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Responses of Avocado Fruit to Insecticidal O_2 and CO_2 Atmospheres

Elhadi M. Yahia and Armando Carrillo-López

Centro de Investigación en Alimentación y Desarrollo, A.C. Apartado Postal 1735, Hermosillo, Sonora, 83000 (México)

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*Atmospheres with ≤0.5% O_2 and ≥60% CO_2 have been shown to be insecticidal. Avocado fruit (*Persea americana *Mill., cv. Hass) were exposed to air and to a modified atmosphere (MA) containing 0.1 to 0.44% O_2 and 50 to 75% CO_2 at 20 °C for 0 to 5 d. The fruits were evaluated every day, immediately after removal from MA and after ripening in air at 20 °C. Fruit exposed to MA for more than 1 d and then ripened in air had exocarp and mesocarp injury. Fruit in MA for more than one day had decreased concentrations of three glycolytic metabolites: glucose 6-phosphate, fructose 6-phosphate and 2-phosphoglycerate. Other metabolites were not affected. Insecticidal MA can only be used as a potential insect control treatment in avocado fruit for periods of 1 d or less.*

Introduction

Mexico, although the world's largest producer of avocados, exports only about 1% of its total production. Mexico's main export markets are Europe and Japan. Export to the U.S.A. is not allowed due to phytosanitary reasons and lack of a quarantine insect control treatment (1).

Atmospheres with very low levels of O_2 (≤0.5%) and/or very high levels of CO_2 (≥60%) have been shown to be insecticidal (2–5). These atmospheres have been commercially used during grain storage (4), and most insects were killed within a period of 2 to 4 d at 21 °C or higher (2,4,5). The use of modified atmosphere (MA) as an insecticidal agent is advantageous because it is economically competitive with chemical fumigation (6), and leaves no chemical residue on the fruit.

Most fresh horticultural crops do not tolerate such extreme (insecticidal) O_2 and CO_2 atmospheres during long-term storage (7), but some can tolerate short exposure periods (8–13). Moderately high levels of CO_2 (5 to 20%) applied to fruits have decreased succinate dehydrogenase activity, induced succinic acid accumulation, disintegration of plastids and cytoplasmic matrix, decreased activities of ATP: phosphofructokinase, PPi: phosphofructokinase and increased contents of fructose 6-phosphate and fructose 2, 6-bisphosphate (14,15).

Insecticidal atmospheres can affect the glycolytic pathway by inducing a shift from aerobic respiration to anaerobic fermentation. These atmospheres allow for the accumulation of ethanol and acetaldehyde in various fruits (8,10,16,17), and an increase in the activity of anaerobic enzymes such as alcohol dehydrogenase, pyruvate decarboxylase and lactate dehydrogenase (12,16,17). However, there are no published reports concerning the effects of insecticidal atmospheres on the glycolytic intermediates in horticultural crops. This information should be helpful in elucidating the mode of action of such atmospheres.

Fruits vary greatly in response and level of tolerance to insecticidal atmospheres. For example, at 20 °C mango tolerated 5 d of atmospheres containing <0.3% O_2 and/or >70% CO_2 (11,13), papaya tolerated 2 d of atmospheres containing <0.4% O_2 (12), while postclimateric 'Bartlett' pears tolerated only 1 d of atmospheres containing 0.25% O_2 and/or 80% CO_2 (16,17). It is important to determine the responses of fresh horticultural crops to such insecticidal atmospheres before they can be recommended for commercial use.

The objective of this study was to determine the tolerance of 'Hass' avocado fruit to MA with a potential use for insect control, and to investigate its effect on the glycolytic intermediates.

Materials and Methods

Materials and handling

Avocado fruits were harvested at Uruapan, Michoacan, Mexico, and transported by air to the laboratory (arrived 1 d after harvest). The fruits were then immediately sorted and selected for freedom of defects and uniformity of size. Eight fruits were frozen (−40 °C) for analysis of glycolytic intermediates, 10 fruits were used for respiration measurements, and another 20 fruits were evaluated for initial quality. The rest of the fruit were stored in air (control) or MA at 20 °C. MA stored fruits were placed in 3.8 L glass jars connected to flow boards and capillary tubings for flow control. MA was accomplished by passing a humidified

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CO_2 atmosphere at a rate of 200 mL/min for 2 h before the jars were sealed to maintain a static atmosphere.

