Growth-regulator-induced Alleviation of Chilling Injury in Green and Red Bell Pepper Fruit during Storage

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Abstract. Bell pepper (*Capsicum annuum* L.) plants were sprayed at full bloom with paclobutrazol (50 and 100 mg·liter⁻¹), uniconazole (20 and 50 mg·liter⁻¹), or mefluidide (20 and 50 mg·liter⁻¹). Mature-green and red fruit were harvested 4 and 8 weeks after spraying, respectively. Paclobutrazol and uniconazole, but not mefluidide, affected plant growth and fruit morphology. All three growth regulators alleviated chilling injury that developed on green and red peppers after 28 days at 2°C. Potassium leakage was lower from tissue disks, and weight loss less, from treated fruit than from control fruit. Ethylene and CO₂ production at 20°C were similar in control and paclobutrazol-treated fruit after 28 days at 2°C. These results are consistent with previous findings that showed these growth regulators are able to increase tolerance to low temperatures in whole plants. Chemical names used: N-[2,4-dimethyl-5-trifluoromethyl sulfonyl amino phenyl acetamide] (mefluidide); (2*RS*, 3*RS*) 1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol (paclobutrazol); (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol (uniconazole).

Triazole plant growth regulators, which include paclobutrazol and uniconazole, inhibit gibberellin biosynthesis and also appear to protect plants against various types of stress, including drought, atmospheric sulfur dioxide, and temperature extremes (Davis et al., 1988; Fletcher and Hofstra, 1988). Triazole compounds protect herbaceous plants from chilling injury (CI) within several days of treatment (Asare-Boamah and Fletcher, 1986; Borochoy and Shahar, 1989; Wang, 1985). Whitaker and Wang (1987) concluded that paclobutrazol did not confer chilling resistance by causing significant alterations in lipid composition. They noted, however, that chilling-induced degradation of membrane lipids, which is symptomatic of CI, was inhibited by paclobutrazol.

Mefluidide, an acetamide, protects plants from CI (Borochoy and Shahar, 1989; Tseng and Li, 1984). Absciscic acid synthesis was proposed as the site of mefluidide action, because this hormone accumulates in large amounts following treatment of corn (*Zea mays* L.) plants with mefluidide even under nonstress conditions (Zhang et al., 1986).

Absciscic acid increases resistance to a range of environmental stresses (Levitt, 1980).

These compounds could possibly protect fruit normally sensitive to low-temperature storage. The present investigation was conducted to determine if these growth regulators, which are effective in reducing CI at the whole-plant level, would also confer low-temperature tolerance to fruit harvested from treated plants.

Materials and Methods

Bell pepper plants ('Maor') were grown in a plastic house and treated at full bloom with sprays of paclobutrazol (50 and 100 mg·liter⁻¹), uniconazole (20 and 50 mg·liter⁻¹), and mefluidide (20 and 50 mg·liter⁻¹) with 0.02% Triton X-100 (Sigma, St. Louis) as a surfactant. The control plants were sprayed with 0.02% Triton X-100 in water. Spraying at this stage has been successfully used to cause earlier and more uniform ripening in tomatoes (*Lycopersicon esculentum* Mill.) and peppers,

allowing for a one-time harvest instead of selective picking (B. Aloni, unpublished; Frost and Kretschman, 1987). Each treatment was applied to three 3-m sections of a row with a plant density of six/meter. One month later, part of the fruit from each section was harvested mature-green, and 2 months later a second harvest was made for red peppers. At the second harvest, only fruit from one concentration (50 mg·liter⁻¹) of each growth regulator were harvested. Twenty fruit per section from each treatment (including the control) were placed at 2°C, 95% relative humidity, for 28 days. At the end of storage, the fruit were examined for CI and rots. CI was evidenced as surface pitting. If a fruit had more than five pits, regardless of size, it was classified as having CI. Rots were due to either *Alternaria alternata* Fr. or *Botrytis cinerea* Pers. ex Fr. Fruit having either or both fungi present were classified as decaying irrespective of the extent of the fungal development.

Upon removal from storage, five fruit each from the control and paclobutrazol treatment were placed at 20°C in individual 1.1-liter jars and CO₂ and ethylene production measured daily. The jars were closed for 2 h and a gas sample of the head space extracted by syringe. Ethylene was measured on a FID-GC (Varian, Sunnyvale, Calif.) with a 1-m alumina column, using N as the carrier gas, and injector, column, and detector temperatures of 80, 50, and 110°C, respectively. Carbon dioxide was determined on a TCD-GC (Varian) with a 2-m Poropak Q column, with He as the carrier gas at ambient temperature.

Weight loss was determined on 40 fruit from control and the paclobutrazol treatment at 50 mg·liter⁻¹. All fruit were weighed at harvest and then, at varying intervals, 10 fruit from each treatment were removed from storage and weighed again. Firmness and elasticity were measured on 10 fruit from each treatment, by placing a 2-kg weight on the fruit for 20 sec and measuring the resulting deformation in millimeters. Twenty seconds after removing the weight, the residual deformation was noted and termed elasticity. Leakage was determined by placing two rinsed 1-cm-diameter disks from three fruit of each treatment separately in 5 ml of distilled water and incubating for 3 h at 25°C. Potassium ions leaking from the disks into the incubation solution were measured with a flame photometer. The disks were then frozen, thawed, and ground in 5 ml of water. The solution was filtered and analyzed for total K. Leakage was expressed as the percentage of total K.

