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Chapter 14

Nitric Oxide and Postharvest Stress of Fruits, Vegetables and Ornamentals

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Abstract Horticultural produce are impacted by a range of abiotic stresses during storage and marketing that accelerate ripening and senescence and thus reduce shelf life. Exogenous application of nitric oxide (NO) by gas fumigation or dipping in a solution of NO-donor compound has been demonstrated to alleviate some of the effects of abiotic stress on a wide range of produce. A key reported beneficial effect of NO treatment is to reduce the production of ethylene. Other reported beneficial effects of NO treatment include a reduced rate of respiration and reduced ion leakage resulting from better maintenance of cellular integrity; reduction in oxidative stress through reduced lipid oxidation and enhanced activity of a range of antioxidant enzymes which have been implicated in defence mechanisms; inhibition of polyphenol oxidase activity associated with reduced internal and surface browning; and alleviation of chilling injury potentially through enhancing the natural antioxidant defence systems which could include endogenous NO. Postharvest application of NO is a potential new technology to reduce losses of horticultural produce during handling and marketing.

Keywords 4-6 Nitric oxide, postharvest; fruit, vegetables, ornamentals

14.1 Introduction

Horticultural commodities such as fruits, vegetables and ornamentals can be considered as being under continuous abiotic stresses as they are still biologically active. Even after harvest they maintain a full range of metabolic sequences in order to maintain cellular integrity. However, the physical act of harvesting has removed the ability of fruits and vegetables to obtain water and nutrients from the parent plant. Thus, fruits and vegetables must utilise the components present at harvest for all subsequent postharvest metabolism and therefore all fruit and vegetables are under abiotic stress. A similar situation of continuous abiotic stress also applies to ornamentals although in parts of the marketing chain, water and nutrients can be supplied by standing stems in a vase solution. The depletion of metabolic reserves inevitably leads to loss of cellular integrity and the onset of various forms of deterioration that a consumer recognises as a loss in quality which may either be physiological or pathological in nature. The rate and nature of this deterioration varies greatly between individual produce and physical conditions of the postharvest environment. However, the increasing distance between production areas and markets, the growing complexity of marketing systems to support international trading and extended domestic marketing to capture better out-of-season prices results in considerable abiotic stress being imposed on produce.

Postharvest research has developed various technologies to minimise deterioration after harvest. These technologies range from environment management (principally of temperature and humidity but also of ethylene which is an important ripening and senescence inducing compound produced by all horticultural commodities), and chemical intervention to inhibit respiration and ethylene action or growth of invading pathogens. While considerable advances have been made in extending the postharvest life of horticultural produce, research continues to find new technologies to better maintain product integrity and quality, particularly if they can utilise naturally occurring compounds.

14.2 Relationship between Endogenous Nitric Oxide and Ethylene

Research into the possibility of using nitric oxide (NO) to minimise the effects of abiotic stress on postharvest horticultural commodities was instigated in the mid 1990s by the late Ya'acov Leshem from Bar-Ilan University in Israel. Leshem was aware of the growing medical interest in the potential therapeutic benefits of NO arising from its involvement in numerous mammalian metabolic sequences and sought to uncover if NO had a parallel role in plants. His initial publication (Leshem and Haramaty 1996) examined a role for NO in plant signalling and reported that adding the NO-donor compound S-nitroso-N-acetylpenicillamine (SNAP) to pea leaves resulted in a greater emission of NO than ethylene, and that both NO and ethylene increased with the addition of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). They suggested that NO may regulate the ethylene production in growing plants. Subsequent studies reported an inverse relationship between endogenous NO and ethylene released from four unripe and ripe fruits and two cut flowers (Leshem et al. 1998) and increased ethylene production during ripening of banana and strawberry was found to coincide with reduced NO emission (Leshem 2000; Leshem and Pinchasov 2000). Leshem et al. (1998) also reported that alfalfa sprouts, heat stressed at 37°C, showed increasing production of NO and decreasing production of ethylene with time suggesting a stress coping role for NO. The documentation of an inverse link between endogenous NO and ethylene, was the trigger that spawned research over the last 15 years to ascertain whether exogenous application of NO to both normal intact produce and the rapidly growing retail market segment of fresh-cut fruits and vegetables would extend postharvest life.

14.3 Postharvest Application of NO

14.3.1 Fumigation with NO Gas

The commercial availability of NO gas cylinders and industry experience of fumigation with 1-methylcyclopropene (1-MCP) to inhibit ethylene action on a range of horticultural produce (Blankenship and Dole 2003) makes NO gas fumigation an obvious potential method of application. However, treatment with the free radical NO gas needs to take

into account the rapid oxidation of NO by atmospheric oxygen, e.g. Snyder (1992) reported a half life of NO in air of 5 sec. In addition, the rate of oxidation of NO increases with decreasing temperature due to increased intermolecular interaction between NO and oxygen molecules (Tsukahara et al. 1999). This led early studies (e.g. Leshem et al. 1998) to treat horticultural produce with gaseous NO in a nitrogen atmosphere. However, oxygen (O₂) is required for the maintenance of normal aerobic metabolism and while produce can tolerate some reduction below the normal atmospheric concentration (about 21% O₂), extended exposure below threshold levels results in anaerobic metabolism and leads to the subsequent development of tissue damage, off-flavours and off-odours (Wills et al. 2007a). This would limit the fumigation time to relatively short periods and imposes additional logistical and equipment requirements on any commercial treatment.

