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# Effect of nitric oxide on ethylene production in strawberry fruit during storage

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**Abstract**

Strawberry is a non-climacteric fruit, with low ethylene production rate after harvest. Its response to nitric oxide (NO), which can be released from sodium nitroprusside (SNP), was studied. We have examined the effect of 1.0, 5.0 and 10.0 μmol $l^{-1}$ SNP aqueous solution on ethylene production, respiration rate, 1-aminocyclopropane-1-carboxylic acid (ACC) content and the activities of ACC synthase and ACC oxidase in post-harvest strawberry (''Fengxiang''). The most remarkable effect was obtained with 5 μmol $l^{-1}$ SNP aqueous solution, which significantly inhibited ethylene production, respiration rate, the activity of ACC synthase and reduced the content of ACC, but did not significantly affect the activity of ACC oxidase. SNP at 10 μmol $l^{-1}$ harmed the fruits; 1 μmol $l^{-1}$ SNP was too low to significantly extend strawberry storage life. It was suggested that NO could decrease ethylene output, through inhibiting ACC synthase activity reducing ACC content.


*Keywords:* ACC oxidase; ACC synthase; Ethylene; Nitric oxide; Sodium nitroprusside; Strawberry

## 1. Introduction

Ethylene is a plant hormone that controls many plant processes, such as seed germination, growth, ripening and senescence. Yang and Hoffman (1984) found that ethylene is produced from methionine via *S*-adenosyl-L-methionine (AdoMet) and the cyclic non-protein amino acid 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is formed from AdoMet, by the action of ACC synthase (ACS), and the conversion of ACC to ethylene is carried out by ACC oxidase (ACO) (Yang & Hoffman, 1984). Ethylene biosynthesis in higher plants is under strict metabolic regulation and is subject to induction by a variety of signals, including mechanical wounding, auxin, and fruit ripening. Inhibitors of ethylene production and/or action, such as 1-methylcyclopropene (Sisler, Serek, Dupille, & Goren, 1999), silver thiosulfate (Serek, Prabucki, Sisler, & Andersen, 1998) and aminoethoxyvinylglycine (Silverman, Petracek, Noll, & Warrior, 2004), will inhibit on delay ethylene biosynthesis, thereby prolonging fruit storage life.

Strawberry is a non-climacteric berry with a limited shelf life. Some works (Kim & Wills, 1998; Tian et al., 2000; Wills & Kim, 1995) showed that ethylene exposure could be a factor in strawberry deterioration. Methods for preventing ethylene accumulation or inhibiting ethylene action on strawberries (Bower, Biasi, & Mitcham, 2003; Sisler & Serek, 1999) would thus be of considerable commercial interest.

Due to its diverse biological activities and general ubiquity, nitric oxide (NO) has been widely studied in animal and botanic research since the 1980s (Delledonne, Xia, Dixon, & Lamb, 1998; Durner, Wendehenne, & Klessig, 1998; Furchgott & Zawadzki, 1980; Ignarro, Buga, Wood, & Byrns, 1987; Koshland, D.E., 1992; Palmer, Ferrige, & Moncada, 1987; Wang et al., 2004). NO is involved in vegetative stress and senescence of horticultural products (Leshem & Haramaty, 1996). Exogenous application of NO, either by direct fumigation in an $O_2$-free atmosphere

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or by means of NO releasing chemicals, such as *N-tert*-butyl-α-phenylnitrone and 3-morpholino sydnonimine, to markedly delay shelf life of strawberry or other horticultural products, has been reported (Leshem & Haramaty, 1996; Leshem & Wills, 1998). The emission of NO was negatively related with ethylene output in the process of maturation and senescence of fruits. Wills, Ku, and Leshem (2000) has demonstrated that the post-harvest life of strawberries could be extended by fumigating with NO. Our objective was to explore the mechanism(s) by which NO regulates ethylene production, based on information on the effects of NO on the activities of ACC synthase and ACC oxidase in strawberry fruits.

## 2. Materials and methods

### 2.1. Plant material and application of NO

Strawberry (*Fragaria ananassa* L.) fruits were obtained from a local orchard and harvested on the day of the experiment, then transported to the laboratory at Shandong Agricultural University, China. The fruits were sorted, so as to select only well-formed, rot-free, unblemished fruits with intact calyxes. Samples of fruits (500 g) were selected from five replicate groups. Each sample was placed in a container without lid.

