Research Journal of Biological Sciences 5 (2): 209-214, 2010
ISSN: 1815-8846

Exogenous Nitric Oxide Negatively Impacts on Ethylene Emissions from Intact and Fresh-Cut Tomato Fruit

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Abstract: The objective of this study was to investigate the effect of Nitric Oxide (NO) on the production of basal and wound-associated stress ethylene (C_2H_4) from intact and fresh-cut tomato fruits, respectively. For this purpose, a non-invasive and online sampling technique based on Laser Photoacoustic Spectroscopy (LPAS) was employed. Pre-treatment of intact Mature Green (MG) tomato fruits with a low concentration (200 ppbv) of NO gas resulted in a significant and steady average reduction of 33% in the basal-level C_2H_4 production to 6.0±0.44 pmol h^{-1} g fwt^{-1} compared to 9.0±0.18 pmol h^{-1} g fwt^{-1} in the non-treated MG control. Moreover, NO gas fumigation of fresh-cut MG tomato fruit slices caused a 60% reduction in peak wound-induced C_2H_4 levels compared to untreated fresh-cut control fruit. These results clearly indicate that NO pre-treatment negatively impacts on both basal and wound-associated stress C_2H_4 emission levels, respectively in both intact and fresh-cut tomato fruits. These results are discussed in the light of possible mechanisms of NO interference with C_2H_4 biosynthesis. Moreover, the potential utilization of NO in controlling stress-induced and undesirable biochemical changes which are known to occur in fresh-cut fruits is highlighted.

Key words: Tomato, nitric oxide, ethylene biosynthesis, laser photoacoustic spectroscopy, fresh-cut fruits, Saudi Arabia

INTRODUCTION

Stress is generally defined as any environmental factor that is potentially unfavourable to living organisms (Levitt, 1972) with the exception of decay (i.e., biotic factors). Thus, quality losses in fresh-cut produce such as fruits and vegetables can be directly or indirectly attributable to a combination of abiotic stress and stress-induced senescence (Lester, 2003). In this context, wounding is one of the major stresses experienced by fresh-cut produce undergoing various forms of processing (e.g., slicing, dicing, chopping, trimming, peeling, coring and/or shredding). Internal and external factors that can affect the wound response include species, cultivar, maturity, storage/processing temperature, cutting protocols, CO_2 and O_2 levels and water vapour pressure (Brecht, 1995; Cantwell and Suslow, 2002). Increasing interest has recently been shown in prepacked/precut fresh fruit and vegetables because of the advantages offered by those commodities to consumers; e.g., convenience, freshness and low calorific content.

However, as a result of the active metabolism of the plant tissues caused by wounding damage and the exposure of cut surfaces to external factors, the produce changes from having a relative stability with a shelf-life of several weeks or months to a perishable product with a very short shelf-life (Lanciotti *et al*., 1999). During climacteric fruit ripening, the burst of autocatalytic ethylene (C_2H_4) co-ordinates and accelerates the ripening process (Alexander and Grierson, 2002). Delaying fruit ripening by reducing C_2H_4 biosynthesis has been a major goal of postharvest physiologists for example via C_2H_4-suppressed transgenic plants (Hamilton *et al*., 1990; Oeller *et al*., 1991; Picton *et al*., 1993).

Ripening of fruits and senescence of flowers can also be controlled using 1-methyl cyclopropene (1-MCP) (Reid and Staby, 2008). However, the transgenic approach is time-consuming, financially-demanding and requires extensive studies and the use of 1-MCP may actually inhibit the occurrence of desirable changes in the produce that are induced by C_2H_4.

Fresh-cut processing and packaging protocols result in stress for fruit and vegetable tissues and much of this has been described as producing a shorter shelf-life for fresh-cut versus intact fruits and vegetables (MarÃa Gil *et al*., 2006). Fresh-cut fruits and vegetables exhibit increased respiration rates and wound-induced C_2H_4 production and increase the surface area per unit volume, thus exacerbating water loss (Toivonen and DeEll, 2002). Thus, development of non-hazardous and efficient procedures for preventing C_2H_4 accumulation or inhibiting C_2H_4 action on fresh-cut fruits would be of considerable commercial interest.

