Reducing Injury of Lettuce from Phosphine Fumigation

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SUMMARY. Fumigation with cylindered pure phosphine free of ammonia has been used commercially at low temperatures in recent years to control pests on harvested fresh fruit and vegetables. However, long fumigation treatments cause injuries to lettuce (*Lactuca sativa*) and prevent its commercial use on lettuce. We evaluated whether absorbents for carbon dioxide (CO₂) and ethylene can prevent injuries to lettuce in phosphine fumigation, hypothesizing that an accumulation of CO₂ or ethylene be responsible for the injuries. Head and romaine lettuce were fumigated in chambers in the presence or absence of CO₂ and ethylene absorbents for 3 days at 2 °C. The use of absorbents prevented lettuce injury associated with fumigation and resulted in higher lettuce quality. In the absence of the absorbents, both head and romaine lettuce sustained significant injuries in the form of brown stain, a typical symptom of CO₂ injuries, and significantly reduced quality as compared with fumigation in the presence of absorbents. The injuries were likely caused by CO₂ based on the facts that injuries were identical to CO₂ injuries and the prevention of the injuries by the use of CO₂ absorbent. The findings of this study have important implications for developing safe and effective phosphine fumigation protocols at low temperature for controlling insect pests on fresh commodities, especially when a long treatment time is required.

United States-exported lettuce faces phytosanitary barriers in overseas markets due to the presence of quarantined pests including western flower thrips (*Frankliniella occidentalis*) for Taiwan, and californian pea leafminer (*Liriomyza langei*) and lettuce aphid (*Nasonovia ribisnigri*) for Japan (Liu, 2008, 2012). Although methyl bromide fumigation is still exempted for quarantine treatment usage while its production is being phased out globally as mandated by the Montreal Protocol, it may not be available in the future. Methyl bromide fumigation also injures lettuce and shortens lettuce shelf life. Therefore, it is critical to develop a safer alternative to meet pest quarantine requirement for exported lettuce.

Phosphine has been used extensively for stored product pest control for over 80 years, and now has emerged as a main methyl bromide alternative (Chaudhry, 1997; Fluck, 1973; Horn et al., 2003). However, there are disadvantages with phosphine fumigation. Phosphine acts slowly against insects and often requires long treatment times to achieve effective control of target pests. For some tolerant pests, fumigations of over 10 d are needed (Hole et al., 1976). Phosphine is more active at higher temperatures and therefore low-temperature phosphine fumigation also takes longer time to control pests (Bond, 1984; Hole et al., 1976; Liu, 2008). Some insects also developed resistance to phosphine (Schlipalius et al., 2002).

Traditionally, phosphine generated from metal phosphides has not

been used for pest control on fresh commodities due to phytotoxicity caused by ammonia, which is cogenerated to modulate the phosphine generating chemical reaction. In recent years, cylindered phosphine gas free of ammonia has been used successfully in Chile to control pests on fresh fruit and vegetables (Horn and Horn, 2004; Horn et al., 2005, 2010). However, information on potential impact on postharvest quality of fresh products is still very limited (Jamieson et al., 2012; Klementz et al., 2005; Liu, 2008, 2011, 2012). One-day fumigation with 1000 ppm phosphine at 2 °C for control of western flower thrips was found to be safe for fruit and vegetables including lettuce (Liu, 2008). Western flower thrips was also controlled effectively without negative impact on lettuce quality in a pallet scale 18-h fumigation of prechilled lettuce under an insulation cover (Liu, 2011). However, a 3-d fumigation with phosphine at a high concentration of 2200 ppm at 2 °C for controlling lettuce aphid resulted in significant injuries to both romaine and head lettuce in the form of brown stains identical to CO₂ injuries (Lipton et al., 1972; Liu, 2012). Because cylindered phosphine is free of ammonia, it is possible that factors other than phosphine may be responsible for the injuries to fumigated lettuce.

