PEST MANAGEMENT GRANTS FINAL REPORT

CONTRACT NO. 99-0220

FIELD TRIALS FOR THE COMBINED USE OF OZONE GAS AND BENEFICIAL MICROORGANISMS AS A PREPLANT SOIL TREATMENT FOR TOMATOES AND STRAWBERRIES

ALAN PRYOR
SOILZONE, INC.

AUGUST 31, 2001

PREPARED FOR CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION

DISCLAIMER - THE STATEMENTS AND CONCLUSIONS IN THIS REPORT ARE THOSE OF THE CONTRACTOR AND NOT NECESSARILY THOSE OF THE CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION. THE MENTION OF COMMERCIAL PRODUCTS, THEIR SOURCE, OR THEIR USE IN CONNECTION WITH MATERIALS REPORTED HEREIN IS NOT TO BE CONSTRUED AS ACTUAL OR IMPLIED ENDORSEMENT OF SUCH PRODUCTS.

ACKNOWLEDGEMENTS - The contributions of Drs. Becky Westerdahl of the Dept. of Nematology and John Duniway of the Dept. of Plant Pathology are gratefully acknowledged. THIS REPORT WAS SUBMITTED IN FULFILLMENT OF CONTRACT NO. 99-0220 - FIELD TRIALS FOR THE COMBINED USE OF OZONE GAS AND BENEFICIAL MICROORGANISMS AS A PREPLANT SOIL TREATMENT FOR TOMATOES AND STRAWBERRIES UNDER THE SPONSORSHIP OF THE CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION. WORK WAS COMPLETED AS OF JULY 31,2001.

TABLE OF CONTENTS

ABSTRACT

This research investigated the effect in tomatoes and strawberries of co-treatment of soils with pre-plant application of ozone gas and post-plant treatment with either fungicidal or nematicidal beneficial microorganisms. Ozone gas was injected 2-3 days before planting into bedded soil through drip tubing buried 3-4" deep along the bed length. In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used in soils heavily infested with root knot nematodes. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used in a field in transition to organic certification. Each type of beneficial microorganism was applied separately by drench application to transplants prior to planting. Each crop also received one additional application of microorganism that was applied to the surface of the soil and plants subsequent to planting.

Tomato harvest yields indicated that use of either microorganism on a standalone basis resulted in generally higher yields compared to untreated or chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a statistically significantly higher yield than untreated or chemically treated controls. Strawberry harvest yields indicated that most treatments produced a numerically higher yield (ranging from 5-15%) compared to untreated controls but none of the increased yields was statistically significant.

EXECUTIVE SUMMARY

Introduction

Tomato and strawberry growers in California are highly reliant on methyl bromide as a soil fumigant. In 1997, use of methyl bromide for strawberries in California amounted to over 4,100,000 lbs. on 20,400 acres. In the same year methyl bromide use in California for tomatoes amounted to over 1,200,000 lbs. on 5,560 acres. Use of methyl bromide is being phased out by 2005 due to concerns of its deleterious long-term effects on the ozone layer. Further, the most effective chemical alternatives, Telone (containing 1,3-dichloropropene) and Vapam (containing metam sodium), include materials that have been placed on California's Proposition 65 list as known carcinogens in recent years and increasingly severe restrictions are being placed on their use. Thus, there is an urgent need for environmentally benign substitutes for preplant soil fumigation.

Paradoxically, ozone gas itself has been recently shown by the project investigators to be effective in increasing yields of crops grown in soils treated with commercially viable quantities of ozone (50 – 400 lbs./acre). This research investigated the effect of preplant co-treatment of soils with both ozone gas and fungicidal and nematicidal beneficial microorganisms that were used subsequently for tomato or strawberry crop production.

Objectives

The following project objectives were successfully completed as part of this research:

Task 1 - Screen the effects of ozone on the selected beneficial microorganisms in the laboratory.

Task 2 - Presample field soils to determine baseline pathogen pressures.

Task 3 - Prepare fields for ozonation and subsequent planting of test crops.

Task 4 - Perform ozonation and addition of microbes and application of additional control chemicals.

Task 5 - Perform soil analysis after treatments.

Task 6 - Determine Crop Yield.

Task 7 - Preparation of Interim and Final Reports.

Methodology

The following describes the different ozone and microbe treatments employed at each trial.

