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USING OZONE FOR CONTROLLING BEAN THRIPS IN THE NAVELS OF ORANGES BEING EXPORTED TO AUSTRALIA

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ABSTRACT

With the uses of methyl bromide becoming fewer as we approach the cutoff year of 2005, new and inventive alternatives to those uses are becoming more important. In 1999, we began to investigate the possibility that ozone could replace some fumigant uses of methyl bromide against postharvest insect pests. We first found that ozone produces mortality throughout the life stages of the insect even though exposure was to the egg or larval stages. We also found that carbon dioxide enhanced the toxic effects of the ozone. We began by establishing the most tolerant stage of two common stored product pests, Indianmeal moth (*Plodia interpunctella* (Hübner)) and confused flour beetle (*Tribolium confusum* du Val). Using an ozone concentration ranging up to 12,000 ppm (v/v), a carbon dioxide concentration of 7 to 15%, a vacuum of –250 mm Hg, and a 2-hour exposure, we found that with both insects, the egg stage was the most tolerant stage. We then began to look for areas where ozone might be used as a treatment to replace methyl bromide. We investigated the possible use of ozone to kill bean thrips, *Caliothrips fasciatus* (Pergande), in the navel of navel oranges where they overwinter and present a problem to the export of California navel oranges to Australia. We found that a combination treatment of ozone, vacuum, and CO_2 killed the overwintering bean thrips hiding in the navel of 'Navel' oranges being exported to Australia. This treatment was found to have no damaging effects on properly waxed fruit.

INTRODUCTION

Insects cause a significant amount of damage to post-harvest commodities each year. One method of dealing with this problem is to fumigate the commodity either in storage or prior to export where the country accepting the commodity requires it to be free of insects of an economic, pestiferous, or quarantine nature. Presently, methyl bromide and phosphine are the fumigants of choice. Phosphine is used when turnover time is not important because it requires from 3 to 5 days, depending on

temperature, to be effective. When rapid turnover is necessary, methyl bromide has been the fumigant of choice because it only requires 2 to 24 hours to be effective against most pests. Our dependence on methyl bromide will end in 2005 with the exception of that used for quarantine uses (Anon. 1997a). This will drastically reduce the import and export uses of methyl bromide on commodities that were estimated to have a total value of $411.9 million dollars in 1997 (Anon. 1997b). Over the past few years, this increasingly imminent loss of methyl bromide has stimulated a frantic increase in research to find alternative fumigants for use on stored commodities.

Several chemicals have been proposed as replacements for methyl bromide. Among those that have shown some promise are methyl iodide, carbonyl sulfide ,sulfuryl fluoride (Vikane®/ProFume®) (Zettler *et al.* 1997, 1999 and Leesch and Zettler 2000) and propylene oxide. In addition, ozone has been considered as having potential to kill pests in commodities in its gaseous form. Ozone was the subject of research of Erdman (1980) on 2 species of stored product insects, confused flour beetle (*Tribolium confusum* (duVal)) and red flour beetle (*Tribolium castaneum* (Herbst)), both of which infest many stored commodities, especially grains and other durable commodities. Recently, the efficacy of ozone as a fumigant against stored product insects in grain was reported. High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* (Motsch.) and red flour beetle, *Tribolium castaneum* (Herbst) and the larval stage of the Indianmeal moth, *Plodia interpunctella* (Hübner), however; the time of treatment was 3 days which is allowable for control fumigations, but not for quarantine fumigation (Kells *et al.* 2001). Besides that research, little has been done to determine whether or not ozone has potential as a fumigant for eliminating pests from post-harvest commodities in the marketing channel.

Due to the spontaneous decay of ozone back to diatomic oxygen, special considerations must be addressed in assessing the use of ozone as a fumigant. All fumigants are held in contact with a commodity using some method of confinement such as a plastic tarpaulin or chamber designed to be gas tight (Bond, 1984). Because ozone concentration decreases continually if not replenished, a flow-through chamber must be considered for its use on commodities. Of course, some of the advantages of using ozone are the on-site generation of the material and the elimination of a need to transport the pre-fumigation to the site as well as the elimination of a need to consider its post-fumigation disposal or collection because it can be reconverted to oxygen on-site.

