The Effects of Refrigerated, Controlled-atmosphere Storage during Marine Shipment on Insect Mortality and Cut-flower Vase Life

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Summary. Refrigerated (2 °C) controlled atmospheres significantly increased the mortality of green peach aphids [*Myzus persicae* (Sulzer)] and western flower thrips [*Frankliniella occidentalis* (Pergande)] in laboratory experiments. However, insect mortality during marine shipment in mixed-load containers at 0.5 °C did not significantly increase in a controlled atmosphere. In laboratory experiments, mortality of green peach aphids ranged from 32.8% in the refrigerated control to 96.8% after storage in 0.10% O₂ for 4 d followed by 7 d in 3% O₂ with 5% CO₂. When

stored under these same conditions, western flower thrips mortality was 71% compared to 16% mortality in the refrigerated control. Following an 11-day marine shipment from California to Guam in a controlled atmosphere, vase life was extended for most of the 20 California cut-flower and foliage products compared to those shipped in the refrigerated air control.

Western flower thrips and green peach aphids are commonly found on floral products grown in California. These insects are obstacles to the export of cut flowers and florist greens from California to Pacific Rim markets because quarantine regulations require imported floral products to be free of live insect pests. The current quarantine treatment for cut flowers and foliage is fumigation, most often with methyl bromide (Plant Protection and Quarantine Treatment Manual, 1992). With Environmental Protection Agency plans to prohibit production and importation of methyl bromide under the Clean Air Act by 1 Jan. 2001, alternative quarantine procedures are needed.

Controlled atmospheres (CAs) differ from air in their proportions of O₂, CO₂, and N₂. Some benefits of storing fresh commodities in a refrigerated CA environment include 1) reducing respiration and delaying senescence to maintain produce quality (Kader, 1992), 2) reducing ethylene production, 3) inhibiting postharvest pathogens [e.g., botrytis rot of roses (Phillips et al., 1985), 4) reducing sensitivity of produce to chilling damage, and 5) controlling surface insects and mites (Carpenter and Potter, 1994; Whiting and van den Heuvel, 1995).

Recent experiments using commercial shipping containers with microprocessor-CAs have demonstrated that a refrigerated CA can kill insect pests while preserving the quality of fresh fruit and vegetables (Gay and Kerber, 1993). In these studies, the CAs inside the shipping containers were maintained at 0.5 °C or 7 °C with >85% relative humidity, which is suitable for storing numerous kinds of fruit, vegetable, and floral products (Hardenburg et al., 1986; McGregor, 1987).

Our objective was to assess the

feasibility of using a refrigerated CA to disinfest floral products of western flower thrips and green peach aphids during marine shipment from California to Pacific Rim destinations.

Materials and methods

Laboratory experiments. Rearing green peach aphids and collecting western flower thrips was performed by Plant Sciences, Watsonville, Calif. Green peach aphids were reared and harvested from mustard (*Brassicasp.*) plants grown in a greenhouse environment. Western flower thrips were collected from landscape iceplant (*Mesembryanthemum crystallinum* L.) flowers. Insects were shipped by express mail in screened plastic vials containing small samples of plant matter. Shipping time was ≈1 to 3 d. The number of live insects in each vial ranged from 8 to 67 for aphids and from 1 to 15 for thrips. On arrival at the Horticultural Crops Research Laboratory, Fresno, Calif., the insects were stored overnight in the shipping vials at 13C. One vial of each insect species was placed into a gas-tight, 0.47-L glass jar receiving a flow-through atmospheric treatment. Five insect vials were evaluated per treatment in each of three experimental replications. Experiments were completed in Dec. 1993 and Feb. and Apr. 1994.

