

RECENT DEVELOPMENTS IN AGRI-FOOD APPLICATIONS OF OZONE

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Summary

The versatility of ozone - in that it is both a strong disinfectant and a strong oxidant that can be applied in the gas and/or liquid phases - plus its fundamental cleanliness and lack of negative environmental impact, means that it is well-suited for many applications in the agriculture and food processing industries. In the gas phase, this versatile chemical can provide an atmosphere for processing, packaging and storage of many agricultural products (fruits, vegetables, meats, poultry, etc.), thereby minimizing the proliferation of spoilage microorganisms and controlling many molds and odors. When applied in the aqueous phase, ozone can be used to treat plant influent water, recycled process water, and wastewaters. Aqueous solutions of ozone in water are used commercially for spray washing of many different agricultural and food products and for sanitizing processing equipment and for plant wash-downs. When combined with ultraviolet radiation and electrolyzed water, ozone provides a new technique for replacing pesticide and insecticide sprays on growing agricultural crops. Ozone plus UV radiation, electrolyzed water and ultrasound allows close to sterilization of foods during processing and packaging, thus providing significant extensions of shelf-lives of fresh and/or processed foods. In turn, this

combination of advanced technologies has created a new concept in restaurants, in which pre-prepared and specially packaged uncooked meals are created in a central processing plant for distribution to restaurants and large institutions where microwave ovens have replaced gourmet chefs and kitchens.

In 2001 the U.S. Food and Drug Administration (FDA) formally approved ozone as an Antimicrobial Agent for direct contact with foods. Since that approval, many commercial applications of ozone in treating many foods have developed.

1. Historical Developments

Although ozone was discovered and named by Schönbein in 1840, applications for food treatment did not develop until much later. A brochure published about 1920 by the German firm Mannesmann-Demag reported studies conducted by the German Imperial Ministry of Health on the microbiological effects of ozone (Mannesmann Demag, 1920 citing Heise, 1917) and led to German approval of ozone for meat storage lockers. An ozone concentration of ca 3 mg m^{-3} (ppm) applied every 3-4 hours was found to be sufficient to destroy more than 95% of individual spores located on the surface of culture media. Later, Kuprianoff (1953) confirmed that the first known use of ozone as a food preservation agent (in the gas phase) was in Köln (Cologne), Germany, for cold storage of meats in 1909.

Hartman (1924) discussed cold storage of eggs in ozone-containing atmospheres. Gane (1933, 1936) found that exposure of ripening bananas to 1.5 and 7 ppm of ozone caused no changes in the rate of banana respiration, and was effective in retarding the rate of banana ripening, but only if the fruit was not within a few days of its period of rapid ripening. Kaess (1936) reported that daily exposure of meats to ozone concentrations of 10 mg/m^{-3} for three hours (at 3°C and 90% relative humidity = RH) extended the lag phase of bacterial development; however, after about 3 days of this treatment discoloration of the freshly cut meat occurred. Klotz (1936) found ozone to be ineffective against the microbes which cause decay of citrus fruits. Salmon and LeGall (1936) found that the storage life of freshly caught fish could be nearly doubled (extended 5 days) by storing them under ice made from ozonized sea water.

Wiberg (1951) noted that "ozone has been used technically, for example in air improvement and sterilization in theaters, schools, hospitals, cold rooms, meat packing houses, and breweries". Heruth et al. (1985) cited several early studies of the uses of ozone atmospheres in the storage of pears, cauliflower, potatoes (ozone entirely stopped the growth of *Phytophthora infestans*) and meat. In the latter case, while the germicidal effect of ozone was restricted to the surface of the meat, "the storage life of beef in a refrigerated state can be increased by to 30 to 40 percent if the beef is kept in an atmosphere of 10 to $20 \text{ mg (O}_3\text{) m}^{-3}$ ".

In Japan, thanks to the pioneering scientific research studies of Shigezo Naitou and his colleagues at the Aichi Industrial Technology Institute, Food Research Center in Nagoya, the introduction of ozone treatments to food manufacturing plants began in 1982. Japanese food processing industries were looking for feasible ways of eliminating or greatly reducing

levels of microorganisms in or on food products and thus producing safer foods. More than 200 scientific studies have been published by Naitou and his associates as of this writing on many aspects of ozone applied to different food processing applications. Unfortunately, most of these papers are published in Japanese, and therefore are not widely read outside of Japan.

