Modified Atmosphere Packaging Minimizes Increases in Putrescine and Abscisic Acid Levels Caused by Chilling Injury in Pepper Fruit

María Serrano,† María Concepción Martínez-Madrid,† María Teresa Pretel,† Fernando Riquelme,*,‡ and Félix Romojaro‡

Centro de Edafología y Biología Aplicada del Segura (CSIC) Avda La Fama No. 1, 30003 Murcia, Spain, and Escuela Politécnica Superior de Orihuela (UPV), Ctra. Beniel, Km 3.2, Orihuela (Alicante), Spain

Bell pepper fruit (*Capsicum annuum* L. cv. Lamuyo) at two stages of maturity, mature green (MG) and mature red (MR), were stored at 2 and 10 °C to investigate physiological changes resulting from chilling injury. The MG fruit showed evidence of chilling injury (surface pitting) when stored at 2 °C, while MR fruit were unaffected by cold. At the same time, differences in the evolution of plant regulators were detected: In chilling-sensitive MG peppers, ethylene evolution and levels of 1-aminocyclopropane-1-carboxylic acid (ACC), putrescine (Put), and abscisic acid (ABA) rose significantly during storage, while in MG fruits stored at 10 °C and MR fruit stored at both 2 and 10 °C the values were low and no changes were observed. Spermidine (Spmd) content remained low in fruits at both maturity stages and at both temperatures. Chilling injury was reduced in MG fruits stored at 2 °C when modified atmosphere packaging (MAP) was used, particularly with the less permeable film. MAP also led to less pronounced increases in ACC, Put, and ABA levels as compared with fruit stored without film.

Keywords: *Abscisic acid; Capsicum annuum; chilling injury; modified atmosphere packaging; pepper; polyamines; ripening*

INTRODUCTION

Chilling injury (CI) is a physiological disorder that arises during the cold storage of many economically important crops that are indigenous to the tropics and subtropics (Saltveit and Morris, 1990). Exposing these chilling-sensitive crops to cold but non-freezing temperatures (<12 °C) causes a myriad of symptoms that include uneven and abnormal ripening, increased water loss and surface pitting, increased CO_2 and ethylene production upon warming, increased susceptibility to certain diseases, and increased permeability of the cellular membranes (Saltveit and Morris, 1990; Wang, 1993; Lelièvre *et al.*, 1995). CI becomes more severe at longer storage times and/or at lower temperatures (Risse *et al.*, 1987). Differences in chilling sensitivity have been reported in tomatoes (Autio and Bramlage, 1986) and pepper (Lin *et al.*, 1993) at different stages of maturity.

One of the most common responses to chilling is the stimulation of ethylene production due to increased ACC synthase activity. ACC synthase catalyzes the transformation of *S*-adenosylmethionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor (Wang, 1987). High polyamine titres have also been observed in plants after exposure to CI and other types of environmental stress, e.g., nutritional stress, osmotic shock, UV radiation, oxygen deficiency, salinity, low pH, and K^+ and Mg^{2+} deficiency (Evans and Malmberg, 1989; Feng and Barker, 1993; Kramer *et al.*, 1991; McDonald and Kushad, 1986; Reggiani *et al.*, 1989; Wang, 1987). Treating fruit with high concentrations of CO_2 (10−40%) before they are cold stored have been shown to reduce CI symptoms in lemon, avocado, and zucchini, as has treatment of avocado by low concentrations of O_2 (Wang and Ji, 1989; Pesis *et al.*, 1994). Also, modified and controlled atmospheres diminish injury symptoms in many fruits (Saltveit and Morris, 1990).

The objectives of this study were to investigate the chilling sensitivity of bell peppers (*Capsicum annuum* L. cv. Lamuyo) at two stages of maturity when stored at 2 °C. Fruit stored at 10 °C were used as a control. In addition, the effect of using modified atmosphere packaging (MAP) was studied in an attempt to ascertain whether an atmosphere containing high levels of CO_2 and low levels of O_2 could reduce CI and the associated physiological responses in peppers stored at 2 °C.

