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Reduction of the attachment, survival and growth of *L. monocytogenes* on lettuce leaves by UV-C stress

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ABSTRACT

Mild stress of leafy greens by UV-C radiation has been reported to stimulate plant defences capable of reducing pathogens on produce surfaces. In this study, the attachment, survival and growth of *Listeria monocytogenes* was investigated on lettuces stressed with mild UV-C radiation (1.3 and 2.6 kJm^{-2}). Attachment of *L. monocytogenes* to UV-C stressed (1.3 kJm^{-2}) lettuce leaves after 1 h was significantly ($p < 0.05$) reduced by 1.4–1.5 log cfu/cm^2. UV-C stress also reduced the survival of *L. monocytogenes* on lettuce by 1.8–1.9 log cfu/g 96 h after inoculation, however a higher dosage of UV-C stress (2.6 kJm^{-2}) did not inhibit the survival of *L. monocytogenes*. The total phenolic compounds in lettuce significantly increased following UV-C stress (1.3 kJm^{-2}) indicating the accumulation of polyphenols might have contributed to the inhibition of *L. monocytogenes* attachment and growth. Appropriate dosage of mild UV-C stress of lettuce can reduce the attachment, survival and growth of *L. monocytogenes* in lettuce and can therefore be explored further for application in fresh produce safety.

1. Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive bacterium which has been implicated in many fresh produce related outbreaks of food borne illness (e.g. Self et al., 2019). Fresh produce related outbreaks of foodborne illness as well as recalls due to pathogenic bacteria such as *L. monocytogenes* continue to be a concern in food safety (New Zealand Food Safety, 2020). There have been nine major fresh produce recalls associated with *L. monocytogenes* (from January to August 2020) in the US (United States Food and Drug Administration, 2020). Within the same time period, four major recalls of fresh produce due to *L. monocytogenes* were reported in New Zealand (NZ) (New Zealand Food Safety, 2020). In our previous studies, we demonstrated the attachment of *L. monocytogenes* to lettuce surfaces as well as lettuce juice supporting the biofilm formation of *L. monocytogenes* on stainless steel coupons (Kyere et al., 2019, 2020a). In addition, we recently found 7% of bagged lettuces sold in supermarkets in a major city in NZ to be contaminated with *Listeria* (Kyere, Qiu, et al., 2020). These recalls as well as contamination of lettuces with *Listeria* in retail outlets supports the need for research to investigate innovative control methods capable of reducing the risk from pathogens in fresh produce. Novel control methods are important in the produce industry since only non-thermal processing techniques can be used to sanitize fresh produce (Jung et al., 2014).

UV radiation (UV-A; 315–400 nm, UV-B; 280–315 nm and UV-C; 200–280 nm) has been used to inactivate microorganisms in the food industry, particularly in liquid foods and juices (Gómez-López et al., 2012). However, the application of UV-A and UV-B requires long exposure of food products to radiation lasting several days to weeks (Aarrouf & Urban, 2020). Hence the use of an appropriate dosage of UV-C with high energy potential for a relatively short period of time has been explored (Koutchma et al., 2016). In recent years, several researchers have investigated the bacteriostatic effect of UV-C radiation in fresh produce (Aarrouf & Urban, 2020; Collazo et al., 2019; Srey et al., 2014). UV-C treatment (390 mJ/cm^2) reduce pre-existing biofilm of *L. monocytogenes* on lettuce leaves by more than 3 log colony forming units (cfu)/cm^2 (Srey et al., 2014). Similarly, UV treatment (30 mW/cm^2) reduced *Salmonella enterica* on lettuce by 1.98 log cfu/cm^2 (Lippman

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et al., 2020). However, direct application of UV radiation used to reduce pathogens already growing on leaf surfaces has sometimes been found to have a damaging effect on leaf quality (Tellez et al., 2016) and can affect the sensory quality of leaves (Tomás-Callejas et al., 2012). An alternative approach that minimises the damaging effect of UV radiation on leaves is mild UV stress of the leaf to generate an antimicrobial response in the plant (Urban et al., 2018). There have been several studies using UV-C stress to stimulate plant defence (Martínez-Sánchez et al., 2019; Urban et al., 2018; Vàsquez et al., 2017). In response to UV-C stress, plants produce phytochemicals and other phenolic compounds as a protective mechanism (Gamage et al., 2014). Some of these phytochemicals such as ascorbic acid and gallic acid found in leafy greens have antibacterial properties (Kang et al., 2018; Zudina et al., 2018). Other phytochemicals are beneficial to leafy greens by preventing diseases, strengthening plant structures such as the cell wall, fortifying plants to withstand unfavourable climatic conditions and increasing shelf life of produce (Urban et al., 2016).

