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Ethylene Inhibits Aflatoxin Biosynthesis in *Aspergillus parasiticus* Grown on Peanuts

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Abstract

The filamentous fungi *Aspergillus parasiticus* and *A. flavus* synthesize aflatoxins when they grow on a variety of susceptible food and feed crops. These mycotoxins are among the most carcinogenic naturally occurring compounds known and they pose significant health risks to humans and animals. We previously demonstrated that ethylene and CO₂ act alone and together to reduce aflatoxin synthesis by *A. parasiticus* grown on laboratory media. To demonstrate the potential efficacy of treatment of stored seeds and grains with these gases, we tested ethylene and CO₂ for ability to inhibit aflatoxin accumulation on Georgia Green peanuts stored for up to 5 days. We demonstrated an inverse relationship between *A. parasiticus* spore inoculum size and the level of toxin accumulation. We showed that ethylene inhibits aflatoxin synthesis in a dose-dependent manner on peanuts; CO₂ also inhibits aflatoxin synthesis over a narrow dose range. **Treatments had not discernable effect on mold growth.** These observations support further exploration of this technology to reduce aflatoxin contamination of susceptible crops in the field and during storage.

Keywords

aflatoxin; peanuts; ethylene; carbon dioxide

1. Introduction

The filamentous fungi *Aspergillus parasiticus* and *A. flavus* synthesize aflatoxin under appropriate environmental conditions on a variety of economically important, susceptible food and feed crops including peanuts, tree nuts, corn, and cottonseed (reviewed in Cary et al. 2000). Aflatoxins are extremely mutagenic and carcinogenic in certain animals and there is a strong epidemiological link between aflatoxin ingestion and hepatocellular carcinoma in humans (reviewed in CAST, 2003). As a result, the International Agency for Research on Cancer designated aflatoxin B₁ a probable human carcinogen (CAST, 2003). Our laboratory

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is focused on developing novel, effective, and practical methods to reduce aflatoxin contamination of food and feed crops.

Sharma et al. (1985) reported that low concentrations (0-1200 ppm) of 2 chloroethyl phosphonic acid (CEPA) stimulated fungal growth and drastically reduced aflatoxin biosynthesis in *A. parasiticus* in laboratory culture. CEPA concentrations at 1400 to 1600 ppm reduced both fungal growth and aflatoxin biosynthesis while CEPA at 3200 ppm eliminated aflatoxin accumulation in either stationary or shake cultures of *A. parasiticus*. Although CEPA frequently is used as an ethylene-generating compound, the authors did not measure ethylene concentration in the culture medium and did not confirm that ethylene was the active compound in these studies. However, this initial study prompted us to examine the potential use of ethylene to reduce aflatoxin contamination of susceptible food and feed crops during storage or in the field because it is food safe, inexpensive to generate, and has a long history of application in agriculture (Roze et al. 2004).

Roze et al. (2004) developed a novel flow through system to monitor the effects of ethylene on aflatoxin biosynthesis by *A. parasiticus* grown on a defined solid culture medium (GMS). Ethylene treatment reduced aflatoxin accumulation in a dose-dependent manner; the greatest reduction (10 fold) was observed when the fungus was treated with 146 ppm ethylene. CO₂ at 0.1% also reduced aflatoxin accumulation (about 5 fold); adding more CO₂ (0.7 or 3.0%) reversed the inhibitory effect. Co-treatment with CO₂ and ethylene resulted in an additive inhibitory effect. Ethylene decreased the level of transcription of the *nor-1* gene in *A. parasiticus* (encodes an early enzyme in aflatoxin synthesis) suggesting that ethylene acts directly or indirectly on aflatoxin promoter function. In this same study, treatment of peanut seeds with a single concentration of ethylene (2 ppm) and/or two concentrations of CO₂ (0.05% or 0.1%) inhibited aflatoxin accumulation up to 5 fold (Roze et al. 2004).

Although these data were promising, it was important to clearly establish an effective ethylene dose range in the laboratory prior to initiating large scale crop storage, green house, or field experiments. The objective of the current study was to optimize efficacy of ethylene treatment by testing a wide range of ethylene concentrations (using the flow through model) for ability to inhibit aflatoxin accumulation by *A. parasiticus* grown on peanuts under conditions that generate much higher levels of toxin accumulation than in the previous study. Ethylene reduced aflatoxin accumulation in a dose-dependent manner (0 to 100 ppm); the highest reduction (6 fold) was observed at 100 ppm ethylene (atmospheric CO₂ levels ~ 0.03%). CO₂ alone at 0.1% inhibited aflatoxin accumulation, however, 0.1% CO₂ blocked the inhibitory effect of ethylene when these treatments were combined.

