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The Use of Gaseous Ozone to Control Pests in Export Commodities

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Abstract: Ozone was tested against several insects and arachnids infesting stored products (coffee beans, navel oranges and grapes). Tests were conducted 1) to determine the usefulness of ozone gas treatments to replace methyl bromide for the control of pests on exports to other countries, 2) to determine any phytotoxic response of the commodity upon which the pest resided and 3) to determine the parameters that provided the highest efficacy and least phytotoxic response of the system. Tests were conducted with all life stages of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), adult bean thrips (BT), *Caliothrips fasciatus* (Pergande), mature female adult black widow spiders (BW), *Latrodectus hesperus* Chamberlin and Ivie and three species of late stage citrus mites, citrus flat mite (CFM), *Brevipalpus lewisi*, citrus rust mite (CRM), *Phyllocoptruta oleivora*, and two-spotted mite, *Tetranychus urticae* (TSM). Results were highly variable dependent on both species and life stage tested. Ozone gas controlled all life stages, except eggs of CBB, at concentrations from ranging from 2,500 to 10,000 ppm (v/v). Insects were tested as well as the commodity on which they are a problem.

Key words: grain storage, controlled atmosphere, ozone, coffee beans, navel oranges, grapes.

Introduction

With the loss of methyl bromide for postharvest pest control in perishable and durable commodities [1], new methods to control or eliminate postharvest pests are urgently needed. Ozone in gaseous form has not received much attention as a replacement for methyl bromide. Ozone has many advantages as a fumigant; 1) it is short lived in most situations and likes to return to its less reactive, more stable form, oxygen; 2) it has been declared a Generally Regarded as Safe (GRAS) compound meaning that it does not require registration to be used; 3) it is highly reactive against living organisms; 4) with the proper equipment, it can be generated on site from either air or oxygen; 5) it is easily converted back into oxygen so that none is emitted into the atmosphere following fumigation; and 6) it leaves no residues except the products of oxidation.

The major disadvantage of ozone is its oxidative action on many materials including some commodities. The advantages of using ozone prompted us to look at four situations where ozone fumigation might solve pest control problems. The first situation involved the use of ozone as a quarantine treatment to eliminate the coffee berry borer (CBB) from coffee beans being imported into Hawaii for roasting and blending. The second was the elimination of adult bean thrips (BT) from the navel of 'Navel' oranges. The thrips overwinter in this winter crop and can be found in the navel when the oranges are exported to Australia. The third situation involved the use of ozone to eliminate 3 species of mites from citrus being exported. And finally, the fourth situation attacked was the killing of adult black widow spiders (BW) from table grapes being exported to Europe and particularly the U. K. Trials with ozone against these pests and the host commodity were undertaken at the San Joaquin Valley Agricultural Sciences Center from 2001 to 2008 and here we give the results of those tests.

Materials and Methods

Exposure of Insects

A small fumigation chamber was used for exposures to ozone. The chamber was constructed from a stainless steel jacketed cylinder, solid on one end and having a 1.5 cm thick polycarbonate circular closure on the other end, held in place by bolts tapped into a flange around the end of the cylinder. The chamber was 56.5 cm long × 26.7 cm diameter with a volume of 31.6 L. Ozone was generated from oxygen in a ClearWater model CD12 unit and a continuous injection of ozone was necessary to

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keep the concentration constant. To monitor and control the concentration of ozone gas within the chamber a Hankin model HA – 100 – GTP – 12 ozone gas analyzer was used. Temperature was controlled by using a cooler circulating a water/antifreeze mixture in the jacket of the chamber when a temperature lower than 21℃ was required. The chamber was equipped with a vacuum pump and needle valves to maintain a negative pressure of about 460 to 508 mm Hg (absolute) during the exposure period. When carbon dioxide was used in combination with ozone, the CO_2 was introduced into the chamber using a rotameter to a concentration of 82% CO_2. Carbon dioxide has often been shown to have a synergistic effect in combination with other fumigants as shown by research done to decrease bacteria in meat[2] and also the fact that CO_2 increases insect respiration[3,4,5,6]. Relative humidity in the chamber followed that of the room at 35% ± 10%. The source, rearing, and exact exposure of the pests investigated follows.

Coffee Berry Borer (CBB)

CBB were supplied by Dr. Maribel Portilla, USDA, ARS, Mississippi State University. All stages of the insect were exposed to an ozone schedule of 10 000 ppm (v/v in air) for 6 h at 10℃ and without additional CO_2. (atmospheric CO_2). Negative pressure in these tests was about 460 mm Hg (absolute). The insects, that were treated, were internal pests infesting their natural host, inside galleries within green parchment coffee berries. Tests were conducted to determine efficacy of the treatment only.