In 1996, Japan and then Australia approved the use of ozone for food applications (EPRI, 1997). In June, 2001, the U.S. Food & Drug Administration, in response to a Food Additive Petition submitted by the Electric Power Research Institute, approved the use of ozone as an Antimicrobial Agent for direct contact with foods of all types U.S. FDA, 2001). In December, 2001, the U.S. Department of Agriculture (USDA) also approved ozone as an Antimicrobial Agent for direct contact with meats, poultry and other agricultural items regulated by the USDA (USDA FSIS, 2001).

Several informative reviews on the subject of ozone and its many applications in the food and agricultural industry are available on this general subject (Rice *et al.*, 1982; EPRI, 1997; EPRI, 2000a; Kim *et al.*, 1999, 2003; Smilanick, 2003; Global Energy Partners, 2004).

1.1. A Word about Units of Ozone Measurement

Smilanick (2003) points out the following important facts about ozone measurements. The units that express concentration of ozone in air and water typically are parts per million (ppm). In water, ppm (mg L^{-1}) is a unit of weight/volume (mg mL^{-1}), while in air, ppm is a unit of volume/volume ($\mu\text{L L}^{-1}$). One liter of water containing a concentration of 1 ppm ozone has many ozone molecules, while one liter of air containing a concentration of 1 ppm ozone contains relatively few. When calculated, equal volumes of 1 ppm of ozone in water contain 500,000 times as many molecules as is the case of 1 ppm ozone in air.

2. Regulatory History and Status of Ozone in Agri-Foods

Prior to mid-1937, there were few or no commercial applications of ozone in food processing or treatment in the United States. The reason was entirely regulatory in nature, and had nothing at all to do with the technology of ozone. The regulatory control over the use of ozone is the Federal Food, Drug and Cosmetic Act, passed in the late 1950s and under which the Food and Drug Administration is required to operate. This Act defines any material that comes in contact with food to be a food additive, which must be approved by the FDA prior to use. The U.S. FDA regulates all foods except meats, poultry and egg products. These last three food categories are regulated by the U.S. Department of Agriculture. However, USDA will not allow the use of any food additive on its regulated foodstuffs unless that additive has received prior FDA approval.

In the early 1980s, the International Bottled Water Association successfully petitioned the FDA to affirm that the application of ozone to disinfect bottled water under specified conditions is GRAS (Generally Recognized As Safe). The conditions included a maximum dosage of ozone of 0.4 mg/L over 4 minutes contact time, and that the water to be treated must already meet the potable water requirements of the U.S. Environmental Protecting

Agency. The FDA approved IBWA's petition for ozone in bottled water, and in 1982 published in the Code of Federal Regulations a formal FDA regulation affirming GRAS Status for use of ozone (U.S. FDA, 1982). Later, the FDA also approved the use of ozone as a sanitizing agent for bottled water treatment lines, under a similar GRAS petition.

Unfortunately, the GRAS approval for ozone disinfection of bottled water in 1982 contained the additional statement [21 CFR 184.1(b)(2)]: All other food additive applications for ozone must be the subject of appropriate Food Additive Petitions. This statement effectively mandates the filing of Food Additive Petitions in order to gain FDA approval for other uses of ozone in direct contact with other foods (FDA has defined bottled water as a food).

2.1. The 1997 EPRI GRAS Declaration

In June 1997, an Expert Panel of Food Scientists convened by the Electric Power Research Institute (EPRI, 1997) concluded the following:

The available information supports the safety of ozone when used as a food disinfectant or sanitizer, and further, that the available information supports a GRAS classification of ozone as a disinfectant or sanitizer for foods when used at levels and by methods of application consistent with good manufacturing practices.

2.2. FDA and USDA Approvals of Ozone - 2001

EPRI's GRAS affirmation gave a clear green light to food processors to test and use ozone for a variety of food processing applications. Nevertheless the lack of specific regulatory approval for ozone by the FDA continued to disturb many food processors and continued to slow the broader acceptance of ozone in the food industry.

FDA recognized this, and also recognized that most applications for ozone in food treatment involve antimicrobial properties of ozone. However, the statement in the 1982 GRAS approval for ozone in bottled water disinfection, All other food applications for ozone must be the subject of appropriate food additive petition(s), continued to impede the commercial development of ozone for food processing applications in the United States.

Consequently, in mid-1999, the FDA suggested that a single food additive petition (FAP) that would provide specific data showing the antimicrobial properties of ozone in a number of food processing applications could be reviewed quickly, and if approved, would overcome the requirement of the 1982 GRAS regulation regarding other food uses for ozone. EPRI, with considerable support from several interested food processing organizations, developed such a FAP and formally filed it with the FDA in August 2000 (EPRI, 2000). FDA approval of this FAP was published June 26, 2001 in the Federal Register (U.S. FDA, 2001).

