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Assessing the potential of unmanned aerial vehicle spraying of aqueous ozone as an outdoor disinfectant for SARS-CoV-2

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ABSTRACT
The COVID-19 pandemic has revealed gaps in our understanding of safe, effective and efficient means of disinfecting high use public spaces. Whilst this creates an opportunity for development and application of innovative approaches such as unmanned aerial vehicle (UAV) based disinfection, unregulated outdoor disinfection using chlorine has led to environmental and public health risks. This study has quantified the efficiency, safety and efficacy of UAV-based spraying of aqueous ozone. Optimised UAV flight characteristics of 4.7 km/h at 1.7 m elevation spraying 2.4 L/min were able to provide >97% and >92% coverage of a 1 m and 2 m wide swath respectively. During spraying operations using 1 mg/L aqueous ozone, atmospheric concentrations of ozone remained within background levels (<0.04 ppm). Highly efficient inactivation of two different isolates of SARS-CoV-2 virus was achieved at aqueous ozone concentrations of 0.75 mg/L after an incubation period of only 5 min, with 0.375 mg/L achieving 82–91.5% inactivation in this time. Exposure of diamondback moth larvae and parasitic wasps to 1 mg/L aqueous ozone did not significantly affect their survivorship. These results indicate for the first time that aqueous ozone may provide the required balance between human and environmental safety and viral inactivation efficacy for targeted application in high risk outdoor settings.

1. Introduction
Since declared by the World Health Organisation (WHO) on March 11, 2020 as a global health crisis, the COVID-19 pandemic has resulted in >100 million infections and >2 million deaths with the daily number of infections and deaths still remaining very high (https://covid19.who.int). The rapid escalation of the COVID-19 pandemic has in many cases led to ad-hoc large-scale dispersion of chlorine or alcohol-based disinfectants across public areas (Fig. 1). Despite recommendations from the WHO that disinfection outdoors is not required (WHO 2020), widespread spraying of public areas has occurred in numerous locations eg China, Russia, India, Philippines and Iran (Nabi et al., 2020; Service 2020). Recent assessments indicate SARS-CoV-2 can remain active on surfaces for up to 28 days (Riddell et al., 2020). High touch areas of partially covered facilities such as sports stadiums and park/playground equipment have been an area of particular concern in many jurisdictions.

Outdoor disinfection is largely unregulated and often sits between medical therapeutic regulation of indoor disinfection and agricultural chemical regulation for outdoor settings. The urgency with which outdoor disinfection is often applied frequently limits the possibility of even the most basic environmental and public health risk assessments being conducted. Any proposed widespread spraying of outdoor spaces must consider the potential for secondary adverse impacts alongside the
The efficacy of outdoor spraying (SanJuan-Reyes et al., 2021). The direct exposure to spraying of chlorine as disinfectant during Ebola outbreaks in West Africa led to significant respiratory, skin and eye impacts on patients, residents and health workers (Mehtar et al., 2016). Whilst there have been no quantitative studies to date on the secondary impacts of outdoor disinfection spraying in response to SARS-CoV-2, potential impacts on water quality of natural systems from increased use of chlorine based disinfectants have been highlighted (Chu et al., 2020). Anecdotal evidence from Chongqing, in southwest China suggests wildlife mortality occurred as a result of high intensity outdoor disinfectant application (Yingzi, 2020). Attention has also been recently drawn to the environmental risks of broad scale outdoor disinfection (Nabi et al., 2020).

Three principal groups of chemical disinfectants are typically used for virus inactivation; alcohol based (>70%), quaternary ammonium and oxidisers (chlorine, hydrogen peroxide or ozone) (Wigginton et al., 2012; Hora et al., 2020). Despite safety and health concerns (Gorguner et al., 2004; Medina-Ramón et al., 2005) chlorine bleach at 1000–5000 mg/L continues to be the most commonly used disinfectant product due to its low cost, ease of use, deodorising and efficacy across a broad spectrum of microorganisms, including viruses (Kampf et al., 2020). Oxidisers such as ozone and hydrogen peroxide in contrast, offer promise as a safer alternative. Environmental and public health risks are alleviated as these oxidisers rapidly convert to oxygen and water without leaving residual toxicity on disinfected surfaces.