For >2 decades, due to its diverse biological activities and general ubiquity, Nitric Oxide (NO) has been extensively studied in animal and plant research (Delledonne *et al*., 1998; Durner *et al*., 1998; Furchgott and Zawadzky, 1980; Ignarro *et al*., 1987; Koshland, 1992; Palmer *et al*., 1987; Wang *et al*., 2004; Aboul-Soud *et al*., 2009). NO is a bioactive molecule implicated in vegetative stress and senescence of horticultural products (Leshem and Haramaty, 1996).

Earlier, it has been reported that exogenous application of NO, either by direct fumigation or via NO-releasing chemicals, significantly extends shelf and post-harvest lives of intact vegetables and climacteric and non-climacteric horticultural products (Leshem and Haramaty, 1996; Leshem and Wills, 1998; Leshem *et al*., 1998; Wills *et al*., 2000; Sozzi *et al*., 2003; Soegiarto and Wills, 2004; Zhu and Zhou, 2007; Zhu *et al*., 2006). Moreover, the emission of NO was negatively correlated with C_2H_4 output during the process of maturation and senescence of intact whole climacteric and non-climacteric fruits (Leshem and Pinchasov, 2000). However, detailed knowledge of the effects of NO treatment on freshly-cut fruits is seriously lacking. Hence, the objective of this study was to investigate the effect of exogenous NO on the production of stress C_2H_4 from intact and fresh-cut tomato fruits via a non-invasive detection using Laser Photoacoustic Spectroscopy (LPAS).

The obtained results are discussed in the light of possible mechanisms of NO interference with C_2H_4 biosynthesis. Moreover, the potential utilization of NO in controlling stress-induced and undesirable biochemical changes that are known to occur in fresh-cut fruits is highlighted.

MATERIALS AND METHODS

Plant growth: Experiments were performed using a near isogenic line of diploid *Solanum lycopersicon* Mill. cv. Ailsa Craig (AC^{++}) plants that have been grown at Sutton Bonington, Leics., UK for over 20 years. All fruits utilised for experiments were grown in Sutton Bonington (Leic., UK) greenhouse facility in December 2003. Basically, plants were grown, maintained and flowers were tagged at anthesis as earlier described (Aboul-Soud and El-Shemy, 2009).

Fruits at Mature Green (MG) stage were detached from the plants with approximately 5 cm of pedicle and subsequently put in a well aerated box to avoid stressing the fruits. The fruits were air-shipped from Nottingham to Nijmegen, the Netherlands in a <8 h duration from dispatch to arrival. Measurements were carried out within hours of arrival.

Fruit treatments: Tomato fruits were initially selected for uniformity of size and freedom from defects, infection and mechanical damage. Mature Green (MG) AC^{++} fruits were all freshly cut for the wounding studies.

All samples were manually processed using disinfected stainless steel knives and plastic cutting boards. MG AC^{++} fruits were systematically cut in slices 10-12 mm thick. Subsequently, the resulting slices were then cut into 4 triangular pieces. For fruit treatment with NO, a calibrated cylinder containing NO at a concentration of 1 ppm, purchased from NMI (National Metrological Institute, the Netherlands) was utilised. Thus, tomato fruits were fumigated overnight with NO gas at a physiological concentration of 200 part per billion volume (ppbv). This was accomplished by flushing fruit samples with a mixture of NO from the calibrated cylinder (NMI, the Netherlands) and ozone-free air at a total flow rate of 2 L h^{-1}. In order to reach 200 ppbv NO, a 5 time dilution of the NO from the calibrated cylinder was attained by mixing 0.4 L h^{-1} NO flow with 1.6 L h^{-1} ozone-free air.

