OZONE AS A POST-HARVEST TREATMENT FOR POTATOES

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ABSTRACT

This project evaluated the potential for using ozone gas as post-harvest treatment for control of disease in stored potatoes. Ozone is a short-lived, highly reactive oxidizing agent with demonstrated potential to control disease-causing microorganisms. Preliminary trials showed that the atmospheric concentration of ozone obtained using commercial ozone generation equipment depended on the room size and reactive demands in the treatment area. Ozone applied to freshly harvested seed-grade potatoes up to 0-20 mg O_3/kg/hr for 1, 7 or 21 days had little significant effect on the incidence or severity of a range of diseases or tuber colour, but did increase tuber weight loss in a dosage-dependant manner. Continuous ozone application (1.9 mg O_3/kg/hr) accentuated weight loss by the stored crop. Application of ozone (10-20 mg O_3/kg/hr) and Purogene® (Chlorine dioxide; 200 ppm) for 1 day at the mid-point of the winter storage period had no effect on disease levels, skin colour or weight loss measured at the end of storage.

When tubers were inoculated with a range of pathogens (*Fusarium sambucinum, F. solani., Phytophthora infestans, Helminthosporium solani*), introduced at wound depths appropriate to each disease, disease levels typically increased, however ozone treatment (20 mg O_3/kg/hr) did not reduce development of any of these diseases.

In the absence of potential interference by the surrounding storage environment, pure cultures of *Fusarium spp., P. infestans* and sclerotia of *Sclerotinia sclerotiorum* were exposed to 45 mg O_3/plate/hr for 1 or 2 days. The ozone treatments had no effect on sporulation of any of the pathogens and did not reduce mycelial growth of *Fusarium spp*. Mycelial growth of *Phytophthora* and sclerotial germination of *Sclerotinia* were suppressed for the duration of the ozone treatment period, however normal growth resumed when the cultures were placed in ozone-free conditions.

Ozone treatments (up to 320 mg O_3/kg/hr for 2 days) did not reduce the sprouting ability of seed potatoes, however ozone treatments (~20 mg O_3/kg/hr for 1, 7 or 21 days) significantly reduced wound periderm thickness of treated potatoes. Treating seed potatoes with ozone (0 or 20 mg O_3/kg/hr for 1 or 2 days) prior to planting typically did not affect plant stand or yield, however under cool, wet conditions, ozone-treated seed potatoes produced poorer stands and yields relative to the controls.

Based on the results obtained for the range of treatments evaluated in this project, ozone appears to have limited potential as a disease management tool in stored potatoes.

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1. INTRODUCTION/RESEARCH OBJECTIVES

The control of storage diseases in potato (*Solanum tuberosum* L.) involves minimising the incidence of disease coming into storage, followed by rigorous control of storage conditions, and the application of chemical treatments designed to minimise disease development and spread. The control of post-harvest diseases by manipulation of storage conditions is complex and costly, relying on constant management of environmental conditions within high quality storage facilities. At one time, fungicides applied after harvest were relied upon to control post-harvest losses to fungal diseases in stored potatoes. However, use of these products has decreased over the last decade due to a loss of efficacy (Kawchuk *et al*., 1994; Holley and Kawchuk, 1996). Alternative effective control options for potato storage diseases are needed. Increasing concerns regarding pollution of the environment and food health and safety are also driving the quest for alternative methods of disease control.

Ozone is a potential alternative method for the control of storage diseases of potato and other horticultural crops. Ozone is a powerful oxidising agent with a demonstrated ability to reduce populations of bacteria and fungi in a diversity of use situations (Horvath *et al*., 1985; Kim *et al*., 1999b). However the potential for using ozone to control diseases during post-harvest management of horticulture crops such as potatoes is not fully understood. The rates of ozone (concentration and duration of application) required to control specific diseases, under varying conditions have not been clearly defined. Side-effects of ozone treatments on crop quality and food value are also largely unknown. The relative efficacy and cost efficiency of ozone treatments, as compared to current chemical treatments must also be evaluated.

The objectives of the project reported in this thesis were to:

1) evaluate the efficacy of ozone as a pre-storage and/or in-storage treatment for disease control in potatoes.
2) determine whether pre-storage and/or in-storage treatment with ozone affects tuber quality characteristics, such as skin colour and weight loss.

3) evaluate the effect of ozone on physiological processes of potatoes such as wound healing, sprouting and seed performance.
4) evaluate how ozone affects specific post-harvest pathogens *in vivo* and *in vitro*.
5) provide an objective evaluation of ozone as a post-harvest treatment and provide treatment recommendations to the industry.

2. LITERATURE REVIEW

Potatoes (*Solanum tuberosum* L.) are an important source of nutrients and energy, globally representing the fourth most important food crop. Historically, successful potato production and storage has had a significant impact on world population growth and movement. The introduction of a highly productive, high quality food source like potatoes to Europe resulted in an unprecedented population growth in the 1500 to 1800's. In the 1800's, the failure of the Irish potato crop due to Late Blight (*Phytophthora infestans* (Mont.) de Bary) resulted in the death by starvation or emigration of millions of people from Ireland and other parts of Europe. Although this disease causes loss of the crop in the field, it is the resulting decay of the crop in storage that is most devastating. Potato production is increasing worldwide, primarily as a function of increasing yields, due to superior agronomic practices and improved disease control. In Canada, the potato industry is expanding rapidly in response to increasing local and export demand for processed potato products.

2.1 Post-harvest Management of Potatoes

The post-harvest management of potatoes is complex, with many interconnected processes that together result in a quality product at the end of the storage period. The management of post-harvest diseases of potatoes requires a high level of control at all stages. Failure to control or minimise disease at any one stage can complicate management in later stages.

Schaupmeyer (1992) and Burton (1966) provide detailed reviews of the post-harvest management of potatoes.

2.1.1 Pre-harvest

Decisions determining post-harvest quality start before the potatoes are planted. Planting disease-free seed reduces the initial disease pressure in the harvested crop, thereby reducing the levels of disease that will eventually go into storage.

During the growing season, management practices are employed which maximise yields of high quality, mature, disease-free product. Maintenance of adequate moisture and soil fertility ensures a vigorous crop of healthy plants. Stressed plants are susceptible to disease in both the field and in storage (Burton, 1966). While irrigation minimises drought stress, it can also influence the type and severity of disease prevalent in the crop. For example, irrigation produces conditions favorable for the development of late blight.

Potatoes are top-killed with chemical dessicants at least 10 days prior to harvest (Schaupmeyer, 1992). Top-killing triggers skin set, and good skin set reduces the number and severity of wounds received during harvest and during post-harvest handling. Wounds are potential entry points for disease organisms like dry rot and soft rot bacteria. Tubers should be harvested when mature, as immature potatoes are more susceptible to disease and can be problematic in storage (Burton, 1966). Tubers should not be left in the field for too long a period after top-kill. Late harvests increase the danger of frost damage, which will influence the susceptibility of the stored potatoes to disease. Late harvests can also increase levels of *Rhizoctonia* black scurf on the tuber surface (Stevenson *et al*., 2001).

Proper adjustment, maintenance and operation of harvest and handling equipment helps minimise post-harvest damage which, in turn, reduces the risk of disease. Cleanup of storages will help to prevent potential contamination of the new crop by disease residues from the previous crop.

2.1.2 Harvest

The ideal harvest temperature for potatoes is between 10 and 15°C. Potatoes should not be harvested when tuber pulp temperatures are less than 5°C, as the cold tubers are prone to shatter bruising. Any type of bruise reduces marketability (Schaupmeyer, 1992) and renders the tubers more susceptible to attack by pathogens such as *Fusarium* dry rot and *Erwinia* soft rot. Tubers harvested in excessively high temperatures are prone to storage breakdown caused by bacterial soft rot and *Pythium*. If harvest conditions result in damage, separation of damaged from healthy potatoes should be attempted to minimise the chance of cross infection during handling and storage (Schaupmeyer, 1992).

Harvesting can cause a significant amount of damage to potatoes. The potatoes pass over a long series of chains and rollers in the harvester which can bruise or abrade potatoes. Harvest equipment should be designed, maintained and operated to reduce harvest damage. Chains should be padded and drops of more than 30 cm (12 inches) should be avoided. Rollers and chains should also be kept full of potatoes, to increase the self-padding effect. Ensuring that machine operators and line workers are well trained will improve the handling of the product (Schaupmeyer, 1992). Potatoes pass through the harvester and are placed into a truck for transport back to grading lines and the storage.

2.1.3 Post-harvest Handling and Grading

Potatoes are graded prior to storage to remove obviously deformed, diseased or damaged tubers. Placing quality potatoes into storage will help ensure that a higher quality of potatoes comes out of storage. Low quality potatoes going into storage serve as an initial inoculum source for disease which can subsequently spread within storage. Low quality potatoes also contribute to a reduction in the overall quality of storage conditions. For example, increased respiration by damaged tubers can lead to pockets where the storage temperature is elevated. These elevated temperatures in turn accelerate moisture loss and development of disease.

As the potatoes are conveyed on rollers into the storage structure, the tubers may be treated with a fungicide. This treatment is designed to both control any disease that may be present on the tubers and also to protect against future infections. The fungicides are typically applied as a liquid spray. For the fungicides to be effective, the potatoes must be completely covered with the chemical protectant. However, the required complete and thorough coverage is difficult to obtain due to the large volumes of potatoes passing into storage. As potatoes are not washed prior to storage, any soil on the tuber surfaces will reduce contact by the protectant fungicides. Application of the fungicides in large volumes of water can help provide thorough and even coating, but excessive moisture on the tuber can increase susceptibility to bacterial disease, which is not controlled by fungicides. Resistance to commonly applied fungicides is also currently a problem. Highly resistant isolates of *Helminthosporium solani* and *Fusarium*

spp. have been found in storages across the Canadian prairies (Kawchuk *et al*., 1994; Errampalli *et al*., 2001).

2.1.4 Post-harvest Curing Period

Storage management of potato involves manipulation of physical and environmental factors (e.g. temperature, ventilation, relative humidity). Careful consideration must go into the structural design, selection and operation of the environmental control systems.

The harvest process inevitably causes wounds which represent an opportunity for pathogen infection. Potatoes, however, have the potential for self-protection via wound healing. To promote this process, freshly harvested tubers are initially stored for approximately three weeks at 15°C with high humidity (>85%) and abundant ventilation. During wound healing, potatoes lay down tissue barriers which reduce disease development and water loss (Patterson and Grey, 1972; Nnodu *et al*., 1982; O'Brien and Leach, 1983).

The elevated temperatures and high relative humidity which promote wound healing (Morris *et al*., 1989; Kim and Lee, 1993) are also ideal for the development of certain post-harvest diseases. The application of chemicals during load-in can help to control pathogen growth during this curing phase. Application of any disease control product once the crop is loaded into the bulk storage is difficult due to the large volumes of product involved. To be effective, treatments must be specially adapted for this type of application.

2.1.5 Long-term Storage

Once tubers have completed the curing process, storage temperatures are dropped at approximately 1°C per day until the desired long-term storage temperature is reached. Seed potatoes are stored at 4°C to minimise disease development and prevent sprouting and ageing. Table potatoes and potatoes destined for processing cannot be stored at this low a temperature due to the undesirable physiological changes that take place at low temperatures. Low temperatures promote the conversion of starch to sugar, which can negatively affect cooking quality. Processing potatoes are stored at 7 to 10°C while table potatoes are stored at 6-7°C. These higher storage temperatures are also more suitable to the development of post-harvest disease.

2.1.6 Post-storage Considerations

At the completion of the storage period, which in Saskatchewan can last up to six months, potatoes are unloaded and shipped to the buyer. Disease levels at this time can significantly affect marketability of seed, table and processing potatoes. All types of potatoes are covered by government-mandated grade standards for disease levels (Schaupmeyer, 1992). Disease problems, which have been kept in a latent state by low storage temperatures, can escalate into major problems once the commodity leaves cold storage and encounters the higher temperature and relative humidity common in retail and market situations.

2.2 Important Post-harvest Diseases of Potato in Saskatchewan

Potatoes are attacked by a wide range of diseases in the field and in storage, with the relative prevalence and economic impact of each disease varying from region to region. Storage losses in potatoes can approach 40 percent, with 5-10% losses common in even the most modern and well-managed facilities (Morris *et al*., 1989; Waterer, Personal Communication). On the Canadian prairies, dry rot (*Fusarium sambucinum* Fckl. f. 6 WR.; *F. solani* (Mart.) App. & Wr.*)*, soft rot (*Erwinia carotovora ssp carotovora* (Jones) Bergey *et al.*; *E. c. ssp atroseptica* (van Hall) Dye) and silver scurf (*Helminthosporium solani* Dur. & Mont.) are the dominant diseases in potato storages (Personal Communication J. Thomson/D. Waterer; Holley and Kawchuk, 1996; Stevenson *et al*., 2001). These diseases cause economic losses by destroying tubers or by decreasing tuber quality during storage. They can also impair seed potato performance and processing potato quality. The relative importance of these diseases varies with the grower, storage conditions and the end use of the potatoes. Rhizoctonia or black scurf (*Rhizoctonia solani* Kühn) is also commonly found on potatoes in Saskatchewan. This disease does not cause storage losses, but may cause a reduction in aesthetic value while also posing a threat to emerging seedlings if the infested potatoes are used as seed. The storage rot of potatoes associated with late blight (*Phytophthora infestans* (Mont.) de Bary) is also problematic in years favouring the establishment of this disease in the fields prior to harvest.

2.2.1 *Erwinia* Soft rot

Erwinia soft rot of potatoes is caused by the pectolytic bacteria *Erwinia carotovora ssp. carotovora* (Jones) Bergey *et al*. and *E. c. ssp. atroseptica* (van Hall) Dye. The gram negative rod-shaped *Erwinia* bacteria are facultative anaerobes. Soft rot can be isolated from the surface or lenticels of most potatoes. Consequently, most potato storages experience some loss to bacterial soft rot, but much more extensive losses can occur when conditions are suitable for disease development.

In storage, decaying tubers infested with bacterial soft rot break down, releasing a bacteria-laden ooze onto adjacent tubers in the storage. In storage, the development of films of water on the tubers produce the anaerobic conditions ideal for development of this disease. Bacterial soft rot often develops as a secondary infection, following primary invaders such as late blight, which create wounds and weaken host tissues. Given suitable conditions and adequate initial infection levels, both the incidence and severity of soft rot will increase as time in storage increases, as the bacteria grow and spread rapidly under suitable conditions.

Control of bacterial soft rot can be accomplished by; 1) using clean seed to reduce inital infection levels. 2) keeping all handling equipment clean to reduce spread of the soft rot bacteria at the various stages of the post-harvest process. 3) reducing wounding and bruising and ensuring adequate post-harvest wound healing. 4) maintaining adequate aeration and storage temperatures less than 10°C will reduce development of the disease throughout the post-harvest process. 5) avoid washing potatoes if possible. If the crop must be washed, it should be thoroughly dried before storage, as bacterial soft rot thrives when tuber surfaces are moist.

2.2.2 *Fusarium* Dry rot

Fusarium dry rot is one of the most important post-harvest diseases of potato, with losses typically ranging from 6 to 25% and reaching 60% in some situations (Stevenson *et al*., 2001). Dry rot is caused by many *Fusarium* species, but *F. sambucinum* Fuckel (syn. *F. sulphureum* Schlechtend.; teleomorph *Gibberella pulicaris* (Fr.:Fr.) Sacc.) and *Fusarium solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth are the most common causes of *Fusarium* dry rot in North America (Stevenson *et al*., 2001). These species can be differentiated by their growth on Potato Dextrose Agar

(PDA). *F. sambucinum* grows very quickly and produces a light orange to salmon coloured pigment, whereas *F. solani* is slower growing and is a bluish purple colour. The more rapid growth of *F. sambucinum* may explain why it is the predominant type of *Fusarium* dry rot infection. The shape of the macroconidia produced by these fungal species also differ somewhat. Macroconidia of *F. solani* are stout, thick-walled and cylindrical, while those of *F. sambucinum* are short, stout, thick-walled and strongly curved (Howard *et al.*, 1994).

Fusarium inoculum is initially found in contaminated soil or in tubers infected with *Fusarium* dry rot spores. *Fusarium* dry rot requires wounds or entry points to infect tubers. Once infection has taken place, the disease continues to develop within infected individual tubers for the duration of the storage period. Slightly darkened, shallow lesions become apparent within one month of infection. As the disease spreads, the infected tissues become sunken and somewhat wrinkled, with concentric rings of discoloured tissue radiating from the initial infection point. Rotted tissues are dry, with cavities lined with mycelium and spores. Tubers may become completely shrivelled after a period of time as the rot advances and the damaged tissues dry. *Fusarium* does not spread between tubers in storage (Stevenson *et al*, 2001).

The application of fungicides at load-in has historically been used to control *Fusarium* dry rot. Efficacy of this treatment has always been limited by the difficulty of obtaining adequate contact between the chemical and the infected area, as the fungicides registered for post-harvest use in potatoes require contact with the target pathogen to be effective. The incidence of fungicide resistance in isolates of *Fusarium* is increasing (Kawchuk *et al*., 1994; Holley and Kawchuk, 1996) and has resulted in most growers abandoning use of thiabendazole (TBZ), which is the most commonly employed post-harvest treatment for potatoes. Maintaining low temperatures throughout the storage period will slow but not halt development of dry rot (Stevenson *et al*., 2001). Other methods of reducing *Fusarium* dry rot include employing good sanitary techniques in pre-planting and post-harvest activities. Any activity that minimises wounding will help reduce losses to *Fusarium* dry rot. Ensuring adequate skin set prior to harvest and adjusting harvest and handling equipment properly will minimise wounding and loss to *Fusarium* dry rot.

2.2.3 *Helminthosporium* Silver scurf

Silver scurf of potatoes caused by the fungus *Helminthosporium solani* Durieu & Mont involves production of characteristic silvery lesions on the skin of the potato. Under high humidity, the lesions become more defined, with a black-speckled or sooty appearance indicating that conidiophores are present. *H. solani* produces brown mycelium in culture, with conidia borne in whorls on septate conidiophores. Spores of *H. solani* are dark brown, fairly large (7-11 by 24-85 μm), have 2 to 8 septa and are tapered at the base (Howard *et al.*, 1994). Damage to the tubers by *H. solani* is superficial, however some increase in moisture loss can occur in heavily infected tubers. In the high temperatures characteristic of retail situations, the lesions can become more obvious, thereby reducing marketability.

Helminthosporium is found in most soils and potatoes are typically contaminated with spores prior to load-in. *H. solani* sporulates under high humidity, triggering the spread of the disease through the air in storage. Fungicides applied to tuber surfaces at load-in can help control this surface disease and prevent its spread in storage. However, isolates of *H. solani* resistant to thiabendazole (TBZ), which is the most commonly employed post-harvest treatment for potatoes, are becoming more common across the Canadian prairies (Holley and Kawchuk, 1996; Errampalli *et al*., 2001). Reducing relative humidity in the storage while maintaining good air circulation immediately after harvest can help minimise infection by *H. solani*. Low temperature storages are also effective at limiting development of this disease (Howard *et al.*, 1994). Silver scurf is increasing in importance on the Canadian prairies (Holley and Kawchuk, 1996; Thomson and Waterer, 1999, 2001), primarily due to the increasing incidence of fungicide resistance and increasing potato production leading to short crop rotations and a subsequent increase in disease pressure.

2.2.4 *Rhizoctonia* Black scurf

Rhizoctonia or black scurf (*Rhizoctonia solani* Kühn.) is a soil-borne Hyphomycetes or Mycelia Sterilia fungus that forms a black scurf (sclerotium) on the skins of potatoes. Differentiation between other isolates of *R. solani* (i.e. those affecting other host groups) is accomplished by observing anastomosis (crossing between hyphae of compatible strains). The *R. solani* that causes black scurf of potatoes belongs to

Anastomosis Group 3 (AG-3). This group of *Rhizoctonia* does not produce spores and has dark brown mycelium, with a characteristic right angle branching pattern (Howard *et al.*, 1994). Sclerotia may be formed in culture by some isolates. The sexual stage of this pathogen is *Thanatephorus cucumeris*.

Rhizoctonia does not spread from tuber to tuber in storage but the lesions (sclerotia) may increase in size during storage (Personal Communication, Jill Thomson). Sclerotia are formed on the tubers late in the season, following vine death (Stevenson *et al.*, 2001). High levels of scurf can reduce marketable yields as tubers with excess levels of surface scurf must be graded out (Bains *et al.*, 2002). Planting of *Rhizoctonia*-infected seed causes stem cankers which decreases seedling vigour. The application of post-harvest chemicals will not reduce levels of this disease.

2.2.5 *Phytophthora* Late blight

Late blight of potato (*Phytophthora infestans* (Mont.) de Bary) can cause extensive losses of potatoes in the field and in storage. Late blight occurs sporadically in Saskatchewan, when conditions are favourable for disease, and extensive storage losses can occur (J. Thomson, Personal Communication). Despite aggressive management in field and storage, extensive storage losses to late blight are relatively common.

Phytophthora is an Oomycete belonging to the phylum Oomycota, class Peronosporomycetidae, order Pythiales, family Pythiaceae. Asexual reproduction predominates, with lemon-shaped, hyaline sporangia produced on branching sporangiophores (Howard *et al.*, 1994). Thick-walled sexual oospores capable of overwintering in the soil are produced in areas where compatible mating types exist. In other areas, the primary disease source is infested tubers used as seed. The disease also overwinters in tubers as mycelium (Howard *et al.*, 1994)

Late blight can also be introduced into a field from cull piles as diseased tubers discarded from the previous crop sprout and produce disease-laden foliage (Agrios, 1997). Under favourable conditions, late blight spores can also blow in from other geographic areas (Howard *et al.*, 1994). Infected areas on foliage or tubers produce sporangia under high humidity and moderate temperatures (10-24°C). Sporangia either penetrate the host directly or produce motile zoospores, which swim in films of water to

encyst and infect the host or are transferred by rain splash to new host plants (Howard *et al*., 1994). Under favourable conditions, late blight can spread very rapidly across a field, resulting in the destruction of the plant canopy. Sporangia and zoospores are washed from infected foliage into the soil to infect the developing tubers. Infected tuber tissues are a foxy red colour. The disease spreads within the tuber, with the rate of spread dependant on environmental conditions. Cool, dry conditions slow the spread of tuber rot. Tubers infected by late blight are prone to invasion by secondary pathogens, such as bacterial soft rot.

Management of late blight requires integrated control of field and storage conditions. Proper disposal of cull tubers is crucial. The relatively dry and cool growing conditions characteristic of Saskatchewan help to restrict initiation and spread of this disease (D. Waterer, Personal Communication). Isolation from other potato production areas is also beneficial in limiting the introduction of late blight. Treating infected seed tubers with fungicides prior to planting can help prevent introduction of later blight into a developing crop. The primary method of control of the foliar stage of late blight is the preventative application of protective fungicides to the foliage of the developing crop. These chemicals must be applied before the disease is present and then the application must be repeated as the crop grows and/or conditions favour disease spread and development. Timely repeated applications are required to provide continuous and thorough coverage. In Manitoba, crops may be sprayed as many as 8 to 10 times per season at a cost of $120 or more per acre (Personal Communication, D.R. Waterer).

Cultural practices, which reduce the duration of leaf wetness periods, will reduce late blight as this disease requires high humidity to sporulate and spread. Timing irrigation to decrease periods of leaf wetness will help reduce disease pressure. Resistance to late blight is limited in commercial cultivars but breeding efforts are underway to enhance resistance (Stevenson *et al*., 2001).

2.3 Disease Management: Methods and Issues

Several of the pathogens of stored potatoes are active in the relatively warm temperatures and high relative humidity conditions recommended during curing of potatoes. It is not uncommon for diseases like dry rot (*F. sambucinum, F. solani*),

bacterial soft rot (*E. carotovora ssp carotovora or E. c. ssp atroseptica*), silver scurf (*H. solani*) and late blight (*P. infestans*) to become established, and in some cases, begin to spread through the stored crop during the curing period. Traditionally, control of the diseases which attack stored potatoes has been accomplished using pre-storage applications of contact fungicides coupled with manipulation of storage environmental conditions such as temperature, relative humidity and air movement. Fungicides or disease control products are often sprayed onto the potatoes at the commencement of the curing period (during loading in) in an effort to slow the development, increase and spread of disease organisms both during the curing period and during subsequent long-term storage. There are relatively few fungicides registered for post-harvest use in potatoes. This has resulted in the development of resistance in many of the pathogens (Kawchuk *et al*., 1994; Platt, 1997; Errampalli *et al*., 2001).