Soegiarto et al. (2003) examined the rate of loss of 30 µl. l⁻¹ NO gas at 20°C from atmospheres containing 0.3% O₂ to 21% O₂ and found that the rate of NO loss was much lower than expected with the half life of NO ranging from about 16 hr in 0.3% O₂ to 3.5 hr in 21% O₂. They also showed that the rate of loss of NO from the atmosphere was much greater in the presence of horticultural produce than from air only, indicating a rapid uptake of NO by produce but the rate of uptake varied considerably between different types of horticultural produce. Thus, NO seems to be sufficiently stable at the concentrations and fumigation times utilised to allow produce to be treated in normal air.

Wills et al. (2007b) subsequently developed a solid mixture that released NO gas in the presence of horticultural produce. They found that the solid NO-donor compound, 2,2'-(hydroxynitrosohydrazino)-bisethanamine (diethylenetriamine nitric oxide, DETANO) (Fitzhugh and Keefer 2000) quantitatively liberated NO gas in the presence of a range of acidic substances including citric acid. A solid mixture of DETANO, citric acid and wheat starch (added as a filler and moisture absorbent) in the ratio of 1:10:20 was found to be stable when stored in dry air. However, in humid air, the absorption of moisture from the atmosphere led to reaction of DETANO with citric acid and the evolution of NO gas. When the dry mixture was placed in a container with strawberry fruit and mushrooms, the moisture given off by produce activated the mixture and resulted in a similar extension in postharvest life as achieved by direct fumigation with

NO gas. The industrial use of such a solid mixture could be through tablets or sachets which are more manageable in a farm or packing house than gas fumigation.

14.3.2 Dipping in Aqueous Solution of NO-donor Compounds

Postharvest treatment by dipping in aqueous solutions (e.g. fungicides) is a common practice in the horticultural industry and is therefore of interest for research into the application of NO. The requirements for any commercial treatment is for the NO-generating compound to be quite stable as a solid, relatively stable in solution but to quantitatively release NO when absorbed by produce. There are now a wide range of NO-donor compounds that are relatively stable in solution with the list being considerably expanded due to medical interest in using NO donors (Hou et al. 1999). However, only a few NO-donor compounds have been used in postharvest horticulture research.

The most commonly examined NO-donor compound is sodium nitroprusside (SNP) ($\text{Na}_2 [\text{Fe} (\text{CN})_5 \text{NO}] 2\text{H}_2\text{O}$). SNP is a transition metal NO complex class of donor compound that releases the NO^+ cation and has found clinical use (Pitkanen et al. 1999; Wang et al. 2002). SNP is soluble in water and relatively stable in solution, resisting oxidation at a neutral or slightly acidic pH (Verner 1974) and is widely available and relatively inexpensive.

DETANO was first utilised in postharvest research by Bowyer et al. (2003) on carnation cut flowers although Noritake et al. (1996) had applied DETANO to growing potato plants and found an effect on potato tubers (see Section 14.4.2). DETANO was selected as a candidate for NO treatment as it was claimed to be the most stable diazeniumdiolate with a half life in solution of 20 hr at 37°C and pH 7.4 and followed first order kinetics in degrading to two molar equivalents of NO^* free radical with the reaction being dependent on solution temperature and pH (Lemairè et al. 1999; Fitzhugh and Keefer 2000). The half-life of 0.1 mM DETANO solution markedly decreases at low pH with Davies et al. (2001) reporting it to be 24 sec in solutions buffered to pH 2.

Only three other NO-donor compounds have been utilised and each in just one postharvest study. These compounds are: Piloty's acid (*N*-hydroxybenzenesulfonamide), *N*-tert-butyl- α -phenylnitron (PBN) and 3-morpholinosyl-nominine (Sin-1).

14.4 Effects of NO on Intact Produce

The postharvest behaviour of horticultural commodities traditionally classifies individual produce into two groups - climacteric and non-climacteric produce. Climacteric produce which can be fruits (such as banana and tomato) or ornamentals (such as carnation) have a definite ripening phase which is characterised by a marked increase in respiration and ethylene production and the produce are transformed into the desirable colour, texture and flavour of edible produce. They are generally harvested at a mature but unripe stage with the aim of preventing ripening occurring until produce reach the wholesale or retail market. Non-climacteric produce which can be fruits, vegetables or ornamentals that do not undergo a marked physiological change after harvest but follow a general senescence pathway which can be accelerated by exposure to enhanced ethylene concentrations. Research with climacteric produce often aims to inhibit the initiation of the ripening process, while studies with non-climacteric produce aim to inhibit general senescence (Wills et al. 2007a).