Fruit evaluation
A sample of nine fruits were taken daily from each of MA and air storage. Three fruits were immediately frozen for analysis of glycolytic intermediates, three fruits were used for respiration measurements and three fruits were evaluated for incidence of exocarp and mesocarp injury. Injury was assessed after the fruit had been held in air at 20 °C for a total holding time of 10 d in MA and air. Exocarp injury was estimated as percentage of affected surface. Mesocarp injury was estimated using a numerical scale where 0 = no injury, 1 = very slight, 2 = slight, 3 = moderate, 4 = severe and 5 = very severe.

Gas analysis
MA gas mixtures (O_2 and CO_2) were monitored daily using a portable O_2 analyser (Mocon LC 700F, Modern Controls, Inc., Minneapolis, MN, U.S.A.), and a Hach Carle AGC 100 gas chromatograph (Hach Carle Chromatography, Loveland, CO, U.S.A.) equipped with a thermal conductivity detector (for CO_2). For respiration measurements, individual fruits were placed in 3.8 L glass jars and ventilated with a continuous flow of air free of CO_2 and C_2H_4 at 210 mL/min, and CO_2 was measured using an infrared CO_2 analyser (Horiba PIR 700, Horiba Instruments, Inc., Irvine, CA, U.S.A.).

Extraction and quantification of glycolytic intermediates
Glycolytic intermediates quantified were: glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (F1,6-P_2), glyceraldehyde 3-phosphate (G3P), 1,3-diphosphoglycerate (1,3 di PGA), 3-phosphoglycerate (3PGA), 2-phosphoglycerate (2PGA), phosphoenol pyruvate (PEP) and pyruvate (PYR). Frozen tissue was lyophilized and ground into a fine powder. Lyophilized powdered avocado mesocarp tissue (0.625 g) in 25 mL perchloric acid (0.6 mol/L) was mixed in a vortex for 1 min, filtered through a Whatman no. 41 filter paper, the filtrate was neutralized (pH 7.0 to 7.4) with potassium carbonate (5 mol/L), centrifuged at 5000 g for 30 min, and filtered again. All extraction procedures were done at 0 °C and the final filtrate was used to quantify intermediates. All glycolytic intermediates were assayed spectrophotometrically in a Perkin Elmer Lamda 3A spectrophotometer at pH 6.7 and 25 °C by following the oxidation-reduction of phosphopyridine nucleotide at 340 nm, and were quantified by standard enzymatic methods (18). The determinations were carried out in triethanolamine (TEA) buffer (pH 7.6). The assay mixture to quantify G6P contained 1.0 mL TEA (0.26 mol/L), 0.1 mL $MgCl_2$ (0.025 mol/L), 0.5 mL extract, 0.05 mL NADP (10 mmol/L) and the reaction was initiated by adding 0.1 unit of G6P dehydrogenase in 0.5 mL buffer. The assay mixture to quantify F6P included the addition of 1.5 units of phosphoglucoisomerase in 0.5 mL buffer to the assay mixture of G6P. The assay mixture to quantify G3P contained 1.0 mL TEA (184 mmol/L), 0.03 mL NADH (10 mmol/L), 0.5 mL extract, 1.6 units of triosephosphate isomerase in 0.3 mL buffer and 1.6 units of glycerol 1-phosphate dehydrogenase in 0.3 mL buffer. The assay mixture to quantify F1,6-P_2 included the addition of 0.6 units of aldolase to the assay mixture of G3P. The assay mixture to quantify 1,3 di PGA contained 1.0 mL TEA (184 mmol/L), 0.015 mL $MgSO_4$ (0.5 mmol/L), 0.015 mL ATP (10 mmol/L), 0.015 mL NADH (10 mmol/L), 0.5 mL extract and 7 units of G3P dehydrogenase in 0.05 mL buffer. Fifteen units of kinase phosphoglycerate in 0.05 mL buffer were added to the latter assay mixture to quantify 3PGA. PYR assay mixture contained 1.0 mL TEA (98 mmol/L), 0.03 mL KCl (60 mmol/L), 0.03 mL $MgSO_4$ (30 mmol/L), 0.03 mL ATP (1.25 mmol/L), 0.03 mL NADH (10 mmol/L), 0.5 mL extract, and 6 units of lactate dehydrogenase in 0.03 mL buffer. Four units of pyruvate kinase in 0.03 mL buffer were added to the latter assay mixture to quantify PEP. Two units of enolase in 0.02 mL buffer were added to the assay mixture of PEP to quantify 2PGA.

