated tape and adult insects, pupae, or old larvae in Petri dishes containing untreated food. During these experiments, it was established that ACP-treated tape or food had no influence on the viability of the insects. All control samples were exposed to the corresponding PTRT of either 0 or 24 h at 15°C. Each experiment involving adult insects, old larvae, and pupae was replicated three times. Experiments with eggs and young larvae were repeated three times; each experiment was performed using duplicate samples ($n = 6$).

For insects' respiration activity and biochemical assays, the indirect mode of plasma exposure and sublethal treatment times were selected. To measure respiration of *T. castaneum*, 30 adult insects (~4 weeks from the day of

eclosion) were exposed to 5 min of indirect treatment and 0 h PTRT (six independent experiments were conducted, $n = 6$). For biochemical tests, 30 adult insects were subjected to treatment for 0, 1, 2, and 5 min, followed by 0 h PTRT (three independent experiments were conducted).

2.4 | Analysis of insects

2.4.1 | Viability assay

The effect of ACP treatment on the viability of *T. castaneum* was assessed in terms of percentage mortality. All insect samples were visually examined using a stereomicroscope (SZ61-TR; Olympus). To assess the effect of ACP on *T. castaneum* egg mortality, the eggs were incubated at 34°C for up to 7 days. The mortality of the eggs was calculated as the number of eggs unhatched/total number of eggs per trial $\times 100\%$. Young larvae were assessed immediately after treatment and corresponding PTRT. To examine the effect of ACP on adult insects, the samples were transferred into *T. castaneum* rearing medium and incubated for 24 h at 34°C. For moribund and surviving insects, the observation was repeated on Day 7. Insects were considered dead if no movement was observed. Mortality of adult insects and young larvae was calculated as the number of dead insects/total number of insects for each trial $\times 100\%$.^[42] To evaluate the effect of ACP on old larvae and pupae viability, treated samples were incubated until adult emergence was observed (6–8 days). The number of adult insects was calculated and the mortality of old larvae and pupae was calculated as $100 - \frac{\text{number of adult emerged}}{\text{total number of pupae per trial}} \times 100\%$.

2.4.2 | Respiration activity

The production of CO₂ was used to measure the respiration activity of *T. castaneum*. To facilitate O₂ and CO₂ measurements, Universal glass bottles containing aluminum screw caps with black rubber seal were used as respiratory chambers. Immediately after 5 min of indirect treatment, 20 live insects were selected and transferred into bottles containing 2.5 g of organic wheat flour. The samples were tightly sealed and left for 18 h at room temperature ($22 \pm 1^\circ\text{C}$) for acclimatization. Survival rates were 100% for both control and treated samples after acclimatization.

The changes in the O₂ and CO₂ gas compositions inside the bottles were monitored using a gas analyzer (Systech Instruments). Gas sampling was performed using a hypodermic needle, inserted through a septum

on the caps of the Universal bottles, at a flow rate of 150 ml/min for 10 s. The instrument is based on an electrochemical sensor to record O₂ and on a mini-IR spectrophotometer to record CO₂ concentrations (%) (accuracy: 0.1% v/v O₂; 2% v/v CO₂).^[43] After completing the measurements, the insects' body weights (mg) for treated and control groups (each group comprised 20 insects) were also recorded. The results represent an average of six independent experiments.

2.4.3 | Biochemical assays

Before biochemical analysis, the weights of insects before and after indirect ACP treatments for 1, 2, and 5 min were recorded. For biochemical tests, 20 living adult insects (insects that moved or responded to tactile stimuli) were homogenized by crushing the beetles with 1 ml of ice-cold phosphate-buffered saline. The homogenates were centrifuged twice for 10 min at 10,000 rpm and the collected supernatant was used for further analyses. Untreated beetles were used as control samples. All nonenzymatic responses and enzymatic activities were determined using a colorimetric method by measuring absorbance at the specific wavelengths on a multimode microplate reader (Synergy HT, Biotek Instruments Inc.; Varioskan™ LUX, Thermo Fisher Scientific™). The absorbance for each individual sample was read in triplicate (three wells per sample). All calculations were performed using the averaged absorbance values obtained from three wells for each individual experiment performed in triplicate.

Total protein content

The insects' sample homogenates were diluted (1:10) and the total protein content was measured using the bicinchoninic acid assay (Thermo Fisher Scientific) according to the manufacturer's instructions. Results were expressed as the total protein content (mg/ml).

Lipid peroxidation/MDA assay

The magnitude of lipid peroxidation, a nonenzymatic oxidative stress marker, was determined by measuring the concentration of malondialdehyde (MDA; a lipid peroxidation byproduct, which is a thiobarbituric acid-reactive substance) according to the procedure described by Wang et al.,^[44] with minor modifications. Briefly, 0.2 ml of 30% trichloroacetic acid (Sigma-Aldrich) was added to 0.2 ml of the supernatant. The mixture was vortexed and centrifuged at 15,000g for 10 min at 4°C. After centrifugation, 0.3 ml of the supernatant was mixed with 0.3 ml of 0.8% thiobarbituric acid (Sigma-Aldrich). The resulting suspension was incubated at 98°C for

60 min and cooled in ice for 5 min. The concentration of MDA in the test solutions was determined by measuring absorbance at 532 nm and comparing it with a standard curve of known MDA (Sigma-Aldrich) concentrations. Results were expressed as the concentration of MDA (μmol) per mg of protein.

SOD

The activity of SOD was determined using the SOD Determination Kit (Sigma-Aldrich) according to the manufacturer's instructions. Results were expressed as percentage SOD activity (%).

GST

The activity of GST was determined using the GST Assay Kit (Sigma-Aldrich) according to the manufacturer's instructions. The sample homogenates were diluted 1:10, and the enzyme-specific activity was determined by measuring absorbance at 340 nm in a UV 96-well plate immediately after preparing the reaction tests and every minute thereafter for 6 min (established as a linear range of the plot) and expressed as $\mu\text{mol}/\text{min}$ per mg of protein.