14.4.1 Effects of NO gas

The first reported postharvest study with exogenous application of NO was conducted by Leshem et al. (1998) who treated strawberry, broccoli, cucumber, Chinese broccoli, kiwifruit and mushroom with 0.05-0.25 $\mu\text{mol.l}^{-1}$ NO gas in a nitrogen atmosphere for 2-16 hr followed by storage in air at 20°C and found a 70-180 % extension of postharvest life compared to untreated produce. Ku et al. (2000) then reported that rate of water loss was reduced by about 20% from eleven fruits and vegetables and five cut flowers following a 24 hr exposure to NO in nitrogen but did not measure the effect beyond this period.

Studies on the effects on NO on climacteric fruit would seek inhibition of ripening as the desired outcome. Indeed numerous studies on a range of climacteric fruits have shown that fumigation with NO gas can regulate ethylene biosynthesis which is the trigger for initiation of ripening (Wills, 2007a). In mango fruit, Zaharah and Singh (2011b) showed that NO fumigation (20 $\mu\text{l.l}^{-1}$ NO in air for 2 hr at 21°C) inhibited ethylene biosynthesis through inhibition of the activity of the ethylene biosynthetic

enzymes 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), leading to reduced level of the immediate precursor of ethylene (ACC) in the fruit pulp. These two key enzymes (ACS and ACO) effectively regulate ethylene production (Alexander and Grierson, 2002). The consequent lower levels of ethylene reduced the activity of fruit softening enzymes (polygalacturonase and pectinesterase) but increased the level of endo-1,4-D-glucanase in pulp tissue during ripening and cool storage. In tomato fruit, Eum et al. (2009b) showed that fruit at different ripening stages were affected by different concentrations of NO fumigation as mediated through different ripening genes.

The regulation of ethylene has significant physiological effects in fruit which significantly affect ripening and senescence. For example in peaches, Zhu et al. (2006) found that fruit treated with 5 and 10 $\mu\text{l.l}^{-1}$ NO gas in air for 3 hr delayed ripening, as measured by fruit firmness, through reduced ethylene biosynthesis. They also reported that NO inhibited lipoxygenase (LOX) activity and suggested the decrease in LOX activity might be a collateral process with the inhibition of ethylene biosynthesis. Flores et al. (2008) also found that peaches fumigated with 5 $\mu\text{l.l}^{-1}$ NO gas in nitrogen for 4 hr at 20°C resulted in a reduced respiration rate. They further showed NO treated fruit remained firmer after storage and the degree of disintegration of cell membranes, as assessed as the percentage of electrolyte leakage, was also lower in fruits treated with NO. Singh et al. (2009) reported that Japanese plums fumigated with NO in air had a reduced rate of respiration and ethylene production rates during ripening at 21°C. They further showed that NO-fumigation caused a 3-4 day delay in ripening as evidenced by restricted skin colour changes and retarded softening. In pears, Sozzi et al. (2003) fumigated mature fruit with 10 $\mu\text{l.l}^{-1}$ NO gas in air for 2 hr and showed decreased ethylene production, while fumigation with 10 and 50 $\mu\text{l.l}^{-1}$ NO for 12 hr delayed skin yellowing but did not affect fruit softening. They concluded that the NO treatments had differential effects on pear ripening and suggested a time $\times$ concentration effect existed for applied NO.

Chilling injury is a major storage problem for some horticultural crops (particularly of crops of tropical and subtropical origin) when they are stored at low temperatures (Wills et al. 2007a). NO has been implicated in improving chilling

tolerance. Zaharah and Singh (2011a) fumigated mangoes with 10, 20 and 40 $\mu\text{l.l}^{-1}$ NO gas in air for 2 hr and showed that NO treatment not only delayed ripening (as defined by softening and colour changes) during storage at 5°C for 2 and 4 weeks but also reduced the incidence of chilling injury. Singh et al. (2009) also reported that Japanese plums fumigated with 10 $\mu\text{l.l}^{-1}$ NO in air showed reduced chilling injury symptoms during storage at 0°C for 6 weeks. Zhu et al. (2010) further found that fumigation with 15 $\mu\text{l.l}^{-1}$ NO gas with intermittent warming cycles of 1 day at 25°C after 14 and 28 days at 5°C was effective in preventing mealiness in peaches during storage at 5°C. Xu et al. (2012) demonstrated that endogenous NO plays a role in chilling injury development. They showed that loquat fruit stored at 1°C induced the accumulation of endogenous NO but pre-treatment with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) not only abolished endogenous NO accumulation but also enhanced chilling injury symptoms. The action of NO in alleviating chilling injury symptoms was ascribed to stimulating the antioxidant defence systems in the fruit.