2.3.1 Chemical Applications

2.3.1.1 Timing and Product Efficacy

Carnegie *et al*. (1986, 1990 and 1998) evaluated the performance of a number of disease control products (including thiabendazole, 2-aminobutane, benzimidazole, imidazole, phenylpyrrole) applied at harvest or at different points in the grading process. Efficacy of the products varied, although better control was consistently observed when the products were applied early in the post-harvest period. Early application addressed the disease problems before the infection had the opportunity to penetrate and spread. Ogilvy (1992) examined a number of pre- and post-harvest chemical treatments for control of silver scurf, black scurf, common scab (*Streptomyces scabies* (Thaxt.) Waksm & Henrici) and skin spot (*Polyscytalum pustulans* (M.N. Owen & Wakef.) M.B. Ellis.). Post-harvest treatment with imazalil and thiabendazole reduced skin spot but did not control silver scurf. Storage temperature management was more effective than any of the fungicide treatments in controlling silver scurf (*H. solani*). Scab was not affected by any treatment. A University of Idaho study (http://www.kimberley.uidaho.edu/potatoes/scurftrt.htm) assessed a wide variety of post-harvest applied chemical treatments, including imazalil, mancozeb, Maxim®, thiabendoazole and combined treatments, for the control of *H. solani* on seed potatoes. Most treatments were ineffective at controlling this disease during storage. Harris (1979) tested a number of chemicals for their ability to provide

soft rot (*Erwinia*) control in wounded potatoes. An unregistered product $5NO_2$-8-OHQ effectively slowed disease development of the soft rot. Products such as Elbadyne (Winston Laboratories) and Chlorine Dioxide (ClO_2) were less effective at reducing disease. Wyatt and Lund (1981) also showed that ClO_2 provided some limited control of bacterial soft rot. Harrison and Franc (1988) demonstrated that several post-harvest chemicals, including captan, thiabendazole and ClO_2 reduced infection of potatoes by *Alternaria solani*.

Oxidate (BioSafe Systems, Connecticut, USA), has been recently introduced for the control of a wide range of storage pathogens. Oxidate contains 27% hydrogen peroxide and is described as a highly-engineered peroxygen formulation. Similar to Purogene®, the biocidal action of Oxidate is based on oxidation reactions (Bio-Safe Communication). Oxidate has many of the same performance characteristics and limitations as chlorine dioxide. It is inactivated by soil and has no residual activity. Oxidate does not require activation or mixing, but is simply a dilution of a corrosive concentrate. It is applied by adding the Oxidate solution to the water used in the storage humidification system. The ventilation system carries water vapour containing the Oxidate through the potato pile. There are no limitations on re-entry or application frequency for Oxidate. However, repeated applications can lead to corrosion of metals. Oxidate has received approval for use on a range of crops in the U.S.A. and has received Emergency Use registration for use on potatoes in several provinces.

A number of studies have evaluated the efficacy of Oxidate in post-harvest applications. Al-Mughrabi (2002) found that Oxidate was effective in preventing the development of dry rot, silver scurf and soft rot, without affecting french frying quality. Repeated applications resulted in some corrosion of metals that came into direct contact with the Oxidate mist. Some promotion of sprouting was also observed after four months of storage with Oxidate treatments. Kirk (2002) showed that Oxidate applications did not differ in level of disease control from water controls in wounded/non-wounded inoculated potatoes.

2.3.1.2 Application Methods

To be effective, fungicides must completely and thoroughly cover the potato, as these products typically must come in contact with the pathogen to exert control. Growers often find it difficult to achieve thorough coverage, as:

A) this requires application of large volumes of the fungicidal treatment to the potatoes. This is undesirable as the associated moisture creates an ideal habitat for many decay organisms and it is also messy.

B) the potatoes must be rolling as they pass by the spray applicator to ensure thorough coverage. The installation of roller systems to the grading line involves a significant additional cost.

C) any soil on the potatoes intercepts and/or inactivates the spray.

2.3.1.3 Cost and Resistance Issues

Post-harvest applications of fungicides for the control of storage diseases are expensive, costing as much as $6 per metric tonne (TBZ). Many of the common post-harvest pathogens of potatoes have developed resistance to Mertect (thiabendazole; TBZ), which is the only post-harvest fungicide presently registered for use on table or processing potatoes in North America (Hide *et al*., 1988; Platt, 1997; Satyaprasad *et al*., 1997; Thomson and Waterer, 1999). This resistance likely reflects repeated use of a single control product. Kawchuk *et al*. (1994) point out that *Fusarium spp*. and *Helminthosporium solani* isolates in the U.S.A. and Canada did not show resistance prior to 1990, but resistance increased dramatically over the last decade. Different species of *Fusarium* and *H. solani* vary in the degree of resistance to TBZ. Szeto *et al*. (1993) found that the efficacy of the registered fungicide thiabendazole (TBZ) in controlling silver scurf of potato in storage varied depending on the presence of some resistant isolates of the pathogen. They found that selected isolates were either susceptible, tolerant or resistant to TBZ. Holley and Kawchuk (1996) studied the distribution of TBZ resistant isolates in Alberta potato storages. Seventy-six and 67 % of southern Alberta farms sampled had TBZ-resistant isolates of *H. solani* and *F. sambucinum* respectively. Northern seed farms had a much lower incidence of resistant isolates. Thomson and Waterer (1999, 2001) found that isolates of *Fusarium* and *Helminthosporium* from Saskatchewan potato farms had varying degrees of resistance to

thiabendazole, however resistance was widespread across the province. Errampalli *et al*. (2001) observed that *Helminthosporium solani* has only recently emerged as an economically significant disease in stored potatoes. The authors attribute this change to the development of resistance to TBZ in *H. solani*. The widespread resistance to TBZ is driving research into alternative control measures for post-harvest disease of potatoes.

2.3.1.4 Health and Safety Issues

The public is becoming increasingly consciousness of health and food safety issues. The environmental impact and consumer safety of any fungicide applied post-harvest to a dietary staple like potatoes is an issue of concern, especially when that product is designed for application within days or weeks of sale. Camire *et al*. (1995) found residues of post-harvest fungicides and sprout inhibitors in potato peels and found that processing did not diminish these residue levels.

Stringent safety and product testing requirements have restricted the range of fungicides registered for post-harvest disease control in potatoes. There is consequently a need for new, effective and environmentally friendly treatments to control storage diseases in potatoes.

2.3.2 Non-chemical Disease Management

Careful handling during harvest and storage (e.g. minimised handling, wound healing) reduces losses to post-harvest disease, through the reduction of wounds. Careful handling also improves post-harvest tuber quality, through reduced water loss. Partial control of post-harvest diseases can be achieved through precise control of storage temperature (low temperature storage) and relative humidity during storage (Butchbaker *et al*., 1972). Low storage temperatures slow development of all post-harvest diseases, but excessively low temperatures (<2°C) also result in chilling injury to the tuber. Potatoes that are destined for processing must be stored at temperatures between 7 and 10°C, as lower temperatures increase sugar content, which results in darker fries and chips. The higher temperatures commonly encountered at bin loading and unloading can increase disease development in stored potatoes. Alternative methods must be employed to control disease in these situations or some degree of loss must be anticipated. Lowering storage relative humidity coupled with increased ventilation rates can reduce the development of bacterial soft rot and silver scurf, but excessively low

relative humidity increases shrinkage of the stored crop. Control of disease through storage management is also costly, as it hinges on the construction of superior buildings and the installation of complex environmental control and monitoring systems.

2.3.3 Wound Healing as a Means of Reducing Disease in Stored Potatoes

During mechanical harvest, potato tubers are often slightly damaged (abrasions, cuts, bruises), particularly if their skins are not completely developed at the time of harvest. The damaged areas are susceptible to infection by pathogens introduced from the surrounding soil (such as *Fusarium spp.*), air or water-borne spores (e.g. *H. solani* or *P. infestans*) or by contact with other infected tubers (e.g. *E. carotovora*). These wound pathogens may develop and/or spread during the subsequent storage, causing significant losses of weight and quality (Morris *et al*., 1989). To decrease losses to post-harvest diseases introduced at wound sites, a curing period is carried out at the commencement of storage. During this two to three week period, the crop is stored at 15°C with abundant volumes of air pumped through the pile. This curing period promotes the development of wound periderm tissue, thus reducing the number of open wounds and potential entry points for pathogenic organisms. The wound periderm layer also reduces water loss from the wound sites.

Stark *et al*. (1994) and Yan and Stark (2000) describe the processes of wound healing and suberisation in detail. Wound healing begins within 24 hours of harvest, with several distinct changes taking place. First, one to three layers of cells at the surface of the wound become more lignified and suberin lamellae are deposited on the inner surfaces of the walls. A wound cambium then develops beneath the initial suberised area and finally, cork cells are produced and became lignified and suberised (Thomson *et al.*, 1995).

The integrity and thickness of the combined suberin and wound periderm layer may determine resistance or susceptibility to disease (Vaughn and Lulai, 1991). The rate and extent of development of the wound periderm layer is affected by environmental factors including temperature and relative humidity. Morris *et al*. (1989) found that the rate of development and final thickness of the wound periderm layer was positively correlated with curing temperature. Kim and Lee (1993) also found that

curing period temperatures of 18°C resulted in faster periderm formation than at 10 or 15°C. High relative humidities also enhanced wound healing.

Many studies have shown that the prompt and thorough formation of wound periderm layers significantly enhanced the resistance of potato tubers to post-harvest disease. Patterson and Gray (1972) showed that wound periderm formation on potato slices restricted the penetration of the underlying tissue by a gangrene-causing pathogen (*Phoma exigua* Desm.). Clarke and Kassim (1977) determined that differences in wound periderm thickness were related to differences in clonal resistance to *Phytophthora infestans*. Nnodu *et al*. (1982) found that tubers were significantly more resistant to post-harvest infection by *Alternaria solani* under conditions favouring wound periderm development. When O'Brien and Leach (1983) compared a numbered clone versus Russet Burbank potatoes for resistance to *Fusarium roseum* 'Sambucinum', they found that wound periderm development, as determined by both the rate of periderm development and the completeness of the layers, was an important disease resistance mechanism. Vaughn and Lulai (1991) studied the involvement of mechanical barriers in potato resistance to *Verticillium dahliae* Kleb. They found that susceptible and resistant cultivars differed in the rate of wound periderm formation, with a faster rate of formation characteristic of resistant cultivars.

2.4 Ozone as an Alternative Post-harvest Treatment

The increasing cost of chemical fungicides, coupled with concerns about the food safety of applying fungicides to food and the development of disease resistance, highlight the need for alternative post-harvest disease control options in stored potatoes.

Ozone has several characteristics that may be used to enhance the post-harvest quality of horticultural commodities. As a chemically reactive compound, ozone exerts significant antibiotic activity on a range of spoilage organisms including fungi and bacteria (Horváth *et al.*, 1985). Ozone is more reactive than chlorine and other commonly employed general disinfectants (Law and Kiss, 1992), and unlike other disinfectants, ozone does not leave any toxic residues (http://postharvest.ucdavis.edu/Produce/Ozone1.shtml; Law and Kiss, 1992), which is beneficial from a food safety perspective. Ozone also works as an ethylene abatement treatment, thereby potentially prolonging the post-harvest life span of ethylene-sensitive

horticultural crops (Dickson *et al.*, 1992). Finally, ozone has been shown to stimulate plant/fruit defence mechanisms, which may also increase post-harvest life of commodities (Kangasjarvi *et al.*, 1994; Sarig *et al.*, 1996).

2.4.1 Chemistry of Ozone

Ozone (O_3) is a chemically reactive oxidising agent with a half-life of 15-50 minutes. The short half-life means that ozone cannot be stored and must be generated on site (Law and Kiss, 1992; http://postharvest.ucdavis.edu/Produce/Ozone1.shtml). Typically, ozone is generated by exposing normal atmospheric oxygen to a high-energy source, such as an energised electrode or an UV light source. This causes the oxygen molecules to split and then reform into the allotrope ozone. Adjusting the energy levels or the amount of oxygen in the airflow controls the concentration of ozone in the resulting air stream. The life span of the ozone molecule varies depending on temperatures and the amount and type of substance being treated. Non-reacted ozone eventually degrades back into oxygen. Higher temperatures favour degradation of ozone (Law and Kiss, 1992), however the effect of temperature on the efficacy of ozone is not known. The reactivity of ozone with different substances varies with the extent of contact between the ozone molecules and the target surface, as well as the reactivity of the target substances (http://postharvest.ucdavis.edu/Produce/Ozone1.shtml; Berg *et al.*, 1964; Hodge, 1998). Organic materials are much more reactive than more inert materials such as plastics and metals (Dave Loewen, Personal Communication). No harmful residues are left by the reaction of ozone (Law and Kiss, 1992); this represents one of the major strengths of ozone as a treatment for foods.

At the cellular level, reactions with ozone alter the function and activity of organelles and cellular components. Staehelin and Hoigne (1985) in Kim *et al*. (1999b) suggested that ozone's efficacy in the control of organisms is either due to a direct reaction with the ozone molecule or is due to reactions with free-radicals produced from ozone. Kangasjarvi *et al*. (1994) suggested that the reaction of ozone with plant tissues results in a decrease in plasma membrane permeability. Scott and Lesher (1963) in Kim *et al*. (1999b) proposed that the double bonds of unsaturated lipids in the cell membrane of bacteria were the principle site of the ozone reaction, leading to cellular lysis and leakage. The targeting of sulfhydryl groups in enzymes by ozone can lead to cell death

(Barron, 1954 cited in Kim *et al*., 1999b). Hodge (1998) indicated that ozone reacted readily with sulfhydryl group-containing proteins, and suggested a range of susceptibility to ozone in amino acids (Menzel, 1971 cited in Hodge, 1998). Ozone can also damage nucleic acids which could affect both cellular function and reproduction of pathogenic species (Scott, 1975 cited in Kim *et al.*, 1999b). Ozone affects cellular respiration by destroying dehydrogenating enzymes (Emafo, 1966 cited in Hodge, 1998). The nature of ozone reactions varies depending on the chemical composition of the target organism, the composition and physical condition of the reaction system and the factors governing contact between ozone and the organism.

2.4.2 Practical Applications of Ozone

2.4.2.1 Environmental Purification

Historically, ozone has been used as a purifying agent for a number of commodities and environments. Ozone is commonly used as an odour removal agent in settings such as night clubs, bars and personal residences (Personal Communication, Dave Loewen, Crystalair, Delta B.C.). During reaction with ozone, pollutants are broken down into their basic molecular components and are thereby neutralised (www.iqx.com). Ozone is used commercially to reduce microbial populations in liquid products ranging from fruit juice to spring water (reviewed in Kim *et al*., 1999b). Ozone has been used as a water purifier in water treatment plants in Europe since the early 1900's (Law and Kiss, 1992). Although ozone is highly insoluble in water, injection of ozone into water is possible (http://postharvest.ucdavis.edu/Produce/Ozone1.shtml). The life span of ozone in water depends on the purity of the water. Roy-Archand and Archibald (1991) found that combined ozone and fungal (mycological degradation tools) treatments of paper mill effluent enhanced colour removal over fungal treatments alone. Korhonen and Tahkanen (2000) tested the efficacy of ozone as a biocide in water from a pulp mill. They found that ozone destroyed about 80 to 90% of aerobic bacteria in cloudy or clear filtrate. Ozone was highly effective in killing viral bodies in recirculation water from a soilless greenhouse operation and moderately successful in the eradication of *Verticillium* microsclerotia (Runia, 1994).

Ozone is commonly used to surface sterilise medical or laboratory equipment. When alumina surfaces fouled with Bovine Serum Albumin (BSA) were treated with

ozone prior to alkali cleansing, BSA desorption was improved by ozone pre-treatments. This reflects the partial decomposition of the BSA by the ozone (Urano and Fukuzaki, 2001). Oizumi *et al*. (1998) compared gaseous versus aqueous ozone applications for sterilisation of dentures. They found that gaseous ozone applications reduced the numbers of cells of several strains of microorganisms to $1/10^5$ within one minute. Ozonated water reduced microorganisms to 1/10 of the original population. Generation of the aqueous ozone application required 35X more ozone per hour than the gaseous application.

2.4.2.2 Agricultural Commodities

Ozone has been used for various purposes on a number of different agricultural commodities in varying situations. The potential to use ozone to protect meat from spoilage and disease-causing microorganisms has been extensively studied (Horváth *et al*., 1985; Reagan *et al*., 1996). Jindal *et al.* (1995) reported that adding ozone to the atmosphere improved the shelf life of poultry in coolers. Horváth *et al*. (1985) stated that briefly exposing stored meats to ozone controlled infectious surface bacteria, particularly at higher storage temperatures.

Horváth *et al*. (1985), Kim *et al*. (1999b) and Xu (1999) have reviewed the use of ozone on fruits and vegetables and other horticultural commodities. Horváth *et al*. (1985) indicate that differing rates of ozone were effective in reducing decay and disease development in a range of horticultural commodities.

Skog and Chu (2001) found that while ozone extended the post-harvest life of broccoli and cucumbers, it had limited utility on mushrooms. Pérez *et al*. (1999) found that gaseous ozone treatments were only partially effective in preventing fungal decay in strawberries held at 20°C after treatment. Labbe *et al*. (2001) found that ozone was ineffective in reducing aerobic plate counts in maple sap. They surmised that this was due to inactivation of the ozone by the high concentrations of sucrose in the sap. Liew and Prange (1994) found that the rate of fungal growth on stored carrots was reduced by up to 50% by gaseous ozone treatments. Ozone treatments suppressed fungal growth in blackberries for up to 12 days, with minimal negative effects on berry quality (Barth *et al*., 1995). Sarig *et al*. (1996) found that gaseous ozone treatments of grapes reduced colony forming unit (CFU) counts, an effect that persisted well after the ozone treatment

ceased. They surmised that the extended disease control effect was probably a result of the stimulation of host resistance, such as the formation of phytoalexins in response to the ozone. Song *et al*. (2000) reported that gaseous ozone treatments in onion storage reduced surface discoloration and mould growth. They found that the number of airborne spores of onion pathogens within the storage area was reduced by the ozone treatment, with no significant changes in internal decay or quality traits of the onions. Ridley and Sims Jr. (1967) observed that ozone treatments significantly reduced the percentage of peach fruit with storage rots (i.e. *Monilinia fruticola* and *Rhizopus spp*.). Ozone also controlled the spread of these rots. Subsequent trials by Ridley and Sims Jr. (1967) found that ozone treatments reduced the percentage surface area with rot caused by *Monilinia fruticola* and *Rhizopus spp.*, but did not reduce rot incidence. Norton *et al*. (1966) showed that gaseous ozone treatments reduced rot in stored cranberries.

The use of ozone as a gaseous versus an aqueous application can influence efficacy and has implications in terms of application methodology. Ozone generation requirements are much higher in aqueous applications due to the limited solubility of ozone in liquid and the shorter half-life of ozone in liquid. Also, not all commodities will tolerate immersion in water or washing. Nonetheless, application of ozonated water has effectively reduced disease levels in some horticultural commodities. Klingman and Christy (2000) developed a sanitation system for whole apples using ozone injected into wash water. Their prototype system reduced *E. coli* levels by 1.25 to 2 $\log_{10}$. Spotts and Cervantes (1992) evaluated the effects of ozonated wash water on control of surface pathogens of pears. Spore germination was inhibited following ozone exposure times of one to five minutes.

While ozone treatments have demonstrated potential to control disease development, the potential effects of the ozone treatment on commodity quality must also be considered. Quality of horticultural commodities such as potatoes is a complex variable determined by the desired end use and the perceptions and opinions of the person measuring it. Potato quality includes aesthetic value or visual appeal, as well as physical characteristics such as flavour, texture and sugar content. Quality may be clearly defined in regulations, or may be defined by the end user, such as a processor or a grower. From an economic perspective, quality determines both the quantity and value

of product available for marketing. The effect of ozone on quality must be considered in assessing its value as a post-harvest treatment. Pérez *et al*. (1999) found that the anthocyanin and sugar content of strawberries was reduced by post-harvest ozone treatments. The vitamin C content of strawberries treated with ozone after harvest was three times higher than the controls. Ong *et al*. (1996) found that ozone washes were as effective as chlorine washes in removing pesticide residues from whole and processed apples. Although Liew and Prange (1994) showed that adding gaseous ozone to the atmosphere in a carrot storage reduced fungal growth on the carrots by up to 50%, the ozone treatments also elevated respiratory rates, increased electrolyte leakage and caused some bleaching of the carrots. These changes reduced post-harvest life-span and quality of the crop. Giacalone *et al*. (1997) showed that adding ozone to the atmosphere in a blueberry storage resulted in greater weight loss from the berries than if CO_2 was added to the storage atmosphere. Barth *et al*. (1995) found that anthocyanin levels in ozone-treated blackberries were not significantly different from controls after 12 days of storage. Colour values were also not significantly affected by ozone treatments. Daniels-Lake *et al*. (1996) found that ozone treatments after harvest did not affect reducing sugar content or sprouting of Russet Burbank potatoes.

Ethylene (C_2H_4) abatement is potentially a mechanism whereby ozone treatments can extend the post-harvest life-span of horticultural commodities. Ethylene accelerates maturation and aging of sensitive horticultural commodities. Ethylene is produced by the commodity, by combustion of petrochemicals in the storage area, or by decay organisms. Removal of this ethylene from the storage atmosphere is commercially accomplished by heated catalyst oxidation or chemiabsorption. These processes are costly and disposal of the absorbents is difficult (Dickson *et al.*, 1992). Ethylene is oxidised by ozone to carbon dioxide, water and oxygen. Dickson *et al*. (1992) tested ozone against standard ethylene absorbing treatments in conditions similar to those found in a commercial storage. They showed that ozone treatment represented an economically feasible alternative ethylene abatement system.

2.4.3 *In vivo* and *In vitro* Interactions Between Ozone and Pathogens

The efficacy of any post-harvest treatment hinges on its ability to control infection and the subsequent development and spread of the key pathogens affecting the

target commodity. Interactions between the commodity, pathogens, the post-harvest treatment and the storage environment will determine the overall efficacy and economics of the pesticidal treatments.

Ozone affects plants and plant products in different ways. As a component of air pollution, ozone may act as a stress, triggering production of defence compounds, such as lignin, phenolics or phytoalexins (Eckey-Kaltenbach *et al*., 1994; Kangasjarvi *et al*., 1994; Sarig *et al*., 1996;). Ozone is produced as a product of industrial processes, including combustion. Ozone pollution effects on plants and plant pathogens have been well studied. Ozone stress may weaken or injure plants, increasing their susceptibility to invasion by pathogens (Manning *et al*., 1969; Heagle, 1977; Holley *et al*., 1985; Tiedemann, 1992; Tonneijck and Leone, 1993; Khan and Khan, 1999). However, by acting as an antagonist of ethylene, ozone may slow aging, thereby increasing resistance to pathogens (Dickson *et al.*, 1992).