Catalase activity

The catalase activity was estimated by the decomposition rate of hydrogen peroxide according to the procedure described by Wang et al.,^[44] with minor modifications. Briefly, to a diluted (1:100) sample, a 1% hydrogen peroxide solution was added. The absorbance of 240 nm was recorded every 1 min for 10 min, and CAT activity was calculated using the following formulas: $K = 1/300 \times (\ln A_3/A_{10})$, where A_3 and A_{10} are the absorbances at 3 and 10 min, respectively, $K_{\text{total}/\text{ml}} = K/\text{sample volume}$, and $K/\text{mg} = K_{\text{total}/\text{mg}}$ protein.

2.5 | Statistical analysis

Statistical analysis was performed using Prism (version 8.2.1; GraphPad Software, Inc.). Apart from the weight of insects, the means of all ACP-treated samples and the corresponding untreated controls were subjected to analysis of variance and compared according to the method of Fisher's least significant difference at the 0.05 confidence level. For the respiration activity, the means of the weight of ACP-treated and untreated insects were determined and compared using a one-sample t and the Wilcoxon test at the 0.05 level. Multiple t tests and the Holm-Sidak method, with $\alpha = .05$, were used for pairwise comparisons to determine the statistical significance between the weights of insects measured before and immediately after indirect ACP treatment for 1, 2, and 5 min.

3 | RESULTS AND DISCUSSION

3.1 | Effect of ACP on the viability of insects

The sensitivity of *T. castaneum* to ACP depended on the mode of plasma exposure, duration of treatment, and PTRT, and the insects' developmental form. The mortality of *T. castaneum* increased with increasing treatment time, which is in agreement with other reports focusing on the insecticidal effects of plasma.^[32,34,37] Higher inactivation rates of insects across the different life stages were achieved with the direct mode of plasma exposure than the indirect mode (Figures 2 and 3, respectively). The direct mode of plasma exposure is characterized by the simultaneous attack of generated charged particles, UV, and short- and long-lived plasma-reactive species (ozone, hydroxyl radicals, and other reactive oxygen and nitrogen species) on the target organisms, whereas with the indirect mode of exposure, the contact of charged particles or an electric field with the target organism is avoided. With direct contact, charged particles can accumulate on the surface and cause electrostatic stress leading to morphological changes and cause cellular destruction through etching effects.^[45] Regardless of PTRT, the direct treatment significantly reduced ($p < .05$) the viability of eggs, and larvae and pupae within 0.16 min (10 s) and 0.5 min (30 s) of treatment, respectively. The mortalities for eggs, young larvae, old larvae, and pupae corresponded to 84.6% and 95.0%, 97.1% and 100%, 80.0% and 96.7%, and 76.7% and 96.7% after 0 and 24 h PTRT, respectively (Figure 2a,b). However, much longer treatments were required to kill adult insects, but more pronounced effects of PTRT were noted, suggesting a different emphasis on the mechanisms of inactivation. Direct treatment for 5 min in conjunction with 0 h PTRT resulted in the mortality of adult populations of 66.7%, whereas 100% mortality was achieved when 5 min of treatment was accompanied by 24 h of PTRT. *Tribolium* larvae were the most sensitive to the direct mode of plasma exposure and 0 h PTRT, followed by eggs, pupae, and adult populations (Figure 2a). Both young and old larvae were completely inactivated after 1 min of treatment, whereas eggs, pupae, and adult insects were inactivated after 2, 5, and 10 min of treatment, respectively (Figure 1a). The order of the insect life stages with respect to their resistance to direct treatment and 0 h PTRT was as follows: adult (10 min) > pupae (5 min) > egg (2 min) > young and old larvae (1 min). Application of extended PTRT (24 h) completely eliminated young larvae after 30 s of treatment, and old larvae and pupae after 1 min of treatment. Furthermore, the direct mode of ACP exposure and longer PTRT in a

