

RESPONSE OF BEIT ALPHA-TYPE CUCUMBERS (*CUCUMIS SATIVUS* L., 'MANAR') TO CONTINUOUS ETHYLENE EXPOSURE

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Abstract. The effect of the continuous exposure of exogenous ethylene on the postharvest quality of Beit Alpha-type ('Manar') cucumbers (*Cucumis sativus* L.) was investigated using commercially grown cucumbers. Cucumbers were stored at 10 °C, 95% relative humidity and exposed to 0 (compressed air only), 1, 5 or 10 ppm of ethylene for 12 days; when quality deterioration was evident at 1 ppm. The concentration of exogenous ethylene had a direct effect on the postharvest life of stored cucumbers. Concentrations of 10 ppm of ethylene reduced the shelf life by more than 50% to less than six days by primarily affecting pulp firmness. Concentrations of 5 ppm had the same effect but it took 9 days for deterioration on pulp integrity to be affected, while fruit exposed to 1 ppm was rendered unmarketable after 12 days due pulp softness and microbial rot. Ethylene had the effect of increasing the incidence of microbial rot on fruit treated with ethylene when compared to the control fruit.

Beit Alpha-type cucumbers are being grown commercially in North Florida and Georgia (Hochmuth, 2003). Total area under cultivation in 2003 in Florida and Georgia was approximately 3 acres (Hochmuth, 2004). This type of cucumber, which are much smaller than the traditional Dutch-type or English-type cucumber, have the potential to become a major greenhouse crop (Shaw, 2003) in the Florida vegetable industry due to their outstanding flavor (Sargent, 2001) and suitability for commercial production in Florida for the fresh cucumber market. Recent studies at the University of Florida have shown that Beit Alpha-type cucumbers such as Manar can be stored with acceptable quality for up to two weeks at 10 °C in rigid clamshells (Villalta et al., 2003). However, aspects such as their sensitivity to external ethylene have not been studied and formed the basis for undertaking this study. Ethylene has both beneficial and deleterious effect (Saltveit, 1998) depending on the commodity. The acceleration of senescence, the enhancing of fruit softening and the promotion of chlorophyll loss (Saltveit, 1998) are among the deleterious effects of ethylene on vegetables that reach commercial maturity at a physiologically immature stage. Dutch-type cucumbers (*Cucumis sativus* L.), also known as English or European cucumbers, produce between 0.1-1.0 $\mu\text{L kg}^{-1} \text{hr}^{-1}$ of ethylene at 20 °C (68 °F) and are highly sensitive to exposure to exogenous ethylene (Kader, 2002). There are no published reports on ethylene sensitivity of Beit Alpha-type cu-

cumbers grown in Florida, which were recently introduced to Florida and are considered a specialty cucumber.

Materials and Methods

Plant Material. Beit Alpha-type cucumbers ('Manar') were grown under commercial conditions in soil-less media (pine bark) in double-poly passively ventilated greenhouses in Wellborn, Florida (Beli Farms, Inc.). Cucumbers were harvested in the afternoon (28 April 2004) and transported to the Postharvest Laboratory at the Horticultural Sciences Department at the University of Florida in Gainesville. Cucumbers were stored overnight at 10 °C, in their original 8-kg, corrugated cartons. Cucumbers were then sorted and graded by size and appearance. Since no quality standards are available for Beit Alpha-type cucumbers in the US, cucumbers were manually graded according to shipper's recommendations (dark green color and free of any visible defects or injuries) and size (diameter of no less than one inch and no more than 1.5 inches). Only premium fruit was used for this experiment. Cucumbers were then surface sanitized by immersion for 90 s in a 150-ppm free-chlorine solution and air-dried. Sanitized cucumbers were then placed in vented, rigid, hinged polystyrene containers, with 10 cucumbers per clamshell. Clamshells were subsequently stored in sealed metal gassing chambers (7 cubic feet volume) at 10 °C \pm 1.0 (50 °F). Gassing chambers were connected to a mixing board using 0.25-inch polyethylene tube. The gas mixture (ethylene and compressed air) was humidified by bubbling it through a 2-liter glass jar with water then introduced into the gassing chambers. Cucumbers were continuously exposed to ethylene at four different concentrations; 0 (control), 1, 5 and 10 ppm (\pm 5%). Total gas flow was monitored using a digital ADM 1000 flow meter (J & W Scientific, Folsom, Calif.) and adjusted to ensure an atmosphere containing less than 2% of CO₂. To ensure consistency of ethylene concentrations in the gassing chambers headspace samples were collected every day and analyzed using a Tracor 540 gas chromatograph (Tremetrics Analytical Division, Austin, Texas), equipped with a photoionization detector, an Alumina F1 column with a mesh size of 80/100. The following quality parameters were measured every three days for 12 d to assess the quality of stored fruit.