Statistical analysis
Analysis of variance and mean separation were calculated using SAS software (19).

Results and Discussion

The respiration rate in air at 20 °C (control) of avocado fruit used in this study started to increase after 6 d and reached a maximum after 9 d (**Fig. 1**). The O_2 concentration in the MA ranged between 0.1 and 0.44%, while that of CO_2 ranged between 50 and 75% (**Fig. 2**). MA from 1 to 3 d heightened the production of CO_2 compared to that of air (control) storage (**Fig. 1**), most likely reflecting anaerobic respiration and injury to the fruit tissue. Production of CO_2 of fruit stored in MA for 4 or 5 d was comparable to that of the control. Storage of avocados in O_2 concentration below 5% may cause a switch to anaerobic processes such as fermentation and alcohol formation, producing CO_2 temporarily and raising its concentration to levels above those in air (20). Studies on 'Fuerte' avocado by Biale (21) have shown that upon transfer from air to N_2 the fruit exhibited a temporary rise in CO_2 evolution for half a day followed by a sharp decline. Upon transfer from anaerobic conditions to air the increase in CO_2 continued, but the fruit discolored rapidly, was attacked by fungi and never softened. Solomos and Laties (22) have observed that when 'Hass' avocado is transferred to N_2, CO_2 evolution increases for about 30 min, declines slowly to a steady state in about 2.5 to 3.0 h, and show no further change for about 30 h.
Fruit ripened in air after MA had exocarp and mesocarp injury (**Fig. 3**). Injury was in the form of tissue discoloration. Severe injury caused mesocarp tissue breakdown and induced fermentation odors. Exocarp injury became apparent after 4 d in MA plus 6 d in air, and increased thereafter. Mesocarp injury was observed in fruits kept in MA for 2 d or more, before