The sealed 0.47-L glass jars were stored inside a walk-in environmental chamber maintained at 2 ± 0.5 °C, with a 16-h light : 8-h dark photoperiod. The atmosphere and relative humidity in flow-through glass jars were maintained as described by Soderstrom et al. (1990). Atmospheres were generated from gas cylinders using a mixing board. Oxygen-deficient atmospheres were made by blending air with N₂ to obtain atmospheres of 0.1%, 0.25%, and 0.5% O₂. Carbon dioxide-enriched, low-O₂ atmospheres were achieved by adding CO₂ to the air and N₂ blend to yield atmospheres containing 10% or 20% CO₂ with 0.1% O₂ (Soderstrom, et al., 1991). Insects were exposed to a primary atmosphere of 0.1%, 0.25%, or 0.5% O₂ with no CO₂ or 0.1% O₂ with either 10% or 20% CO₂ for 4 d. All treatments, except the 0.1% O₂ treatment with no CO₂, then were exposed for 7 d to a secondary atmosphere of 3% O₂ with 5% CO₂. Insects used as controls were exposed to 2 °C air for 11 d. The total treatment exposure time of 11 d was

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similar to the duration of a marine shipment from California to Guam.

Vials containing insects were placed into their treatment jars every half hour and were exposed for 4 d to their respective primary atmosphere treatment. The gas-mixing board then was modified to deliver the secondary atmosphere for an additional 7 d. After the seven additional d, the insects were retrieved from the vials every half hour. Mortality counts were started 1 h after removal from the secondary atmosphere to allow for insect revival. When the sealed glass jars were disconnected from the humidified, flow-through system, wet paper towels were placed in the jars to avoid insect desiccation. Mortality counts were conducted by hand, using a vacuum aspirator to retrieve insects from the vials.

The following analytical procedures were conducted separately for aphid and thrips data in each replication: percent mortality values were computed for each treatment by dividing the total number of dead insects in each of the five treatment vials by the total number of insects in the five treatment vials and multiplying by 100. Data were transformed to logits to ensure homogeneity of variances and then were subjected to an analysis of variance (ANOVA) with treatment (levels 1 to 6) as the source of variation. Since 100% aphid mortality was observed in the third replication across all treatments, the ANOVA was run excluding this replicate. Plant Sciences reported that the aphid culture was weak at the time of shipment for replicate 3 (Matt Blechman, personal communication). Means for significant treatments were separated using

Duncan's multiple range test (BMDP Statistical Software, Westwood, Calif.).

Guam shipments. Boxes of fresh-cut flowers and florist greens in standard 48-inch packs with gel refrigerant (Cold Ice; Cold Ice, Oakland, Calif.) were obtained on separate dates in Dec. 1993 from commercial San Francisco Bay area flower growers and shippers and were transported by air-conditioned car to the port in Oakland, Calif. The flower boxes were loaded into three refrigerated marine containers, each containing either fresh fruit (coded A1) or vegetables (B1 and C1) for shipment to military bases in Guam. The flower boxes were placed on top of boxed fruit and vegetables in each container. Three screened, 1-quart glass containers with 27 to 99 green peach aphids on mustard leaves and 33 to 179 western flower thrips on iceplant flowers then were placed into the front, middle, and rear of each shipping van. Samples of floral products from each box were transported by air-conditioned car from the Oakland, Calif., port to a laboratory at California Poly State Univ. and were placed into vases of water and dextrose-based floral preservative (Floralife; Floralife, Burr Ridge, Ill.) as a control for vase-life studies. Laboratory conditions were $20 \pm 2^\circ\text{C}$, with natural window light and $35\% \pm 5\%$ relative humidity.

Atmospheres inside the marine containers were established by TransFresh Corp., Salinas, Calif., using the Tectrol system, which flushes storage containers with N_2 to reduce O_2 levels, followed by CO_2 injection with gas-blending manifolds. Oxygen sensors control N_2 release or introduc-

tion of fresh air to maintain O_2 at the desired concentration. Since damaging CO_2 levels may be generated by respiring produce, the Tectrol unit uses a CO_2 scrubber (hydrated lime) to absorb excess CO_2 . All gas levels are controlled precisely by a microprocessor, allowing for adjustments in the gas mixture to be made in transit (Kader, 1992). The temperature inside the marine containers was maintained at 0.5°C . A primary atmosphere of 2% O_2 with 0.75% CO_2 and 0.5% O_2 with 20% CO_2 was established, respectively, for 4 d in the mixed fruit (A1) and mixed vegetable (B1) marine containers. After 4 d, a secondary atmosphere of 5% O_2 with 1% CO_2 and 3% O_2 with 5% CO_2 was established, for the remaining transit duration in fruit and vegetable containers, respectively. Air was maintained in the second vegetable (C1) container throughout the transit duration (11 to 14 d) in both Guam shipments. Trip logs from each shipment revealed problems with atmosphere maintenance in B1 containers. A malfunctioning microprocessor led to a 4-day primary atmosphere in container B1, shipment 1, of 24 h of 0.6% O_2 with 19% CO_2 , then 58 h of 20% O_2 with 19% CO_2 , and finally 14 h of 0.2% O_2 with 0% CO_2 . The container sensors read 0.2% O_2 with 0% CO_2 for the secondary atmosphere, but this was probably incorrect (Ken Rodde, TransFresh Corp., personal communication). In shipment 2, the B1 container leaked in transit, causing the primary 4-day atmosphere to be 28 h of 2% O_2 with 15% CO_2 , followed by 68 h of 6% O_2 with 10% CO_2 . The secondary atmosphere was recorded as 13% O_2 with 5% CO_2 for 7 d.

