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The Effect of H_2O_2 Application during Production on Greenhouse Pepper Shelf Life

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ABSTRACT

Greenhouse-grown sweet peppers (*Capsicum annuum* L.) are commercially harvested at full color and stored at 10°C or above to avoid chilling injury. This study was undertaken to determine whether a H_2O_2 pre-treatment during production could reduce postharvest loss due to storage decay. Pepper fruit were stored at 2.5, 5, 7.5 or 10°C for 28 days, and then transferred to 21°C for an additional three days. Each fruit was visually inspected every three to four days during storage. When a fruit showed signs of incipient decay, the days elapsed between that day and the date of harvest was recorded as the shelf life. There were two experiments. In Experiment 1, a one-time application of 500 ppm H_2O_2 was imposed over 2~3 days in each of three production seasons (early, mid- and late). Shelf life was determined for three weekly harvests in each of three seasons. Effects of H_2O_2 were apparent through interactions with other factors. The H_2O_2-treated fruit had longer shelf life than control fruit when they were stored at 2.5°C or 5°C, depending on production season and on the number of weeks following H_2O_2 application. In Experiment 2, a single application of H_2O_2 for 5 days was followed by three weekly harvests. H_2O_2 prolonged the shelf life as a main effect. Our results indicated that pre-harvest application of 500 ppm H_2O_2 potentially prolonged the shelf life of greenhouse peppers either as a main effect or through interaction with other factors (i.e. under different specific experimental conditions). A single application of H_2O_2 seemed to be adequate for up to three weeks.

Keywords: *Capsicum annuum*, chilling injury, oxidative stress, postharvest, pre-harvest, storage

INTRODUCTION

Greenhouse-grown sweet pepper fruits are chilling sensitive (Lin *et al*. 1993), and the recommendation is storage at 7.2°C (45°F) or above for maximum shelf life (Jovicich *et al*. 2009). Chilling injury of stored sweet peppers leads to decay and other quality deterioration (McColloch 1962).

Chilling injury is associated with oxidative stress, accumulating high levels of H_2O_2 as a result (Sevillano *et al*. 2009). Zucchini squash fruit produced more H_2O_2 at 0°C than at 10°C (Gualanduzzi *et al*. 2009). H_2O_2 has dual effects: at high concentration it causes cell death, and at lower levels acts as a signaling compound (Dat *et al*. 2000). These dual effects were exemplified by experimental data on wheat: first, as oxidant, responsible for lipid peroxidation, membrane injury, pigment bleaching, protein/enzyme inactivation, and second, as an inducer of antioxidant enzymes glutathione reductase and catalase, which are responsible for scavenging H_2O_2 (Sairam and Srivastava 2000).

Chilling injury can be reduced by postharvest treatments such as heat shock (Whitaker 1994) and intermittent warming (Wang and Baker 1979), suggesting that one type of moderate stress may induce tolerance to stress of other kinds (Sevillano *et al*. 2009). These results indicated that there is crosstalk between induced resistance to biotic and abiotic stress (Bostock 2005; Fujita *et al*. 2006). H_2O_2 is one of the key signaling compounds (Neill *et al*. 2002) to induce tolerance to chilling injury. When plants respond to environmental stress, H_2O_2 is generated (Neill *et al*. 2002) which triggers the activation of antioxidant systems (e.g. Gualanduzzi *et al*. 2009) and leads to tolerance to subsequent chilling. This was proven in experiments where application of exogenous H_2O_2 led to elevated antioxidant enzyme activity (Prasad *et al*. 1994; Sairam and Srivastava 2000). Other evidence also exists, in that exogenous H_2O_2 reduced chilling injury of mung bean seedlings (Saleh 2007). Our own results showed that a pre-treatment of H_2O_2 or NaCl reduced severity of chilling injury in excised sweet potato shoots (Lin and Block 2009). Similarly, exogenous H_2O_2 induced salt and heat tolerance of rice seedlings (Uchida *et al*. 2002). However, there is no literature regarding the effect of H_2O_2 on the shelf life of greenhouse-grown sweet peppers. In this study, we attempted to use exogenous H_2O_2 as a pre-harvest treatment to prolong postharvest shelf life of greenhouse-grown sweet peppers.