Although ozone may either increase or decrease plant susceptibility to pathogens, increasing ozone concentrations beyond ambient levels typically slows or reduces post-harvest disease development. This suggests that ozone is reacting with the pathogen. The effect of ozone on pathogen viability, growth and dispersal has been studied extensively for many pathogens, both in field studies and *in vitro*, using pure cultures. Heagle (1973) outlined ozone pollution effects on plant pathogens. In many cases, exposure to ozone reduced pathogenicity or disease severity. In a few cases, disease was increased by ozone pollution. These differences appear to be related to the impact of the ozone stress on the disease susceptibility of the host crop. Heagle and Strickland (1972) found that ozone treatments reduced the infection capability of *Erysiphe graminis* (DC.) Merat *f.sp. hordei* Em. Marchal conidia when colonies were treated during sporulation and spore incubation. At ozone concentrations that were damaging to barley (*Hordeum vulgare* L.) plants, the authors found that colony and spore mass length of *E. graminis f.sp. hordei* were increased. Heagle and Key (1973) observed that hyphal growth and urediospore production by *Puccinia graminis* Pers. were reduced by low concentrations of ozone. However, when ozone was applied to the wheat plants prior to inoculation, it caused plant injury and a corresponding increase in disease levels. Resh and Runeckles (1973) found that diurnal applications of low concentrations of ozone reduced bean rust

(*Uromyces phaseoli* G. Winter) uredial size, however, spore numbers were increased and secondary infection by the pathogen was common. Heagle (1977) found that corn (*Zea mays* L.) inoculated with *Helminthosporium maydis* Nisikado Race T, grown in the presence of low concentrations of ozone had larger lesions and more sporulation than non-exposed plants. If ozone rates were increased and applied post-inoculation, sporulation decreased. Krause and Weidensaul (1978b) found that ozone treated geraniums exhibited necrotic lesions; however the lesions were not colonised by *Botrytis cinerea* Pers. By contrast, untreated leaves inoculated with *Botrytis* were colonised. Tiedemann (1992) observed that ozone treatments altered the susceptibility of wheat plants to infection by rust and powdery mildew diseases, depending on the growth stage of the plants. In young wheat plants, the number of powdery mildew colonies and conidia were increased by moderate ozone concentrations; however, this effect was reversed by higher concentrations of ozone. No response to ozone concentration was apparent in mature plants. Rust symptoms were increased by ozone treatments, especially as plant maturity increased. Holley *et al*. (1985) found that ozone treatments increased the colonisation of potato leaves by *Alternaria solani* Sorauer. *Alternaria* typically attacks tissues weakened by age or environmental stress (Holley *et al*., 1985; Howard *et al*., 1994). Khan and Khan (1999) evaluated the effects of varying applications of ozone on the development of powdery mildew of cucumber (*Sphaerotheca fuliginea* (Schltdl). Ozone treatments caused some damage to cucumber (*Cucumis sativa*) plants, however, the authors found that fungal colonisation was less apparent in ozone-treated, inoculated plants, especially as ozone concentrations increased. Conidia were smaller and had lower germination rates at the higher ozone rates.

Ozone treatments are effective in the reduction of water-borne pathogens such as bacteria and fungal bodies. Gaubert *et al*. (2000) reported that the treatment of raw sewage water significantly reduced the bacterial load of the filtered output water. A number of studies have examined the ability of ozone and other disinfectants to remove *Cryptosporidium parvum* from water. Ozone treatments increased initial primary disinfection rates compared to chlorine treatments (Rennecker *et al*., 2000). Somiya *et*

al. (2000) determined that ozone attacked intact *Cryptosporidium* oocysts before other stages.

In a plant or field situation, plant: pathogen: ozone interactions can be altered or masked by the influence of external environmental factors. Consequently, the study of ozone treatments *in vitro* allows a better basic understanding of ozone and pathogen interactions.

In an *in vitro* study, Whistler and Sheldon (1989) compared ozone treatments to traditional formaldehyde treatment methods to disinfect poultry hatcheries. Numerous bacterial and fungal pathogens were tested. The authors found that ozone treatments reduced bacterial loads by 4-7 $\log_{10}$ and fungal loads by greater than 4 $\log_{10}$. The authors concluded that ozone treatments were capable of reducing pathogen loads in this type of application but were not as effective as formaldehyde. The removal of external factors facilitated the comparison of the treatments.

A study by an air purification company (www.airpure.com/mold.html) found that ozone treatments significantly reduced levels of bacteria (*Staphylococcus, Salmonella, E. coli*) and fungi (*Candida* [yeast], *Aspergillus*) placed onto non-reactive stainless steel rings.

Scherm *et al*. (1980) found that ozonated water (3.8 mg $O_3\ l^{-1}$) inactivated *Bremia lactucae* Regel sporangia within one minute of application *in vitro*. Lower rates of ozone (2.5 mg $O_3\ l^{-1}$) resulted in inhibition of germination, with no effect apparent at the lowest rate tested (0.5 mg $O_3\ l^{-1}$).

Shargawi *et al*. (1999) examined the effects of negative air ions and ozone on *Candida albicans* (C.P. Robins) Berkhout survival *in vitro*. Growth of *C. albicans* was inhibited by increased exposure time, which also increased ozone concentration. They assumed an involvement of ozone in this inhibition and proposed that the microbiocidal effects of negative air ions are linked with production of ozone.

Dyas *et al*. (1983) tested the ability of a domestic-sized ozone generator to control various bacterial and fungal pathogens that can cause health problems in humans. Plates of various pathogens were exposed to levels of ozone determined by room size, ranging from 0.3 ppm to 0.9 ppm in small cabinets to much lower levels in larger rooms. In the small cabinet trials, ozone treatments reduced microbial

populations by several fold within the first few hours of treatment. The rate of decline in CFU slowed as time passed. In larger rooms, the amount of ozone generated by the commercial units was incapable of reducing pathogen levels. Komanapalli and Lau (1998) found that *in vitro* ozone treatments reduced *Escherichia coli* and *Candida albicans* viability by several fold, however these microbes were not completely eliminated by the ozone treatment. Bacteriophages were much more sensitive to ozone.

Previous studies indicate that ozone has the potential to limit the growth and development of some plant pathogens, although there is significant variation between pathogens, in terms of their relative resistance to ozone treatment (Hibben and Stotsky, 1969; Horváth *et al*., 1985; Hodge, 1998). The ability of a fungus to resist any control treatment often reflects structural or chemical characteristics. Hibben and Stotzky (1969) observed a wide range in efficacy of ozone treatments as a means for preventing spore germination of a number of fungal species. They noted that larger spores with pigmentation were insensitive to ozone treatments up to 100 pphm (parts per hundred million; 1 ppm), although longer exposure to higher concentrations did provide some control. Smaller, hyaline spores were more sensitive to ozone applications.

A treatment need not kill the pathogen to be effective; inhibition of the synthesis of compounds essential to pathogenicity or a reduction in other pathogenic factors are also effective means to reduce damage. For example, ozone may reduce the pathogenicity of pathogens or the severity of pathogen attack on product quality. Chatterjee and Mukherjee (1993) found that ozonation of aflatoxin B_1 (AFB_1)-contaminated food crops reduced the immunity impairing action of the AFB_1.

Krause and Weidensaul (1978a) observed that *Botrytis cinerea* conidia grown in ozonated conditions exhibit both reduced germination and pathogenicity. They also found that germ tube lengths were reduced by the ozone treatment. The authors did not find any difference between *in vivo* and *in vitro* responses to ozone.

Treshow *et al*. (1969) found that fumigation with 10 pphm (parts per hundred million; 0.1 ppm) ozone significantly reduced the radial growth and spore production of *Colletotrichum lindemuthianum* Shear. but had no effect on *Alternaria oleraceae* J. Milb. cultures. Higher ozone concentrations (60 pphm; 0.6 ppm) inhibited radial growth of the *Alternaria* cultures, however spore production was accelerated while spore

viability was unaffected. The authors observed the loss of pigments and formation of light-refractive globules in the hyphae of *Colletotrichum* in response to ozone application. A 25% decrease in the average neutral-lipid content was observed in ozonated cultures, suggesting that contact with ozone suppressed synthesis of the lipids essential to the structure of cellular membranes.

James *et al*. (1982) found that some *Fomes annosus* (Fr.) Cke. isolates showed reduced growth rates and conidial production under ozone concentrations >0.10 ppm. Surviving cultures removed from ozone resumed almost normal rates of growth and sporulation. Spore germination and germ tube length were reduced as ozone concentration and duration of exposure increased. The colonising ability of the cultures was reduced following treatment with very high ozone levels (up to 0.45 ppm).

Rich and Tomlinson (1968) observed that *Alternaria solani* (Ell. & G. Martin) Sor. conidiophores treated with ozone stopped elongating and often had swollen and/or collapsed apical cells. Conidiophores resumed elongation when the cultures were removed from the ozone chambers but tended to grow on a different angle, as a function of the damaged tip cells. Further testing using an α-iodacetamide treatment (sulfhydryl-binding) showed that ozone treatments reduced sulfhydryl content relative to the non-treated hyphal mats.

Foegeding (1985) showed that the spore coat was important in determining the reaction of *Bacillus* and *Clostridium* spore populations to ozone treatments. Ozone rapidly inactivated 90 to 99 % of *Bacillus cereus* T spore populations in 15 minutes, whereas only 60% inactivation of *B. stearothermophilus* ATCC 1518 was achieved in the same time. When *B. cereus* were treated to remove or reduce the integrity of their coat proteins, 99.995% inactivation was achieved in 15 minutes. The author therefore theorised that the primary spore coat plays an important role in protecting pathogens against ozone.

Hodge (1998) states that resting state spores are 10 to 15 times more resistant to ozone than vegetative bacteria cells. Hodge (1998) indicates that the presence of multiple layers (including a thick cortex, a multilayered protein spore coat and an exposorium) increases resistance of bacterial spores to ozone. Bacterial species also differed in terms of ozone sensitivity (Hodge, 1998). Viruses are typically readily

destroyed by ozone, owing to the single protein layer in their protective coats (Hodge, 1998). Bacterial resistance to ozone increases (10 to 100X) when they are attached to the surface of produce (Hodge, 1998). Attachment involves the formation of extracellular organic molecules (Hodge, 1998). The presence of these organic films reduces the efficacy of ozone disinfection (Notermans and Kampelmacher, 1995 cited in Hodge, 1998).

Restaino *et al*. (1995) compared the susceptibility of a number of bacterial and fungal (yeast and mould) species to ozonated water. They determined that there were differences between gram-negative and gram-positive bacteria as well as yeasts and moulds. The ability of ozone to reduce the number of viable cells was reduced by the addition of organic matter to the solution. This reflects the decrease in residual ozone concentration that occurs as a result of the introduction of increasing amounts of reactive materials.

2.4.4 Ozone Effects on Plant Resistance to Disease

Eckey-Kaltenbach *et al*. (1994), Kangasjarvi *et al*. (1994) and Sarig *et al*. (1996) found that exposure to ozone may enhance plants' inherent resistance to disease. Sarig *et al*. (1996) found that grapes exposed to various ozone treatments exhibited increased phytoalexin production, which protected the fruit from fungal pathogens. Eckey-Kaltenbach *et al*. (1994) found that ozone treatments induced parsley to produce several defence compounds. Kangasjarvi *et al*. (1994) reviewed plant responses to ozone treatment, noting several changes in the phenylpropanoid and lignin biosynthesis pathways resulting in increased levels of flavanoids, phytoalexins, and lignins. Lignification and wound periderm formation are important factors in disease resistance in potatoes (Clarke and Kassim, 1977; Nnodu *et al.*, 1982; Vaughn and Lulai, 1991).

2.4.5 Effect of Ozone on Wound Healing

The wound healing process is critical in maintaining potato quality and minimising losses, as it prevents or slows disease development and decreases water loss. During the curing period (approx. three weeks at 15°C), potatoes lay down suberised tissue layers at the wound sites (Thomson *et al*., 1995). The rate of development and the degree to which this layer develops can mean the difference between susceptibility and

resistance to many post-harvest pathogens (Clarke and Kassim, 1977; Nnodu *et al*., 1982; Vaughn and Lulai, 1991).

The potential effects of ozone on wound periderm formation and structure must be examined before it can be adopted as a post-harvest treatment in potatoes. Ozone is highly reactive and the organic compounds comprising the wound layers of potatoes, may be vulnerable to oxidisation. A number of studies have evaluated the potential of ozonation as a means to accelerate biodegradation of wood products high in lignin (Kaneko *et al*., 1983; Kang *et al*., 1995; Korai *et al*., 2001). Kaneko *et al*. (1983) suggested that ozone was a promising agent for use in wood bleaching operations, as ozone appeared to selectively react with lignin compounds. Kang *et al*. (1995) found that lignin compounds had somewhat of a protective effect on carbohydrate degradation by ozone, through competition for ozone reaction. They also suggested that ozone-lignin reactions may produce radicals that promote carbohydrate degradation.

Bono *et al*. (1985) also showed that ozone treatments altered the structure of lignin in wood samples. They found that ozone treatments were more efficient than gamma-rays in increasing cellulose accessibility. They suggest that this may be the result of increased delignification, through direct lignin degradation and through lignin solubilisation. These changes lead to greater susceptibility to fungal degradation. These studies suggest that ozone may influence the disease resistance of potatoes by altering wound periderm formation. Lapierre *et al*. (1996) studied the composition of the suberised layer of potato wound periderm to determine the resemblance between this layer and the lignin in woody angiosperm cell walls and xylem. They determined that there were certain commonalities and similarities, however chemical proportions and ratios were not identical in the two types of lignin-containing compounds.

Booker and Miller (1998) examined the effect of ozone treatments (as a pollutant) on phenylpropanoid metabolism and phenolic compound biosynthesis (e.g. lignin and suberin) in soybean (*Glycine max*) leaves. They showed that exposure to ozone increased lignin pathway products, but histochemical and other analysis showed no change in lignin or suberin levels as a function of exposure to ozone.

The effect of ozone on wound periderm in potatoes is not entirely clear, but there is the distinct potential for some ozone effects on this important tissue.

2.4.6 Ozone Treatment Considerations

2.4.6.1 Concentration and Duration of Exposure

The movement of ozone gas over commodity surfaces is limited only by the rate and uniformity of airflow within the storage; there should therefore be no coverage problems if there is sufficient air movement. This represents a significant advantage over disease control products applied as a liquid spray, as sprays do not always provide complete coverage. Application of sprays to stored product can also be somewhat problematic as the resulting increase in humidity and surface moisture can actually create disease problems.

Ozone application involves a range of treatment variables that must be optimised. Concentration and duration of exposure variables must be considered. Ozone concentrations are primarily determined by the amount of ozone being generated in a given time period, relative to the volume of the application space and/or the volume or quantity of produce being treated. The temperature and the relative reactive demand of the materials in the application chamber determines the rate of loss of ozone. Ozone concentration is consequently not static, but relatively stable concentrations can be achieved using variable rate application technology coupled with an ozone sensing system (Dave Loewen, Personal Communication). The greater precision offered by this type of system comes at a substantial additional cost, as the required control/sensing systems are complex.

Although the duration of ozone application is more readily controlled by operators, there is residual ozone left following termination of active ozone generation, therefore the duration of treatment is always somewhat longer than the duration of generation.

The combination of ozone concentration and duration of exposure determines the overall amount of ozone that contacts the produce. The total amount of ozone (concentration by duration of exposure) applied generally determines the reactive power of the treatment (Hibben and Stotsky, 1969; Liew and Prange, 1994). Hibben and Stotzky (1969) evaluated the relationship between ozone concentration and duration of exposure by exposing a number of fungal species to varying ozone concentrations for varying amounts of time. The fungal species responded differently to the varying ozone

treatments. In most species, application of higher concentrations of ozone reduced the time of exposure required to decrease spore germination. Similarly, effective inhibition of spore germination could be achieved with lower ozone concentrations providing longer exposure periods were used. The total amount of ozone required to achieve a set level of control (concentration by duration of exposure) varied between species.

Spotts and Cervantes (1992) also tested the effectiveness of ozone as a function of concentration and duration of exposure. They found that the LD_{95} (the dose at which 95% of the population died) values for the spores of *Botrytis cinerea* Pers.:Fr., *Mucor piriformis* E. Fisch. and *Penicillium expansum* Link were 1 µg/ml (1 ppm) for a five-minute exposure compared to 1.5 - 2.5 µg/ml (1.5-2.5 ppm) for a one-minute exposure. This indicated that the effect of total ozone exposure was not linear.

The relationship between concentration and duration of exposure will determine which method of application provides the best overall efficacy and cost efficiency. The most effective combination of ozone treatments must, in turn, be determined for each pathogen/commodity combination.

2.4.6.2 Residual Activity and Reaction Capabilities of Ozone

Chemicals applied to control post-harvest diseases may only react with surface pathogens at the time of contact, with no residual capacity, or they may be absorbed by the produce and act systemically. This difference in the modes of action helps determine the duration of efficacy of the product. Chemical fungicides used for post-harvest disease control are typically designed to react for extended periods (weeks to months), providing protection against both existing pathogens and any pathogens that arrive after the time of application. Eventually these chemicals are inactivated by soil or by chemical/microbial degradation. The rate of application of these products is determined by manufacturers' recommendations relating to disease pressure, environmental conditions and the mode of action of the chemical (protectant versus systemic). The duration and degree of protection provided is also affected by disease pressure and conditions before and after the time of application.

Even under the most ideal conditions, ozone is highly reactive and has a very short half-life (15-50 min). The half-life is further shortened by the presence of reactive materials, such as the commodity, debris or storage structures. Ozone degrades to

oxygen (O_2), which has no residual disease control activity (http://postharvest.ucdavis.edu/Produce/Ozone1.shtml; Law and Kiss, 1992).

Various authors have shown that the relative protective/reactive capability of ozone is limited to the surface of the commodity. Spotts and Cervantes (1992) demonstrated that ozone was relatively ineffective for controlling pathogens introduced in deep wounds (i.e. deeper than scrapes or bruises). Deeper penetration by pathogens is often associated with deep punctures and well-established infections. Berg *et al*. (1964) showed that the efficacy of ozone as a means of destroying microorganisms was increased by using ultrasonics to break up clumps of the target microorganisms. This would indicate that ozone does not penetrate dense clumps or into cellular masses, but rather is restricted to surface reactions.

2.4.7 Other Considerations

2.4.7.1 Safety Considerations

Several characteristics of the ozone molecule represent potential hazards or drawbacks when considering its use as a treatment for disease control in potato storages. Applicator/worker safety is critical with all agri-chemicals. Exposure to ozone can irritate the eyes, nasal passages and lungs, and cause shortness of breath. Prolonged exposure to low concentrations of ozone or brief exposure to more elevated levels can damage the respiratory system, leading to pulmonary oedema or other illness (http://ccinfoweb.ccohs.ca/). The eight-hour exposure limit for ozone is 0.08 ppm, and exposure to higher levels for shorter periods can result in the above problems. Exposure to levels higher than 1 ppm is not recommended for any duration of time and exposure to 50 ppm is potentially lethal within 30 minutes (http://ccinfoweb.ccohs.ca/). Ozone monitoring devices are available to insure safe application. The odour of ozone is very distinctive and easy to detect. Ozone levels higher than 1 ppm smell like chlorine and become increasingly acrid well below the toxic exposure limit (Personal Communication, Dave Loewen, Crystalair). Respirators are advised in areas where ozone is being applied (http://siri.org/msds; http://ccinfoweb.ccohs.ca).

2.4.7.2 Effect of Ozone on Materials and Structures

Potential negative effects of ozone on mechanical and non-organic surfaces in the storage and surrounding area must be considered. Prolonged exposure of non-

resistant materials (e.g. rubber) to ozone can result in degradation and weathering, similar to that observed due to prolonged exposure to UV light. PVC plastic materials and some metals may also be affected with prolonged exposure to ozone (Dave Loewen, Crystalair, Personal Communication). Equipment exposed to ozone can deteriorate over time without proper maintenance and replacement. Materials such as rubber or plastic hoses and fittings vary in their chemical resistance and proper consideration should be given when using ozone in concentrated or prolonged applications. Other post-harvest products such as Purogene® (chlorine dioxide) are also known corrosives, and can lead to deterioration of storage materials (Norikane *et al.*, 2000). Ozone applied at the rates recommended for purification of residential or commercial environments do not lead to any appreciable breakdown or damage (Dave Loewen, Crystalair, Personal Communication).

2.4.7.3 Cost

Cost is a critical component when calculating the relative merit of various management options. Fungicide application costs vary with the number of applications and the chemical involved. TBZ costs $6.40 per tonne when applied at the Canadian label rate, while Dithane (mancozeb), costs $11.06 per tonne at the label rate. Purogene® (chlorine dioxide, ClO_2) costs approximately $2.50 per ton for a pre-storage spray treatment followed by monthly applications over five months. Ozone (O_3) generation costs are primarily the cost of electricity required to run the generator at the desired output concentration. The remainder of the cost is for the ozone generation unit, which vary greatly in cost, depending on capacity and supplier (Dave Loewen, Crystalair, Personal Communication). Additional control equipment (i.e. sensors, etc.) also increase the cost of ozone systems. Cost calculations (power only) for an ozone generation system from a Saskatchewan farm would be approximately $0.0075/tonne of potatoes for a pre-storage application.

Ozone appears to be a lower cost alternative for the post-harvest control of disease in potatoes, however effective rates and evaluation of treatment efficacy must be determined.

3. PRELIMINARY TRIALS: OZONE GENERATION AND DEGRADATION PROFILES

3.1 Introduction

Ozone (O_3) gas is highly reactive chemical oxidiser produced by the splitting of oxygen molecules in a high energy-demand reaction. The ozone reaction is as follows:

$$3O_2 + \text{energy} = 2O_3$$

The energy required to produce the ozone reaction can come from a variety of sources. Ozone is produced in nature during lightning storms and is commonly produced commercially using electrical energy sources. Law and Kiss (1992) describe the mechanisms of ozone generation in detail. Commercially available ozone generators vary greatly in their production capacities and the accuracy of control of ozone outputs. The quantity of ozone produced in a generator depends on two main factors; the concentration of oxygen in the air passing over an energy source and the amount of energy used to generate the reaction. Increasing the amount of oxygen available for reaction will increase the amount of ozone produced by a given generator. This can be accomplished by using a purified oxygen source or by slowing the airflow over the energy source and out of the generator. These same factors are used to control the ozone concentrations in a given application. Ozone generators capable of producing as little as several milligrams of ozone per hour to as much as hundreds of grams per hour are available.

As a highly reactive gas, ozone has several characteristics that present problems in situations where it is going to be applied. Ozone has a very short half-life (15-50 minutes), degrading back to oxygen if no reaction takes place. Higher temperatures increase the rate of degradation. Ozone must therefore be generated on-site. Ozone is highly reactive, combining with both organic and inorganic substances to produce generally non-toxic substances (www.iqx.com). The amount of degradation or loss of ozone is dependent upon the "ozone demand" or reactivity of a given application

environment (Kim *et al*., 1999b). To achieve and maintain a given concentration of ozone, one must match the ozone generation rate to the degradation and reaction rates. Control of ozone levels in an environment can be accomplished by coupling an ozone metre to an ozone generator and a series of electronic controllers which trigger generation of ozone to maintain ozone levels within a pre-determined range. This type of equipment is costly and can vary in accuracy (Dave Loewen, Personal Communciation).

Ozone has excellent reactive potential in biological reactions, however the efficacy of an ozone application will vary depending on a number of factors. First, the amount of ozone applied will affect the outcome of the application. The total amount of ozone applied increases with; a) an increased concentration of ozone or b) an increase in the duration of the application. The efficacy of ozone application generally corresponds to the total amount of ozone applied (Liew and Prange, 1994). Varying the concentration and duration of exposure on a number of fungal spore samples showed that high ozone concentrations for short periods of time or low ozone concentrations for longer periods of time resulted in similar degrees of control (Hibben and Stotsky, 1969). Liew and Prange (1994) varied ozone application rates by adjusting the duration of exposure to the set ozone concentrations. The biologically effective concentration of the ozone application is also affected by the pathogen. Pathogens vary in chemical resistance to ozone (e.g. bacteria vs. fungi; hyaline spores vs. darker spores), which will significantly influence the amount of ozone required for effective application (Hibben and Stotsky, 1969; Hodge, 1998).

To produce an effective application of ozone, the dynamics of the ozone reaction within a designated environment must be understood. The capacity of the generation system must be tested in the designated application environment.

The objectives of this experiment were to:

1) determine the generation capacity of commercial ozone generators under different reaction regimes in treatment facilities suited for potatoes.
2) map the generation and degradation curves of ozone under different reaction regimes to determine the best application method for the available ozone generation equipment and storage facilities.

3.2 Materials and Methods

Crystalair Canada Inc. (Delta, BC) supplied custom-built corona-discharge type ozone generators capable of generating up to 600 mg O_3/hr. Tests were conducted using one, two or three generators (600, 1200 and 1800 mg O_3/hr) in an empty storage room (~18 cu. metres) at 15°C. This temperature represents the standard for the pre-storage potato curing period. The room was sealed, but was equipped with dual internal air circulation fans. The walls were lightweight plastic or galvanised metal, which are fairly resistant to reaction with ozone (Dave Loewen, Personal Communciation; Chemical reactivity tables, VWR CanLab Catalogue, 1999). Ozone levels were measured using an ozone monitor with a 0-100 ppm measurement range (Model Series #AMC 1100 Armstrong Monitoring Corp., Nepean, ON, Canada). This monitor was suspended in the centre of the room, to evaluate ozone levels within the airflow channels of the room. The ozone levels were recorded every minute until changes became less rapid. Once ozone levels had plateaued, the ozone generators were turned off and the degeneration or degradation process was monitored until no ozone was detected. Depending on the number of ozone generators present, ozone levels were monitored for up to one week.