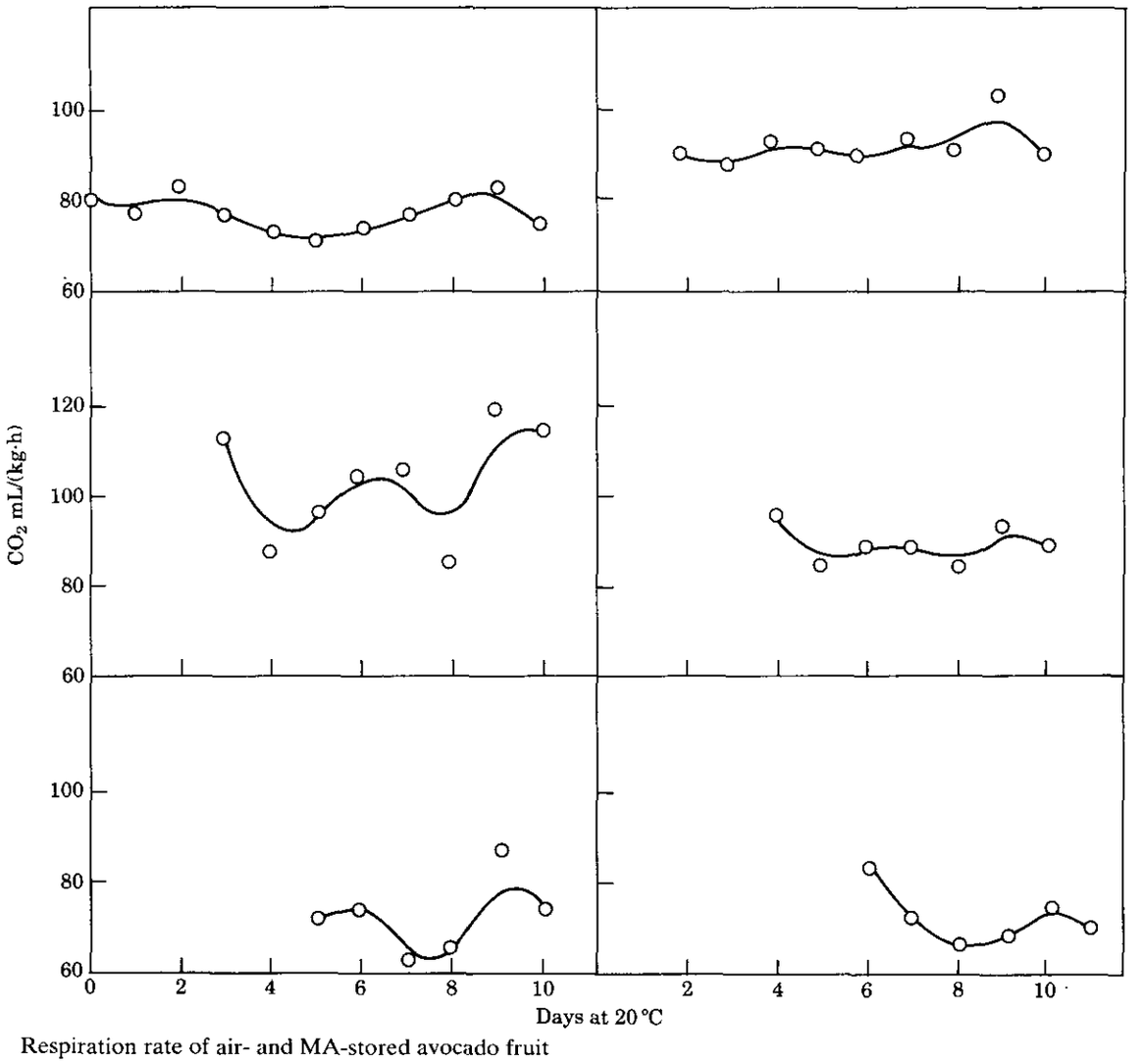

Fig. 1 Respiration rate of air- and MA-stored avocado fruit

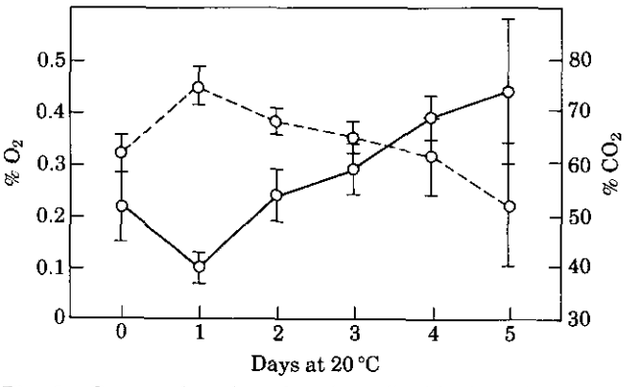

Fig. 2 Oxygen (——) and carbon dioxide (– – –) changes during MA storage. Vertical bars represent S.E. of the mean

ripening in air. This indicates that avocado fruit tolerates these insecticidal MA for less than 2 d. Yahia and Kader (10) found that 'Hass' avocado tolerated only 1 d in atmospheres containing 0.25% O_2 and/or 80% CO_2 (balance N_2); exocarp and mesocarp injury had developed after 2 d, and was more severe due to high CO_2 than due to low O_2. Biale and Young (23) stated that 'the intact avocado fruit is highly sensitive to anaerobic conditions unlike other fruits which are able to switch to fermentative metabolism when deprived of oxygen'. Very high CO_2 and/or very low O_2 causes injury in most fresh horticultural crops. Relative tolerance of avocado to insecticidal atmospheres is comparable to post-climateric 'Bartlett' pears (16,17), less tolerant than papaya (12), and much less tolerant than mango (11,13).

