0101

Advances in Postharvest Pest Control on Perishable Commodities Using Ultralow Oxygen Treatment and Low Temperature Phosphine Fumigation

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Abstract: Recent research in postharvest pest control on fresh fruits and vegetables for export markets have resulted in promising ultralow oxygen (ULO) treatments and low temperature phosphine fumigation treatments for a variety of pests on different commodities. Lettuce aphid (*Nasonovia ribisnigri*), western flower thrips (*Frankliniella occidentalis*), and black widow spiders (*Latrodectus hesperus*) were successfully controlled on head lettuce, broccoli, and table grapes respectively, without negative impact on product quality. Tolerance of lettuce to ULO treatment varied among cultivars and was also affected by pre-treatment storage. One week storage under normal or CA conditions prevented injury to lettuce by the subsequent ULO treatment for control of western flower thrips. In general, shorter treatment at higher temperature had less impact on lettuce quality as compared with longer treatment at lower temperature for control of western flower thrips. Low temperature fumigation with diluted pure phosphine gas was effective for control of western flower thrips on lettuce, broccoli, asparagus, and strawberries without negative effects on product quality. A successful commercial trial in a refrigerated reefer container also demonstrated the efficacy of low temperature phosphine fumigation for control of the thrips and its safety for the postharvest quality of all products. Complete control of lettuce aphid and leafminer (*Liriomyza langei* Frick) were also achieved with low temperature phosphine fumigation without negative effects on lettuce quality. These advances provided promising controlled atmosphere and fumigation treatments for commercial development. The ULO treatment also has potential to be used for postharvest pest control on organic products and facilitates international trade of organically produced agricultural commodities.

Introduction

Fresh fruits and vegetables present a unique challenge for postharvest pest control because of their requirements for cold storage to preserve quality and their sensitivity to fumigants. Typically, chemical fumigations for pest control of fresh fruits and vegetables requiring cold storage are conducted at ambient temperatures. The warm up of products for fumigation treatment compromises product quality and shelf life. Most vegetables are fragile and delicate and susceptible to injury by fumigants. For lettuce, fumigation with methyl bromide for pest control causes injury and quality reduction. The lack of safe and effective treatment for postharvest pest control hinders export of U. S. lettuce and other fresh products to overseas markets. The global phase out of methyl bromide production also heightens the need to develop alternative treatments. Although its use for pest quarantine treatment is exempt from phasing out at present, this status may change in the future. On the other hand, organic products have gained popularity around the world in the recent years. Postharvest pest quarantine treatments which are compatible with organic products would be needed for international trade of organic products.

There are a wide range of alternatives being studied for postharvest pests control on perishables, including controlled atmosphere (CA), irradiation, temperature treatment, and alternative fumigants (Mangan and Hallman 1997, Mitcham 2001, Fields and White 2002). Most CA studies focus on combinations of reduced oxygen and elevated CO_2 for pest control. But the efforts over the last 20 years have yielded little progresses due to adverse effects on product quality (Mitcham et al. 2001, 2003). Some products such as iceberg lettuce are very sensitive to CO_2 (Lipton et al. 1972). Recent studies on CA treatment with ultralow oxygen (ULO treatment hereafter) have resulted in successful control of lettuce aphid, western flower thrips, and black widow spider on different fresh products (Liu 2005, 2007, 2008a, 2008b; Liu et al. 2008).

Among alternative fumigants, pure phosphine fumigation at low temperature has been used successfully to control quarantined pests

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on fresh fruits and vegetables in Chile (Horn and Horn 2004, Horn et al 2005) and is being studied for pest quarantine treatments on a variety of fresh commodities in several countries (Klementz et al 2005, Liu 2008c). Our research also shows that fumigation with bottle pure phosphine gas at low temperature is promising for insect control on vegetables. In this paper, recent and current research on ULO treatment and low temperature phosphine fumigation are presented and discussed.

Ultralow Oxygen Treatments

ULO treatment for insect control on lettuce. ULO treatment was studied for control of lettuce aphid, *Nasonovia ribisnigri* (Mosley) and leafminer, *Liriomyza langei* Frick, quarantined pests in Japan, on iceberg lettuce and for control of western flower thrips, *Frankliniella occidentalis*, a quarantined pest in Taiwan, China, on lettuce and broccoli. ULO treatments with different durations, temperatures, and oxygen levels were tested to determine responses of the insects and effects on product quality. Selected ULO treatments were tested on lettuce or broccoli to verify their efficacy in insect control and preservation of product quality.