Lettuce stressed with a UV-C radiation dose of 0.85 kJ m^{-2} increased the resistance of lettuce to *Botrytis cinerea* L. (grey mould), a fungal pathogen (Vàsquez et al., 2017). UV-C stress (1.5 kJ m^{-2}) reduced mesophilic bacterial growth on spinach by 0.5–2 log cfu/g (Martínez-Sánchez et al., 2019). UV-C stress (2.6 and 5.2 kJ m^{-2}) increased some phytochemicals in broccoli such as sulforaphane nitrile which were capable of inhibiting *L. monocytogenes* growth (Gamage, 2015). These reports suggest the potential for UV stress to be used in controlling pathogenic bacteria on leafy greens. However, no studies have specifically investigated the effects of UV-C stress of lettuce on *L. monocytogenes*.

This is the first report of the effect of UV-C stress of pre-harvest lettuce on *L. monocytogenes* attachment, survival and growth. We also show the importance of optimising the dose to avoid damage to the leaf while generating an antimicrobial stress response.

2. Materials and methods

2.1. Preparation of lettuce

Lettuce plants were grown in the Plant Growth Unit of Massey University, Palmerston North, New Zealand. Buttercrunch lettuce seeds (*Lactuca sativa* L. *var. capitata*) were grown under the same conditions as described previously (Kyere et al., 2019). Lettuce plants were grown for four weeks in a greenhouse at an average temperature of 20 °C. The soil-grown lettuce seeds were sown in potting mix in thin rows at a depth of 5 mm with a distance of 35 cm between each row (Sanguandeekul, 1999).

2.2. UV-C treatment

UV-C treatment was carried out with the UV equipment previously described by Gamage et al. (2014). The UV equipment consists of four germicidal UV-C lamps (Philips ultraviolet TUV 30W/G30T8, Holland) with an output of 253.7 nm. Whole lettuce plants rooted in pots of potting mix were placed under the UV lamps at a distance of 50 cm from the lamps. This was measured with a tape measure (Jobmate, 8 m × 25 mm, New Zealand). Plastic racks were used to support lettuce samples to maintain the desired distance when it was necessary. The UV light intensity was measured with an ILT1400 radiometer photometer (International Light Technologies, USA). The duration of UV radiation exposure to the lettuce leaves of two and 5 min corresponded to doses of 1.3 kJm^{-2} and 2.6 kJm^{-2} respectively. The UV-C lamps were turned on for at least 30 min before exposure to lettuce leaves and briefly turned off while putting leaves in the chamber. All lettuce plants were stressed with UV after sunset. Non-UV stressed plants were used as controls. Three independent experiments with three lettuce plants each were conducted. Scanning electron microscopy (SEM) images of both lettuce types (UV stress and non-UV stress) were taken after UV treatment to check if the UV dose used had any negative effect on leaf surface structures. After UV stress, all plants were kept in the greenhouse with the same growth conditions described above.

2.3. Bacterial strains and inoculum preparation

The inoculum was prepared as previously described by Kyere, Foong, et al., 2020, p. 109114. *L. monocytogenes* O8A07 and O8A08 (cabbage isolates) from the New Zealand Institute for Plant and Food Research Limited (PFR) culture collection were maintained at −80 °C on protective beads with Brain Heart Infusion (BHI) Broth (Becton, Dickinson & Company, Le Pont de Claix, France) and 20% (v/v) glycerol. Frozen culture was first transferred to 9 ml BHI broth at 30 °C for 12–14 h with agitation at 120 rpm (Gallery Orbitron Shaker, INFORS HT, Germany) and then 1 μL sub-cultured in BHI broth (9 ml) for an additional 12–14 h at 30 °C before use.

L. monocytogenes isolates were centrifuged at 4400 g for 10 min at room temperature (Eppendorf Centrifuge 5702, Hamburg, Germany). Phosphate Buffered Saline (PBS, Code OPM343, Fort Richard Laboratories, Auckland) was used to wash the cell pellet and then resuspended in 0.1% sterile Buffered Peptone Water (BPW, GranuCult™, Merck KGaA, Billerica MA, USA). Serial dilutions were made in BPW to obtain a final cell count of approximately 10^5 cfu/ml which was used as the initial inoculum.

2.4. Inoculation of lettuce for attachment

Lettuce leaves were collected 24 h after UV stress. The attachment assay used previously (Kyere et al., 2019) was followed. Leaves were cut into 2 cm by 2 cm squared with a sterile cutter. Leaves were rinsed in sterile distilled water and placed under a laminar flow cabinet for 30 min to dry. The 2 cm by 2 cm leaves were inoculated with 500 μL inoculum to cover the whole surface. The *L. monocytogenes* inoculum was left on the leaf surface for 5 min, 30 min and 60 min for attachment. After the attachment exposure times, lettuce leaves were washed with 250 ml sterile distilled water with an approximate flow rate of 0.05 L/s for 1 min to remove unattached cells. SEM images of *L. monocytogenes* attached to both UV and non-UV stressed lettuce were taken after every attachment exposure time.