In December 2001, the USDA's Food Safety Inspection Service (FSIS) approved ozone for use on meat and poultry products, including treatment of ready-to-eat meat and poultry products just prior to packaging (USDA FSIS, 2001).

Formal regulatory approval by the FDA and by the USDA/FSIS for the use of ozone as an Antimicrobial Agent in direct contact with foods cleared away the regulatory hurdle that had impeded application of ozone to foods in the United States, and reassures food processing firms wishing to improve the qualities of their products by approaches involving ozone.

2.3. A Recommended Ozone Evaluation Protocol

Rice and Graham (2002a,b) recommended that the following steps be taken whenever a food processor becomes seriously interested in testing ozone for microorganism control:

1. Select food item or process to be treated with ozone.
2. Identify specific spoilage microorganisms that will be involved (not all foods are spoiled by the same microorganisms).
3. Establish ozone or process performance required (how many logs of inactivation of the targeted microorganisms are required; how much extension of shelf life is required; how clean must a recycled process water be, etc.).
4. Check published literature - start with the Food Additive Petition. If insufficient data are available (as expected), then conduct laboratory studies on those microorganisms to determine ozone dosages and conditions for their inactivation.
5. Apply conditions to food/process and confirm results.
6. Determine cost-effectiveness.

In the Food Additive Petition submitted to the FDA, is a table showing ozone dosage/exposure data reported in specific studies. These data are useful as guidance to the prospective ozone user, with the caution that the user must determine the minimum ozone dosage/exposure level necessary to accomplish the intended effect (Good Manufacturing Practice). At the same time, the prospective user should determine the maximum ozone dosage/exposure level that will cause damage to the agricultural or food product being treated. If ozone is evaluated in this manner for each potential application, the user will have a comfortable operating range of ozone dosage/exposure. This will allow the user to specify ozone treatment conditions that will always ensure attaining ozone's intended effect(s) while also ensuring that excess ozone sufficient to damage the food product will be avoided.

3. Agricultural Uses for Ozone (Parmenter *et al.*, 2004)

Because of its versatility (ability to be applied in the gas and/or aqueous phases) plus its powerful oxidizing and disinfecting actions, ozone has found applications in many agricultural areas. Some of its uses include treatment of irrigation water, livestock and poultry drinking water and wastewater, soil treatment, weed control, odor control in animal housing, dairies, slaughterhouses and fish processing plants, and pest control during storage of grain, livestock and poultry feed. A sequential combination of electrolyzed water, aqueous ozone, and ultraviolet radiation is applied to growing crops. The consequence of these treatments is to stimulate chemicals (such as salicylic acid, jasmonic acid, ethylene and the like) within the growing plants that impart a Systemic Acquired Resistance to insects and microorganisms for a significant period of time. In turn, such PhytO3 Tech

treatments (a recent Swiss development) eliminate the need for spraying insecticides and pesticides during their growing periods (Steffen and Rice, 1998a).

3.1. Livestock and Poultry Drinking Water

Ozone can be used effectively to treat livestock and poultry drinking water. It is generated onsite and then injected into the feed water by one of several commercially available techniques. Ozone acts as an antimicrobial agent against bacteria, viruses, and parasites, and oxidizes organic substances and coagulates suspended solids. Ozonation sometimes is combined with filtration to remove the oxidized contaminants from the water supply and reduce turbidity levels. Ozonated drinking water leads to improved health, resulting in greater feed efficiency, and higher productivity in animals.

The use of ozone for purifying livestock and poultry water can yield impressive results in terms animal health and survival rates. Healthier animals often are more productive and achieve greater weights. For example, ozone treatment systems for drinking water have resulted in increased milk production by dairy cows and increased egg production by hens. In addition, several poultry farms have seen slight gains in poultry weight since installing ozone systems.

Table 1 summarizes a specific case study in which drinking water for dairy cows was ozonated (Rice, 2003a). Prior to installation of the ozone system, the dairy cows were given well water with impurities such as high levels of hydrogen sulfide to drink. After ozone treatment, hydrogen sulfide levels were reduced to zero, and the odor and levels of other impurities, such as iron, manganese, and organic load, were reduced to acceptable levels. Milk production increased a sizeable amount thanks to ozone - from an average of 62 lb/day/cow prior to ozone, to 88 lb/day/cow soon after ozone, to 100 lb/day/cow after several months of ozone treatment.