Appearance. Appearance of stored cucumbers was rated using a subjective scale (Table 1) from 1 to 9; 9 representing field fresh fruit, 3 the marketability threshold and 1 representing inedible fruit. Fruit with dark green external color and free of defects and decay received higher ratings while fruit that exhibited yellowing and/or shriveling or decay received lower ratings.

Weight loss. Weight loss was determined by weighing 10 individual cucumbers per treatment every 3 d as well as after being transferred from its original storage temperature to 20 °C (68 °F) for 24 h. Weight loss is expressed as a percent of the initial fresh weight.

Fruit firmness. Fruit firmness was assessed on equatorial slices using an Instron® Universal Testing Instrument Model 4411 (Instron Corporation, Canton, Mass.) equipped with a

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Table 1. Appearance Rating Scale for Beit Alpha-type Cucumbers Stored at 10 °C.

9	Field Fresh	Dark green color, waxy appearance, turgid
7	Good	Minor defects are present but not objectionable. Slight shriveling of the stem end, firm (doesn't yield when flexed)
5	Fair	Slight yellowing is evident, stem-end and blossom end shriveling is slightly evident
3	Unmarketable	Pale yellow external color, severe water loss (shriveling, leathery skin), severe shriveling of the stem and blossom ends.
1	Inedible	

3.0-mm diameter probe with a crosshead speed of 50.0 mm min⁻¹, a 5-kg load cell and a 7-mm displacement. Firmness was evaluated at the mesocarp area (between the epidermis and locular tissue, approximately 2 mm from the epidermis) of similarly sized, transverse, equatorial slices of fruit. Fruit slices were obtained using a double-bladed cutting instrument with an 11-mm separation between blades, which produced slices identical in thickness. Two firmness measurements were taken per slice, and averaged to obtain a final value. Each slice was obtained from a different cucumber (n = 5).

Color evaluation. Both external and internal color was assessed using a CR-400 Chroma Meter (Konica Minolta Sensing Co., Japan). External color readings were taken on an equatorial spot predetermined and marked before placing the fruit in storage. Two measurements (on opposite sides of the fruit) were taken and averaged to obtain a final value for each fruit. Internal color was measured on the sliced mesocarp tissue, as with external color, two measurements were taken per slice (n = 5) and averaged to obtain a final value, as with firmness each slice was obtained from a different cucumber.

Respiration. The rate of respiration of the stored fruit was measured on fruit stored in sealed 1-liter plastic respiration chambers. Three cucumbers, weighing approximately 150 gm, were placed in each of three respiration chambers per treatment. Two headspace samples per container were withdrawn and averaged to obtain a final value. Samples were withdrawn every 3 d using a 1-mL disposable hypodermic syringe and analyzed for carbon dioxide content using a Gow Mac, series 580 gas chromatograph (Gow Mac Instruments Co., Bridgewater, N.J.) equipped with a thermal conductivity detector). Respiration rates were expressed as mg CO₂/kg fruit per hour.

