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A comparison of low intensity UV-C and high intensity pulsed polychromatic sources as elicitors of hormesis in tomato fruit

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ABSTRACT

Postharvest hormetic treatment of mature green tomato fruit (*Solanum lycopersicum* cv. Mecano) with high intensity pulsed polychromatic light (HIPPL) significantly delayed ripening to levels comparable to those achieved using a conventional low intensity UV-C (LIUV) source. A 16 pulse HIPPL treatment reduced the ΔTCI (tomato colour index) by 50.2% whilst treatment with a LIUV source led to a reduction of 42.8%. Moreover, the 16 pulse treatment also induced disease resistance in the fruit to *Botrytis cinerea* with a 41.7% reduction in disease progression compared to a 38.1% reduction for the LIUV source. A single 16 pulse HIPPL treatment was found to significantly reduce disease progression on ripe fruit with a 28.5% reduction in comparison to 13.4% for the LIUV treatment. It is shown here that delayed ripening and disease resistance are local responses in side-treated tomato fruit for both LIUV and HIPPL treatments. Finally, utilising a 16 pulse HIPPL treatment would reduce treatment times from 370 s for LIUV sources to 10 s per fruit – a 97.3% reduction.

1. Introduction

The portion of the electromagnetic spectrum between 10 and 400 nm is referred to as the ultraviolet light region (UV). Within this region, wavelengths between 100 and 315 nm are known as 'germicidal UV'. Germicidal UV is used extensively to directly inactivate a range of micro-organisms in a number of different media including both solids and liquids (Shama, 2014). Some three decades ago research began to be undertaken in inducing UV-C hormesis in fresh produce (Lu et al., 1987). Since then UV-C treatment has been performed on a wide range of produce, as reviewed by Shama and Alderson (2005), Ribeiro et al. (2012) and Turtoi (2013). Hormesis is a phenomenon in which low doses of a potentially damaging agent bring about a beneficial response in the organism undergoing treatment. The beneficial effects of UV-C hormesis have been demonstrated for numerous types of fresh produce including both climacteric and non-climacteric fruit, tubers, salads and brassicas (Ranganna et al., 1997; D'hallewin et al., 1999; Costa et al., 2006; Pongprasert et al., 2011; Kasim and Kasim, 2012). Such effects include, but are not limited to, pathogen resistance, delayed ripening and improved nutritional content (Shama and Alderson, 2005; Ribeiro et al., 2012; Turtoi, 2013).

It has been estimated that in the UK 45% of all purchased salad and 26% of fruit is disposed of post retail (WRAP, 2012). Losses in storage, however, can be attributed to spoilage pathogens, senescence and transpiration (Maharaj et al., 1999). Crop-dependant pre and postharvest losses of 8–15% occur annually due to spoilage pathogens (Oerke, 2006). Losses of tomato fruit (*Solanum lycopersicum*)- the tenth most economically important non-meat food commodity- however, are particularly high as fruits are prone to chilling injury (Morris, 1982; FAOSTAT, 2015).

UV-C hormesis has been shown to induce disease resistance against a wide range of pathogens- and is achieved through both phytoalexin production and delayed ripening (Ben-Yehoshua et al., 1992; D'hallewin et al., 1999, 2000; Mercier et al., 2000; Romanazzi et al., 2006; Charles et al., 2008a). Many phytoalexins are phenolic compounds that act both as light quenchers, absorbing damaging wavelengths of light, and antioxidants that prevent reactive oxygen species (ROS) mediated cellular damage (Pietta, 2000; Sourivong et al., 2007; Lev-Yadun and Gould, 2009). It would appear, therefore, that it is their dual function which allows the build-up of resistance against plant pathogens in response to UV-C stress. Furthermore, specific pathogenesis-related (PR) proteins have also been shown to increase in concentration following

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hormetic UV-C treatment; these include chitinases and β-1,3-glucanases which interact directly with pathogens to reduce their viability (Charles et al., 2009).

The vast majority of previous studies on UV-C hormesis have been conducted with low pressure mercury sources that emit UV light with a peak emission at 254 nm at relatively low intensities, henceforth referred to as low intensity UV-C (LIUV). The long treatment times required by LIUV sources explains in part why there has been reluctance by the horticulture sector to adopt this form of treatment. To take a specific example, there is consensus on the average UV-C dose (3.7 kJ/m^2) necessary to induce hormetic effects in tomato fruit (Liu et al., 1993 & Maharaj et al., 1999). Using low pressure mercury sources at an intensity of 20 W m^{-2} would require an exposure time of approximately six min per fruit. Furthermore, the requirement for complete surface irradiation to induce the beneficial effects on certain types of produce both complicates the treatment procedure and extends the treatment time (Mercier et al., 2000). The recent advent of high intensity pulsed polychromatic light (HIPPL) sources with considerable emission in the UV region could result in a substantial reduction in treatment time from minutes to seconds.