Tomato Treatments

Untreated Control
Bacillus chitinosporus
Burkholderia cepacia
250 lb. O_3/acre
50 lb. O_3/acre
50 lb. O_3/acre w/ *B. cepacia*
50 lb. O_3/acre w/ *B. chitinosporus*
Telone II
Metam Sodium
Telone EC

Strawberries Treatments

Untreated Control
Trichoderma harzianum

Pseudomonas fluorescens
400 lb. O_3/acre
100 lb. O_3/acre
400 lb. O_3/acre w/ *T. harzianum*
400 lb. O_3/acre w/ *P. fluorescens*
100 lb. O_3/acre w/ *T. harzianum*
100 lb. O_3/acre w/ *P. fluorescens*

Plot sizes were 20 ft. x 32 inches for tomatoes (6 replicates per treatment) and 15 ft x 52 inches for strawberries (3 replicates per treatment).

Ozone gas was injected through drip tubing buried 3-4" deep into preirrigated or moistened soil that averaged 11-13% for the tomatoes and 12-14% for the strawberries.

In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used in soils heavily infested with root knot nematodes. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used in an organically certified field. Each type of beneficial microorganism was applied separately by drench application to transplant roots prior to planting. Each crop also received one additional application of microorganism (except for *Pseudomonas fluorescens* in strawberries) that was applied to the surface of the soil subsequent to planting. The microorganisms were applied in an amount and concentrations recommended by the manufacturers.

In the tomato field trial, end-of-season harvest yields, root gall ratings, and soil pathogen counts were obtained as well and soil beneficial microorganism obtained during the growing season. In the strawberry field trial, weekly harvest yield were obtained as well and soil beneficial microorganism obtained during the growing season.

Results and Conclusions

Crop yields and soil pathogen and beneficial microorganism concentrations were monitored to gauge the effectiveness of the treatments.

Tomato harvest yields indicated that use of either microorganism on a standalone basis resulted in generally higher yields compared to untreated or chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a significantly higher yield than untreated or chemically treated controls. Strawberry harvest yields indicated that most treatments produced a small yield increase (ranging from 5-15%) compared to untreated controls but none of the increased yields was statistically significant.

Outreach and Budget

A field day was held on March 21st at Mallard Bend Farms in which the ozonation technology was displayed and discussed in conjunction with organic farming practices. The Field Day was held as part of a seminar on sustainable farming practices sponsored by the Ecological Farming Association and the USDA Natural Resources Conservation Service.

The project was completed within budget. A no-cost extension of time was made to complete the strawberry field trial.

REPORT

Methodology

Two field trials were performed under these research contracts. The crops, California location, and collaborators are listed below.

Crop	Location	Collaborator
Tomatoes	Irvine	Dr. B. Westerdahl, UCD
Strawberries	Camarillo	Conway Farms Dr. John Duniway, UCD

All ozone was injected through standard 1/2" PVC drip tubing (Drip In, Madera, CA) with 0.5 gph emitters on 12" spacing. Tubing was buried 3.0 – 4.0" deep in 40" (tomatoes) or 52" (strawberries) beds. Injection tubing so used was left in place throughout the duration of the trial and was used for subsequent irrigation in the tomato trial. A single drip tube centered in the bed was used for the tomato trials. Two tubes were used for each strawberry bed with each tube placed 6-8" from the edge of each bed. Both field trial applications utilized ozone produced in air. Ozone treatments were under moistened conditions generally at about half of field capacity and proceeded planting by 1-5 days. Plots were laid out in randomized blocks. Upon harvest of the crops, yields were segregated and weighed.

Ozone gas was injected into preirrigated or moistened soil that averaged 11-13% for the tomatoes and 12-14% for the strawberries. Plot sizes were 20 ft. x 32 inches for tomatoes (6 replicates per treatment) and 15 ft x 52 inches for strawberries (3 replicates per treatment). The following describes the different ozone and microbe treatments employed at each trial.