MATERIALS AND METHODS

Plant materials

There were two experiments in which the cultivar, length of H_2O_2 application, time between H_2O_2 application and harvest, production season and the number of harvests differed (details below). Storage temperatures were the same in both experiments. These two experiments were designed to reveal the main effect of H_2O_2 and its possible interactions with the various factors mentioned above. Two separate phases of each experiment are described. One is pre-harvest H_2O_2 application in the greenhouse, and the other is individual fruit sampling for storage shelf life. There were 8 treatment combinations, consisting of 2 H_2O_2 treatments (H_2O_2 vs control) × 4 storage temperatures (2.5, 5, 7.5 and 10°C).

Greenhouse H_2O_2 treatments

Commercial cultural practice was followed for greenhouse pepper crop production (Portree 1996). Feed solution contained 10.98 mM N (as NO_3), 1.32 mM P, 7.35 mM K, and 2.68 mM Ca, along with micro-nutrients and Fe. In general, both greenhouse experiments were a 2 × 2 factorial arrangement in a split plot design. This 2 × 2 experiment consisted of 2 cultivars in combination with 2 H_2O_2 treatments, resulting in 4 greenhouse treatments. In each experiment there were 96 plants of each cultivar, totalling 192

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plants in a greenhouse of 78 m^2 growing area. The greenhouse was divided into north and south sections with 8 north-south rows in each. Two adjacent rows constituted a replicate, totalling 4 replicates in an east-west direction. Each of two cultivars (main plot) were randomly assigned into north and south sections with the two H_2O_2 treatments randomly assigned to each of two rows (subplot). Each cultivar-H_2O_2 treatment combination had 12 plants in 6 bags for each replicate. Fully coloured fruits (> 90% surface area) were harvested from each of the 4 greenhouse treatments and pooled for storage trials.

Storage life

Fruits from all 4 replicates of each greenhouse treatment (2 cultivars and 2 H_2O_2 combinations) were pooled to ensure sufficient numbers for storage trials at 4 storage temperatures. In these two experiments, fruit were stored for 28 days at 2.5, 5, 7.5 or 10°C which was followed by 3-day recovery at 21°C. Each fruit was considered an experimental unit. Twice a week (every 3 to 4 days), each fruit was visually inspected for signs of decay. When a fruit showed the symptom of incipient decay, the days elapsed between that day and the date of harvest was designated as the shelf life. The data on shelf life were analyzed as a completely randomized design.

Experiment 1

In 2007, a 500 ppm H_2O_2 application over 2-3 days was imposed on the same plants in each of three production seasons (early, mid-season, and late). Seeds of the red cultivar '4Ever' (Enza Zaden, the Netherlands) and the yellow cultivar 'Baselga RZ' (Rijk Zwaan, the Netherlands) were sown on 23 January and transplanted on 5 March 2007 into a 35L sawdust bag with two transplants per bag. The experiment with 4 replicates was arranged in a split-plot design. Cultivars and H_2O_2 treatments were randomly assigned as a 2 cultivars × 2 H_2O_2 factorial experiment. There were three consecutive harvests per season. In the early season, H_2O_2 was applied on 22 May, and three harvests occurred on 29 May, 5 and 12 June, 2007. A similar schedule for H_2O_2 application and three subsequent weekly harvests was repeated in the mid-season (H_2O_2 applied on 24 July and fruit harvested on 31 July, 7 and 14 August) and in late season (H_2O_2 applied on 2 October and fruit harvested on 9, 16 and 23 October). For each of 3 seasons, there were 12 treatments consisting of 2 cultivars × 2 H_2O_2 treatments × 3 weekly harvests. Upon each weekly harvest, 4 fruit each were randomly selected and assigned to 4 storage temperatures (2.5, 5, 7.5 and 10°C).

Experiment 2

In 2008, the 500 ppm H_2O_2 application was imposed over 5 days. Seeds of the red pepper cultivar '4Ever' and yellow cultivar 'Baselga RZ' were sown on 21 February. Seedlings were transplanted into a 78 m^2 greenhouse on 29 May. Two cultivars and H_2O_2 treatments were randomly assigned as a 2 cultivars × 2 H_2O_2 treatments factorial experiment as in Experiment 1. On 6 October 2008, 500 ppm H_2O_2 was incorporated into the nutrient solution and administered over 5 days while control plants remained on regular solution. There were three harvests on 14, 21 and 28 October. Fruit of each cultivar and H_2O_2 combination were pooled and 4 fruit each were randomly assigned to each of 4 storage temperatures (2.5, 5, 7.5 and 10°C) as in Experiment 1.