2.5. Inoculation of lettuce for survival and growth studies

Lettuce leaves were collected for *L. monocytogenes* survival and growth studies 24 h after UV stress. Lettuce leaves were sprayed with 5 log cfu/ml *L. monocytogenes* inoculum. For spray inoculation, a 500 ml LabServ sprayer (Thermo Fisher Scientific, New Zealand) was used three times to spray lettuce leaves in a horizontal position 6 cm above the base in a biosafety cabinet. Initial investigations in our laboratory revealed that approximately 1.5 ml of inoculum was applied to each lettuce plant by spraying three times for 2 s. Leaves were allowed to dry in a laminar flow cabinet for 60 min. After drying, 10 g of lettuce leaves from plants growing in five different pots were tested for the number of initial *Listeria* inoculum attached. Both control (non-UV) and UV stressed lettuce leaves with the *Listeria* inoculum were transferred to a 90% relative humidity chamber at 20 °C. *L. monocytogenes* survival and growth on lettuce plants were enumerated after every 24 h for four days to maintain the quality of lettuce leaves.

2.6. Enumeration of L. monocytogenes to monitor attachment, survival and growth on lettuce leaf surfaces

Enumeration for *L. monocytogenes* attachment followed the procedure described previously (Kyere et al., 2019). Leaf squares (2 cm by 2 cm) were aseptically placed into 9 ml 0.1% sterile peptone water filled with 10 g of glass beads. *L. monocytogenes* cells were released from lettuce leaf surfaces, mixing by vortex for 2 min. Serial dilutions were

plated on Palcam agar (Code 1440, Fort Richard Laboratories, Auckland) and plates were incubated for 48 h at 30 °C.

For the enumeration of *L. monocytogenes* survival and growth on lettuce leaves, the method described previously (Kyere, Qiu, et al., 2020) was used. 10 g of lettuce from each pot were homogenised with 90 ml of 0.1% peptone water (GranuCult®, Merck, KGaA, Germany) using a Stomacher Lab Blender (AES-Chemunix) for 120 s at a speed of 250 rpm. Serial dilutions of the homogenates were plated on Palcam agar. Plates were incubated for 48 h at 30 °C.

2.7. Scanning electron microscopy

The attachment of *L. monocytogenes* on UV stressed and non-UV stressed lettuce leaves was confirmed by SEM. SEM images of both UV stressed and non-UV stressed lettuce leaves were observed before they were inoculated with *L. monocytogenes*. The attachment of *L. monocytogenes* on UV stressed and non-UV stressed lettuce was also observed after every exposure time. Lettuce leaves were sent for imaging after each time culture period. Lettuce leaves were prepared for SEM as described previously (Kyere, Foong, et al., 2020, p. 109114), and viewed under the FEI Quanta 200 Environmental scanning electron microscope (FEI Company, Hillsboro, OR) at 20 kV.

2.8. Determination of total phenolic compounds

Total phenolic content was determined using the modified Folin-Ciocalteu reagent method (Oh et al., 2009). 10 g of fresh lettuce leaves were homogenised in 60 ml 80% (v/v) acetone with a homogeniser (Breville CG2B, Australia) for 2 min. The homogenate was incubated in darkness at 4 °C overnight. It was then centrifuged at 3000 g for 2 min with an Eppendorf centrifuge (5702 Eppendorf AG Hamburg, Germany). 270 μL H_2O, 1.5 ml 1/10 dilution Folin-Ciocalteu reagent (Sigma-Aldrich, USA) and 1.2 ml 7.5% (w/v) Na_2CO_3 was added to 100 μL of the homogenate. The homogenate was mixed by vortex for 30 s and incubated in a water bath at 45 °C for 15 min. Absorbance was read at 760 nm and total phenols were quantified using a freshly made gallic acid standard curve. Each sample (UV stressed and non-UV stressed lettuce leaves) was tested in triplicate.

2.9. Statistical analysis

All experiments were done with three biological repeats with each composing of three technical repeats. An analysis of variance (ANOVA) was performed at a significance level of $p < 0.05$ using Minitab Statistical Software (Minitab version 17, State College, Pennsylvania, USA) and Tukey's test was applied to determine significant differences. The numbers of *L. monocytogenes* cells attached to the leaves were converted to log cfu/cm^2 or/g.