Bean Thrips (BT)

BT were reared at our laboratory in Parlier, CA. Only the adult stage of thrips was of concern. Tests were conducted at 625, 1 250, 2 500, and 5 000 ppm (v/v) for 2 hr at 5℃ and with 102% CO_2. The insects treated were "external" pests infesting the navel of navel oranges. Although not always or easily visible inside the navel, the navel affords no barrier to penetration of the ozone gas reaching the target pest. Tests were conducted to determine both efficacy of ozone to control thrips and phytotoxicity of the oranges to exposure to ozone gas.

Citrus Mite Species (CM)

Three species of mites found on California-grown citrus were provided by Dr. Joseph Morse, U. C. Riverside, CA. The mites were grown on small, green lemons and different stages of development (egg to adult) were treated as external pests on the young, green fruit. The mites were exposed to three schedules: Treatments were conducted as described for BT above at schedules of 5 000 ppm (v/v) for 2 h, 10 000 ppm for 2 h, and 10 000 ppm for 4 h, providing CT products of 10 000, 20 000, and 40 000 ppm h.

Black Widow Spiders (BW)

Black widow spiders were collected from the field during nighttime hours in and around Fresno, California. They were brought into the laboratory, transferred individually to 7 – dram plastic vials with snap-cap lids. The vials had many 1 mm holes in the top, bottom, and sides to allow for free exchange of gas during treatment. The spiders were fed lepidopterous larvae and tests were conducted within one to two days from field collection. Mature or nearly mature female spiders were fumigated. All tests were conducted at a 1 – hour exposure time at 31℃ at several concentrations of ozone gas and with or without the addition of 102% CO_2.

Results

All stages of the CBB, except eggs, were controlled when exposed to 10 000 ppm ozone gas under a vacuum of 460 mm Hg, at 13℃ for 6 hours without additional CO_2. Visual tests and taste tests of green parchment coffee beans treated with the same ozone schedule were done earlier at a different facility and showed no adverse effects from the ozone treatments. BT were controlled from 1 250 to 2 500 ppm ozone in combination with 82% CO_2 for 2 hr at 5℃. Damage was minimal, less than 10% at all dosages even up to 5 000 ppm (CT = 10 000 ppm hr) when waxed (carnauba or shellac) and packed oranges were exposed to ozone gas. However, the severity of damage increased with increasing gas concentrations with field run oranges that had not been waxed. Tests with BWs showed ozone to be more efficacious alone, without the addition of CO_2 gas. Exposures of one hour provided 95% control at CT products of about 10 000 ppm hr and 7 000 ppm hr with or without 10% CO_2, respectively. Grapes were quite tolerant to ozone gas. The only response of grapes to ozone was the observation that the rachis of the berries sometimes showed small, hairline streaks running parallel with the rachis after a period of cold storage following treatment. Citrus mites were extremely tolerant to ozone gas in air as discussed below.

Coffee Berry Borer Infesting Green Coffee Beans

Ozone gas was shown to be an effective treatment controlling all life stages of CBB, except eggs, with an exposure schedule of 10 000 ppm for 6 hours at 13 ± 3℃ (55 ± 5°F) or above and at 508mm Hg. (absolute). Tests were replicated until ≥35 000 larvae, pupae, or adults were treated and observed. Since all stages of CBB are very prone to desiccation in dehydrated green parchment coffee, it is presently unclear if the eggs pose a threat to the coffee industry and thus may not be important to control with fumigation. In observations of eggs that we used in our tests, it was clear that eggs desiccate quickly and might not survive the shipping of berries to another country. Coffee tasting tests with coffee made with ozone-treated beans indicated that no off-flavor or odours resulted from the ozone treatment (Jack Armstrong USDA – ARSpersonal communication, 2006).

Table 1. Survival of coffee berry borer, *Hypothenemus hampeI* (Ferrari) (Coleoptera: Scolytidae) exposed to ozone gas at 10 000 ppm for 6 hours at 13℃ and –12" Hg.

