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1-MCP Reduces Development of Chilling Injury Symptoms in Yellow Summer Squash

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Summer squash (*Cucurbita pepo* L.) is a high value fresh produce item, but its marketability is limited by sensitivity to low temperature, which varies among different types of summer squash. In particular, symptoms of chilling injury (CI) develop quickly and are highly visible in the yellow type of summer squash at temperatures below 12 °C and the product quickly becomes unappealing to consumers, rendering it unmarketable. Ethylene production has been associated with development of CI. The purpose of this research was to observe the effect of 1-methylcyclopronene (1-MCP; SmartFresh™), which blocks ethylene action, on CI development in yellow summer squash fruit. 'Colorado 601' yellow summer squash that had been treated or not with 1-MCP were held at 1 °C or 10 °C for 1 or 2 weeks, then transferred to 20 °C for 2 days. Ethylene production was reduced by 1-MCP at both storage temperatures as well as after transfer to 20 °C. Chilling injury symptoms, including pitting, lenticel discoloration, and surface scald, increased in severity during storage at both temperatures, but more so at 1 °C, and CI symptoms were reduced by 1-MCP. Electrolyte leakage (EL) was increased by 1-MCP after 2 weeks at 1 °C, but EL was reduced by 1-MCP after both 1 and 2 weeks at 10 °C. Electrolyte leakage appears to be an indicator of senescence rather than CI in yellow summer squash. Treatment with 1-MCP extended the shelf life of yellow summer squash by limiting both CI and senescence and thereby maintaining postharvest quality factors.

Summer squash is a highly perishable product, with its marketability limited by its sensitivity to mechanical injury as well as storage conditions, such as temperature and relative humidity. In particular, yellow summer squash is quite sensitive to chilling injury (CI). The chilling threshold temperature for green summer squash cultivars including 'Zucchini' has been reported to be 5 °C while that of yellow cultivars has been reported to vary from 7.2 °C to 10 °C (Nunes et al., 2003; Ryall and Lipton, 1979; Sherman et al., 1987; Suslow and Cantwell, 1998). Fruit injured by CI show increased water loss, especially upon transfer to non-chilling temperatures (McCollum, 1989). The light yellow color of yellow summer squash makes CI symptoms easily visible. Thus, compared with green summer squash cultivars, the yellow types may become unappealing to consumers sooner after harvest when the two types are held at the same temperature.

Summer squash fruit are sensitive to ethylene, with exposure resulting in increased respiration rate, accelerated softening, and external and internal chlorophyll loss (for green cultivars); those effects have been shown to be overcome by treatment with 1-methylcyclopropene (1-MCP) (Lee et al., 2006). The compound 1-MCP is an inhibitor of ethylene perception that binds irreversibly to the ethylene-binding protein (Sisler and Serek, 1997). The commercial formulation of 1-methylcyclopropene is marketed as SmartFresh℠ Quality System (AgroFresh, Inc.). It has been suggested that CI symptom development is associated with the onset of ethylene synthesis (Ben-Amor et al., 1999; McCollum, 1989). Thus, there may be commercial interest in using 1-MCP to control CI in yellow summer squash.

The purpose of this research was to investigate the effect of 1-MCP on CI symptom development in yellow summer squash fruit. We also examined the effects of storage duration at chilling and non-chilling temperatures on weight loss, rate of respiration, ethylene production, and electrolyte leakage (EL) in yellow summer squash fruit that had been treated or not with 1-MCP.

Materials and Methods

Plant material. Yellow summer squash fruit, cv. Colorado 601, were obtained on the day of harvest in October 2006 from Accursio and Sons, Inc., Homestead, FL, and transported to the Postharvest Laboratory in Gainesville in an air-conditioned vehicle. The fruit were held overnight at 10 °C then those to be used for the experiment were selected on the basis of uniformity of size and minimal defects.

Treatments. A total of 54 fruit were selected, of which 48 were weighed and distributed equally among 24 plastic containers (2.25 L). The containers with fruit were then equally distributed into storage rooms at either 1 °C or 10 °C. The remaining six fruit were used for initial measurement of EL. At each temperature, the 2.25-L containers were distributed equally between two, 179-L (71 x 71 x 35.5 cm) chambers, one of which was used for 1-MCP treatment and the other as the control (without 1-MCP). After 1 and 2 weeks of storage at 1 °C or 10 °C, squash samples were transferred to 20 °C for observation of CI symptom development.

1-MCP application. Gaseous 1-MCP was produced by adding 0.447g of SmartFresh powder (0.14% formulation, SmartFresh,

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AgroFresh, Inc. Philadelphia, PA.) to 100 mL deionized water in a 100 mL vial that was immediately sealed. Squash fruit were treated with 1 ppm 1-MCP in the sealed 179-L chambers at either 1 °C or 10 °C for 12 h. Control fruit were subjected to the same conditions without exposure to 1-MCP. The chambers were opened at the conclusion of 1-MCP treatment.