Wu et al (2012) found that fumigation of the climacteric Chinese bayberry (*Myrica rubra*) fruits with 20 $\mu\text{l.l}^{-1}$ NO gas in nitrogen for 2 hr inhibited ethylene production and disease incidence, and delayed the decrease in firmness, total phenolics and DPPH radical-scavenging activity. They also suggested that NO might maintain the balance between the formation and detoxification of reactive oxygen species (ROS) and enhance the resistance of tissues to decay. Indeed NO seems to play a regulatory role in the antioxidant balance within the fruit. Flores et al. (2008) also measured a range of antioxidant enzymes and systems and showed that fumigation with NO seemed to have a beneficial effect on the oxidation equilibrium and the antioxidant capacity of peach fruit. Zhu et al. (2008) also found NO in solution significantly reduced the accumulation of malondialdehyde (MDA), superoxide and hydrogen peroxide, delayed the decrease in vitamins C and E, maintained the content of soluble solids, inhibited the activity of LOX and peroxidase (POD) and increased the activity of superoxide dismutase (SOD) and catalase (CAT) in kiwifruit during storage.

Research on the effect of NO gas on non-climacteric produce was reported by Wills et al. (2000) who examined strawberries stored at 20°C and 5°C in humidified air

containing 0.1 $\mu\text{l.l}^{-1}$ ethylene and found that fumigation with NO gas in nitrogen resulted in an increase in postharvest life with the most effective concentration being 5 to 10 $\mu\text{l.l}^{-1}$ NO at both temperatures. Soegiarto and Wills (2004) fumigated broccoli, green beans and bok choy (*Brassica chinensis*) with NO gas in air for 2 hr then stored produce at 20°C in air containing 0.1 $\mu\text{l.l}^{-1}$ ethylene. All produce showed an extension in postharvest life through inhibition of yellowing but the optimum concentration of NO differed markedly, being about 50 $\mu\text{l.l}^{-1}$ for green bean and bok choy and 4000 $\mu\text{l.l}^{-1}$ for broccoli. Eum et al. (2009a) later reported that NO fumigation with 1000 $\mu\text{l.l}^{-1}$ in nitrogen for 5 hr delayed yellowing and retarded the onset of chlorophyll degradation in broccoli florets.

Soegiarto and Wills (2006) examined the interaction of NO fumigation and modified storage atmospheres with strawberries and iceberg lettuce. The effect of low O₂ was examined by fumigating with 10 and 100 $\mu\text{l.l}^{-1}$ NO gas in air and 2% and 5% O₂ in air followed by storage at 10°C for strawberries and 5°C for lettuce in the same atmosphere. They found an increase in postharvest life due to NO and to storage in low O₂ but when both treatments were applied concurrently there was no additional increase in postharvest life. The effect of elevated carbon dioxide (CO₂) and reduced O₂ was also examined by placing produce in a sealed polyethylene bag and injecting NO gas at 10 and 20 $\mu\text{l l}^{-1}$ into the bag. The atmosphere stabilised after 7 days at about 20% CO₂ and 3% O₂ for strawberries and 5% CO₂ and 13% O₂ for lettuce. The results showed that the postharvest life was enhanced by NO fumigation and was further enhanced by co-application of the modified atmosphere (Soegiarto and Wills, 2006).

The role of NO in improving the tolerance of non-climacteric fruit against chilling injury was examined by Yang et al. (2011). They treated cucumber fruit with 25 $\mu\text{l.l}^{-1}$ NO for 12 hr at 20°C then stored the treated fruit at the chilling temperature of 2°C for 15 days. The application of NO reduced the increases in membrane permeability and lipid peroxidation associated with chilling injury and delayed the increases in both the superoxide anion (O₂^{•-}) production rate and hydrogen peroxide content. The NO-treated fruit exhibited significantly higher activities of SOD, CAT, ascorbate peroxidase (APX), POD and higher DPPH-radical (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity than

control fruit during the storage. The overall results suggest that NO enhanced chilling tolerance in cucumber fruit by improving the antioxidative defence system.

Zhu et al. (2009) found that Chinese winter jujube fumigated with 20 $\mu\text{l l}^{-1}$ NO gas in nitrogen at 22°C for 3 hr inhibited polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities. Indeed, they also showed that treatment with different NO concentrations from a saturated solution diluted to less than 1 $\mu\text{mol.l}^{-1}$ exhibited inhibitory effects on *in vitro* PPO and PAL activities in a dose-dependent manner.

Dong et al. (2012) found that mushrooms fumigated with 10, 20 and 30 $\mu\text{l.l}^{-1}$ NO gas in air for 2 hr had increased antioxidant activities as determined by assays of reducing power, chelating effect on ferrous ions, scavenging effect on hydroxyl free radicals, and DPPH radical scavenging activity. Furthermore, they found that NO fumigation significantly enhanced phenolic and flavonoid contents and stimulated the activities of PAL and chalcone synthase.

14.4.2 Effects of NO Donor Compounds

The most studied NO donor compound with climacteric fruit has been SNP. Sis et al. (2012) showed a 1 mM SNP aqueous dip treatment for 5 min could prolong the postharvest life of peaches with a reduced ethylene production rate and increasing firmness and antioxidant enzymes activity. Zhang et al. (2008) found reduced flesh browning in plum during storage at 2°C following dipping in 1 mM SNP for 3 min but no treatment had any effect on PPO activity. Lai et al. (2011) showed that a 1mM SNP dip applied to tomatoes delayed ripening and enhanced resistance to fungal pathogens such as *Botrytis* spp. They further showed that the activity of antioxidant enzymes in NO-treated fruit was higher late in the storage period compared to the control. They concluded that NO suppressed ethylene biosynthesis, stimulated antioxidant enzymes activity and regulated the expression of age-related genes.