2. Materials and methods

2.1. Fungal strains and inoculation methods

A. parasiticus D8D3, an isogenic strain derived from the wild type aflatoxin producer *A. parasiticus* SU-1 (ATCC 56775), contains the GUS (*uidA*; encodes β -D-glucuronidase) reporter fused to the *nor-1* promoter (Chiou et al. 2002).

Stocks of D8D3 conidiospores were prepared and stored frozen (-80°C) until use as described previously (Chiou et al. 2002). Spore concentrations ranging from 10^2 conidiospores/g peanuts to 10^5 conidiospores/g peanuts were inoculated onto peanuts, depending on the experiment. Spores were diluted with sterile, deionized, distilled water (ddH₂O) to achieve the desired spore concentration. One hundred μl of the diluted spore suspension was added to 4 grams of peanuts in a sterile, conical centrifuge tube; spores and peanuts were mixed by light agitation. Inoculated peanuts were then placed into a sterile Petri plate lined with sterile filter paper.

Uninoculated peanuts incubated under the same conditions served as the negative control. Two ml of sterile, ddH₂O were added to wet the filter paper prior to adding the inoculated peanuts.

2.2. Peanut cultivar

Experiments were conducted using the near isogenic, Georgia Green peanut cultivar (kindly provided by Dr. Corley Holbrook, USDA-ARS, Tifton, GA). Peanuts were obtained with the shells removed. Prior to inoculation, peanuts were washed in distilled water 3 times for approximately 5 seconds each. Testa were removed by hand and the peanuts air-dried in a chemical fume hood for at least 2 h.

2.3. Growth conditions, flow through system

Fungal growth experiments were conducted in desiccator jars using a novel flow through system described previously (Roze et al. 2004). For each treatment, three Petri plates with inoculated peanuts were placed into one chamber resulting in triplicate samples. Where no CO₂ and/or ethylene was added, CO₂ or ethylene scrubbers (5 g soda lime for CO₂; 5 g KMNO₄ for ethylene) were placed in the growth chamber to minimize effects due to either production of these gases by the fungus or to external contamination by these gases (Roze et al. 2004). Desiccator jars carrying inoculated, uninoculated, and no treatment control peanuts were incubated for 5 d (unless otherwise noted) in the dark at 30°C; air/gas mixtures were water saturated by passing through a sparger containing distilled water. **Mold growth was determined by visual inspection.**

2.4. Aflatoxin extraction

Aflatoxins were extracted using AOAC official method 990.34 specifically developed for peanuts, corn, and cottonseed with slight modifications (AOAC). After incubation, inoculated peanuts were removed using heat-sterilized forceps, ground in a sterile mortar and pestle, and the resulting material was placed into a 50 ml conical centrifuge tube (Corning Inc., Corning, NY 14831). Eight ml of methanol-water (80% v/v) was added to each tube and the suspension was mixed by vortexing for 3 min. Tubes were allowed to sit for 15 min; the liquid phase was removed to a fresh tube which was centrifuged at 2500 rpm, at 10°C for 10 minutes (IEC PR-600 Centrifuge, Thermo Electron Corp., Marietta, OH) to remove spores, mycelia, peanut materials, and other solids. The supernatant, now virtually free from foreign materials, was collected into a scintillation vial (wide mouth 20 ml low background glass vial, Research Products International Corp., Mt. Prospect, IL).

Supernatants were evaporated (Meyer N-Evap Analytical Evaporator (Organomation Associates, Inc., South Berlin, MA) under a stream of nitrogen (99.8% oxygen free) in a hot water bath (approximately 50°C). Aflatoxin was resuspended in 1 ml, methanol-water (70% v/v). Samples were stored at 4°C for at least 1 d prior to analysis. Just before analysis, mixtures were placed into 1 ml screw-cap micro centrifuge tubes and centrifuged for 5 min (13,000 rpm, room temperature) to remove solids. This AOAC approved procedure routinely extracted greater than 80% of the aflatoxin from the peanut samples.