Lettuce aphid was subjected to ULO treatments with 0.015% -0.025% O_2 at 1, 5, and 10℃ for 1 to 3 days. Complete control of lettuce aphid was achieved in a 3 day treatment at 1℃, 2 day treatment at 5℃, and 1 day treatment at 10℃ (Table 1). In large-scale tests in 562 liter box chambers with commercial iceberg lettuce, a 2 day treatment at 6℃ and a 3 day treatment at 3℃ with 0.015% -0.025% O_2 achieved complete control of lettuce aphid. No negative effect on lettuce quality was detected after two weeks of post-treatment storage. Therefore, the selected treatments have potential in postharvest control of lettuce aphid on iceberg lettuce.

Western flower thrips was found to be more tolerant to ULO treatment than lettuce aphid. ULO treatments with different durations, temperatures, and much lower oxygen levels were studied to determine effective treatments for the control of the thrips. Thrip mortality increased with reduced oxygen level and increased treatment time and temperature. At 0.003% O_2, over 99.6% mortality rates of thrips was achieved in three ULO treatments of 2, 3, and 4 days at 10, 5, and 1℃ respectively (Table 1). Although there were no injuries to lettuce surface leaves and there was no reduction in visual quality for treated lettuce, about 9 to 33% of lettuce heads sustained injury to heartleaves. The 2 day ULO treatment with 0.003% O_2 produced the lowest injury rate to heartleaves and the injury increased with increased treatment duration. The amount of injured leaves was small (<2 g per head). There were also some variations among the lettuce cultivars in susceptibility to heartleaf injury by ULO treatments.

Pre-treatment storage of lettuce was also studied to determine whether it affected lettuce tolerance to ULO treatment. For control of western flower thrips lettuce was stored under normal atmosphere and under controlled atmosphere (CA) with about 3% O_2 at low temperature for one week and was then compared with fresh lettuce for their response to 2 day ULO treatment with 0.003% O_2 at 10℃.. Lettuce which had been stored for one week under normal or CA condition tolerated the ULO treatment while over 30% of fresh lettuce sustained minor injury to heartleaves. Therefore, pre-treatment storage at low temperature enhanced tolerance of lettuce to the subsequent insecticidal ULO treatment. A sequential combination of CA storage and the ULO treatment was demonstrated to be effective against western flower thrips and lettuce aphid and safe for all seven lettuce cultivars tested. The study indicated that ULO treatment can be made safer for lettuce through pre-treatment storage to increase lettuce tolerance.

Leafminer flies were more tolerant to ULO treatment than western flower thrips and no successful ULO treatment was developed for leafminer flies (Table 1). Previous CA treatments which resulted in over 95% mortality of leafminer flies caused only 14% -44% mortality of leafminer pupae (Liu 2003). Therefore, ULO does not seem to be suitable for leafminer control.

ULO treatment for thrips control on broccoli. ULO treatments at a low temperature of 1℃ were studied for controlling western flower thrips on ice-packed broccoli. Complete control of thrips was achieved by a 5 day ULO treatment with 0.003% O_2. Oxygen level affected efficacy of ULO treatment. At a higher oxygen level of 0.03%, a 6 day treatment killed approximately 85% of thrips while a 10 day treatment killed all thrips. The 5 day ULO treatment with 0.003% O_2 was successfully tested on iced commercial broccoli of several cultivars without any noticeable negative effects on shelf-life and postharvest quality. The production of off-odor mainly methanethiol was a major concern for ULO treatment. The 5 day ULO treatment for thrips control did not result in a detectable off-odor. A 10 day ULO treatment with a higher oxygen level did result in the production of off-odor at the end of the treatment. The 5 day ULO treatment provided a safe and effective alternative to methyl bromide fumigation for postharvest control of western flower thrips on broccoli.

ULO treatment for black widow spider control on table grapes. Western black widow spider, *Latrodectus hesperus* Chamberlin & Ivie, was subjected to ULO treatments at different temperatures. Complete control of the spiders was achieved in 1 day ULO treatments with 0.5% O_2 or lower at 1℃ and in 1 day low oxygen (2%) treatment at 15℃. Oxygen level and temperature greatly affected spider mortality. At 1℃, as oxygen level was decreased from 2% to 0.5%, spider mortality increased from 0 to 100%. At 2% O_2, as temperature was increased from 1 to 15℃, spider mortality increased from 0% to 100%. The 1 day ULO treatment with 0.5% O_2 at 1℃ was tested on harvested table grapes of the 'Thompson Seedless' and Flame Seedless' varieties. The treatment had no negative effects on grape quality. Because of the relatively short treatment time, effectiveness at low storage temperature and the easily attained oxygen level, we conclude that the ULO treatment has good potential to be implemented commercially for control of black widow spiders on harvested table grapes.