Chilling injury symptoms. The squash fruit were evaluated for presence and severity of CI symptoms using a 1 to 5 rating scale modified from that of Wang (1996) in which 1 = no symptoms and 5 = severe symptoms (Fig. 1). The symptoms of CI in yellow summer squash include water-soaked and sunken areas (i.e., pitting) on the surface; the pitted areas may subsequently be colonized by saprophytic fungi.

Electrolyte leakage (EL). Samples for measuring EL were taken initially, at the end of the first week of storage and at the end of the second week. Pericarp disks (four per fruit) were removed with a cork borer (8 mm in diameter) from the region of the largest fruit transverse diameter, rinsed briefly with deionized water, blotted on slightly moistened filter paper, and then incubated in 10 mL of 200 mM mannitol solution for 4 h with shaking in a capped polypropylene tube (50 mL). Twelve disks (four disks from three different squash fruit) were placed in each tube. Immediately upon placement of disks in tubes, the solution conductivity was measured with a conductivity bridge (YSI-31A; Yellow Springs, OH) equipped with a conductivity cell (model 3100; Yellow Springs, OH). The conductivity was measured again after 4 h of incubation. Afterward, the disks and solutions were frozen and thawed and the conductivity was measured once again to estimate total electrolyte content. Electrolyte leakage was expressed as a percentage of total electrolyte content (freeze-thaw) after correction for initial solution conductivity.

Respiration and ethylene production. Respiration and ethylene production were measured every 2 d during storage at 1 °C and 10 °C (days 1, 3, 5, 7, 9, 11, and 13). Squashes were sealed in 2.25-L plastic containers (two squashes per container, six containers per treatment) for 1.0 h. For CO_2 and C_2H_4 determination, 1-mL samples of the headspace atmosphere were removed using gas-tight syringes inserted through rubber septa in the container lids. The CO_2 was analyzed on a gas chromatograph (series 580; Gow-Mac, Bridgewater, NJ) equipped with a thermal conductivity detector (TCD). The carrier (helium) flow rate was 30 mL/min. The detector current was set at 90 mA and the detector and injector were operated at 23 °C and 24 °C, respectively. The C_2H_4 was analyzed on a gas chromatograph (Tracor, Austin, TX) equipped with a photoionization detector (PID). The carrier gas (helium) flow rate was 25 mL/min. The oven was set at 50 °C and the detector at 100 °C and the injector was operated at 35 °C. The CO_2 production was expressed as mL/kg/h and the ethylene production as μL/kg/h.

Fig. 1. Rating scale for chilling injury from 1 = none to 5 = severe (left to right). Definitions were as follows: 1 = no signs of CI; 2 = trace pitting and discoloration; 3 = slight pitting, discoloration and scald; 4 = moderate pitting, scald, abrasion, and lenticel damage; 5 = severe symptoms of CI (water-soaking and browning).

Weight loss. The weights of six individual squash fruit per treatment were measured initially and after 7 and 14 d of storage. The results were expressed as a percentage of the initial weights.

Results and Discussion

Chilling injury symptoms. After 2 days at 20 °C, slight development of surface pitting, peel discoloration, and abrasion were observed on both 1-MCP and control fruit previously stored for 1 week at 1 °C or 10 °C (Table 1). Symptoms of CI were more severe on 2-week-stored fruit, for which moderate lenticel damage and scalding were also observed (Fig. 2). The 1-MCP-treated fruit stored at 1 °C or 10 °C developed significantly less severe symptoms of CI than control fruit (Fig. 2). Decay incidence was very low (< 5%) and was observed at the end of 10 °C storage and after transfer to 20 °C following 2 weeks for both 1 °C and 10 °C storage. The decay incidence did not differ between treatments and thus did not appear to be related to CI, but was reduced by 1-MCP treatment.

Weight loss. At both 1 and 10 °C, there was a significant correlation between weight loss and storage duration. The weight loss gradually increased with time and treatment with 1-MCP not significantly influencing weight loss (Table 1). Similar results were obtained for the two storage temperatures, indicating either that the vapor pressure deficit in the storage rooms was similar, which is not likely, or more likely that CI at

Table 1. Effect of 1-MCP on weight loss and electrolyte leakage of yellow summer squash during storage at 1 °C and 10 °C.