Statistical procedures

The 2 (cultivar) × 2 (H_2O_2 treatments) factorial experiments were conducted with a split plot design during the greenhouse production period to ensure each treatment was randomly located throughout the greenhouse. However, there was often not a sufficient number of coloured fruit available on a single day for replicates from each treatment. Fruits from 4 replicates were therefore pooled according to 4 treatment combinations, and randomly assigned to each of 4 storage temperatures. Each fruit was treated as an experimental unit. Analysis of variance on shelf life was carried out as a completely randomized design using Proc GLM of SAS package (SAS version 9.1.3, SAS Institute, Cary, NC, USA). Eight treatment combinations based on 2 pre-harvest H_2O_2 applications and 4 storage temperatures were collectively considered as a single source of variance analyzed for each experiment. When treatment main effects were significant, means of H_2O_2 and control for each storage temperature were separated by comparing Least Square Means (LSMeans).

RESULTS

Experiment 1

H_2O_2 was applied over 2~3 days in each of three production periods (**Table 1**). Each H_2O_2 application was followed by 3 weekly harvests (week-1, week-2 and week-3). The analysis of variance (ANOVA) indicated that there was no main effect of cultivar or cultivar-treatment interaction on shelf life, therefore the data of 2 cultivars were combined, resulting in 8 fruits (experimental units) for each of 8 treatment combinations, consisting of 2 H_2O_2 × 4 storage temperatures. ANOVA also revealed a significant interaction of season-week-treatment (Pr = 0.0014). First, we separated data into early, mid- and late seasons. Then we further analyzed the interaction between weekly harvests and 8 treatment combinations. We focused on effects of weeks after application (week-1, -2 and -3), H_2O_2 (500 ppm H_2O_2 vs control), and storage temperatures (2.5, 5, 7.5 and 10°C) in these 3 analyses (**Table 1**). We first describe whether there is any main effect, and then we describe interactions in detail.

Table 1 Pre-harvest application of 500 ppm H_2O_2 to nutrient solution and 4 storage temperatures affect the shelf life of greenhouse peppers. There were 3 consecutive weekly harvests during each of 3 production seasons in Experiment 1 (2007).

Season	Weeks	Shelf life								Average[Z]
		Control				H_2O_2				
		2.5°C	5°C	7.5°C	10°C	2.5°C	5°C	7.5°C	10°C	
Early	Week-1	22.9[Y]	19.3	13.5	9.0	29.9(***)[Y]	25.6(**)[Y]	15.1	8.1	17.9
	Week-2	21.9	18.8	11.6	7.0	25.1	25.1(***)	12.4	10.4	16.6(*)
	Week-3	19.1	14.1	8.5	7.8	20.9	13.8	11.1	9.8	13.1(***)
	Average	21.3	17.3	11.2	7.9	25.3(***)	21.6(***)	12.9	9.4	
Mid	Week-1	21.8	21.0	9.9	7.4	24.8	20.4	10.8	7.0	15.4
	Week-2	20.8	17.8	9.8	8.1	20.4	20.8	9.9	7.8	14.4
	Week-3	15.5	12.9	10.6	8.5	20.8(**)	14.5	10.6	9.3	12.8(***)
	Average	19.3	17.2	10.1	8.0	22.0(*)	18.5	10.4	8.0	
Late	Week-1	22.0	19.9	8.3	5.0	26.8(*)	17.4	8.8	5.0	14.1
	Week-2	18.8	15.0	8.6	6.0	27.8(***)	22.5(***)	10.0	4.5	14.1
	Week-3	25.9	13.4	7.8	5.0	26.6	19.4(**)	9.5	5.4	14.1
	Average	22.2	10.1	8.2	5.3	27.0(***)	19.8(**)	9.4	5.0	

[Z] The least squares means (n=8) of week-2 or week-3 are compared with week-1 harvest.
[Y] The least squares means (n=8) are compared between control and H_2O_2 when stored at the same temperature.
*, **, *** designated statistical significance at p=0.05, 0.01, and 0.001 levels.