Electrolyte Leakage. Four cores (9 mm long by 7 mm wide) of mesocarp tissue were excised from transverse slices using a No. 5 brass cork borer. Samples were excised from five different cucumbers. The mesocarp cores were cleaned of torn tissue by gently rinsing the cylinders with deionized water before placing them in plastic vials containing 35 mL of 0.25 moles isotonic mannitol solution. Isotonic solution was determined as per Appendix 1. The samples were then placed in a shaker for 4 h and then the EC of the bathing solution was measured using a temperature-compensated YSI 3100 conductance bridge (YSI Inc., Yellow Springs, Ohio). The samples were then frozen and subsequently boiled for 30 min and allowed to cool to room temperature before obtaining a final EC measurement of the bathing solution. Electrolyte leakage was expressed as a percent of the total ion leakage.

Data analysis. Data collected were analyzed using SAS software (SAS Institute Inc., Cary, N.C.). All data presented were subject to a Duncan's Multiple Range Test using a P value of <0.05.

Results and Discussion

Appearance. After 3 d in storage, there was no significant difference in the appearance of cucumbers exposed to any of the concentrations (0, 1, 5 and 10 ppm). The same held true for cucumbers constantly exposed to ethylene for 6 days. At 6 d in storage, fruit stored at 1, 5 or 10 ppm had similar appearance to the control fruit. All groups had very good, dark green color with no visible defects such as shriveling, water soaking or microbial rot, with all four groups scoring a respectable 8 in a scale from 1 to 9 (9 representing field fresh fruit and 1 representing inedible fruit). However, the external appearance of fruit exposed to ethylene deteriorated after being transferred to 20 °C for 24 h. After this transfer period, fruit exposed to 1, 5 or 10 ppm showed slight yellowing and moderate stem-end shriveling while the control fruit retained its pre-transfer visual appearance. Quality, as measured by external appearance, continued to decline throughout the storage period but lagged behind changes in electrolyte leakage and firmness.

Significant differences in external appearance were not observed until the 9th day of storage. Fruit continuously exposed to either 5 or 10 ppm of ethylene reached the marketability threshold (based on appearance only) after 9 d in storage, while fruit exposed to 1 ppm rated a 5 (above the marketability threshold) and the control fruit retained very good external appearance (8 rating), with no visible signs of deterioration after 9 d in storage. Fruit exposed to 5 or 10 ppm of ethylene rated an average of 3 after 9 d of continuous exposure to ethylene, below the marketability threshold (based on visual appearance only). Marketability threshold, based solely on appearance, was reached at 12 d in storage by fruit exposed to 1 ppm of ethylene. However, this fruit had been rendered unmarketable by significant losses in firmness prior to 12 d. The control fruit (0 ppm) retained its acceptable external appearance beyond 12 d in storage at 10 °C. Microbial rot was first observed on fruit that had been exposed to 10 ppm of ethylene for 9 d and transferred to 20 °C for 24 h. More severe microbial infection was observed after 12 d in storage in fruit that was exposed to 1, 5 or 10 ppm. There were no significant differences among these groups, and all had a 90% infection rate. Control fruit on the other hand did not have any visible signs of microbial growth after 12 d in storage.

Weight Loss. Exposure to continuous ethylene had no effect on the weight loss of cucumber fruit throughout the 12-d storage period. Significant differences in weight loss were not observed on fruit that had been constantly stored at 10 °C and exposed to any of the four ethylene concentrations (Table 2). Weight loss increased from an average of 0.86% (all four ethylene concentrations) at 3 d in storage to an average of 1.02% (all four ethylene concentrations) after 12 d in storage at 10 °C only. Significant differences in weight loss were only observed when the fruit was transferred to 20 °C for 24 h in an ethylene free environment and a relative humidity of 90%. Weight loss after this 24-h transfer period was significantly higher in fruit previously exposed to 10 ppm (3.17%) than fruit exposed to 0, 1 or 5 ppm, a four-fold increase when compared to 6 d of storage. Weight loss also increased dramatically when fruit that had been held for 9 d at 10 °C in ethylene and transferred to 20 °C for 24 h, ranging from 3.2 to 4.7%, with no significant differences among treatments.

Firmness. Cucumbers retained their original firmness, regardless of the ethylene concentration, up to the third day in storage, ranging from 14.7 to 15.2 Newtons (Fig. 2). Signifi-

Table 2. Weight loss (% of fresh weight) of Beit Alpha-type cucumbers exposed to different ethylene concentrations.