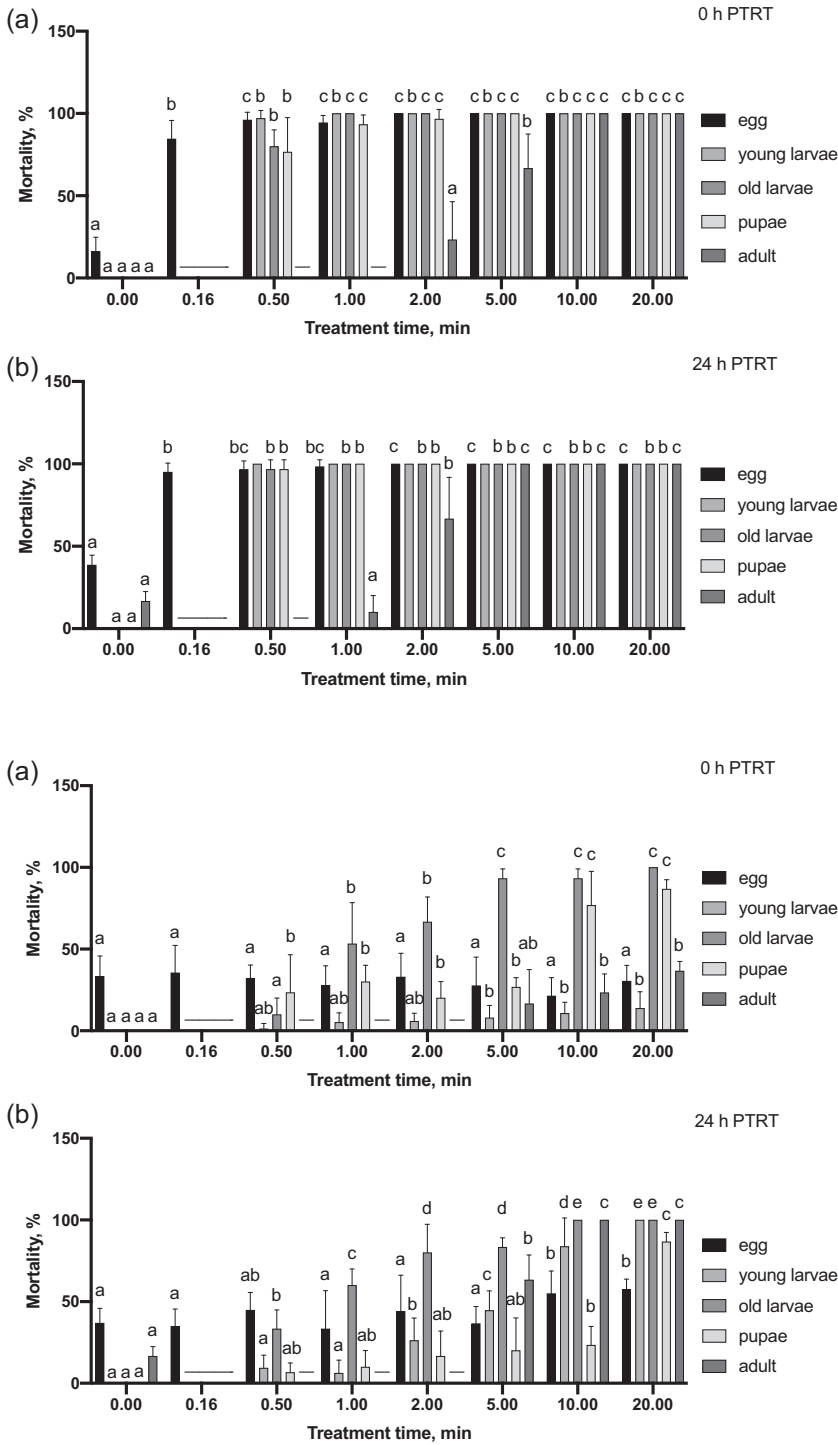


FIGURE 2 Effect of the *direct* mode of atmospheric cold plasma exposure on the viability of *Tribolium castaneum* at different life stages (egg, young larvae, old larvae, pupae, and adult). Mortality of insects presented as a function of treatment time and post-treatment retention time (PTRT): (a) 0 h and (b) 24 h. –, not tested. Error bars indicate the standard deviation. Different letters represent significant differences at the level of $p < .05$

FIGURE 3 Effect of the *indirect* mode of atmospheric cold plasma exposure on the viability of *Tribolium castaneum* at different life stages (egg, young larvae, old larvae, pupae, and adult). Mortality of insects presented as a function of treatment time and post-treatment retention time (PTRT): (a) 0 h and (b) 24 h. –, not tested. Error bars indicate the standard deviation. Different letters represent significant differences at the level of $p < .05$

contained environment resulted in higher inactivation rates of all the insect populations studied (Figure 1b). This is due to the reactive plasma components, the action of which was further extended using a contained plasma treatment approach. The observed results are of direct relevance for the treatment of stored grain commodities, and are in agreement with our previous reports, which demonstrated that mode of plasma exposure and PTRT in a contained environment played a key role in

achieving biocidal effects.^[46] Regardless of PTRT, adult populations were more resistant to direct treatment than the other life stages examined. The order of resistance was as follows: adult (5 min) > eggs (2 min) > old larvae and pupae (1 min) > young larvae (0.5 min).

Using the indirect mode with no PTRT had no significant effects on the viability of eggs regardless of the treatment time (0.16–20 min) (Figure 3a). The survival of pupae, old larvae, young larvae, and adult insects was

significantly reduced after 0.5, 1, 5, and 10 min of treatment, respectively, in comparison with the corresponding controls ($p < .05$). However, complete inactivation was achieved only for old larvae and only after the longest treatment of 20 min. This treatment time resulted in mortalities of pupae, adult, and young larvae of 93.3%, 36.7%, and 13.8%, respectively (Figure 3a).

Combining 10 min of indirect treatment and 24 h of PTRT completely eliminated old larvae, and adult insects and young larvae after 20 min of treatment, while eggs and pupae resisted indirect treatment, with the mortality corresponding to 18.9% (relative to the untreated control) and 86.7%, respectively (Figure 3b). With indirect treatment and 24 h PTRT, the most resistant populations were eggs and pupae (the order of resistance: egg and pupae [>20 min] $>$ young larvae [20 min] $>$ old larvae and adult insects [10 min]).

The difference in the level of sensitivity between the eggs, larval, pupal, and adult stages to plasma treatment is linked to the difference between respiration rates at lifecycle stages. This increases during the development from the egg to the prepupal stage, then decreases during pupation. Furthermore, respiration can be higher in young larvae than in older ones.^[47] Morphological characteristics, such as type of cuticle (soft or hard)^[48] and properties of the cuticular layers (waxes, chitin, sclerotizing agents, and protein content) of the insect at each stage,^[49,50] can also have a protective effect. The susceptibility of insects of different developmental stages to plasma varies in the literature. Ratish Ramanan et al.^[36] investigated the influence of voltage, exposure time, and electrode gap on the insecticidal effects of HV-DBD ACP operating between 1 and 10 kV and yielded 100%, 80%, and 100% mortality rates of *T. castaneum* eggs, larva, and adult, respectively, after 15 min of treatment using optimized voltage and distance between electrodes. *Plodia interpunctella* meal moth larvae were more sensitive than pupae to atmospheric pressure plasma jet treatment, which could be due to the pupae sclerotized cuticle-protecting effect.^[33] Human lice (*Pediculus humanus*) were more resistant to plasma generated by a comb-like device than their eggs, but the age of the eggs also had an influence, with older eggs demonstrating higher resistance.^[51] Similar results were observed for eggs of *Tribolium* spp. in response to UV irradiation treatment, where younger eggs were more sensitive to UV rays than older ones.^[52] Their work also reports that *Cadra cautella* eggs were more resistant than *Tribolium* eggs at all ages and exposure periods. Furthermore, Yao et al.^[17] reported differences in susceptibility to other insecticides (methoprene and pyriproxyfen) between *Tribolium* species, with *Tribolium confusum* being more tolerant than *T. castaneum*. There

is huge crop and regional diversity in the type species and lifecycle stage of pests that pose risks for food supplies, but also wide opportunities for flexible modes and stages of application of cold plasma for insect pest control. This study demonstrates that different lifecycle stages can influence successful inactivation and also highlights the need for process optimization where treatments are aligned with retention of other properties such as grains' functionality. Further studies are recommended for other species and their respective lifecycle stages to identify successful applications of ACP for integrated pest management strategies across the agri-food sector.