Stage	Total[1]	Live	P	95% LL	95% UL
Egg	6086	887	0.146	0.137	0.155
Larva[2]	46,782	0	0	—	—
Pupa[3]	39,947	0	0	—	—
Adult	35,222	0	0	—	—

[1]Estimated number treated.
[2]First and second instar larva pooled.
[3]Pupa and prepupa pooled

Bean Thrips in Navel of 'Navel' Oranges

Ozone gas effectively controlled BT infesting the navel of 'Navel' oranges. In small chamber tests, we observed 100% mortality after 2 hours at 1 250 ppm ozone (2 500 ppm hr) at 20℃ or at 2 500 ppm (5 000 ppm hr) at 5℃ under vacuum (508 mm Hg-absolute) and in combination with 8% –10% CO_2. However, experiments on a commercial scale required 5 000 ppm for 2 hours (10 000 ppm hr) (all else the same) to obtain 100% control of BT.

There are two reasons the test failed at lower exposures in the commercial chamber: 1) the ozone generator was undersized resulting in a prolonged ramp time to setpoint, and 2) the air circulation system of the chamber was not designed to force the ozone atmosphere through the large palletized load and to efficiently distribute and penetrate the load with ozone gas. We observed minimal or no damage to navel oranges in years of testing with ozone gas until tests conducted in 2005. This crop year was classified as a "weak rind" year and samples of packed, waxed navel oranges taken from various packinghouses in the central San Joaquin Valley were damaged more severely than previously observed as a result of exposure to ozone gas at 5 000 ppm for 2 hours at 5℃ at 508 mm Hg (absolute) with CO_2. Several different types of symptoms were recorded and described.

Tests were conducted in 2006 to determine if low or high rates of application of wax will protect (diminish or eliminate) the oranges from damage due to exposure to ozone gas. Results are shown in Figure 1. All the processing waxes added to the field oranges, except one, were effective in reducing phytotoxic effects on theNavel' oranges. This indicates that the oranges should be treated after waxing in the processing procedures. We hope to revisit this project in the future with better designed large chambers in the hopes of demonstrating efficacious treatments at lower doses of 1 250 to 2 500 ppm for 2 hr and alleviating the problem of damage to the commodity as well.

Citrus mites on Navel Oranges

Mites proved to be quite tolerant to ozone gas in air even at very high doses. Three species of CM were exposed to ozone gas: 1) citrus flat mite (FM), *Brevipalpus lewisi*, 2) citrus rust mite (RM), *Phyllocoptruta oleivora*, and 3) two-spotted spider mite (TM), *Tetranychus urticae*. Mites infesting bean plants were exposed to ozone gas at 5 000 ppm for 2 hours, 10 000 for 2 hours, or 10 000 ppm for 4 hours (CT product = 10 000, 20 000, or 40 000 ppm hr, respectively). Other parameters were the same as described above for BT. Mortality data showed CFM and CRM most tolerant with TSM most susceptible to ozone. Based on 95% mortality as an acceptable level of control at a schedule of 5 000 ppm for 2 hours, only the two-spotted mite is likely to be controlled using ozone gas.

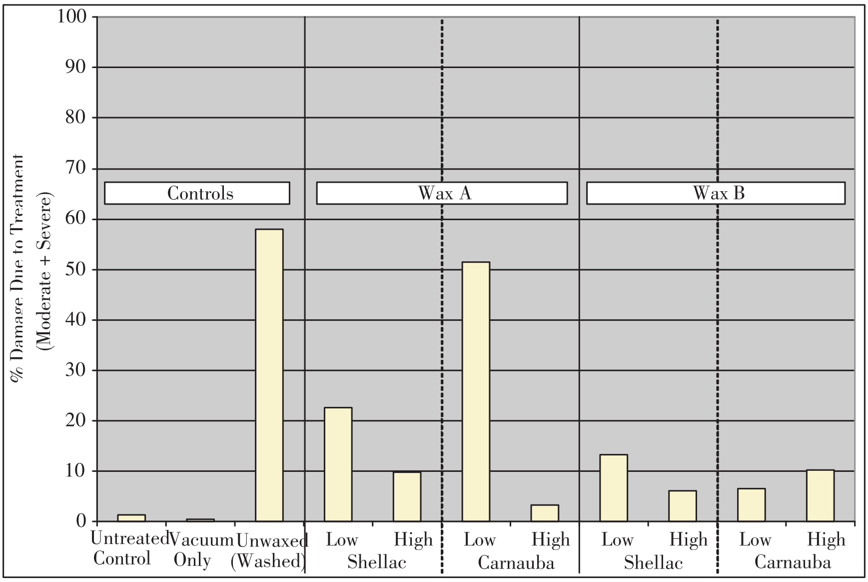

Fig. 1 Phytotoxic response of navel oranges following a 2 – hour exposure to ozone gas (ppm) and 8 2% CO_2 and 508 mm Hg (absolute) at 5 or 20℃: After 21 days storage at 1℃.