3. Results and discussion

3.1. The effect of UV-C stress on L. monocytogenes attachment, survival and growth to lettuce surface

The attachment of *L. monocytogenes* (O8A07 and O8A08) to non-UV stressed lettuce was significantly higher ($p < 0.05$) than UV stressed lettuce at 30 and 60 min attachment times (Fig. 1). There was no significant difference between the two strains in terms of their attachment to both non-UV stressed and UV stressed lettuce. *L. monocytogenes* attachment to non-UV stressed lettuce showed a time-dependent response with attachment increasing slowly with increasing exposure times. Exposure of non-UV stressed lettuce to 5 log cfu/ml *L. monocytogenes* for 60 min resulted in an approximately 2.6 log cfu/cm^2 attachment. However, exposure of UV stressed lettuce to 5 log cfu/ml of *L. monocytogenes* resulted in 1.1–1.2 log cfu/cm^2 attachment (Fig. 1) and did not increase with time. Attachment of both O8A07 and O8A08 *L. monocytogenes* strains was reduced on UV stressed lettuce (Figs. 1 and 2).

L. monocytogenes survival and growth on non-UV stressed lettuce was also significantly higher ($p < 0.05$) than UV stressed lettuce after 24, 48 and 96 h (Fig. 3). After 60 min drying, approximately 3 log cfu/g of the *L. monocytogenes* survived on the non-UV stressed leaves whereas only 2 log cfu/g survived on the UV stressed leaves. *L. monocytogenes* numbers on non-UV stressed lettuce grew to about 4.6–4.7 log cfu/g after 96 h whereas *L. monocytogenes* on UV stressed lettuce only grew to 2.7–2.8 log cfu/g after 96 h. These results suggest that *L. monocytogenes* is less likely to attach and grow on UV stressed lettuce than non-UV stressed lettuce.

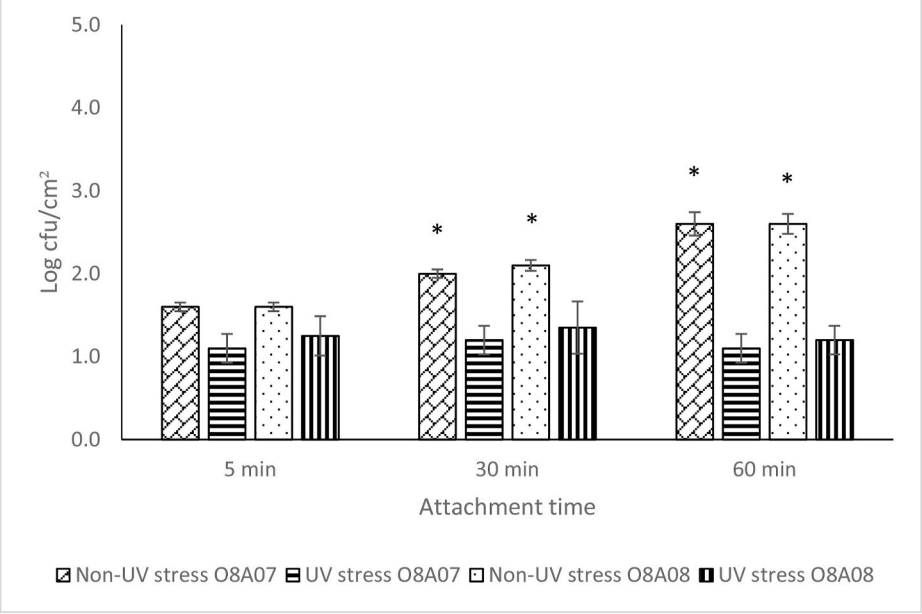

Fig. 1. Effect of UV-C stress (1.3 kJm^{-2}) on *L. monocytogenes* (O8A07 and O8A08) attachment (log cfu/cm^2) to lettuce leaves at exposure times of 5 min, 30 min and 60 min. Data is represented by the mean of three biological repeats with standard deviation error bars. Statistical differences were determined with ANOVA and Tukey's test at $p < 0.05$. Bars with an asterisk (*) denote a significant difference.