To evaluate the impact of reactive materials on ozone levels, the test was repeated using three ozone generators (1800 mg O_3/hr) with 350 kg of newly harvested potatoes in the room. Potato tubers were not washed or treated in any way prior to testing.

3.3 Results

In the trials where ozone was added to an empty room, the generation of ozone followed a generally asymptotic curve, with ozone concentration increasing rapidly initially and then slowing as the ozone concentrations approached the maximum obtainable concentration (Fig. 3.1a). Increasing the number of ozone generators increased the maximum levels of ozone in the room (Table 3.1; Fig. 3.1b,c). The degradation curves were approximately the same shape as the generation curves, except the time required to return to zero ozone was shorter than that observed for the initial generation curves. The number of generators used influenced the peak ozone levels obtained, with more units resulting in an arithmetic increase in peak ozone concentrations (Table 3.1).

The T_{50} value, or the time required for ozone levels to reach half of the maximum levels increased with the number of generators present (Table 3.1). Vmax values, or the rate which maximum or minimum levels were achieved were similar for two and three generators, however the peak ozone concentration was higher for the three generators (Table 3.1). Degradation T_{50} values were similar to generation values (Table 3.1).

Because of the relatively non-reactive composition of room materials, the major cause of loss of ozone during the degradation period would likely be the spontaneous degradation of the ozone molecules back to oxygen.

When the room contained reactive surfaces such as potatoes and the associated dirt/debris, the ozone generation curve was distinctly lower and each incremental increase in the ozone levels took longer to achieve (Table 3.1; Fig. 3.1c). Ozone degradation was also very rapid when reactive materials were present (Table 3.1; Fig. 3.1c).

Table 3.1 Ozone accumulation/degradation V_{max} and T_{50} values for several generators

Ozone	Generation			Degradation		
	O_3 Maximum (ppm)	**Time to O_3 Maximum (hr) V_{max}**	**T_{50} (hr)***	**O_3 Maximum (ppm)**	**Time to O_3 Minimum (hr) V_{max}**	**T_{50} (hr)****
1 generator (600 mg O_3/hr)	2	2.0	0.18	2	2.0	0.45
2 generators (1200 mg O_3/hr)	8	23.2	0.70	8	10.0	0.70
3 generators (1800 mg O_3/hr)	14	25.2	1.45	14	7.0	0.97
3 generators w/ potatoes	8	215.5	72.2	8	3.0	0.62

*T_{50} = time to 50% of maximum ozone concentration; **T_{50} = time to 50% of starting concentration
V_{max} = rate at which maximum or minimum level is achieved

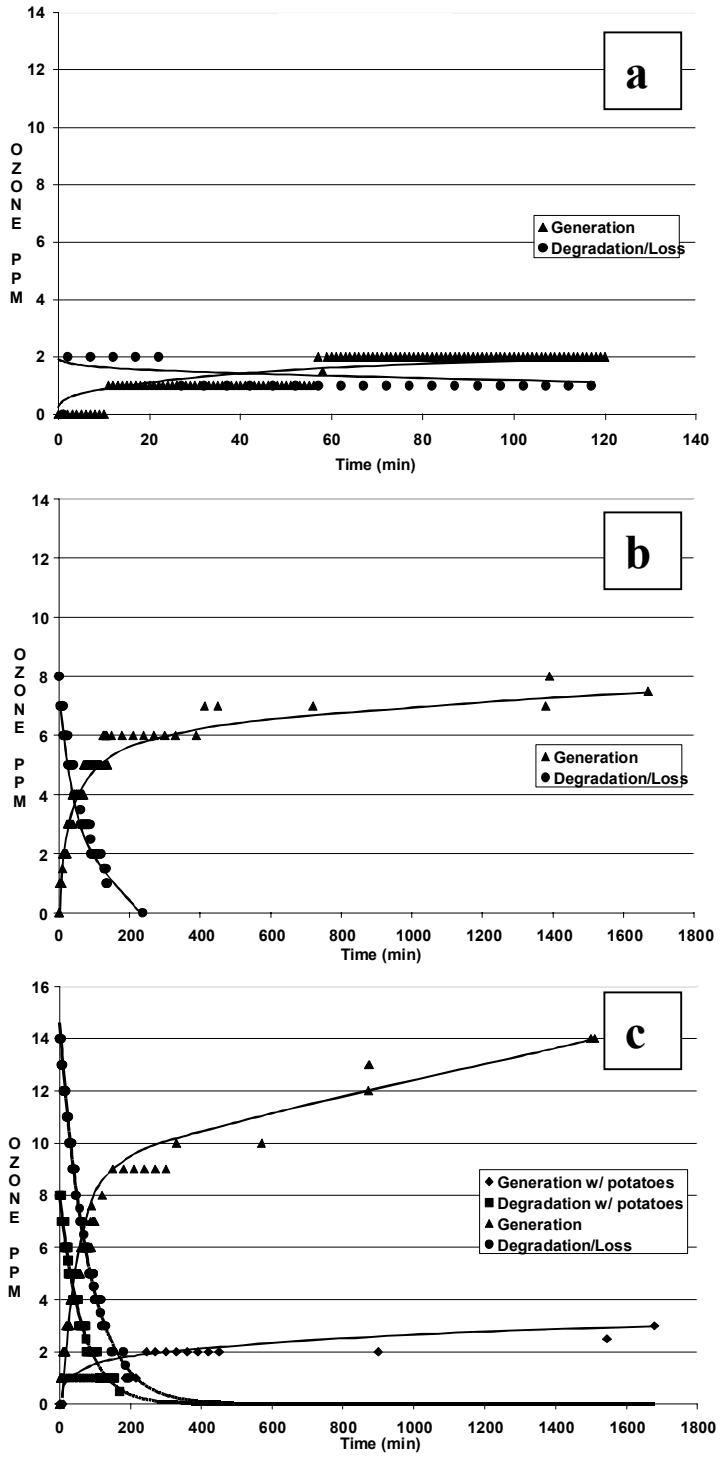

Figure 3.1 Ozone generation/degradation curve for; a) a single generator (600 mg O_3/hr output), b) two generators (1200 mg O_3/hr), c) three generators (1800 mg O_3/hr output with or without 350 kg of potatoes) in an 18 m^3 room.

3.4 Discussion and Conclusions

Ozone is an unstable and highly reactive compound, with a residence time duration determined by environmental conditions and the availability of reactive substances. Achieving a prescribed ozone concentration in any environment involves balancing ozone application with degradation and reaction. Ozone application is therefore a continuous process, requiring constant addition of ozone to reach and maintain target levels (Figure 3.1a,b,c). In this trial ozone levels within storage climbed rapidly at the onset of treatment, then the rate of increase slowed. The maximum concentration and the rate of increase were related to the number of generators present in the room. Klingman and Christy (2000) observed similar generation curve characteristics when they ozonated water for apple treatment.

When the ozone source was removed, ozone concentrations dropped rapidly initially, with a reduction in degradation rates as time passed. The addition of a quantity of potatoes to the storage rooms resulted in a dramatic reduction in the maximum peak ozone concentrations achieved as well as an increase in the degradation rate following removal of the ozone source. This reflects an increase in the availability of reactive surfaces.

Sarig *et al*. (1996) measured ozone levels in a treatment chamber for grapes. The presence of grapes in the chamber reduced the levels of ozone measured at the exit of the treatment chamber (or the peak ozone concentration achieved) compared to when grapes were not present. In a review by Kim *et al*. (1999b), the impact of the presence of organic materials or high reactive demand materials (such as debris or high levels of pathogenic propagules) is discussed. This review emphasises that the amount of ozone required to reach specific ozone concentrations can vary greatly, depending on the ozone demand of the test system. Restaino *et al*. (1995) reported that death rates of microorganisms during ozone treatment were significantly reduced with the addition of organic materials to the treatment solution. Venosa (1972) cited in Kim *et al*. (1999b) suggested that in order to ensure application accuracy, the levels of ozone going in and coming out should be monitored in an ozone application rather than simply the application concentration.

Liew and Prange (1994) addressed the issue of residual ozone concentrations following termination of ozone application. They found that residual ozone levels were related to the initial ozone concentration and temperature. Higher initial concentrations and low storage temperatures maximised residual ozone levels. As storage temperatures alter ozone activity, tests must be conducted at compatible temperatures.

Given the dynamic nature of the interaction between the ozone and reactive surfaces and materials, an alternative approach for generating comparable treatment levels would be to provide a set amount of ozone applied per unit weight of the commodity. This represents a simpler approach than attempting to achieve and maintain a set concentration, as it eliminates the need for a complex and expensive system designed to maintain specific ozone concentrations. Instead, generators with specific capacities could be used, with treatment levels determined by the amount of materials present (mg O_3/kg/hr). This application style is similar to a rate-based system of chemical application and avoids error factors such as atmospheric disruptions, variable reaction rates, temperature effects, etc.

Ozone generators vary greatly in their generation capabilities. Similarly, pathogens vary greatly in their resistance to ozone. This necessitates customisation of the ozone application based on commodity, equipment, pathogen, etc. Preliminary trials demonstrated the ability of the supplied generators to produce ozone concentrations of up to 100X the human daily exposure limit, even with reactive materials present (Table 3.1). Biologically active levels of ozone vary according to the commodity and pathogen, therefore it is difficult to determine if the supplied generators were producing biologically active amounts of ozone. Horváth *et al*. (1985) state that ozone applications of 7 to 8.4 ppm were effective in controlling late blight in potatoes. Other studies give a wide range and variety of effective ozone concentrations and exposure times. Ozone rates in the main trials will be determined initially by the generation capability of supplied generators and then adjustments to treatment concentrations and duration of application will be made. As highly complex equipment is required to achieve specific concentrations, a rate-based application system was selected for use in subsequent experiments.

4. PRE-STORAGE APPLICATION OF OZONE

4.1 Introduction

Control of post-harvest diseases of potatoes has historically been achieved through management of storage conditions (Butchbaker *et al*., 1972) and application of chemical disease-control products to the potatoes as they come into storage (Carnegie *et al*., 1998; Harrison and Franc, 1988; Ogilvy, 1992). Concerns about pesticide residues left on the potatoes, coupled with increasing incidence of potato pathogens developing resistance to approved pre-harvest fungicides (Hide *et al*., 1988; Platt, 1997; Satyaprasad *et al*., 1997; Thomson and Waterer, 1999) has created a need for new effective, environmentally friendly measures for managing post-harvest diseases in potatoes.

Ozone (O_3) represents a potentially useful tool for disease control in stored potatoes. The highly reactive ozone molecules exert significant antibiotic activity on a range of disease-causing organisms, including fungi and bacteria (Kim *et al.*, 1999b). As a gas, ozone can move in and around produce piled within storage, potentially providing thorough, uniform disease control. Potato storages are designed to provide air flow throughout the piled potatoes. Ozone can be added into the ventilation system, where it is pumped throughout the storage. Ozone degrades to oxygen (O_2), leaving behind no toxic residues. This is beneficial from a food safety perspective, especially with increasing public concern about food quality and pesticide residues (Camire *et al*., 1995). Ozone also works as an ethylene abatement device and has been shown to prolong the post-harvest life span of various horticultural crops (Dickson *et al.*, 1992). Ozone may also stimulate plant defence mechanisms, again potentially increasing the post-harvest life span of stored commodities (Kangasjarvi *et al.*, 1994; Sarig *et al.*, 1996).

The short life span of ozone molecules necessitates constant generation if the treatment objective involves continuous exposure. The lability of ozone also makes it difficult to reproducibly treat a commodity with a prescribed concentration of

ozone.

Ozone treatments involve a combination of the rate of application or the concentration of ozone being generated and the amount of time that the commodity or pathogen is exposed to that level of ozone. The total amount of ozone represents the product of the concentration and duration of exposure. Spotts and Cervantes (1992) evaluated the effect of a number of ozone concentration by duration of exposure treatments on the germination of fungal spores and the development of disease in pears. They found that high concentrations of ozone with short durations of exposure (1.5 & 2.5 μg O_3/ml for 1min) reduced spore germination to the same degree as low concentrations of ozone with longer durations of exposure times (1.0 μg O_3/ml for 5min). Both the total amount of applied ozone and the application regime (i.e. duration of exposure, etc.) may affect the success of the ozone treatment. An appropriate concentration by duration of exposure regime must therefore be determined for each commodity and each treatment objective.

Ozone application appears to have little or no residual effect. For example, Liew and Prange (1994) found that ozone applications reduced mycelial growth of *Botrytis* and *Sclerotinia* in inoculated carrots, however the pathogen was not killed and resumed growth when ozone applications ceased. The lack of residual control presents the possibility for re-growth if control is not complete. Consequently, repeated or continuous applications at low concentrations must be considered. Alternatively, increasing the duration of exposure to ozone can break down barriers and increase the efficacy of treatments (Hodge, 1998). Potato storage design allows for a continuous application of ozone after potatoes enter storage. Continuous or repeated applications of ozone could potentially increase the level of control provided and suppress the build up of disease. Song *et al*. (2000) applied ozone continuously to onions for the duration of the storage period and found that airborne spore counts and surface symptoms of onions were reduced.

When developing any new agrochemical product or process, treatment effects on produce quality must be evaluated along with associated treatment costs.
The objectives of this experiment were therefore to:

1) evaluate the efficacy of pre-storage ozone application for the control of post-harvest pathogens of potato as a function of duration of exposure and ozone concentration.
2) evaluate the efficacy of continuous application of low concentrations of ozone throughout the storage period.
3) determine if ozone application affects tuber quality characteristics.

4.2 Materials and Methods

The trials were conducted on field-run potatoes obtained from commercial growers in Saskatchewan. This ensured that the handling practices and disease characteristics of the experimental material were representative of "real world" situations. In the Fall 1999, two potato cultivars (Norland and Russet Burbank) were obtained from Hyland Seed Potato Farms, Scott, SK while in Fall 2000, one cultivar (Norland) was obtained from Barrich Farms, Outlook, SK. Norland is a red-skinned, early-maturing table potato cultivar that is very sensitive to *Fusarium* dry rot during storage. Russet Burbank is a late-maturing russet-type potato that is somewhat more resistant to post-harvest disease than Norland. The potatoes had been recently harvested and had not completed wound healing prior to the onset of the trial. Upon delivery, the potatoes were counted (20) into 5kg mesh bags and weighed in preparation for treatment.

The ozone was supplied using custom-made corona-type ozone generators supplied by Crystalair Canada Inc. (Delta, BC), capable of generating approximately 600 mg O_3 per hour. The tubers were held in 18m^3 cool storage rooms equipped with circulating fans. Rooms were relatively non-reactive, being built of plastic and metal materials. The rooms were held at 15°C to simulate the pre-storage curing period. Rooms contained zero, one, two or four of the generators, resulting in ozone generation rates of 0, 600, 1200 or 2500 mg per hour (based on preliminary generation tests, Chapter 3; 2.5 g O_3/hr generator). In the first year, the rates of ozone tested were calculated to be approximately 0, 2.8, 5.5 and 11.6 mg O_3/kg/hour. This was calculated based on an estimated average potato weight of 142 g. The potatoes were treated at these rates for 1, 7 or 21 days. Twenty-one days represents the standard curing period for potatoes (Schaupmeyer, 1992).

Based on the limited disease control observed in the first trial, it was decided that the rates of ozone applied should be increased in the second trial. The same exponential treatment scale was used, but the rates were increased to 0, 5, 10 or 20 mg O_3/kg/hr. Ozone generator capacities were such that a reduction in the number of cultivars was required, to allow for other treatment adjustments (i.e. addition of continuous ozone treatments).

In both years, potatoes were sampled immediately following the ozone treatments and again after three and six months storage at 4°C which is the standard regime for the storage of seed potatoes (Burton, 1966). Potatoes were evaluated for changes in disease level, physiological quality (skin colour) and weight loss. Weight loss was evaluated by weighing each treatment bag before the ozone treatment and then prior to washing at all subsequent sampling dates. The tubers were gently hand-washed to remove dirt and chemical residues, which might interfere with the accuracy of the colour and disease evaluations. Tubers were not scrubbed to the point of damaging the skin or removing surface disease. Following washing, tubers were placed on paper towels to air dry.

In the first year, the potatoes were examined for levels of *Fusarium spp*., *Erwinia carotovora*, and *Rhizoctonia solani*, which cause dry rot, soft rot and black scurf, respectively. In the second year, *Helminthosporium solani* (silver scurf) was added to the list of diseases evaluated, as this is a significant post-harvest disease in Western Canada. The effect of ozone treatments on the viability of *Rhizoctonia* sclerotia was also examined.

The number of tubers per treatment bag (total 20 tubers) with soft rot (*Erwinia sp.*) was recorded following weighing and any soft rot-damaged tubers were then discarded. In the first trial year, 15 potatoes from each treatment bag were individually scored for percent surface area infested with *Rhizoctonia solani*, using a predetermined scoring chart (developed by W.C. James for the Canadian Food Inspection Agency). Possible scores included 0, 1, 5, 10 and 15 % of the tuber surface area affected by *Rhizoctonia*. The same tubers were then sliced three times, perpendicular to the growth axis of the tuber, to provide four slices. These slices were rated for the approximate

percent of dry rot per slice, using a ranking system of 0 – 6 corresponding to a range of disease per slice. The ranks were as follows:

0	0 percent of slice with rot
1	1 to 5 percent of slice with rot
2	5 to 10 percent of slice with rot
3	10 to 25 percent of slice with rot
4	25 to 50 percent of slice with rot
5	50 to 75 percent of slice with rot
6	More than 75 percent of slice with rot

The amount of dry rot per slice was averaged over the four slices.

In the second trial year, soft rot incidence was determined as in the previous trial and ten tubers were then rated for percent surface area affected by *Rhizoctonia* and the percentage of the tuber damaged by *Fusarium* dry rot. To test the survival of *Rhizoctonia* sclerotia following ozone treatments, at least five sclerotia were isolated from the selected tubers from each treatment replicate. These sclerotia were placed onto Water Agar (WA) media plates and incubated for 24 hours at room temperature, at which time the percent germination of the sclerotia was determined. If the required five sclerotia could not be obtained, the number of sclerotia was noted and the percentage germination calculated accordingly. To evaluate the potential of ozone to control the spread and development of silver scurf (*Helminthosporium solani*), five tubers from each treatment replicate were incubated for 3 weeks at room temperature in polyethylene tubs lined with water-saturated paper towel. These conditions are ideal for the development of the spore-bearing structures of *Helminthosporium solani*. The percent surface area covered with *Helminthosporium* silver scurf lesions was determined at the end of the incubation period based on 0, 1, 5, 10, 15, 25, 50, or 75 % of the surface area infected (Based on a CFIA key for Scab Severity).

In both trial years, skin colour of the tubers was evaluated using a Miniscan XE colorimeter (Hunter Assoc., Reston, VA), modified with a foam collar to insure a light-tight fit between the potato and the sensor. Ten tubers from each treatment replicate were analysed. The “L”, “a” and “b” values were recorded. Table 4.1 describes the meaning of each colour value.

Table 4.1 Interpretation of Hunterlab Colorimeter Values

COLORIMETER VALUE	MEANING
L	Denotes the lightness of the objects on a 0-100 scale, with white equalling 100 and black equalling 0
A	Refers to the red and green colour ranges, with positive "a" values denoting red and negative "a" values denoting green colour.
B	Refers to the yellow and blue colour ranges, with positive "b" values denoting yellow and negative "b" values denoting blue

Where appropriate, the data were transformed to reduce variability of experimental error and to normalise the data. Data for the percent surface area or percent rot affected by the various diseases (*Helminthosporium, Rhizoctonia, Fusarium*) were transformed using square root transformations [SQRT (#+0.5)] as determined by reference to Gomez and Gomez (1984). The experiment was conducted utilising a Completely Random Design with a four (rates) by three (duration of exposure) factorial array of treatments and repeated measures. Each treatment was replicated four times in the first year and three times in the second year. The cultivars were treated and analysed separately. This experiment was conducted twice, but as treatments were not consistent in the two trials, the data were analysed separately.

The data were analysed via the GLM procedure of the SAS statistical analysis package (SAS Institute Inc.). Main effect means were compared using Least Significant Difference (LSD) tests (p=0.05) based on the significance of the analysis of variance (ANOVA), which will show large treatment differences.

The first two trials focussed on ozone application during the wound healing period. Continuous application of low concentrations of ozone over the duration of the storage period could further enhance disease control. To test this hypothesis, in the second trial, potatoes that had received the previously described pre-storage ozone treatments were also treated with approximately 1.9 mg O_3/kg/hr continuously for the duration of the 4°C storage period. These tubers were also sampled three and six months after treatment. At each sampling period, potatoes receiving the continuous ozone treatment were compared to those stored under the standard ozone-free conditions for differences in disease levels and quality as previously described.

Approximately four to eight weeks after the initiation of the long-term ozone treatment, one of the ozone generators failed. This failure was not immediately detected

and ozone generation stopped for a period of approximately four to eight weeks. Upon discovery of the problem, the generator was replaced and the ozone application was continued.

4.3 Results

4.3.1 Disease Analysis

4.3.1.1 Short-term Pre-storage Ozone Treatments

In the first trial, the incidence and severity of all diseases were quite low throughout the storage period (Tables 4.2 & 4.3). *Rhizoctonia* levels and the percent incidence of *Erwinia* soft rot decreased significantly with time in storage in both Norland and Russet Burbank potatoes (Tables 4.2 & 4.3). The severity of *Fusarium* decreased over time in Norland potatoes (Table 4.2). Altering the ozone concentration had no impact on disease levels in either cultivar (Tables 4.2 & 4.3). Longer exposure to ozone decreased the percent surface area covered by *Rhizoctonia*. The percent incidence of *Erwinia* soft rot in Norland potatoes varied significantly with duration of exposure treatment (Table 4.2), however differences were variable and of limited value.

Table 4.2 Analysis of variance and main effect means for the effect of ozone treatments on disease variables of Norland potatoes (Trial 1)

SOURCE	DF	SQRT *Rhizoctonia* MS	SQRT *Fusarium* MS	DF	SQRT Soft Rot MS
CONCENTRATION (C)	3	1.04	5.19	3	0.09
EXPOSURE (E)	2	2.72**	3.71	2	0.23**
SAMPLE TIME (S)	2	34.28***	13.47***	2	0.31***
C*S	6	1.26*	2.80	6	0.09
E*S	4	0.65	4.46	4	0.11
C*E	6	0.84	4.11	6	0.07
C*E*S	12	0.68	4.19*	12	0.10*
REP	108	0.67***	2.54***		
ERROR	2008	0.36	0.17	108	0.06

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**

SQRT = square root transformed

MEANS‡

CONCENTRATION (mg O_3/kg/hr)	*Rhizoctonia* % Surface Area	*Fusarium* Rating (0-6)	Soft Rot % Incidence
0	2.01	0.37	0.36
2.8	1.75	0.27	0.28
5.5	2.14	0.33	0.03
11.6	2.00	0.26	0.17
EXPOSURE (DAYS)	**% Surface Area**	**Rating (0-6)**	**% SR**
1	2.16a†	0.38	0.13b
7	2.01a	0.30	0.44a
21	1.76b	0.24	0.06b
SAMPLE TIME (MONTHS)	**% Surface Area**	**Rating (0-6)**	**% SR**
0	2.93a	0.45a	0.48a
3	1.67b	0.21b	0.06b
6	1.34c	0.27b	0.08b

‡ Means are original data; † Mean separation is based on transformed data;
Values followed by the same letter are not significantly different (*p*=0.05)

Table 4.3 Analysis of variance and main effect means for the effect of ozone treatments on disease variables of Russet Burbank potatoes (Trial 1)

SOURCE	DF	SQRT *Rhizoctonia* MS	SQRT *Fusarium* MS	DF	SQRT Soft Rot MS
CONCENTRATION (C)	3	2.09	4.18	3	0.12
EXPOSURE (E)	2	0.33	4.68	2	0.07
SAMPLE TIME (S)	2	65.48***	3.38	2	2.02***
C*S	6	0.81	4.68*	6	0.06
E*S	4	3.07**	4.91*	4	0.03
C*E	6	0.91	3.47	6	0.07
C*E*S	12	0.54	3.87	12	0.11
REP	108	1.28***	2.57***		
ERROR	2009	0.40	0.17	108	0.11

, **, = significant at p=0.10, 0.05 or 0.01 respectively**

SQRT = square root transformed

MEANS‡

CONCENTRATION (mg O_3/kg/hr)	*Rhizoctonia* % Surface Area	*Fusarium* Rating (0-6)	Soft Rot % Incidence
0	2.35	0.30	0.42
2.8	2.69	0.26	0.53
5.5	2.96	0.17	0.28
11.6	2.59	0.26	0.50
EXPOSURE (DAYS)	**% Surface Area**	**Rating (0-6)**	**% SR**
1	2.73	0.29	0.31
7	2.66	0.27	0.58
21	2.55	0.20	0.40
SAMPLE TIME (MONTHS)	**% Surface Area**	**Rating (0-6)**	**% SR**
0	4.10a†	0.22	0.94a
3	2.05b	0.27	0.29b
6	1.80b	0.26	0.06b

‡ Means are original data; † Mean separation is based on square root transformed data
Values followed by the same letter are not significantly different (p=0.05)

Fusarium levels and the percent incidence of *Erwinia* soft rot in the Norland potatoes were significantly affected by the interaction between ozone concentration by duration of exposure and this relationship changed over time (Figures 4.1 & 4.2).