Ozone gas is routinely used as an effective disinfectant in the medical industry. In uncontrolled outdoor environments, the gaseous phase of ozone is neither practical nor safe due to potential for exposure of operators to harmful concentrations of ozone (>0.1 ppm). Ozone can be dissolved in water to form ozonated water, or ‘aqueous ozone’, which can deliver a liquid form of ozone to surfaces as a disinfectant. The aqueous form of ozone has been utilised as a disinfectant in wastewater treatment (Von Sonntag and Von Gunten 2012), food and livestock industries (Aslam et al., 2019), and as a commercial disinfectant (Martinelli et al., 2017). Studies have also demonstrated the effectiveness of aqueous ozone as a hand sanitiser in hospital settings, with improved efficacy and lower secondary impacts (eg skin irritation) compared to traditional alcohol-based sanitisers (Breidablik et al., 2019). Aqueous ozone can effectively inactivate viruses through disrupting the proteins and lipids of the virus spikes (Tizaoui, 2020). However, the instability of aqueous forms of ozone (20 min half-life) has limited the widespread uptake as a commercial disinfectant. Recent advancements in understanding aqueous ozone chemistry (Eriksson 2005) has led to commercially available ozone generators producing a stabilised form of aqueous ozone, increasing its stability to several hours.

The widespread disruption from the COVID-19 pandemic has driven innovation in the utilisation of autonomy, robotics and artificial intelligence across a range of settings (Zeng et al., 2020). Numerous jurisdictions globally have re-purposed agricultural spraying unmanned aerial vehicles (UAVs) to support disinfection efforts in response to COVID-19. To date, this effort has largely been reactionary with little pre-planning, testing or assessment of efficacy. A range of disinfectant solutions (chlorine (1000–5000 ppm), alcohol, hydrogen peroxide) have been utilised alongside early trials of high intensity UV light for indoor applications. However, the lack of systematic testing coupled with significant concerns around public and environmental safety of widespread chlorine bleach spraying, has limited the effectiveness and continuation of these UAV disinfection programs.

Using conventional disinfection delivery systems in outdoor areas with complex structures is a major challenge due to health and safety risks to applicators, as well as the logistics of moving large volumes of liquid in confined areas. In addition, where rapid application of disinfectant is required (eg between sporting or entertainment events), this presents a challenge to current manual delivery systems. Furthermore, the scaling of adequate and rapid disinfectant coverage using existing manual delivery systems requires greatly increased person hours. The advent of aerial spraying using UAVs represents a feasible approach to overcome these challenges, as a single system is able to provide rapid coverage within complex outdoor structures.

Here we present the first quantitative assessment of the potential of utilising unmanned aerial vehicles to disinfect complex outdoor environments, demonstrating the efficacy of the aqueous form of ozone to inactivate SARS-CoV-2 and show its environmental safety in two experimental insect models.

2. Materials and methods

This study assessed three core areas; efficiency of spray coverage, environmental and operator safety, and efficacy in inactivation of SARS-CoV-2 using aqueous ozone.

2.1. Spray coverage

A critical component to ensure both effective and efficient UAV based spraying of disinfectant is to assess coverage and drift of spray under different flight conditions. Test strips of 6 m × 5 m 160 gsm geofabric (Grunt Non-Woven Geotextile Membrane, Preston, Australia) were used to quantify both coverage and density of spray from the UAV (DJI Agras MG-1, Shenzhen, China). Two hundred mL of red food dye (Pillar Box Red Food Colour, Queen, Brisbane, Australia) was added to the 10 L spray tank of water and trials were conducted using flight speeds of 4.7 km/h and 5.8 km/h. Spray heights of 1, 1.7 and 2 m were tested as well as a combination of coarse or mist nozzles either mounted below the motors or on a rigid boom. Following each spray trial a high resolution aerial image was taken from 10 m elevation using a UAV (DJI Mavic 2 Pro, Shenzhen, China). These high resolution images with 3 mm pixel size were georectified (ArcGIS 10.5, ESRI) prior to further analysis.