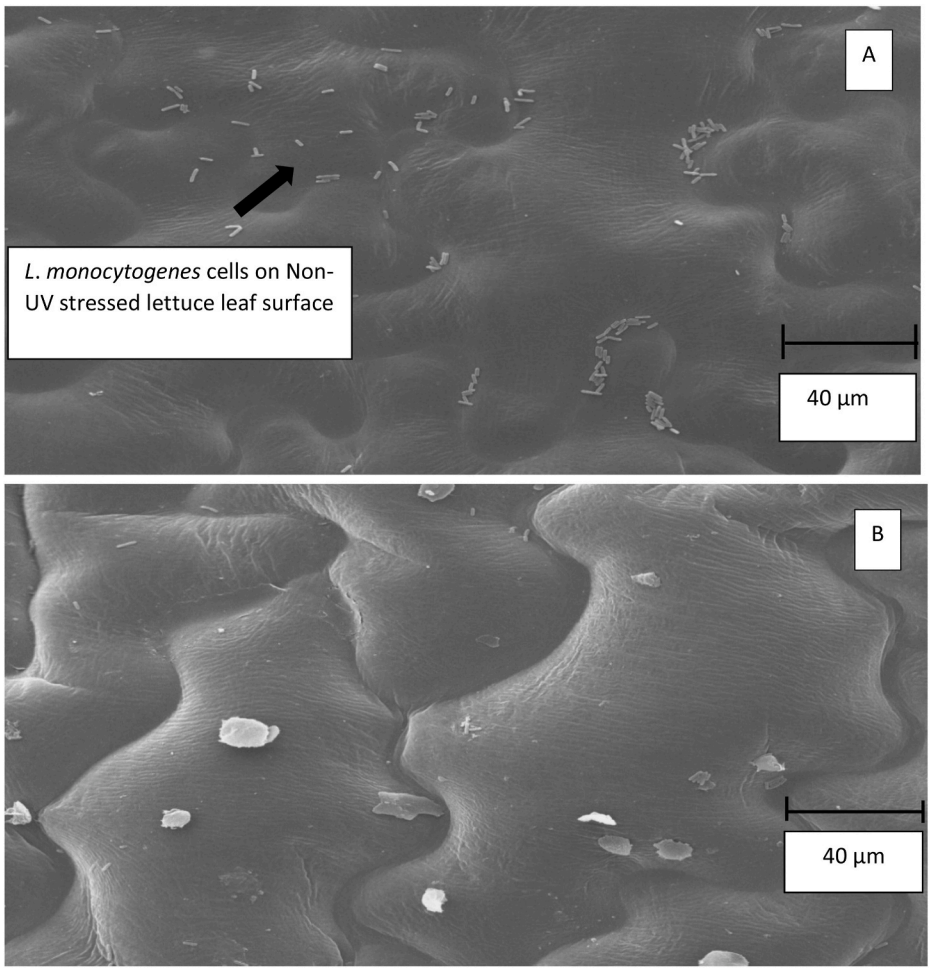

Fig. 2. SEM images of *L. monocytogenes* attachment on Non-UV stressed and UV stressed (1.3 kJm^{-2}) lettuce leaves after 60 min exposure time. A: Non-UV stressed and B: UV stressed lettuce. Images were taken with 1500× magnification with a scale of 40 µm.

SEM images of both non-UV and UV stress lettuce did not show any structural differences (data not shown), which suggests the dosage used did not influence leaf surface properties. However, a UV dosage of 2.6 kJm^{-2} resulted in lettuce leaves with brown spots 24 h after stressing (Supplementary Fig. 1) and the growth of *L. monocytogenes* on lettuce stressed with 2.6 kJm^{-2} was not inhibited but was actually slightly increased after 96 h (Fig. 4). Rather than evoking a stress response, the higher dose of UV damaged the lettuce leaves. There is therefore a need to optimise the UV dose to achieve a protective stress response but avoid damage to the lettuce tissue that may increase the colonization of lettuce by *L. monocytogenes*.

Most studies on the effect of UV stress on leafy greens have focused on plant fungal pathogens (Ouhibi et al., 2015; Vàsquez et al., 2017). UV-C (0.85 kJm^{-2}) stress of lettuce leaves inhibited the growth of *Botrytis cinerea* (a fungal pathogen) on lettuce leaves (Vàsquez et al., 2017). UV-C stress resulted in 10–20% reductions in fungal lesions on lettuce surfaces by the 6th and 7th day after inoculation (Vàsquez et al., 2017). Similarly, Ouhibi et al. (2015) reported a significant inhibition in the growth of *Sclerotinia minor* (a fungal pathogen) by UV stress on lettuce by the 4th day after inoculation.

There have been some studies on the effect of UV stress on the growth of human pathogenic bacteria on leafy greens. Gamage et al. (2014) reported that an aqueous extract from UV stressed (5.2 kJm^{-2}) broccoli significantly reduced growth of *L. monocytogenes* after 16 h. The relatively high UV dose (5.2 kJm^{-2}) that they used would have damaged our lettuce plants as shown in our results. Broccoli branchlets are structurally robust (Lopez-Sanchez et al., 2011) which may explain why the 5.2 kJm^{-2} dose did not have an adverse effect on broccoli plants.