Ethylene Concentration (ppm)	Days in Storage			
	3 ^z	6 ^z	9 ^z	12 ^z
0	0.47 b	0.84 a	0.86 a	0.87 b
1	0.67 ab	0.72 a	0.92 a	1.18 a
5	0.74 a	0.87 a	0.76 a	1.03 ab
10	0.56 ab	0.74 a	0.90 a	0.98 ab

^zColumns with different letters are significantly different at $P < 0.05$, according to Duncan's Multiple Range Test.

cant differences were observed on the 6th day of storage; fruit exposed to 10 ppm were significantly softer, having lost more than 50% of the original firmness. At this time the fruit was no longer marketable even though visual appearance and external color were still acceptable. Fruit exposed to 1 ppm of ethylene had lower firmness values than the control fruit but were not significantly different than fruit exposed to 5 ppm. Fruit exposed to 0, 1 and 5 ppm had good visual appearance and external color and were still marketable at this point. Firmness continued to decline in all four treatments throughout the 12-d storage period, with the biggest changes observed in ethylene-treated fruit. At 9 d in storage, fruit exposed to 5 ppm had lost 81% of the initial firmness and were no longer marketable at this point, even though external appearance and color remained acceptable. Cucumbers exposed to 1 ppm remained marketable after 9 d in storage with no significant differences in firmness when compared to the control fruit. At 12 d in storage, fruit exposed to 1 ppm of ethylene had lost 63% of the initial firmness and were no longer marketable at this point due to microbial rot and pulp softness. Control fruit remained marketable, free of microbial rot, with very good external appearance and acceptable firmness (65% of initial firmness). Fruit softening is one of the ripening processes that is most sensitive to ethylene (Lelièvre, 1997) and it is promoted as part of the ripening process (Saltveit, 1998). However softening is an undesirable

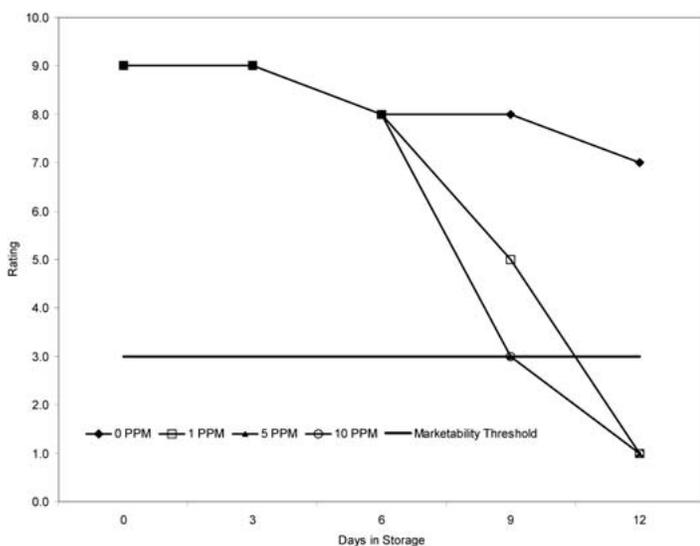


Fig. 1. Changes in external appearance ratings (1 to 9) of Beit Alpha-type cucumbers during storage at 10 °C and exposed to different ethylene concentrations (5 and 10 ppm follow the same values).