In the 3-d low-temperature phosphine fumigation as reported by Liu (2012), the lettuce was sealed in fumigation chambers for 3 d without air exchange. This might have resulted in significant changes in atmosphere composition as lettuce consumes oxygen and produces CO₂ and other volatiles such as ethylene. After harvest, head lettuce produces CO₂ at 17 mg·kg⁻¹·h⁻¹ at 5 °C (Saltveit, 2004) and releases ethylene at <0.1 µL·kg⁻¹·h⁻¹ at 20 °C (Kader and Kasmire, 1984). Lettuce is also very sensitive to both

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
3.7854	gal	L	0.2642
3.3864	inch(es) mercury	kPa	0.2953
0.4536	lb	kg	2.2046
28.3495	oz	g	0.0353
0.001	ppm	mg·g ⁻¹	1000
1	ppm	mg·kg ⁻¹	1
1	ppm	µL·L ⁻¹	1
6.8948	psi	kPa	0.1450
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

CO₂ (Kader and Morris, 1977) and ethylene (Kim and Wills, 1995; Schouten, 1985). Exposure to 1% CO₂ or 0.1 ppm ethylene may cause injuries to sensitive lettuce cultivars under unfavorable atmospheric conditions (Lipton et al., 1972; Morris et al., 1974). For lettuce, CO₂ causes brown stains (Lipton et al., 1972), and ethylene causes russet spotting (Morris et al., 1974). Cultivars vary significantly in their sensitivities to CO₂ and ethylene (Brecht et al., 1973; Cantwell and Suslow, 2001). In this study, we conducted low-temperature phosphine fumigation treatments of head and romaine lettuce with and without CO₂ and ethylene absorbents to determine whether scrubbing CO₂ and ethylene can reduce or prevent fumigation associated injuries to lettuce. Lettuce injuries were also analyzed for associations with lettuce weight and quality.

Materials and methods

LETTUCE AND CHEMICALS. Vacuum-cooled commercial head and romaine lettuce grown in Salinas Valley were stored for 1 d at 2 °C after harvest at Tanimura & Antle Fresh Foods (Salinas, CA) and were then transported to the U.S. Department of Agriculture and immediately used in fumigation treatments. All head lettuce was individually wrapped in perforated plastic sleeves. Romaine lettuce was packed in perforated bags in groups of three. Seven lettuce cultivars were used in these studies: two head lettuce cultivars (Telluride and Spyglass) and five romaine lettuce cultivars (Green Forest, Sun Valley, Zuma, Sparx, and Absolute). A phosphine sample (1.6% pure phosphine balanced with nitrogen) in a compressed gas cylinder from Cytec Canada (Niagara Falls, ON, Canada) was used. CO₂ absorbent soda lime (SodaSorb; W.R. Grace, Cambridge, MA) and ethylene absorbent potassium permanganate (Power Pellets; Ethylene Control, Selma, CA) were bought commercially.

USE OF CO₂ AND ETHYLENE ABSORBENTS. Amounts of CO₂ and ethylene absorbents used in each test were determined based on projected production of CO₂ and ethylene by lettuce. Head lettuce produces CO₂ at 17 mg·kg⁻¹·h⁻¹ at 5 °C (Saltveit, 2004). Soda lime can absorb 294 mg·g⁻¹ CO₂ at 20 °C (W.R. Grace, unpublished

data). Head lettuce releases ethylene at <0.1 μL·kg⁻¹·h⁻¹ at 20 °C (Kader and Kasmire, 1984). The ethylene absorbent can absorb 7.4 μL·g⁻¹ ethylene at 20 °C (Ethylene Control, unpublished data). Since there were no data of ethylene release and CO₂ respiration rates for head and romaine lettuce at 2 °C, we used one and half times of the CO₂ and ethylene release rates at the higher temperatures to calculate amounts of CO₂ and ethylene absorbents. Therefore, for 1 kg lettuce, 6.24 g soda lime was used to absorb the estimated amount of CO₂ release [(17 mg·kg⁻¹·h⁻¹ × 72 h)/(294 mg·g⁻¹)] × 1.5. For ethylene absorption, 1.46 g ethylene absorbent was used [(0.1 μL·kg⁻¹·h⁻¹ × 72 h) × 1.5/(7.4 μL·g⁻¹)] for 3-d fumigation. The quantities of CO₂ and ethylene absorbents were sufficient based on the observation that soda lime did not change color from white to deep purple, and the color of ethylene absorbent did not change from purple to brown color by the end of fumigation.