Treatment of fresh produce with HIPPL has been shown to increase the concentration of anthocyanins and total phenolics along with improving colour in nethouse-grown fig, *Fiscus carica* (Rodov et al., 2012). Both LIUV and HIPPL treatments have been shown to significantly increase the total lycopene, carotenoid and phenolic content as well as the antioxidant activities of tomato fruit (Liu et al., 2009, 2012; Pataro et al., 2015). HIPPL has also been shown to increase anthocyanin and Vitamin D_2 levels in mushrooms, *Agaricus bisporus* (Oms-Oliu et al., 2010; Koyyalamudi et al., 2011).

The aim of this study was to investigate whether HIPPL sources were able to delay colour change during ripening and induce resistance against *B. cinerea* on mature green tomatoes. Treatments were also conducted with a LIUV source as a basis for comparison. Further experiments were undertaken to establish whether it was necessary to irradiate the entire fruit surface for successful elicitation of delayed colour change and disease resistance. Additionally, treatments using both types of source, HIPPL and LIUV, were conducted to assess their ability to induce disease resistance on red ripe fruit, as an increasing number of tomato growers are harvesting at this stage due to high consumer demand.

2. Materials and methods

2.1. Plant material

Mature green and red ripe tomato fruit, cv. Mecano, were grown in the glasshouse at APS Salads (UK) and delivered at ambient temperature to the University of Nottingham within 24 h of harvesting. Fruit were then sorted to remove fruit showing deviations from the desired developmental stage or uniformity in size. Fruit showing any surface damage were also discarded.

2.2. UV treatment

Upon arrival tomatoes were randomly assigned to treatment groups and treated at room temperature on the same day. LIUV treatments were carried out using a U-shaped amalgam UV source (UVI 12OU2G11 CP15/469) obtained from Dr Hőnle AG, Gräfelfing, Germany, with peak emission at 254 nm and mounted within an anodised aluminium parabolic reflector. Doses of 3.7 kJ/m^2 were delivered at an intensity of 20 W m^{-2} following the procedures of Charles et al. (2008a). Intensity was measured with a portable radiometer (Model UVX, UVP Instruments, Cambridge) fitted with a 254 nm sensor.

HIPPL treatments were carried out with a XENON LH-840 16" ozone-free B lamp powered by an RT-847 cabinet and RC-802 controller, supplied by Lambda Photometrics (Harpenden, Herts). The source produced 505 J of energy per pulse with a pulse width of 360 μs at 3.2 pulses per second. Spectral emission of the source lay between 240 nm and 1050 nm. Fruit were placed at a distance of 10 cm from the window of the lamp housing. Through extrapolation of the manufacturer's data an estimated 4.6 kJ/m^2/pulse was delivered at fruit level.

Fruit received exposure on two sides through 180° axial rotation. For experiments aimed at determining whether full tissue exposure was necessary for inducing disease resistance, fruit were treated from one side only. Following treatment fruit were immediately stored in the dark until subjected to surface sterilisation. For sterilisation tomatoes were immersed in 2% Sodium hypochlorite (Sigma-Aldrich) for approximately 5–10 s to prevent growth of naturally occurring microorganisms during the incubation period. Fruit were then rinsed three times in sterile distilled water (SDW), dried and immediately incubated in the dark at 13 °C to prevent photoreversal. Fruit were stored for 10 d in high humidity boxes with relative humidity >98%.

2.3. Colour measurement

Tomato colour was monitored to determine ripening progression (Lopez Camelo and Gomez, 2004; Corcuff et al., 2012). Measurements were conducted using a calibrated CR-200 Chroma meter (Konica Minolta) in L*a*b* mode. Readings were taken at a single point directly facing the source and at a 90° axial rotation from that point. A second colour measurement was taken using the same reference points at 10 days post treatment (DPT). Tomato colour index (TCI), was then calculated (Hobson, 1987), (Eq. (1)). The two measurements were then used to calculate the change in TCI over 10 d.

$$\mathrm{TCI} = \frac{2000a)}{L\sqrt{a^2 + b^2}} \quad (1)$$

Tomato colour index (TCI) formula where L = lightness, a = red-green and b = blue-yellow values (Hobson, 1987).

2.4. Pathogen maintenance and inoculum preparation

A strain of *Botrytis cinerea*, originally isolated from the genus *Rosa*, was obtained from The University of Nottingham's own culture collection. Cultures were grown at room temperature on potato dextrose agar (Sigma-Aldrich) supplemented with Penicillin G sodium salt (Sigma-Aldrich) at 33 mg/L and Streptomycin sulphate salt (Sigma-Aldrich) at 133 mg/L. A calibrated spore solution was made from 10 to 14 d old cultures. Briefly, Petri dishes were flooded with 15 mL of SDW supplemented with 0.03% Tween 20. Spores were released by gentle agitation and then filtered through a double layer of muslin cloth and vortexed vigorously to release conidia from conidiophores. The spore solution was then centrifuged at 184*g* in a Centaur 2 (MSE) for 10 min and the supernatant discarded. The pellet was re-suspended in SDW, vortexed and centrifuged again at 184*g* for a further 10 min following which the supernatant was discarded. The pellet was re-suspended in SDW and a haemocytometer was used to obtain the desired spore concentration.

2.5. Inoculation and lesion measurement

At 10 DPT fruit were inoculated with *B. cinerea*. This interval was selected on the basis of the work of Charles et al. (2008a, 2008b, 2008c) who showed near optimal induction of resistance occurred