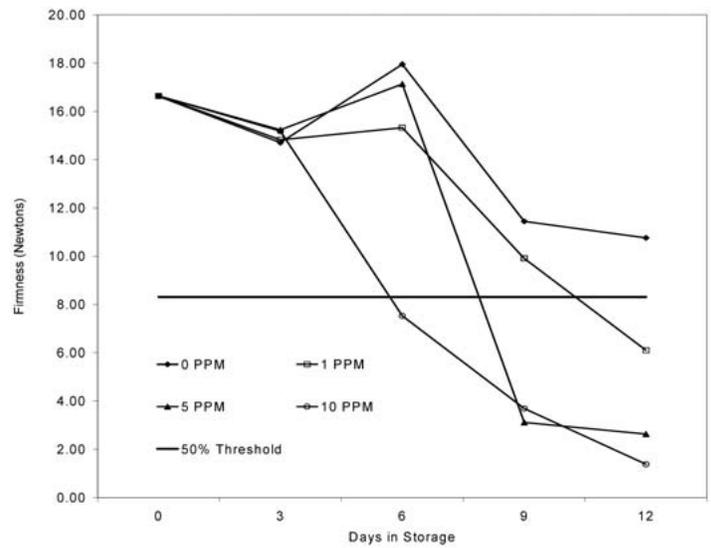


Fig. 2. Changes in mesocarp firmness (Newtons) of Beit Alpha-type cucumbers during storage at 10 °C and exposed to different ethylene concentrations.

effect in the case of cucumbers, which are consumed at a physiologically immature stage and do not need to undergo through a ripening process to become edible (such as bananas). Ethylene has also been shown to be partially responsible for pulp softening in climacteric fruits, such as cantaloupe Charantais melons (Flores et al., 2001). Softening of placental tissue has also been reported as an effect of exogenous ethylene on watermelon (Karakurt and Huber, 2002).

Color. Hue angle represents a more objective assessment of chlorophyll loss than a visual rating using a subjective scale. After 3 d in storage there were no differences in the external color of cucumbers stored at any of the four concentrations of ethylene (Fig. 3). At 6 d however, differences in external color were evident in fruit stored at 1, 5, and 10 ppm when compared to the control fruit (0 ppm). Fruit stored at 1, 5 and 10 ppm had significantly lower (more yellow) hue angle

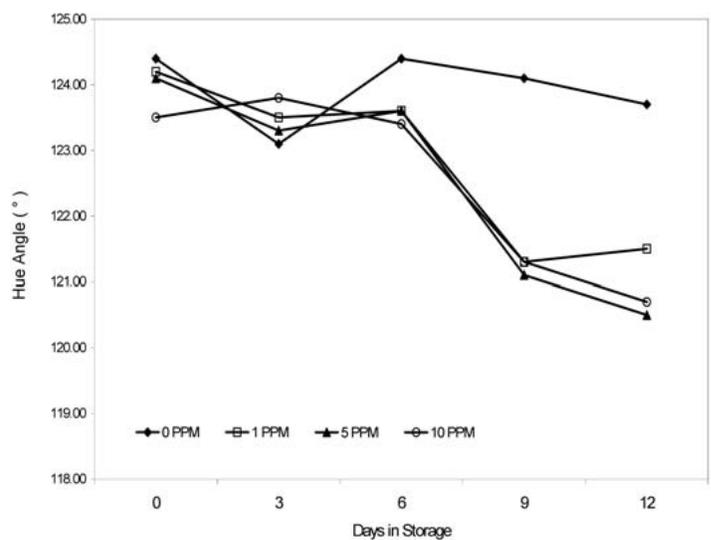


Fig. 3. Changes in external color (hue angle) of Beit Alpha-type cucumbers during storage at 10 °C and exposed to different ethylene concentrations.

values than the control fruit (0 ppm). Hue angle continued to decrease throughout storage on fruit continuously exposed to ethylene, and at 9 d, ethylene-exposed fruit had significantly lower hue angles than the control fruit. The same pattern was observed in fruit stored for 12 d, where the control fruit retained a dark green color with significantly higher hue angles than fruit exposed any of the ethylene concentrations. The level of ethylene (1 to 10 ppm) in the environment, in this experiment, did not affect the rate at which fruit yellowed, as measured by surface color measurements. Ethylene promotes the breakdown of chlorophyll (Lelièvre, 1997) due to an increased *de novo* synthesis of chlorophyllase (Jacob-Wilk et al., 1999). The effect of exogenous ethylene on color was slower and not as severe at the concentrations tested on Beit Alpha-type cucumbers, as the effect on pulp integrity.

There were no consistent significant differences in internal (mesocarp) tissue color throughout the storage period. Hue angle measurements of mesocarp tissue were slightly lower after 12 d in storage but there were no significant differences among treatments. Fruit from all treatments lost 2 to 3% of their initial hue angle values. However, slight yellowing of internal tissue was detectable upon visual inspection in fruit stored for 12 d at 10 ppm.