Linear Spectral Unmixing, a sub-pixel classification method was used

![Image](https://via.placeholder.com/150)

Fig. 1. Disinfectant spraying of public spaces in response to COVID-19 outbreaks in: (A) Kolkata, India and (B) Moscow, Russia (Source: Shutterstock).
to calculate the intensity of the spray. The process offers superior results where the feature size is relatively smaller than the pixel size and with limited spectral bands (Kamal and Phinn 2011). As a result of spectral unmixing, fractional cover maps were generated and a rule-based feature extraction model was applied to identify the concentration of the red dye in each pixel. The fractional images provide endmember-based data with pixel values ranging between 0 and 1. Pixels with complete coverage of red dye had high pixel value, whereas the areas with less or no spray will provide values close to zero. The areas were then divided into the four distinctive spread classes of high, moderate, low and no spread (Fig. 2).

2.2. Ozone generation and stability

The stability of dissolved ozone in four solutions were assessed: Brisbane municipal tap water pH = 7.4; Brisbane municipal tap water filtered through a cation exchange resin (Tersano SAO-24, referred here as Municipal-Tersano) pH = 3.2; deionised water pH = 7.1; and deionised water filtered through a cation exchange resin (Tersano SAO-24, referred here as Deionised-Tersano), pH = 3.1. Five liters of each solution was ozonated for 10 min by recirculating through an industrial ozone generator (Grenof, Brisbane, Australia). The ozone generator uses corona discharge to produce 5 g/h of ozone which is introduced into the water flow via a venturi injector nozzle. Dissolved ozone concentrations were monitored using a Cronos Ozosense Analyser (Process Instruments, Burnley, UK) every 10 min for the first 4 h and every 30 min for the following 4 h.

2.3. Virus inactivation assays

Cell line and virus isolates: Vero E6, African green monkey kidney cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and maintained at 37°C with 5% CO₂. Two different isolates of SARS-CoV-2, QLD02 (GISAID accession EPI_ISL_407,896) and QLD935 (GISAID accession EPI_ISL_436,097) were obtained from the Queensland Department of Health as passage 2 in Vero E6 cells and passaged once more in Vero E6 cells to generate virus stocks. The virus titres were then determined by immuno-plaque assay (IPA) as described below.

Virucidal activity assay: viral isolates, each with an estimated

![Fig. 2. Spatial coverage assessment of UAV based spraying across 5 m × 6 m test strips. Flight height (h) and speeds (v) of different spatial coverage assessments were recorded. A) h = 2 m, v = 5.8 km/h, and 4 sprinkler nozzles, B) h = 2 m, v = 4.7 km/h, and 4 mist nozzles, C) h = 1 m, v = 4.7 km/h, and 4 coarse nozzles, D) h = 1.7 m, v = 4.7 km/h, and 2 mist + 2 coarse nozzles, E) h = 1.7 m, v = 4.7 km/h, and 4 offset mist nozzles, F) h = 1.7 m, v = 4.7 km/h, and 4 on boom coarse nozzles, G) h = 1.7 m, v = 4.7 km/h, and 4 on boom mist nozzles, H) example of UAV spraying red food dye on geofabric during coverage trials. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image-url)
1–5x10^5 foci forming units (FFU)/mL, were prepared in 1.8% NaCl solution in deionised and filtered water (pH = 6.0). 100 μL of each viral isolate (estimated 1–5x10^4 FFU) were then mixed with equal volumes of different concentrations of aqueous ozone solutions (3 mg/L, 1.5 mg/L, and 0.75 mg/L) to yield final concentrations of 1.5 mg/L, 0.75 mg/L and 0.375 mg/L. Aqueous ozone solutions used for viral assays were generated using Municipal-Tersano water as described above and ozone concentrations monitored (Cronos Ozonese Analyser) in control solutions without virus for the duration of incubations. The mixtures were incubated at room temperature for 5 min or 30 min. After incubation, 20 μL was transferred to 180 μL of DMEM with 2% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin (DMEM-2%, PBS-P/S), mixed and 25 μL of this virus solution was then added to a well of confluent monolayer of Vero E6 cells in 96 well plates for infection. Approximately 4 x 10^5 Vero E6 cells per well were seeded in 96-well plates a day prior viricidal assay. Cells were then incubated with the virus for 30 min at 37°C and 175 μL of overlay media (M199, 5% FBS, 1% carboxymethyl cellulose, 100 U/mL penicillin, 100 μg/mL streptomycin) was then added. Infected cells were incubated in overlay media for 14 h at 37°C and 5% CO2. Overlay media was then removed and cells were fixed with 80% acetone solution for 30 min at ~20°C.