MATERIALS AND METHODS

Plant Material. Bell pepper (*Capsicum annuum* L. cv. Lamuyo) fruits were harvested from greenhouse-grown plants in Murcia (Spain) and taken to the laboratory (day 0 of the experiments). Experiment I used fruits at two stages of ripeness, mature green (MG) and mature red (MG). Fifty-four peppers of each stage were kept at 2 °C and another 54 fruits of each stage were kept at 10 °C, making a total of four lots, which were stored in darkness and with a relative humidity of 90%. Nine fruits were taken weekly from each lot over a period of 6 weeks. These fruits were used to measure color and ethylene production before being divided into three samples of three peppers each. Fruit were sliced, frozen in liquid N_2, and triturated prior to the determination of ACC, polyamines, and ABA. In experiment II, storage at 2 °C of MG peppers in MAP was studied using two polymer films (A and B) made of microperforated polypropylene (35 μm thickness) with the following characteristics (Sidlaw Packaging-P-Plus; Avon, U.K.): they were not selectively permeable (i.e., each one allowed the passage of O_2 and CO_2 at the same rate). The permeability of film A for both O_2 and CO_2 was 10 000 mL m^{-2} 24 h^{-1} and that of film B was 80 000 mL m^{-2} 24 h^{-1} for both gases; water permeability of both films was 4 g m^{-2} 24 h^{-1}. Twenty-three bags (25 × 30 cm) of each plastic film

* Author to whom correspondence should be addressed (telephone 34-68-215717; fax 34-68-266613; e-mail friquelm@natura.cebas.csic.es).

† UPV.

‡ CSIC.

Table 1. Ethylene Production (nL g^{-1} h^{-1}) at 20 °C of Mature Green (MG) and Mature Red (MR) Peppers following Cold Storage at 2 and 10 °C[a]

storage temp	storage time (weeks)	time at 20 °C after cold storage			
		1 h	3 h	6 h	24 h
MG at 2 °C	1	0.33 ± 0.04	1.96 ± 0.21	2.93 ± 0.32	0.35 ± 0.02
	3	0.55 ± 0.06	1.78 ± 0.19	2.34 ± 0.29	0.43 ± 0.05
	6	0.43 ± 0.07	2.05 ± 0.21	2.57 ± 0.31	0.39 ± 0.06
MR at 2 °C	1	0.22 ± 0.03	0.19 ± 0.03	0.23 ± 0.03	0.20 ± 0.01
	3	0.24 ± 0.02	0.20 ± 0.01	0.23 ± 0.02	0.22 ± 0.01
	6	0.27 ± 0.04	0.18 ± 0.02	0.28 ± 0.03	0.32 ± 0.03
MG at 10 °C	1	0.29 ± 0.04	0.27 ± 0.03	0.30 ± 0.02	0.28 ± 0.02
	3	0.31 ± 0.02	0.25 ± 0.04	0.30 ± 0.01	0.27 ± 0.03
	6	0.27 ± 0.04	0.29 ± 0.03	0.31 ± 0.02	0.25 ± 0.02
MR at 10 °C	1	0.30 ± 0.02	0.25 ± 0.03	0.24 ± 0.01	0.28 ± 0.03
	3	0.24 ± 0.03	0.28 ± 0.03	0.23 ± 0.02	0.26 ± 0.02
	6	0.31 ± 0.03	0.25 ± 0.02	0.28 ± 0.02	0.27 ± 0.01

[a] Data are the mean ± SE of determinations made for nine fruits independently.

were prepared in which a silicone septum was placed. Three peppers (≈700 g) were enclosed in each bag 24 h after the fruits reached the laboratory. Similar samples were placed on trays in the open air as control. The O_2 and CO_2 concentrations in the atmosphere inside the bags were determined weekly inside five bags of each film. In addition, after 2, 4, and 6 weeks, three bags of each film and three control batches were taken and used to measure ethylene production at 20 °C for 24 h. Next, the three fruits from each bag of film A and film B were sliced, frozen in liquid N_2, and ground to obtain three homogeneous samples for each treatment. Free ACC, conjugated ACC, polyamines, and ABA were determined for these samples. The same was done for each batch of control fruit.