### 2.2. NO treatments

Sodium nitroprusside (SNP), the donor of NO, was purchased from Beijing Shuanghe Medicine Ltd. Co. Four strawberry samples were dipped in 1.0, 5.0 or 10.0 μmol $l^{-1}$ SNP aqueous solutions for 2 h at 25 °C. The control was treated with water. The fruits were dried with cold gas and stored in air at room temperature (25 °C).

### 2.3. Rot index of fruits

In each treatment 50 fruits were selected for investigating the number of rotten fruits. All fruits were classified in four ranks by the extent of rot: 0, fruits were not rotten; 1, the rotten surface was less than 1/3; 2, the rotten surface was between 1/3 and 2/3; 3, the rotten surface was more than 2/3. The rot index was expressed as the following equation:

$$\text{Rot index} = \frac{\sum(\text{rank} \times \text{quantity})}{4 \times 50} \times 100\%.$$

### 2.4. Measurement of respiration rate

The respiration rates were measured using an EGM-4 $CO_2$ Analyzer (PP Systems, UK) calibrated earlier with standard $CO_2$. Strawberry fruits (500 g) were placed in a 10 l airtight chamber for 2 h, and air was passed through the chamber. The ratio of $CO_2$ was measured and the $CO_2$ production was calculated. The results were expressed as mmol $CO_2$ $kg^{-1}$ FW $h^{-1}$.

### 2.5. Measurement of ethylene production

Ethylene production was measured by incubating 500 g strawberry fruits in a 10 l airtight chamber for 2 h at room temperature (25 °C). A 5 ml sample of the headspace gas was withdrawn, using a gastight syringe, and injected into a gas chromatograph (GC-9A, Shimadzu, Japan) fitted with a GDX-502 column and a FID detector, column temperature 70 °C, injection temperature 120 °C. The carrier gas was $N_2$ with a flow rate of 40 ml $min^{-1}$. The rate of ethylene production was expressed as nmol $C_2H_4$ $g^{-1}$ FW $h^{-1}$. After the measurement of ethylene production, 100 g of strawberries were chosen randomly and frozen in liquid nitrogen for the following measurements.

### 2.6. Measurement of ACC synthase activity

Frozen strawberry pulp tissues (10 g) were homogenized in an extraction buffer, as described by Kato, Hayakawa, Hyodo, Ikoma, and Yano (2000). The homogenate was centrifuged at 25,000*g* for 20 min at 4 °C and the supernatant dialyzed for 12 h with 2 mmol $l^{-1}$ Hepes buffer, pH 8.5, containing 0.1 mmol $l^{-1}$ dithiothreitol, and 0.2 μmol $l^{-1}$ pyridoxal phosphate. The eluent was assayed for ACS activity with the method of Lizada and Yang (1979). ACS activity was expressed as μl $C_2H_4$ $g^{-1}$ FW $h^{-1}$.

### 2.7. Measurement of ACC oxidase activity

The ACO activity was measured in vitro. The homogenate (10 g pulp tissue) was prepared in extraction buffer as described by Moya-Leon and John (1994). The ACO activity was expressed as μl $C_2H_4$ $g^{-1}$ FW $h^{-1}$.

### 2.8. ACC content measurement

Frozen strawberry pulp (2 g) was homogenized in 10 ml of 80% ethanol at 4 °C and the sample was centrifuged at 13,000*g* for 25 min. The supernatant was evaporated under vacuum at 50 °C. Residues were taken up in distilled water and stored at 4 °C for one night. Aqueous solution (1 ml) was with drawn and ACC contents were measured by the method of Lizada and Yang (1979).

### 2.9. Statistical analysis

Each experiment was repeated three times and statistical analyses were performed using Microsoft Excel software. *P*-values were determined by *t*-test. Standard errors of the means were shown for the data points in each figure.