Installation Location	Application	Well Water Problems	Results After Ozone
Dairy Farm, Paulding, OH	treating well water used for dairy cattle drinking water	odoriferous; contained H ₂ S, iron, manganese + organic load	Reduced H ₂ S levels to zero; Reduced Fe, Mn & organic load to acceptable levels; Increased milk production from 62 lb/day/cow before ozone to 88 lb/day/cow soon after ozone, to 100 lb/day/cow after several months

Source: R.G. Rice, AOzone and Ozone/UV in Sanitation and Food Production, May 28, 2003, Powerpoint presentation

Table 1. Ozonation of Drinking Water for Dairy Cattle - Summary of a Case Study

Performance data for poultry given ozonated drinking water show positive results as well. Case study findings from three poultry farms show that water quality was improved after conversion to ozone purified water (EarthSafe Ozone, Inc., ~2004). Specifically, iron levels dropped from a high of 3.8 ppm to less than 0.3 ppm, manganese levels dropped from a high of 0.60 ppm to less than 0.05 ppm, and total bacteria levels dropped from a high of greater than 100 ppm to less than 2 ppm. Because of the cleaner water, survival rates and average bird weights increased, although the increases were very modest. The average bird weight increased by about 2.5 % across the three farms, and the percentage of live birds increased from an average of 96% to 97%. Healthier birds equate to greater profits for poultry producers.

A similar study by the Agriculture and Food Technology Alliance of Global Energy Partners, Inc. (2004) in which poultry drinking water and flock data were compared before and after ozonation and filtration of the water showed that poultry production data and mortality were not greatly affected by ozonation. However, the ozonation-filtration system did decrease variable water costs as well as reduce fouling of emitters (GEP, 2004).

3.2. Livestock and Poultry Wastewater Treatment (GEP, 2004)

Ozone can mitigate some of the concerns associated with livestock and poultry wastewater, including pathogens in wastewater streams and lagoon water, odors, and costly water use and treatment. By reducing odors and pathogens, ozone can improve the livestock and poultry living environment and the health and safety of farm personnel.

Ozone can be used to treat lagoon waters by pumping into the top foot or so of the lagoon's surface to reduce pathogen levels and odors associated with the lagoon water. Wastewater exiting barns and animal operations also can be treated prior to entry into lagoons to keep odors and pathogen levels lower in lagoons. In some livestock and poultry applications, wastewaters can be reused if treated with ozone. For example, water used to mist and water cattle can be recycled and reprocessed with ozone in order to lower water consumption and wastewater treatment costs.

3.2.1. Performance Results

Watkins et al. (1997) have shown that treatment of waste with concentrations of 1-3 g L⁻¹ of ozone destroys phenolics, indolics and other metabolites that are produced by bacteria in swine manure and cause odor. They also found that, for the concentrations tested, ozone reduced but did not eliminate pathogenic microorganisms. Ozone's efficacy at a given concentration is affected by the contaminant loading and other characteristics of the wastewater such as pH. Lightly loaded wastewater will be cleaned more thoroughly than heavily loaded wastewater for a given concentration of ozone.

Various ozone manufacturers, livestock and poultry producers, and universities are testing the use of ozone for animal wastewater treatment with favorable results (Vansickle, 1999; TriO3 Industries, Inc., 2004). Odor reductions are particularly encouraging.

3.3. Irrigation Water Treatment

Ozone has been shown to work well for smaller irrigation applications such as in drip systems and for hydroponic farming. However, there is not much definitive information currently available for large-scale irrigation systems.

Ozone can improve water quality, help enable water recycling and reuse, and can clean irrigation lines and emitters. Some researchers believe it may also increase penetration of irrigation applied to crops. Ozone currently is most applicable to small-scale irrigation systems. In destroying microorganisms that affect crop health, ozone also can reduce organic loadings, hydrogen sulfide levels, and stabilize pH. It is beneficial over chlorine for water treatment in that it does not produce trihalomethanes (THMs) and it is generated on-site. For agricultural production, ozone is advantageous because it is generated on-site and can be used to treat water supplies without the worry of chemical storage and handling. It is relatively safe to use as long as measures are taken to prevent exposure to toxic levels.

One example of employing ozone for treating irrigation water involves a case study with hydroponic tomatoes (Rice, 2003a). In this study, ozone treatment was used to improve the quality of well water for irrigating the tomatoes. Prior to ozone treatment, the well water had a hydrogen sulfide concentration of 60 ppm and a pH of 7.3. In addition, the rejection rate of tomatoes was 40% due to blossom end rot. Ozone treatment reduced the hydrogen sulfide concentration to 0 ppm, lowered the pH to 7.04 by reducing organic load and producing H₂SO₄, and reduced the rejection rate to less than 3%. The total tomato yield increased by more than 300%. By stabilizing the pH, the fertilizer consumption also decreased by 25%. Because of the outstanding benefits, the payback period for the ozone system ended up being less than 6 months.