Direct application of UV treatment on pathogenic bacteria in leafy greens inhibits bacteria growth by damaging bacterial DNA. This leads to the formation of dimers between pyrimidine residues in the DNA strands thereby halting DNA replication (Montgomery & Banerjee, 2015; Yin et al., 2013). The reduction in the number of *L. monocytogenes* (1.8–1.9 log) on lettuce by UV stress as shown by our results is similar to the reduction that has been achieved by some studies on the application of UV to plants already colonised by bacteria. For example, UV treatment (0.43 Jcm^{-2}) reduced the biofilm formation of *L. monocytogenes* on Romaine lettuce by 1.19 log cfu/ml after 24 h (Montgomery & Banerjee, 2015). The reductions observed in the present trials were higher than that observed by direct UV application to the bacteria on plant surface as reported by (Martínez-Hernández et al., 2015). A UV-C dose of 2.5 kJm^{-2} reduced *L. monocytogenes* on broccoli by only 0.72 log (Martínez-Hernández et al., 2015). This shows the need for further investigations into how UV-C radiation can be used to cause a stress response in fresh produce as this can have a greater effect on reducing pathogen contamination without causing damage to leaf tissues.

The effect of mild UV stress on leafy greens to prevent pathogen colonization has been reported to be influenced by several factors including leaf structures such as cuticles, stomata and epidermis (Fan et al., 2017). UV stress of leaves can change the leaf surface structure by damaging the epidermal covering (Vàsquez et al., 2017). Depending on the extent of damage, this can either be beneficial or harmful to pathogenic bacteria. For example, it was reported that UV stress (3.4 kJm^{-2}) weakened the epidermal protection of lettuce leaves resulting in a

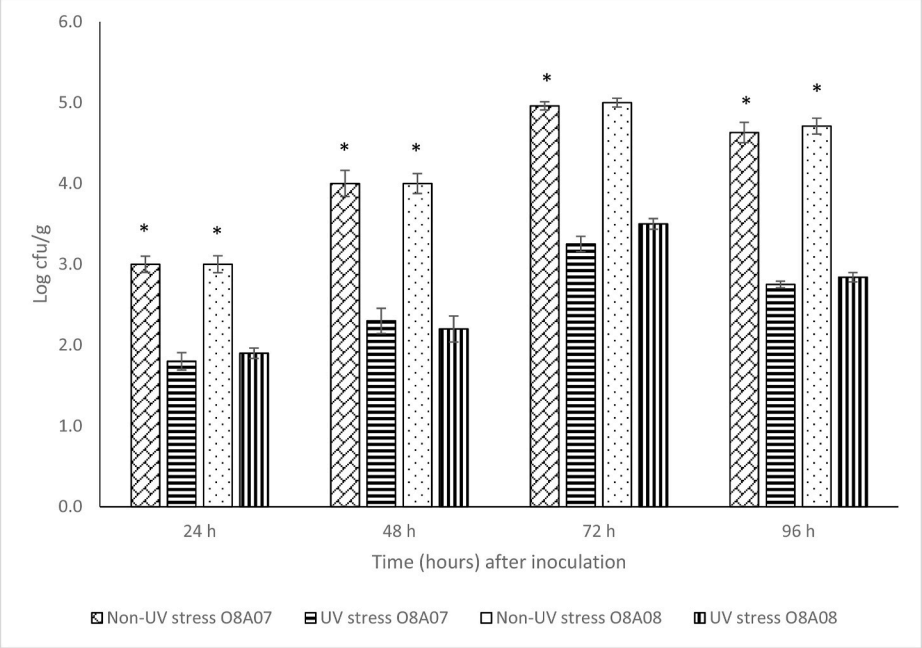

Fig. 3. Effect of UV-C stress (1.3 kJm^{-2}) on *L. monocytogenes* (O8A07 and O8A08) survival and growth (log cfu/g) on lettuce leaves. Data is represented by the mean of three biological repeats with standard deviation error bars. Statistical differences were determined with ANOVA and Tukey's test at $p < 0.05$. Bars with an asterisk (*) denote a significant difference.