Analytical Determinations. To measure ethylene production rate, fruits were transferred to 20 °C and individually enclosed in 1.0-L glass jars hermetically sealed with a rubber stopper. After 1 h, 1 mL of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a 3-m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. This was expressed in nanoliters of ethylene evolved per gram of tissue per hour (nL g^{-1} h^{-1}). In experiment II, to quantify CO_2 and O_2 into the bags, 1 mL of their internal atmosphere was drawn out with a syringe by passing the needle through the silicone septum of the film surface, and then it was injected in a Shimatzu GL 14A chromatograph with catarometric detector. The column temperature was 55 °C, and injector and detector temperature were 110 °C.

Free and conjugated ACC were extracted with trichloroacetic acid and quantified as described previously (Pretel *et al.*, 1995). Two separate extractions were made from each sample, and for each extraction ACC was quantified in triplicate. Polyamines were extracted with perchloric acid and analyzed by the benzoilation method as previously reported (Serrano *et al.*, 1995), using the same number of extractions as for ACC but quantifying each extraction in duplicate. The results are expressed as nanomoles per gram fresh weight. ABA was quantified by an enzyme-linked immunosorbent assay (ELISA), as described previously (Serrano *et al.*, 1995). Once again two extractions were made from each sample. ABA content was estimated from the standard curve prepared for each plate. Four dilutions were prepared for each extract, and at least three of them fell onto the curve obtained for the ABA standard. Results are expressed as nanomoles per gram fresh weight.

RESULTS

Experiment I: Storage of MG and MR Peppers at 2 and 10 °C. The MG fruit remained green throughout storage at 2 °C, while the same fruit kept at 10 °C underwent the characteristic color change from green to red. This change became more pronounced after 3 weeks of storage, and after 6 weeks 80% of the fruit surface was totally red. One day after their exposure to ambient temperature (20 °C), CI symptoms appeared as surface pitting in MG fruit stored at 2 °C. The surface area of the fruit showing such damage increased with longer periods of storage at this temperature (20% after 1 week, rising to 60% after 6 weeks of storage). However, neither MG fruit stored at 10 °C or MR fruit stored at 2 and 10 °C showed these symptoms even after several days of exposure to ambient temperature regardless of storage time.

Ethylene production of MG peppers stored at 2 °C rose 3 and 6 h after they were transferred to 20 °C, but after 24 h it dropped to levels found 1 h after removal. No differences between the ethylene production rate of these peppers after 1, 3, and 6 weeks of storage at 2 °C were found. However, ethylene production by MG fruit stored at 10 °C and MR fruit stored at both 2 and 10 °C remained low, with no increase being observed after their removal from cold storage (Table 1). This suggests that there is a connection between the appearance of CI damages in pepper fruits and the stimulation of their ethylene production when they are transferred to 20 °C.

Free ACC levels were 0.34 nmol g^{-1} fresh weight in recently picked MG peppers and 0.33 nmol g^{-1} fresh weight in MR, while the conjugated ACC contents were 0.67 and 0.68 nmol g^{-1} fresh weight, respectively. Neither free ACC or conjugated ACC levels showed significant changes in MR peppers stored at either temperature or in MG peppers stored at 10 °C. After 6 weeks of storage of MG peppers at 10 °C, their free and conjugated ACC levels were 0.36 and 0.71 nmol g^{-1} fresh weight, respectively; in MR peppers, these levels were 0.29 and 0.72, for peppers stored at 10 °C, and 0.35 and 0.71 nmol g^{-1} fresh weight for peppers stored at 2 °C. On the other hand, ACC levels in MG peppers kept at 2 °C rose considerably during storage, most of the increase being in free ACC (Table 2). These results illustrate the increased activity of the ACC synthase enzyme in MG peppers induced by low temperatures.