PHOSPHINE FUMIGATION PROCEDURES. Fumigations were conducted in 442-L sealed metal chambers at 2 °C in walk-in coolers. The temperature was monitored with temperature/relative humidity data loggers (Onset Computer Corp., Pocasset, MA). In each test, two fumigation treatments, one with CO₂ and ethylene absorbents and the other without the absorbents, were conducted in two fumigation chambers. Two cartons of romaine lettuce from two cultivars and two cartons of head lettuce of the two cultivars were sealed in each chamber after removing nine heads of romaine lettuce and six heads of head lettuce from each carton, respectively, to be stored in cartons at 2 °C in a cooler as controls. Lettuce was weighed and the amounts of ethylene and CO₂ absorbents used were calculated based on the weight of lettuce. Each sealed chamber was vacuumed to ≈-15 inches mercury pressure first with a vacuum pump to prevent pressure build up in the chamber when phosphine gas sample is released into the chamber, and then a volume of about 68 L of 1.6% phosphine sample was released into each fumigation chamber. As phosphine sample has 1.6% phosphine balanced with nitrogen, 18 L of oxygen gas from a preloaded foil bag was

released into the fumigation chamber to maintain the normal oxygen level in the fumigation chamber. The outside air was then released into the chamber through a one-way valve to balance air pressure. The chambers were then kept sealed for the duration of fumigation treatments.

The volume of phosphine sample was calculated based on differential pressure readings on a 1-gal sample cylinder. A pressure of 264 psi in the sample cylinder is equivalent to the volume of 68 L phosphine sample, and results in about 2500 ppm initial phosphine concentration based on an earlier study (Liu, 2012). Phosphine concentration was monitored using a gas chromatograph (HP 6890N; Agilent Technologies, Santa Clara, CA) equipped with a flame photometric detector. The gas chromatograph procedures were the same as described previously (Liu, 2012). The samplings were conducted 30 min after the fumigation, every early morning and later afternoon after that, and at the end of the experiment, for a total of eight times. During the 3-d fumigation, the average concentration of phosphine was 2218 ppm for fumigation without CO₂ and ethylene absorbents, and 2083 ppm for fumigation with absorbents.

After the 3-d fumigation, the fumigation chamber was vented by a ventilation system for at least 3 h. The lettuce was then stored at 2 °C in a walk-in cooler together with the controls for 14 d. Visual quality of lettuce for marketability was then evaluated based on Morris et al. (1974) using the score system of Kader et al. (1973) with quality scores ranging from 1 (extremely poor) to 9 (excellent) and 3, 5, and 7 representing poor with excessive defects, fair with slightly to moderately objectionable defects, and good with minor nonobjectionable defects, respectively. Scores 2, 4, 6, and 8 were also used for quality between the full scores as in a previous study (Liu, 2008). All quality evaluations were conducted by one person to maintain consistency. Head lettuce was evaluated for external quality and injuries after being weighed individually. Each lettuce head was cut twice longitudinally on the vertical plane to inspect for internal quality and injuries. Romaine lettuce was evaluated for external quality and injuries and then cut once vertically to inspect for

internal quality and injuries. Presence and absence of injuries were recorded as 1 and 0 for statistical analysis.

In each test, 96 heads of head lettuce in four cartons and 144 heads of romaine lettuce in four cartons were used. The test was replicated four times. A total of 384 heads of head lettuce in 16 cartons and 576 heads of romaine lettuce in 16 cartons were used.

DATA ANALYSES. A total 3840 quality and injury data points from head lettuce and romaine lettuce were used for statistical analysis. The external and internal quality scores, percentages of lettuce injuries, and weights of head lettuce were analyzed using the fit model platform of JMP statistical discovery software (version 10.0; SAS Institute, Cary, NC). All data were subjected to one-way analysis of variance (ANOVA) followed by a Tukey's honestly significant difference (HSD) multiple range test for comparison of means of treatments. The mean and standard errors published here were not transformed. Significant differences were considered at the $P \leq 0.05$ level.