Real-time monitoring of C_2H_4 emission with LPAS: C_2H_4 production was monitored in real time via LPAS, employing a sensitive laser-based C_2H_4 detector in combination with a gas flow through system, essentially as described by Cristescu *et al*. (2002). A schematic diagram of the setup is shown in Fig. 1. Briefly, the system consists of a line-tunable CO_2 laser that emits 9-11 μm infrared light into a Photoacoustic Absorption Cell (PAC) where C_2H_4 is detected.

The C_2H_4 gas mixtures are very sensitively measured by the laser-based C_2H_4 detector at tens of pptv levels (parts per trillion volume = 1: 10^{12}) due to the distinct fingerprint-like spectrum of C_2H_4 in the CO_2 laser wavelength range (Brewer *et al*., 1982). Tomato fruits are placed into closed glass cuvettes and continuously flushed with carrier gas at constant flow of 2 L h^{-1}. A system of electric valves allowed three cuvettes with biological samples to be measured alternatively (Fig. 1a). Each cuvette was measured for about 15 min. When not being measured, the gas flow through the cuvette was maintained but was vented into the (Fig. 1b). The obtained C_2H_4 levels corresponding to an empty cuvette were subtracted.

Ethylene emission from tomato fruits was related to the production rate by multiplying the measured value with the flow rate (2 L h^{-1}) and divided by the fresh weight; the results were expressed in pmol h^{-1} per g fresh weight (fwt). Each experiment was repeated at least three times, each of which produced essentially similar results. The data shown in Fig. 2 and 3 are of a typical experiment.

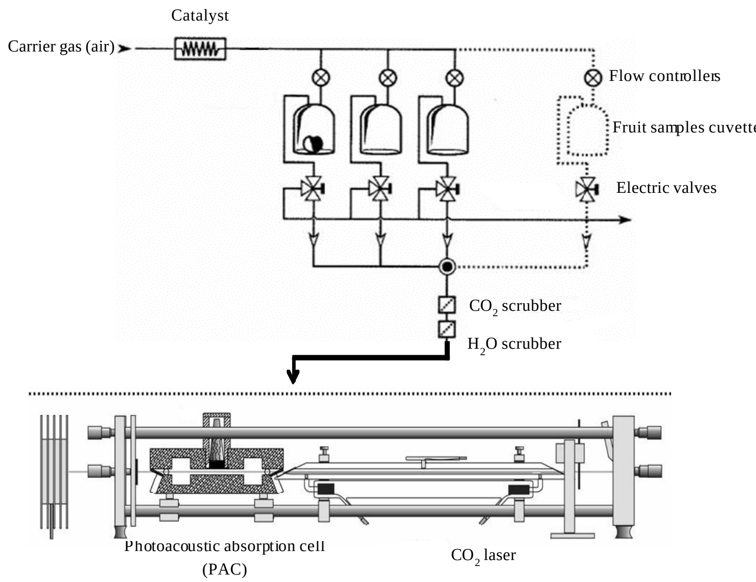

Fig. 1: Experimental setup for ethylene detection using LPAS; Gas sampling system. Compressed air is passed through a catalyst to obtain hydrocarbons-free air and used to flush the cuvettes containing the tomatoes at constant flow rate of 2.0 L h^{-1} (regulated by flow controllers). Each cuvette is connected alternatively to the measuring system by electric valves. Scrubbers with KOH and $CaCl_2$ were placed before the PAC in order to reduce the CO_2 and the water content in the gas flow; LPAS-based system. A CO_2 laser is used as laser source in combination with a photoacoustic absorption cell (PAC) for real-time measurement of C_2H_4