The application of ozone for improving irrigation penetration and cleaning tubes and emitters is relatively new. Further research is required to evaluate performance. Ozone is being investigated as a potential method for reducing levels of microorganisms and other impurities that can clog or contaminate irrigation pipes and emitters (Natl. Org. Standards Board, 2002).

Stong *et al.* (2001) also conducted laboratory experiments to determine the effects of ozone on soil physical and chemical properties. Results showed that repeated application of ozone to soils led to changes in hydraulic conductivity and reduction in soil hardness as a result of ozonation. The leachate from the ozonated soils had lower pH, higher electrolyte concentrations, and appreciably more soluble organic matter, nitrate, and ammonium ion compared to the air-treated soils.

The control of corky root on tomato caused by *Pyrenochaeta lycopersici* generally has been based on soil fumigation with methyl bromide. The use of this product has been banned since 2005 because of its heavy environmental effect. Ciccacese *et al.* (2007a) described a research project to determine and demonstrate the efficacy of soil treatment with ozone in the control of corky root on tomatoes in a greenhouse and in an open field, as an alternative to methyl bromide.

In the trial carried out in the greenhouse, ozone, produced by an ozone generator, was applied to the soil by drip sub-irrigation (20 cm) using drip lines equipped with water emitters (4 L h^{-1}) every 30 cm. The ozone was applied as gas in moistened soil or dissolved in irrigation water. In the open field trial, the ozone was applied dissolving it in irrigation water by drip sub-irrigation with drip lines set on the mulched soil. Plots were distributed in a randomized block design with four replications for each treatment. Untreated plots were used as controls. In both trials the severity of corky root was significantly reduced in ozone-treated plots compared to controls, either on main and secondary roots. In the open field trial a significant difference was found between the two ozone treatments, as the severity of corky root in plots treated by drip irrigation was higher than sub-irrigation treated plots. In the two experiments all ozone treatments significantly increased tomato marketable yields compared to untreated controls (Ciccarese *et al.*, 2007a).

3.4. Soil Fumigation with Ozone

Soils contain items such as weeds, insects, nematodes and fungi. All of these affect plant health and yields. One common approach to overcoming these problems is to fumigate the soils with methyl bromide. However, methyl bromide damages the stratospheric ozone layer and is a suspected carcinogen. Therefore it has been phased out for agricultural applications in industrialized countries. Environmentally friendly alternatives to methyl bromide and other hazardous chemical agents are needed, and ozone is one serious candidate.

The main advantages of ozone are pathogen destruction, possible increase in nutrient availability, absence of residue, on-site production, thus eliminating storage, handling, and disposal of hazardous chemicals and chemical containers. Nor is ozone regulated as a pesticide by the U.S. Environmental Protection Agency under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Research into the use of ozone as a soil fumigant is still in its early stages. Much of the work through 2004 was conducted by Pryor (1996; 1997; 2001b). In 1998, Pryor worked with EPRI and the California Energy Commission's Public Interest Research Program (PIER) to conduct field trials of ozone treatment for a variety of crop and soil types under a range of climatic conditions (EPRI, 1999). The types of crops fumigated with ozone included tomatoes, carrots, strawberries, sugar beets, broccoli, prunes, sweet potatoes, and peaches. Results showed that application of 50 to 400 lbs of ozone per acre through either drip tube emitters (for row crops) or probes (for orchard replants) generally reduced negative impacts from soil pathogens and increased plant yields. The results further indicate that the ozone may increase nutrient availability to the plants due to its oxidation of soil organics; however, more work is required to verify this effect. Pryor has since extended his work to include the evaluation of ozone for weed control (Pryor, 2001a).

Studies have shown that ozone injection under plastic mulch may be capable of controlling weeds with multiple pre-plant applications of 2 lbs per acre (EPRI, 1999; Pryor, 2001a).