Low Temperature Phosphine Fumigation

Phosphine fumigation for control of western flower thrips. Fumigation with diluted pure phosphine at a low temperature of 2℃ was studied to control western flower thrips and to determine its effects on the quality of treated lettuce, broccoli, asparagus, and strawberry. Complete control of thrips was achieved in ≥18 hour fumigation treatments with ≥ 250 ppm phosphine (Table 2). One day fumigation treatment with 1 000 ppm phosphine was tested on lettuce and broccoli. One day fumigation treatments with 500 ppm and 1 000 ppm phosphine were tested on asparagus and strawberry. Visual quality of lettuce, broccoli, and asparagus was evaluated after 2 weeks of post-treatment storage. Strawberry quality was evaluated immediately after fumigation and after 1 week of post – treatment storage. For all the above mentioned products, there were no significant differences in postharvest quality between the treatments and the controls and there were no injuries caused by the fumigation treatments. Therefore, phosphine fumigation at low temperature is promising for postharvest control of western flower thrips on lettuce, broccoli, asparagus, and strawberry.

A commercial scale fumigation trial was conducted in a 40 ft (12.19 m) reefer container with half-full load of iceberg and romaine lettuce and small loads of broccoli, asparagus, and strawberries. Gaseous phosphine was injected into the container through the fresh air vent using the Horn Dyluphos System (Horn et al. 2005). The treatment lasted 18 hours with an average phosphine concentration of about 600 ppm at 2℃. The treatment was terminated by 4 repeated 30 min aerations separated by a 30 min interval with the door closed to maintain a stable temperature in the container. Phosphine level in the head space at the end of the aeration was < 0.3 ppm. The treated products were sealed in the container for additional 3 days to simulate shipping and phosphine level in the container was monitored. One day after treatment termination, phosphine level was below the detection limit of 0.01 ppm of ultralow phosphine detector tubes. In the trial complete control of thrips was achieved. The quality of lettuce, broccoli, and asparagus was evaluated after two weeks of post-treatment storage. Strawberry quality was evaluated immediately after fumigation and after one week of post-treatment storage. There were no negative effects on the quality of any of the treated products. The results from the trials indicate that it is feasible to conduct commercial fumigation with pure phosphine at a low temperature in reefer containers for pest quarantine control.

Phosphine fumigation for control of lettuce aphid and leafminer on iceberg lettuce. Lettuce aphid and leafminer flies and pupae were subjected to low temperature phosphine fumigation treatments. Leafminer flies were much more susceptible to phosphine fumigation than leafminer pupae. At 500 ppm of phosphine, complete control of leafminer flies was achieved in the 24 h treatment and complete control of leafminer pupae was achieved in the 72 h treatment. At 1 000 ppm of phosphine, all treatments ranging from 12 h to 72 h achieved complete control of leafminer flies and 48 h or longer treatments resulted in complete control of leafminer pupae. At 2 000 ppm of phosphine, all treatments ranging from 24 h to 72 h resulted in complete control of leafminer pupae and the 24 h treatment also resulted in complete control of leafminer flies (Table 2).

Lettuce aphid was more tolerant to phosphine fumigation than the leafminer. The 48 h treatment with 2500 ppm phosphine and 72 h treatment with 2000 ppm phosphine did not completely control the lettuce aphid. The 72 h treatment with 2500 ppm phosphine and the 48 h treatment with 3400 ppm phosphine, did achieve complete control of lettuce aphid (Table 2). Both treatments were safe for iceberg lettuce. Yet, given the very high phosphine concentrations and long treatment durations used, there could be problems in implementing such treatment practically due to the cost and the feasibility of maintaining such a high phosphine level. More research is needed to improve the phosphine fumigation treatment for control of lettuce aphid.

Discussion

The large variations in susceptibility to ULO treatments among pest species tested indicate limitations as well as opportunities of ULO treatment for postharvest pest control. Aphids and thrips can be controlled effectively and safely by ULO treatment on lettuce and broccoli respectively. However, pre-treatment storage is needed to prevent injury to iceberg lettuce for control of western flower thrips using ULO treatment. Low temperature is not effective (Table 1). with ULO treatment for the leafminer, The most remarkable finding is that black widow spiders can be easily controlled with a short ULO treatment. The treatment has good potential of being adopted commercially because the oxygen level used in the treatment is not very far from the low oxygen used in CA storage of table grapes. Like most other postharvest treatments, ULO is not suitable for all pests. However, effective treatments can be developed for pests within certain pest groups that have been demonstrated to be susceptible to ULO treatment. Many other pest groups have yet to be explored for susceptibility to ULO treatment. The present examples of success seem to suggest that ULO treatment is a promising alternative for postharvest pest control. More research efforts are warranted to explore ULO treatment for a wide variety of pests on various fresh commodities and to develop successful ULO treatments for commercial applications.