This research was undertaken to determine if ozone could eliminate live adult bean thrips, *Callothrips fasciatus* (Pergrande) overwintering in the navel of oranges being exported to Australia. Over the past few years, some shipments of 'Navel' oranges have been rejected because of the presence of adult bean thrips deep in the navel of oranges where they attempt to survive the winter in a relatively protected environment. In earlier experiments with Indianmeal moths and confused flour beetles, it was shown that a low concentration of CO_2 and vacuum increased

mortality (Leesch, 2002). Thus we conducted these experiments using from 7 to 10% CO_2 and a vacuum of -250 mm Hg (Torr).

MATERIALS AND METHODS

Ozone treatments at reduced pressure and with carbon dioxide

When reduced pressure was introduced to the experimental design, a new chamber was required. Fortunately, Cosmed Group, Inc. donated a chamber designed to test the efficacy of ozone under reduced pressure and with carbon dioxide. The chamber consisted of a 2 cylindrical tubes of 316 stainless steel turned in a horizontal direction such that one fit inside the other with a space in between to allow for a fluid circulation for the control of temperature. A plate was welded to one end of the 2 tubes to form a closure with the inner tube being equidistant from the outer tube. On the other end a 11cm Lexan® plate was held in place by wing nuts attached to threaded studs protruding from a stainless steel plate that sealed that end of the concentric tubes an formed the water jacket for the temperature control with recirculating coolant. Insects were placed in the inner tube (chamber) by removing the Lexan® cover. Inside the chamber, was a recirculating fan mounted at the stainless closure end of the chamber and a temperature/humidity sensor. The chamber was fitted with ports to allow the introduction of ozone/carbon dioxide/oxygen, air and for the removal of the air/ozone/carbon dioxide to maintain the reduced pressure. Ozone was produced by passing a stream of oxygen/carbon dioxide through a corona discharge tube on a model CD/12 ozone generator manufactured by ClearWater Tech, LLC (San Luis Obispo, CA). Constant ozone concentration was maintained using a model OzoMeter® ozone detection unit manufactured by Hankin Atlas Ozone Systems, Ltd.(Scarborough, Ontario, Canada). A feedback from the high alarm turned the amperage on or off of the corona discharge tube which allowed the control of concentration by $\pm$5%. The speed of concentration attainment and its over-shoot could be controlled by varying the amperage applied to the corona discharge tube. Temperature was controlled by heating and recirculating water in a closed system formed by the concentric cylinders forming the chamber. Tests conducted with reduced pressure in this chamber were all conducted at 32° C in an attempt to reduce the exposure time to something that could be used in a quarantine situation.

Exposure of insects

First, adult thrips were exposed in small cloth bags. Adult thrips were collected with a hand aspirator and placed in moist cloth bags. Bags were kept moist but not wet in

order to keep the thrips from desiccating during treatment. The bags with thrips inside were placed on the shelf inside the chamber.

Following the exposure of the test insects in the bags, exposure was done in oranges by collecting thrips in a nylon mesh screen bubble anchored over the navel of oranges. Thrips were introduced at room temperature under the screen over the navel and then the temperature of the oranges was dropped slowly over several hours to 5°C in order to drive the thrips into the navel to escape the cold. After 24 hours of standing at the cold temperature, 8 to 12 oranges were exposed to the ozone treatment.

In large-scale treatments, insects were prepared as in the exposures in oranges. Selected boxes within each pallet load were opened and 5 oranges with thrips were introduced following the removal of the same number of oranges. Boxes were replaced back into the load and stacked in the original configuration. Four to eight pallets were then placed in a 3,600 cu. ft. chamber and fumigated with the appropriate dosage for the treatment time.

Insect exposure and dosages and evaluation of insect mortality

In the small chamber studies, insects were exposed to dosages ranging from 1250 ppm ozone to 5,000 ppm ozone for 2 hours under -250 mm Hg (Torr) and 10% CO_2. In the large-scale tests, insects were exposed to 2,500 ppm ozone in the first test and 5,000 ppm ozone in the second test with the vacuum and CO_2 remaining the same as for the small chamber tests. Following the 2 hour treatments, the vacuum was released and the chamber was evacuated of all ozone (approximately 10 minutes).