Respiration. Respiration rates were measured every three days until the 9th day in storage, after which time bacterial and fungal infections were clearly visible on the ethylene-treated fruit. After 3 d in storage (Fig. 4), fruit that was continuously gassed with ethylene showed significantly higher respiration rates than the control fruit (0 ppm). This pattern continued at 6 d in storage; however at 9 d in storage, the control fruit had significantly higher respiration rates than the fruit exposed to 1 or 10 ppm of ethylene. Respiration rates of control fruit continued to increase from the first measurement at 3 days through the third measurement at 9 days in storage, at which point it reached similar levels of the treated fruit.

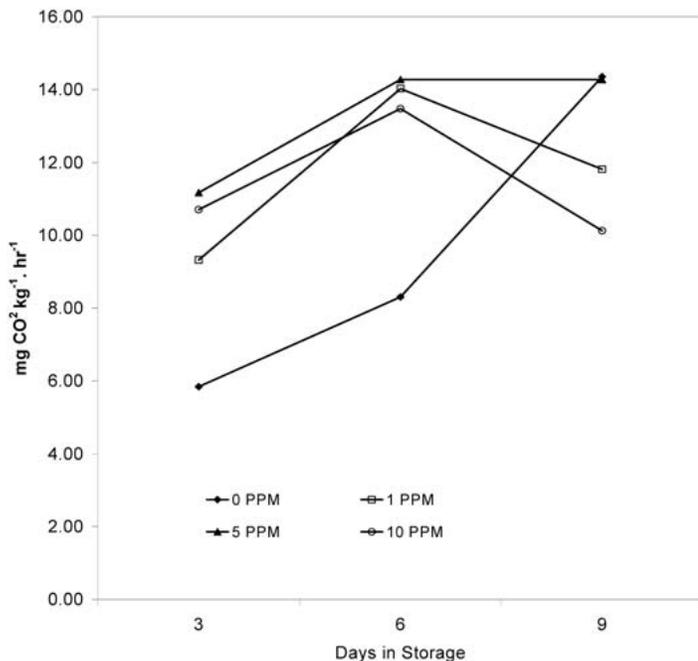


Fig. 4. Changes in respiration (mg. CO₂·kg⁻¹·hr⁻¹) of Beit Alpha-type cucumbers during storage at 10 °C and exposed to different ethylene concentrations.

Electrolyte Leakage. Ion leakage was measured to determine the relative health of cell membranes, since increased rates in electrolyte leakage are correlated to changes in membrane permeability (Knowles et al., 2000). The concentration of ethylene had a direct effect on the rate of electrolyte leakage (Fig. 5). These results are consistent with similar findings in watermelon fruit (Elkashif and Huber, 1988) and Charentais cantaloupe melons (Flores et al., 2001). Ion leakage was the first parameter indicative of quality loss in cucumber fruit exposed to continuous ethylene gassing. After three days in storage fruit that was exposed to either 5 or 10 ppm of ethylene had significantly higher ion leakage than fruit exposed to either 1 or 0 ppm. Throughout the storage period, the rate of ion leakage was lowest in the control fruit (0 ppm). After 12 d in storage, although the rate of ion leakage in the control fruit almost doubled the rate of 9.5% seen at harvest, it was still significantly lower than the increase seen in ethylene-treated fruit. Ion leakage in fruit exposed to 1 ppm increased five-fold after 12 d in storage while fruit exposed to 5 or 10 ppm increased seven-fold.

Conclusion and Recommendations

Based on the data obtained from this experiment it can be concluded that environmental concentrations of ethylene as low as 1 ppm adversely effected the shelf life of Beit Alpha-type cucumbers after 12 d. It can be speculated that adverse effects could be observed in fruit exposed to 1 ppm in less than 12 d if the fruit is subjected to further stress such as ambient temperatures above 10 °C. Pulp firmness was the attribute that was most severely affected by exogenous ethylene. At higher exposure concentrations (10 ppm), other adverse effects, such as pulp softening, were evident by six days in storage. Once again, adverse effects could perhaps be seen earlier if the stored fruit suffers other types of stress. Beit Alpha-type cucumbers should be handled and stored in an ethylene-free environment.