With respect to the possible mechanisms of action of gaseous plasma on insects, Donohue et al.^[32] suggested that these might be the nervous and/or the neuromuscular system altered by the plasma metabolic rate. Ferreira et al.^[53] hypothesized that the interaction of nonthermal plasma with the membranes of various organs of *Drosophila melanogaster* can have interrupting effects on endocrine system functionality. Plasma-generated reactive oxygen species (ROS) can cause the destruction of hydrocarbons in the cuticular lipid layer of insects and dehydration effects leading to insect death.^[36] Interestingly, the change in the color from red to yellow was observed in sample homogenates (Figure S1), although these changes were not apparent in treated adult insects before homogenization. Discoloration could be due to pigment degradation through the plasma bleaching effect. Ozone treatment is capable of causing significant changes in the fatty acid composition and the destruction of natural pigments.^[27] Dehydration itself could be among the factors influencing the body color in *T. castaneum* adult insects, with hydrated populations being significantly darker than dehydrated ones.^[50] Starvation was shown to irreversibly affect the body color of the pea aphid, which shifted from red to pale under the stress, and it was associated with the change in pigment composition and their content.^[54] The authors highlighted that the body color change could be an adaptation mechanism to the environmental stress and a strategy that aphids use for the storage and consumption of energy reserves. The change in the color of insects' homogenate suggests the occurrence of complex biochemical modifications due to plasma exposure, but whether this is an effective stress response is unclear. In the case of insect eggs, plasma can interfere with embryogenesis due to the electrostatic accumulation of charges and by penetrating the egg's outer membrane.^[36] El-Aziz et al.^[33] reported plasma-oxidizing effects observed through changes in enzymatic activity and induction of oxidative stress. Plasma-generated ROS are considered to be responsible for the above mentioned effects. Ozone, as one of the main long-lived

plasma-reactive species, is a powerful oxidizing agent that is highly toxic at physiological and cellular levels to all living organisms, with already demonstrated potential to be used for controlling stored product insects.^[55–57] It is capable of reacting with any biomacromolecule, including lipids, proteins, nucleic acids, and carbohydrates, causing oxidative damage and leading to cell death.^[58] The mode of action of ozone is through the insect's respiratory system, which explains the higher sensitivity of adult and larval insect stages, which have higher respiratory activity, than of eggs and pupae, with lower respiration rates.^[27] Similarly, in the current work, eggs and pupae were the most resistant to the indirect mode of treatment, suggesting that insects' respiratory system could be the target of the indirect mode of ACP exposure; however, physical mechanisms as a result of electrostatic stress and etching from the action of plasma-reactive species and charged particle attack could also be involved in direct plasma contributing to higher insecticidal effects.

3.2 | Effect of ACP on the respiration activity of insects

In this study, the level of carbon dioxide as a measure of the respiratory activity of insects was recorded after 18 h of acclimatization under atmospheric conditions. The gas composition inside the chambers containing wheat flour only (without insects) measured at time 0 and 18 h was 20.5% and 0% for O₂ and CO₂, respectively. Insect populations exposed to a sublethal plasma dose (indirect 5 min of treatment) showed a significantly lower respiration rate compared with the control sample ($p < .05$), as a lower CO₂ output and a proportionally higher level of O₂ for the treated samples were recorded (Figure 4a). A positive correlation was observed between respiratory activity and the insect's body weight. The weight of the control samples was on average 0.0385 ± 0.0011 g, whereas a significantly lower weight, 0.0341 ± 0.0011 g, was recorded for the treated samples ($p < .05$) (Figure 4b).

Lu et al.^[59] reported that the respiration rate of insects exposed to ozone can be biphasic. The insects were able to breathe discontinuously, that is, with a lower respiration rate, to reduce the uptake of toxic chemicals, thereby minimizing oxidative stress, and then showed an increase in respiration and/or metabolic rates that may support the insect's faster recovery, which could be one of the resistance mechanisms associated with active exclusion of the gas. In another work, reduced respiration rates and therefore reduced uptake of the fumigant were correlated with higher resistance levels to phosphine in resistant insect populations compared with their susceptible counterparts.^[60] The respiratory patterns largely depend on the metabolic rate of insects, which in turn may be affected by multiple factors.^[61] The possible effects of dehydration^[62] and etching from the action of plasma-reactive species and charged particle attack could physically injure insects and thus impact insects' metabolic rate, leading to the reduction in respiratory activity. This could also interfere with normal physiological processes, including feeding, which could lead to the reduction in the body mass of insects as observed in this study. In fact, higher insect body mass and respiration rate could indicate higher energy reserves for insecticide resistance.^[63] In contrast to our results, Sousa et al.^[64] showed no correlation between the two indices in their study on the resistance of *T. castaneum*, *Rhyzopertha dominica*, and *Oryzaephilus surinamensis* to ozone. Importantly, the dehydration process, which is largely associated with the action of plasma-generated species, alone can lead to significant physiological injuries and insect death.^[65] Ferreira et al.^[53] reported an abnormal larvae behavior with a lack of normal feeding reflex post plasma discharge. Some of these larvae died after several days, while others continued to show abnormal behavior up to 30 days without further development into an adult stage, and finally died. Further investigations exploring the relationship between reduced respiration rate and body

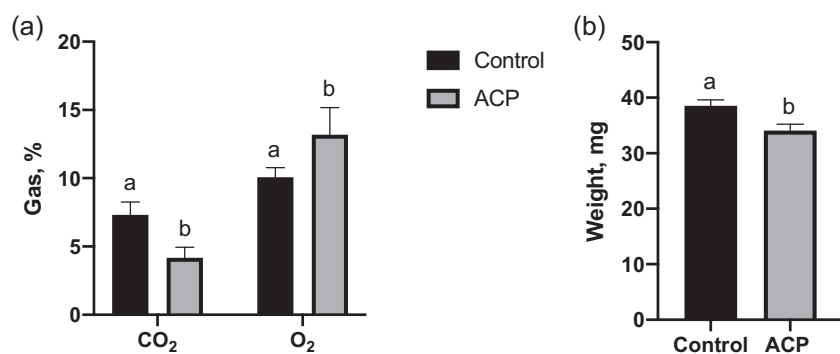


FIGURE 4 Effect of indirect 5 min of atmospheric cold plasma (ACP) treatment on (a) respiration and (b) weight of adult insects. Mean values ($n = 6$) of the control group were compared with the ACP-treated group. Vertical bars indicate the standard deviation. Different letters represent significant differences at the level of $p < .05$

mass and the potential impact of cold plasma mechanisms on insects signaling systems are warranted to understand effective strategies to manage insects' resistance to plasma.