## 3. Results

### 3.1. Changes in rot index

The effect of NO on the rot index of strawberry is shown in Fig. 1. It was found that the controlled fruits and the

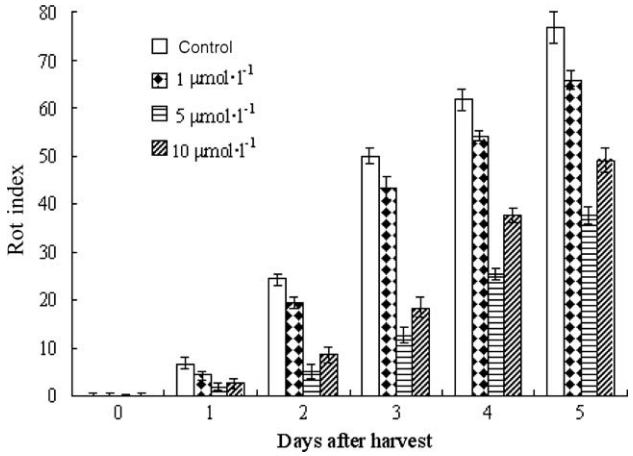



Fig. 1. Rot index of strawberry fruits treated with different concentration of SNP. Data are presented as means ± SE ($n = 5$).

strawberry fruits treated with 1 μmol $l^{-1}$ SNP started to rot at day 1 after harvest. For all treatments, the rot index began to increase after day 3. Especially, the 1 μmol $l^{-1}$ SNP-treated fruits ($P = 0.0001$ at day 3) and the control ($P = 0.0005$ at day 3) increased significantly. Rot indices of fruits treated with 5 and 10 μmol $l^{-1}$ SNP were significant lower ($P = 0.0007$ at day 3), and increased more slowly than the control. The rot index of 10 μmol $l^{-1}$ SNP-treated fruits was higher than 5 μmol $l^{-1}$ SNP at day 5 ($P = 0.0018$).

### 3.2. Changes in respiration rate and ethylene production

The respiration rates of strawberry fruits treated with NO and the control were measured during 5 days stored at 25 °C (Fig. 2). In contrast to the control, the respiration rate for the fruits treated with 1 μmol $l^{-1}$ SNP was not different significantly, but decreased for the fruits treated with 5 and 10 μmol $l^{-1}$ SNP on the first day of storage and then a gradual increase was observed for all the fruits after day 1 of storage. The 5 μmol $l^{-1}$ SNP treatment significantly inhibited ($P = 0.00025$) the respiration of strawberry at room temperature. The respiratory rate at day 5 was about 84% of the control. SNP at 1 μmol $l^{-1}$ did not affect significantly the respiration of strawberry ($P = 0.19$). SNP at 10 μmol $l^{-1}$ inhibited ($P = 0.017$) the respiration of strawberry, but the inhibition was lower than that of 5 μmol $l^{-1}$ SNP ($P = 0.00025$).

Ethylene production increased during storage for all SNP-treated fruits and the control (Fig. 3). The changed pattern was similar to that of the respiration rates. SNP treatments decreased ethylene production; 5 μmol $l^{-1}$ and 10 μmol $l^{-1}$ SNP treatments significantly ($P = 0.01$) slowed ethylene production down. No obvious difference existed between the control and 1 μmol $l^{-1}$ SNP ($P = 0.73$).

### 3.3. Changes in ACC content

Changes in ACC content in the pulp of the control and the SNP-treated strawberry fruit are shown in Fig. 4. The initial ACC content was 3.9 nmol $g^{-1}$ FW and the observed changes during storage for the control and all of the treatments were clearly different. ACC levels between the control and fruits treated with 1 μmol $l^{-1}$ SNP did not significantly vary during the five days of storage. They reached a peak on day 3 ($P = 0.0007$) and then there was a significant decline during the rest of the storage ($P = 0.001$). However, the ACC content in the 5 and 10 μmol $l^{-1}$ SNP-treated strawberry fruit pulp was lower than the control ($P = 0.0015$ and $P = 0.0052$, respectively) in the first three days. ACC levels kept high and were more than that of the control at day 4 ($P = 0.0034$) and then decreased.