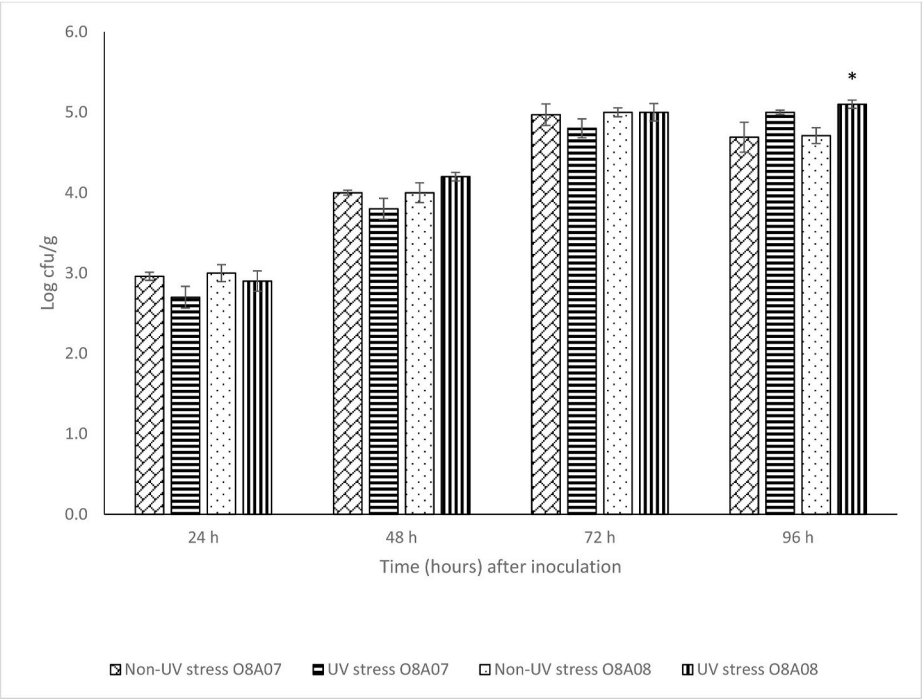

Fig. 4. Effect of UV-C stress (2.6 kJm^{-2}) on *L. monocytogenes* survival and growth on lettuce leaves. Data is represented by the mean of three biological repeats with standard deviation error bars. Statistical differences were determined with ANOVA and Tukey's test at $p < 0.05$. Bars with an asterisk (*) denote a significant difference.

greater colonization by *Botrytis cinerea* L. (a fungal pathogen). Similarly, a UV dosage of 24 kJm^{-2} damaged baby spinach tissues resulting in greater colonization with *L. monocytogenes* (Escalona et al., 2010). On the other hand (Syamaladevi et al., 2015), found a UV dose of 1.03 kJm^{-2} reduced *Penicillin expansum* (a fungal pathogen) by 1.8 log on apple surfaces without affecting the epidermis. UV stress has also been found to affect leaf surface properties such as hydrophobicity and contact angle measurements (Syamaladevi et al., 2015). Greater UV doses are needed to achieve microbial reductions on produce with rough surfaces. For example, a greater UV dose was required to achieve similar reduction in *P. expansum* on strawberry as compared to apples (Syamaladevi et al., 2015). In addition to these factors mentioned above, other properties such as leaf surface microflora, leaf age and source of leaf may also be factors in the effects of UV stress (Urban et al., 2016).

3.2. The effect of UV-C stress on total phenolic content in lettuce leaves

We tested the total phenolic content of UV stressed and non-UV stressed lettuce leaves and observed a significant increase ($p < 0.05$) in the total phenolic content of UV stressed lettuce (Fig. 5). This indicates an accumulation of the total polyphenols in lettuce as a stress response. Although the exact mechanism for reduced attachment, survival and growth on UV stressed leaves is unknown, it is likely related to this increase in polyphenols. Other researchers have also reported an increase in total polyphenol content in lettuce and other fresh produce after UV stress (Lee et al., 2014; Ouhibi et al., 2015; Tsormpatsidis et al., 2008). Some major polyphenols in lettuce that accumulate in response to UV stress include chlorogenic acid, chicoric acid, caffeic acid and quercetin-3-O-glycoside (Gamage et al., 2014; Wargent et al., 2015). Some of these polyphenols are bactericidal to foodborne pathogens (Lou et al., 2011). A study to investigate the antibacterial activity of chlorogenic acid on different foodborne pathogens; *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella* Typhimurium was conducted by (Lou et al., 2011). Chlorogenic acid effectively inhibited the growth of all tested pathogens with minimum inhibitory concentrations ranging from 20 to 80 μg/ml. Chlorogenic acid increases plasma membrane permeability leading to the breakdown of membrane function. In extreme cases, this leads to leakages in cellular cytoplasmic components (Lou et al., 2011). Caffeic acid has also been reported to have inhibitory effects on several foodborne pathogens (Wen et al., 2003; Zhang et al., 2020). A combination of caffeic acid (1.5 mg/ml) with fosfomycin (50 mg/L) significantly reduced the growth of *L. monocytogenes* with a minimum inhibitory concentration of 2.25 mg/L (Zhang et al., 2020). Inhibitory effects of caffeic acid against *L. monocytogenes* have also been reported in other studies (Pernin et al., 2019; Vallejo et al., 2020).