3.3 | Oxidative stress response

Among the ROS, ozone, atomic oxygen, singlet oxygen, superoxide, peroxide, and hydroxyl radicals, which are generated within air cold plasma, have been demonstrated to play a crucial role in cellular death.^[45] On the contrary, ROS (singlet oxygen, superoxide anion, hydroxyl radical, and hydrogen peroxide) are also products of normal cellular aerobic metabolism, but generated at low to moderate concentrations. Under the influence of environmental (physical, chemical) and physiological (diseases, injury) stressors, there can be an enhanced production of ROS and thus oxidative stress occurs, resulting in peroxidation of lipids, oxidative damage of proteins and nucleic acids, and dysfunction of cells and organs, leading to insects' death.^[66] Thus, in living organisms, system responses are in place where both endogenous and exogenous ROS are scavenged by enzymatic and nonenzymatic antioxidant defense systems to preserve cell structures and biomacromolecules from oxidative stress.^[58,67] Among enzymatic defenses, SOD, CAT, and GST have been reported to be the main antioxidant enzymes for a range of insects.^[68] In the cell membrane, polyunsaturated fatty acids are the primary targets for ROS stimulating the process of lipid peroxidation.^[58]

In the present study, the extent of oxidative stress achieved from plasma exposure on lipid peroxidation was monitored by measuring the concentration of MDA. The MDA content recorded in plasma-treated insect samples was positively correlated with the increase in the plasma treatment time. The concentration of MDA recorded after 1–5 min of treatment was significantly higher ($p < .05$) than the value of the untreated control (Figure 5a). A significant increase ($p < .05$) in GST activity with increasing treatment time from 1 to 2 and 5 min was also observed (Figure 5b). However, in contrast to MDA results, 5 min of treatment reduced the level of GST over 2 min of treatment. GST contributes to antioxidant defense by conjugating reactive species and detoxifying lipid peroxidation products and also plays a major role in insecticidal resistance mechanisms.^[69–71] An increase in the MDA content, indicating higher concentrations of lipid peroxidation products in samples exposed to 2 and 5 min of treatment, can explain the increase in the activity of GST. Similar results were observed in response to plasma generated by an

atmospheric pressure plasma jet, which affected lipid peroxidation levels, and the activity of GST in the last instar larvae of *P. interpunctella*.^[33]

In the present study, there was no significant change in the activity of SOD observed for all treatment times studied, although a slight decrease in enzymatic activity after 1 and 5 min of plasma exposure was noted (Figure 5c). Similarly, no significant difference in the relative activity of CAT was observed after ACP treatment (Figure 5d). SOD converts a superoxide anion radical into molecular oxygen and hydrogen peroxide, while hydrogen peroxide is further scavenged and decomposed into water by catalase;^[67,72] therefore, an increase in the activities of these enzymes would be expected. The current results suggest that neither CAT nor SOD played a significant role in protecting *T. castaneum* against oxidative damage of ACP. Another possible explanation for this observation could be that the toxicity of the reactive species generated from higher plasma doses and ROS produced by cellular metabolism in response to treatment could lead to irreparable oxidative damage to the enzymes, rendering them dysfunctional. This could also explain the reduction in the activity of GST after prolonged plasma exposure (2 and 5 min). The demonstrated reduction in the respiratory activity and thus the reduced metabolic rate could also contribute to such a response. Holmstrup et al.,^[56] evaluating the effects of ozone on *T. castaneum*, demonstrated that transcription of genes involved in protection against treatment, including *SOD*, *CAT*, and *GST*, was either decreased or unchanged after ozone exposure, indicating the general decrease in the metabolism post treatment. Other insecticides, deltamethrin and pirimiphosmethyl, were shown to reduce the activity of enzymatic defense systems in *Tribolium* species.^[72]

Proteins play a major role in the synthesis of detoxifying enzymes and help to detoxify the entered toxicants.^[73] In this study, a significant reduction in the protein content ($p < .05$) was observed in all plasma-treated samples in comparison with untreated controls (Figure 5e), while the weight of the treated groups did not change significantly in comparison with the corresponding controls (Figure 5f). This can be due to the direct action of plasma-oxidizing species or endogenously produced ROS, which could result in reduced metabolism and lack of energy resources for protein synthesis. Under oxidative stress, proteins/enzymes can be impaired by carbonylation, loss of structural integrity, and thus functionality.^[66,67] Shonouda et al.^[73] observed a reduction in the protein content in *Anaceana globulus* in association with the toxicity of heavy metals, which was related to the loss in the activity of glutathione peroxidase and CAT enzymes. Another explanation for the