The levels of Put in freshly picked MG and MR fruit were 85 and 42 nmol g^{-1} fresh weight, respectively. These did not change significantly in MR fruit throughout storage at either 2 or 10 °C or in MG fruit stored at 10 °C. In MG peppers stored at 2 °C, Put concentration rose substantially, reaching 10 times the original level after 6 weeks (Figure 1). Spmd levels varied between 60 and 43 nmol g^{-1} fresh weight during storage, but no effect of either temperature or ripeness stage was observed. Spermine (Spm) levels were low and decreased slightly during storage from 20 to 15 nmol g^{-1} fresh weight. ABA levels were 0.20 and 0.95 nmol g^{-1}

Table 2. Free, Conjugated, and Total ACC Levels (nmol g^{-1} Fresh Weight) in Pericarp of Mature Green Peppers during Storage at 2 °C[a]

weeks in storage	free ACC	conjugated ACC	total ACC
0	0.34 ± 0.05	0.67 ± 0.13	1.01 ± 0.16
1	0.51 ± 0.10	0.77 ± 0.09	1.28 ± 0.15
2	0.79 ± 0.07	0.81 ± 0.11	1.60 ± 0.21
3	0.97 ± 0.10	0.86 ± 0.20	1.83 ± 0.26
4	1.35 ± 0.28	0.92 ± 0.07	2.27 ± 0.25
5	1.63 ± 0.12	0.97 ± 0.08	2.60 ± 0.26
6	1.89 ± 0.39	1.00 ± 0.03	2.89 ± 0.38

[a] Data are the mean ± SE of two separate extractions made from each sample and each extraction quantified in triplicate. Three samples were taken weekly with three peppers per sample.

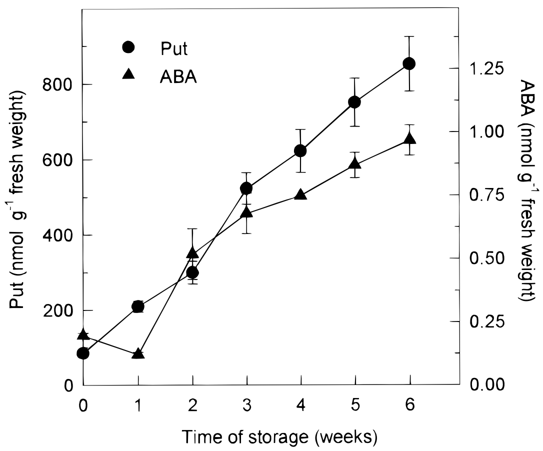

Figure 1. Putrescine (Put) and ABA levels (nmol g^{-1} fresh weight) in pericarp of mature green pepper fruit during storage at 2 °C. Bars indicate SE of the means.

fresh weight in freshly picked MG and MR fruit, respectively. The ABA levels in MG fruit stored at 2 °C rose significantly (Figure 1), reaching about 1.0 nmol g^{-1} fresh weight after 6 weeks, while there was no significant change in MR stored at this temperature or in fruit of either stage of ripeness stored at 10 °C (data not shown).

Experiment II: Storage of MG Peppers at 2 °C in Modified Atmospheres. The use of MAP considerably reduced CI, particularly in peppers stored in the less permeable film (film A), in which less than 10% of the surface area showed pitting after 6 weeks, while in control peppers (stored in open air: 21% O_2 and 0.04% CO_2), 60% of the surface area was affected by CI at this moment. This decrease in CI was less pronounced when film B was used since 40% of the pepper surface area showed pitting after 6 weeks at 2 °C. Inside the bags of film A, the levels of O_2 and CO_2 were 16.1% and 4.5%, respectively, while for film B they were 18.5% and 0.2%, respectively (Figure 2), showing that higher permeability results in a smaller modification of the bag's atmosphere.

The increase in ACC levels in MG peppers stored at 2 °C was less pronounced in fruit kept under film A (Table 3), in which the CO_2 levels were higher (Figure 2), while in MG peppers stored under film B the levels of free and conjugated ACC increased during storage as did in control peppers (Table 3). The MAP also affected ethylene production in peppers a few hours after transfer to 20 °C. The ethylene emission rate of the peppers stored under film A for 2, 4, and 6 weeks, remained between 0.20 and 0.30 nL g^{-1} h^{-1} after transfer to 20 °C, while peppers exposed to the air and those kept under film B showed ethylene emission rates as high as 3.0 nL g^{-1} h^{-1} 6 h after being transferred to

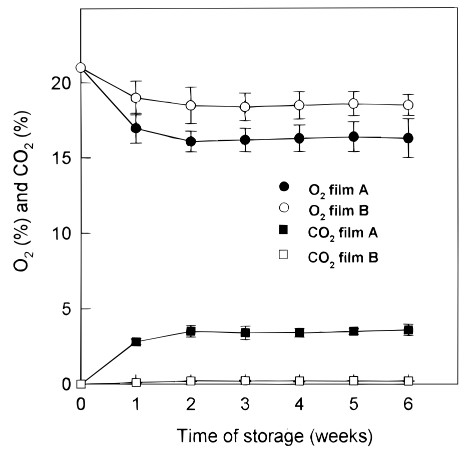

Figure 2. CO_2 and O_2 levels (%) inside the bags of films A and B during storage of mature green peppers at 2 °C. Data are the mean ± SE of the determinations made in five bags of each film.