Tomato Treatments

Untreated Control
Bacillus chitinosporus
Burkholderia cepacia
250 lb. O_3/acre
50 lb. O_3/acre
50 lb. O_3/acre w/ *B. cepacia*
50 lb. O_3/acre w/ *B. chitinosporus*
Telone II
Metam Sodium
Telone EC

Strawberries Treatments

Untreated Control
Trichoderma harzianum
Pseudomonas fluorescens
400 lb. O_3/acre
100 lb. O_3/acre
400 lb. O_3/acre w/ *T. harzianum*
400 lb. O_3/acre w/ *P. fluorescens*
100 lb. O_3/acre w/ *T. harzianum*
100 lb. O_3/acre w/ *P. fluorescens*

Laboratory exposures of all microbes were performed prior to the field trials and all were shown to be susceptible to ozone exposure compared to untreated controls (See Appendix A). As a result of this information, the researchers determined that the most advantageous mode of delivery of the microorganisms would be through a root drench application and additional spray application to the surface of the soil subsequent to ozonation.

Bacterial and fungal isolates were applied as a stand-alone treatment and in conjunction with ozone applications. In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used. Each type of beneficial microorganism was applied separately by drench application to tomato transplant roots 2 weeks prior to planting and to strawberry transplant roots immediately prior to planting. Each crop also received one additional application of microorganism (except for *Pseudomonas fluorescens* in strawberries) that was applied to the surface of the soil subsequent to planting. The microorganisms were applied in an amount and concentrations recommended by the manufacturers as follows:

Bacillus chitinosporus - The *Bacillus chitinosporus* culture was provided by Natural Resources Group of Woodlake, CA. Two weeks prior to planting, transplant drench solution was prepared to a concentration of 3.8 x 10exp5 CFU/ml. Approximately 1 gallon of microbe solution was added as a drench to a flat of 200 tomato transplants, allowed to soak for 20 minutes, and the excess drained. Post planting solution of the same concentration was prepared two weeks after planting. 0.75 ml of this solution was immediately further diluted to 250 ml of solution and sprayed uniformly onto the surface of each plot followed by approximately 0.5 gallon of water applied in the same manner.

Burkholderia cepacia - The *Burkholderia cepacia* culture was provided by Stine Microbial Products of Adel, IA. Two weeks prior to planting, transplant drench solution was prepared to a concentration of 3.4 x 10exp5 CFU/ml. Approximately 1 gallon of microbe solution was added as a drench to a flat of 200 tomato transplants, allowed to soak for 20 minutes, and the excess drained. Post planting solution of the same concentration was prepared two weeks after planting. 0.75 ml of this solution was immediately further diluted to 250 ml of solution and sprayed uniformly onto the surface of each plot followed by approximately 0.5 gallon of water applied in the same manner.

Pseudomonas fluorescens - The *Pseudomonas fluorescens* culture was provided by Dr. John Duniway of the Department of Plant Pathology at UC Davis. Transplant dip solution was prepared to a concentration of 5.0 x 10exp9 bacteria/gallon. Bare root strawberry transplant roots were fully submerged in the solution for 10 minutes on October 20 and immediately planted. No post planting application of this microbe was made.

Trichoderma harzianum strains (5) - A mixture of *Trichoderma harzianum* strains were provided by NBT of Seville, Spain. Transplant dip solution was prepared to a concentration of 3.4 x 10exp10 conidia/gallon. Bare root strawberry transplant roots were fully submerged in the solution for 2 minutes on October 20 and immediately planted. Post planting solution was prepared to a concentration of 8.9 x 10exp9 conidia/gallon. 15 ml of solution was applied directly to the exposed root crown of each transplant on January 2.