The use of SNP has been found beneficial in the elucidation of chilling injury. Zhao et al. (2011) showed that a 0.02 mM SNP dip protected tomatoes from cold injury by inducing NO accumulation and expression of a C-repeat/dehydration-responsive element (CRT/DRE)-binding factors (CBFs) which play a prominent role in cold response regulation. They showed SNP treatment protects tomatoes from cold injury by

inducing expression of LeCBF1 and that nitric oxide synthase (NOS) activity may play a role in NO accumulation associated with cold tolerance in tomato fruit.

For non-climacteric produce, Zhu and Zhou (2007) dipped strawberries in 5 $\mu\text{mol.l}^{-1}$ SNP in water for 2 hr at 25°C and found it inhibited ethylene production, respiration rate, activity of ACS and reduced the level of ACC. Unfortunately the storage quality of the fruit in this trial was not quantified. Cheng et al. (2009) showed that mature green banana slices treated with 5 mM SNP solution had reduced ethylene production which was associated with reduced ACO activity and expression of the *MA-ACO1* gene. Duan et al. (2007) reported that dipping longan fruit in 1 mM SNP for 5 min inhibited pericarp browning and pulp breakdown and reduced PPO activity and the level of MDA, a marker of lipid peroxidation (Mittler, 2002), but SNP treatment maintained higher levels of total soluble solids and ascorbic acid during storage. Liu et al. (2012) found that Glorious oranges dipped in 30, 50 and 100 $\mu\text{mol.l}^{-1}$ SNP for 10 min maintained a higher level of titratable acidity, soluble protein, ascorbic acid and reducing sugar, and a lower weight loss and soluble solids concentration as well as retarded ripening.

The initial study with DETANO was by Noritake et al. (1996) who applied DETANO to growing potato plants and found it induced accumulation of the phytoalexin rishitin in potato tubers. The result suggests a key role for NO in the synthesis of the defence mechanism in plants possibly in response to abiotic stress. The only postharvest study with DETANO on intact fruit and vegetables was by Jiang et al. (2011) who dipped button mushrooms for 10 min in DETANO and stored produce in sealed modified atmosphere biorientated polypropylene bags at 4°C for up to 16 days at 4°C. They found that treatment with 1 mM DETANO maintained a higher level of firmness, delayed browning and cap opening, promoted the accumulation of phenols, ascorbic acid and reduced the increases in both respiration and hydrogen peroxide content. Furthermore, the DETANO dip inhibited PPO activity and increased the activity of the antioxidant enzymes CAT, SOD and APX throughout storage period. Unfortunately, Jiang et al. (2011) did not measure the effect of the treatments without MA packaging and hence it is not known whether the effects of the NO and MAP treatments were additive.

Postharvest application of DETANO has also been reported for cut flowers (see Section 15.6) and fresh-cut produce (see Section 15.5)

14.5 Effects of NO on Fresh-Cut Produce

Due to increased consumer interest, fresh-cut produce is a growing sector of the food service industry, however, fresh-cuts are more perishable than intact produce arising from significant abiotic stresses during preparation which involves removal of protective epidermal cells. This leads to reduced shelf-life due to an increase in respiration, ethylene evolution, water loss, alterations in flavour and aroma and enhanced surface browning (Watada et al. 1996; Artes et al. 1998).

The development of browning on the cut surfaces of apple slices was examined by Pristijono et al. (2006). They fumigated Granny Smith apple slices with NO gas in air and showed a delay in the onset of browning on the apple surface during storage at 0°C with the most effective treatment being fumigation with 10 $\mu\text{L.l}^{-1}$ NO for 1 hr. They also reported that Royal Gala, Golden Delicious, Sundowner, Fuji and Red Delicious apple slices stored at 10 °C and fumigated with 10 $\mu\text{L.l}^{-1}$ NO for 1 hr was effective in inhibiting surface browning. Pristijono et al. (2008) further examined the effect of DETANO on browning of apple slices and found that dipping in 10 mg.l^{-1} DETANO in pH 6.5 phosphate buffer for 1 min was the most effective treatment in inhibiting surface browning of apple slices stored at 0°C with the postharvest life more than double that of untreated slices. They showed it was necessary to buffer the DETANO solution to be slightly acidic, probably due to the apple vacuolar acids leaking into the dip solution and degrading the DETANO before it was absorbed into the apple slice. Pristijono et al. (2008) also compared the effect of dipping in DETANO solution and fumigating with NO gas and found that DETANO was more effective than NO gas in inhibiting browning development. Huque et al. (2013) confirmed that dipping in a buffered DETANO solution was more effective than fumigation with NO gas in inhibiting surface browning at 5°C. They also reported that DETANO and NO gas resulted in a lower level of total phenols, inhibition of PPO activity, reduced ion leakage and reduced rate of respiration but had no significant effects on ethylene production or lipid peroxide level as measured by MDA and hydrogen peroxide levels with DETANO having a greater effect than NO