Immunoplaque assay (IPA) staining: Viral foci in fixed infected cells were detected by the cross-reacting recombinant mouse monoclonal antibody CR3022 against SARS-CoV Spike protein (ter Meulen et al., 2006) as described (Amarilla et al., 2021). Briefly, fixed cells were blocked for 1 h at 37°C by adding 150 μL of blocking solution (Pierce™ Clear Milk Blocking buffer, Thermo Scientific, USA). Concentrated blocking solution was diluted in phosphate-buffered solution (pH = 7.4) containing 0.05% Tween 20 (PBS-T). Subsequently, blocking solution was removed and 50 μg/well of primary antibody (CR3022) in blocking solution was added to each well and incubated for 1 h at 37°C. Cells were then washed 3–5 times with PBS-T, and 20 ng/well of conjugated secondary antibody (IRDye, LI-COR, USA) in blocking solution was added and incubated for 1 h at 37°C. Cells were then washed 3–5 times with PBS-T, dried and scanned using the LI-COR Biosciences Odyssey Infrared Imaging System (Odyssey CLx, LI-COR, USA). Foci were visualised by Image Studio Lite software, counted, and viral titres were calculated based on dilution factor and expressed as FFU/mL (Log10).

2.4. Environmental and operator safety

Operator safety is a key consideration for any disinfection operation, therefore a field test was undertaken to examine atmospheric ozone levels during UAV spraying operations. The Australian occupational limit for atmospheric ozone over an 8 h period is 0.1 ppm and it is important to ensure pilots as well as support crew are not exposed to atmospheric ozone levels above this limit during aerial spraying operations. An atmospheric ozone logger (Aeroqual Inc., Series 500 – Portable Ozone Monitor) was placed 20 cm above ground level directly in the spray zone to capture the likely maximum atmospheric ozone levels during spray operations. Spray tests using 1.5 mg/L aqueous ozone were undertaken on May 20, 2020 at a spray height 2 m above the ground with a spray duration of 2 min to maximise local atmospheric ozone levels.

2.5. Insect assays

A laboratory population of diamondback moth (Plutella xylostella L. (Lepidoptera: Plutellidae) (Silva and Furlong, 2012) was used. Ported Brassica oleracea cv Sugarloaf plants (7-leaf stage) were infested with 10 early third instar larvae which were allowed 2 h to establish feeding sites prior to experiments. Single larvae-infested plants were then placed into clean, mesh cages (25 x 25 x 25 cm) and treated with one of the following aqueous solutions, aqueous ozone (1 mg/L), NaOCl bleach (4000 ppm), hydrogen peroxide (0.25%) (Oxivir Five 16, Diversey, Sydney, Australia), deltamethrin (10 ppm), or a water control. Concentrations of hydrogen peroxide and deltamethrin were based on manufacturer recommendations, 1 mg/L ozone was used based on typical concentration generated by commercial ozone generators (eg Tersano Lotus Pro and Grenof), 4000 ppm of NaClO bleach was utilised as within the range of 2000–5000 ppm utilised by UV disinfection operations elsewhere. All solutions were applied using a hand-held sprayer that dispensed 0.2 mL of liquid per depression of the spray trigger. Each plant was treated with 5 sprays of the test solution (a total of 1 mL) and care was taken to cover all leaf surfaces. Each treatment was replicated 4 times. Plants and larvae were left in cages for 2 h before all surviving larvae from each plant were carefully transferred to single labelled Petri dishes (9 cm diameter) together with a treated leaf from the plant. Petri dishes were incubated (25 ± 1°C; 70–80% RH; 12:12 L: D) for 72 h when survivorship was assessed.