MA for 2 d or more decreased the concentration of G6P, F6P and 2PGA compared with air, while the concentration of G3P and 1,3 di PGA decreased in fruit exposed to MA for 5 d (**Fig. 4**). No significant differences were observed in the content of the rest of the intermediates. Crossover plots for the changes in the concentration of the glycolytic intermediates between air and MA (data not shown) failed to show any significant effect of MA on any of the control sites. Crossover plots have been used to identify, and to show the effect on control sites in a pathway (24), where a 'forward crossover' with increasing flux indicates an activated control site, while a 'reverse crossover' with decreasing flux indicates an inhibited control site.

Metabolic changes associated with very high CO_2 and/or very low O_2 atmospheres include harmful increase in acidity (15), accumulation of fermentation products such as ethanol and acetaldehyde (8,10,16), and a *de novo* synthesis and/or activation of anaerobic proteins

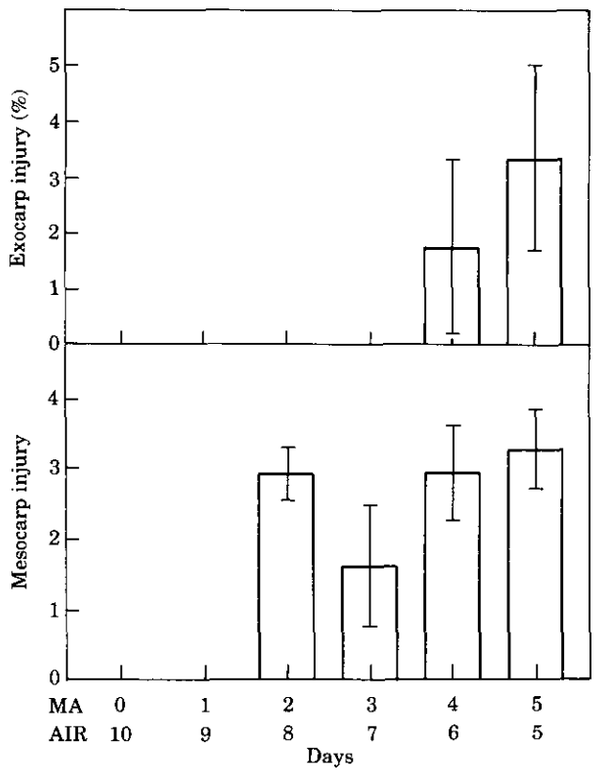

Fig. 3 Exocarp and mesocarp injury of MA-stored fruit. Vertical bars represent S.E. of the mean

such as alcohol dehydrogenase, pyruvate decarboxylase, and lactate dehydrogenase (10,12,25). High CO_2 effect has been based on decreasing succinate dehydrogenase activity resulting in accumulation of succinate (26). However, CO_2 can also cause changes in intracellular pH (27), or can have direct effects on metabolic activities where some proteins, for example, can be fractionated in the presence of CO_2 (28). Changes in levels of glycolytic intermediates in response to hypoxia and anoxia have been reported in higher plants (22,29–32). These studies have reported an increase in glycolytic flux, and have pointed to phosphofructokinase and pyruvate kinase as key regulatory enzymes. Activation of glycolysis under anoxia has also been explained in terms of a decrease in energy charge (33). High CO_2 (10%) increased the concentration of F6P, reduced the concentration of fructose 2,6-bisphosphate, but did not affect the rest of the glycolytic intermediates in pear fruit (31). Anoxia resulted in a 2.3- to 3.0-fold increase in the rate of glycolysis in 'Hass' avocado (22). Solomos and Laties (22) reported that anaerobiosis (established by flushing with a continuous flow of N_2) caused a 10- to 50-fold increase in fructose diphosphate, slightly increased G6P, and resulted in a sharp fall in 3PGA. This is not in accordance with our results. Anoxia reported by Solomos and Laties (22) was due to insufficient O_2, while that reported by us is due to a combination of both insufficient O_2 and excess of CO_2. Insufficient O_2 and excess CO_2 seem to have different mechanisms by which anaerobiosis and associated injury in avocado fruit are initiated (17). Work is still needed to elucidate the mode of action of insecticidal atmospheres.