On arrival in Guam, floral products from the A1 and B1 containers were placed in vases with Floralife solutions and stored at room temperature with natural window lighting. Vase life was evaluated after 1, 3, 5, 7, 10, and 14 d of storage and was determined based on visual quality, discoloration, wilting, flower bud

Table 1. Insect mortality data for refrigerated (2°C), controlled atmosphere laboratory experiments^z

Treatment no.	Four d in primary atmosphere (% O_2 /CO ₂)	Seven d in secondary atmosphere (3% O_2 /5% CO ₂)	Mean mortality (%) ^{y, x}		Mean no. insects/vial	
			Aphids	Thrips	Aphids	Thrips
1	0.10/0	No	95.1 $\pm$ 1.3 a	36.3 $\pm$ 7.3 c	30.8 $\pm$ 4.9	11.0 $\pm$ 2.7
2	0.10/0	Yes	96.8 $\pm$ 0.6 a	71.1 $\pm$ 4.9 b	24.9 $\pm$ 3.3	10.9 $\pm$ 1.7
3	0.25/0	Yes	95.1 $\pm$ 1.0 a	53.4 $\pm$ 6.1 bc	33.8 $\pm$ 4.6	12.7 $\pm$ 2.8
4	0.50/0	Yes	82.9 $\pm$ 3.5 b	49.7 $\pm$ 4.6 bc	31.9 $\pm$ 5.2	14.6 $\pm$ 2.8
5	0.10/10	Yes	95.4 $\pm$ 1.7 a	83.0 $\pm$ 4.1 ab	32.4 $\pm$ 3.5	8.47 $\pm$ 1.5
6	0.10/20	Yes	96.0 $\pm$ 0.9 a	77.3 $\pm$ 5.6 b	24.3 $\pm$ 2.3	10.0 $\pm$ 1.9
Control	Air	No	32.8 $\pm$ 7.6 c	16.0 $\pm$ 4.3 d	31.8 $\pm$ 4.1	13.9 $\pm$ 2.5

^zInsects were subjected to the primary atmosphere for 4 d followed by immediate placement in a secondary atmosphere for 7 d before mortality evaluation.

^yMeans within columns followed by the same letter are not significantly different at $P \geq 0.05$ by Duncan's multiple range test.

^xSE of the mean.

Table 2. Insect mortality data in refrigerated (0.5 °C) controlled atmosphere during marine shipment from California to Guam. Data represent mean percent mortality for three replicates of insects per container.

	Shipping date 1						Shipping date 2					
	Mixed	Mixed	Control	Mean no. insects/rep.			Mixed	Mixed ^w	Control	Mean no. insects/rep.		
	fruit (A1) ^z	vegetable (B1) ^y	(C1) ^x	A1	B1	C1	fruit	vegetable	C1	A1	B1	C1
Aphids	97	64	96	57	36	26	48	63	39	69	57	42
Thrips	1.5	2	0	44	51	56	37	81	0	176	89	118

^zOxygen at 2% with 0.75% CO₂ for 4 d, and 5% O₂ with 1% CO₂ for an additional 7 to 10 d.

^yOxygen at 0.6% with 19% CO₂ for 24 h, 20% O₂ with 19% CO₂ for 58 h, and 0.2% O₂ with 0% CO₂ for 14 h. Secondary atmosphere uncertain.

^xFresh-air atmosphere for trip duration.