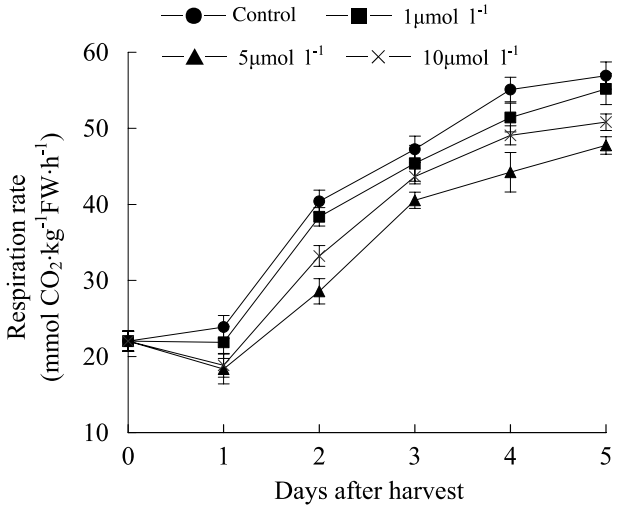



Fig. 2. Changes of respiratory rate ($CO_2$ evolution) of strawberry with NO treatment. Data are presented as means ± SE ($n = 5$).

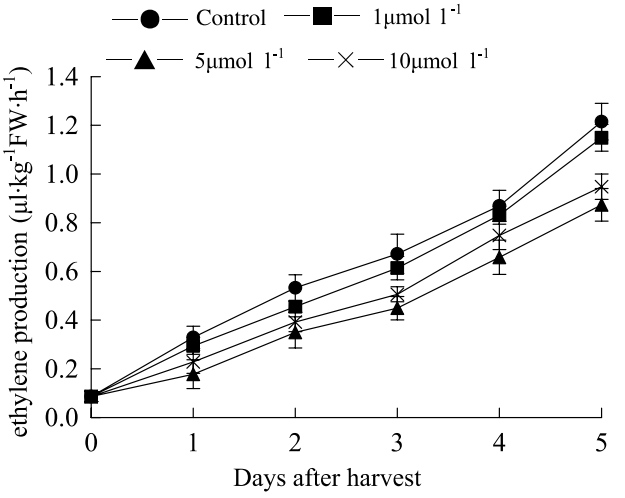



Fig. 3. Changes of ethylene production of strawberry with NO treatment. Data are presented as means ± SE ($n = 5$).

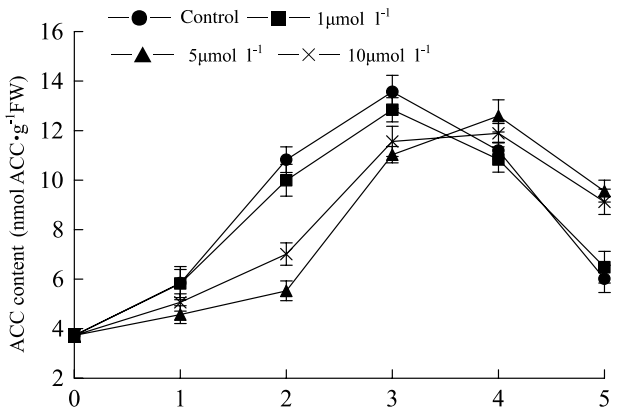



Fig. 4. Changes of ACC content of strawberry with SNP treatment. Data are presented as means ± SE ($n = 5$).

### 3.4. Changes in ACS activity

The ACS activity was analyzed in pulp of the control and the SNP-treated strawberry fruits during storage (Fig. 5). The ACS activity of the control the and 1 μmol $l^{-1}$ SNP-treated fruits increased by factors of 7 and 6.5, respectively, over the first three days and then gradually decreased over the last two days. The ACS activities of the fruits treated with 5 and 10 μmol $l^{-1}$ SNP were lower than the control at the same time from day 0 ($P = 0.016$ and $P = 0.018$, respectively) to day 3 ($P = 0.0015$ and $P = 0.001$, respectively). However, the ACS activity of these fruits exceeded that of the control and reached the maximum at day 4 ($P = 0.013$); a gradual decline then followed. The effect of 5 μmol $l^{-1}$ SNP was greater than that of 10 μmol $l^{-1}$ SNP ($P = 0.0028$). The difference between the ACS activities in the control and the 1 μmol $l^{-1}$ SNP-treated fruits was not significant.

### 3.5. Changes in ACO activity

In vitro ACO activity gradually increased from the beginning of storage, and reached a peak at day 3 for the control and 1 μmol $l^{-1}$ SNP-treated fruits and at day 4 for 5 and 10 μmol $l^{-1}$ SNP-treated fruits (Fig. 6). There was no obvious difference in ACO activities in the first three days ($P = 0.60$) for all of the fruits. However, the ACO activity in the fruits treated with 5 and 10 μmol $l^{-1}$ SNP exceeded the control at day 4 ($P = 0.003$ and $P = 0.007$, respectively). No significant difference ($P = 0.82$) existed between 1 μmol $l^{-1}$ SNP-treated fruits and the control during storage.