Table 3. Free, Conjugated, and Total ACC Levels (nmol g^{-1} Fresh Weight) in Mature Green Peppers Stored at 2 °C for 4 Weeks[a]

		free ACC	conjugated ACC	total ACC
before storage		0.34 ± 0.02	0.67 ± 0.05	1.01 ± 0.18
after storage	control	1.69 ± 0.39	0.80 ± 0.14	2.49 ± 0.38
	film B	1.58 ± 0.24	0.75 ± 0.17	2.33 ± 0.37
	film A	0.89 ± 0.02	0.61 ± 0.14	1.50 ± 0.27

[a] Fruit were non-packaged (control) or in films A and B. Data are the mean ± SE of two separate extractions made from each sample and each extraction quantified in triplicate. Permeability of film A for O_2 and CO_2 was 10 000 mL m^{-2} 24 h^{-1} and that of film B was 80 000 mL m^{-2} 24 h^{-1}.

20 °C (Table 4), although this subsequently fell (to 0.2 nL g^{-1} h^{-1}), as was observed in experiment I for these peppers (Table 1).

Put and ABA levels gradually increased during cold storage both in fruits exposed to air and in those under film B, while a smaller increase was recorded in peppers stored in the less permeable film (Figures 3 and 4). However, the levels of the polyamines Spmd and Spm were low and fell slightly during cold storage (from 60 to 42 and from 25 to 15 nmol g^{-1} fresh weight, respectively), with no significant differences being found between the fruit conserved in MAP and those exposed to air.

DISCUSSION

The results show that the susceptibility of greenhouse-grown bell peppers to CI depends on their stage of ripeness since the CI symptoms only appear in MG peppers stored at 2 °C, in accordance with the results of Lin *et al.* (1993) in cv. Doria and cv. Bison peppers and of Autio and Bramlage (1986) in tomatoes. These results are of commercial importance since they show that it is possible to store MR fruit at lower temperatures than MG fruit and so keep them longer, although Lurie *et al.* (1995) found that both green and red bell peppers showed symptoms of CI after 28 days at 2 °C. The results also show that MAP can be used for storing peppers at low temperatures, diminishing the appearance of CI symptoms and permitting longer storage periods. Meir *et al.* (1995) found that the storage of bell peppers at 3 °C in polyethylene bags extended their quality and shelf life. Also, low O_2 (2−5%) and high

Table 4. Ethylene Production (nL g^{-1} h^{-1}) at 20 °C in Mature Green Peppers following Storage at 2 °C[a]

storage conditions	storage time (weeks)	time at 20 °C after cold storage			
		1 h	3 h	6 h	24 h
film A	2	0.21 ± 0.05	0.23 ± 0.07	0.20 ± 0.03	0.24 ± 0.02
	4	0.29 ± 0.07	0.25 ± 0.06	0.21 ± 0.01	0.28 ± 0.03
	6	0.31 ± 0.08	0.28 ± 0.05	0.20 ± 0.05	0.30 ± 0.06
film B	2	0.18 ± 0.03	1.72 ± 0.13	2.89 ± 0.16	0.21 ± 0.03
	4	0.15 ± 0.01	1.90 ± 0.07	3.00 ± 0.17	0.31 ± 0.02
	6	0.21 ± 0.03	2.17 ± 0.09	3.13 ± 0.15	0.27 ± 0.03
control	2	0.25 ± 0.07	2.05 ± 0.21	2.95 ± 0.10	0.28 ± 0.05
	4	0.19 ± 0.05	1.81 ± 0.18	3.10 ± 0.15	0.31 ± 0.05
	6	0.21 ± 0.03	2.13 ± 0.10	2.87 ± 0.12	0.27 ± 0.06

[a] Fruit were non-packaged (control) or packaged in films A and B. Data are the mean ± SE of determinations made for nine fruits independently. Permeability of film A for O_2 and CO_2 was 10 000 mL m^{-2} 24 h^{-1} and that of film B was 80 000 mL m^{-2} 24 h^{-1}.