Results

EFFECTS OF CO₂ AND ETHYLENE ABSORBENTS ON LETTUCE RESPONSES TO PHOSPHINE FUMIGATION. The presence of absorbents for CO₂ and ethylene in the 3-d phosphine fumigation prevented injuries associated with phosphine fumigation and resulted in better lettuce quality. Typical injuries associated with fumigated lettuce were translucent sunken and coalesced or individual lesions with brown stain along margins. There was no interaction between treatments and lettuce types based on ANOVA (Table 1). Therefore, head lettuce and romaine lettuce were analyzed together for the impact of phosphine fumigation on lettuce quality (Fig. 1). There were no significant differences in external injury or external lettuce quality scores between treatment without absorbents and the control. However, phosphine-fumigated lettuce with absorbents had 7.8% heads with external injury. This was significantly lower than the 15.0% and 14.6% injuries for the control and the treatment without absorbents ($P < 0.01$). Phosphine-treated lettuce with absorbents also had a significantly higher external quality score (7.6) as

Table 1. Analysis of variance of effects of the presence of carbon dioxide and ethylene absorbents in 3-d phosphine fumigation treatments and lettuce types (head and romaine on lettuce quality and injury ratings after 14 d posttreatment storage at 2 °C (35.6 °F)).

Source	df	<i>F</i> (<i>P</i>) values for quality		<i>F</i> (<i>P</i>) values for injury	
		External	Internal	External	Internal
Treatment	2	9.54 (<0.0001)	14.75 (<0.0001)	5.54 (0.005)	4.60 (0.010)
Type	1	0.01 (0.940)	1.17 (0.279)	0.01 (0.888)	41.27 (<0.0001)
Treatment × type	2	0.06 (0.945)	0.63 (0.532)	0.44 (0.642)	1.08 (0.341)

compared with those for the control (7.4) and the treatment without absorbents (7.4) ($P < 0.0001$) (Fig. 1). The fumigation with absorbents had a significantly lower percentage of heads with internal injuries (8.4%) and significantly higher internal quality (8.2) than the 14.1% internal injury and the 7.8 internal quality score for the treatment without absorbents respectively ($P < 0.05$). The internal injury of 14.1% for fumigation without absorbents was not significantly higher than the injury level for the control ($P > 0.05$). However, the internal quality score (7.8) for fumigation without absorbents was significantly lower than those for the control (8.0) and the fumigation with absorbents (8.2) ($P < 0.0001$) (Fig. 1).

Head lettuce and romaine lettuce were also analyzed separately for sensitivity to the fumigation treatments (Fig. 2). For head lettuce, ANOVA showed that the external and internal quality scores of lettuce from the treatment with absorbents (scores of 7.6 and 8.2) were significantly higher than those from the treatment without absorbents (scores of 7.4 and 7.7) but were not significantly different from those for the control (scores of 7.4 and 8.0) ($P < 0.01$). The 6.8% of head lettuce with external injuries for the treatment with absorbents was significantly lower than the 17.1% of injuries for the control but not significantly lower than the 14.2% of injuries for fumigation without absorbents ($P < 0.05$). There was no significant difference in internal injury among the treatments ($P = 0.085$), even though 24.3% of lettuce had sustained injuries for fumigation without absorbents as compared with 15.5% and 14.8% of injuries in fumigation with absorbents and the control (Fig. 2). The weight of head lettuce was not significantly different among the control (731.8 g), the treatment without

Fig. 1. Effects of 3-d phosphine fumigation treatments with and without carbon dioxide and ethylene absorbents on combined head and romaine lettuce. Quality and injuries were evaluated 14 d after fumigation. The quality scores range from 1 (extremely poor) to 9 (excellent) with 7 as good quality with minor nonobjectionable defects as described in the materials and methods. Means with the same letters in each group were not significantly different based on Tukey's honestly significant difference multiple range test at $P > 0.05$.

absorbents (724.5 g), and the treatment with absorbents (699.4 g) ($P = 0.085$).