2.5. Aflatoxin analysis

Aflatoxin was quantified by Enzyme Linked Immuno-Sorbent Assay (ELISA) as described previously (Pestka et al. 1980). Aflatoxin B₁ (AFB₁) and rabbit anti-AFB₁ were purchased from Sigma (St. Louis, MO). Horse Radish Peroxidase (HRP)-AFB₁ conjugate was prepared in our laboratory (Skory et al. 1993). This ELISA detects primarily AFB₁ although there is measurable cross reactivity with other aflatoxins. A standard curve was generated using aflatoxin concentrations ranging from 0.001 ng/ml to 10 ng/ml. Aflatoxin concentrations reported for each treatment group represent the mean of peanuts from three independent Petri

plates. Prior to analysis, samples were diluted appropriately to generate samples that fell within the linear range of the standard curve. Error bars represent standard error of the means of these triplicate samples. All experiments were conducted a minimum of two times.

Thin Layer Chromatography (TLC) was used to confirm ELISA data and to demonstrate inhibitory effects on accumulation of both B and G aflatoxins. Peanut extracts (5 µl sample per lane) and known quantities of aflatoxin standards (Sigma) were spotted on Partisil® LHPKD silica gel TLC plates (60Å, 10 × 10cm, 200µm thick, plates; Whatman Inc., Clifton, NJ) while minimizing spot size. Plates were resolved using a 95% chloroform-acetone (v/v) solvent system and analyzed under UV light. TLC plates were photographed using a Kodak DC 290 Zoom Digital Camera and band intensity calculated using Kodak 1D 3.6 Image Analysis software. Band intensity and R_f values for bands in peanut extracts were compared with values for the aflatoxin standards to establish relative concentrations and identity. Background intensity was subtracted from each band to obtain a true measure of band intensity. TLC and ELISA methods have been used extensively in our laboratory to quantify aflatoxins (Chiou et al., 2002; Miller 2003; Roze et al. 2004).

2.6. Analysis of gas concentrations

Gases (medical air, CO₂, and ethylene/air mixtures) were purchased from Linde gas (Cleveland, OH). In certain experiments, ethylene/air mixtures were prepared in our laboratory by mixing medical air with ethylene, to achieve the appropriate final ethylene concentration for use in the flow through system. No significant differential effects were observed for purchased gas mixtures or mixtures made in our laboratory.

O₂, CO₂, and ethylene concentrations were monitored by Gas Chromatography (GC) using two different instruments; one for ethylene (Carle AGC series 400, HACH Carle Chromatography, Loveland, CO) and the other for CO₂ or O₂ (ADC.225.MK3, The Analytical Dev. Co. Ltd., Hoddesdon, England). Standards were analyzed prior to analysis of each set of samples. Sample concentrations were calculated based on concentration of standards. Samples were obtained from the in-port and out-port of each desiccator.

2.7. Statistical analyses

In measuring the effect of inoculum size on aflatoxin accumulation, the data did not follow normal distribution and did not display equal variances. ANOVA for heterogeneous variances was used to identify significant differences between treatment groups. All other data displayed a normal distribution with equal variances; one-way ANOVA followed by Bonferroni's multiple comparison test was used to identify significant differences between treatment groups.

3. Results and discussion

3.1. Effects of inoculum size on aflatoxin accumulation on peanuts

Sharma et al. (1985), and Clevstrom and Ljunggren (1983) reported that inoculum size strongly influences the quantity of aflatoxin that accumulates during fungal growth. For example, a 2-fold reduction in inoculum increased aflatoxin accumulation 8 to 10 fold. This observation prompted us to determine the effect of inoculum size on aflatoxin accumulation on peanuts in our flow-through model.

The peanuts used in initial studies (Roze et al. 2004) were bought locally and the cultivar was unknown. Peanut cultivar Georgia Green provided by USDA-ARS in Tifton, GA was used in the current study providing more control over host genotype. This is a commonly used, commercially sold, peanut cultivar.

Four inocula sizes were tested (10^5 , 10^4 , 10^3 , and 10^2 conidiospores/g peanuts) on peanuts incubated under standard growth conditions. Aflatoxin concentration increased with decreasing inoculum size (Figure 1). The lowest inoculum (10^2 conidiospores/g peanuts) generated highest aflatoxin levels (1,000 $\mu\text{g/g}$ peanuts). However, mycelium did not completely coat the peanuts within 5 d with an inoculum of 10^2 conidiospores/g whereas peanuts were evenly coated by mycelium at 5 d with an inoculum of 10^3 conidiospores/g. Therefore, we carried out further experiments with an inoculum of 10^3 conidiospores/g peanuts as a conservative approach to make certain that ethylene and/or CO_2 treatment was effective under conditions generating high levels of aflatoxin accumulation. Uninoculated peanuts did not develop apparent mold growth nor accumulate detectable aflatoxin under these growth conditions.

