RESULTS OF 2 YEARS OF FIELD TRIALS USING OZONE GAS AS A SOIL TREATMENT

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Introduction – Ozone (O_3) is a light, colorless gas which has a characteristic pungent odor. Naturally, it is produced from diatomic oxygen in the atmosphere by ultraviolet light and electrical discharges (lightning). These phenomena produce the protective "ozone layer" in the upper stratosphere that is at risk , in part, due to methyl bromide. Paradoxically, ozone itself may be an effective tool for controlling soil borne plant pathogens and improving soil microflora. Due to its reactivity, ozone is a very effective biocide and its use has been approved in a number of agricultural and food processing applications. These include post harvest fumigation for storage of certain fruits and non-perishable commodities, wash waste treatment for fruits infested with surface pests, and as a structural treatment for food storage areas.

Ozone is unstable and rapidly breaks within minutes when dissolved in water or within hours in the gaseous state. Thus, ozone cannot be stored and transported and must be produced onsite for immediate use. For commercial purposes, ozone is produced from oxygen in ambient air through an electrical discharge process with relatively simple pieces of equipment known as ozone generators. Because the byproduct of ozone reaction or decomposition is simple diatomic oxygen, ozone is increasingly viewed as a possible environmentally benign alternative to more persistent and/or toxic fumigants in agricultural applications. Other potential benefits of ozone treatment of soil include:

1. No Transportation, Storage, or Discharge of Hazardous or Toxic Chemicals
2. No Environmentally-Persistent Chemicals Left in Soil
3. No Reentry, Permitting, or Use Restrictions
4. Minimum Human Acute and Chronic Toxicity
5. No Human Carcinogenicity or Teratogenicity
6. No Broad Spectrum Environmental Toxicity

SoilZone has been broadly investigating the use of ozone gas as a soil treatment agent in field trials for 3 years. This study reports the results of several of these field trials performed in carrots, tomatoes, and strawberries. These specific trials were chosen to report because they have been functionally repeated for 2 years in a row in the same field thus lending more validity to the results.

TOMATO FIELD TRIAL RESULTS

1997 Tomato Field Trials - These trials were performed in a field heavily infested with root knot nematodes at the University of California South Coast Extension Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl of the University of California at Davis Department of Nematology. Ozone was injected in early July with and without pre- and post-treatment irrigation at the rate of 250 lb./acre through underground drip tubing buried 2.5 – 3.5 inches deep in the center of 32” furrows. Tomato seedlings were planted 3 weeks later and the total yield and number of root galls were compiled at the end of the September harvest. Each treatment consisted of 6 replicated 20 ft. plots in randomized blocks. The increase or decrease in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 1).

Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	Yes	No	+ 79.5 %
250 lb. O_3	No	No	+ 14.5 %
250 lb. O_3	No	No	- 1.0 %
250 lb. O_3	Yes	Yes	- 1.9 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Alternative Fumigant
250 lb. O_3	Yes	Yes	+ 12.5 % vs. Telone II
			- 19.8 % vs. Vapam

Statistically, the extent of nematode root galling was no lower in the higher yielding ozone treated plots than in the untreated control plots despite the improved yield in the ozone treated plots (see Figure 2). This indicates that other biological factors (possibly increased nutrient availability) in addition to the biocidal aspects of ozone treatment may also be important in plant yield.

1998 Tomato Field Trials - These field experiments were again performed at the UC South Coast Field Station with Dr. Becky Westerdahl. The methods of application were functionally identical to those used in the experiments performed the previous year. In the 1999 experiments the effects of varying dosages were tested as well as the effect of coinjecting carbon dioxide to increase soil penetration by the ozone. All plots were irrigated prior to ozonation to about 10% moisture level unless otherwise indicated. None of the plots received post-treatment irrigation as did some plots the prior year. The increase in yield resulting from each ozone treatment compared to the untreated control is shown below (also see Figure 3).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	+ 44.2 %
250 lb. O_3 w/o Preirrigation	+ 35.1 %
50 lb. O_3 w/ 100 lb. CO_2	+ 30.0 %
250 lb. O_3 in O_2	+ 22.1 %
50 lb. $O_{3,}$	+ 17.6 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant

250 lb. O_3	+ 17.1 % vs. Telone II - 1.4 % vs. Vapam

CARROT FIELD TRIAL RESULTS

1997 Carrot Field Trials - These experiments were also performed at the UC South Coast Field Station with Dr. Westerdahl. Ozone was injected into plots in the same manner as the tomato trials followed by carrot seed planting 3 weeks later. The increase in marketable yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 4).

Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	Yes	Yes	+ 64.8 %
250 lb. O_3	Yes	No	+ 60.1 %
250 lb. O_3	No	Yes	+ 46.3 %
250 lb. O_3	No	No	- 25.5 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Ozone Marketable Crop Yield Compared to Alternative Fumigant
250 lb. O_3	Yes	Yes	- 39.4 % vs. Telone II - 17.8 % vs. Vapam

1998 Carrot Field Trials – These trials were performed in a manner similar to the prior year with only minor dosage variations. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 5).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
50 lb. $O_{3,}$	+ 92.2 %
250 lb. O_3	+ 92.0 %
250 lb. O_3 in O_2	+ 53.6 %
50 lb. O_3 w/ 100 lb. CO_2	+ 45.4 %
250 lb. O_3 w/o Preirrigation	- 9.1 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below. The total yield (including nematode damaged produce) was greatest in the 250 and 50 lb./acre ozonated plots possibly indicating increased nutrient uptake and growth in the ozonated plots (see Figure 6).

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant

50 lb. O_3	- 20.8 % vs. Telone EC
	- 19.2 % vs. Vapam

STRAWBERRY FIELD TRIAL RESULTS

1997-98 Strawberry Field Trials - This experiment was performed at a research site maintained by the California Strawberry Commission in Watsonville, CA in conjunction with Dr. John Duniway of the UC Davis Department of Plant Pathology. The soils were heavily infested with *Verticillium* sp. fungi. In November of 1997, ozone was injected at the rate of 400 lb. per acre through two drip tubes buried about 3.5 - 4.0" deep approximately 5" off center in 42" beds. Ozonation applications were made with and without pre-inoculation with Bioworks T-22 *Trichoderma* fungi granules at the rate of 100 lbs./acre, Transplant planting followed 5 days later. In early June 1998 an additional midseason application of 15 lb./acre was made to those ozonated plots that had been previously inoculated with the *Trichoderma sp.* fungi. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 7).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
400 lb. O_3, w/100 lb. *Trichoderma* T-22	+ 96.9 % (w/ 1x 15 lb./acre O3 midseason)
400 lb. O_3	+ 51.1 %
100 lb. *Trichoderma* T-22	+ 35.2 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant
400 lb. O_3, w/100 lb. *Trichoderma* T-22	- 5.0 % vs. Methyl Bromide

1998-99 Strawberry Field Trials – This experiments were performed at the same site and in nearly the same manner as above. The differences included burying the drip tube at 6" depth vs. 3.5-4.0" the previous year and additional treatments as described below.. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 8).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
400 lb. O_3, w/100 lb. *Trichoderma* T-22	+ 11.3 %
100 lb. O_3 w/100 lb. *Trichoderma* T-22	+ 9.7 % (w/ 3x 5-15 lb./acre O3 midseason)
400 lb. O_3,	+ 6.1 %
400 lb. O_3, w/100 lb. *Trichoderma* T-22	- 6.0 % (w/ 3x 5-15 lb./acre O3 midseason)
100 lb. O_3	- 6.5 % (w/ 3x 5-15 lb./acre O3 midseason)

DISCUSSION AND CONCLUSIONS

Ozone treatments in both tomatoes and carrots in 1997 and 1998 showed the highest yield with pretreatment irrigation. Almost all of the ozone treated plots that received a pretreatment irrigation (to about half of field capacity) showed numerical increases in marketable yield compared to untreated controls. All 1997 ozone treatments in carrots and tomatoes were at a rate of 250 lb./acre. In 1998, several treatments of 50 lb./acre were also tried. When ozone was applied at a 50 lb./acre basis on a standalone basis in the carrot trials, it was almost as effective as a 250 lb./acre treatment in increasing yield. When ozone was injected in tomatoes at 50 lb./acre in conjunction with CO2, it was slightly more effective in increasing yield than a 250 lb./acre treatment.