For romaine lettuce, ANOVA showed that there were no significant differences in internal and external quality scores between the treatment without absorbents (7.3, 7.9) and the control (7.4, 8.0), but the quality scores of fumigated lettuce with absorbents (7.6, 8.2) were significantly higher than those for the control and fumigated lettuce without absorbents ($P < 0.005$). There were no significant differences among the treatments in the percentage of external and internal injuries ($P > 0.1$). The external injuries were 13.6%, 8.6%, and 14.9%

Fig. 2. Effects of 3-d phosphine fumigation treatment with and without carbon dioxide and ethylene absorbents on head and romaine lettuce. Quality and injuries were evaluated 14 d after fumigation. The quality scores range from 1 (extremely poor) to 9 (excellent) with 7 as good quality with minor nonobjectionable defects as described in the materials and methods. Means with the same letters in each group were not significantly different based on Tukey's honestly significant difference multiple range test at $P > 0.05$.

for control lettuce, treatments with and without absorbents, respectively. The internal injuries were 4.6%, 3.6%, and 7.2% for control lettuce, treatments with and without absorbents, respectively (Fig. 2).

VARIATION AMONG LETTUCE CULTIVARS IN SUSCEPTIBILITY TO INJURIES BY PHOSPHINE FUMIGATION. There were considerable variations among lettuce cultivars in responses to phosphine fumigation treatments as demonstrated by 'Telluride' and 'Spyglass' (Fig. 3). A total of 10 cartons of 'Telluride' and six cartons of 'Spyglass' were used in the study. For 'Telluride', phosphine-fumigated lettuce with absorbents had significantly higher external quality compared with the fumigated lettuce without absorbents ($P < 0.01$). There were no significant differences among the treatments in internal quality ($P = 0.265$) or internal injury ($P = 0.370$) (Fig. 3). For 'Spyglass', no significant differences were found among treatments in external quality scores ($P = 0.373$) or external injury percentages ($P = 0.997$). The fumigated lettuce with absorbents had significantly better internal quality as compared with the lettuce fumigated

without absorbents ($P < 0.005$), and significantly less internal injuries as compared with the lettuce without absorbents ($P < 0.05$) (Fig. 3). The fumigated lettuce without absorbents had the lowest internal quality and the highest level of internal injury, but the quality scores and injury percentages were not statistically significantly different from the control.

In the absence of absorbents, fumigated head lettuce 'Telluride' had a significantly higher internal quality ($P < 0.0001$) and a lower percentage of internal injury ($P < 0.0001$) than 'Spyglass' (Table 2). The weights of the two cultivars were not significantly different ($P = 0.234$). In the absence of absorbents, fumigated romaine lettuce 'Sun Valley' showed the lowest score of external quality, while 'Absolute' had the highest score and the difference was significant ($P < 0.05$). 'Green Forest' did not have internal injury, while 'Zuma' showed considerable internal injuries ($P = 0.052$) (Table 2). The order of sensitivity of romaine lettuce to internal injury from tolerant to susceptible was 'Green Forest' > 'Absolute' > 'Sparx' > 'Sun Valley' > 'Zuma'.

CROSS RELATIONSHIPS AMONG LETTUCE WEIGHT, QUALITY, AND INJURIES. For head lettuce fumigated in the absence of CO_2 and ethylene absorbents, the internal quality and injury were significantly correlated with lettuce weight. ANOVA showed that the heavier the lettuce, the higher the percentage of lettuce heads with internal injuries and the lower the internal quality. The average weights for head lettuce with internal quality scores of 7, 8, and 9 were 793.8, 736.5, and 674.3 g, respectively, and the differences in weight were significantly different with lower internal quality corresponding to heavier weight of lettuce ($P < 0.01$) (Table 3). The average weight of 769.3 g for head lettuce with internal injury was also significantly higher than the 712.3 g for the average weight of lettuce without internal injuries ($P < 0.05$). On the other hand, the lettuce weight had no significant correlation with external quality ($P = 0.263$) or external injury ($P = 0.760$) (Table 3). There were, however, highly significant correlations between the external injury and external quality ($P < 0.0001$), and between internal injury and internal quality

Fig. 3. Effects of 3-d phosphine fumigation treatment with and without carbon dioxide and ethylene absorbents on head lettuce cultivars Telluride and Spyglass. Quality and injuries were evaluated 14 d after fumigation. The quality scores range from 1 (extremely poor) to 9 (excellent) with 7 as good quality with minor nonobjectionable defects as described in the materials and methods. Means with the same letters in each group were not significantly different based on Tukey's honestly significant difference multiple range test at $P > 0.05$.