3.2. Dose response to ethylene

Roze et al. (2004) reported that ethylene at the only concentration tested (2 ppm) reduced aflatoxin accumulation on peanuts approximately 5 fold with an inoculum (10^6 conidiospores/g) that resulted in accumulation of low levels of aflatoxin B₁ (11 $\mu\text{g/g}$ peanuts). To optimize the effective ethylene dose for treatment of peanuts (inhibition of toxin accumulation), we tested 5 ethylene concentrations (2 ppm, 10 ppm, 25 ppm, 70 ppm, and 100 ppm) using an inoculum (10^3 conidiospores/g) that resulted in 10 fold higher levels of aflatoxin accumulation (100 $\mu\text{g/g}$ peanuts). Because fungal growth was previously shown to result in CO_2 accumulation in the flow through system (Roze et al. 2004, and data not shown), a CO_2 scrubber (soda lime) was included where appropriate to eliminate inhibitory effects due to excessive CO_2 accumulation.

Analysis of gas concentrations was conducted at the beginning of the experiment and at d 1, 2, and 5 (Table 1). The data showed that the scrubber maintained CO_2 levels at or just below atmospheric levels (approximately 0.03 to 0.04 %) and effectively removed excess CO_2 produced by the respiration of the fungus.

The greatest effect on aflatoxin accumulation was obtained at 100 ppm ethylene, although statistically significant differences were not observed between treatments containing 25 ppm, 70 ppm, and 100 ppm ethylene (Figure 2). These treatments reduced aflatoxin accumulation approximately 5 fold while 2 ppm and 10 ppm ethylene reduced aflatoxin accumulation by 2 and 3 fold, respectively. **Treatment had not discernable effect on mold growth as determined by visual inspection.** TLC confirmed ELISA data and demonstrated that accumulation of both B and G aflatoxins was inhibited to similar degrees (data not shown).

3.3. Effects of ethylene, CO_2 , and ethylene combined with CO_2 on aflatoxin accumulation in peanuts

Previously we observed additive effects of ethylene and CO_2 treatment on inhibition of aflatoxin accumulation in culture media (Roze et al. 2004). To analyze effects of combined gases on peanuts, we used the following treatments: air, 0.1% CO_2 , 2 ppm ethylene, 100 ppm ethylene, 0.1% CO_2 + 2 ppm ethylene, and 0.1% CO_2 + 100 ppm ethylene. Gas concentrations were measured on d 1, 2, 3, and 5 (not shown). As in the ethylene dose-response experiment, the CO_2 scrubber effectively maintained CO_2 levels at or slightly below atmospheric levels.

The largest effect on reduction of aflatoxin B₁ was observed with 100 ppm ethylene (Fig. 3). Treatment with CO_2 alone (0.1%) generated a similar inhibitory effect on aflatoxin accumulation. However, unlike the situation with culture media, co-treatment of peanuts with both ethylene (either 2 or 100 ppm) and CO_2 (0.1%) did not show a statistically significant inhibitory effect. TLC confirmed the ELISA data (not shown). **Treatment had not discernable effect on mold growth as determined by visual inspection**

Our goal is to identify novel, safe, practical, and effective means to reduce aflatoxin contamination in susceptible food and feed crops. We focused initial attention on the use of food-safe gases; an ideal gaseous compound for application in food would be inexpensive, easy to apply, would yield no residue, and would be effective at low concentrations. Ethylene is characterized by these properties. It also has been used effectively in a variety of agricultural applications for decades and was found to have no negative impact on the nutritive value of certain fruits (reviewed in Kays and Beaudry, 2002).

Using our model system, ethylene reduced both aflatoxin B and G accumulation in *A. parasiticus* grown on peanuts with an efficacy at or above that observed when the fungus was grown on laboratory media. Growth of *A. parasiticus* (like other fungi) is associated with production of CO₂ and other volatiles, and uptake of oxygen. To minimize these effects, our experiments were performed in a flow through system that allowed us to isolate the effects of ethylene from effects of changes in concentration of other gases in the system.