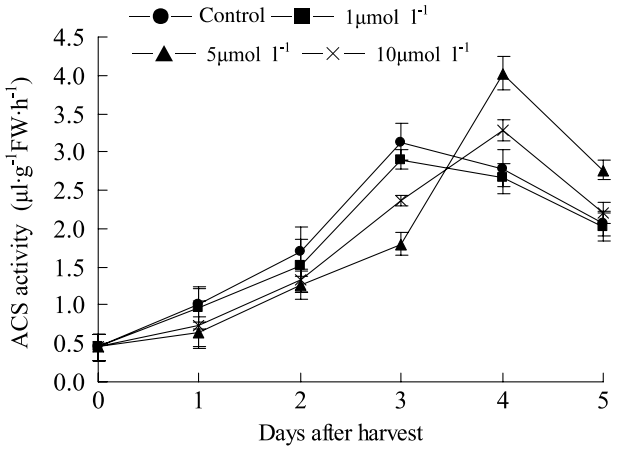



Fig. 5. Changes of ACC synthase activity of strawberry with NO treatment. Data are presented as means ± SE ($n = 5$).

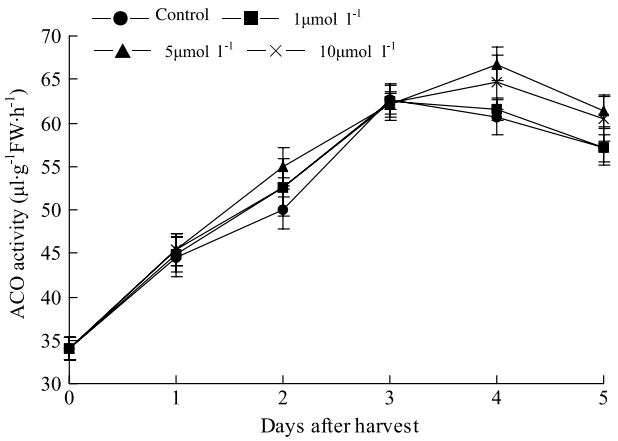
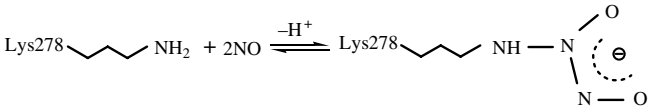



Fig. 6. Changes of ACC oxidase activity of strawberry with NO treatment. Data are presented as means ± SE ($n = 5$).

## 4. Discussion

Yang and Hoffman (1984) reported that the level of ACS was a limiting factor in the ethylene production cycle. The putative ACS catalytic mechanism for conversion of SAM to ACC comprised four essential stages (Huai et al., 2001): an internal aldimine intermediate with enzyme lysine, an external aldimine intermediate with SAM, SAM conversion, and transaldimination to release ACC. As an inter- and intracellular messenger involving in a vast of physiological and pathophysiological processes, NO interacts directly with biological molecules at low concentrations (Wink & Mitchell, 1998). ACC was released upon transaldimination by the Lys278 of ACS to complete the catalytic process as shown in Fig. 7. We think NO possibly reacts with the $-NH_2$ group of Lys278 as in the following equation (Drago & Paulik, 1960):




This process prevents to release ACC by the transaldimination reaction and deactivates ACS.

The present results showed that 5 μmol $l^{-1}$ SNP could extend the post-harvest life of strawberry fruits, confirming the research of Wills et al. (2000). The respiratory rate and ethylene production of strawberry fruits were correlated with fruit senescence, as were also found by Wu, Gu, Tai, and Liu (1992). In 5 μmol $l^{-1}$ SNP-treated strawber-