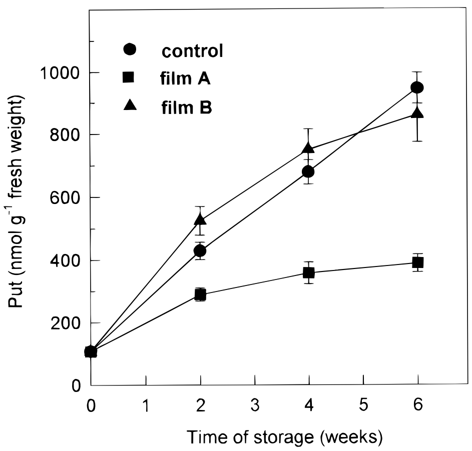

Figure 3. Putrescine levels (nmol g^{-1} fresh weight) in pericarp of mature green peppers during storage at 2 °C inside the bags of films A and B. Peppers stored without film served as control. Bars indicate SE of the means.

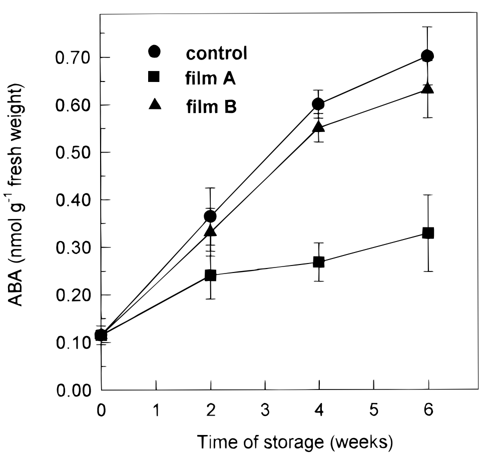

Figure 4. ABA levels (nmol g^{-1} fresh weight) in pericarp of mature green peppers during storage at 2 °C inside the bags of films A and B or without film (which served as control). Bars indicate SE of the means.

CO_2 (up to 20%) can alleviate chilling injury symptoms in bell peppers (Luo and Mikitzel, 1996).

The increase in ethylene production and ACC levels found in chilling-injured peppers is similar to the response observed in other fruits (Chan *et al.*, 1985; Lelièvre *et al.*, 1995). However, the increase in ethylene production at 20 °C in MG fruit that had been cold stored and in ACC levels in these fruits during storage at 2 °C was much less when film A was used, under which equilibrium levels of 4.5% of CO_2 and 16.1% of O_2 were reached. This suggests that a sufficiently high concentration of CO_2 can inhibit the ACC synthase enzyme.

Put levels were also related to CI since they only rose in MG peppers stored at 2 °C in the air and in the more permeable film, such fruit being the only ones to show CI symptoms. Various authors (Reggiani *et al.*, 1989; Kramer *et al.*, 1991; Feng and Barker, 1993) have suggested that Put increases as a result of cold storage-provoked stress and that Put could probably protect tissue from this stress. Furthermore, in several studies, exogenously added polyamines have been shown to protect plant tissue from the detrimental effects of several types of stress, including chilling (Kramer *et al.*, 1991). It has been proposed that polyamines act as free radical scavengers and that they stabilize membranes by means of ionic interactions to provide protection against environmental stress (Drolet *et al.*, 1986).

This study has shown that MG peppers stored at 2 °C in film A exhibited fewer CI symptoms and smaller increases in Put levels, which suggests that the increase in Put observed in fruit damaged by the cold is not a mechanism to protect the tissue from this stress but simply a consequence of this damage. Furthermore, it has been shown that MAP with a sufficiently high level of CO_2 (4.5%) can protect the tissue against CI, reducing the manifestation of the above-mentioned physiological alterations. However, it must be pointed out that a too high CO_2 concentration (60%) may have the contrary effect, since this provokes another kind of stress and the levels of ethylene, ACC, and Put may increase (Mathooko *et al.*, 1995). There was also a correlation between the appearance of CI symptoms and increased ABA levels since both rose significantly in MG peppers stored at 2 °C in the air and in the more permeable film (film B), while these increases were much less pronounced in MG fruit under film A.