The status of ozone as a soil fumigant replacement for methyl bromide was presented by Stong and Amrhein (2002). Concerns exist that ozone will oxidize soil organic matter,

causing undesirable changes to physical and chemical soil properties. The authors conducted laboratory studies to determine the changes to physical and chemical properties of nine California agricultural soils where ozone was tested as a fumigant. Changes in clay dispersion, swelling, saturated hydraulic conductivity, and hardness were measured as a function of repeated ozone treatment, and analyses were conducted of the chemical composition of leachates from ozone- and air-treated soils. These studies showed that ozonation of the soil reduced the pH in all soils, and reduced hydraulic conductivity in soils with low clay content and increased the hydraulic conductivity in high clay soils. Organic matter was degraded, shown by increased dissolved organic carbon and decreased soil organic carbon content. Ozone also affects SOM-metal complexes in soil (SOM = soil organic matter), shown by increased concentrations of Al, Fe, Mn, Na, Ca, Ba, Sr, Li, and K in leachates of ozonated soils. Ozonation increased chelation by SOM, concentrations of ammonia, nitrate, and phosphorus in leachates of ozonated soils; and cation exchange capacity. These studies suggest that under specific guidelines, ozone can be used in soils with any SOM content, but in every case field studies should be conducted to quantify the N and P additions so fertilization management adjustment can be made. Due to the ability of ozone to mobilize metals, it should not be used in soils that have large concentrations of dangerous heavy metals. Crop management should include return of crop residue to the soil to replenish solubilized SOM loss with ozonation.

3.5. The PhytO3 Ozone-UV-Based Residual-Free Crop Protection Technology

In 2005, the Swiss agricultural engineer Steffen announced the development of a system that is designed to treat growing crops with materials that are not chemical in nature, leave no residues to harm the plants or to find their way into the environment, but stimulate the development, inside the plants, of chemicals that cause the plant to resist attacks from insects and microorganisms. The phenomenon has been termed Systemic Acquired Resistance (SAR) (Steffen, 2005a,b, Steffen and Rice, 2008a).

Mounted on a tractor are the three key materials that are applied sequentially, but almost simultaneously. First, a solution of electrolyzed water is sprayed onto the plants. Immediately thereafter is sprayed a water solution containing up to 8 mg/L of dissolved ozone, and this is followed by exposure to UV radiation - see Figure 1.

Steffen and Rice (2005, 2008a) reviewed what is known about ozone, UV radiation and active oxygen species and their roles in triggering the development of SAR inside growing plants, and then hypothesized that what may be occurring with the PhytO3 Tech system is a sophisticated method of inserting low levels of hydrogen peroxide into the plants, which then quickly decomposes into other active oxygen species, known to trigger SAR. The initial spray of electrolyzed water serves to shock the plants, and causes their stomata (breathing pores on leaf undersides) to open. The aqueous solution of ozone follows, and some of this is absorbed by the plants, entering the stomata. The immediate application of UV-C radiation then reacts with aqueous ozone to produce hydrogen peroxide (Peyton and Glaze, 1988), which in turn is destroyed by more UV radiation, producing active oxygen species (including hydroxyl and oxygen free radicals).



Figure 1. The PhytO3 Tech boom sprayer (Steffen, 2005)

Regardless of the specific mechanisms involved, the significant point is that the PhytO3 Tech procedure does in fact stimulate SAR in growing plants, thereby eliminating the necessity for sprays of chemical insecticides and pesticides (Steffen and Rice, 2005, 2007a,b, 2008a). Since chemical sprays are eliminated, there are significant cost savings to the farmer who uses PhytO3 Tech, and the return on investment is essentially one-crop period in the first year (Steffen and Rice, 2005, 2007a,b, 2008a).

Chan *et al.* (2007b) described the responses of five species of blade-leaf growing vegetables (Choi Sum, Pak Choi (big), Pak Choi, Mustard green head, and Chinese spinach) irrigated with two levels of ozone-containing water (low level: 0.5 mg L^{-1} ; high level: 1.5 mg L^{-1}). The effects on leaf area, fresh weight, chlorophyll content, antioxidative enzymes and auxins were studied. Leaf area had positive responses under low level ozone-containing water treatment, while the effect was less prominent after high ozone-containing water treatment.

Irrigation by low level ozone-containing water for one month increased the fresh weight of Pak Choi and Chinese spinach, while negative effect was found in high level ozone-containing water treatment. Compared with well water conditions, there is no significantly change in chlorophyll content in most species in both levels of ozone treatments.

The activity of superoxide dismutase (SOD) was significantly stimulated by low level ozone-containing water, while catalase (CAT) did not show significant changes except in Chinese spinach. Glutathione (GSH) and ascorbic acid (AsA) contents were more or less increased, along with accumulated H_2O_2 induced by increasing SOD. Increasing Abscisic acid (ABA) combined with increasing SOD indicates oxidative stress on plants. No tendency in ABA change with elevating ozone concentration was found. The level of indole-3-acetic acid (IAA) had different changes in different species, which confirmed that the effect of ozone-containing water differed on the growth of different plants and growth stages.