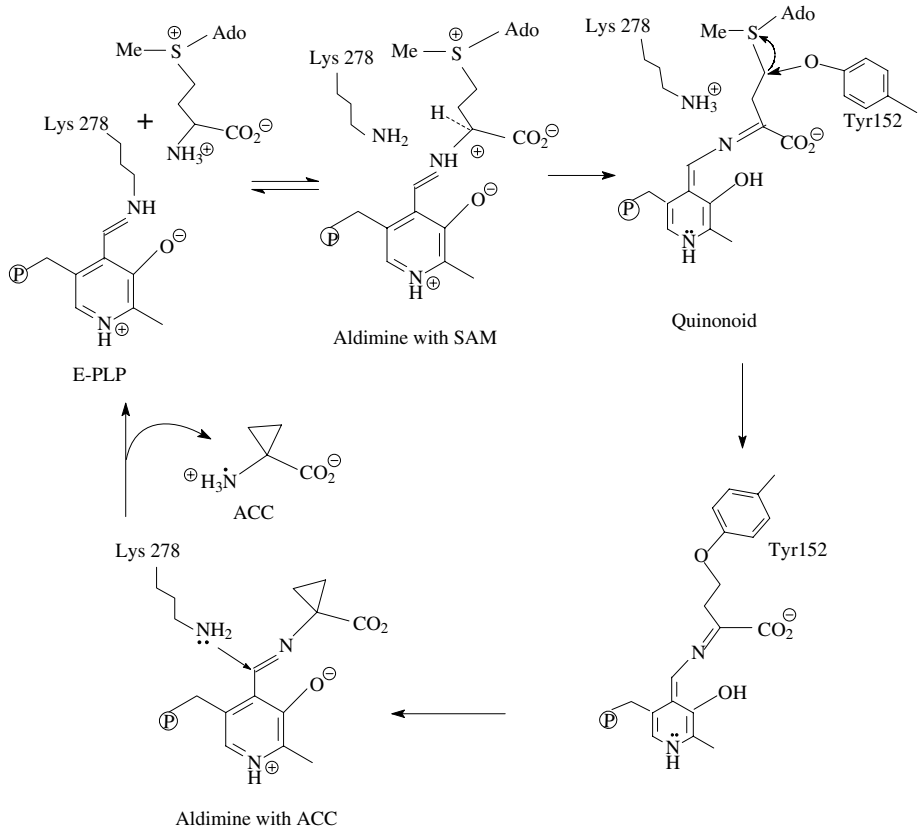



Fig. 7. The putative ACS catalytic mechanism for conversion of SAM to ACC.

ries, ethylene production and ACC content were decreased. The activities of ACS and ACO were also inhibited, in particular that of ACS. SNP at 10 μmol $l^{-1}$ could harm the fruits, demonstrating the toxicity of NO. These effects are similar to the observations of Leshem (Leshem & Wills, 1998; Leshem, Wills, & Ku, 1998). Bowyer and Wills (2003) suggested that the mode of action of NO differed from the current commercial treatments, such as silver thiosulfate and 1-methylcyclopropene. Based on these results, we tentatively state that NO treatment did not affect the conversion of ACC to ethylene, but prevented ACC synthesis, probably due to ACS deactivation. Hence, NO might inhibit ethylene biosynthesis. However, further work should be carried out to prove the mechanism.

## References


Bower, J. H., Biasi, W. V., & Mitcham, E. J. (2003). Effects of ethylene and 1-MCP on the quality and storage life of strawberries. *Postharvest Biology and Technology, 28*, 417–423.

Bowyer, M. C., & Wills, R. B. H. (2003). Delaying postharvest senescence of cut flowers using nitric oxide. *A Report for the rural industries research and development corporation*. RIRDC Publication.

Delledonne, M., Xia, Y. J., Dixon, R. A., & Lamb, C. (1998). Nitric oxide function as a signal in plant disease resistance. *Nature, 394*, 585–588.

Drago, R. S., & Paulik, F. E. (1960). The reaction of nitrogen (II) oxide with diethylamine. *Journal of American Chemical Society, 82*, 96–98.

Durner, J., Wendehenne, D., & Klessig, D. F. (1998). Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proceedings of the National Academy of Sciences, 95*, 10328–10333.

Furchgott, R. F., & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acethylcholine. *Nature, 288*, 373–376.

Huai, Q., Xia, Y., Chen, Y., Callahan, B., Li, N., & Ke, H. (2001). Crystal structures of 1-aminocyclopropane-1-carboxylate (ACC) synthase in complex with aminoethoxyvinylglycine and pyridoxal-5′-phosphate provide new insight into catalytic mechanisms. *Journal of Biological Chemistry, 276*, 38210–38216.