Overall, the effects of mixing carbon dioxide with ozone gas were mixed. In the case of the tomato trials, coextensive use of carbon dioxide with ozone resulted in increased yield. The opposite effect was seen in the carrot trials.

Both ozone treatments (with and without pre-inoculated *Trichoderma* sp. fungi) in the 1997-98 growing season showed substantial increases in yield compared to untreated controls. The average yield from the best ozone treatment was only slightly less than that of the methyl bromide treated control. (Note that both the untreated and methyl bromide treated controls in this trial were physically separate from the ozone treated plots by several hundred feet). The best yielding ozone treatment in 1997-98 (I.e. 400 lb./acre ozone w/ *Trichoderma* sp. fungi) also showed an accelerated yield increase immediately after receiving a single midseason ozone dosage of 15 lb./acre. This was in spite of showing modest levels of phytotoxicity in the form of lower leaf burn in a number of plants receiving the midseason application.

In 1998-99, an average 10% increase in yield was seen in the ozone treated plots with the exception of two of the three treatments receiving midseason applications of ozone. These 2 plots receiving 3 midseason ozone treatments resulted in average yield decline of about 5-7%. It is believed that this yield reduction is directly correlated with the amount of phytotoxicity suffered by these plants upon midseason ozone application. It is further believed that the escaping ozone was due to slight leaks in the type of push-on compression fitting used to connect the tubing in the various plots. A screw type compression fitting with an inert O-ring has been subsequently used in other trials which has seemingly eliminated all leaks. Further work needs to be performed to properly defined the correct dosage levels that yield the maximum growth response without phytotoxicity. The reduced increases in yield in the other ozone treated plots compared to the untreated control in 1998-99 were due to a number of factors. In the 1998-99 trials, the ozone injection tubing was buried at 5.5-6" beneath the surface as compared to 3-3.5" in 1997-98. Based on other trials in other crops, the author now believes that a more shallow drip tube depth provides greater control in the all important top few inches of soil into which new transplants are placed. Further, in 1998-98, the trial was conducted in ground that had been fumigated with methyl bromide the previous year. In the prior year's trial, the soil had not been recently fumigated. Combined with the substantially reduced temperatures in the 1998-99 growing season, this contributed to greatly reduced soil pathogen pressures that reduced the apparent differences between the untreated and ozonated plots. As an example, the untreated controls in 1998-98 produced over 2,000 g of strawberries per plant whereas in 1997-98 the untreated control plots had produced an average of less than 600 g of berries per plant.

In summary, the results of these field trials generally demonstrate the potential effectiveness of ozone treatment of soil in increasing plant yield in these crops. Much additional work is necessary to be able to accurately predict the specific growth response achieved by ozonation in different crops grown in different soil types with different pathogens and different climatic conditions.