Roze et al. (2004) demonstrated that *nor-1* gene expression was decreased to non detectable levels when the fungus was treated with 0.6, 5.6, or 12.6 ppm ethylene on a defined laboratory medium suggesting that ethylene affects aflatoxin biosynthesis at the level of gene transcription. Treatment of the fungus with 1-methyl-cyclopropene (MCP), an inhibitor of the ethylene response in plants, reversed the inhibitory effect of ethylene (Roze et al. 2004) prompting the hypothesis that ethylene affects toxin synthesis via an ethylene receptor that functions as part of a signal transduction pathway.

Flaishman and Kolattukudy (1994) observed that *Colletotrichum* responds to ethylene produced either by climacteric fruits or to ethylene produced by the ethylene-generating compound, ethephon. In *Colletotrichum*, ethylene induces appresoria production in the fungus, a structure that plays a role during the infection process to enable the fungus to obtain nutrients from the host. Amagai (1987) indicated that ethylene induced macrocyst formation in the slime mold *Dictyostelium mucoroides*. These studies suggest that the ability of *A. parasiticus* to synthesize and respond to ethylene may have a biological basis in the life cycle of the fungus. **Based on the data reported in our current study, one likely biological response is regulation of the timing and level of aflatoxin synthesis.**

CO₂ has previously been reported to inhibit growth and aflatoxin synthesis in *A. parasiticus* (reviewed in Zaika and Buchanan, 1987) although the mechanism is still unknown. In bacteria, CO₂ has negative effects on various biochemical pathways and this results in the anti-microbial properties attributed to CO₂ (Daniels et al. 1985).

In summary, these studies demonstrate that ethylene and CO₂ show promise in the battle to control aflatoxin contamination. Future studies will measure efficacy using a variety of *A. parasiticus* and *A. flavus* isolates, will include other aflatoxin susceptible crops (corn, cotton, treenuts), and will scale-up the quantity of stored plant material.

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References

- Amagai A. Regulation of the developmental modes of *Dictyostelium mucoroides* by cAMP and ethylene. *Differentiation* 1987;36:111–115. [PubMed: 2834253]
- AOAC Official Methods 990.34. Aflatoxins B₁, B₂, and G₁ in Corn, Cottonseed, Peanuts, and Peanut Butter. In: Horwitz, W., editor. *Official Methods of Analysis of AOAC International*. II. AOAC International; Gaithersburg, MA: p. 7-8.

- Cary, JW.; Linz, JE.; Bhatnagar, D. Microbial foodborne diseases: mechanisms of pathogenesis and toxin synthesis. Technomic Publishing Co; Lancaster, PA: 2000. Aflatoxins: Biological significance and regulation of biosynthesis; p. 317-361.
- Chiou CH, Miller M, Wilson DL, Trail F, Linz JE. Chromosomal location plays a role in regulation of aflatoxin gene expression in *Aspergillus parasiticus*. Appl Environ Microbiol 2002;68:306-315. [PubMed: 11772640]
- Clevstrom G, Ljunggren H, Tegelstrom S, Tideman K. Production of aflatoxin by an *Aspergillus flavus* isolate cultured under a limited oxygen supply. Appl Environ Microbiol 1983;46:400-405. [PubMed: 6414371]
- Council for Agricultural Science and Technology. Mycotoxins: Risks in Plant, Animal, and Human Systems. Task Force Report 139; Ames, IA: 2003.
- Daniels JA, Krishnamurthi R, Rizvi SSH. A review of effects of carbon dioxide on microbial growth and food quality. J Food Prot 1985;48:532-537.
- Flaishman MA, Kollatukudy PE. Timing of fungal invasion using host's ripening hormone as a signal. Proc Nat Acad Sci 1994;91:6579-6583. [PubMed: 11607484]
- Kays, SJ.; Beaudry, RM. Techniques for inducing ethylene effects. In: Reid, MS., editor. Symposium on Manipulation of Ethylene Responses in Horticulture. XXII International Horticultural Congress; Davis, CA: p. 77
- Miller, M. PhD Thesis. Michigan State University; East Lansing, MI: 2003. Transcriptional regulation of the *Aspergillus parasiticus* aflatoxin biosynthetic pathway gene *nor-1*.
- Pestka JJ, Gaur PK, Chu FS. Quantitation of aflatoxin B₁ and aflatoxin B₁ antibody by an enzyme-linked immunosorbent microassay. Appl Environ Microbiol 1980;40:1027-1031. [PubMed: 7006504]
- Roze LV, Calvo AM, Gunterus A, Beaudry R, Kall M, Linz JE. Ethylene modulates development and Toxin biosynthesis in *Aspergillus* possibly via an ethylene sensor-mediated signaling pathway. J Food Prot 2004;67:438-447. [PubMed: 15035355]
- Sharma A, Padwal-Desai SR, Nadkarni GB. Possible implications of reciprocity between ethylene and aflatoxin biogenesis in *Aspergillus flavus* and *Aspergillus parasiticus*. Appl Environ Microbiol 1985;49:79-82. [PubMed: 3919643]
- Skory CD, Chang P-K, Linz JE. Regulated expression of the *nor-1* and *ver-1* genes associated with aflatoxin biosynthesis. Appl Env Microbiol 1993;59:1642-1646. [PubMed: 8517755]
- Trail F, Mahanti N, Rarick M, Mehig R, Liang SH, Zhou R, Linz JE. A physical and transcriptional map of an aflatoxin gene cluster in *Aspergillus parasiticus* and the functional disruption of a gene involved early in the aflatoxin pathway. Appl Environ Microbiol 1995;61:2665-2673. [PubMed: 7618880]
- Zaika LL, Buchanan RL. Review of compounds affecting the biosynthesis of aflatoxins. J Food Prot 1987;50:691-708.