Ignarro, L. I., Buga, G. M., Wood, K. S., & Byrns, R. E. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences, 84*, 9265–9269.

Kato, M., Hayakawa, Y., Hyodo, H., Ikoma, Y., & Yano, M. (2000). Wound-induced ethylene and expression and formation of 1-amino-cyclopropane-1-carboxylate synthase, ACC oxidase, phenylalaninne ammonia lyase and peroxidase in wounded mesocarp tissue of *Cucurbita maxima*. *Plant and Cell Physiology, 41*, 440–447.

Kim, G. H., & Wills, R. B. H. (1998). Interaction of enhanced carbon dioxide and reduced ethylene on the storage life of strawberries. *Journal of Horticultural Science and Biotechnology, 73*, 181–184.

Koshland, D. E. (1992). The molecular of the year. *Science, 258*, 1861.

Leshem, Y. Y., & Haramaty, E. (1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. Foliage. *Journal of Plant Physiology, 148*, 258–263.

Leshem, Y. Y., & Wills, R. B. H. (1998). Harnessing senescence delaying gases nitric oxide and nitrous oxide, a novel approach to postharvest control of fresh horticultural produce. *Biologia Plantarum, 41*, 1–10.

Leshem, Y. Y., Wills, R. B. H., & Ku, V. V. V. (1998). Evidence for the function of the free radical gas-nitric oxide ($NO^{centerdot}$) – as an

endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry, 36*, 825–833.

Lizada, M. C. C., & Yang, S. F. (1979). A simple and sensitive assay for 1-amino cyclopropane-1-carboxylic acid. *Analytical Biochemistry, 100*, 140–145.

Moya-Leon, M. A., & John, P. (1994). Activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ethylene-forming enzyme) in the pulp and peel of ripening bananas. *Journal of Horticultural Science, 69*, 243–250.

Palmer, R. M., Ferrige, A. G., & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature, 327*, 524–526.

Serek, M., Prabucki, A., Sisler, E. C., & Andersen, A. S. (1998). Inhibitors of ethylene action affect final quality and rooting of cuttings before and after storage. *HortScience, 33*, 153–155.

Silverman, F. P., Petracek, P. D., Noll, M. R., & Warrior, P. (2004). Aminoethoxylglycine effects on late-season apple fruit maturation. *Plant Growth Regulation, 43*, 153–161.

Sisler, E. C., & Serek, M. (1999). Compounds controlling the ethylene receptor. *Botanical Bulletin of Academia Sinica, 40*, 1–7.

Sisler, E. C., Serek, M., Dupille, E., & Goren, R. (1999). Inhibition of ethylene response by 1-methylcyclopropene and 3-methylcyclopropene. *Plant Growth Regulation, 27*, 105–111.

Tian, M. S., Prakash, S., Elgar, H. J., Young, H., Burmeister, D. M., & Ross, G. S. (2000). Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. *Plant Growth Regulation, 32*, 83–90.

Wang, X., Tanus-Santos, J. E., Reiter, C. D., Dejam, A., Shiva, S., Smith, R. D., et al. (2004). Biological activity of nitric oxide in the plasmatic compartment. *Proceedings of the National Academy of Sciences, 101*, 11477–11482.

Wills, R. B. H., & Kim, G. H. (1995). Effect of ethylene on postharvest life of strawberries. *Postharvest Biology and Technology, 6*, 249–255.

Wills, R. B. H., Ku, V. V. V., & Leshem, Y. Y. (2000). Fumigation with nitric oxide to extend the postharvest life of strawberries. *Postharvest Biology and Technology, 18*, 75–79.

Wink, D. A., & Mitchell, J. B. (1998). Chemical biology of nitric oxide, insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radical Biology and Medicine, 25*, 434–456.

Wu, Y. M., Gu, G. Q., Tai, G. F., & Liu, Y. (1992). The role of ABA and Ethylene in the ripening and senescence of strawberry fruits. *Acta Phytophysiologica Sinica, 18*, 167–172.

Yang, S. F., & Hoffman, N. E. (1984). Ethylene biosyntheses and its regulation in higher plants. *Annual Review of Plant Physiology, 35*, 155–189.