Pupae of the diamondback moth parasitoid Diadegma semiclausum Hellén (Hymenoptera: Ichneumonidae) were supplied by Biological Services (Loxton, SA, Australia) and placed in nylon mesh rearing cages (45 x 45 x 45 cm) for eclosion. Upon emergence adult insects were fed on 10% (w/v) honey solution and then 10 males were transferred to a clean, mesh cage (25 x 25 x 25 cm) which was positioned in front of a window so that insects rested on the rear wall. 1 mL of test solution was applied to insects resting on the back wall of the cage from 5 sprays of the hand-held sprayer as described above. Each treatment was replicated 4 times. After treatment, parasitoids remained in the cages which were then placed on the laboratory bench under ambient conditions (20–25°C; natural light); mortality was assessed 24 h later.

2.6. Statistical analyses

For the viricidal activity assay, comparison between groups were performed using Mann-Whitney test. For the diamondback moth and D. semiclausum experiments, survival probability (SP) for each replicate calculated as: \( SP = \frac{\text{number of surviving individuals}}{\text{number of surviving individuals} \times \text{number of individuals missing}} \). Data were subject to one-way ANOVA and treatment means were compared by Tukey multiple comparison tests. All statistical tests were performed using GraphPad Prism V 8.4.3 (San Diego California USA, www.graphpad.com).

3. Results

3.1. Spray coverage

Analyses of high-resolution images of the coverage effectiveness revealed that higher flight speeds of 5.8 km/h with sprinkler nozzles only achieved 15.8% coverage in the central 1 m swath below UAV (Fig. 2A). A slower flight speed of 4.7 km/h and intermediate elevation of 1.7 m delivered the best combination of coverage and density (Fig. 2D–G). Using these flight characteristics with four misting nozzles or four coarse nozzles on a fixed boom achieved 99.9% coverage of the central 1 m swath (Fig. 2F and G). The coverage effectiveness was gradually decreased from 94% to 83% for the central swath of 2 and 3 m respectively (Fig. 2F and G). A significant coverage decline was observed with 67–73% across the central 4 m swath (Fig. 2F and G). This demonstrated the coverage effectiveness is highly variable depending on height, speed and nozzle type.

3.2. Ozone stability

Ozone is highly unstable in the dissolved phase in water, which is dependent on pH, temperature and presence of metal ions and other impurities (Eriksson 2005). We assessed the stability of dissolved ozone in four different preparations of water (Brisbane municipal tap water, Brisbane municipal tap water with Tersano filtering, deionised water, and deionised water with Tersano filtering). The solubility of ozone in municipal water was the lowest of the four solutions assessed, with 3.5
mg/L reached after 10 min of ozonation (Fig. 3). The stability of ozone in municipal water was substantially lower than in other solutions, with a half-life of 8 min and reaching <0.3 mg/L after 20 min. Deionised water, Municipal-Tersano, and Deionised-Tersano all had higher stability with half-lives of 120 min, 150 min and 165 min respectively (Fig. 3). Aqueous ozone solutions prepared in Municipal-Tersano water were selected for viral and insect assay treatments due to limited availability of deionised water for future commercial applications. Degassed controls used in viral and insect assays were left for 24 h for ozone to reduce <0.01 mg/L.

### 3.3. Viral inactivation efficacy

The current pandemic of SARS-CoV-2 provides an ideal example to use aqueous ozone as a disinfectant. Two clinical isolates of SARS-CoV-2, an early Australian isolate QLD02 sampled from a patient on January 30, 2020, and the more recent isolate QLD935 sampled from a patient on March 25, 2020 were tested here for inactivation by aqueous ozone. QLD935 isolate represents the currently dominating virus isolate with a characteristic D614G mutation in a Spike protein which potentially increases virus infectivity (Korber et al., 2020). Both viral isolates were efficiently inactivated by as little as 5 min incubation with 1.5 mg/L and 0.75 mg/L of aqueous ozone with more than 1.7 log<sub>10</sub> reduction (Fig. 4A). Thirty minutes incubation produced similar results for these ozone concentrations with complete inactivation of both virus isolates (Fig. 4B). In fact, no virus was detected at all in treatments with these ozone concentrations with the limit of detection being 2.9 log<sub>10</sub> FFU/ml (400 FFU/ml). Further reduction in ozone concentration to 0.375 mg/L decreased efficiency of inactivation (Fig. 4), however, we still observed 91.5% (1.07 log<sub>10</sub>) inactivation for QLD02 isolate and 82% (0.74 log<sub>10</sub>) for QLD935 virus isolate after 5 min incubation (Fig. 4A), or 92% (1.09 log<sub>10</sub>) inactivation for QLD02 virus isolate and 84.5% (0.81 log<sub>10</sub>) inactivation for QLD935 virus isolate after 30 min incubation (Fig. 4B).

