Postharvest Senescence and Deterioration of ‘Thoroughbred’ and ‘Carlo’ Green Beans (*Phaseolus vulgaris* L.) in Response to 1-Methylcyclopropene

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Abstract. Nonclimacteric commodities produce low levels of ethylene yet remain quite sensitive to the growth regulator. 1-Methylcyclopropene (1-MCP; SmartFresh Quality System), an inhibitor of ethylene action, allows analysis of the effects of endogenous ethylene on the senescence of nonclimacteric commodities during storage. Two commercial cultivars (Thoroughbred and Carlo) of fresh green beans (*Phaseolus vulgaris* L.) were treated with 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP followed by storage at 7 °C. 1-MCP was effective in delaying color change, brown spot incidence, and watersoaking in both cultivars. 1-MCP was effective at delaying yellowing as indicated by a decline in surface hue and chroma; however, lightness was not significantly different between control and 1-MCP-treated beans. Respiration in both cultivars was suppressed, but ethylene production was stimulated late during storage in response to 1-MCP. The appearance of brown spot, a surface disorder possibly reflecting low-temperature injury, was delayed by ≈ 5 days, and the progression of the disorder was reduced in 1-MCP-treated beans. The incidence of watersoaking in ‘Carlo’ was reduced by 50% in 1-MCP-treated compared with control beans. 1-MCP alleviated symptoms of senescence and chill injury of green beans during long-term storage, implicating a role for endogenous ethylene in the senescence of nonclimacteric commodities after harvest.

Green or common beans (*Phaseolus vulgaris* L.) are harvested at a physiologically immature stage of development. Growth is rapid at the time of harvest and beans exhibit comparatively high respiration rates, even when held at low temperatures (Watada and Morris, 1967). Like with other nonclimacteric fruits and fruit-vegetables, green beans produce very low quantities of ethylene postharvest (Wills and Kim, 1996). For many nonclimacteric fruits, the impact of ethylene is generally believed to be limited to cases in which external levels of the gas exceed threshold values for induction of accelerated yellowing, increased microbial growth, physiological disorders, and development of undesirable flavors (Kader, 1985). In a detailed survey of ethylene responses in a range of nonclimacteric fruit and vegetables, the threshold concentrations for ethylene-induced responses were below 5 $\text{nL}\cdot\text{L}^{-1}$

(Wills et al., 1999). Biological activity at these levels would suggest that the low levels of ethylene produced by nonclimacteric fruits and vegetables might be sufficient to accelerate senescence and related disorders during prolonged storage. Consistent with this idea, pretreatment with 1-methylcyclopropene (1-MCP; SmartFresh Quality System, Agro-Fresh, Inc., Rohm and Haas, Philadelphia, PA), a potent inhibitor of ethylene perception and action (Serek et al., 1994; Sisler, 2006), significantly delayed symptoms of senescence in air-stored nonclimacteric watermelon (*Citrullus lanatus* Thunb. Matsum and Nakai) (Mao et al., 2004) and cucumber (*Cucumis sativus* L.) fruits (Lima et al., 2005). The ethylene production capacity of watermelon (Elkashif et al., 1989) and cucumber fruits (Nilsson, 2005) ranges from 50 to 100 $\text{nL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in the range of production values for green beans (Wills and Kim, 1996).

The purpose of the present study was to determine if postharvest storage life and quality in green beans could be extended by prestorage treatment with the ethylene action inhibitor 1-MCP.

Materials and Methods

Plant material and 1-methylcyclopropene treatment. Fresh green beans (*Phaseolus*

vulgaris L.) cultivars ‘Thoroughbred’ and ‘Carlo’ were obtained from commercial packing houses in south Florida on the days of harvest and transported to Gainesville within 4 h. The beans in their original packing containers were transferred to 7 °C and held overnight. The next day, the beans were sorted for uniform size and color and freedom from visible defects. After selection, the beans were placed in ventilated plastic containers (1.7 L, Model 3991A-4; Tupperware, Orlando, FL) that maintained an internal relative humidity of 93 to 95% [monitored with humidity sensors (H08-003-02; Onset Computer Corp., Bourne, MA)]. Treatments were distributed as a completely randomized design with 15 replicates per treatment, each replicate consisting of 50 green beans (274.5 ± 15.2 g fresh weight). Five of the replicates from each treatment were used for ethylene and respiration measurements, and the remaining 10 replicates were used for hue, chroma, and brown spot measurements.