Cultivars

There was no main effect of cultivar or cultivar-treatment interaction on shelf life (above). The data of two cultivars were combined for further analysis.

Season

In general, early season had longer shelf life than mid- and late season with shelf life of 15.9, 14.1 and 14.4 days, respectively. However seasonal effect was complicated by interaction of season-week-treatment.

Weekly harvests

On average, pepper shelf life at week-1 was longest (15.8 days), followed by week-2 (15.0 days), and then week-3 (13.3 days). Again, it was complicated by the interaction of season-week-treatment.

Treatment combinations (2 H_2O_2 × 4 storage temperature)

There was significant treatment effect on shelf life (Pr<0.001). Treatment effect itself, in fact, is the interaction of 2 H_2O_2 × 4 storage temperatures. Further, treatment effect was complicated by an interaction of season-week-treatment (**Table 1**). H_2O_2 effect on shelf life was directly compared with control (n=8) under specific harvest season, weekly harvest, and storage temperature. In the early season, H_2O_2 improved shelf life of week-1 harvest with fruit stored at 2.5 or 5°C and shelf life of week-2 harvest stored at 5°C (**Table 1**). In the mid-season sampling, H_2O_2 only improved shelf life of week-3 harvest when fruits were stored at 2.5°C. In the late season crop, H_2O_2 improved shelf life of week-1 harvest stored at 2.5°C, week-2 harvest stored at 2.5 or 5°C, and week-3 harvest of fruits stored at 5°C (**Table 1**).

Experiment 2

In 2008, the 500 ppm H_2O_2 application was extended to 5 days (**Table 2**). The main effect of H_2O_2 on shelf life was significant (p=0.0297): shelf life increased from 13.8 days (control) to 15.8 days (H_2O_2 treatment). The main effect of harvest was also significant (p<0.001): average shelf life decreased from 17.8 days on harvest-1 to 13.4 and 13.2 days, harvest-2 and -3, respectively. There was no interaction between harvest and H_2O_2.

DISCUSSION

Cultivars

In Experiment 1 (H_2O_2 application for 2 days in each season), there was no cultivar effect on shelf life, nor interaction between cultivar and treatment combinations (H_2O_2-storage temperature). In Experiment 2 (application of H_2O_2 for 5 days), no cultivar difference in response to H_2O_2 was observed (**Table 2**). In short, no cultivar effect on shelf life was observed in both experiments. It is similar to our previous report where there was no cultivar-temperature interaction in chilling injury of greenhouse peppers (Lin *et al*. 1993). Yet the lack of an effect of cultivar is somewhat unexpected, because red and yellow peppers behaved differently in fruit coloration (Lin and Hill 2007) and fruit yield patterns (Lin and Hill 2008; Lin *et al*. 2009). Further, a cultivar effect on postharvest shelf life and quality was also observed in tomato fruit (Whitaker 1994). In contrast to other cultivars of tomatoes, one specific cultivar 'Rutgers' did not respond to heat treatment to reduce chilling injury of greenhouse tomato fruit, but instead slow ripening induced chilling tolerance. Many new greenhouse pepper cultivars are expected to be developed and released to growers by private companies. It is possible that the specific H_2O_2 treatment regime used in this study may not necessarily be as effective with all future cultivars.