It was concluded by Chan *et al.* (2007b) that using low level ozone-containing water irrigation can induce positive effects on vegetables under greenhouse conditions, while high ozone-containing water treatment may lead to a strong oxidation stress on vegetables; adverse effects were observed on antioxidant and fresh weight.

4. Food Storage Applications for Ozone (Parmenter *et al.*, 2004)

Chemical pesticides are widely used in food storage to control insects, fungi, rodents, and other pests. These pests can damage food supplies in a number of ways. For example, insects destroy stored crops by eating them and defecating on them. Defecation in turn enables fungal growth. Certain types of fungi are particularly problematic. For example, *Fusarium* and *Aspergillus* produce pathogenic mycotoxins that can harm animals or humans. Fungal growth also can ruin the taste of stored crops, as odors from the fungi are readily absorbed by food. It is estimated that 5 to 10% of the world's food production is destroyed each year by insects; in some countries the loss may be as much as 50% (Purdue News, 2003).

For pest control during food storage, air containing gaseous ozone is introduced to the storage environment. One approach is to use high ozone concentrations for short durations; another approach is to use low ozone concentrations for extended periods of time.

Tests conducted with ozone to kill Indian meal moth and diapausing codling moth larvae in crop storage required 400-500 ppm of ozone for 4 to 5 hours (EPRI, 2002). Other tests with confused flour beetle and saw-toothed grain beetle achieved complete mortality with 5 ppm of ozone over a 3 to 5 day period (Mason *et al.*, 1996a). Similarly, continuous exposure to 5 ppm ozone inhibited surface growth of *A. flavus* and *F. moniliforme* and also eliminated sporulation and aflatoxin production (Mason *et al.*, 1996b). (Note that ozone can destroy toxin-producing microorganisms, but does not destroy the toxins already produced.)

Mendez *et al.* (2002) studied the fumigation of grains (rice, popcorn, soft and hard red winter wheats, soybeans and corn) with ozone. Storage bins containing these grains and a known number of insects were fumigated with ozone in two applications, and the quality of food products made with ozone-treated grain was evaluated. It was found that all species of insects were destroyed by ozone treatment, except immature weevils, who hide within kernels. Ozonated grains were found to have essentially the same features as non-ozonated grains, in terms of milling, making flour, and being used to make bread. No significant differences were found in the nutritional and metabolic values of amino acids and essential fatty acids in the ozone-treated grains.

Maier *et al.* (2005) conducted field trials at the pilot storage bin facility of the Purdue University Post-Harvest Education & Research Center in July 2003 with corn, at an organic rice storage facility in California in September 2003, and at a barley farm storage facility in Idaho in December 2003. The basic setup for ozonation at these sites consisted of generating ozone gas, introducing it at the top of the storage bin, drawing it to the plenum using a suction fan, and recirculating ozone back into the bin head space. An ozone concentration of 50 ppm in the plenum was attained and maintained for a period of three days to achieve insect mortality comparable to phosphine fumigation. The concept of two

phases of ozonation and the air flow rates needed to achieve the required treatment levels of 50 ppm were confirmed in field trials utilizing commercially available ozone generators.

The primary objective of these field trials was to determine the efficacy of ozonation to control insect pests and inhibit the growth of fungal spores, bacteria, and other pathogens. Inhibiting or eliminating fungal spores reduces production of mycotoxins that can be toxic to humans or mammals when ingested. Pre- and post-ozonation tests on grain samples also were conducted to determine the effect on end-use parameters like popping volume of popcorn, fatty acid and amino acid composition, milling characteristics of wheat and corn, and stickiness (adhesiveness) of rice. Results of these field trials demonstrated that ozonation not only effectively controls stored product pests (like maize weevil, red flour beetle, and Indian meal moth) but also addresses biosecurity issues of mycotoxins and pathogens by inhibiting or eliminating growth of fungal spores and pathogens without detrimental effect on the end-use quality of grain.

Kells *et al.* (2001, 2005) evaluated the efficacy of ozone as a fumigant to disinfect stored maize. Treatment of 8.9 tonnes (350 bu) of maize with 50 ppm ozone for 3 days resulted in 92-100% mortality of adult red flour beetle, *Tribolium castaneum* (Herbst); adult maize weevil, *Sitophilus zeamais* (Motsch); and larval Indian meal moth, *Plodia interpunctella* (Hübner); and reduced by 63% the contamination level of the fungus *Aspergillus parasiticus* (Spear) on the kernel surface.