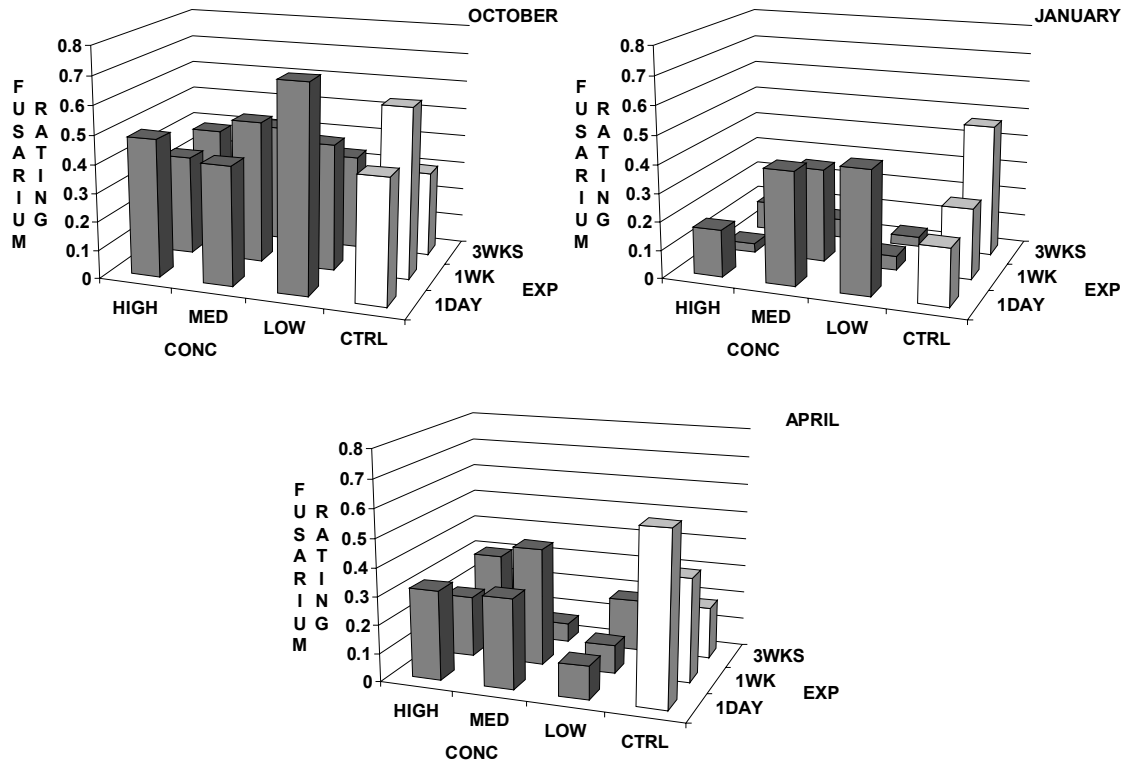

Figure 4.1 Average *Fusarium* ratings at differing points in the storage period of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.36; Jan=0.29; Apr=0.37]

These interactions appeared to be driven by changes in the control treatments and are therefore of limited relevance to the study of ozone effects. In the ozone-treated Norland potatoes, levels of *Fusarium* dry rot decreased after the initial sample time, with no significant change through the remainder of storage (Fig. 4.1). Increased exposure to ozone, whether by increased concentration or duration of exposure decreased incidence of *Erwinia* soft rot levels in Norland potatoes at the first sampling date. However at subsequent sampling dates, no differences were detected between ozone treatments and control treatments. *Erwinia* soft rot levels decreased with time in storage (Fig. 4.2).

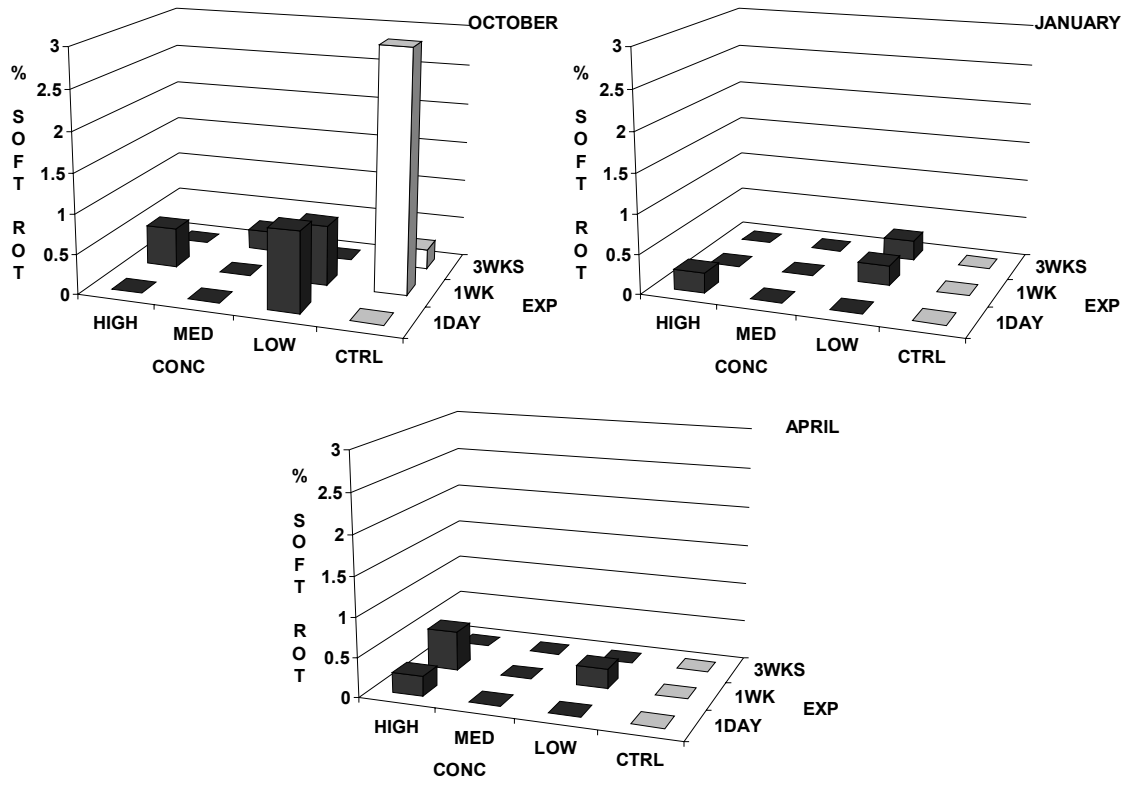

Figure 4.2 *Erwinia* soft rot incidence (percent tubers damaged) during storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=1.40; Jan=0.30; Apr=0.31]

In the second trial, disease levels were again low overall (Table 4.4). *Fusarium* dry rot increased significantly with time in storage, while levels of *Rhizoctonia* black scurf and *Helminthosporium* silver scurf on the tuber surface did not change over time, nor did the viability of the *Rhizoctonia* sclerotia (Table 4.4). Incidence of *Erwinia* soft rot was negligible in the second trial and this data was not presented.

Altering the ozone concentration had no impact on *Fusarium*, *Rhizoctonia* or *Helminthosporium* levels (Table 4.4). Longer exposure to ozone significantly decreased the percent surface area affected by *Rhizoctonia*. The viability of the *Rhizoctonia* sclerotia was not significantly affected by ozone treatment (Table 4.4).

Table 4.4 Analysis of variance and main effect means of the effect of ozone treatments on disease variables of Norland potatoes (Trial 2)

SOURCE	DF	SQRT *Rhizoctonia*	SQRT *Fusarium*	DF	SQRT Silver Scurf	DF	*Rhizoctonia* Sclerotia Germination
		MS	MS		MS		MS
$CONC^N$ (C)	3	1.03	0.04	3	0.41	3	687.29
EXPOSURE (E)	2	2.47***	0.00	2	0.09	2	498.96
SAMPLE TIME (S)	2	0.67	0.41***	1	0.00	1	486.92
C*S	6	0.70	0.01	3	0.05	3	552.71
E*S	4	0.45	0.04	2	0.07	2	83.20
C*E	6	0.43	0.01	6	0.47	6	314.75
C*E*S	12	0.41	0.01	6	0.10	6	536.23
REP	72	0.53***	0.03	48	0.21		
ERROR	972	0.37	0.03	288		45	610.21

, **, = significant at p=0.10, 0.05 or 0.01 respectively**

SQRT = square root transformed; $CONC^N$ = Concentration

MEANS‡

$CONC^N$ (mg O_3/kg/hr)	*Rhizoctonia* % Surface Area	*Fusarium* Rating (0-6)	Silver Scurf % Surface Area	*Rhizoctonia* % Germination
0	1.31	0.62	0.86	78.1
5	1.62	0.61	0.46	94.4
10	1.41	0.60	0.31	91.2
20	1.30	0.58	0.42	89.3
EXPOSURE (DAYS)	**% Surface Area**	**Rating (0-6)**	**% Surface Area**	**% Germination**
1	1.46a	0.60	0.62	93.6
7	1.58a	0.60	0.37	86.7
21	1.20b	0.60	0.55	85.5
SAMPLE TIME (MONTHS)	**% Surface Area**	**Rating (0-6)**	**% Surface Area**	**% Germination**
0	1.51	0.57b		
3	1.30	0.57b	0.59	91.2
6	1.42	0.66a	0.43	86.0

‡ Means are original data; † Mean separation is based on square root transformed data
Values followed by the same letter are not significantly different (p=0.05); $CONC^N$ = Concentration

4.3.1.2 Continuous Ozone Treatments

The analysis of variance and main effect means for the disease levels of potatoes treated with ozone both prior to and throughout storage is presented in Table 4.5. Continuous ozone application data cannot be compared statistically to pre-storage ozone treatment data for the second trial, as the treatments were not replicated. Therefore only differences in treatment trends will be discussed.

Disease ratings for potatoes that received the continuous ozone applications appeared to be lower than for potatoes that received only the pre-storage application (Table 4.6). Differences between pre-storage treatment components were not as

apparent in the continuous applications as in some of the pre-storage treatments. For example, the effect of different ozone treatments on silver scurf surface severity was similar for all concentrations and durations of exposure in tubers that had received continuous ozone treatments. In some cases, such as the viability of *Rhizoctonia* sclerotia, response to ozone treatments was reversed, i.e. viability appeared to decrease over time in continuous ozone compared to the increase that was generally observed with pre-storage ozone treatments (Table 4.6).

Table 4.5 Analysis of variance for the effect of continuous and pre-storage ozone treatments on disease variables of Norland potatoes

SOURCE	DF (DF SS/RS)	SQRT *Rhizoctonia*	SQRT *Fusarium*	SQRT Silver Scurf	*Rhizoctonia* Sclerotia Germ[n]
		MS	MS	MS	MS
CONC[N] (C)	3	0.55	0.01	0.08	1395.7
EXPOSR (E)	2	0.56	0.02	0.01	2578.1*
SAMPLE TIME (S)	1	0.20	0.21*	1.90***	2511.4
C*S	3	0.87	0.00	0.31*	1054.5
E*S	2	1.38	0.11	0.03	1061.4
C*E	6	1.22	0.04	0.22	1289.2
C*E*S	6	0.49	0.04	0.21	1650.4
REP	48	0.72***	0.06	0.12	
ERROR	648 (288/45)	0.38	0.04	0.12	991.9

*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively

SQRT = square root transformed; CONC[N] = Concentration; EXPOSR = Exposure

Table 4.6 Main effect means for the effect of pre-storage ozone ($PreO_3$) and continuous ($ContO_3$) treatments on disease variables of Norland potatoes

	Rhizoctonia% Surface Area		*Fusarium* Rating (0-6)		Silver Scurf % Surface Area		*Rhizoctonia* % Germination	
CONC[N] (mg O_3/kg/hr)	$PreO_3$	$ContO_3$	$PreO_3$	$ContO_3$	$PreO_3$	$ContO_3$	$PreO_3$	$ContO_3$
0	1.31	1.09	0.62	0.20	0.86	0.43	78.1	78.9
5	1.62	1.45	0.61	0.19	0.46	0.49	94.4	90.4
10	1.41	1.22	0.60	0.22	0.31	0.43	91.2	72.2
20	1.30	1.00	0.58	0.16	0.42	0.53	89.3	70.8
EXPOSURE (DAYS)	**% Surface Area**		**Rating (0-6)**		**% Surface Area**		**% Germination**	
1	1.46a	1.09	0.60	0.18	0.62	0.45	93.6	67.0b
7	1.58a	1.37	0.60	0.21	0.37	0.47	86.7	80.9ab
21	1.20b	1.11	0.60	0.19	0.55	0.50	85.5	86.7a
SAMPLE TIME (MONTHS)	**% Surface Area**		**Rating (0-6)**		**% Surface Area**		**% Germination**	
0	1.51		0.57b					
3	1.30	1.19	0.57b	0.26a	0.59	0.67a[†]	91.2	84.9
6	1.42	1.19	0.66a	0.16a	0.43	0.28b	86.0	72.0
OVERALL PRE vs. CONT	**1.25**	**1.19**	**0.15**	**0.19**	**0.51**	**0.47**	**88.5**	**78.3**

‡ Means are original data; † Mean separation based on square root transformed data;
Values followed by the same letter are not significantly different (p=0.05); CONC[N] = Concentration

4.3.2 Colour Analysis

4.3.2.1 Short-term Pre-storage Ozone Treatments

In the first trial, general skin colour characteristics of both cultivars (Norland and Russet Burbank) changed in a similar manner. As time in storage increased, the potatoes got darker (lower Hunter L), less red (lower Hunter "a") and more yellow (higher Hunter "b") (Table 4.7).

In Norland potatoes, ozone concentrations significantly increased skin lightness (Hunter L) and redness (Hunter "a") compared to controls (Table 4.7). Longer exposure to ozone also significantly increased skin redness in Norland potatoes (Table 4.7). The combined effects of the treatment variables (concentration and duration of exposure) on the Hunter L values for Norland potatoes varied over the storage period (Table 4.7; Fig. 4.3). The combined effect of the treatment variables had a significant effect on skin redness and yellowness which stayed consistent over time (Table 4.7; Fig. 4.4).

Table 4.7 Analysis of variance and main effect means for the effect of ozone treatments on skin colour of Norland and Russet Burbank potatoes (Trial 1)

SOURCE	DF	Norland			Russet Burbank		
		L	a	b	L	a	b
		MS	MS	MS	MS	MS	MS
CONC^N (C)	3	9.54***	2.06**	0.02	27.77***	0.49**	8.03***
EXPOSURE (E)	2	2.45	9.06***	0.53	25.48***	0.92***	6.47***
SAMPLE TIME (S)	2	34.36***	31.16***	16.69***	51.64***	2.36***	3.38***
C*S	6	1.08	0.29	0.29	2.54	0.21	0.74
E*S	4	4.46**	1.51	0.67*	22.35***	0.43**	4.22***
C*E	6	1.29	2.03**	0.62*	5.13*	0.16	1.03
C*E*S	12	2.74**	0.83	0.41	2.42***	0.17***	0.64***
REP	108	1.40***	0.77***	0.30***	2.42***	0.17***	0.64***
ERROR	1296	0.56	0.24	0.17	1.08	0.08	0.31

, **, = significant at p=0.10, 0.05 or 0.01 respectively; CONC^N = Concentration**

MEANS‡

CONCENTRATION (mg O_3/kg/hr)	Norland			Russet Burbank		
	L	a	b	L	a	b
0	21.3b	3.28b	2.89	22.7c	1.87b	4.37c
2.8	21.7a	3.40ab	2.91	23.0b	1.94a	4.57b
5.5	21.6a	3.42a	2.90	23.3a	1.94a	4.65ab
11.6	21.7a	3.46a	2.91	23.3a	1.95a	4.71a
EXPOSURE (DAYS)	**L**	**a**	**b**	**L**	**a**	**b**
1	21.5	3.31b	2.94	22.9b	1.88b	4.48b
7	21.5	3.31b	2.88	23.0b	1.92ab	4.54b
21	21.6	3.55a	2.89	23.3a	1.97a	4.70a
SAMPLE TIME (MONTHS)	**L**	**a**	**b**	**L**	**a**	**b**
0	21.8a	3.69a	2.69b	23.5a	1.97a	4.51b
3	21.6b	3.25b	2.97a	22.9b	1.95a	4.54b
6	21.3c	3.24b	3.04a	22.8b	1.84b	4.67a

‡ Means are original data; Values followed by the same letter are not significantly different (p=0.05)

Immediately after application of the ozone treatments, higher rates of ozone (higher concentration and longer exposure to ozone) appeared to increase Norland tuber lightness. Later in the storage period, this effect was less apparent as the tubers darkened with time (Fig. 4.3).

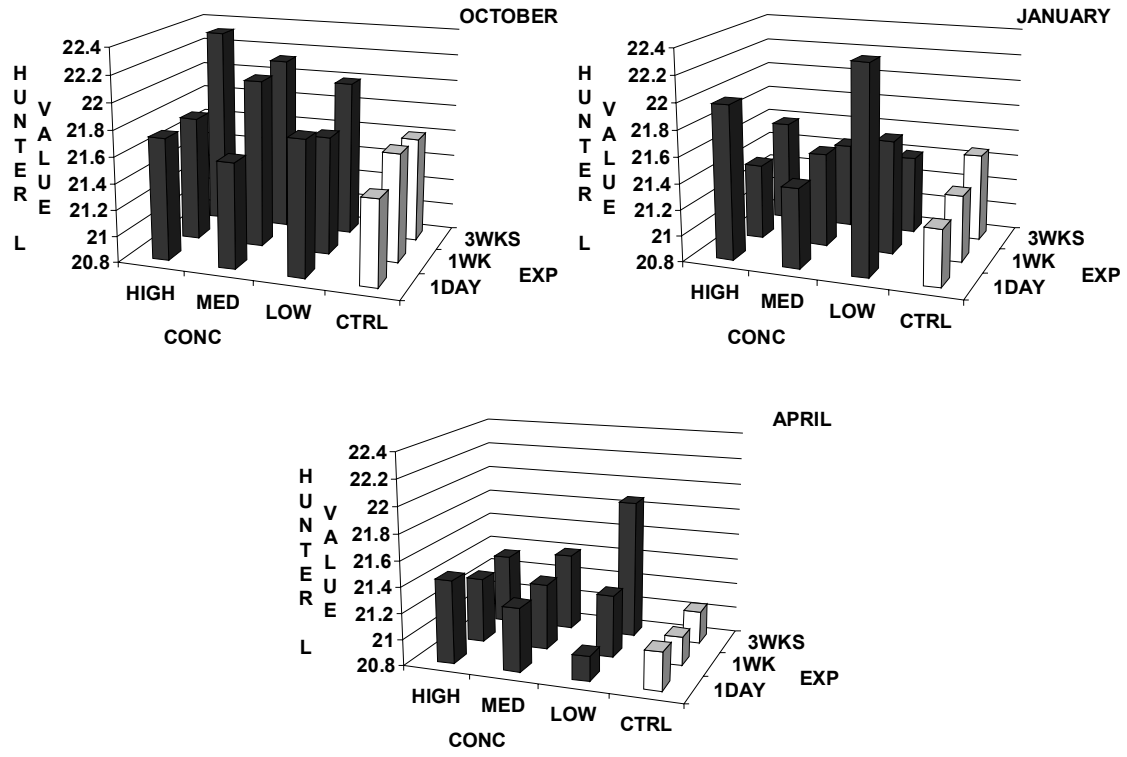

Figure 4.3 Lightness (Hunter L) values during storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.53; Jan=0.50; Apr=0.59]

The interaction between ozone concentration and duration of exposure to ozone was significant for redness (Hunter "a") and yellowness (Hunter "b") of Norland potatoes (Table 4.7; Fig. 4.4). Longer exposure to ozone appeared to be the key factor in changes to skin redness. Longer duration of exposure increased redness with increasing ozone concentration (Fig. 4.4). There was no consistency in response of Hunter "b" values to the interaction between ozone concentration and duration of exposure, as changes in skin yellowness (Hunter "b") were highly variable, with no consistent trends (Fig. 4.4.).

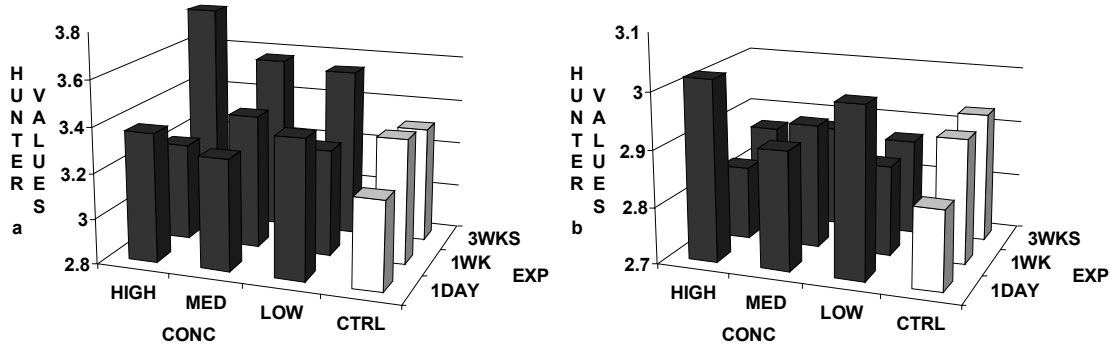

Figure 4.4 Redness (Hunter "a") and yellowness (Hunter "b") values of Norland potatoes treated prior to storage with ozone at varying concentrations and for differing exposure periods [LSD a=0.39; b=0.25]

In Russet Burbank potatoes, treatment with ozone again significantly increased tuber lightness and redness compared to the controls (Table 4.7). Increasing ozone concentration significantly increased skin lightness and yellowness compared to the control. Longer exposure to ozone also significantly increased skin lightness. The effects of the duration of ozone exposure and the interaction between ozone concentration and duration of exposure varied at differing points in the storage for all colour variables (Table 4.7; Figures 4.5, 4.6 & 4.7).

In Russet Burbank potatoes, Hunter L values at the first sampling date increased with total ozone applied (concentration by duration of exposure). This treatment effect was lost by the January sampling date, as all treatments darkened consistently with time in storage (Fig. 4.5).