Results

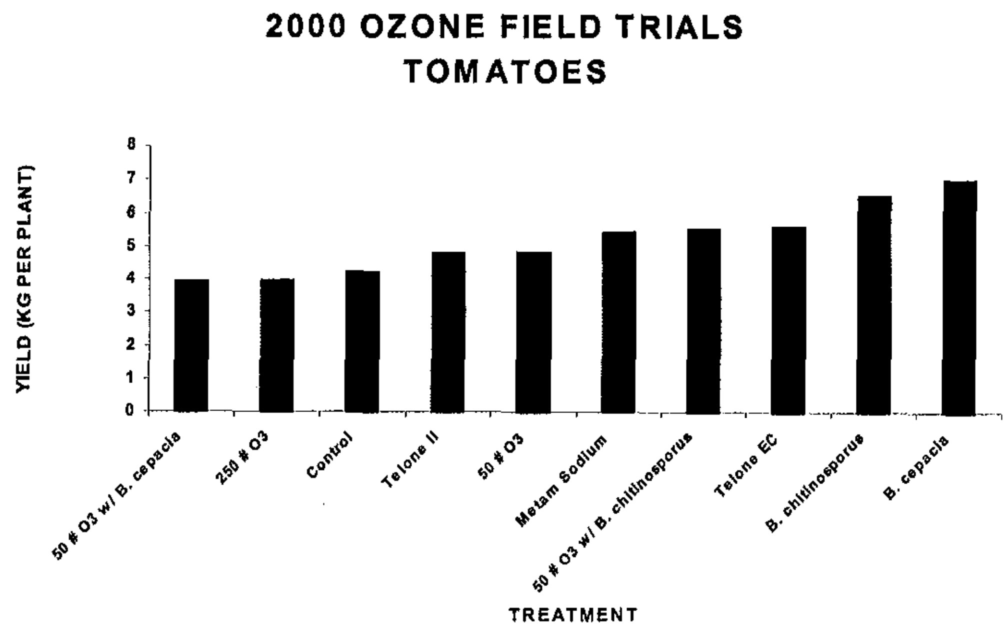
Tomatoes

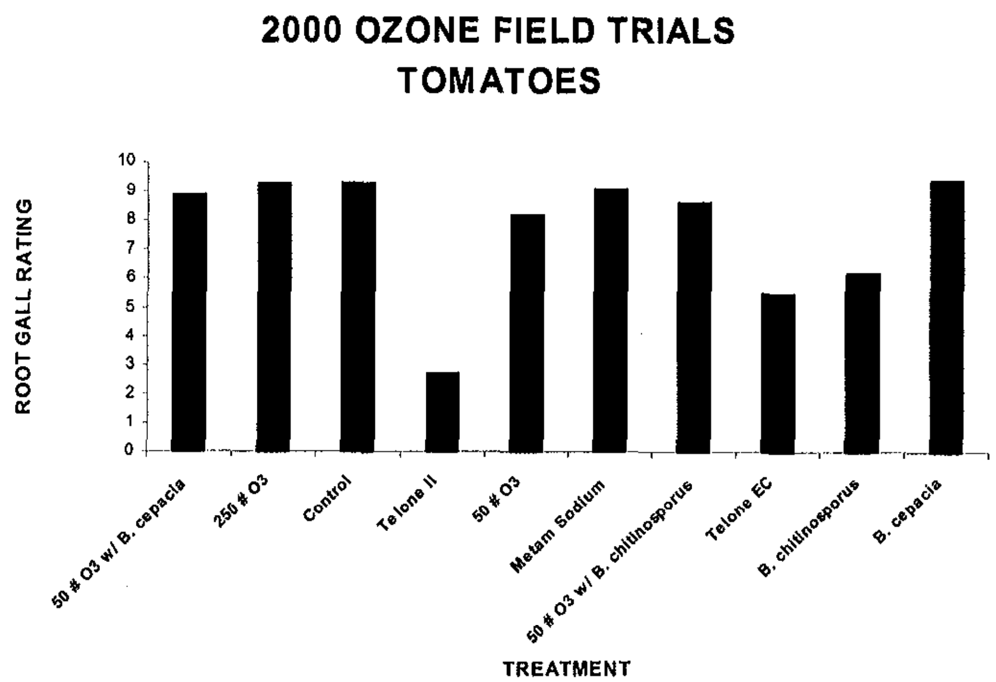
The increase or decrease in yield and Root Gall Rating resulting from each treatment compared to untreated and chemically treated controls is shown below.

Effects of ozone and *Bacillus chitinosporus* and *Burkholderia cepacia* on yield and root gall rating in the tomato field trial

Treatment	kg/Plant	% +/- Control	Gall Rating
50 lb. O_3/acre w/ *B. cepacia*	3.956 - a	-7.4%	8.933 - cd
250 lb. O_3/acre	4.012 - ab	-6.1%	9.333 - d
Control	4.272 - ab	0.0%	9.333 - cd
Telone II	4.840 - ab	13.3%	2.733 - a
50 lb. O_3/acre	4.892 - ab	14.5%	8.267 - cd
Metam Sodium	5.484 - ab	28.4%	9.133 - d
50 lb. O_3/acre w/ *B. chitinosporus*	5.608 - ab	31.3%	8.667 - cd
Telone EC	5.688 - ab	33.1%	5.533 - b
B. chitinosporus	6.576 - bc	53.9%	6.267 - cd
B. cepacia	7.096 - c	66.1%	9.467 - d