The containers were placed in 174-L steel chambers and provided with either air or gaseous 1-MCP at 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ for 24 h at 7 °C. 1-MCP gas was generated by mixing SmartFresh powder (0.14% formulation; Agro-Fresh, Inc.) and 50 mL distilled water in a 100-mL flask. The flasks were placed in the chambers that were immediately sealed. After 12 h, the chambers were opened, ventilated, and the treatment repeated. After the air or 1-MCP treatments, the beans in plastic containers were removed from the chambers and placed in storage for up to 22 d at 7 °C.

Ethylene production and respiration rate. Ethylene and CO_2 were measured using five of the replicate 1.7-L containers described, each containing 50 green beans. The ventilated containers were sealed for 3 h and 1.0- and 0.5-mL headspace samples were removed with a syringe for CO_2 and ethylene measurements, respectively. CO_2 was determined using a Gow-Mac (Series 580, Bridge Water, NJ) gas chromatograph (GC) equipped with a thermal conductivity detector and a 1219 $\times$ 3.18-mm, 80/100-mesh Porapak Q column. The carrier gas (helium) flow rate was 30 $\text{mL}\cdot\text{min}^{-1}$. The detector and injector were operated under ambient conditions (24 to 26 °C) and the oven was at 40 °C. Ethylene was measured using a GC (Hewlett Packard 5890, Avondale, PA) equipped with an activated alumina stainless steel column (914 $\times$ 3.18 mm, 80/100 mesh) and flame ionization detector. The carrier gas (nitrogen) was 30 $\text{mL}\cdot\text{min}^{-1}$, air 300 $\text{mL}\cdot\text{min}^{-1}$, and hydrogen 30 $\text{mL}\cdot\text{min}^{-1}$. Injector, oven, and detectors were operated at 200, 70, and 250 °C, respectively. Ethylene and CO_2 were quantified by using standard gas mixtures of 0.9 $\mu\text{L}\cdot\text{L}^{-1}$ and 1.02%, respectively.

Color (hue angle, chroma, lightness). Color parameters were measured on 50 beans (five from each of the 10 replications) at a point midway along the bean axis using a colorimeter (Minolta CR-400, Ramsey, NJ) with an 8-mm diameter, illuminant C, and CIE $L^*a^*b^*$ color scale. A white calibration

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tile ($L^* 97.75$, $a^* -0.43$, $b^* +2.09$) was used to calibrate the colorimeter before each measurement and hue and chroma were calculated using the formulae [$\arctan(b^*/a^*)$] and [$(a^{*2} + b^{*2})^{1/2}$], respectively.

Brown spot and watersoaking development. Chilling injury is a postharvest physiological disorder that is manifested as brown spots or streaks on the surface of beans and watersoaking that is manifested as translucency of internal tissues. The development of brown spots or streaks was continually monitored through 19 d and is reported as the percent of 500 green beans exhibiting browning for each treatment. For the examination of watersoaking, 150 control and 150 1-MCP-treated 'Carlo' beans were cut transversely at the fruit equator after 19 d of storage and is reported as a percent of beans exhibiting any degree of watersoaking.

Statistical analysis. Data sets were subjected to GLM using SAS statistical software (version 8; SAS Institute, Cary, NC) to control the effect of 1-MCP treatment and storage duration. Comparisons between samples were made using least significant differences among processing treatments ($P \leq 0.05$). Data are presented as the mean $\pm$ SEM.

Results and Discussion

Respiration rate and ethylene production. The respiratory changes observed during storage at 7 °C for 'Thoroughbred' and 'Carlo' green beans, monitored as CO₂ production, are shown in Figure 1. In 'Thoroughbred', the respiration rates of control and 1-MCP-treated green beans increased to $117 \pm 18.2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and $83 \pm 6.0 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively, over 15 d of storage. During this period, 1-MCP-treated beans showed reduced ($P \leq 0.05$) respiration rates with values ranging from 30 to 40% lower than those of controls. After 16 d at 7 °C, the respiratory rates of control beans declined to levels observed in 1-MCP-treated 'Thoroughbred' beans. In 'Carlo', the respiration rates of control beans were $\approx 70 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ through 14 d after which respiration declined 21%. In contrast, the respiration rate of 1-MCP-treated 'Carlo' beans at 12 d at 7 °C was approximately half that observed at 4 d ($86 \pm 3.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Thereafter, the respiration rate of 1-MCP-treated 'Carlo' beans remained statistically unchanged.