### 3.4. Operator safety

The safety of UAV operators was assessed by comparing background ozone levels to in-situ ozone sensors within spraying operation. Ambient ozone levels at the nearby (less than 7 km) Southport air quality monitoring station (https://apps.des.qld.gov.au/air-quality/) ranged between 0.002 and 0.04 ppm over the duration of the sampling month (Fig. 5A). Local measurements from the spray area showed background levels to be 0.015 ppm immediately prior to testing (Fig. 5B). The highest reading recorded during spraying of stabilised aqueous ozone was 0.026 ppm within the spray zone (Fig. 5B). Monitoring of atmospheric ozone levels 5 m downwind from the spray zone showed concentrations similar to background levels of 0.015 ppm. Based on these initial findings, the atmospheric exposure to ozone during spraying is unlikely to be a health concern for operators as the observed levels all lay within the range for background ambient levels.

### 3.5. Environmental safety

Environmental safety is an important consideration for disinfection operations. We evaluated the potential effect of aqueous ozone and other disinfectants on the survivorship of larvae of the worldwide crucifer crop pest, the diamondback moth, and adults of its parasitoid, D. semicalassum, which has been widely introduced for biological control of the pest (Furlong et al., 2013). Parasitoids are typically far more sensitive to insecticides and other xenobiotic chemicals than their insect hosts (Devine and Furlong 2007; Kim et al., 2019) and as such their responses can serve as important bio-indicators of xenobiotic compounds in the environment (Furlong et al., 2004). Bleach and hydrogen peroxide significantly affected the survival of diamondback moth larvae (Fig. 6A: F<sub>4,15</sub> = 10.5, P < 0.01, respectively) but aqueous ozone and the insecticide deltamethrin did not (P > 0.05) (Fig. 6A). Similarly, application of bleach (P < 0.01), hydrogen peroxide (P < 0.01), and deltamethrin (P < 0.01) significantly affected the survival of adult D. semicalassum (Fig. 6B, F<sub>4,15</sub> = 18.3, P < 0.001) while aqueous ozone did not (P > 0.05).

### 4. Discussion

Disinfection in healthcare settings is a critical component to infection control, supported by a range of standard procedures and PPE ensuring minimal risk of transmission. In contrast, disinfection procedures in outdoor settings and public places are not standardised. COVID-19 pandemic has seen disinfection chemicals applied in unprecedented volumes and in poorly understood or regulated settings such as outdoors and in public areas. This has led to unintended consequences on the environment in some instances (Nabi et al., 2020), and a placebo effect or “disinfection theatre” in others (Lowe 2020).

As the pandemic transitions to local community transmission, developing effective yet safe disinfection protocols will be critical to enable large scale outdoor events to proceed safely. Examples from Italy have demonstrated that high density community events, such as sporting matches, can lead to rapid escalation of community transmission and are a critical gap in knowledge (Sassano et al., 2020). The complexity and scale of surfaces in sporting stadiums and the short turn around between events limits the potential of targeted manual disinfection of high touch surfaces. Recent work demonstrating SARS-CoV-2 may remain active on surfaces for up to 28 days highlights the need to investigate new approaches for disinfection (Riddell et al., 2020). Aerial spraying of traditional disinfectants (eg chlorine bleach) introduces too many environmental and human health risks to make it a viable option in reducing COVID-19 transmission risk. The potential of ultraviolet (UV) radiation has recently been demonstrated as a cost-effective sterilizing method for SARS-CoV-2 (Bianco et al., 2020), however the integration of high energy UV systems on aerial platforms is less developed than spraying systems. Aqueous ozone may provide the ideal balance between efficacy, cost effectiveness and safety to be utilised at scale for disinfection control of large community gatherings. Recent studies showed effectiveness of ozone spraying in the inactivation of aerosolized airborne viruses (Dubuis et al., 2020) indicating that in addition to intended inactivation of SARS-CoV-2 contaminated surfaces aqueous ozone spraying may also aid in inactivating SARS-CoV-2 potentially remaining in the air after large community gatherings such as sporting events.