(Separation of means performed with Fisher's Protected LSD at .05 Significance Level for previous and all subsequent analyses)


2000 OZONE FIELD TRIALS
TOMATOES
YIELD (KG PER PLANT)
0
1
2
3
4
5
6
7
8
50 # O3 w/ B. cepacia
250 # O3
Control
Telone II
50 # O3
Metam Sodium
50 # O3 w/ B. chitinosporus
Telone EC
B. chitinosporus
B. cepacia
TREATMENT


2000 OZONE FIELD TRIALS
TOMATOES
ROOT GALL RATING
0
1
2
3
4
5
6
7
8
9
10
50 # O3 w/ B. cepacia
250 # O3
Control
Telone II
50 # O3
Metam Sodium
50 # O3 w/ B. chitinosporus
Telone EC
B. chitinosporus
B. cepacia
TREATMENT

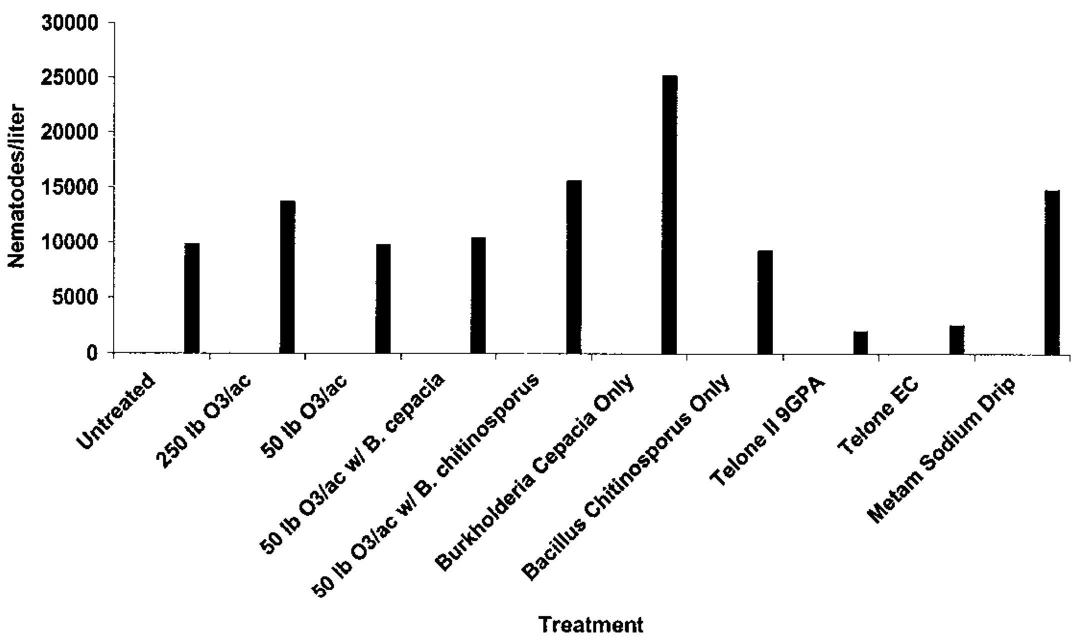
Soil levels of *Bacillus chitinosporus* and *Burkholderia cepacia* 4 weeks after planting

TREATMENT	*B. cepacia* (cfu/gdw soil)	*B. chitinosporus* (cfu/gdw soil)
Untreated control	348,000	32,000,000
Microbe Only	305,000	47,000,000
Microbe + Ozone @ 50 lb./acre	331,000	36,000,000

Effect of Treatments on Soil Root Knot Nematodes at Harvest

Treatment	RK Nemas / Liter
Untreated Control	9,940
250 lb. O_3/acre	13,790
50 lb. O_3/acre	9,870
50 lb. O_3/acre w/ *B. cepacia*	10,490
50 lb. O_3/acre w/ *B. chitinosporus*	15,650
Burkholderia cepacia	25,300
Bacillus chitinosporus	9,350
Telone II	2,140
Telone EC	2,660
Metam Sodium	14,930