It therefore seems that, like Put, the ABA increase in pepper might be a result of the stress caused by CI and not as a protection against such damage since MG peppers stored under film A, as stated above, showed fewer CI symptoms and lower increases in Put and ABA levels during storage at 2 °C. If ABA and Put were protection agents of chilling damage, these compounds would have increased as CI was reduced by MAP. This observation coincides with that of Lafuente *et al.* (1991), who found no relation between ABA levels and CI tolerance in cucumber seedlings. In tomatoes stored at CI-inducing temperatures, there was an increase in ABA levels during storage (Ludford and Hillman, 1990), although this increase was not associated with the appearance of CI symptoms (Kubik *et al.*, 1992). Dallaire *et al.* (1994) suggested that ABA did not play an essential role in the development of freezing tolerance in intact plants of wheat. However, the levels of ABA were increased in zucchini squash by temperature conditioning at 10 °C for 2 days, after which the severity of CI was reduced. Similarly, CI was reduced when zucchini were treated with exogenous ABA before storage (Wang, 1991), suggesting that ABA could protect tissues from CI damage. According to these comments, the increases in Put and ABA levels in chilling injured peppers found in this work might well have been an unsuccessful attempt to prevent CI. Thus, the role of ABA and polyamines in the development of CI is not totally clear, and the experimental results found by different authors are contradictory, perhaps as a consequence of the physiological differences between the different plant organs studied.

LITERATURE CITED

Autio, W. R.; Bramlage, W. J. Chilling Sensitivity of Tomato Fruit in Relation to Ripening and Senescence. *J. Am. Soc. Hortic. Sci.* **1986**, *111*, 201−204.

Chan, H. T.; Sanxter, S.; Couey, H. M. Electrolyte Leakage and Ethylene Production Induced by Chilling Injury of Papayas. *HortScience* **1985**, *20*, 1070−1072.

Dallaire, S.; Houde, M.; Gagné, Y.; Saini, H. S.; Boileau, S.; Chevrier, N.; Sarhan, F. ABA and Low Temperature Induce Freezing Tolerance Via Distinct Regulatory Pathways in Wheat. *Plant Cell Physiol.* **1994**, *35*, 1−9.

Drolet, G.; Dumbroff, E. B.; Legge, R. L.; Thompson, J. E. Radical Scavenging Properties of Polyamines. *Phytochemistry* **1986**, *25*, 367−371.

Evans, P. T.; Malmberg, R. L. Do Polyamines Have Roles in Plant Development? *Annu. Rev. Plant Physiol.* **1989**, *38*, 155−178.

Feng, J.; Barker, A. V. Polyamine Concentration and Ethylene Evolution in Tomato Plants Under Nutritional Stress. *HortScience* **1993**, *28*, 109−110.

Kramer, G. F.; Norman, H. A.; Krizek, D. T.; Mirecki, R. M. Influence of UV-B Radiation on Polyamines, Lipid-Peroxidation and Membrane-Lipids in Cucumber. *Phytochemistry* **1991**, *30*, 2101−2108.

Kubik, M. P.; Buta, J. G.; Wang, C. Y. Changes in the Levels of Abscisic Acid and its Metabolites Resulting from Chilling of Tomato Fruits. *Plant Growth Regul.* **1992**, *11*, 429−434.

Lafuente, M. T.; Belver, A.; Guye, M. G.; Saltveit, M. E. Effect of Temperature Conditioning on Chilling Injury of Cucumber Cotyledons. *Plant Physiol.* **1991**, *95*, 443−449.

Lelièvre, J. M.; Tichit, L.; Fillion, L.; Larrigaudière, C.; Vendrell, M.; Pech, J. C. Cold-Induced Accumulation of 1-Aminocyclopropane-1-Carboxylate Oxidase Protein in Granny Smith Apples. *Postharvest Biol. Technol.* **1995**, *5*, 11−17.