Although aqueous ozone has been utilised in limited health care
settings, uptake across the cleaning and disinfection industry has been limited by a number of factors including stability of ozone in solution, presence of ozone-consuming compounds such as organic substances, and of by-products from ozone action on organic compounds (Khadre et al., 2001). In our experiments, deionised and filtered water showed the best performance in providing extended ozone solubility and stability. The highest antiviral activity also required virus dilution in NaCl solution prepared in deionised and filtered water. Virus preparations in other solutions, such as complete cell culture media with or without foetal bovine serum were inactivated less efficiently (data not shown). Whether these factors could potentially reduce the efficacy of SARS-CoV-2 inactivation by aqueous ozone spraying in the settings of large community gatherings remains to be determined. Field trials of aqueous ozone spraying in large stadiums using model microbial or- ganisms as the readout will provide highly valuable data to further assess the potential of aqueous ozone as an effective disinfectant.

Background levels of exposure to high atmospheric ozone and particulate matter concentrations have been linked to increased transmission and severity of COVID-19 (Fattorini and Regoli, 2020; Zhu et al., 2020; Bontempi, 2020a, 2020b). The low atmospheric concentrations of ozone released during outdoor spraying indicates these broader relationships between ozone pollution and transmission and severity of COVID-19 will not be influenced by disinfection spraying. Despite the human health risks associated with ozone exposure (Ito et al., 2005; Wang et al., 2020), our field trials suggest aqueous ozone sprayed in outdoor settings maintains atmospheric concentrations below regulatory levels, thus ensuring operator safety.

5. Conclusions

For the first time, we have demonstrated aqueous ozone at 0.75 mg/L is highly effective at inactivation of SARS-CoV-2 virus after a 5 min incubation, with 0.375 mg/L achieving 82–91.5% inactivation in this time. Although effective at inactivating the virus, aqueous ozone did not affect survivorship of two experimental insect models. Through optimizing UAV flight and spray nozzle characteristics, >97% coverage of outdoor surfaces can be achieved. Whilst challenges remain to ensure effectiveness of aqueous ozone under a range of environmental settings to achieve regulatory approvals, this work has clearly demonstrated the potential of new approaches to disinfection of public spaces for management of COVID-19. However, it is critical to recognise that disinfection approaches are only one tool in the effective virus transmission
Fig. 5. A) Atmospheric ozone concentrations from Southport monitoring station during May 2020. B) Atmospheric ozone concentrations at both Southport and in-situ logging during spraying operations on May 20, 2020. Southport station data retrieved from QLD Government air quality monitoring database. Field logger data collected in-situ during UAV spraying operation using Aeroqual Inc., Series S500 – Portable Ozone Monitor.

Fig. 6. (A) Survival (72 h) of diamondback moth larvae and (B) Survival (24 h) of Diadegma semiclausum males after spraying with water, aqueous ozone (1 mg/L), bleach (4000 ppm), hydrogen peroxide (0.25%) and insecticide (deltamethrin, 10 ppm). Bars indicate standard error, and asterisks above bars indicate statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001).
management of large public gatherings, and public behavioral change as well as security/volunteer staff training are important contributors to this management process (Ludvigsen and Hayton 2020).

Author contributions

BT initially conceived the concept. SA, AG, AAA, SHW, MMH, PRY, MJF and AAK designed the study and co-wrote MS. ND and NH assisted with ozone generation and co-wrote MS. AAA, DJJS, YXS and NM performed the experiments, AAA, SHW, DJJS, DW, AAN and AN analysed and compiled the data. SHW, MMH and MJF designed and conducted tests on insects.

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.

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