On the basis of the results obtained in this study we conclude that 'Hass' avocado fruit are very sensitive to

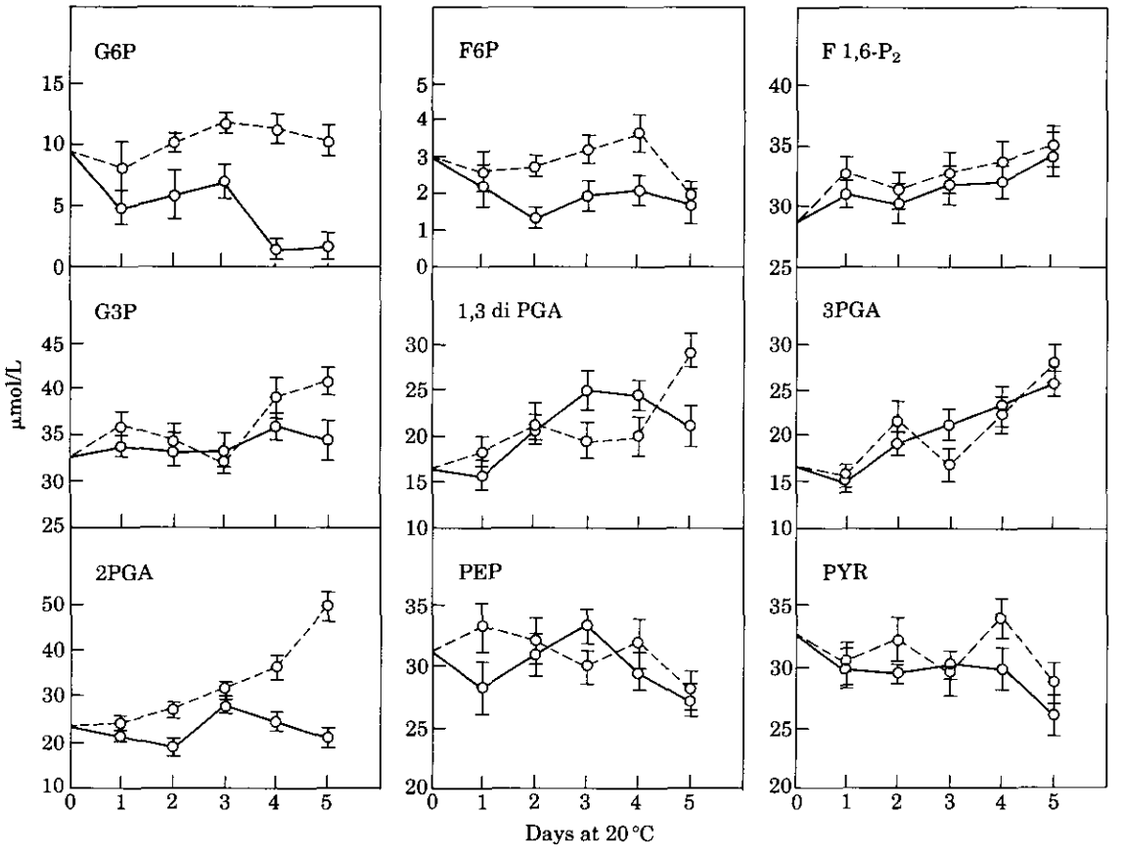

Fig. 4 Changes of glycolytic intermediates in air (– – –) and MA (——) stored avocado fruit. Vertical bars represent S.E. of the mean

insecticidal MA. Therefore, this MA regime can be used for 'Hass' avocado as an insect quarantine treatment provided that disinfestation is accomplished within 1 d or less at 20 °C.

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