Figure 1.

Effect of inoculum size on aflatoxin accumulation by *A. parasiticus*. Each inoculum (10^5 , 10^4 , 10^3 , 10^2 conidiospores/g peanuts) was grown on 4 grams of raw peanuts in the dark for 5 days at 30°C. Aflatoxins from three replicate samples were extracted and analyzed by ELISA.

Aflatoxin B1 is reported as µg toxin/per gram of peanuts. Bars represent standard error of the means for each treatment. a is statistically different than b ($p < 0.05$).

Figure 2.

Effect of ethylene concentration on aflatoxin accumulation by *A. parasiticus* grown on peanuts. *A. parasiticus* D8D3 (10^3 conidioisporos/g peanuts) was grown on Georgia Green peanuts in the flow through system for 5 days at 30°C in the dark. Aflatoxins from three replicate samples were extracted and analyzed by ELISA. Bars represent standard error of the means for each treatment. a is statistically different than b ($P < 0.05$).

Figure 3.

Effect of CO₂, ethylene, and a mixture of ethylene and CO₂ on aflatoxin accumulation by *A. parasiticus* grown on Georgia Green peanuts. *A. parasiticus* D8D3 (10³ conidioispor/g peanuts) was grown on raw peanuts in the flow through system for 5 days at 30°C under dark. Aflatoxins from three replicate samples were extracted and analyzed by ELISA as described in Methods. Bars represent standard error of the means for each treatment. a is statistically different than b (P<0.05).

Table 1
Gas concentrations in ethylene dose response experiment. A. CO₂ concentrations. B. Ethylene concentrations. ND denotes no detectable peaks.

Ethylene Treatment	Measured CO ₂ concentration (%)							
	Day							
	0		1		2		5	
Air	In-port	ND	In-port	ND	In-port	ND	In-port	ND
2 ppm	Out-port	ND	Out-port	ND	Out-port	ND	Out-port	ND
10 ppm	In-port	ND	In-port	ND	In-port	ND	In-port	ND
25 ppm	Out-port	ND	Out-port	ND	Out-port	ND	Out-port	ND
70 ppm	In-port	ND	In-port	ND	In-port	ND	In-port	ND
100 ppm	Out-port	ND	Out-port	ND	Out-port	ND	Out-port	ND
A.	In-port	ND	In-port	ND	In-port	ND	In-port	ND
Out-port	ND	ND	ND	ND	ND	ND	ND	ND
B.								
Ethylene Treatment	Measured ethylene concentration (ppm)							
	Day							
	0		1		2		5	
Air	In-port	ND	In-port	ND	In-port	ND	In-port	ND
2 ppm	Out-port	ND	Out-port	ND	Out-port	ND	Out-port	ND
10 ppm	In-port	1.5	In-port	2.08	In-port	1.92	In-port	1.83
25 ppm	Out-port	5.80	Out-port	9.17	Out-port	8.40	Out-port	8.35
70 ppm	In-port	22.15	In-port	31.80	In-port	30.10	In-port	26.03
100 ppm	Out-port	63.70	Out-port	83.92	Out-port	74.03	Out-port	71.61
B.	In-port	88.30	In-port	111.90	In-port	107.70	In-port	108.52
Out-port	ND	ND	ND	ND	ND	ND	ND	ND

* , sampling location.