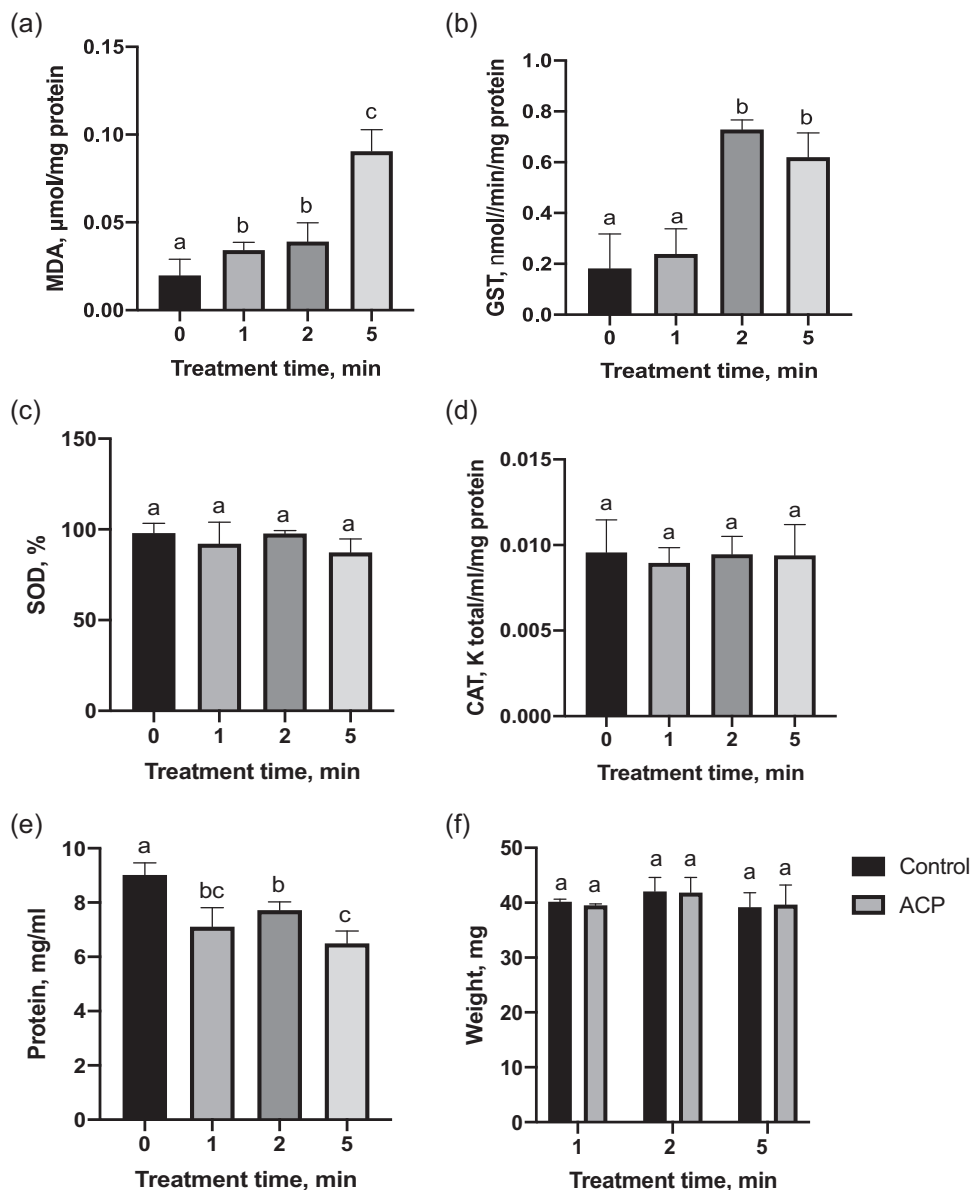


FIGURE 5 Concentration of (a) malondialdehyde (MDA), activity of (b) glutathione *S*-transferase (GST), (c) superoxide dismutase (SOD), and (d) catalase (CAT), (e) total protein content, and (f) weight of insects either untreated (0 min or control) or exposed to 1, 2, and 5 min of indirect atmospheric cold plasma (ACP) treatment. Vertical bars indicate the standard deviation. Different letters represent significant differences at the level of $p < .05$

lower protein content is their consumption in the process of scavenging of ROS.^[33] Generally, the analysis of oxidative stress markers correlates with plasma-induced mortality of insects, suggesting that higher plasma doses cause severe oxidative damage to insects and that oxidative stress-related mechanisms resulting from the action of both endogenous and exogenous ROS could be possible mechanisms of plasma target toxicity.

Overall, this study demonstrated that a contained CP reactor using ambient air could be effectively applied against target insect populations, with process and target characteristics identified that can be exploited in addition

to demonstrated antimicrobial and growth regulation potential,^[74,75] to enhance efficacy for agricultural and food sectors. For successful application of cold plasma as a multi-target approach for stored grain preservation, the potential role of the plasma-generated reactive species to act as allelochemicals (kairomones) and the interaction between the grain microbiome and insects should be further considered. It is known that some microbes can be pathogenic to insects, while others can be used as a food source.^[76,77] Furthermore, insect and microbial attacks on plants can cause changes in plant molecular physiology and biochemistry, thus modifying

plant quality and repellence/attraction defense mechanisms.^[78] Cold plasma has the potential to be tailored to microbe–plant–insect interactions as a means of sustainable biocontrol at different insect lifecycle stages. However, efforts should be made to elucidate the long-term effects in terms of the emergence of resistant strains, cross-species responses, and minimal nontarget or bystander effects to develop a safe alternative for insect pest control for integrated pest management in the safe storage of grains.

4 | CONCLUSIONS

Contained HV-ACP treatment in air was effective against *T. castaneum*, which demonstrates the potential for ensuring grain stability in storage. Within relatively short treatment times from 0.5 to 5 min, 100% mortality of *T. castaneum* at various developmental stages was achieved, with adults being the most resistant stage to direct treatment. Exposure to sublethal ACP treatments reduced the respiration rate in adult populations, which was positively correlated with insects' weight. Lipid peroxidation levels increased with increasing treatment time and an enhanced activity of GST was also observed. However, no changes in the levels of SOD and CAT activity were recorded. Considering insect target adaptability and resistance processes, further studies are necessary to understand long-term plasma effects as well as plant–microbiome–insect interactive responses to exploit this technology for sustainable biocontrol in the agricultural sector.

ACKNOWLEDGMENTS

This study was funded by the Science Foundation Ireland (SFI) under Grant Number 14/IA/2626 and by the Erasmus+ study exchange for Robin van Cleynenbreugel.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Dana Ziuzina was involved in the conceptualization, methodology, investigation, and writing of the original draft. Robin van Cleynenbreugel and Claudio Tersaruolo were involved in the methodology. Paula Bourke was involved in the conceptualization, supervision, methodology, review, writing and editing.

DATA AVAILABILITY STATEMENT

Data are available upon request from the authors.