	Day 7		Day 14	
	Control	1-MCP	Control	1-MCP
1 °C storage				
Weight loss (%)	2.2 ± 0.5	2.3 ± 0.5	5.6 ± 1.7	4.1 ± 1.4
Electrolyte leakage[z]	–11.3 ± 22.8	–7.9 ± 17.5	26.0 ± 17.7	53.8 ± 20.8
10 °C storage				
Weight loss (%)	3.0 ± 1.3	2.0 ± 0.7	6.7 ± 2.9	5.0 ± 2.4
Electrolyte leakage[z]	36.8 ± 20.8	–8.3 ± 24.8	69.2 ± 23.5	19.9 ± 16.4

[z]Percentage compared to the initial value: average = 84.6 and standard deviation = 11.4

Fig. 2. Comparison of chilling injury symptom development after storage for 2 weeks at 10 °C (left two fruit) vs. 1 °C (right two fruit) plus 2 days at 20 °C, with 1-MCP treatment (+) or without 1-MCP (–).

1 °C increased the fruit susceptibility to losing weight so that the rate of weight loss at 1 °C was similar to the rate of weight loss at 10 °C.

Electrolyte leakage. Electrolyte leakage from pericarp disks decreased by approximately 8% in 1-MCP-treated squash after 1 week for both 1 and 10 °C storage (Table 1). However, in the control fruit EL decreased by 11.3% after 1 week at 1 °C, but increased by 36.8% after 1 week at 10 °C (Table 1). These results suggest that no CI was incurred as a result of the 1-week exposure to 1 °C, in agreement with the lack of visible CI symptom development.

By contrast, EL after 2 weeks diverged significantly between treatments. Interestingly, EL was reduced by 1-MCP compared to the control after 2 weeks storage at 10 °C (19.9% versus 69.2% EL; Table 1), but 1-MCP treatment resulted in increased EL after 2 weeks at 1 °C compared with the control fruit (53.8% versus 26%). This observation suggests that ethylene perception is somehow involved in the maintenance of membrane integrity during chilling of yellow summer squash fruit. The higher EL that occurred after 10 °C storage compared to 1 °C storage was probably senescence-related and this was reduced by 1-MCP treatment.

Respiration and ethylene production. At 1 °C, the respiration of both control and 1-MCP-treated fruit gradually increased during storage, reaching peaks on days 9 and 13, respectively [3.52 mL/kg/h of CO_2 (control) and 3.96 mL/kg/h of CO_2 (1-MCP)] (Fig. 3). However, at 10 °C the respiration rate of the 1-MCP-treated fruit gradually decreased during storage, although there were significant and sharp increases of respiration on day 3 (8.6 mL/kg/h of CO_2) and day 13 (7.8 mL/kg/h of CO_2) (Fig. 4). Similar behavior was observed for control fruit on day 3 (8.4 mL/kg/h of CO_2). In general, the respiration of the 1-MCP-treated fruit remained at the same level as the control fruit during storage at both 1 °C and 10 °C.

At 1 °C, there was no ethylene production detected in the control or 1-MCP treatments (Fig. 5). Ethylene production by both control and 1-MCP-treated fruit declined during storage at 10 °C with increases observed only on day 3 [1.9 μL/kg/h of C_2H_4 (control) and 1.05 μL/kg/h of C_2H_4 (1-MCP)] (Fig. 6).

1-MCP significantly reduced respiration and ethylene production in squash fruit after transfer from 1 °C to 20 °C, but did

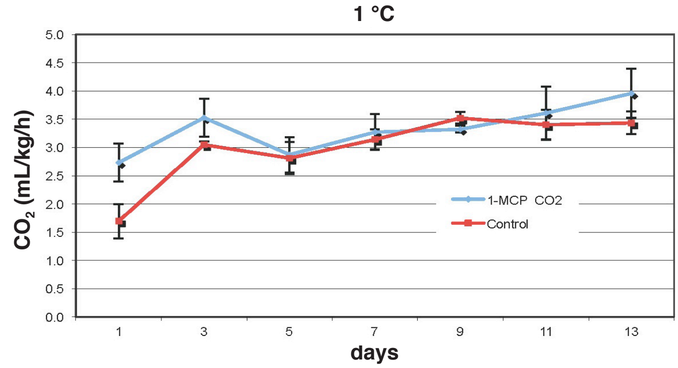

Fig. 3. Respiration (CO_2 production; mL/kg/h) of yellow summer squash fruit during storage at 1 °C.