Ozone fumigation of maize occurred in two distinct phases. Phase 1 was characterized by rapid degradation of the ozone and slow movement through the grain. In Phase 2, which occurred once the molecular sites responsible for ozone degradation became saturated, the ozone flowed freely through the grain with little degradation. The rate of saturation depended on the velocity of the ozone air stream.

The optimum apparent velocity for deep penetration of ozone into the grain mass was 0.03 m s^{-1} , a velocity that is achievable in typical storage structures with current fans and motors. At this velocity 80% of the ozone penetrated 2-m into the column of grain in 0.8 day during Phase 1, and within 5 days a stable degradation rate of $1 \text{ ppm } 0.3 \text{ m}^{-1}$ was achieved. Optimum velocity for Phase 2 was 0.02 m s^{-1} . At this velocity, 90% of the ozone dose penetrated 1.7-m in less than 0.5 day. These data demonstrate the potential efficacy of using ozone in managing stored maize and possibly other grains.

Leesch and Tebbets (2007) reported ozone fumigation studies of a number of insects associated with harvested fruits, testing ozone as an alternative to methyl bromide. Oranges exported from the USA sometimes contain over-wintering adult bean thrips, *Caliothrips fasciatus* (Pergande), in the navels of the oranges. Ozone was tested as a fumigant to rid the oranges of the adult thrips. Susceptibility was established on naked thrips and then on oranges with bean thrips in the navel. All adult thrips in the oranges were killed upon exposure to 2,500 ppm of ozone or 5,000 ppm for two hours. Large-scale tests showed that some adults survived at 2,500 ppm but not at 5000 ppm. Thus, small and large-scale tests showed that adult thrips could be controlled using ozone.

Leesch and Tebbets (2007) also tested ozone to control the stages of coffee berry borer, *Hypothenemus hampei* (Ferrari), (CBB) in coffee beans. The most tolerant stage of the borer was the egg. All stages of the CBB but eggs could be controlled with ozone in combination with slight vacuum.

A sometimes hitchhiker in table grapes being exported abroad from the USA is the black widow spider, *Lactrodectus hesperus*, Chamberlin & Ivie. Leesch and Tebbets (2007) initiated tests to determine if ozone could be used effectively to achieve 100% mortality of the spider in exported grapes. Ozone can eliminate the adult spiders effectively, whether or not CO₂ was added to the ozone.

Although ozone has been shown to be an effective pesticide in certain food storage applications and in laboratory environments, more research is necessary to further its development in this arena. For example, LD₅₀ and LD₁₀₀ values (at which 50% and 100%, respectively, of test microorganisms are destroyed) need to be developed. Moreover, it is important to note that ozone must have direct contact with insects and fungi in order to react with them. Therefore, techniques to ensure adequate mixing throughout the environment and exposure to surfaces where the pests reside are critical.

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Biography Sketch

Dr. Rip G. Rice is President/CEO of Rice International Consulting Enterprises, located in Sandy Spring, Maryland, USA, and specializing in ozone technologies, particularly with respect to Agri-Foods as well as water and wastewater treatment. He served as Ozone Resource to the Electric Power Research Institute Expert Panel which declared ozone to be Generally Recognized as Safe for food applications in 1997. He was the senior author of the Food Additive Petition submitted by the ERRI to the FDA to request approval of Ozone as an Antimicrobial Agent for direct contact with and treatment of all types of foods. This petition was approved June 26, 2001. Dr. Rice advises food processors and other interested parties about how to evaluate ozone for various purposes in the Agri-Food industries including the many combinations of ozone with other technologies (UV radiation, ultrasound, electrolyzed waters, modified air packaging, etc.), in both gas and aqueous phases, in food processing and handling plants. He is a frequent lecturer on these subjects at meetings of the International Ozone Association (IOA) and other trade associations.

Dr. Rice co-founded the International Ozone Institute (now the IOA) in 1973, was its President during 1982-1983, Editor-in-Chief of *Ozone: Science & Technology*, the Journal of the IOA and Editor-in-Chief of *Ozone News*, the newsletter of the IOA. He has authored more than 120 papers on various aspects of ozone technology, and has edited or co-edited 21 books, proceedings or monographs in ozone technology. In 1995, Dr. Rice received the Morton J. Klein Memorial Award for outstanding service to the IOA. He has chaired the IOA-Pan American Group Agri-Food Task Force since its formation in 2003.

In 1999, Dr. Rice co-founded the International Ultraviolet Association, and served as Editor-in-Chief of *IUVA News*, IUVA's newsletter, for its first several years.