Evaluation of orange quality changes caused by treatments

Oranges were evaluated for phytotoxicity following exposure to ozone gas after 7- and 21-days storage at 5°C. Oranges were split into the following categories of physical damage based on marketability by visual inspection: 1) None, 2) Minor, 3) Moderate, 4) Severe, 5) Mold, or 6) Damage Due to Ozone Treatment. Results were expressed as a percentage of the total number of fruit evaluated. Untreated, control fruit were included for comparison.

RESULTS AND DISCUSSION

Insect mortality

Thrips exposed in bags responded to treatments as shown in Table 1. Results showed only 2 survivors or 99.7% mortality at 1,250 ppm and 100% mortality at 2,500 ppm with thrips either in moist, cloth bags or in oranges. Following exposure in small-scale laboratory tests with insects in the navel of oranges, insects responded to commercial-scale tests as shown in Table 2. In the first large-scale test, the predicted dosage of 2,500 ppm for 2 hours resulted in less than 100% mortality. Due to the reactive nature of ozone gas and the presence of the large organic load in a commercial-sized treatment, it was postulated the ozone gas was not penetrating at sufficient concentrations to control the test insects on and in the navel oranges. Therefore, it was decided to repeat the test and increase the dose two-fold to 5,000 ppm. Because there was no pattern of mortality that depended upon where in the pallet load the insects were located it was speculated that the penetration to the insects in each box was the reason that complete kill was not achieved. By increasing the ozone concentration to 5,000 ppm for the 2 hours, 100% mortality was achieved throughout the load (Table 2) when the large-scale test was repeated. .

Fruit quality following treatment

Oranges exposed to selected doses of ozone at either 5 or 20°C reacted as shown in Figure 1. Those oranges that were waxed on the processing line were protected against phytotoxic response at dosages up to 10,000 ppm. Unwaxed oranges began to show some adverse effects at 1,250 ppm and the effect intensified as the concentration of ozone rose. From the observations made, it was clear that waxing the oranges prior to treatment protected the oranges indicating that the ozone treatment should be applied only to packed, waxed fruit. Furthermore, these particular oranges were very late season fruit and had received a relatively light application of wax. A carefully applied wax should provide adequate protection to navel oranges exposed to ozone at concentrations that will control bean thrips.

In the large-scale tests, no fruit was deemed damaged by the treatment. One orange that was not waxed did show moderate damage as expected from our studies on fruit in the chamber tests. In all the fruit that was tested and later sold commercially, no phytotoxic effects were noted or reported on waxed, treated fruit.

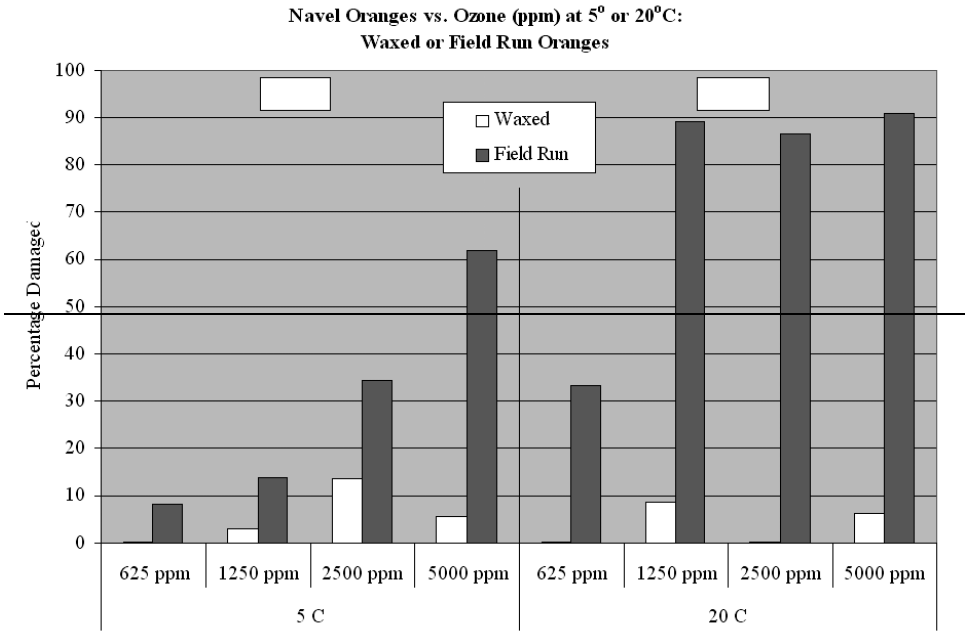

Figure 1. Phytotoxic response of navel oranges following a 2-hour exposure to selected concentrations (ppm) of ozone gas, 7-9% CO_2, and –250 mm Hg vacuum at 5 or 20°C: Evaluations were made after 21 days storage at 1°C.