2000 OZONE FIELD TRIALS
TOMATOES



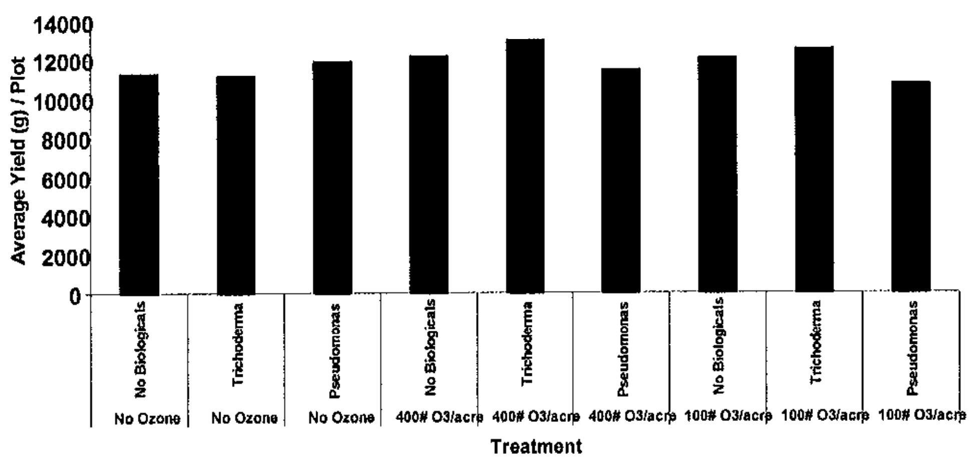
Strawberries

The increase or decrease in yield resulting from each treatment compared to untreated controls is shown below.

<u>Effects of ozone and *Trichoderma harzianum* and *Pseudomonas fluorescens* on yield in the strawberry field trial</u>

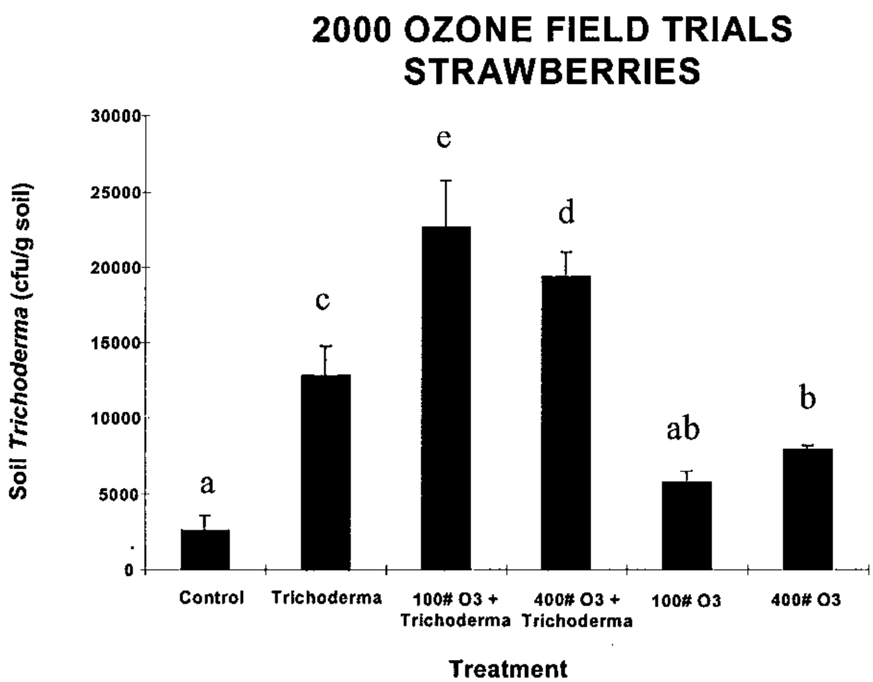
Ozone	Yield g / Plot	% of Control
Untreated Control	11378 - a	100%
Trichoderma harzianum	11267 - a	99%
Pseudomonas fluorescens	11968 - a	105%
400 lb. O_3/acre	12246 - a	108%
100 lb. O_3/acre	13090 - a	115%
400 lb. O_3/acre w/ *T. harzianum*	11573 - a	102%
400 lb. O_3/acre w/ *P. fluorescens*	12149 - a	107%
100 lb. O_3/acre w/ *T. harzianum*	12598 - a	111%
100 lb. O_3/acre w/ *P. fluorescens*	10854 - a	95%

2001 OZONE FIELD TRIALS
STRAWBERRIES



Post Treatment Soil Colonization by Trichoderma (CFU/Gram Wet Soil)