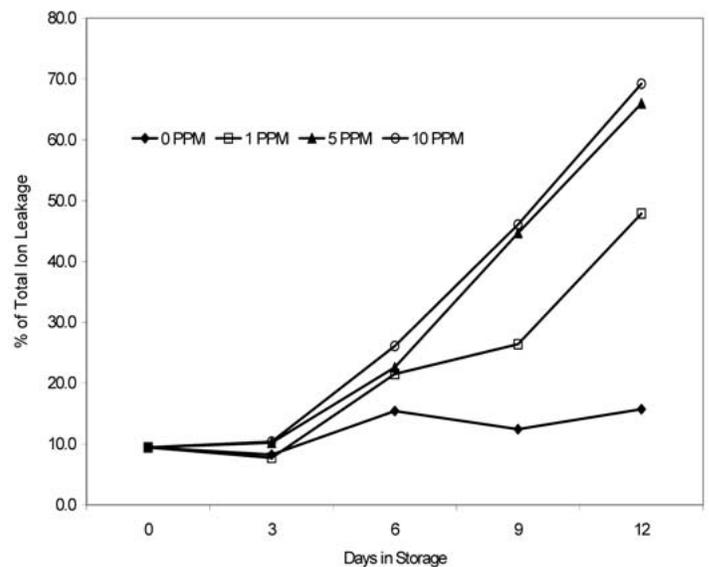


Fig. 5. Changes in electrolyte leakage (% of total electrolyte leakage) of mesocarp tissue of Beit Alpha-type cucumbers during storage at 10 °C and exposed to different ethylene concentrations.

It can also be concluded that external appearance as a sole means of judging quality can be misleading when assessing fruit that has been exposed to exogenous ethylene; the deleterious effects of ethylene will be expressed faster in other attributes such as firmness and cell permeability. Fruit exposed to exogenous ethylene had higher membrane permeability as evidenced by the higher rates of electrolyte leakage observed in the stressed fruit. A loss of chlorophyll, as determined by external hue angle measurements, was observed in all four treatments during storage but was lower in the control fruit; however, an increase in the environmental concentration of ethylene from 1 to 10 ppm did not accelerate the rate of chlorophyll loss. Fruit exposed to ethylene developed higher incidences of microbial rot while the control fruit did not show any visual signs of microbial rot even after 12 d in storage. The sensitivity threshold of Beit Alpha-type cucumbers is more than likely below 1 ppm ethylene and its saturation level at or above 10 ppm. However, further experiments with a wider range of ethylene concentrations are needed to prove this hypothesis.

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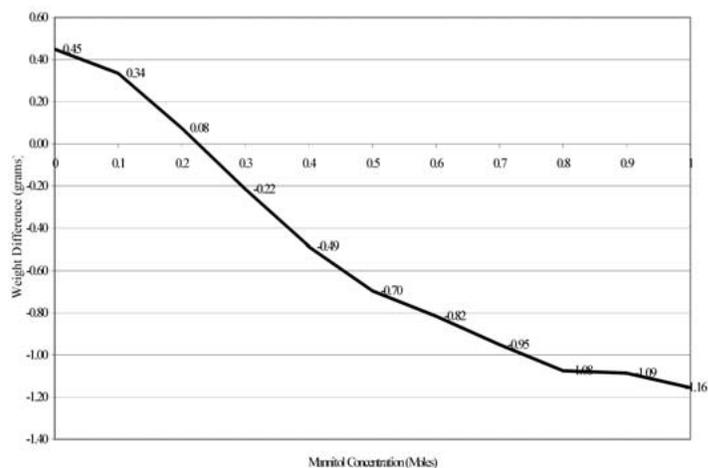
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Appendix 1. Weight Differential of Mesocarp Tissue of Beit Alpha-type Cucumbers After 4 Hours at Different Mannitol Solutions, Used to Determine Isotonic Bathing Solution