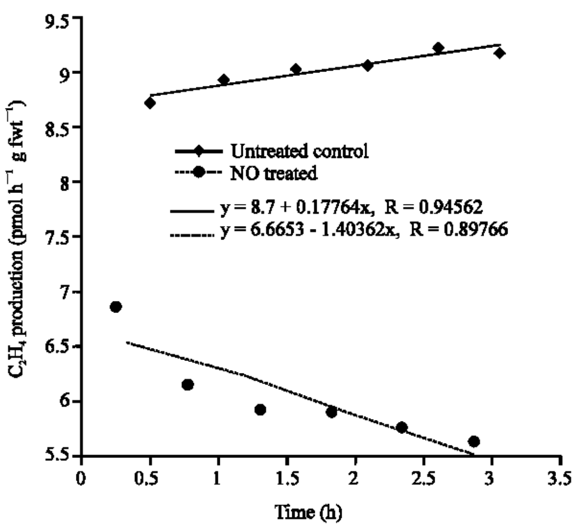

Fig. 2: Ethylene production in intact MG tomato fruits pre-treated with NO compared to untreated intact control, as detected using LPAS. NO-treated fruits were fumigated with 200 pbbv according to materials and methods. Each sample cuvette was measured for approximately 15 min. This figure shows averaged C_2H_4 emissions of typical data obtained over a 3 h monitoring period. The experiment was repeated twice with similar results

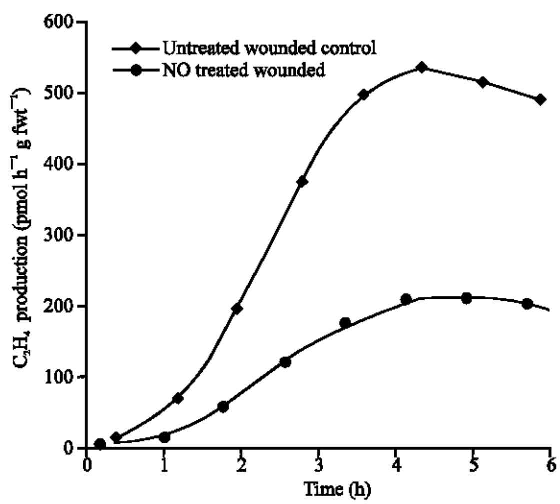

Fig. 3: Ethylene production by fresh-cut MG tomato fruits pre-treated with NO compared to untreated fresh-cut control, as detected using LPAS. NO-treated fruits were fumigated with 200 pbbv according to materials and methods. MG fruits were manually processed using disinfected stainless steel knives and were systematically cut in slices 10-12 mm thick. Subsequently, resulting slices were then cut into 4 triangular pieces

RESULTS AND DISCUSSION

NO emissions were negatively linked to C_2H_4 production in intact climacteric and non-climacteric fruits (Leshem and Pinchasov, 2000). This raised the prospect that NO could have a role in the postharvest behaviour of horticultural produce, as exogenous NO fumigation has been shown to extend postharvest shelf-life and storage of intact fruits (Leshem and Wills, 1998; Leshem *et al*., 1998; Wills *et al*., 2000). However, a direct causal relationship between NO and C_2H_4 has not been investigated earlier, whether in intact or fresh-cut fruits. In this research, The researchers examined the direct effect of short-term application of NO gas, at low concentration on levels of C_2H_4 production in tomato fruit. For this purpose, a direct-trace gas, non-invasive and online sampling technique based on LPAS was employed. Thus, C_2H_4 emissions from MG fruits which had been pre-treated with NO gas at a concentration of 200 ppbv were monitored in real-time via LPAS. Ethylene production from intact tomato fruits at three different developmental stages (namely: Mature Green (MG), Breaker (B) and B+5) was monitored.