CONCLUSIONS

Commercial-scale tests showed that a treatment of 5,000 ppm ozone gas with 10% CO_2 and –250 mm Hg (Torr) for 2 hours at a temperature of 10°C or higher controlled bean thrips inside the navel of navel oranges. Furthermore, if only packed fruit that has received an adequate application of wax will protect the oranges from any damage due to exposure to ozone at concentrations that will control bean thrips.

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TABLE 1
Efficacy of ozone gas to control bean thrips: Small chamber tests (USDA, Parlier, CA, 2003)

Pallet No.	Outside Navel						Inside Navel					
	No. Live		No. Dead		% Mortality		No. Live		No. Dead		% Mortality	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1,250 ppm, 2 hours, bean thrips inside cloth bags												
Total	489	2	90	571	15.5	99.7	--	--	--	--	--	--
2,500 ppm, 2 hours, bean thrips inside cloth bags												
Total	253	0	11	391	4.2	100.0	--	--	--	--	--	--
2,500 ppm, 2 hours, bean thrips infesting navel oranges												
Total	479	0	76	584	13.7	100.0	174	0	12	160	6.5	100.0

TABLE 2
Efficacy of ozone gas to control bean thrips infesting navel oranges: Commercial-scale tests (Sparks, NV, 2004)

	Outside Navel						Inside Navel					
Pallet	No. Live		No. Dead		% Mortality		No. Live		No. Dead		% Mortality	
No.	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
2,500 ppm, 2 hours, Test # 1, 05/06/2004, 8 pallets packed, waxed navel oranges												
1		15		92		86.0		10		18		64.3
2		9		86		90.5		5		17		77.3
3		12		88		88.0		7		14		66.7
4		30		81		73.0		9		6		40.0
5		10		89		89.9		5		15		75.0
6		21		83		79.8		9		8		47.1
7		13		92		87.6		9		17		65.4
8		7		86		92.5		5		15		75.0
Total	103	117	13	697	11.2	85.6	28	59	3	110	9.7	65.1
5,000 ppm, 2 hours, Test # 2, 06/11/2004, 4 pallets packed, waxed navel oranges												
1		0		76		100.0		0		29		100.0
2		0		109		100.0		0		21		100.0
3		0		109		100.0		0		20		100.0
4		0		85		100.0		0		25		100.0
5 *		0		67		100.0		0		53		100.0
Total	47	0	18	446	27.7	100.0	21	0	5	148	19.2	100.0

* 'Pallet' 5 represents boxes containing infested fruit placed open on top of the four pallets of oranges inside the chamber.

TABLE 3
Phytotoxic response of navel oranges exposed to commercial-scale ozone treatments
(Sparks, NV, 05/06/2004)

	Fruit Damage (any)											
	None		Minor		Moderate		Severe		Mold		Due to Treatment *	
	C	T	C	T	C	T	C	T	C	T	C	T
<u>2,500 ppm, 2 hours, Test # 1, 05/06/2004</u>												
No 7-day post-treatment evaluation												
21-day post-treatment evaluation												
Number of Fruit	24	31	24	27	15	9	5	1	3	3	0	0
Percent of Total	33.8	43.7	33.8	38.0	21.1	12.7	7.0	1.4	4.2	4.2	0.0	0.0
<u>5,000 ppm, 2 hours, Test # 2, 06/11/2004</u>												
7-day post-treatment evaluation												
Number of Fruit	20	21	14	13	7	8	0	0	4	2	0	1*
Percent of Total	44.4	46.7	31.1	28.9	15.6	17.8	0.0	0.0	8.9	4.4	0.0	2.2
21-day post-treatment evaluation												
Number of Fruit	21	26	11	12	6	6	0	0	2	4	0	0
Percent of Total	52.5	54.2	27.5	25.0	15.0	12.5	0.0	0.0	5.0	8.3	0.0	0.0

* Single orange damaged due to exposure to ozone (moderate damage) was an unwaxed fruit somehow found among the packed, waxed fruit

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