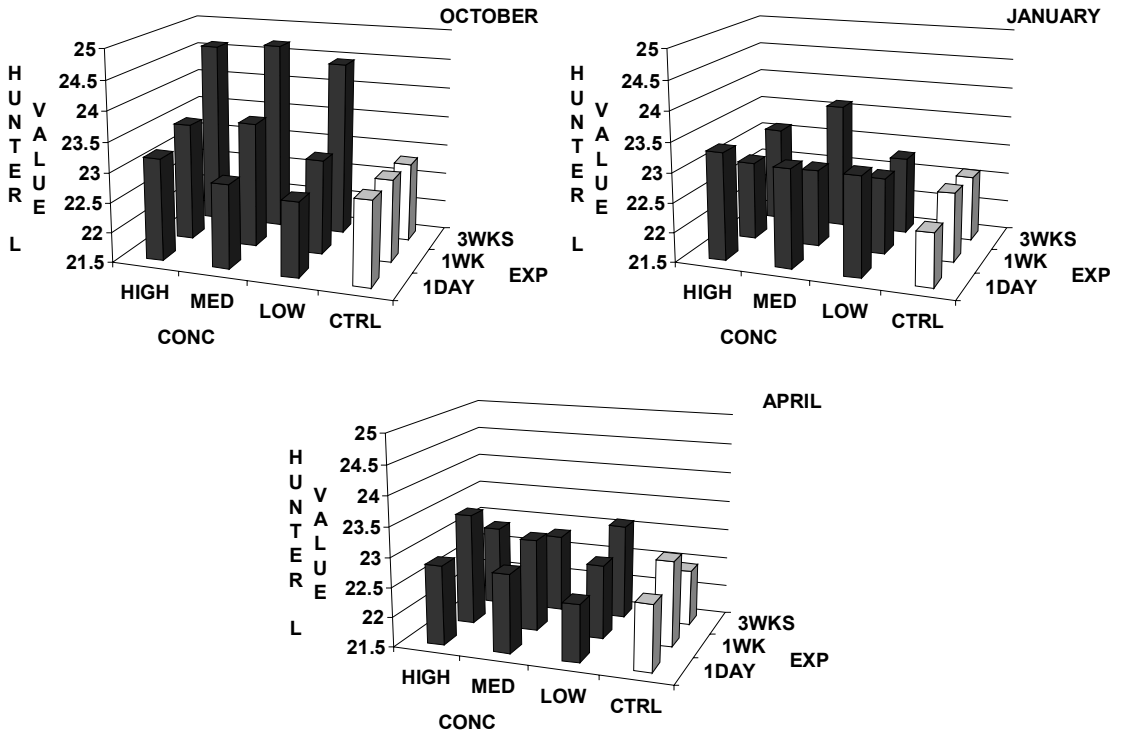

Figure 4.5 Lightness (Hunter L) values during storage of Russet Burbank potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.80; Jan=0.57; Apr=0.72]

Similarly, increasing total amounts of ozone (concentration by duration of exposure) increased skin redness (Hunter "a") of Russet Burbank potatoes immediately after application. By the mid-storage period, skin redness had decreased for all treatments, however treatment differences observed in the first sample period were still visible (Fig. 4.6). All treatment effects on tuber redness were lost by the end of the storage period.

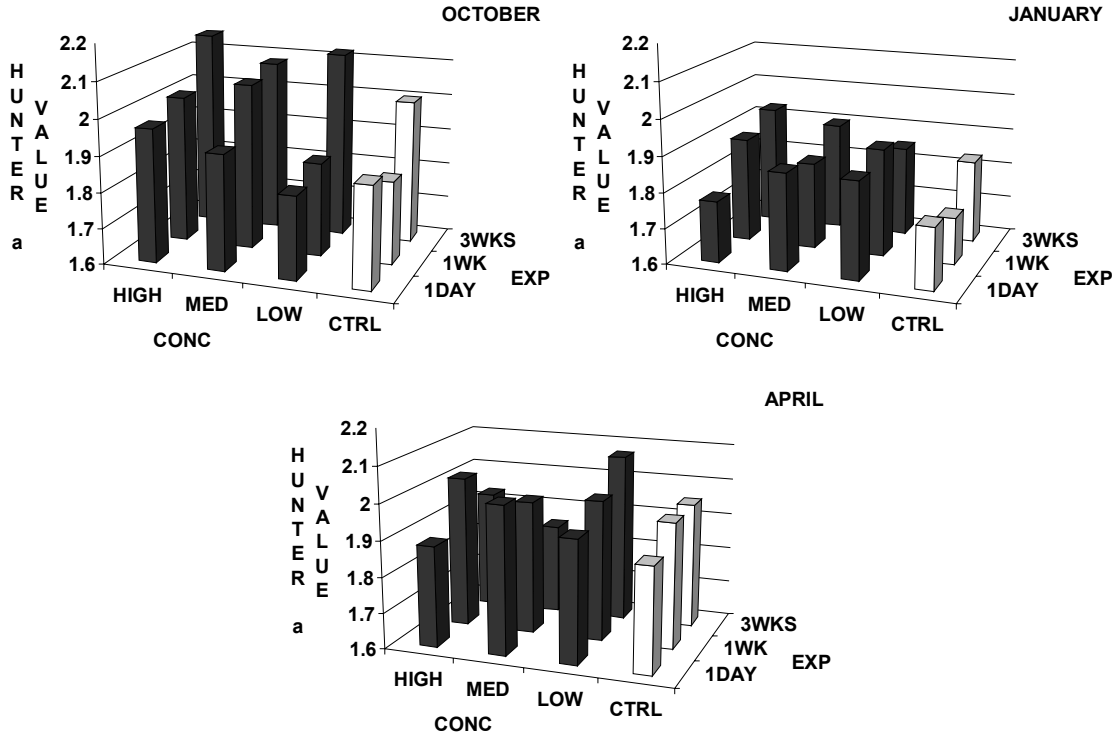

Figure 4.6 Redness (Hunter "a") values during storage of Russet Burbank potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.23; Jan=0.16; Apr=0.17]

Higher ozone rates (concentration by duration of exposure) dramatically increased skin yellowness (Hunter "b") values of Russet Burbank potatoes immediately after treatment. This effect was most apparent early in storage, however differences became more variable as time in storage passed (Fig. 4.7).

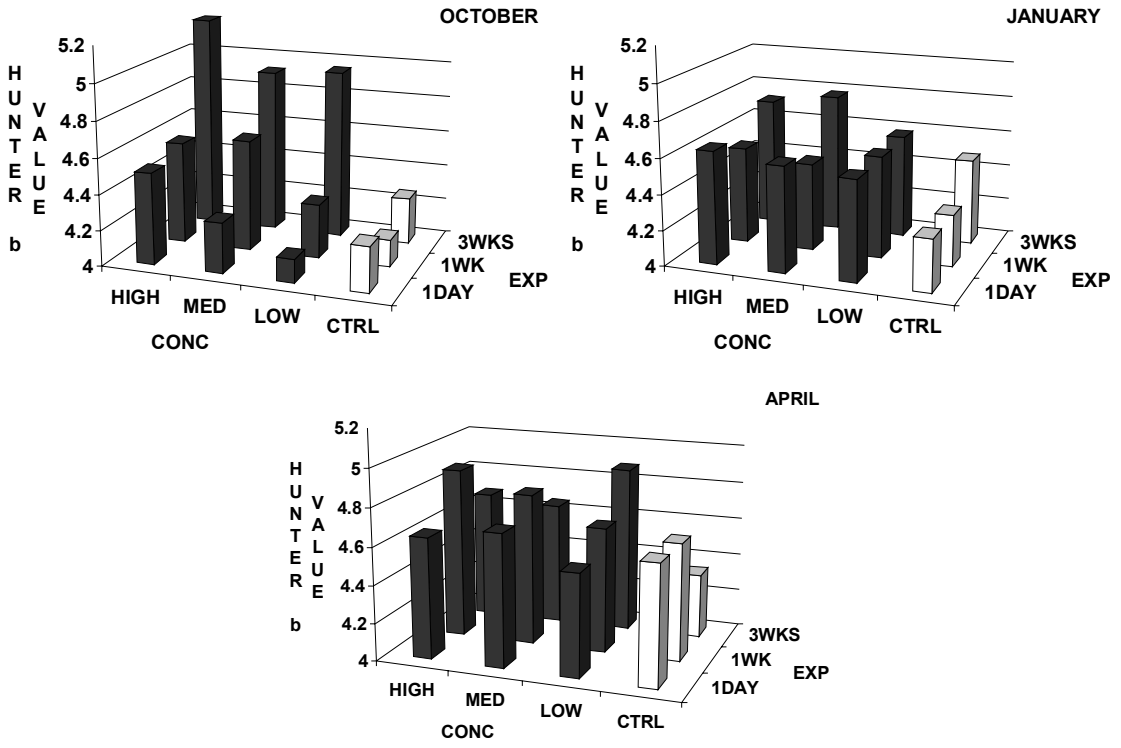

Figure 4.7 Yellowness (Hunter "b") values during storage of Russet Burbank potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.47; Jan=0.27; Apr=0.32]

In the second trial, which was restricted to the red-type Norland potatoes, the potatoes darkened during the first three months of storage, but then lightened towards the end of storage (Table 4.8). Tubers were significantly less red (Hunter "a") and more yellow (Hunter "b") in the latter part of the storage period. These general colour changes were consistent with the observations from the first trial.

Variations in ozone concentration did not affect colour variables in this trial. Longer exposure to ozone generally decreased lightness (Hunter L) values, although some variatiability was observed. Longer exposure to ozone increased skin redness (Hunter "a") of the Norland potatoes, but did not affect tuber yellowness (Hunter "b") (Table 4.8). This effect on skin redness was also observed in Trial 1.

Ozone treatments (ozone concentration by duration of exposure) affected lightness (Hunter L) and redness (Hunter "a") values (Table 4.8; Fig. 4.8) of the Norland potatoes. These interactions were not consistent across the sample periods.

Table 4.8 Analysis of variance and main effect means for the effect of ozone treatments on skin colour of Norland potatoes (Trial 2)

SOURCE	DF	L	a	b
		MS	MS	MS
CONC^N (C)	3	2.66	0.26	0.41*
EXPOSURE (E)	2	11.17***	2.47**	0.28
SAMPLE TIME (S)	2	144.15***	17.05***	16.12***
C*S	6	2.56	1.40*	0.27
E*S	4	2.61	2.78***	0.35
C*E	6	3.65*	2.03**	0.30
C*E*S	12	2.08	0.79	0.21
REP	72	1.74***	0.71***	0.19
ERROR	972	0.80	0.39	0.19

, **, = significant at p=0.10, 0.05 or 0.01 respectively; CONC^N = Concentration**

	MEANS‡		
CONCENTRATION (mg O_3/kg/hr)	**L**	**a**	**b**
0	22.47	3.92	3.97a
5	22.45	3.87	3.88b
10	22.48	3.94	3.91ab
20	22.66	3.91	3.90ab
EXPOSURE (DAYS)	**L**	**a**	**b**
1	22.52ab	3.87b	3.94
7	22.69a	3.86b	3.91
21	22.33b	4.01a	3.89
SAMPLE TIME (MONTHS)	**L**	**a**	**b**
0	23.62a	4.16a	3.73c
3	21.99c	3.75b	3.87b
6	22.33b	3.83b	4.14a

‡ Means are original data; Values followed by the same letter are not significantly different (p=0.05)

Longer exposure to ozone appears to have decreased lightness (Hunter L), while higher concentrations (with shorter exposures) appear to have slightly increased lightness (Fig. 4.8). Norland potatoes that received a longer exposure to ozone appeared to be more red (Hunter "a") than treatments receiving a shorter exposure to ozone (Fig. 4.8). The differences in colour were not substantial.

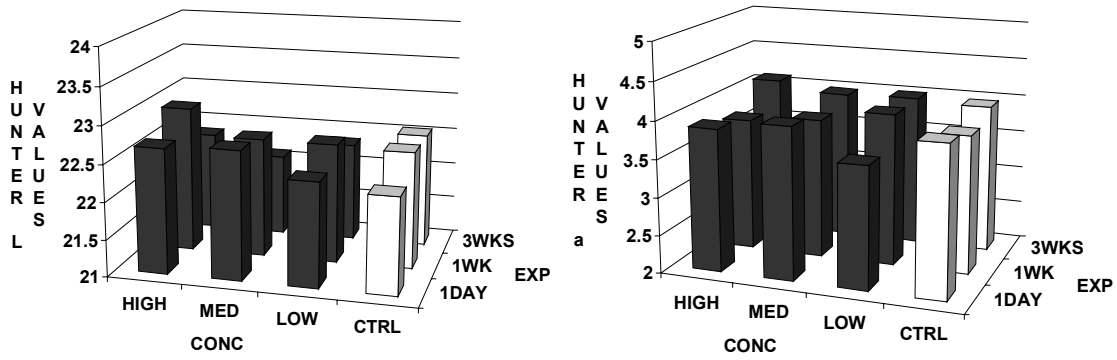

Figure 4.8 Lightness (Hunter L) and redness (Hunter "a") values of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD L=0.67; a=0.45]

4.3.2.2 Continuous Ozone Treatments

Skin colour responses to continuous ozone application could not be statistically compared to pre-storage ozone treatments due to a lack of replication (Table 4.9), however apparent differences in treatment trends will be discussed.

Tubers exposed to continuous ozone appeared to be more red and yellow (higher Hunter "a" and "b" values) and darker (lower Hunter L values) than tubers only treated with ozone prior to storage (Table 4.10). Continuous application of ozone appeared to accentuate the effects of the pre-storage ozone treatments (Table 4.10). The intensity of the red and yellow skin colour increased distinctly with time in storage in potatoes continuously exposed to ozone, while a less obvious colour change occurred in tubers treated solely prior to storage (Table 4.10).

Table 4.9 Analysis of variance for the effect of continuous ozone treatments on skin colour of Norland potatoes

SOURCE	DF (DF CONT)	L	a	b
		MS	MS	MS
CONC^N (C)	3	3.66	1.73**	0.40
EXPOSURE (E)	2	13.73**	4.99***	1.78***
SAMPLE TIME (S)	2	17.61**	11.06***	13.29***
C*S	6	0.76	0.40	0.06
E*S	4	13.45**	0.15	0.75*
C*E	6	4.38	1.45**	0.52*
C*E*S	12	1.75	0.39	0.39
REP	72 (48)	4.17***	0.58***	0.27***
ERROR	97 (648)	0.71	0.37	0.15

*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively; CONC^N = Concentration

Table 4.10 Main effect means for the effect of pre-storage ozone ($PreO_3$) and continuous ($ContO_3$) treatments on skin colour of Norland potatoes

	L		a		b	
CONCENTRATION (mg O_3/kg/hr)	**$PreO_3$**	**$ContO_3$**	**$PreO_3$**	**$ContO_3$**	**$PreO_3$**	**$ContO_3$**
0	22.47	21.96	3.92	4.14b	3.97a	4.04
5	22.45	21.86	3.87	4.13b	3.88b	4.00
10	22.48	21.95	3.94	4.20ab	3.91ab	4.03
20	22.66	22.19	3.91	4.34a	3.90ab	4.14
EXPOSURE (DAYS)	**L**		**a**		**b**	
1	22.52ab	21.91ab	3.87b	4.09b	3.94	4.04b
7	22.69a	21.80b	3.86b	4.15b	3.91	3.96b
21	22.33b	22.26a	4.01a	4.36a	3.89	4.13a
SAMPLE TIME (MONTHS)	**L**		**a**		**b**	
0	23.62a		4.16a		3.73c	
3	21.99c	22.15a	3.75b	4.08b	3.87b	3.91b
6	22.33b	21.83b	3.83b	4.33a	4.14a	4.18a
OVERALL PRE vs. CONT	**22.16**	**21.99**	**3.79**	**4.20**	**4.01**	**4.04**

‡ Means are original data; Values followed by the same letter are not significantly different (p=0.05)

4.3.3 Weight Loss

4.3.3.1 Short-term Pre-storage Ozone Treatments

In Trial 1, weight loss increased over time in storage in both cultivars (Table 4.11). Weight loss was higher in Russet Burbank potatoes than Norland potatoes (Table 4.11). In both cultivars, as ozone concentration and duration of exposure increased, the percent weight loss increased significantly (Table 4.11). In Norland potatoes, any concentration of applied ozone increased weight loss compared to the controls, while higher ozone concentrations were required to increase weight loss in Russet Burbank potatoes (Table 4.11). The interaction between ozone concentration and duration of exposure significantly increased weight loss in both cultivars at all of the storage stages sampled (Table 4.11; Figures 4.9 and 4.10).

Table 4.11 Analysis of variance and main effect means for the effect of ozone treatments on weight loss of Norland and Russet Burbank potatoes (Trial 1)

SOURCE	Norland		Russet Burbank	
	DF	Square Root Weight Loss	DF	Square Root Weight Loss
		MS		MS
CONCENTRATION (C)	3	0.71***	3	1.10***
EXPOSURE (E)	2	0.32***	2	0.71***
SAMPLE TIME (S)	2	7.99***	2	4.75***
C*S	6	0.04	6	0.07
E*S	6	0.38	6	0.37
C*E	4	0.03***	4	0.03**
C*E*S	12	0.12***	12	0.12**
ERROR	109	0.04	109	0.08
*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively				
Square Root = square root transformed				
		MEANS‡		
		Norland		Russet Burbank
CONCENTRATION (mg O_3/kg/hr)		% Wt Loss		% Wt Loss
0		4.08b†		5.29c
2.8		5.21a		5.97b
5.5		5.63a		6.26ab
11.6		5.82a		7.27a
EXPOSURE (DAYS)		% Wt Loss		% Wt Loss
1		4.37b		5.47a
7		5.45a		6.41a
21		5.70b		6.73b
SAMPLE TIME (MONTHS)		% Wt Loss		% Wt Loss
0		3.34c		4.35c
3		5.28b		6.68b
6		6.95a		7.54a

‡ Means are original data; † Mean separation based on square root transformed data;
Values followed by the same letter are not significantly different (p=0.05)

The effects of ozone concentration, duration of exposure and time in storage on weight loss appeared to be additive (Figures 4.9 & 4.10). As time in storage increased, the effect of higher ozone concentrations or longer duration of exposure prior to storage on weight loss appeared to increase, suggesting a permanent wounding.

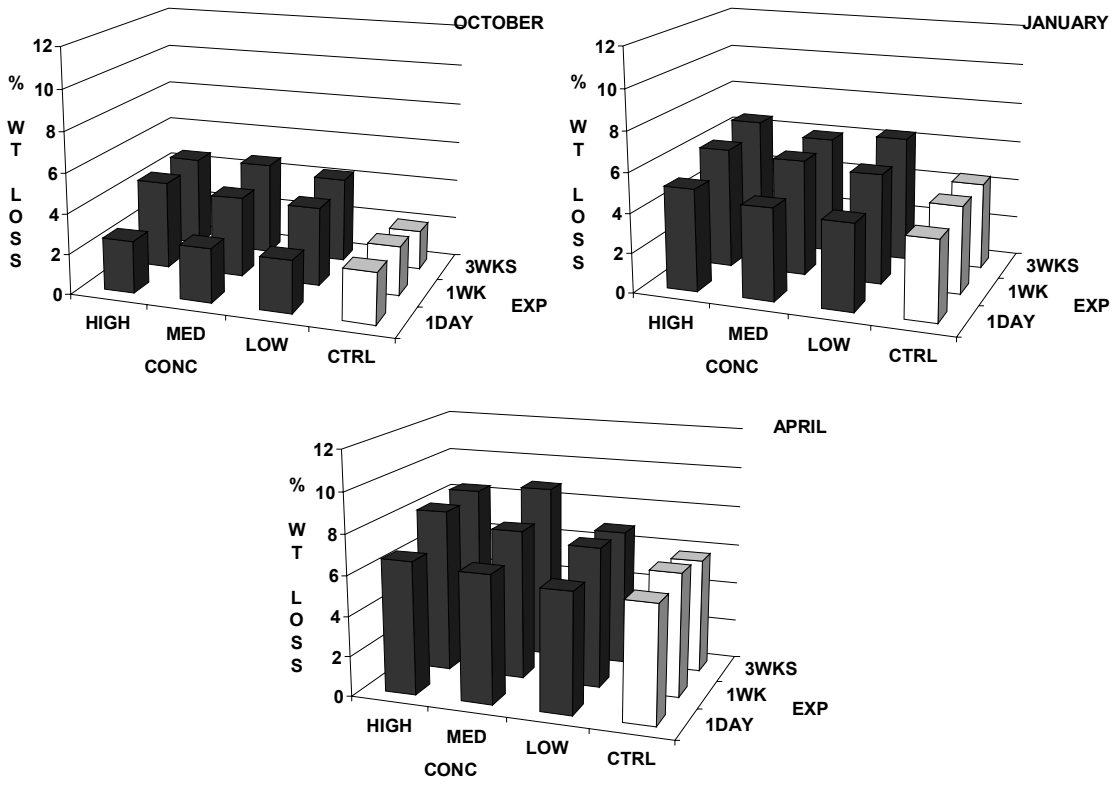

Figure 4.9 Changes in percent weight loss over time in storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=0.78; Jan=0.82; Apr=1.37]

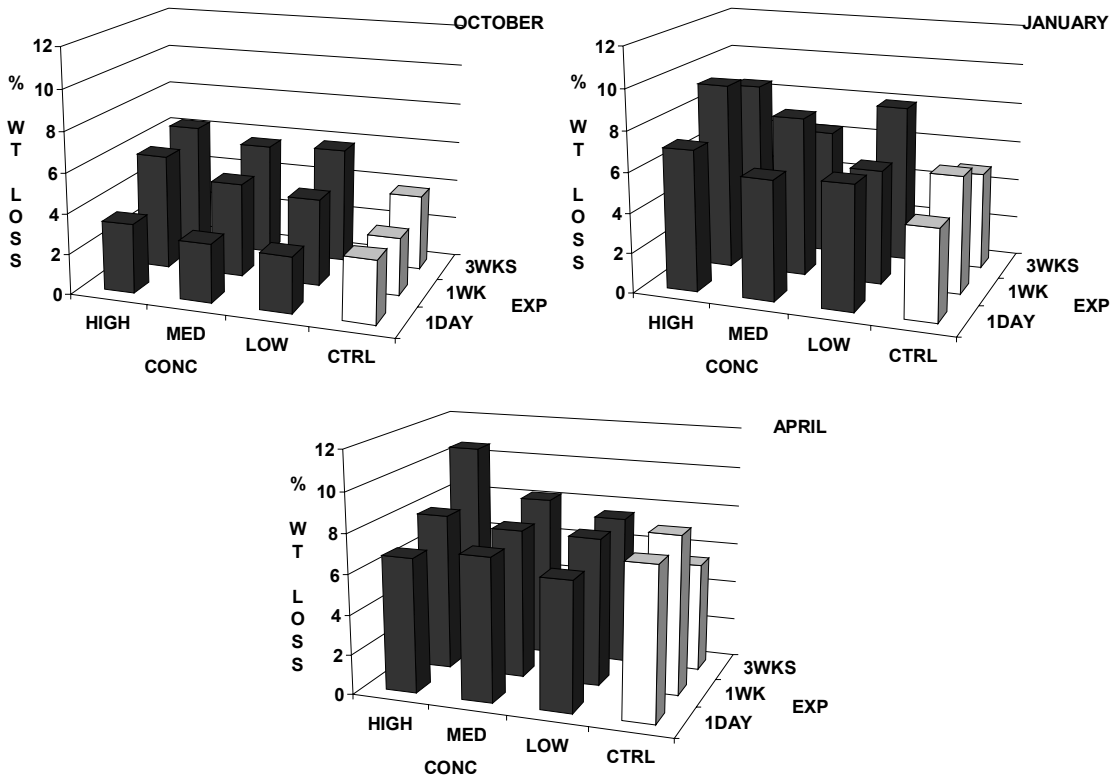

Figure 4.10 Changes in percent weight loss over time in storage of Russet Burbank potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD Oct=1.24; Jan=2.65; Apr=2.26]

In the second trial, weight loss in Norland potatoes again increased with storage time and in response to treatment with ozone (Table 4.12). Longer exposure to ozone (three weeks) increased weight loss relative to the shorter exposure periods (Table 4.12).