Table 2. Variations among cultivars of head and romaine lettuce in quality score and injury level in response to 3 d phosphine fumigation without carbon dioxide and ethylene absorbents after 14 d posttreatment storage at 2 °C (35.6 °F).

Type	Cultivar	Heads (no.)	Wt [mean ± SE (g)] ^z	Quality score [mean ± SE (1–9 scale)] ^y		Injury level [mean ± SE (%)] ^x	
				External	Internal	External	Internal
Head	Spyglass	56	741.4 ± 18.5	7.4 ± 0.1	7.1 ± 0.2	8.9 ± 3.9	44.6 ± 6.7
	Telluride	92	717.0 ± 11.3	7.4 ± 0.1	8.1 ± 0.1	17.4 ± 4.0	12.0 ± 3.4
	ANOVA ^w df (1, 146)	<i>F</i>	1.43	0.11	16.06	2.05	23.08
		<i>P</i>	0.234	0.736	<0.0001	0.155	<0.0001
Romaine	Sun Valley	84		7.1 ± 0.1 b	8.0 ± 0.1 a	23.8 ± 4.7 a	9.5 ± 3.2 ab
	Sparx	27		7.4 ± 0.2 ab	7.6 ± 0.1 a	11.1 ± 6.2 a	7.4 ± 5.1 ab
	Zuma	30		7.5 ± 0.2 ab	7.7 ± 0.2 a	10.0 ± 5.6 a	16.7 ± 6.9 a
	Green Forest	54		7.4 ± 0.1 ab	7.8 ± 0.1 a	11.1 ± 4.3 a	0 b
	Absolute	27		7.7 ± 0.1 a	8.0 ± 0.1 a	3.7 ± 3.7 a	3.7 ± 3.7 ab
	ANOVA ^w df (4, 217)	<i>F</i>		3.16	1.63	2.41	2.39
		<i>P</i>		0.015	0.167	0.051	0.052

^z1 g = 0.0353 oz.

^yThe quality scores range from 1 (extremely poor) to 9 (excellent) with 7 as good quality with minor non-objectionable defects as described in the materials and methods. For romaine lettuce with more than two cultivars, the same letter within a column is not significantly different based on Tukey's honestly significant difference (HSD) multiple range test at $P > 0.05$.

^xThe injury level is percentage of the number of lettuce heads with injuries. For romaine lettuce with more than two cultivars, the same letter within a column is not significantly different based on Tukey's HSD multiple range test at $P > 0.05$.

^wAnalysis of variance among cultivars of each lettuce type for weight of head lettuce and for external and internal quality scores and injury levels of head and romaine lettuce. Degrees of freedom from *F* test reported in the format (numerator df, denominator df).

($P < 0.0001$) in head lettuce (Table 4). For romaine lettuce, except external injury and internal quality, other qualities and injuries were significantly related to each other ($P < 0.01$) (Table 4).

Discussion

Phytotoxicity has been the main obstacle for use of phosphine fumigation on fresh products (Hatto et al., 1982; Leesch, 1984; Weller and Graver, 1998). For most fumigations

with phosphine generated from metal phosphide, ammonia which is cogenerated to modulate phosphine-producing chemical reaction is considered as the main cause for the phytotoxicity to fresh commodities and has prevented

use of phosphine on fresh products. However, long fumigation treatments of 3 d with cylindered phosphine free of ammonia also causes injuries to lettuce, and potentially affects commercial use of low-temperature pure phosphine fumigation against tolerant pests on fresh commodities (Liu, 2012). This study demonstrated that injuries associated with long phosphine fumigation treatments can be prevented by including CO₂ absorbent in the fumigation chambers. This method has a potential to be used to develop safer phosphine

fumigation treatments for postharvest pest control on many sensitive fresh commodities such as lettuce. The injuries on lettuce were likely caused by an accumulation of CO₂ based on the facts that injuries were identical to CO₂ injuries and injuries were prevented by the use of CO₂ absorbent in the fumigation chambers.