^wOxygen at 2% with 15% CO₂ for 1 d, 6% O₂ with 10% CO₂ for 3 d, and 13% O₂ with 5% CO₂ for additional 7 to 10 d.

opening, and off-odors. Vase life data for flowers shipped on both dates were subjected to an ANOVA with container type and flower type as the sources of variation. Flowers from the mixed vegetable, fresh-air containers generally arrived in unmarketable condition and, thus, were not evaluated for vase life. Insect mortality in glass jars in each container was determined in Guam by counting live and dead insects and calculating percent mortality as described for laboratory experiments. Insect mortality data were subjected to an ANOVA with container and shipping date (replication) as the sources of variation.

Results and discussion

Laboratory experiments. The mortality of thrips and aphids after storage in any of the six, refrigerated CAs evaluated in our study was significantly higher than that of the air control (Table 1). For example, aphid mortality was between 83% and 96% after storage in a CA compared to 33% in the air controls. The mortality of thrips was between 36% and 83% after CA storage compared to 16% in air controls. Aphid mortality was higher when insects were exposed to a primary atmosphere of 0.1% or 0.25% O₂ than when exposed to 0.5% O₂ (treatments 2, 3, compared to treatment 4). Adding 10% or 20% CO₂ to the 0.1% primary O₂ atmosphere (treatments 5 and 6 compared to treatment 2) did not increase aphid mortality significantly. This suggests that at refrigerated temperatures, low O₂ is more stressful to aphids than elevated CO₂. The 7-day storage in a secondary atmosphere did not increase aphid mortality significantly (treatment 1 compared to treatment 2). Reid and Cantwell (1994) reported that 100% mortality of green peach aphids occurred after >21 d exposure to 0.5% O₂ at 0 °C. Adding 10% CO₂ to this

atmosphere reduced the time to 100% mortality by ≈7 d. However, reducing the O₂ level from 0.5% to 0.02% reduced the time to 100% aphid mortality by 7 to 14 d, indicating that very low O₂ levels were more lethal to green peach aphids than moderately elevated CO₂ atmospheres at 0C.

The mortality of western flower thrips was lower than that of green peach aphids in each of the CA treatments and air controls, suggesting that thrips are more difficult to kill under the conditions evaluated in our study than green peach aphids. Thrips mortality in CA was significantly higher when a 7-day secondary atmosphere was applied than when only a 4-day primary atmosphere was applied (treatment 1 compared to treatment 2). Storage in 0.1% O₂ resulted in higher mortality than storage in 0.25% or 0.50% O₂ (treatment 2 compared to treatments 3 and 4). Adding 10% or 20% CO₂ to the 0.1% primary O₂ atmosphere (treatments 5 and 6 compared to treatment 2) did not increase thrips mortality significantly. Reid and Cantwell (1994) reported that 100% mortality of western flower thrips occurred after 21 d exposure to 0.5% O₂ at 0 °C. Adding 10% CO₂ to this atmosphere reduced the time to 100% thrips mortality by 14 d. Reducing the O₂ level from 0.5% to 0.02% also reduced the time to 100% thrips mortality by up to 7 d. This indicates that elevated-CO₂ atmospheres were more lethal to thrips than low-O₂ atmospheres at 0 °C.

Better insect control may be obtained from higher levels of CO₂ at higher temperatures, as with New Zealand flower thrips where larval mortality was 100% after 24 h of exposure to 36 °C in 80% CO₂ and 20% N₂. Adult mortality approached 100% in the same atmosphere at 48 °C (Carpenter et al., 1996). Many flower and some vegetable species tolerated

exposure to 80% CO₂ with a balance of air at 20 °C for 24 h, and this treatment killed all green peach aphids and western flower thrips during a pilot test for commercial air shipment (Cantwell et al., 1995).

Guam shipments. Green peach aphid mortality during CA marine shipments to Guam was significantly higher on the first shipping date (mean = 86%) than on the second shipping date (mean = 50%) (Table 2). There were no significant differences in mortality of green peach aphids among the three shipping containers. Western flower thrips mortality during CA marine shipments was significantly higher on the second shipping date (mean = 39%) than on the first shipping date (mean <2%) (Table 2). The atmospheres inside each of the three shipping containers had a different effect on aphid and thrips mortality during shipments 1 and 2. In shipment 1, aphid mortality was highest in the mixed fruit (A1) CA container and lowest in the mixed vegetable (B1) CA container, whereas in shipment 2, mortality was highest in the mixed vegetable CA container. In both shipments, thrips mortality was highest in the mixed vegetable (B1) CA container, where O₂ levels were lowest and CO₂ levels highest; thrips mortality was lowest in the fresh air (control) container.