gas. In addition, they found that apple slices dipped in aqueous chlorogenic acid solution enhanced browning development but subsequent dipping in DETANO solution negated the effect of chlorogenic acid while fumigation with NO gas gave partial relief. Huque et al. (2013) suggested that an increase in phenols occurs on the apple surface soon after cutting, possibly as a defensive mechanism of the apple to limit damage to surface cells and the effectiveness of NO to inhibit surface browning may relate to minimising the level of phenols active on the cut surface possibly in conjunction with a reduced PPO activity.

Huque et al. (2013) also found that the NO-donor compound, Piloty's acid inhibited browning with the optimum aqueous dip solution concentration being 100 mg.l⁻¹. They compared the optimum concentrations of 500 mg l⁻¹ SNP and 100 mg.l⁻¹ Piloty's acid dissolved in water with 10 mg l⁻¹ DETANO in phosphate buffer and fumigation with NO gas to inhibit browning. While all forms of NO extended the postharvest life of apple slices, the order of effectiveness was DETANO > SNP > NO gas = Piloty's acid.

The effect of NO gas and DETANO on fresh-cut iceberg lettuce was examined by Wills et al. (2008). They found development of browning on the cut surface during storage at 0°C was inhibited with the optimal treatments being dipping in 500 mg.l⁻¹ DETANO in water for 5 min and fumigation with 500 µl.l⁻¹ NO for 1 hr with DETANO more effective than NO gas. The use of a water dip compared to the need for a buffered dip with apple slices was attributed to the higher pH of lettuce tissue not degrading DETANO in solution. Huque et al. (2011) extended the findings to four other types of fresh-cut lettuce (*Lactuca sativa*), Green Oak, Green Coral, Baby Cos, and Butter lettuces, during storage at 5°C. They reported that fumigation with NO gas for 2 hr, or dipping in a solution of DETANO dissolved in 0.01 M phosphate buffer pH 6.5 or dipping in an aqueous solution of SNP, all inhibited the development of browning. The optimum treatments to suppress surface browning were dipping in 500 mg.l⁻¹ DETANO or SNP and fumigation with 100 µl l⁻¹ NO gas, with the donor compounds more effective than NO gas. Dipping lettuce slices in 500 mg l⁻¹ SNP was considered to be the most feasible commercial option due to the stability of SNP in unbuffered water.

Zhu et al. (2009) fumigated pre-climacteric peach slices with a 5 μM NO solution for 10 min followed by storage at 10°C for up to 10 days. Treatment with NO suppressed the increased rate of leakage and thus maintained compartmentalisation between the enzymes and their substrates. They also reported that NO increased PAL activity and total phenol content, and inhibited PPO and POD activity. PPO is a complex enzyme with two copper ions in its active centre and they speculated that NO could react with the copper of PPO to form copper–nitrosyl complexes which could change the normal structure of the active site in PPO and thus reduce PPO activity

NO may also be a promising method for maintaining the quality of peeled bamboo shoots. Yang et al. (2010) showed that bamboo shoots dipped in 0.5mM SNP for 1 hr had delayed onset of external browning during storage for 10 days at 10°C. SNP also inhibited PPO, POD and PAL activity and maintained total phenol content during storage. Furthermore SNP treatment inhibited the synthesis of lignin and cellulose and delayed tissue lignification.

14.6 Effects of NO on Ornamentals

Leshem et al. (1998) assessed postharvest senescence of Pink Sim and White-Sim carnation flowers when continually exposed to a solution containing the NO-donor compounds *N*-*t*-butyl- α -phenylnitrone (PBN) and 3-morpholinosyl-nonimine (Sin-1) in the presence of the senescence promoting ethylene precursor, ACC. They found the NO releasing compounds inhibited the action of ACC as observed by petal infolding and wilting.

Bowyer et al. (2003) treated White Sim carnation cut flowers with NO by fumigation with gas in nitrogen for 1 hr and by standing flower stems in an aqueous DETANO solution for 24 hr. All flowers were then stored at 20°C in the presence of 0.1 $\mu\text{l.l}^{-1}$ ethylene. They found that both NO gas and DETANO increased vase life with the optimum concentrations being 1 $\mu\text{l.l}^{-1}$ for NO gas and 10 mg l^{-1} for DETANO. Buffering the solution to pH 6.5 gave a further small but significant increase in vase life. While no direct comparison was made of their relative effectiveness, the data suggests DETANO may give a greater extension in vase life than NO gas. Badiyan et al. (2004) further explored the potential of DETANO with eight flower types with a span in range of