Table 4.12 Analysis of variance and main effect means for the effect of ozone treatments on weight loss of Norland potatoes (Trial 2)

SOURCE	DF	Square Root Weight Loss MS
CONCENTRATION (C)	3	0.22***
EXPOSURE (E)	2	0.28***
SAMPLE TIME (S)	2	8.88***
C*S	6	0.05*
E*S	4	0.01
C*E	6	0.07***
C*E*S	12	0.01
ERROR	71	0.02

, **, = significant at p=0.10, 0.05 or 0.01 respectively**

Square Root = square root transformed

	MEANS‡
CONCENTRATION (mg O_3/kg/hr)	**% Wt Loss**
0	3.85b†
5	4.50a
10	4.64a
20	4.56a
EXPOSURE (DAYS)	**% Wt Loss**
1	4.03b
7	4.32b
21	4.80a
SAMPLE TIME (MONTHS)	**% Wt Loss**
0	2.05c
3	4.53b
6	6.57a

‡ Means are original data; † Mean separation based on square root transformed data; Values followed by the same letter are not significantly different (p=0.05)

Weight loss was higher in ozone treated potatoes than in the controls. Differences between the ozone and control treatment were more distinct at the first sampling time and at the end of the storage period (Fig 4.11).

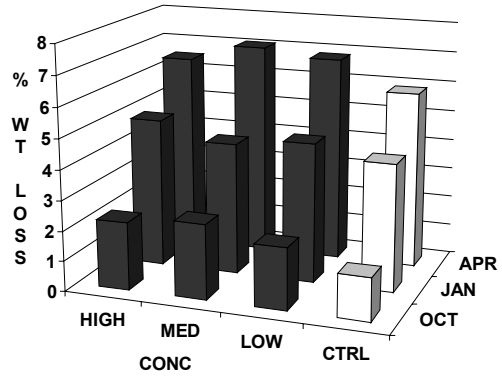

Figure 4.11 Changes in percent weight loss over time in storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations [LSD Oct=0.52; Jan=0.68; Apr=0.85]

Weight loss appeared to increase as a function of either increased ozone concentration or duration of exposure to ozone (Fig. 4.12).

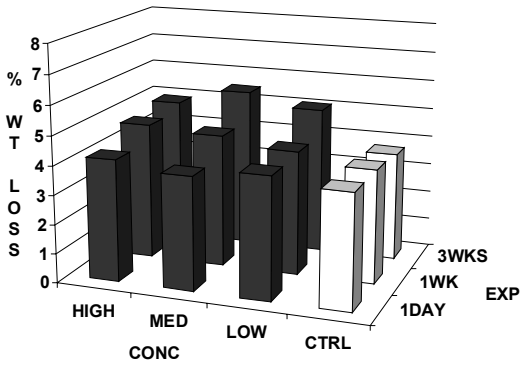

Figure 4.12 Weight loss in Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods [LSD=1.18]

4.3.3.2 Continuous Ozone Treatments

Weight loss in Norland potatoes was higher under continuous ozone than when the ozone treatment was limited to a pre-storage treatment (Table 4.13).

Table 4.13 Analysis of variance for the effect of continuous and pre-storage ozone treatments on weight loss in Norland potatoes

SOURCE	DF (DF CONT)	Square Root Weight Loss MS
CONCENTRATION (C)	3	0.07**
EXPOSURE (E)	2	0.07**
SAMPLE TIME (S)	2 (1)	3.23***
C*S	6 (3)	0.01
E*S	4 (2)	0.05*
C*E	6	0.03
C*E*S	12 (6)	0.01
ERROR	71 (48)	0.02
*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively		
Square Root = square root transformed		

Table 4.14 Main effect means for the effect of continuous and pre-storage ozone treatments on weight loss of Norland potatoes

	Percent Weight Loss	
CONCENTRATION (mg O_3/kg/hr)	Pre-storage O_3	Continuous O_3
0	3.85b†	6.83b
5	4.50a	7.44a
10	4.64a	7.63a
20	4.56a	7.41a
EXPOSURE (DAYS)	% Wt Loss	% Wt Loss
1	4.03b	7.11b
7	4.32b	7.64a
21	4.80a	7.23b
SAMPLE TIME (MONTHS)	% Wt Loss	% Wt Loss
0	2.05c	
3	4.53b	5.92b
6	6.57a	8.74a
OVERALL MEAN	5.57	7.33

‡ Means are original data; † Mean separation based on square root transformed data; Values followed by the same letter are not significantly different (p=0.05)

4.4 Discussion and Conclusions

Throughout the growing season, during harvest and during storage, potatoes are exposed to pathogens. This exposure can result in the development of disease both in the field and during storage. Management practices have been established to minimise disease initiation and subsequent development and spread in storage. Standard practices include careful handling of the potatoes including design and operation of harvest and handling equipment to minimise damage. Obviously diseased or damaged tubers are culled before they enter the storage. Care is taken to ensure proper healing of wounds during the curing period. Subsequent storage conditions are also designed to minimise

disease development while maximising tuber quality. Despite this multi-facetted approach to disease control, storage losses are still significant and post-harvest treatments, such as fungicides or alternative treatments like ozone, are required.

This trial evaluated the potential to use ozone to control various pathogens commonly encountered in the storages of Saskatchewan. The ozone was applied during the curing period which follows harvest. This trial evaluated dosage effects on disease control while also looking for any impact on tuber quality.

Ozone applications had little impact on the incidence or severity of any of the post-harvest diseases monitored in this trial. In large part, this reflects the relatively low incidence and severity of disease, both at the initial time of treatment and during subsequent storage. The potatoes used in this trial were bin-run material produced by commercial seed growers in Saskatchewan. The relatively low disease levels reflect good field management practices. The ozone treatments did not appear to kill any pathogens present on the newly harvested potatoes, but their growth and spread appear to have been temporarily slowed. Liew and Prange (1994) found that ozonating carrots slowed fungal development, with the degree of growth control increasing with increasing rates and durations of exposure to ozone. They found that the growth of the pathogens resumed when treated carrots were moved to an ozone-free environment. The *Fusarium* dry rot lesions observed in this experiment tended to be associated with deep wounds caused by harvest equipment. Ozone is known to be relatively ineffective at controlling deep-seated pathogens (Spotts and Cervantes, 1992) or in situations where the presence of other reactive materials may dampen the effect of ozone (Labbe *et al*., 2001).

Typically, the incidence and severity of post-harvest disease in stored potatoes increases with time in storage. This reflects the fact that time allows disease to spread/develop and that the disease resistance of the commodity decreases as they age. The storage conditions used in this trial mimicked those that commercial growers use. The levels of disease generally did not increase with time in storage, indicating that the proper storage conditions are effective in reducing or controlling disease. In some cases, disease severity actually decreased over time in storage. These results likely reflect natural variability in disease levels.

Pérez *et al*. (1999) found that strawberries treated with ozone prior to sale developed mould more rapidly than untreated fruit, suggesting that ozone was damaging the commodity. In this study, treatment with ozone did not appear to stimulate disease development or alter tuber susceptibility to attack by pathogens, although ozone treatments used in this trial may have been damaging to the stored crop, as indicated by increases in weight loss.

While reducing the development and spread of disease was the primary objective of the ozone treatments, the effect of these treatments on commodity quality and consumer safety must also be considered. Liew and Prange (1994) found that ozone treatments increased lightness values in carrots, while Barth *et al*. (1995) did not observe any change in lightness of ozonated blackberries. In this study, treatment with ozone caused subtle changes in the skin colour of the tubers. In general, ozone treatments increased redness (Hunter "a") of Norland potatoes and yellowness (Hunter "b") of Russet Burbank potatoes. These types of changes would not be negative for these cultivars. The treatment effects on skin colour became less noticeable as time passed. Continuous ozone applications further enhanced the skin redness of the red Norland. Although the ozone treatments in this trial significantly affected colour variables based on assessment with the Hunter Colorimeter, these effects were not apparent to the naked eye and are therefore of questionable relevance from a marketing perspective.

Any weight loss during storage of potatoes is undesirable as it reduces marketable yields since potatoes are sold by weight. Weight loss is particularly unacceptable if it leads to visible softening and wrinkling. In this study, ozone treatments might be expected to decrease weight loss if they controlled disease, but similarly might increase weight loss if the treatments damaged the tubers. Ozone treatments increased weight loss, proportionate to the amount of applied ozone. The ozone concentration was not as important to weight loss as the duration of exposure. While weight loss was significantly increased by ozone treatments, differences between ozonated and control potatoes were not obvious, as no wrinkling was observed. Liew and Prange (1994) observed an increase in electrolyte leakage in carrots treated with ozone, which suggests that the ozone was damaging cell membranes. The increase in weight loss observed in this trial may also reflect a similar damage to cellular

membranes. In contrast, Daniels-Lake *et al*. (1996) treated potatoes with very high concentrations of ozone with no observed decrease in quality or damage to the tubers.

Traditionally, fungicides are applied to potatoes at the commencement of storage, while the tubers are being loaded into the storage facilities. For these treatments to be effective, they need to either provide complete control or some continuous residual activity, as they cannot be reapplied once potatoes are piled. By contrast, ozone provides no residual activity but, as a gas, ozone can be added to the ventilation stream and pumped throughout the piled potatoes. This creates the potential to treat with ozone throughout the storage period. This may be especially useful for the control of diseases that have the potential to spread during storage (i.e. silver scurf). In this study, continuous ozone applications did not appear to provide any additional disease control over that obtained by pre-storage treatment, but continuous applications increased weight loss and exacerbated changes in skin colour. Song *et al*. (2000) observed that ozone applications over the storage period of onions did not reduce internal decay of treated onions, however, surface symptoms of disease were significantly reduced, as were the levels of airborne spores in the storage.

In summary, exposing potatoes to ozone treatments, either for short periods prior to storage or continuously throughout storage, had limited positive effects in terms of reducing post-harvest disease. The ozone treatment selected may have been ineffective. Alternatively, experimental conditions may not have been conducive to the observation of ozone effects due to insufficient disease levels or utilisation of storage conditions non-conducive to disease development. To determine which of these was the case, future trials could utilise higher rates of ozone, either through increased concentration or duration of exposure or potentially the timing of the application. Greater initial incidence of disease could be achieved by inoculating the tubers, while subsequent storage conditions could be adjusted to favour disease development.

The ozone treatments caused some changes in tuber quality, such as increased weight loss and subtle colour change, but these were of limited commercial significance. Continuous applications of ozone did not improve disease control appreciably, but did increase weight loss and exacerbate colour change compared to pre-storage treatments.

This would mitigate against continuous exposure to ozone unless some disease control benefits were clearly demonstrated.

5. OZONE APPLICATION DURING STORAGE

5.1 Introduction

During storage of potatoes, pathogens introduced onto the tubers in the field or at harvest may develop and spread, causing significant reduction in marketable yields and quality. Pathogens carried through the storage period on seed potatoes can also contaminate the subsequent crop. Low storage temperatures (~4°C) represent the primary post-harvest management tool used to control the growth and development of storage pathogens in potatoes (Burton, 1966). Low temperatures decrease the rate of growth and subsequent spread of pathogens, while increasing the crop's disease resistance by maintaining crop vigour (Burton, 1966). However low temperatures cannot be employed or achieved at all points in the post-harvest process. Higher temperatures occur during curing, reconditioning, transit and in retail situations, thereby creating the potential for the development of disease. During low temperature storage, hot spots may also develop in the storages due to the heat produced by respiration of the crop or by disease organisms. These hot spots represent an ideal microenvironment for pathogen growth.

Post-harvest applied fungicides are used to supplement or substitute for the disease suppression provided by low temperature storage. Ideally, fungicides applied as a spray to the tuber surface during loading into storage create a long-lasting barrier to disease infection. The degree of control achieved reflects the activity of the product against the range of pathogens encountered in storage, as well as the uniformity of coverage and subsequent storage conditions.

Control of post-harvest diseases in potatoes via spray application of fungicides is not an option once the crop has been loaded into the storage bins. Application of volatile disease control agents through the ventilation system however represents an alternate disease control option once the storage is loaded. Post-loading treatments could be particularly useful for combating the development of isolated pockets of disease or for problems not apparent at loading.

Any disease control agents applied during storage must be able to penetrate the mass of potatoes, resulting in an even distribution of effective levels of the active ingredient throughout the entire pile. These products also must be safe for the crop, staff and facility and ideally can be effectively applied through the existing ventilation system.

Purogene® (Biocide International Inc., Norman, OK), oxidate and ozone (O_3) represent biocides potentially suited for in-storage application to potatoes. Purogene® is an inactive liquid sodium chlorite solution which when reacted with citric acid produces 2% chlorine dioxide gas and other chlorine species. Chlorine dioxide is a strong oxidiser and is commonly used in the disinfection of water in Europe (Kim *et al*., 1999a) and has been found to have good bactericidal activity (Dychdala 1991 cited in Kim *et al*., 1999a). In potatoes, Purogene® is recommended for application as a spray during load-in and then as repeat applications through the humidification system during the storage period (Bio-Cide Communication, 2000). The suggested number and timing of applications are not specified on label recommendations, however repeated applications are required as the product has relatively little residual activity. In Saskatchewan, potato growers have typically applied Purogene® twice during a typical 6-month storage period (D. Waterer, Personal Communication). Chlorine dioxide is highly soluble in water and is not supposed to leave residues on the commodity, however washing prior to consumption is recommended. Some care must be taken during the activation stage of the preparation process, as the fumes can be dangerous to humans. A two-hour ventilation period is recommended prior to re-entry of treated areas. Repeated use of this product may potentially result in wear on equipment and structures as ClO_2 is highly reactive (Norikane *et al*., 2000).

Purogene® received emergency use registration for post-harvest application on potatoes in Canada in 1999, 2000 and 2001, but the industry did not continue to request emergency clearance to use this product beyond 2002 (D. Waterer, Personal Communication). Although Purogene® is labelled as effective against a range of post-harvest pathogens of potatoes, including dry rot, bacterial soft rot, late blight and silver scurf, experimental testing and commercial use have revealed mixed results. Purogene® produced few positive effects on a range of pathogens in storage trials conducted in

Alberta (Holley, unpublished data). Tsai *et al*. (2001) showed that 7.8 ppm of available chlorine dioxide (Oxine) for 10 minutes reduced *Erwinia carotovora* populations. A 40% reduction in spoilage was achieved by exposing potatoes to activated Oxine with 100 ppm available chlorine dioxide. This study evaluated a number of application methods, including insertion of the Oxine into the air stream and application using a cool-mist humidifier. Concentrations applied varied, as did the results, however the authors indicated some potential for use of this product in potato storages. Kirk (2002) found that Purogene® applications to wounded/non-wounded and inoculated potatoes were not consistently better than the untreated control treatment in terms of reduction of disease infection.

Ozone (O_3) is also a chemically reactive gas, which exhibits significant antibiotic activity (Hovàrth *et al*., 1985; Kim *et al*., 1999b; Xu, 1999). Ozone rapidly degrades to oxygen leaving no residues on or in the crop. Ozone must be generated continuously on-site to maintain biologically active concentrations. Ozone can be pumped into storages through standard ventilation systems. There are worker safety considerations when using ozone, as both short or long-term exposure to ozone is potentially harmful (http://ccinfoweb.ccohs.ca/). Ozone can react with equipment and construction materials within a storage structure, eventually causing weathering and breakdown. Ozone can also potentially cause negative effects on the commodity, depending on the crop species and levels of ozone applied (Pérez *et al*., 1999; Liew and Prange, 1994).

The objective of this experiment was to compare the effect of in-storage treatments with ozone and Purogene® on storage diseases and the post-storage quality of potatoes.

5.2 Materials and Methods

The first trial began in the fall of 1999. Norland and Russet Burbank potatoes (See Chapter 4 for descriptions of the potatoes tested) were stored in mesh bags at 4°C for three months after harvest. The potatoes were then treated for one day at 15°C with either ozone (~10 mg O_3/kg/hr), 200 ppm of Purogene® or were not treated (control). The Purogene® was applied with a humidification system, while ozone was generated with an ozone generator (See Chapter 3&4 for descriptions of the ozone generators). The Purogene® rate corresponded to label recommendations. There is no label

recommendation for Purogene® relating to a recommended timing of application or duration of treatment. The treatment duration of 24 hours was chosen arbitrarily as a suitably short application duration. The ozone rates corresponded to the high ozone concentration used in the pre-storage ozone trial conducted in the same year (Chapter 4). Potatoes were treated at 15°C to correspond with the temperature used in the pre-storage applications. Following treatment, the potatoes were returned to the 4°C cold storage for another three months. At the end of this storage period, disease levels and tuber quality were evaluated as described previously (Chapter 4). The data was analysed using the GLM procedure of SAS (See Chapter 4 for details).

In the second trial conducted beginning in the fall of 2000, only Norland potatoes were examined and the ozone rate was increased to 20 mg O_3/kg/hr, due to the lack of treatment effects on disease in the previous trial. This higher rate corresponds to ozone concentrations used in the pre-storage trial conducted that year. All other experimental parameters, data collection and analysis methods were similar to the previous year.

5.3 Results

5.3.1 Disease Analysis

In both trials, disease levels were low, reflecting good storage conditions and the use of high quality potatoes. In the first trial, the in-storage treatments did not influence *Rhizoctonia* severity in either cultivar, however the Purogene® treatments significantly reduced *Fusarium* dry rot levels in Russet Burbank compared to the control treatments (Table 5.1). *Fusarium* levels in Norland potatoes were not affected by the in-storage treatments (Table 5.1).

Table 5.1 Analysis of variance and main effect means for the effect of in-storage treatments on disease variables for Norland and Russet Burbank potatoes (Trial 1)

		Norland		Russet Burbank	
SOURCE	**DF**	**SQRT *Rhizoctonia***	**SQRT *Fusarium***	**SQRT *Rhizoctonia***	**SQRT *Fusarium***
		MS	**MS**	**MS**	**MS**
TREATMENT	2	0.96	0.43	0.36	0.64**
REP	9	0.48	0.24	1.81***	0.13
ERROR	168	0.32	0.20	0.36	0.22
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**					
SQRT = square root transformed					
		MEANS‡			
		Rhizoctonia	***Fusarium***	***Rhizoctonia***	***Fusarium***
TREATMENT		**% Surface Area**	**Rating (0-6)**	**% Surface Area**	**Rating (0-6)***
CONTROL		1.78	0.53	2.08	0.83a†
PUROGENE		0.97	0.65	1.92	0.20b
OZONE		1.37	0.23	2.43	0.40ab

‡ Means are original data; † Mean separation is based on square root transformed data; Values followed by the same letter are not significantly different (p=0.05)
* 0-6 rating described in Chapter 4

In the second study, in-storage treatments with ozone or Purogene® did not result in any disease reduction relative to the untreated controls (Table 5.2).

Table 5.2 Analysis of variance and main effect means for the effect of in-storage treatments on disease variables for Norland potatoes (Trial 2)

SOURCE	**DF**	**SQRT *Rhizoctonia***	**SQRT *Fusarium***	**DF**	**SQRT Silver Scurf**	***Rhizoctonia* Sclerotia Germination**
		MS	**MS**		**MS**	**MS**
TREATMENT	2	0.32	0.01	2	0.41	325.00
REP	9	0.44	0.01		0.15	
ERROR	108	0.34	0.01	48	0.18	219.44
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**						
SQRT = square root transformed						
			MEANS‡			
		Rhizoctonia	***Fusarium***		**Silver Scurf**	***Rhizoctonia***
TREATMENT		**% Surface Area**	**Rating (0-6)***		**% Surface Area**	**% Germination**
CONTROL		0.95	0.08		0.25	95.0
PUROGENE		1.38	0.06		0.65	82.5
OZONE		0.90	0.10		1.05	100.0

‡ Means are original data; * 0-6 rating described in Chapter 4

5.3.2 Colour Analysis

In the first trial, ozone treatments increased skin redness (Hunter "a") of Norland potatoes relative to when Purogene® was used (Table 5.3). A similar increase in redness was seen when ozone was applied prior to storage (Chapter 4). Fading of the characteristic red skin colour is common during storage of Norland potatoes and it appears that ozone treatments may slow this process.

Ozone treatments also significantly increased skin yellowness (Hunter "b") of Russet Burbank tubers compared to tubers receiving the control or Purogene® treatments (Table 5.3).

Table 5.3 Analysis of variance and main effect means for the effect of in-storage treatments on skin colour of Norland and Russet Burbank potatoes (Trial 1)

		Norland			Russet Burbank		
SOURCE	**DF**	**L**	**a**	**b**	**L**	**a**	**b**
		MS	**MS**	**MS**	**MS**	**MS**	**MS**
TREATMENT	2	1.15	1.38**	0.08	1.43	0.10	1.22**
REP	9	1.03**	0.30	0.31**	5.43	0.18***	0.20
ERROR	108	0.48	0.33	0.14	4.07	0.06	0.25

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**

MEANS

	Norland			Russet Burbank		
TREATMENT	**L**	**a**	**b**	**L**	**a**	**b**
CONTROL	21.0	3.07ab	2.93	22.2	1.80	4.38b
PUROGENE	21.2	2.91b	3.02	22.5	1.86	4.46b
OZONE	21.4	3.28a	2.96	22.4	1.90	4.71a

Values followed by the same letter are not significantly different (p=0.05)

In the second trial, in-storage treatment with ozone or Purogene® had no effect on the skin colour of the Norland potatoes (Table 5.4). Most of the colour values in the second trial were higher than those recorded in the first trial. This difference may reflect differences in seed source and growing conditions between the two test seasons.

Table 5.4 Analysis of variance and main effect means for the effect of in-storage treatments on skin colour of Norland potatoes (Trial 2)

SOURCE	DF	L	a	b
		MS	MS	MS
TREATMENT	2	0.59	0.67	0.29
REP	9	7.13***	1.45***	0.32**
ERROR	108	0.70	0.51	0.15
*, **,*** = significant at *p*=0.10, 0.05 or 0.01 respectively				
MEANS				
TREATMENT		L	a	b
CONTROL		22.08	3.08	3.96
PUROGENE		22.21	3.23	4.10
OZONE		22.32	3.33	4.11

5.3.3 Weight Loss

Weight loss was not significantly affected by in-storage application of ozone or Purogene® in either trial (Table 5.5 and 5.6).

Table 5.5 Analysis of variance and main effect means for the effect of in-storage treatments on weight loss by Norland and Russet Burbank potatoes (Trial 1)

SOURCE	DF	Norland	DF	Russet Burbank
		Square Root Weight Loss		Square Root Weight Loss
		MS		MS
TREATMENT	2	0.17	2	0.05
ERROR	9	0.23	8	0.10
*, **,*** = significant at *p*=0.10, 0.05 or 0.01 respectively				
Square Root = square root transformed				
		MEANS‡		
TREATMENT		% Weight Loss		% Weight Loss
CONTROL		8.61		7.68
PUROGENE		6.78		6.66
OZONE		6.12		7.80

‡ Means are original data.

Table 5.6 Analysis of variance and main effect means for the effect of in-storage treatments on weight loss by Norland potatoes (Trial 2)

SOURCE	DF	Square Root Weight Loss
		MS
TREATMENT	2	0.01
ERROR	9	0.02
*, **,*** = significant at *p*=0.10, 0.05 or 0.01 respectively		
Square Root = square root transformed		
		MEANS‡
TREATMENT		% Weight Loss
CONTROL		4.60
PUROGENE		4.25
OZONE		4.41

‡ Means are original data

5.4 Discussion and Conclusions

In-storage treatments with ozone or Purogene® provided little in the way of protection from storage disease in this experiment. In large part, this reflects the fact that disease levels were low and storage conditions were non-conducive to disease development. In one trial and one cultivar, Purogene® provided slight control of *Fusarium* relative to the control treatment. Although *Fusarium* is a serious storage disease, the significance of this level of control was marginal. Purogene® applied as a spray to Norchip potatoes prior to storage reduced the infection of tubers by *Alternaria solani* (early blight) in low disease pressure situations (Harrison and Franc, 1988). Holley (Personal Communication) showed that Purogene® was ineffective for control of a range of fungal diseases (*Rhizoctonia* black scurf, common scab (*Streptomyces scabies*), *Fusarium* dry rot and *Helminthosporium* silver scurf). In some cases Holley found that Purogene® treatments actually increased the incidence and severity of storage disease when tubers were washed prior to treatment with Purogene®. This increase in disease incidence might be attributed to wounds occurring during washing. However, it is also possible that the absense of the protective layer of soil is increasing the chemical activity of the Purogene®, due to a lack of inactivation by the soil. Holley used treatment methods and rates similar to those used in this study, but multiple applications of Purogene® were employed. Disease levels were also low and variable in Holley's study, making it difficult to develop conclusive data.