Table 1. Incidence of chilling injury (CI) and decay in green and red bell pepper fruit from plants treated with growth regulators at bloom. Peppers were examined after storage for 28 days at 2°C.

Growth regulator	Concn (mg·liter ⁻¹)	Fruit with CI (%)		Fruit with decay (%)	
		Green	Red	Green	Red
None	0	85	79	15	41
Paclobutrazol	50	41	63	5	46
	100	12	---	4	---
Uniconazole	20	11	---	3	---
	50	37	5	9	15
Mefluidide	20	16	---	0	---
	50	27	0	0	0
LSD _{0.05}		8.3	7.2	4.3	8.7

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Results and Discussion

Paclobutrazol and uniconazole affected plant growth and fruit morphology, while mefluidide did not. The fruit from both triazole growth regulator treatments were less elongated than control or mefluidide-treated fruit, and the plants were smaller and more compact. However, there was no effect on number of fruit per plant (data not shown).

All the growth regulators decreased the incidence CI, manifested as surface pitting, appearing on green or red peppers after 28 days at 2C (Table 1). The growth regulators also decreased the amount of rots developing after storage, except for paclobutrazol on red peppers. The red peppers were more susceptible to rots than the green, perhaps because they were more mature. Mefluidide was particularly effective in preventing rots in both green and red peppers. The major causal organism was identified as *Alternaria alternata*, with a minor contribution by *Botrytis cinerea*.

The firmness of red peppers, measured as resistance to deformation, was not significantly affected by the growth regulators, either at harvest or during storage (Table 2). However, elasticity was greater in control peppers than in those treated with paclobutrazol or mefluidide at harvest, but after storage only the difference between the control and those from the paclobutrazol-treated plants was significant. However, when paclobutrazol-treated and control green peppers were measured weekly, the firmness decreased (deformation increased) and elasticity increased more in control than in treated fruit (Table 3). Weight loss of the control peppers during this period was greater than that of those treated with paclobutrazol. The slopes of the changes in firmness, elasticity, and weight loss between control and paclobutrazol-treated peppers were significant at $P \leq 0.05$. Weight loss has been used as an indicator of CI in citrus (Cohen et al., 1994) and appears to be applicable in the case of peppers as well.

Another measure of tissue damage caused by CI is membrane permeability (Levitt, 1980). Leakage from treated peppers at harvest averaged 19.5%. After 28 days of storage, leakage from control fruit had increased by nearly 50%. Leakage from control fruit was higher than from treated fruit, with the exception of red fruit from paclobutrazol-treated plants (Table 4). The latter treatment also did little to alleviate CI in the red fruit.

In some tissues, the appearance of CI is accompanied by a transient burst of ethylene production (Wang and Adams, 1982). In the case of green peppers, this was not apparent, and monitoring control and paclobutrazol-treated fruit after removal from storage gave

Table 2. Firmness and elasticity of red bell pepper fruit at harvest and after storage. Plants had been treated with growth regulators (50 mg-liter⁻¹) at full bloom. The fruit were stored for 28 days at 2C, 95% relative humidity, and measured at removal from storage.

Growth regulator	Firmness ^a (mm)		Elasticity ^b (mm)	
	Harvest	Storage	Harvest	Storage
None	9.4	11.6	3.4	4.9
Paclobutrazol	7.5	10.5	2.4	3.6
Uniconazole	9.3	10.3	3.3	4.2
Mefluidide	8.5	11.4	2.4	4.3
LSD _{0.05}	1.92	2.22	1.00	1.09

^aLower values indicate greater resistance to deformation, i.e., firmer fruit.

^bLower values indicate closer return to original, undeformed height, i.e., more elastic fruit.

Table 3. Firmness, elasticity, and weight loss of green peppers harvested from nontreated and paclobutrazol (Paclo.)-treated (50 mg-liter⁻¹) plants following storage at 2C for various intervals.

Storage period (days)	Firmness (mm)		Elasticity (mm)		Wt loss (%)	
	Control	Paclo.	Control	Paclo.	Control	Paclo.
0	9.4	9.7	3.0	2.4	---	---
7	9.3	10.1	3.0	2.6	1.84	1.81
20	10.2	9.0	4.5	2.9	4.63	3.43
28	12.1	9.1	5.5	3.7	5.74	3.36
Slope	0.1	-0.05	0.08	0.04	0.20	0.12

very similar patterns of ethylene and CO₂ evolution (data not shown).

In summary, paclobutrazol, uniconazole, and mefluidide all increased low-temperature tolerance of green and red bell pepper fruit. The development of CI on the nontreated fruit was accompanied by increased weight loss and K leakage, whereas these features were reduced by the growth regulators. Further work is needed to determine the means whereby these compounds, which are given to the plant at bloom, have a sufficient residual effect to protect the fruit from low temperature after harvest.

Table 4. Potassium leakage of green and red peppers from plants sprayed with 50 ppm of various growth regulators at full bloom. The fruit were stored for 28 days at 2C, 95% relative humidity. Leakage at harvest averaged 19.5% with no differences among fruit from the various treatments.

Growth regulator	K ⁺ leakage (% of total) from fruit	
	Green	Red
None	30	27
Paclobutrazol	24	26
Uniconazole	23	21
Mefluidide	23	24
LSD _{0.05}	0.7	0.7

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