H_2O_2 effects

Our data illustrated that the effect of H_2O_2 on pepper shelf life can be detected as an interaction with other factors as in Experiment 1 (**Table 1**) or as a main effect as in Experiment 2 (**Table 2**). In Experiment 1, when H_2O_2 was imposed for 2~3 days, the effect of H_2O_2 was observed in an interaction of season-week-treatment (Pr = 0.0014), where treatments consisted of a combination of 2 H_2O_2 and 4 storage temperatures. It is expected that growing season (i.e. early, mid- and late season) has an influence on shelf life in general. However, it is rather surprising that season influenced the pattern of shelf life of 3 consecutive weekly harvests (**Table 1**). In the early season, shelf life was reduced significantly from week-1 harvest to week-2 and -3. In mid-season, only week-3 harvest was reduced as compared to week-1 harvest. In late season, no such reduction in shelf life was observed. When season and weekly harvest are detailed, an effect of H_2O_2 was primarily observed on fruits stored at 2.5 or 5°C. In short, H_2O_2 prolonged shelf life of pepper stored at 2.5 or 5°C. Storage temperatures of 2.5 and 5°C are considered to be the cause of chilling injury and result in subsequent decay and short shelf life. Our preliminary results indicated that the chilling injury occurred at 2.5 and 5°C, because of substantial increase in decay when they returned to 21°C for 3 days after 28 days storage at 2.5 or 5°C as compared to those stored at 7.5 or 10°C (unpublished data). Chilling injury symptoms are often expressed more rapidly after returning to higher temperature than when maintained at low storage temperatures (Wang and Baker 1979). Our observation provides some evidence that H_2O_2 possibly alleviated chilling injury when pepper fruits were stored at 2.5 or 5°C. The observed interaction of season-week-treatment (i.e. season-week-H_2O_2-temperature) implies the significant effect of H_2O_2 on shelf life exists under a specific set of conditions consisting of season, week and storage temperature. Similarly, H_2O_2 reduced chilling injury of sweet potato shoots under long photoperiod but not under short photoperiod (Lin and Block 2009). This beneficial effect of H_2O_2 was confirmed as a main effect in Experiment 2 (**Table 2**), where H_2O_2 was imposed for 5 days. The difference in H_2O_2 effect observed in these two experiments may have been due to the fact that 500 ppm of H_2O_2 was applied for 2~3 days in Experiment 1 and for 5 days in Experiment 2. However, H_2O_2 application for a period longer than 5 days may not be necessary. Our preliminary observations of H_2O_2 application for 93 days did not show any better effect than those observed in Experiment 1 and 2 (unpublished data). Despite the fact that H_2O_2 benefited shelf life in different ways, as a main effect or through interaction with other factors, both experiments illustrated the potential benefits of pre-harvest H_2O_2 application in prolonging post-harvest shelf life of stored greenhouse peppers.

Table 2 Harvest date and H_2O_2 affected shelf life of greenhouse peppers in Experiment 2 (2008)[Z].

Harvest	Cultivar	Shelf life	
		0 ppm H_2O_2	500 ppm H_2O_2
Harvest 1	Red	15.4 [Z]	19.4
Harvest 2	Red	11.9	14.1
Harvest 3	Red	12.1	14.4
Harvest 1	Yellow	17.8	18.5
Harvest 2	Yellow	12.5	14.1
Harvest 3	Yellow	12.9	14.3
Significance	Pr > F		
Harvest	<0.0001	***	
Cultivar	0.6112	ns	
H_2O_2	0.0297	*	
Harv x Cv	0.9791	ns	
Harv x H_2O_2	0.9725	ns	
Cv x H_2O_2	0.3770	ns	
Harv x Cv x H_2O_2	0.8114	ns	

[Z] Shelf life represents an average of 16 fruit.
[ns], *, **, *** designated non-significant or significant at p=0.05, 0.01, and 0.001 levels, respectively.

SUMMARY

Two important findings were discovered in this study. First, it appears that pre-harvest H_2O_2 treatment during greenhouse production can potentially prolong postharvest storage shelf life of greenhouse peppers. This may have been the first report on the effects of H_2O_2 application during production on subsequent shelf life of sweet peppers. Shelf life is often studied in relation to postharvest procedures such as heat treatments. Only recently has the influence of growing conditions become a potential tool for improving postharvest shelf life. For example, elevated growing temperatures can improve chilling tolerance of greenhouse-grown cucumbers (Kang *et al*. 2002). Our study confirms such a possibility. Secondly, pre-harvest treatments such as H_2O_2 can result in more moderate and indirect effects due to interactions with other factors. These beneficial effects may be seen as a main effect (i.e. Experiment 2) or as an interaction (i.e. Experiment 1). Therefore, further experiments are needed to verify the conditions required for effective H_2O_2 application as part of hydroponics production systems. In view of H_2O_2 being a signalling molecule in stress physiology (Neill *et al*. 2002), its effects on peppers may not be limited to shelf life extension. H_2O_2 may also be applied for other types of beneficial effects, such as tolerance to other biotic and abiotic stresses, and fruit quality enhancement (Uchida *et al.* 2002). However, specific conditions under which H_2O_2 may be beneficial should be verified experimentally prior to any commercial applications.

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