Three days of phosphine fumigation at 2 °C in the absence of CO₂ and ethylene absorbents caused injuries to both head and romaine lettuce and resulted in significant quality degradation. The injuries were in the

forms of sunken lesions with brown margins, identical to injuries caused by CO₂ (Lipton et al., 1972). Lettuce has a very low rate of ethylene production (Kader and Kasmire, 1984). The typical symptoms of ethylene-induced injuries are russet spotting (Lipton et al., 1972) and were absent in this study. Based on symptoms of injuries, it was likely that most injuries found on lettuce were caused by CO₂ accumulation and prevention of such injuries during the fumigation was due to the CO₂ absorbent.

Lettuce sensitivity to CO₂ in postharvest storage and transportation has been studied extensively (Brecht et al., 1973; Kader and Morris, 1977; Ke et al., 1993; Lipton et al., 1972; Stewart and Uota, 1976). This study also indicated that there were considerable variations among different lettuce cultivars in sensitivity to CO₂. For example, head lettuce ‘Spyglass’ was 3.7 times more susceptible to internal injury than ‘Telluride’, the latter being relatively tolerant to injury. For romaine lettuce, ‘Green Forest’ did not have internal injury, while 16.7% of ‘Zuma’ had considerable internal injury. These findings agree with previous reports of variations among lettuce cultivars in sensitivity to CO₂ and its impact on quality and shelf life (Brecht et al., 1973; Couture et al., 1993).

It is also interesting to note that fumigation treatment in the presence of the absorbents resulted in significantly lower levels of injuries on lettuce surface leaves as compared with controls. Immediately after harvest, lettuce still has high levels of physiological activities and such activities

Table 3. Comparisons of head weights among head lettuce with different quality scores and between head lettuce with and without injuries for 3 d phosphine fumigation treatment without absorbents for carbon dioxide and ethylene after 14 d posttreatment storage at 2 °C (35.6 °F).

Source	Score (1 to 9 scale) ^z or condition	Heads (no.)	Wt [mean ± SE (g)] ^y	F (P) values (ndf, ddf) ^x
External quality	6	4	648.4 ± 35.3a	1.26 (0.263) (2, 140)
	7	68	739.2 ± 14.5a	
	8	71	714.7 ± 14.7a	
Internal quality	7	22	793.8 ± 22.6a	7.22 (0.008) (2, 125)
	8	60	736.5 ± 13.5a	
	9	46	674.3 ± 17.6b	
External injury	Normal	127	725.0 ± 11.1a	0.09 (0.760) (1, 146)
	Injury	21	733.7 ± 19.6a	
Internal injury	Normal	112	712.3 ± 10.7b	6.31 (0.013) (1, 146)
	Injury	36	769.3 ± 22.0a	

^zThe quality scores range from 1 (extremely poor) to 9 (excellent) with 3, 5, and 7 representing poor with excessive defects, fair with slightly to moderately objectionable defects, and good with minor non-objectionable defects, respectively.

^yThe average weights for head lettuce with the three highest quality scores were compared. The numbers of lettuce heads with the three highest quality scores represented 96.6% and 86.5% of total numbers of lettuce heads used for external and internal quality evaluations, respectively. Mean weights followed by the same letters in each group were not significantly different based on Tukey’s honestly significant difference multiple range test at $P > 0.05$; 1 g = 0.0353 oz.

^xDegrees of freedom from F test: ndf = numerator df, ddf = denominator df.

Table 4. Analysis of variance for correlations among quality scores and injury levels for head and romaine lettuce from 3 d phosphine fumigation treatment without carbon dioxide and ethylene absorbents after 14 d posttreatment storage at 2 °C (35.6 °F).