Figure 1 - 1997 TOMATO YIELD

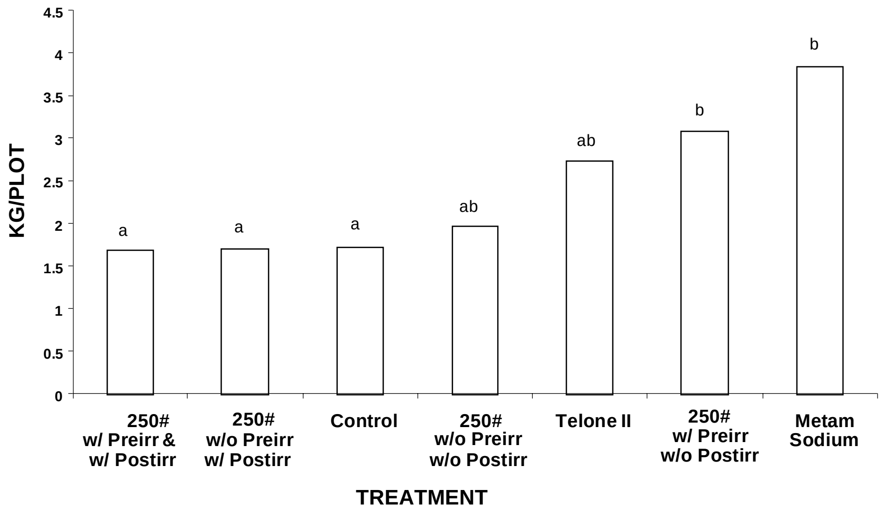

Figure 2 - 1997 TOMATO ROOT GALLING

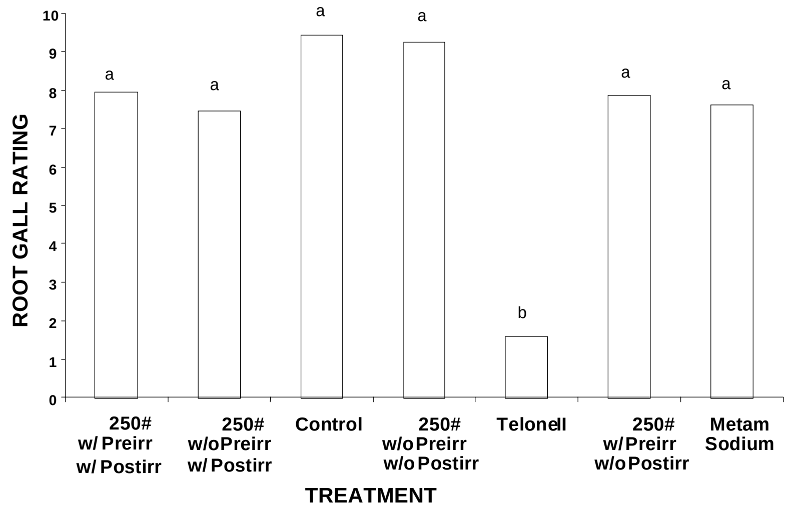

Figure 3 - 1998 TOMATO YIELD

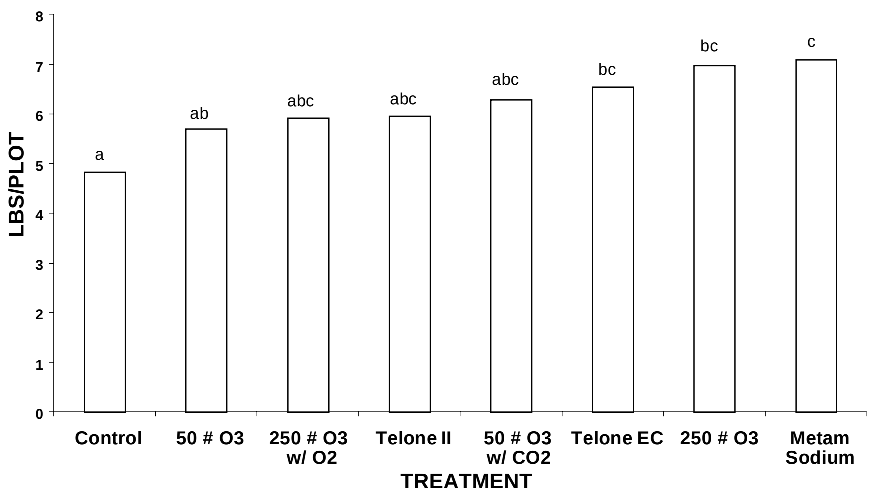

Figure 4 - 1997 CARROT MARKETABLE YIELD

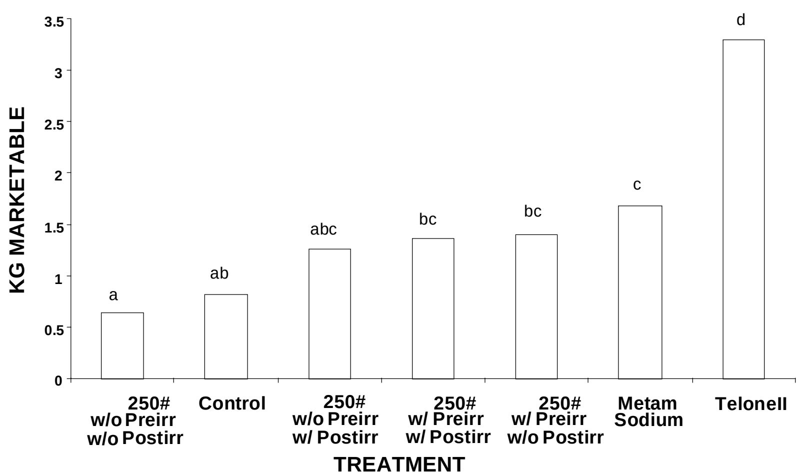