Table 3. Mortality of selected species of citrus mites exposed to ozone gas

Dose (ppm v/v)	Time (h)	CT (ppm h)	Percent mortality: Flat Mite (CFM)	Rust Mite (CRM)	2 – spotted Mite (TSM)
5,000	2	10 000	83.7	89.8	97.7
10 000	2	20 000	92.5	96.8	99.2
10 000	4	40 000	91.4	99.4	99.8
0 (control)	0	0	9.7	20.0	12.5
Total number treated/observed (estimated):			2 000 – 2 500	100 000 – 150 000	300 – 500

Black Widow Spiders on Table Grapes

The dosage-response curve was established for the adult females of the black widow spider. Mature adult female BWs were exposed to varying concentrations of ozone gas for 1 hour at 5℃ (40 F) at 508 mm Hg (absolute) with or without the addition of 10% CO_2. Lethal dosages to kill 50 or 95 percent of the BW population were calculated using regression analysis from probit percent mortality and log dose transformed data. Ozone gas performed better against BW without the presence of additional CO_2, i. e., at atmospheric CO_2 (< 0. 1%). It was determined that a CT treatment of about 7 000 ppm hr with ozone alone would kill all BW in grape clusters. In the presence of 10% CO_2, a CT of 10,000 ppm hr was needed to obtain the same level of control at 95% mortality (LD_{95}) (Table 4). Grapes were quite tolerant to ozone gas. In experiments with grapes in these and other tests, the only response of grapes observed was a slight streaking of the rachis of the berries characterized by small, hairline streaks running parallel with the rachis after a period of cold storage following treatment. We observed no berry shatter or other adverse effects in grapes from the ozone treatments.

Table 4. Estimated lethal dosages (CT = ppm hr) of ozone gas to control adult black widow spiderswith or without the addition of CO_2 gas.

	With CO_2 (10%) (±95% CL)	Without CO_2 (0. 1%) (±95% CL)
LD50	1 950 (1 630 – 2 290)	1 455 (1 216 – 1 717)
LD95	10 131 (7 538 – 15 657)	7 144 (5 387 – 10 691)

Discussion

Ozone gas has promise as an alternative fumigant against surface pests, possibly on a wide range of commodities, but with limited distributional, penetrative and ovicidal properties[7,8,9]. There are many unanswered questions concerning the interaction of the different physical parameters associated with an ozone treatment schedule and the optimal levels and hierarchy of each to enhance the effectiveness of the treatment and diminish any phytotoxic response of the commodity due to exposure to ozone gas.

In our testing of BT in oranges, and BW in grapes, where only adults are the target, ozone proved to be a good choice as a fumigant. In the studies with CBB, ozone gas was effective against all stages at a reasonable treatment schedule. However, ozone was ineffective against CBB eggs. The lack of toxicity to insect eggs was found earlier with stored product insects and may be a deficiency of ozone against insects[8,10]. For protecting grain against insect infestation, it has been shown that ozone used in a flow-through system over a period of days may be very efficacious while not damaging the nutritional characteristics of the grain[11,12,13]. One study tested the chronic effects of ozone on grain insects and found a definite efficacy over a period of days or months[14]. However, use of fumigants in most horticultural crops is for export and quarantine purposes. That means that the time a commodity spends under fumigation can only be a matter of hours, not days, so that these fumigations must be designed to be efficacious in a short time without damaging the commodity being treated. Other variables included time of exposure, temperature, pressure (vacuum), and the presence or absence of carbon dioxide (CO_2) need further investigation to determine the contribution of each to the toxicity of ozone to arthropods.

There are a few disadvantages associated with ozone, including its strong oxidizing power and its short half-life in situations where it can oxidise biotic materials which requires that ozone be continually applied during treatment. However, ozone has several advantages as a fumigant. It can be generated on site, is easy to catalyze back into oxygen so that no toxic material is being emitted to the atmosphere, leaves no residue, is considered a GRAS (Generally Considered As Safe) compound, at treatment levels discussed in this paper, ozone reduces microbial load of fungal and mold spores, increasing shelf-life of fresh produce, and is not flammable at concentrations and temperatures used in commodity fumigation. Clearly, ozone has a place in commodity protection and quarantine treatments and further research will define its role as a fumigant.

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