Typically, C_2H_4 levels of 16.93±2.72, 151.01±18.33 and 718.19±42.16 pmol h^{-1} g fwt^{-1} were produced by MG, B and B+5 fruits, respectively over 13 h period. These values fall within the expected normal C_2H_4 evolution range for each respective developmental stage. Strikingly, pre-treatment of intact MG tomato fruits with 200 pbbv NO gas resulted in a significant steady reduction in C_2H_4 production level (Fig. 2). Specifically, an overall average reduction of 33.33% in C_2H_4 production level was observed when intact MG tomato fruits were fumigated with NO gas (6.0±0.44 pmol h^{-1} g fwt^{-1}), as compared to non-treated MG control (9.0±0.18 pmol h^{-1} g fwt^{-1}) (Fig. 2). It is noteworthy that the reduction in C_2H_4 emission becomes greater the longer the experiment continues being 6.61 and 5.63 pmol h^{-1} g fwt^{-1} at 0.77 and 2.86 h, respectively (Fig. 2).

In addition, to test the effect of NO on wound induced C_2H_4 levels, fresh-cut MG tomato fruits were employed. Interestingly, NO gas fumigation caused approximately 60% reduction in peak C_2H_4 levels in fresh-cut MG tomato fruits as compared to untreated fresh-cut control (Fig. 3). Taken together, these results clearly indicate that NO pre-treatment negatively impacts on C_2H_4 production levels in both intact and fresh-cut tomato fruits. A possible explanation of this observed phenomenon is that NO negatively interferes with the enzymatic activity of one or more key enzymes in the C_2H_4 biosynthetic pathway, thereby influencing maturation and senescence of plant tissue. Hence, it is suggested that both gases act antagonistically.

The chemical nature of NO results in transition metals (e.g., Fe, Cu, Zn) and proteins containing thiol groups being important targets for this molecule (Wendehenne *et al*., 2001). Thus, it has earlier reported that NO inhibits the activity of aconitase (Fe-S) and haem-containing enzymes (e.g., Catalase and peroxidase) resulting in the modulation of reactive oxygen species generation and detoxification (Clarke *et al*., 2000; Ferrer and Barcelo, 1999). In strawberry, a non-climacteric fruit, with low C_2H_4 production rate after harvest, it has been reported that NO could decrease C_2H_4 production through inhibition of 1-Aminocyclopropane-1-Carboxylic acid (ACC) synthase activity but not ACC oxidase; thus, reducing ACC content (Zhu and Zhou, 2007). Thus, NO treatment was hypothesized to have no effect on the conversion of ACC to ethylene; it only prevented ACC synthesis through ACS deactivation (Zhu and Zhou, 2007). Moreover, another report showed that NO directly acts by down-regulating C_2H_4 synthesis through S-nitrosylation of Methionine Adenosyltransferase (MAT1) in Arabidopsis plants (Lindermayr *et al*., 2006).

The attachment of NO leads to the inhibition of MAT1 activity and results in the reduction of the pool of the C_2H_4 precursor S-Adenosylmethionine (SAM) (Lindermayr *et al*., 2006). In this research, it was shown for the first time that exogenous NO negatively modulates C_2H_4 production in fresh-cut MG tomato fruits (Fig. 3).

In this context, it has been previously reported that NO donors strongly inhibit the gene expression of wound signalling-associated genes (e.g., proteinase inhibitor) in young excised tomato plants (Orozco-Cardenas and Ryan, 2002). Hence, it is possible that NO can potentially be used in controlling stress-induced and undesirable biochemical changes that are known to occur in fresh-cut tissues, including fruits. Taken together, the key to achieving improved shelf-life, quality and nutritional status in fresh-cut products is to understand the mechanism of wound-induced change in the tissue physiology and metabolism. Once the mechanism of the wound-induced change is understood, then approaches to delay, inhibit or ameliorate the stress can be reliably developed.

Further studies are needed to determine the protective effect of NO on fruit nutritional value, quality parameters and on the synthesis of undesirable compounds with toxic or allergenic properties.

ACKNOWLEDGEMENTS

This research was supported by the European Union (EU) EU-FP6-Infrastructures-5 program, project FP6-026183 'Life Science Trace Gas Facility'. Thanks are due to Lucas Laarhoven and Simona Cristescu for the technical training with LPAS, set-up and data collection.

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