Respiratory responses of nonclimacteric commodities to 1-MCP do not appear to follow a discernible pattern. In addition to green beans, nonclimacteric commodities exhibiting suppressed respiration in response to 1-MCP include broccoli (*Brassica oleracea* L.) florets (Fan and Mattheis, 2000a) and lime (*Citrus latifolia* Tan.) fruit (Jomori et al., 2003). Respiration was unaffected by 1-MCP treatment of nonclimacteric commodities, including coriander (*Coriandrum sativum* L.) leaves (Jiang et al., 2002), strawberry (*Fragaria ananassa* Duch.) fruit (Tian et al., 2000), carrot (*Daucus carota* L.) roots (Fan and Mattheis, 2000b), and cucumber fruit (Nilsson, 2005). Sweet cherry (*Prunus avium*

Fig. 1. Respiration of (A) 'Thoroughbred' and (B) 'Carlo' green beans treated with 0 (air) or 0.5 $\mu\text{L} \cdot \text{L}^{-1}$ 1-methylcyclopropene. After treatment, beans were stored for up to 22 d at 7 °C. Each point represents the mean of five replications (50 beans per replication) $\pm$ SE of the mean. The least significant difference bar shown in the top left corner represents the significant difference at the 0.05 level for the treatments-time interaction.

L.) fruit exhibited no respiratory response to 1-MCP but did show a suppression in respiratory increases induced by exposure to ethylene (Gong et al., 2002). In contrast, grapefruit (*Citrus paradisi* Macf.) fruit respiration was significantly enhanced in response to 1-MCP (McCollum and Maul, 2007). The differences in respiratory responses noted for these nonclimacteric commodities are likely the result of differences in organ maturity and morphology.

Ethylene production was not detectable in either cultivar through 8 d of storage (data not shown). Thereafter, ethylene production was detected in 'Thoroughbred' at values ranging from 20 to 40 $\text{nL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in control beans and 100 to 150 $\text{nL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in 1-MCP-treated beans. Ethylene was undetectable throughout

storage in 'Carlo' beans regardless of treatment. The slightly promotive effects of 1-MCP on ethylene production in 'Thoroughbred' is consistent with observations for the nonclimacteric leafy vegetables coriander (Jiang et al., 2002) and parsley (*Petroselinum crispum* Mill.) leaves (Ella et al., 2003), sweet cherry fruit (Gong et al., 2002), and grapefruit (McCollum and Maul, 2007), all of which showed enhanced ethylene production after 1-MCP exposure. Ethylene production in parsley (Ella et al., 2003) exceeded 20 $\mu\text{L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in response to 10 $\mu\text{L} \cdot \text{L}^{-1}$ 1-MCP for 12 h and for 40 $\mu\text{L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in grapefruit (McCollum and Maul, 2007) in response to 300 $\text{nL} \cdot \text{L}^{-1}$ 1-MCP for 24 h, ethylene production rates were comparable in magnitude to levels produced in ripening climacteric

fruits. The enhanced ethylene production of these leafy vegetables and grapefruit did not circumvent the beneficial effects of 1-MCP treatment, however, because ethylene insensitivity persisted.

Surface hue and chroma. Hue angle and chroma values for 'Thoroughbred' and 'Carlo' green beans during storage at 7 °C are shown in Figure 2. Initial hue angle values were within the range of 118° to 120° (Fig. 2A–B), indicative of dark green surface color. During storage, a decline in hue angle, indicative of yellowing, was observed for both cultivars with significant differences between the control and 1-MCP-treated beans becoming evident after 6 d at 7 °C. The divergence in hue angle continued during storage with control beans declining to approximately 115.1 ± 0.7° in 'Thoroughbred' and 114.8 ± 0.5° in 'Carlo' after 19 d of storage. In contrast, 1-MCP-treated beans showed persistent hue angle values, remaining at 118.1 ± 0.1° in 'Thoroughbred' and 117.5 ± 0.2° in 'Carlo' after 19 d.

Initial chroma values were 30.4 ± 0.2 for 'Thoroughbred' and 31.9 ± 0.30 for 'Carlo' (Fig. 2C–D) indicative of the intense green color of the beans. The chroma of 'Thoroughbred' and 'Carlo' declined during storage with

differences between control and 1-MCP-treated beans remaining insignificant for the first 9 d in 'Thoroughbred' and 10 d in 'Carlo', during which time chroma values declined to 28.4 ± 0.4 in 'Thoroughbred' and 29.0 ± 0.3 in 'Carlo'. During further storage, chroma values diverged between treatments for both cultivars with controls declining to approximately 26.9 ± 0.41 in 'Thoroughbred' and 27.8 ± 0.5 in 'Carlo' after 19 d. In contrast, 1-MCP-treated beans showed persistent chroma values, declining slightly to 29.0 ± 0.40 in 'Thoroughbred' and 29.5 ± 0.30 in 'Carlo' after 19 d of storage. Lightness values were not significantly different between control and 1-MCP-treated beans for either cultivar throughout storage (data not shown).