The enhanced tolerance of lettuce through pre-treatment storage suggests that lettuce susceptibility to ULO treatment can be modified significantly. This is possibly due to acclimation to postharvest storage. Other fresh commodities may exhibit similar characteristics of increased tolerance to extreme CA conditions including ULO after a certain length of acclimation to postharvest storage. Therefore, future research should consider modifying postharvest physiology of the fresh products in developing CA-based postharvest pest control technology rather than focusing solely on efficacy in controlling target pests.

The results of low temperature phosphine fumigation research are very encouraging. The successful control of western flower thrips, leafminer flies and pupae, and lettuce aphid with low temperature phosphine fumigation indicates that phosphine is a promising as an alternative to methyl bromide for postharvest pest control on perishables. In comparison with methyl bromide fumigation, pure phosphine fumigation under low temperature has the advantage of keeping products at storage temperature, thereby avoiding negative effects on product quality associated with warming up of the products as in methyl bromide fumigation. However, for pests like lettuce aphid, long treatment time and high phosphine level is nec essary to achieve successful control. This makes low temperature phosphine fumigation less practical. In comparison, lettuce aphid can be controlled more easily with ULO treatment.

Longer treatment at lower phosphine concentrations was more effective in controlling insects than low temperature phosphine fumigation as demonstrated with western flower thrips. Similarly, low dose phosphine fumigation with longer treatment time was suggested for controlling aphids on cut flower because of its higher efficacy as compared with shorter fumigation with higher phosphine concentrations (Karunatne et al. 1997).

Phosphine is very toxic to humans and health problems related to exposure to low levels of phosphine were also reported (Bond 1984, Chaudhry 1997, Brautbar and Howard 2002). However, studies on phosphine residue in perishable products are very limited. Phosphine fumigated fruits were reported to have mild off-taste and the off-taste disappeared after 5 – 6 days of storage (Horn and Horn 2004). For phosphine fumigated table grapes, no phosphine could be detected after they were processed to release phosphine residue 9 days after fumigation by chromatography with detection limit of 0.003 ppm (Klementz et al. 2005). In the U. S., the maximum residue limit (MRL) for phosphine is 0. 1 ppm for stored products and 0. 01 ppm for fresh and processed food stuff. Phosphine has low solubility in water (Bond 1984). Even though phosphine has low solubility in water, phosphine residue may still be a health concern (Chaudhry 1997). For low temperature phosphine fumigation of fresh commodities, because of longer treatment time and high water content of the products, there is a need for more research on phosphine residue in fresh products. Research efforts are needed to quantify the amount of phosphine absorbed by the products and how fast and how much of the absorbed phosphine will dissipate to ensure that phosphine fumigated fresh commodities do not impose a risk to human health.

In summary, both ULO treatment and low temperature phosphine fumigation showed promise in postharvest pest control on fresh commodities. For ULO treatment, its effectiveness against most pest species and effects on the quality of most perishable commodities have not been explored. Recent studies also showed limitations of ULO treatment in term of ineffectiveness against some groups of pests and its negative impact on product quality. There are also needs for efforts from industry to develop the ULO treatment for commercial use. However, given that the ULO treatment is compatible with organic products and there is a heightened awareness of health risks related to chemical pesticides, ULO treatment is an important technique to be studied and developed for postharvest pest control on perishable commodities. For low temperature phosphine fumigation, successful use of the technology in Chile and our studies all indicate that it is very promising in postharvest pest control on fresh commodities. However, more research is needed to determine phosphine residue and its fate in treated products to ensure their safety to human health Currently, there are also a lack of low temperature fumigation chambers or suitable commercial coolers for low temperature fumigation treatments. Fumigation in refrigerated shipping containers seems to offer a viable alternative for commercial use of low temperature phosphine fumigation.

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Table 1. Comparison of insect mortality in response to ultralow oxygen treatments

Temp (℃)	Time (day)	O_2 (%)	Mortality (%)		
			Lettuce aphid	Western flower thrips	Leafminer flies
1	2	0.015	95		
	3	0.015	100		
		0.003		85	
	5	0.05	99	51	43
		0.003		100	
5	1	0.015	90		
	2	0.015	100		
	3	0.003		100	90
10	1	0.015	100	65	
	2	0.003		100	86

Table 2. Comparison of insect mortality in response to fumigation treatment with pure phosphine at 2℃

Time(h)	PH_3 (ppm)	Mortality (%)			
		Western flower thrips	Leafminer flies	Leafminer pupae	Lettuce aphid
12	500	89.2	90.4		
	1000	98.5	100.0		
18	250	100.0			
	500	100.0			
24	500	100.0	100.0	91.3	
	1000	100.0	100.0	98.2	
	2000		100.0	100.0	
48	500			99.7	86.1
	1000			100.0	89.3
	2000				95.8
	3400				100.0
72	500				98.1
	1000				99.2
	2000				99.5
	2500				100.0

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