Figure 5 - 1998 CARROT MARKETABLE YIELD

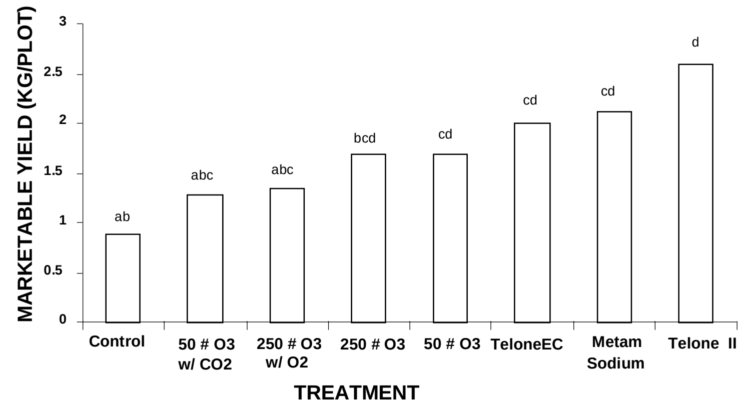

Figure 6 - 1998 CARROT TOTAL YIELD

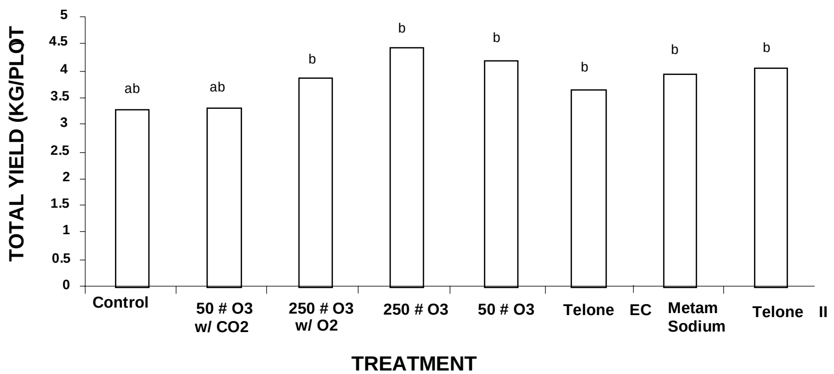

Figure 7 - 1998 STRAWBERRY MARKETABLE YIELD

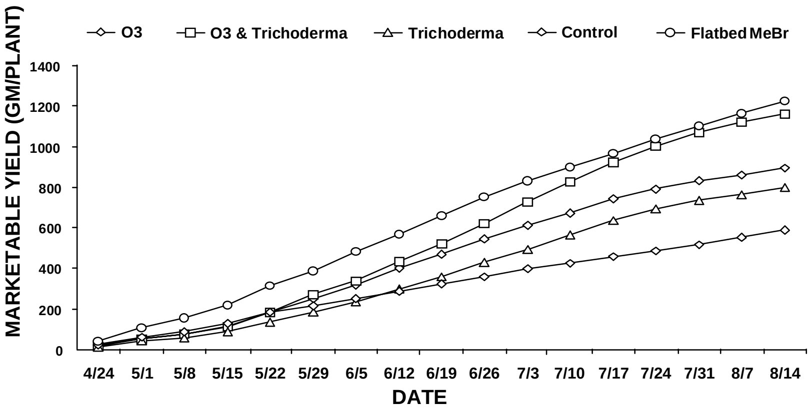

Figure 8 - 1999 STRAWBERRY MARKETABLE YIELD