ORCID

Paula Bourke  <http://orcid.org/0000-0002-5607-8021>

REFERENCES

- [1] J. M. Awika, *ACS Symp. Ser.* **2011**, 1089, 1.
- [2] I. Mahmood, S. R. Imadi, K. Shazadi, A. Gul, K. R. Hakeem, *Effects of Pesticides on Environment*, Springer International Publishing, Switzerland, **2016**.
- [3] C. Srivastava, S. Subramanian, *Indian J. Entomol.* **2016**, 78, 53.
- [4] T. S. Payne, *Harvest and Storage Management of Wheat. Bread Wheat: Improvement and Production*, FAO Plt. Prod. Prot. Ser. 30. Curtis BC **2002**.
- [5] S. D. Eigenbrode, N. A. Bosque-Pérez, T. S. Davis, *Annu. Rev. Entomol.* **2018**, 63, 169.
- [6] T. S. Yun, S. Y. Park, J. Yu, Y. Hwang, K. J. Hong, *Plant Pathol. J.* **2018**, 34, 356.
- [7] S. Navarro, *J. Agric. Eng.* **2012**, 49, 13.
- [8] V. Silva, H. G. J. Mol, P. Zomer, M. Tienstra, C. J. Ritsema, V. Geissen, *Sci. Total Environ.* **2019**, 653, 1532.
- [9] C. Bolzonella, M. Lucchetta, G. Teo, V. Boatto, A. Zanella, *Glob. Ecol. Conserv.* **2019**, 20, e00699.
- [10] M. A. Randhawa, A. Ahmed, M. S. Javed, *Wheat Contaminants (Pesticides) and their Dissipation during Processing*, Academic Press, San Diego, USA **2014**.
- [11] F. P. Carvalho, *Food Energy Secur.* **2017**, 6, 48.
- [12] H. Benhalima, M. Q. Chaudhry, K. A. Mills, N. R. Price, *J. Stored Prod. Res.* **2004**, 40, 241.
- [13] M. A. G. Pimentel, L. R. D. Faroni, M. D. Batista, F. H. da Silva, *Pesqui. Agropecu. Bras.* **2008**, 43, 1671.
- [14] S. Rajendran, V. Sriranjini, *Stewart Postharvest Rev.* **2008**, 3, 1.
- [15] D. Richard-Molard, *Bulk Storage of Grain*, Academic Press, San Diego, USA **2003**.
- [16] R. Gałęcki, T. Bakuła, M. Wojtacki, K. Żuk-Golaszewska, *J. Stored Prod. Res.* **2019**, 83, 117.
- [17] J. Yao, C. Chen, H. Wu, J. Chang, K. Silver, J. F. Campbell, F. H. Arthur, K. Y. Zhu, *J. Stored Prod. Res.* **2019**, 84, 101524.
- [18] M. Deb, D. Kumar, *Ecotoxicol. Environ. Saf.* **2020**, 189, 109988.
- [19] B. Singh, A. Kaur, *LWT–Food Sci. Technol.* **2018**, 87, 93.
- [20] O. Ramos-Rodríguez, J. F. Campbell, S. B. Ramaswamy, *J. Stored Prod. Res.* **2006**, 42, 241.
- [21] B. Malaikozhundan, J. Vinodhini, *Microb. Pathog.* **2018**, 114, 139.
- [22] C. P. de Bortoli, J. L. Jurat-Fuentes, *Curr. Opin. Insect Sci.* **2019**, 33, 56.
- [23] L. K. W. Wijayarathne, F. H. Arthur, S. Whyard, *J. Stored Prod. Res.* **2018**, 76, 161.
- [24] P. G. Fields, N. D. G. White, *Annu. Rev. Entomol.* **2002**, 47, 331.
- [25] P. Agrafioti, C. G. Athanassiou, B. Subramanyam, *J. Stored Prod. Res.* **2019**, 81, 100.
- [26] J. A. Hardin, C. L. Jones, E. L. Bonjour, R. T. Noyes, R. L. Beeby, D. A. Eltiste, S. Decker, *J. Stored Prod. Res.* **2010**, 46, 149.
- [27] M. W. Byun, H. S. Yook, O. J. Kwon, *J. Food Sci. Technol.* **1997**, 32, 221.
- [28] G. J. Hallman, *J. Stored Prod. Res.* **2013**, 52, 36.
- [29] M. Kostyukovsky, A. Trostanetsky, E. Quinn, *Isr. J. Plant Sci.* **2016**, 63, 7.
- [30] P. Bourke, D. Ziuzina, D. Boehm, P. J. Cullen, K. Keener, *Trends Biotechnol.* **2018**, 36, 615.
- [31] A. Los, D. Ziuzina, S. Akkermans, D. Boehm, P. J. Cullen, J. Van Impe, P. Bourke, *Food Res. Int.* **2018**, 106, 509.