Lin, W. C.; Hall, J. W.; Saltveit, M. E., Jr. Ripening Stage Affects the Chilling Sensitivity of Greenhouse-Grown Peppers. *J. Am. Soc. Hortic. Sci.* **1993**, *118*, 791−795.

Ludorf, P. M.; Hillman, L. L. Abscisic Acid Content in Chilled Tomato Fruit. *HortScience* **1990**, *25*, 1265−1267.

Luo, Y.; Mikitzel, L. J. Extension of Postharvest Life of Bell Peppers with Low Oxygen. *J. Sci. Food Agric.* **1996**, *70*, 115−119.

Lurie, S.; Ronen, R.; Aloni, B. Growth-Regulator-Induced Alleviation of Chilling Injury in Green and Red Bell Pepper Fruit During Storage. *HortScience* **1995**, *30*, 558−559.

Mathooko, F. M.; Kubo, Y.; Inaba, A.; Nakamura, R. Induction of Ethylene Biosynthesis and Polyamine Accumulation in Cucumber Fruit in Response to Carbon Dioxide Stress. *Postharvest Biol. Technol.* **1995**, *5*, 51−65.

McDonald, R. E.; Kushad, M. M. Accumulation of Putrescine During Chilling Injury of Fruits. *Plant Physiol.* **1986**, *82*, 324−326.

Meir, S.; Rosemberger, I.; Aharon, Z.; Grinberg, S.; Fallik, E. Improvement of the Postharvest Keeping Quality and Colour Development of Bell Pepper (cv. "Maor") by Packaging with Polyethylene Bags at a Reduced Temperature. *Postharvest Biol. Technol.* **1995**, *5*, 303−309.

Pesis, E.; Marinausky, R.; Zauberman, G.; Fuchs, Y. Prestorage Low-Oxygen Atmosphere Treatment Reduces Chilling Injury Symptoms in "Fuerte" Avocado Fruit. *HortScience* **1994**, *29*, 1042−1046.

Pretel, M. T.; Serrano, M.; Amorós, A.; Riquelme, F.; Romojaro, F. Non-Involvement of ACC and ACC Oxidase Activity in Pepper Fruit Ripening. *Postharvest Biol. Technol.* **1995**, *5*, 295−302.

Reggiani, R.; Hochkoeppler, A.; Bertani, A. Polyamines in Rice Seedlings under Oxygen-Deficit Stress. *Plant Physiol.* **1989**, *91*, 1197−1201.

Risse, L. A.; Chun, D.; Miller, W. R. Chilling Injury and Decay of Film-Wrapped and Conditioned Bell Peppers During Cold Storage. *Trop. Sci.* **1987**, *27*, 85−90.

Saltveit, M. E., Jr.; Morris, L. L. Overview of Chilling Injury of Horticultural Crops. In *Chilling Injury of Horticultural Crops*; Wang, C. Y., Ed.; CRC Press: Boca Raton, FL, 1990; pp 3−15.

Serrano, M.; Martínez-Madrid, M. C.; Riquelme, F.; Romojaro, F. Endogenous Levels of Polyamines and Abscisic Acid in Pepper Fruits During Growth and Ripening. *Physiol. Plant.* **1995**, *95*, 73−76.

Wang, C. Y. Changes of Polyamines and Ethylene in Cucumber Seedlings in Response to Chilling Stress. *Physiol. Plant.* **1987**, *69*, 253−257.

Wang, C. Y. Effect of Abscisic Acid on Chilling Injury of Zucchini Squash. *Plant Growth Regul.* **1991**, *10*, 101−105.

Wang, C. Y. Approaches to Reduction of Chilling Injury of Fruits and Vegetables. *Hortic. Rev.* **1993**, *15*, 63−95.

Wang, C. Y.; Ji, Z. L. Effect of Low-Oxygen Storage on Chilling Injury and Polyamines in Zucchini Squash. *Sci. Hortic.* **1989**, *39*, 1−7.

Received for review November 12, 1996. Revised manuscript received February 3, 1997. Accepted February 3, 1997.[⊗] Work supported by funds from the Comisión Interministerial de Ciencia y Tecnología, Proyect ALI-95-0134.

JF960866H

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.