Treatment	Average (cfu/g wet soil)
Control	2,600 -a
Trichoderma sp.	12,778 - c
Trichoderma sp.+ 100lbs ozone/acre	22,667 - e
Trichoderma sp.+ 400lbs ozone/acre	19,333 - d
100lbs ozone/acre	5,800 - ab
400lbs ozone/acre	7,933 - b



Discussion and Conclusions

Tomato harvest yields indicated that use of either microorganism on a standalone basis produced the highest yields compared to untreated and chemically treated controls. Ozone at 50 lb. O_3/acre on a standalone basis or in conjunction with *B. chitinosporus* resulted in higher yields (approximately 15% and 30%, respectively) compared to untreated controls and was generally equivalent to chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a significantly higher yield than untreated and all chemically treated controls.

The overall differences in yields between ozone treatments and untreated controls were substantially less this year than in previous year, however.

Following is the results of applications of 250 lb. and 50 lb. ozone per acre in tomatoes at the same site in previous years compared to the results achieved in 2000.

<u>Comparative Tomato Yields</u>

Treatment / Year	1997	1998	1999	2000
250 lb. 03/acre	+ 79 %	+ 44 %	+ 20 %	-6.5%
50 lb. 03/acre	NA	+ 19 %	+ 17 %	+15.5%

The differences are partly due to the overall increase in yield in the untreated plots that had the effect of reducing apparent differences in yield compared to years in which soil borne pathogens pressures were greater (i.e. yields in untreated controls were particularly low in 1997 and 1998). The decreased differences in yields also seen between chemically treated and untreated controls over the recent years are also indicative of reduced pathogen pressures. However, it was recently determined that soils from different parts of the field in which these field trials were performed have substantially different characteristics with respect to moisture retention and their ability to allow ozone to pass through the soil without breaking down into oxygen. This may also have been a contributing factor and further research into the underlying causes of these discrepancies needs to be performed.

Colonization of soil by the bacteria, root gall rating, and final soil nematode concentrations were not accurate predictors of final yields. The yields in the standalone biological treatments were the highest in the trial yet also showed little colonization of the soil and generally high root gall ratings and root knot nematode soil counts at the end of the trial.

Strawberry harvest yields indicated that most treatments produced a small increase in yield (ranging from 2-15%) compared to untreated controls but none of the increased yields was statistically significant.

Good colonization of the soil by the *Trichoderma* sp. fungi was achieved with all Trichoderma treatments showing statistically increased soil levels compared to untreated plots. Trichoderma added to ozone treated plots also showed statistically significant increases compared to Trichoderma used on a standalone basis. The uniformity of yield over the ozone treated plots indicates that soil variation was probably not a factor in these field trials.

<u>Problems Encountered and Solutions</u>

i) Tomatoes - Tomato transplants raised for the project at the UC South Coast Extension Station suffered an infestation immediately prior to the planned planting. Almost all alternative sources of transplants in the marketplace were depleted due to the late date in the planting season. Acceptable alternatives were finally obtained but they were larger than planned due to the late date. Extra efforts were made by the various researchers and staff to ensure that a successful planting occurred and very few transplant losses were noted.

ii) Strawberries - Space for the planned strawberry field trials in Watsonville was not available as anticipated. An acceptable alternative site in Camarillo was secured although soil pathogens were not present as originally represented by the grower. The late date securing the site prevented confirmation of the pathogens in the soil prior to planting because of the length of time required for Verticillium or Phytopthora analyses.

APPENDICES

A. **Effects of Ozone on Microbes in a Laboratory Experiment**

I. **Effects of ozone on *Bacillus chitinosporus* and *Burkholderia cepacia* in a laboratory experiment**

TREATMENT	*B. cepacia* (cfu/gdw soil)	B. chitinosporus (cfu/gdw soil)
Untreated control	6,740,000	3,190,000
Ozone @ 50 lb./acre	5,570,000	3,450,000
Ozone @ 250 lb./acre	3,260,000	1,930,000

II. **Effects of ozone on Pseudomonas *fluorescens* and *Trichoderma harzianum* in a laboratory experiment**

TREATMENT	*P. fluorescens* (cfu/gdw soil)	*T. harzianum* (cfu/gdw soil)
Untreated control	9,600,000	12,100,000
Ozone @ 100 lb./acre	11,500,000	13,300,000
Ozone @ 400 lb./acre	6,900,000	14,700,000