sensitivity to ethylene. Chitchat snapdragon, Bellissimo delphinium, Regan chrysanthemum, Golden Brush tulip, Manovale gerbera, Specisiom Simphon oriental lily, Carnavale rose and Blue Magic iris cut flower stems were placed in a solution of 10 and 100 mg.l⁻¹ DETANO buffered to pH 6.5 and after 24 hr were placed at 20°C in air containing 0.1 µl.l⁻¹ ethylene. All flowers showed an extension in vase life over control flowers but with a wide range in level of extension. The greatest benefit of NO treatment was obtained with gerbera and the least was with chrysanthemum and rose. Zeng et al. (2011) studied the effect of SNP on Monte carnation cut flowers and found dipping in 0.1 mmol.l⁻¹ SNP extended the vase life and maintained fresh weight during storage. They concluded that NO could delay petal wilting in carnation cut flowers, maintain water metabolism, cell membrane stability and the activity of antioxidative enzymes (SOD, POD, CAT and APX) as well as eliminate ROS.

14.7 Mode of Action of NO on Postharvest Produce

The initiation of postharvest research with NO was based on the findings by Leshem and Haramaty (1996) on pea leaves that NO and ethylene had an antagonistic relationship. The ability of NO to inhibit ethylene production through inhibition of the ethylene biosynthetic enzymes, ACS and ACO, resulting in a reduced level of ACC has now been demonstrated for a range of climacteric and non-climacteric produce and can be promoted as one of the key effects of NO that inhibit ripening and delay senescence. However, it is noted that Huque et al. (2013) did not find any reduction in ethylene production in the NO-induced inhibition of browning of apple slices. They attributed the lack of effect of NO as due to the apples being post-climacteric and thus having a substantial production of ethylene. Nonetheless, the data would suggest that ethylene is either not a direct causative factor in surface browning of apples or NO can inhibit browning through other modes of action.

A common finding of many studies with applied NO is a reduced the rate of respiration. This is consistent with findings of Millar and Day (1996) and Zottini et al. (2002) that NO affects the function of mitochondria in plant cells and reduces cell respiration by inhibiting the cytochrome pathway. NO can then be ascribed an anti-senescent action which could arise from a general reduction in the rate of cellular

metabolism. Whether this occurs via inhibition of ethylene action or independently needs to be determined. Other effects such as reduced ion leakage could be ascribed as a side benefit of better maintenance of cellular integrity through reduced general metabolism.

The role of NO in reducing oxidative stress and its involvement in plant signalling (Besson-Bard et al. 2008) would seem to also apply to postharvest tissue. Reduced lipid oxidation caused by ROS is purported to be a major cause of membrane deterioration in plant tissues (Mittler, 2002). Reduced levels of biomarkers for lipid peroxidation, MDA and hydrogen peroxide, have been found for various postharvest produce while NO has also been found to stimulate the activity of a range of antioxidant enzymes and enhance PAL activity which has been implicated in defence mechanisms. Indeed, the stimulation of the phytoalexin, rishitin in growing potato tubers by NO (Noritake et al., 1996) may be a NO-moderated response to either abiotic stress or pathogenic invasion. Work is needed to examine whether postharvest NO application can stimulate phytoalexins in other tissues and thereby reduce the need for chemical biocides to combat microbial infections. It is noted that Lazar et al. (2008) in an *in vitro* study found that NO gas inhibited spore germination, sporulation and mycelial growth of three common postharvest fungi, *Aspergillus niger*, *Penicillium italicum* and *Monilinia fructicola*. NO may thus have a dual role in reducing the spread of postharvest diseases.

Inhibition of PPO activity by NO has been demonstrated in many intact and fresh-cut produce and was associated with reduced internal and surface browning. Zhu et al. (2009) speculated that NO could react with the copper to produce a copper-nitrosyl complex that was metabolically inactive. There may be applications with processed fruits and vegetables where browning of juices and purees during storage is a common problem. Pre-treatment with NO to reduce PPO activity may enhance the storage life of the processed product. The action of NO on the phenolic substrates for PPO action is not clear as the various studies give conflicting data with some showing a reduced level of phenols while others show an enhanced level.

Alleviation of chilling injury in a range of produce by NO is a recent expansion of the mode of action of NO. Current suggestions are that the beneficial effect of NO is due to enhancing the natural antioxidant defence systems. In addition, the data on loquat fruit by Xu et al. (2012) indicate that endogenous NO production is a natural defence

mechanism triggered when produce are subject to abiotic chilling stress. Application of exogenous NO would therefore seem to be enhancing a natural response.

The findings of the only study by Soegiarto and Wills (2006) where NO and a modified atmosphere were simultaneously applied (with appropriate air controls) showed that exposure to both NO and elevated CO₂ gave an added extension in postharvest life of strawberries and lettuce over the extension due to the individual component whereas exposure to NO and reduced O₂ gave no added benefit. The findings suggest that NO and CO₂ have a different mode of action while that of O₂ and NO is similar and hence not additive. The benefits of modified atmospheres are generally considered to be through an inhibition of respiration via a feedback signal from the respiratory gases.