The persistence of hue angle and chroma values in 1-MCP-treated beans reflects the maintenance of a more intense green appearance in contrast to the muted and yellowed color of the control beans. As was observed in harvested coriander (Jiang et al., 2002) and parsley leaves (Ella et al., 2003), and processed broccoli florets (Gong and Mattheis, 2003), treatment of nonclimacteric vegetables (including green beans) with 1-MCP slows postharvest degradation of chlorophyll, preventing postharvest yellowing.

Surface browning and watersoaking. The incidence of surface brown spots during storage of 'Thoroughbred' and 'Carlo' green beans is shown in Figure 3. In both cultivars, the appearance of brown spots and streaks was significantly delayed by 1-MCP treatment. In addition to earlier onset, the development of brown spots in both cultivars progressed more quickly in control beans compared with 1-MCP-treated beans. Treatment differences were readily evident after 6 d, increasing with further storage. In 'Thoroughbred', brown spot incidence was negligible through 12 d of storage in 1-MCP-treated beans compared with ≈80% for control beans. In 'Carlo', brown spot incidence at 12 d was ≈20% in 1-MCP-treated green beans compared with 60% in controls.

The development of brown surface spots and streaks might have reflected chilling injury (CI) (Watada and Morris, 1966), because the storage temperature used (7 °C) is only slightly higher than the induction threshold temperature (5 to 7 °C, depending on cultivar) for this disorder in green beans (Cantwell et al., 2007). The suppression of brown spot incidence and rate of development of the disorder are consistent with reports of 1-MCP-mediated alleviation or

Fig. 2. Color change of (A, C) 'Thoroughbred' and (B, D) 'Carlo' green beans treated with 0 (air) or 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-methylcyclopropene. After treatment, beans were stored at 7 °C. Each point represents the mean of 15 replications $\pm$ SE of the mean. The least significant difference bar shown in the bottom left corner represents the significant difference at the 0.05 level for the treatments-time interaction.

Fig. 3. Cumulative incidence of surface brown spots and streaks of (A) 'Thoroughbred' and (B) 'Carlo' green beans treated with 0 (air) or 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-methylcyclopropene (1-MCP). After treatment, beans were stored for up to 22 d at 7 °C. Each point on the graph represents the percent of beans with brown spots or streaks per 500 beans. —, sigmoidal regression equation for 'Thoroughbred' control, $f = 96.71 / (1 + e^{-(x-10.12)/1.349})$, $r^2 = 0.99$; equation for 'Thoroughbred' treated with 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, $f = 10.24 / (1 + e^{-(x-17.20)/1.496})$, $r^2 = 0.98$; — equation for 'Carlo' control, $f = 100.3 / (1 + e^{-(x-11.70)/1.715})$, $r^2 = 0.99$; equation for 'Carlo' treated with 0.5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, $f = 95.60 / (1 + e^{-(x-15.77)/2.386})$, $r^2 = 0.99$.

delay of low temperature-induced browning in pineapple (*Ananas comosus* L.) (Selvarajah et al., 2001) and avocado (*Persea americana* Mill.) fruits (Herskovitz et al., 2005; Pesis et al., 2002). In contrast, 1-MCP-treated peach (*Prunus persica* L.) fruit stored for 3 or 6 weeks at 5 °C had more severe internal browning than untreated fruit (Fan et al., 2002).

Bean watersoaking, another symptom of CI, monitored only in 'Carlo' after 19 d storage, was also significantly suppressed by 1-MCP (data not shown). After 19 d at 7 °C, the percentage of fruits exhibiting watersoaking was 31% for 1-MCP-treated versus 64% for the control. Although there is little evidence that suppression of ethylene action ameliorates the sensitivity or tolerance of chill-sensitive commodities to low-temperature injury, it seems evident that expression of some symptoms of the disorder can be significantly delayed.

In summary, the data show that suppression of ethylene action delayed yellowing or loss of green color, reduced respiration, slightly enhanced ethylene production, and delayed the incidence of disorders, including brown spot and watersoaking, for two common commercial green bean cultivars. The data are consistent with previous reports (Wills et al., 1999, 2000) that threshold values for ethylene-induced disorders in non-climacteric commodities are lower than has heretofore been recognized and that reductions in ethylene perception can result in significant delays in symptoms of senescence and deterioration.

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