Type	Source		F (P) values of quality		F (P) values of injury	
			External	Internal	External	Internal
Head df (1, 146) ^z	Quality	External				
		Internal	2.05 (0.155)			
	Injury	External	87.15 (<0.0001)	0.63 (0.429)		
		Internal	0.49 (0.486)	208.57 (<0.0001)	0.37 (0.546)	
Romaine df (1, 220)	Quality	External				
		Internal	17.16 (<0.0001)			
	Injury	External	216.35 (<0.0001)	3.75 (0.054)		
		Internal	11.32 (0.001)	183.58 (<0.0001)	7.14 (0.008)	

^zDegrees of freedom from F test reported in the format (numerator df, denominator df).

subside over time during cold storage. This is illustrated by high rates of CO₂ production initially after harvest that gradually declined to a low level in about 4 d postharvest in cold storage (Martínez and Artés, 1999). In this study, lettuce was fumigated 1 d after harvest and was expected to have a high CO₂ production rate during the fumigation. Wrapping lettuce heads in plastic sleeves with a few small ventilation holes and packing lettuce heads in cartons also restrict dispersion of CO₂ from lettuce heads. This may be the reason that the exposure to absorbents only during the fumigation resulted in less injuries, which were evaluated 14 d after treatment. The reduced injury in phosphine fumigation with CO₂ absorbent as compared with the control suggests that CO₂ absorbent may also be beneficial to use in preserving lettuce postharvest quality during cold storage.

Head lettuce had a much higher percentage of heads with internal injuries than romaine lettuce (Fig. 2). This is likely due to the closed, round shape structure of head lettuce that may reduce the diffusion of CO₂ from the center of head lettuce to its surfaces as compared with the open structure of romaine lettuce. Heavier heads also tend to be more compact and therefore further hinder CO₂ escape as compared with lighter and often softer heads. A previous study showed that firm heads of lettuce are more susceptible to physiological disorders (Saltveit, 2004). The internal injury was the main factor affecting the internal quality score as indicated by the highly significant correlation between them (Table 4). Similarly, the significant correlations between injury and quality score of both exterior and interior indicate that the injury was the main factor affecting lettuce quality score (Table 4).

In this study, the same volume of 1.6% phosphine sample was released into each fumigation chamber. However, during the 3-d fumigation, the average concentration of phosphine was 2218 ppm for the treatment without ethylene and CO₂ absorbents and 2083 ppm for the treatment with absorbents. The average concentration of phosphine for lettuce with ethylene and CO₂ absorbents was ≈6% lower as compared with the fumigation without absorbents. Phosphine does not react with CO₂ absorbent,

soda lime (Pratt and Reuss, 2004), but it is unknown whether phosphine interacts with the ethylene absorbent, potassium permanganate. For phosphine fumigations, phosphine exposure period was more critical in comparison to dosage for insect control (Hole et al., 1976; Liu, 2008). Therefore, even if the absorbents may slightly reduce phosphine concentration, they are not expected to have a substantial influence on the efficacy of the fumigation treatment.

Low-temperature pure phosphine fumigation has been used to control fresh fruit and vegetable insects (Horn and Horn, 2004; Horn et al., 2003, 2005, 2010; Liu, 2008, 2011, 2012). Pure phosphine fumigation at low temperature caused injuries to fumigated lettuce if the exposure time is too long. Previous studies showed that 3-d pure phosphine fumigation caused lettuce injuries (Liu, 2012), while 24 h phosphine fumigation treatment was safe to lettuce (Liu, 2008). Lower temperatures require longer fumigation periods to control pests compared with that at higher temperatures (Hole et al., 1976; Liu 2008). Longer phosphine fumigation time is also needed for control of more tolerant pests, such as lettuce aphid (Liu, 2012). The longer fumigation period, however, will be more likely to cause accumulation of plant volatiles such as CO₂ and ethylene. The results from this study demonstrate that long fumigation with pure phosphine at low temperatures can be made safe by scrubbing plant volatiles such as CO₂ and ethylene with absorbents. The findings have important implications in developing safe and effective low-temperature phosphine fumigation treatment for postharvest pest control on lettuce as well as on other fresh commodities. However, more research is needed to determine the effects of prolonged hermetic sealing as in low-temperature phosphine fumigation on atmosphere composition, and postharvest quality of fresh commodities including lettuce.

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