- [32] K. V. Donohue, B. L. Bures, M. A. Bourham, R. M. Roe, *J. Econ. Entomol.* **2006**, *99*, 38.
- [33] M. F. A. El-Aziz, E. A. Mahmoud, G. M. Elaragi, *J. Stored Prod. Res.* **2014**, *59*, 215.
- [34] R. Mahendran, K. Ratish Ramanan, R. Sargunam, R. Sarumathi, *J. Agric. Eng.* **2016**, *3*, 37.
- [35] L. G. Carpen, C. Chireceanu, M. Teodorescu, A. Chiriloaie, A. Teodoru, G. Dinescu, *Rom. J. Phys.* **2019**, *64*, 1.
- [36] K. Ratish Ramanan, R. Sarumathi, R. Mahendran, *J. Stored Prod. Res.* **2018**, *77*, 126.
- [37] A. M. Hassan, T. M. Sileem, R. S. Hassan, *Brazilian J. Biol.* **2019**, *6984*, 1.
- [38] S. K. Pankaj, N. N. Misra, P. J. Cullen, *Innovative Food Sci. Emerging Technol.* **2013**, *19*, 153.
- [39] T. Moiseev, N. N. Misra, S. Patil, P. J. Cullen, P. Bourke, K. M. Keener, J. P. Mosnier, *Plasma Sources Sci. Technol.* **2014**, *23*, 065033.
- [40] V. Milosavljević, P. J. Cullen, *Eur. Phys. J. Appl. Phys.* **2017**, *80*, 20801.
- [41] A. Los, D. Ziuzina, D. Boehm, P. J. Cullen, P. Bourke, *Innovative Food Sci. Emerging Technol.* **2017**, *44*, 36.
- [42] H. Lu, J. Zhou, S. Xiong, S. Zhao, *J. Insect Physiol.* **2010**, *56*, 1356.
- [43] N. Misra, K. M. Keener, P. Bourke, J. Mosnier, P. J. Cullen, *J. Biosci. Bioeng.* **2014**, *118*, 177.
- [44] L. Wang, S. Cui, Z. Liu, Y. Ping, J. Qiu, X. Geng, *PLOS One* **2018**, *13*, 1.
- [45] P. Bourke, D. Ziuzina, L. Han, P. J. Cullen, B. F. Gilmore, *J. Appl. Microbiol.* **2017**, *123*, 308.
- [46] D. Ziuzina, S. Patil, P. J. Cullen, K. M. Keener, P. Bourke, *J. Appl. Microbiol.* **2013**, *114*, 778.
- [47] M. Emekci, S. Navarro, E. Donahaye, M. Rindner, A. Azrieli, *J. Stored Prod. Res.* **2002**, *38*, 413.
- [48] L. C. Perkin, B. Oppert, *PeerJ* **2019**, *7*, e6946.
- [49] S. Missios, H. C. Davidson, D. Linder, L. Mortimer, A. O. Okobi, J. S. Doctor, *Insect Biochem. Mol. Biol.* **2000**, *30*, 47.
- [50] M. Y. Noh, K. J. Kramer, S. Muthukrishnan, R. W. Beeman, M. R. Kanost, Y. Arakane, *Dev. Biol.* **2015**, *399*, 315.
- [51] L. Ten Bosch, B. Habedank, D. Siebert, J. Mrotzek, W. Viol, *Int. J. Environ. Res. Public Health* **2019**, *16*, 1.
- [52] S. I. Faruki, D. R. Das, A. R. Khan, M. Khatun, *J. Insect Sci.* **2007**, *7(36)*, 1.
- [53] M. Ferreira, J. G. L. Gomes, M. S. Benilov, M. Khadem, *Plasma Med.* **2016**, *6*, 115.
- [54] X. X. Wang, Z. S. Chen, Z. J. Feng, J. Y. Zhu, Y. Zhang, T. X. Liu, *Front. Physiol.* **2019**, *10*, 1.
- [55] A. A. İşikber, S. Öztekin, *J. Stored Prod. Res.* **2009**, *45*, 159.
- [56] M. Holmstrup, J. G. Sørensen, L. H. Heckmann, S. Slotsbo, P. Hansen, L. S. Hansen, *J. Stored Prod. Res.* **2011**, *47*, 378.
- [57] A. H. Sousa, L. R. A. Faroni, G. N. Silva, R. N. C. Guedes, *J. Econ. Entomol.* **2013**, *105*, 2187.
- [58] M. Iriti, F. Faoro, *Water, Air, Soil Pollut.* **2008**, *187*, 285.
- [59] B. Lu, Y. Ren, Y. Zhou Du, Y. Fu, J. Gu, *J. Insect Physiol.* **2009**, *55*, 885.
- [60] M. A. G. Pimentel, L. R. D. A. Faroni, M. R. Tótolá, R. N. C. Guedes, *Pest Manage. Sci.* **2007**, *63*, 876.
- [61] M. Mänd, R. Karise, *Open Access Insect Physiol.* **2015**, *5*, 31.
- [62] C. Traba, J. Liang, *Biofouling* **2011**, *27*, 763.
- [63] R. N. C. Guedes, E. E. Oliveira, N. M. P. Guedes, B. Ribeiro, J. E. Serrão, *Physiol. Entomol.* **2006**, *31*, 30.
- [64] A. H. Sousa, L. R. D. A. Faroni, R. N. C. Guedes, M. R. Tótolá, W. I. Urruchi, *J. Stored Prod. Res.* **2008**, *44*, 379.
- [65] G. Lopez-Martinez, J. B. Benoit, J. P. Rinehart, M. A. Elnitsky, R. E. Lee, D. L. Denlinger, *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **2009**, *179*, 481.
- [66] D. Kodrik, A. Bednarova, M. Zamanova, N. Krishnan, *Int. J. Mol. Sci.* **2015**, *16*, 25788.
- [67] D. G. M. George, A. M. R. Gatehouse, *Int. J. Curr. Microbiol. Appl. Sci.* **2013**, *2*, 485.
- [68] C. Li, B. Xu, Y. Wang, Z. Yang, W. Yang, *Entomol. Exp. Appl.* **2014**, *151*, 19.
- [69] S. P. Singh, J. A. Coronella, H. Beneš, B. J. Cochrane, P. Zimniak, *Eur. J. Biochem.* **2001**, *268*, 2912.
- [70] A. Che-Mendoza, R. P. Penilla, D. A. Rodriguez, *African J. Biotechnol.* **2009**, *8*, 1386.
- [71] Z. Alias, *The Role of Glutathione Transferases in the Development of Insecticide Resistance*, Intech Open Science **2016**.
- [72] I. Plavšín, T. Stašková, M. Šerý, V. Smýkal, B. K. Hackenberger, D. Kodrik, *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2015**, *170*, 19.
- [73] M. L. Shonouda, L. M. El-Samad, E. H. Mokhamer, N. Toto, *J. Entomol.* **2016**, *13*, 122.
- [74] A. Los, D. Ziuzina, S. Akkermans, D. Boehm, P. J. Cullen, J. Van Impe, P. Bourke, *Food Res. Int.* **2018**, *106*, 509.
- [75] A. Los, D. Ziuzina, D. Boehm, P. J. Cullen, P. Bourke, *Plasma. Process. Polym.* **2019**, *16*, 1.
- [76] N. Magan, V. Sanchis, D. Aldred, *The Role of Spoilage Fungi in Seed Deterioration*, CRC Press, **2003**.
- [77] B. Wielkopolan, A. Obrepalska-Stepłowska, *Planta* **2016**, *244*, 313.
- [78] A. Noman, M. Aqeel, M. Qasim, I. Haider, Y. Lou, *Sci. Total Environ.* **2020**, *699*, 134181.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: D. Ziuzina, R. van Cleynenbreugel, C. Tersaruolo, P. Bourke. Cold plasma for insect pest control: *Tribolium castaneum* mortality and defense mechanisms in response to treatment. *Plasma Processes Polym.* 2021, e2000178.
<https://doi.org/10.1002/ppap.202000178>