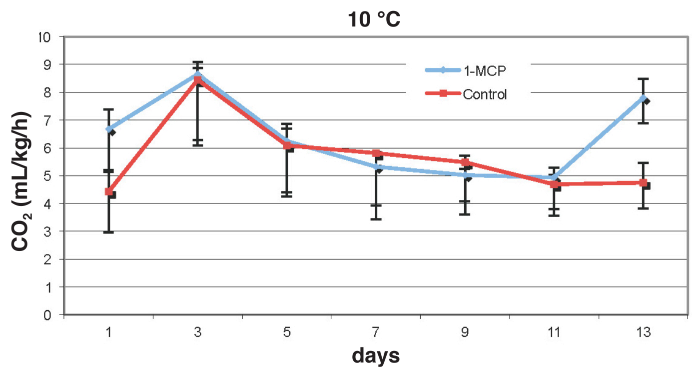

Fig. 4. Respiration (CO_2 production; mL/kg/h) of yellow summer squash fruit during storage at 10 °C.

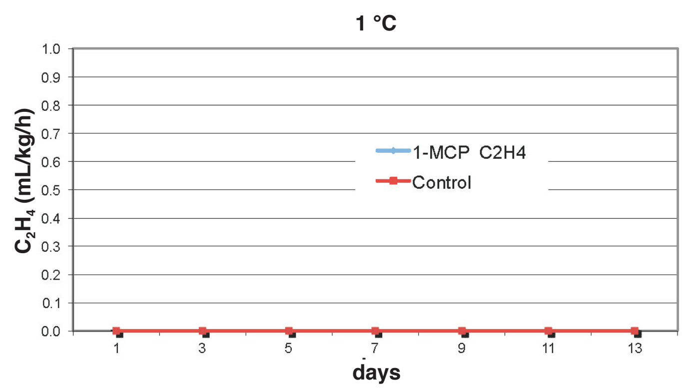

Fig. 5. Ethylene production (μL/·kg/h) by yellow summer squash fruit during storage at 1 °C.

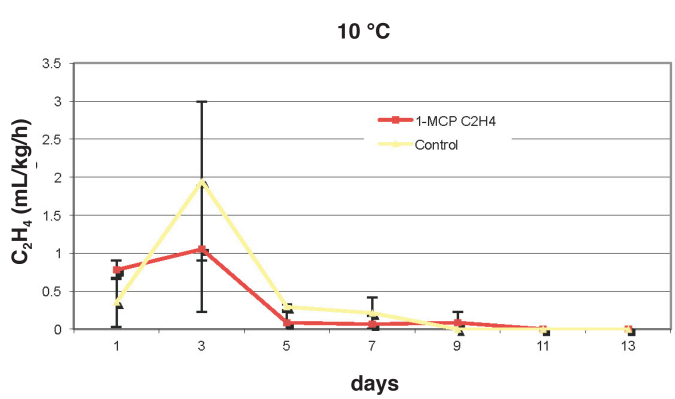

Fig. 6. Ethylene production (μL/·kg/h) by yellow summer squash fruit during storage at 10 °C.

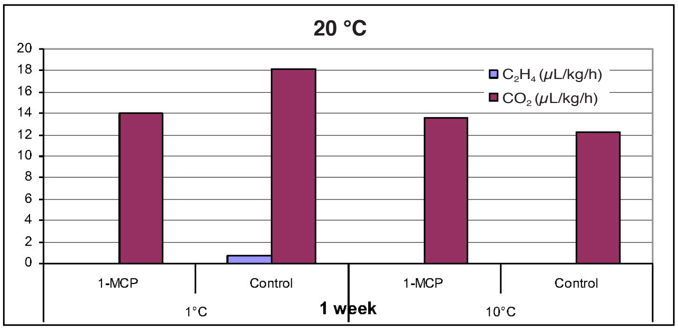

Fig. 7. Respiration (CO_2 production; mL/kg·h[1]) and ethylene production (µL/kg/h) by yellow summer squash fruit after storage for 1 week at 1 °C or 10 °C plus 2 days at 20 °C.

not significantly affect post-storage respiration and ethylene production after transfer from 10 °C to 20 °C (Fig. 7).

Conclusion

Chilling injury symptoms (pitting, lenticel discoloration, and surface scald) increased in severity during storage at both 1 °C and 10 °C, but the symptoms were much more severe at 1 °C, and development of these CI symptoms were reduced by 1-MCP. Weight loss was enhanced at 1 °C relative to 10 °C, presumably related to CI, but it was not affected by 1-MCP. Senescence-related electrolyte leakage was reduced by 1-MCP at the slightly-chilling temperature of 10 °C, but 1-MCP treatment actually resulted in increased EL at the chilling temperature of 1 °C despite the reduction in visible CI symptoms. This suggests that EL is not related to CI in yellow summer squash fruit. Increased ethylene production following transfer from 1 °C to 20 °C was also inhibited by 1-MCP. Thus, treatment with 1-MCP can limit some ethylene-related symptoms of both CI and senescence in yellow summer squash, but the results suggest that ethylene is not directly related to CI.

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