Quercetin has been found to reduce the attachment and biofilm formation of *L. monocytogenes*. 0.2 mM quercetin significantly ($p < 0.05$) reduced *L. monocytogenes* attachment to stainless steel coupons by 1.48 log cfu/cm^2. Also, a 0.2 mM concentration significantly reduced *L. monocytogenes* biofilm cells by 1.96 log cfu/cm^2. The ability of quercetin to inhibit *L. monocytogenes* attachment and biofilms was attributed to its ability to inhibit nucleic acid synthesis (Vazquez-Armenta et al., 2018).

The application of UV-C for improving plant yields as well as helping plants to resist plant diseases is been utilized with the recent formation of companies such as BioLumic, New Zealand and CureUV, Florida, USA. This technology can also be tested in the fresh produce industry by treating pre-harvest leaves with appropriate doses of UV-C since the results indicate a reduction in the *L. monocytogenes* population. In addition, there is a potential for the technology to be explored during post-harvest practices to observe the effect in the polyphenol content in leafy greens.

4. Conclusion

We report for the first time the ability of UV-C stress to reduce the attachment and growth of *L. monocytogenes* on lettuce leaves. Total polyphenol compounds in UV stressed lettuce increased compared to the non-UV stressed lettuce. Some of the polyphenols are known to inhibit *L. monocytogenes* growth which might explain the observed reductions. The application of UV-C should be further explored as a potential pathogen control in the fresh produce industry. Exposure of pre-harvest lettuce in the field to UV-C radiation at the right dosage could significantly reduce the attachment and growth of *L. monocytogenes*. Further studies to identify the polyphenols responsible for *L. monocytogenes* reduction as well as studying the effect of UV-C stress on the gene expression of lettuce genes and the effect of these genes on *L. monocytogenes* growth will be worth investigating. The results from this study indicate the potential benefit of applying UV-C stress in the fresh produce industry to improve food safety.

Credit author statement

Emmanuel Kyere is the PhD student who did most of the practical work and wrote the manuscript.

David Popovich provided advice on the detection of the phenolic compounds.

Jon Palmer provided guidance on UV treatment of plants for microbial control based on some work he completed with another student on broccoli.

Jason Wargent is an expert in UV treatment of plant material – for enhancement of plant growth. He provided guidance on the UV treatment used in this study.

Graham Fletcher is New Zealand's foremost expert in food safety in

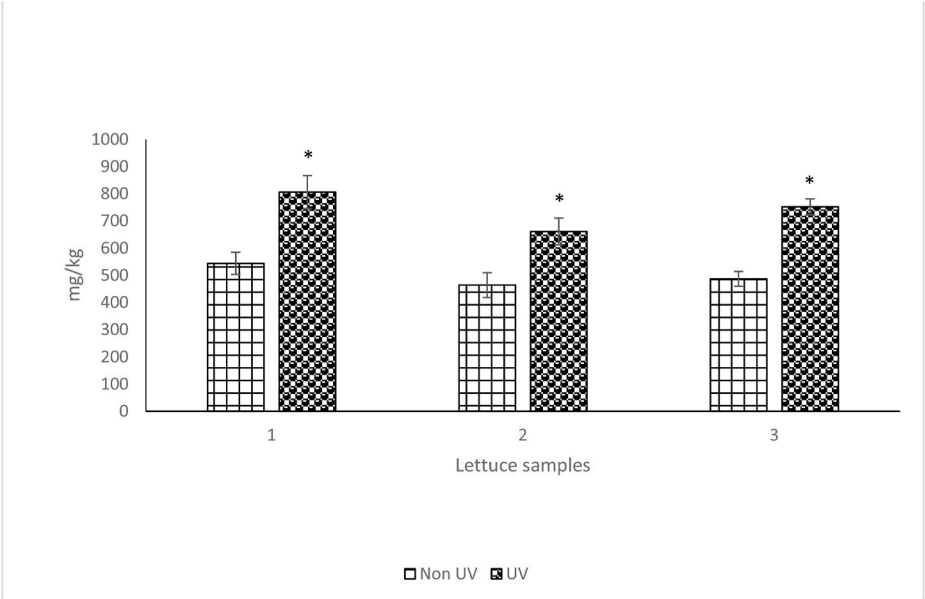

Fig. 5. Effect of UV-C stress (1.3 kJm^{-2}) on total phenolic content in lettuce leaves. Data is represented by mean of three biological repeats with standard deviation error bars. Statistical differences were determined with ANOVA and Tukey's test at $p < 0.05$. Bars with an asterisk (*) denote UV-C stressed lettuce with significantly higher polyphenols than non-UV stressed lettuce.

fresh produce and provided advice on *Listeria* colonization of fresh produce.

Steve Flint was the chief supervisor for this PhD project providing expertise in biofilm development and in *Listeria monocytogenes*.

Jon, **Jason** and **Graham** were co-supervisors for this PhD project.

Declaration of competing interest

There is no conflict of interest associated with this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111528.

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