Despite the use of NO as a gas and various donor compounds in the reported studies, all forms gave a beneficial effect for inhibiting postharvest changes and therefore extending shelf life. However, the different forms of NO degrade to release different NO moieties. DETANO, Piloty's acid and NO gas release the NO[•] free radical and SNP releases the NO⁺ cation (Zamora et al. 1995; Hou et al. 1999; Saavedra and Keefer 2002). It might be expected that each NO moiety has different reactivity on produce metabolism with the moiety more directly linked to the key metabolic action(s) being more effective in inhibiting postharvest change. Since all NO compounds produced some beneficial effect, it would seem likely that the released NO moieties are inter-convertible to some extent.

Direct comparison of the effectiveness of different forms of NO has only been conducted with fresh-cut apples and lettuces and then only in relation to development of surface browning. The applicability of conclusions drawn from these studies to ripening and general senescence of intact produce must therefore be made with some caution. For apple slices, Huque et al. (2013) found all four NO forms inhibited development of browning with DETANO > SNP > NO gas = Piloty's acid while for the four lettuces evaluated, Huque et al. (2011) found all three forms of NO examined inhibited browning with SNP = DETANO > NO gas. As an applied treatment, fumigation with NO gas is therefore less effective than dipping in DETANO and SNP solutions.

However, in determining the relative effectiveness of each NO form, the effect of the solvent must also be considered. In the above studies, DETANO was dissolved in

phosphate buffer, and SNP and Piloty's acid in water while NO gas has no solvent. Since both phosphate buffer and water alone inhibit browning, the relative effectiveness NO gas and DETANO would be, respectively, higher and lower than that found for the actual applied treatments. For the lettuces, Huque et al. (2011) calculated the effectiveness of NO as a percentage of the respective control treatments as $\text{SNP} > \text{NO gas} > \text{DETANO}$.

While a range of effects of NO on metabolism of horticultural produce have been identified, it is not clear whether the various actions of NO are at the chemical enzymic or genetic level, and which are the key reactions that may trigger a cascade of supporting reactions. However, a growing number of studies are beginning to examine the effects of NO at the genetic level. Zhao et al. (2011) demonstrated that an elevated NO content up-regulated the expression of a cold regulation gene (LeCBF1) and also induced antioxidative processes to scavenge ROS resulting in treatment improved cold tolerance in tomatoes in cold storage. While Eum et al. (2009b) showed that NO has the potential to regulate ethylene biosynthesis. It is conceivable that NO may be acting on a range of systems which have different levels of importance in different produce and in response to differing abiotic stresses.

Additional studies would also seem to be required to determine the level and activity of endogenous NO at the stage of commercial maturity of individual horticultural produce and therefore whether exogenous NO is accentuating an active system. The early studies by Leshem (2000) indicated that the level of endogenous NO in mature produce is relatively low. Studies that utilise NO scavengers as used by Xu et al. (2012) would seem to be useful. Examination of the interaction between applied ethylene and NO would appear worthwhile given the central role of ethylene in promotion of ripening and general senescence. It could be reasoned that NO has a major role in maintaining cellular integrity and organisation during produce development and maturation but is replaced by the deteriorative effect of ethylene in mature produce which have reached the end of their developmental life. Application of exogenous NO could then be analogous to applying a juvenile hormone to prevent ageing.

14.8 Commercial Usage

Commercial postharvest usage of NO on fruit and vegetables would require approval from the regulatory agencies in the respective countries. Since NO is synthesised by plants it can be considered as a naturally occurring material which would assist in gaining regulatory approval. The human synthesis of NO and the use of various forms of NO in therapeutic applications should also be positive factors. However, plants and mammals do not have totally identical pathways for metabolism of NO. Of particular interest is the presence in higher plants of nitrate reductase which is a key enzyme that catalyses the conversion of nitrate to nitrite (Yamasaki and Sakihama 2000). Nitrites have the potential to be converted to nitrosamines which are potent carcinogens (Walker 1990). The addition of NO gas or individual donor compounds would need to be shown to not generate nitrosamines. The use of NO with ornamentals does not pose such a regulatory hurdle and should be easier to obtain.

Use of NO gas would require gas-tight fumigation chambers but this is not an insurmountable issue particularly if added to air. A more convenient delivery system could be along the lines proposed by Wills et al. (2007b) of solid mixture that releases NO gas when placed with horticultural produce (see Section 14.3.1). Tablets or sachets of the mixture would be placed in packages in a packing house. The package would not need to be sealed as the steady release of NO gas over a few days would be rapidly absorbed by the surrounding produce.

Of the NO donors presently evaluated, SNP would seem to offer the most potential due to its stability in water which eliminates the need for addition of a buffer. Water dipping is a common practice in many packing houses to clean and/or move produce around the packing house. DETANO has been shown to be more effective than SNP for some produce but is unstable at even mildly acidic pH (Hrabie et al. 1993). The quality of water used in commercial operations is variable and would require the use of a buffered solution to ensure a near neutral pH is maintained throughout dipping. For ornamentals, the addition of DETANO or some other solid NO-donor compound to vase water would seem to offer a commercial opportunity.

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