One reason for the discrepancy between the insect mortality data in the Guam shipments and those of the laboratory experiments is that marine container temperatures were ≈1.5 °C cooler than those in the laboratory experiments. Since CA is generally more insecticidal at higher temperatures than at lower temperatures (Carpenter et al., 1996), we would expect somewhat higher mortality in the laboratory experiments than in marine shipments. In fact, aphid mortality was higher (83%) in treatment 4 (0.5% O₂) of the laboratory experiments (2 °C)

than it was in the B1 container in shipments 1 (64%) and 2 (63%). Aphid mortality in the fresh-air marine container during shipment 1 (96%) was much higher than that of laboratory controls (33%). Thrips mortality was lower than expected in all marine containers in shipment 1 but more similar to laboratory experimental results in shipment 2.

We observed a high level of parasitism among the aphids in all three containers in shipment 1, which accounted for the high aphid mortality in the fresh-air control and overall higher aphid mortality in shipment 1 vs. shipment 2. Leakage of container atmosphere and microprocessor software problems also contributed to the variable insect mortality during the Dec. 1993 shipments. Thus, it is difficult to draw firm conclusions regarding insect control in the CA containers during marine shipment. Produce in CA vans generally arrived in excellent condition with limited vegetable damage.

Ratings for the vase life of the 20 floral products are shown in Table 3. Flowers shipped in the refrigerated air containers were unmarketable on arrival in Guam. The average vase life of the flowers shipped in the CA containers was 8.48 and 8.80 d for flowers shipped in the mixed fruit and veg-

etable CA containers, respectively, compared to 12.27 d for nonshipped flowers. For most species, vase life was comparable to or exceeded that for floral products air-shipped from California to Guam based on discussions with Guamanian florists. Not surprisingly, vase life varied significantly among the 20 flowers and greens evaluated. Further vase-life studies under laboratory conditions are needed to determine specific tolerances of floral products to the most promising insecticidal atmospheres.

Conclusion

Our research has demonstrated the potential for using low O₂, elevated CO₂, or both in refrigerated CAs during surface shipment of mixed loads of highly perishable commodities. Although optimum treatment conditions were not identified in our study, results indicate that refrigerated CAs can be lethal to aphids and thrips and that they have potential for use as a quarantine treatment for these insects. Insecticidal CA treatments may provide the basis for preshipment or in-transit control of insects on a variety of fruit, vegetables, and cut flowers. An exclusive shipment of floral products also might improve vase life beyond that reported here for mixed-load marine containers.

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Table 3. Average vase life (d) for 20 flowers and greens that were marine-shipped from California to Guam in refrigerated, controlled atmosphere containers.

Plant	Shipment 1 (1 Jan. 1993)			Shipment 2 (7 Dec. 1993)		
	A1	B1	Cal Poly ^z	A1	B1	Cal Poly
Annual statice	7	7	12	5	7	10
Carnation, pink	7	14	15	10	10	15
Carnation, red	14	10	15	10	10	15
Cushion poms	10	10	15	10	10	15
Delphinium	5	3	7	10	10	15
Enchantment lily	5	10	12	5	5	10
Eucalyptus	14	14	15	10	10	15
Gypsophila	10	10	7	10	10	15
Heather	14	14	15	10	10	15
Dutch Iris	7	5	7	3	7	---
Ming fern	7	7	15	---	10	15
Monte casino aster	10	14	12	10	10	15
Protea, pink mink	14	14	15	7	5	15
Queen Anne's lace	10	10	12	7	7	10
Rose, fancy pink	5	3	7	3	3	5
Rose, fancy red	10	10	7	7	7	10
Rose, sweetheart	5	3	12	3	5	10
Spider mums	14	14	15	10	10	15
Stock	10	10	12	10	7	15
Waxflower	7	7	7	---	10	15

^zFlowers held in control at Cal Poly State Univ. during shipments 1 and 2.

^yDid not open.