Purogene® appears to be more effective against bacterial diseases such as bacterial soft rot than fungal diseases. Tsai *et al*. (2001) found that applying chlorine dioxide to potato storage at 200 and 400 ppm reduced spoilage due to bacterial soft rot by 50 to 65 % compared to the controls. Lower rates (than 200 and 400 ppm) had higher rates of spoilage. Purogene® used as a water treatment or water wash was effective in reducing bacterial loads on seafood (Kim *et al*., 1999a). Bacterial soft rot was not a common problem in the current study, therefore the efficacy of the in-storage treatments against bacterial soft rot could not be determined. However, bacterial soft rot is a common and serious problem in stored potatoes.

Limited efficacy of in-storage treatments may reflect insufficient concentration or duration of exposure or perhaps a need for multiple applications. In this study, the

disease control treatments were only applied for a brief period (24h) on a single occasion at the mid-point of the storage period. Efficacy may be related to dosage, which is determined by application time and concentration. Ozone can be applied continuously in storage, however the results presented in Chapter 4 did not indicate any benefit of this application. Purogene® can also be applied at any time in storage. Purogene® is registered for use "as needed", indicating that it may be re-applied numerous times during the storage period. Purogene® may be applied at a rate of 16 ml/ton/month as a humification application. Repeated applications of Purogene® increase costs and as noted by Holley (Personal Communication), may result in increased disease levels due to crop injury. This potential for negative effects on the crop coupled with associated damage to storage structures is a deterrent to increasing the concentration or frequency of applications of products like Purogene®. Norikane *et al*. (2000) studied the corrosive capacity of chlorine dioxide on materials commonly found in potato storage. The recommended rate of 100 ppm was corrosive enough to alter the appearance and cause weight loss in several metals commonly employed in potato storage structures.

In summary, a single, mid-season application of Purogene® or ozone to stored potatoes had little impact on disease development, or crop quality. The response to in-storage treatments was likely limited by 1) low disease levels, reflecting a combination of minimal inoculation and ideal storage conditions and 2) limited treatment time. Longer exposure or higher concentrations of the treatment agents, in conjunction with greater disease pressure and less favourable storage conditions might increase the treatment response. However, if higher dosages are required for effective disease control, the cost and potential for negative effects would increase. Further study of these in-storage treatments is required prior to recommending either treatment for this type of application.

6. INFLUENCE OF OZONE ON WOUND HEALING AND PERFORMANCE OF SEED POTATOES

6.1 Introduction

During the course of harvest, grading and post-harvest handling operations, potatoes are often cut or scraped by the machinery. These wounds represent a potential opening for the entry of disease organisms, such as *Fusarium spp.*. Wounds are also an avenue for loss of moisture in storage, resulting in decreased potato quality and yield. Morris *et al*. (1989) cited several studies that estimated wastage losses approaching 40% due to mechanical wounds. Potatoes have the ability to heal wounds, preventing even greater losses (Schippers, 1971 cited in Morris *et al*., 1989). During wound healing, the tubers form protective tissue layers that serve as a barrier against invasion by disease pathogens while also slowing water loss (Nnodu *et al*., 1982; O'Brien and Leach, 1983; Vaughn and Lulai, 1991).

Morris *et al*. (1989) summarised the process of wound healing in potato tubers as an initial deposition of suberin on healthy cells adjacent to the injured surface, followed by the deposition of several wound periderm cell layers beneath the suberised layer. The rapidity of development, along with the thickness of the combined suberin and wound periderm layers, may determine the tuber's susceptibility to disease. Development of the wound periderm layer is affected by environmental factors, including temperature and relative humidity (Morris *et al*., 1989; Kim and Lee, 1993). Growers are recommended to hold the crop at 15°C and 95% relative humidity for several weeks after harvest to promote wound healing (Burton, 1966; Schaupmeyer, 1992). However, the temperatures and relative humidity that promote potato curing are also suitable for establishment and growth of many post-harvest diseases. A balance must therefore be achieved between wound healing and disease control, as many post-harvest problems can be traced back to invasion and development during the wound healing period. Application of fungicides prior to loading is a commonly employed method for reducing disease development during wound healing and subsequent longterm storage. Similarly,

minimising the duration of the curing period and monitoring the status of the pile for disease development can potentially reduce disease development during curing.

Ozone has been proposed as a potential alternative to fungicides for the protection of potatoes from infection by disease during both the wound healing and long-term storage periods. A concern regarding the use of ozone is that the biological activity of ozone on wounded surfaces could potentially interfere with the healing process. Ozone has been used to degrade lignin in wood processing applications (Bono *et al*., 1985). Booker and Miller (1998) found that ozone treatments increased phenylpropanoid pathway products (i.e. lignin and suberin) in soybean leaves. Ozone has also been found to react with cellular membranes, potentially disrupting cellular activity and increasing cellular leakage (Kangasjarvi *et al*., 1994; Scott and Lesher, 1963 cited in Kim *et al*., 1999b).

Potatoes are propagated vegetatively, using sections of tubers as "seed". The quality and yield of the crop are strongly influenced by seed quality. Good quality seed produces a uniform and complete stand. Fungicides are often applied to the seed just prior to planting to prevent decay after planting due to seed and soil-borne pathogens. These treatments are costly, toxic to applicators and of only limited efficacy (Thomson and Waterer, 1999). Ozone has been shown to have biocidal capabilities and has the potential to provide non-toxic (to applicators) control of pathogens present on tuber surfaces (Chapter 4). The concept of using ozone as a pre-planting treatment to decrease seed-borne diseases has not been evaluated. A potential concern regarding the use of ozone as a seed treatment is that ozone is a highly reactive substance and may adversely react with tuber buds (eyes), potentially reducing sprouting and impacting the subsequent performance of the crop.

The objectives of this experiment were to:

1) evaluate the effect of ozone application on wound periderm formation.
2) evaluate the effect of ozone application on seed potato performance (sprouting, seed potato vigour).

6.2 Materials and Methods

6.2.1 Effect of Ozone on Wound Periderm Formation

In preliminary trials, Russet Burbank potatoes were cut in half and exposed to ozone at approximately 22 mg O_3/kg/hr at 20°C for one or two days. Following the ozone treatment, the tubers were held at room temperature (20 to 23°C) for approximately two weeks to allow for wound healing. Tubers were then cut perpendicular to the cut surface and the thickness of the wound periderm was measured with a ruler. Tubers exposed to standard ozone-free air were used as controls.

Based on the results from the preliminary trial, a second suberizing experiment was conducted using Norland potatoes. Tubers were surface sterilised in a 2% sodium hypochloride solution for five minutes and then rinsed in distilled water to minimise disease development during the wound healing period. Tubers were then cut in half and exposed to approximately 22 mg O_3/kg/hr for one day, one week or three weeks at 15°C. These ozone application rates represent rates similar to those used in other experiments in this project. Upon completion of each ozone application period, the tubers were placed in an ozone-free, 15°C environment for the remainder of the three-week curing period. Following completion of the curing period, two thin slices were cut from the centre of each tuber piece and fixed in FAA solution [10% formaldehyde (38-40% purity), 5% glacial acetic acid, 50% ethyl alcohol and 35% distilled water] for one hour. The fixed slices were stained with Sudan III stain [1:20 stain stock solution (1% w/v Sudan III stain in 95% ethyl alcohol) and FAA] for 45-60 minutes. The slices were then rinsed and held in distilled water until measured (Nielsen, 1973). This treatment stained the periderm/suberin layers reddish-brown and facilitated its differentiation from other tissue layers. The thickness of the periderm layer was determined at 12X magnification using an ocular micrometer. The slices were measured in two places and the resulting ocular units were averaged and converted to µm. Treatments were replicated four times and represented a Completely Random Design (CRD). The wound periderm thickness data were analysed using the GLM procedure of SAS.

6.2.2 Ozone as a Pre-planting Seed Treatment

Potato seed that had been stored under commercial conditions for up to six months were received from the same sources as in the main ozone trial (See Chapter 4).

The potatoes were Elite III grade, with disease levels typical of this generation of seed. These potatoes were treated with ozone at different concentrations within 48 hours of planting. The tubers were planted into the University of Saskatchewan Potato Fields (Saskatoon, SK) in a Randomised Complete Block Design (RCBD) layout, with four replicates. Each treatment replicate involved an eight-metre row with 30 cm between plants within the row. The trials were planted in mid-May and subsequently managed using standard production practices. Stand counts were taken periodically. At the end of the season, tubers were mechanically harvested and graded to determine marketable and non-marketable (oversize and undersize) yields. Tubers were not rated for disease levels in these trials.

In the first year, Norland seed (Hyland Seed Potato Farms, Scott, SK; average size ~113g) heavily infected with *Fusarium* dry rot were cut and treated with ozone at 40 or 60 mg O_3/kg/hr for a period of eight hours. This treatment represented an initial assay in small volume application chambers (plastic tubs), with the ozone application rates determined by generator output capacity. The control treatment involved treating the seed with a commercial fungicidal seed treatment (Metiram).

In the second year, cut seed pieces of Norland and Russet Burbank potatoes (Hyland Seed Potato Farms, Scott, SK) were treated with zero, 5, 10 and 20 mg O_3/kg/hr for either one or two days. These rates of ozone corresponded to the rates used in the other ozone trials conducted in this year. As in the previous year, the control treatment involved treating the tubers with a commercial fungicidal seed treatment (Metiram).

In the third year, only Norland seed potatoes (Barrich Farms, Outlook, SK) were evaluated and a non-treated control was added to contrast the ozone treatments and the chemical seed treatment control (Metiram). All other aspects of the study and treatment intervals were similar to the previous year (e.g. ozone rates, etc.).

In each year, the effect of ozone treatments on final stand counts were evaluated and then the final stand counts were used as a covariant in the yield analysis to evaluate the involvement of stand in yield responses. Data were analysed using the GLM procedure of SAS. Years were not compared due to the differences in treatments used.

6.2.3 Effect of Ozone on Sprouting in Seed Potatoes

In preliminary evaluations, tubers that had received the pre-planting seed treatments (ozone or Metiram) were evaluated for their sprouting characteristics. Cut seed pieces of Norland and Russet Burbank potatoes were treated with the ozone rates previously described for the second year seed treatments. Following treatment, the seed was stored at room temperature to promote sprouting. Sprout counts, and the weight of the sprouts produced by each potato (five from each treatment), were evaluated after 2-3 weeks.

In a second trial, ten whole de-sprouted Norland seed potatoes were treated with 0, 20, 40, 80, 160 and 320 mg O_3/kg/hr for two days. The initial number of "eyes" was counted and the number of eyes that had sprouted was determined for each tuber (numbered at start) after four, eight and 14 days of storage at room temperature. The overall final sprout weight was collected and the final sprouting percentage was calculated. Each treatment was replicated four times. All data were analysed using the GLM procedure of SAS.

6.3 Results

6.3.1 Effect of Ozone on Wound Periderm Formation

Based on visual observations made in the preliminary trial it appeared that the ozone treatment increased wound periderm thickness. However, in the second trial, the wound periderm thickness was significantly decreased by ozone treatment (Table 6.1). The duration of exposure to ozone did not influence periderm thickness.

Table 6.1 Analysis of variance and main effect means for the effect of ozone treatments on wound periderm thickness of Russet Burbank potatoes

SOURCE	DF	Wound Periderm MS
REP	3	241161
CONCENTRATION (C)	1	11557993***
REP*C	3	295893
EXPOSURE (E)	2	561152
C*E	2	116166
REP(C*E)	12	761949***
ERROR	216	117569
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**		
		MEANS
CONCENTRATION		**Thickness (μm)**
CONTROL		1506.8a
OZONE		1067.9b
EXPOSURE (DAYS)		**Thickness (μm)**
1		1238.9
7		1239.2
21		1384.1

Values followed by the same letter are not significantly different (p=0.05)

6.3.2 Ozone as a Pre-planting Seed Treatment

Pre-planting treatments with ozone had no effect on the plant stand compared to the control treatments in the first year of trials (Table 6.2). The ozone treatments did not affect Norland potato stand counts in the second year, but the highest ozone rates significantly reduced counts in the final year of trials (Table 6.2; Fig 6.1). Stand of Russet Burbank potatoes was reduced by the highest concentration pre-planting ozone treatments in the second year of trials (Table 6.2; Fig 6.1).

Table 6.2 Analysis of variance and main effect means for the effect of ozone seed treatments on final stand count of Norland and Russet Burbank potatoes

SOURCE	1999		DF	2000		DF	2001
	Norland			Norland	Russet Burbank		Norland
	DF	MS		MS	MS		MS
REP	3	4.42	3	7.37	9.08	3	8.11
TRT	3	7.75	6	2.57	13.54*	5	40.07***
ERROR	9	3.47	18	5.51	6.03	15	4.58

MEANS

TRT*	1999 N Final Stand (%)	TRT	2000 N Final Stand (%)	2000 RB Final Stand (%)	TRT	2001 N Final Stand (%)
		FUNG	81	91a	CTRL	100a
CTRL	82	L O_3 1D	79	84ab	FUNG	98a
L O_3	77	L O_3 2D	84	88a	L O_3 1D	97a
H O_3	74	M O_3 1D	86	80ab	L O_3 2D	99a
FUNG	72	M O_3 2D	84	84ab	H O_3 1D	98a
		H O_3 1D	82	76b	H O_3 2D	74b
		H O_3 2D	81	76b		

Values followed by the same letter are not significantly different (p=0.05)
* L=Low, M=Medium, H=High, FUNG = Fungicide

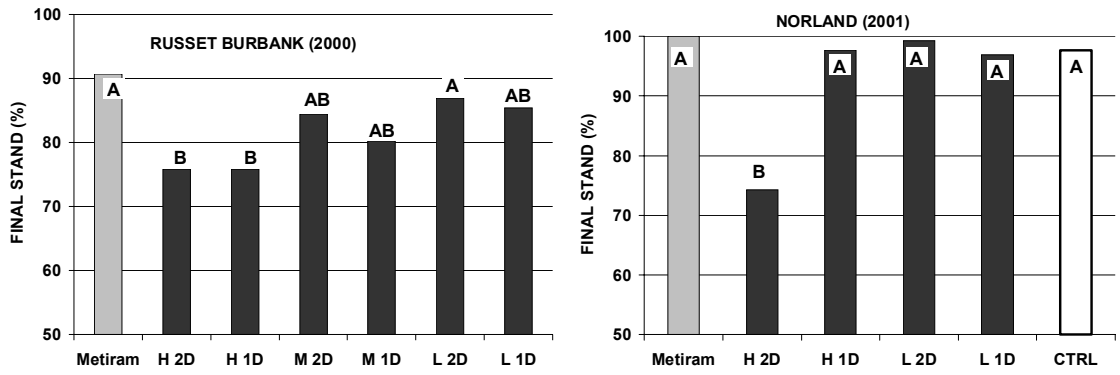

Figure 6.1 Effect of ozone treatments on final stand count of Russet Burbank (2000) and Norland (2001) potatoes [For each graph, columns with the same letter are not significantly different, p=0.05; H2D=20 mg O3/kg/hr for 2days; H1D=1day; M2D=10 mg O3/kg/hr for 2days; M1D=1day; L2D=5 mg O3/kg/hr for 2days; L1D=1day]

In the 1999 trial, marketable yields obtained by planting ozone-treated Norland seed potatoes did not differ significantly from the controls (Table 6.3). In 2000, Russet Burbank potatoes treated prior to planting with the higher ozone concentrations had

significantly lower marketable yields than the chemical treated controls. The test of stand as a covariant with yield was significant (Table 6.3), suggesting that the reduction in stand (Table 6.2) associated with the ozone treatment resulted in a corresponding reduction in marketable yield. In 2001, none of the pre-planting treatments had a significant effect on marketable yields of Norland potatoes (Table 6.3).

Table 6.3 Analysis of covariance and main effect means for the effect of ozone seed treatments on marketable yield (kg/8m plot) of Norland and Russet Burbank potatoes

SOURCE	DF	1999 Norland MS	DF	2000 Norland MS	2000 Russet Burbank MS	DF	2001 Norland MS
REP	3	14.76	3	44.78	64.43	3	61.28***
TRT	3	3.57	6	79.62	244.12***	5	11.13
COUNT	1	50.78	1	0.39	621.18***	1	0.60
ERROR	8	30.90	17	82.48	41.62	14	8.76

*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively

MEANS

TRT*	1999 N Mkt Yld (kg/plot)	TRT	2000 N Mkt Yld (kg/plot)	2000 RB Mkt Yld (kg/plot)	TRT	2001 N Mkt Yld (kg/plot)
		FUNG	25.5	33.4a	CTRL	29.2
CTRL	34.7	L O_3 1D	29.0	22.9bc	FUNG	28.1
L O_3	32.7	L O_3 2D	29.9	21.5bc	L O_3 1D	28.9
H O_3	29.6	M O_3 1D	40.8	28.4ac	L O_3 2D	30.8
FUNG	30.3	M O_3 2D	37.9	22.8bc	H O_3 1D	27.7
		H O_3 1D	32.5	14.9c	H O_3 2D	22.0
		H O_3 2D	29.3	19.2bc		

Values followed by the same letter are not significantly different (p=0.05)
* L=Low, M=Medium, H=High, FUNG = Fungicide

6.3.3 Effect of Ozone on Sprouting in Seed Potatoes

The preliminary trial indicated that treating cut seed with ozone had no negative effects on tuber sprouting. In the second trial, four days after treatment, potatoes exposed to 80 or 320 mg O_3/kg/hr had a significantly higher percentage of their eyes with obvious sprouts than did the control or 20 mg O_3/kg/hr treatments (Table 6.4). No differences between treatments were apparent at any other time in the experiment. Final sprout weights were not affected by the ozone treatments (Table 6.4).

Table 6.4 Analysis of variance and main effect means for the effect of ozone treatments on the sprouting of Norland potatoes

SOURCE	DF	Final Sprout Wt	% SPROUTED		
			DAY 4	DAY 8	DAY 14
		MS	MS	MS	MS
O_3 CONC	5	2.11	3999.02**	93.55	210.88
REP	18	1.30***	1163.39***	438.15***	104.50**
ERROR	216	0.42	459.27	201.34	59.06

*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively

% Day#=% of all eyes which had sprouted

MEANS

[OZONE] (mg O_3/kg/hr)	Wt (g) at 14D	% SPROUTED		
		4 Days	8 Days	14 Days
0	2.00	46.3bc	80.9	94.2
20	1.58	43.2c	78.9	95.1
40	1.64	52.0abc	79.4	94.8
80	1.49	65.1a	82.0	92.8
160	2.05	61.5ab	78.5	98.6
320	1.69	66.7a	81.9	98.3

Values followed by the same letter are not significantly different (p=0.05)

6.4 Discussion and Conclusions

The curing phase of the post-harvest process allows potatoes to develop wound periderm layers at wound points formed during harvest and grading. Wound periderm layers serve as a barrier to infection by disease and to moisture loss during storage. Rapid and complete formation of the wound periderm layer enhances potato health, disease resistance and quality. In a preliminary trial, ozone applications appeared to increase wound periderm layer thickness, but in a more rigorous trial, ozone applications significantly reduced wound periderm layer thickness. Bono *et al.* (1985) showed that ozone treatments altered the structure of lignin in wood samples. Ozone may be affecting wound periderm formation by altering the lignin formation patterns in the periderm layer. Booker and Miller (1998) showed that ozone treatments increased the products of the lignin pathway, but histochemical and other analysis showed no change in lignin or suberin levels as a function of exposure to ozone. Although the functional significance of the reduction in periderm thickness was not determined from a disease perspective, it is possible that this reduction would result in an increase in disease, especially due to the role of wound periderm layers in disease resistance (Nnodu *et al.*, 1982). If ozone treatments slow or inhibit the formation of wound periderm, this would mitigate against its use during the curing period. Ozone-treated tubers did not appear to have been adversely affected in terms of increased disease or excessive weight loss in

this study, however the tests were of a limited duration and did not examine the long term effects in stored potatoes. The potential for ozone treatments to increase weight loss has been demonstrated in other trials (see Chapter 4). Further studies should consider the relative disease resistance of periderm layers formed with or without ozone, as well as the effect of ozone on pre-formed wound periderm (i.e. during an in-storage application). The effects of ozone on wound periderm development and percent infection during the curing period under intensive pathogen pressure would also potentially provide information on the effect of ozone on ozone:wound periderm:pathogen interactions.

Pre-planting treatment of seed tubers with fungicides is a common practice in commercial potato production. Ideally this treatment ensures a uniform stand of healthy and vigorous plants, as seed piece decay is reduced. In this study, ozone treatment of seed potato pieces occasionally resulted in a reduction in stand relative to seed treated with standard fungicides. This reduction in stand translated into a reduction in yields in some cases. Wet and cool field conditions in that year would have increased disease pressure on the seed pieces and the developing seedlings. Otherwise ozone treatments were comparable to the standard fungicide control treatment. Ozone is only effective at eliminating contaminants present on the surface of seed at the time of application. Ozone does not provide any residual control activity while commercial seed treatments are designed to deliver long-term protection against both seed and soil-borne diseases.

Ozone application did not significantly or systematically alter sprouting of seed potatoes. There was occasionally a slight increase in the rate of sprouting, particularly at high ozone concentrations. Any acceleration of sprouting could be a stress response reflecting accelerated aging of the potatoes by exposure to ozone. Aging or other stresses break tuber dormancy in potatoes (Burton, 1966). Daniels-Lake *et al*. (1996), who evaluated ozone as an alternative to commercial sprout inhibitors, found that ozone treatments (up to 100 ppm) did not reduce sprouting or visible tuber quality in stored potatoes. Any accelerated loss of dormancy would be undesirable in most situations as sprouting during storage reduces tuber quality through changes in tuber physiology and increased water loss. Sprouted potatoes are also physically more difficult to remove from storage. There might however be some potential use for ozone as a seed priming

or dormancy break treatment on potato cultivars that have an excessively long dormancy period (i.e. Russet Burbank). This could be examined in future research.

7. FUNGAL INOCULATION AND OZONE TREATMENT

7.1 Introduction

Efforts to determine the efficacy of ozone as a means of controlling post-harvest disease in potatoes are complicated by variability in the type, incidence and stage of development of the diseases typically found in recently harvested potatoes. In turn, the amount, type and stage of disease present on potatoes in storage varies with the grower, growing season, initial seed contamination, and a number of post-harvest management factors. In previous chapters, the potatoes used in the ozone trials were from commercial seed potato growers. These potatoes had low and variable disease levels, which created difficulties in getting consistent disease responses to ozone treatments. Trials with standardised disease loads would assist in efforts to determine whether ozone can reduce the spread and development of storage pathogens.

Fusarium sambucinum, *Phytophthora infestans* and *Helminthosporium solani* are the most important causes of disease in stored potatoes on the Canadian prairies. *Fusarium* requires a wound to infect the tuber (Howard *et al*., 1994). *Fusarium* develops slowly during storage, eventually leading to a deep-seated dry rot. *Phytophthora infestans* is capable of more rapid and invasive infection, with no requirement for a wound (Howard *et al*., 1994). *Helminthosporium solani* also requires no wound and is capable of spreading in storage. Significant incidence of any of these post-harvest pathogens can cause economic losses through reduction in quality of marketable product.

The location and intensity of pathogen infection is related to the nature of the pathogen, the barriers to infection or resistance that the crop possesses and the method and timing of inoculation (Agrios, 1997). The ability of post-harvest treatments like ozone to counteract deep-seated or well-established disease infections is unclear. With deep-seated, well-established infections it is difficult to achieve contact between the treatment agent and the pathogen, unless the treatment agent is incorporated into the host tissues (i.e. systemic). Oranges which were scratch inoculated with *Penicillium* were not

protected from decay if exposed to ozone, however sporulation of the mould was reduced somewhat, which would reduce further spread (Klotz, 1936 cited in Harding Jr., 1968). Liew and Prange (1994) showed that ozone treatments were ineffective at arresting the development of *Sclerotinia* introduced into a wound in a carrot. Ozone has been shown to be more effective in control of surface infections, as compared to wound-introduced pathogens (Spotts and Cervantes, 1992). Berg *et al*. (1964) showed that breaking up clumps of microorganisms increased the efficacy of ozone applications, as this increased the amount of surface area in contact with the ozone.

Early application of post-harvest treatments improves disease control in stored potatoes, presumably due to the early contact between control agents and the pathogens (Carnegie *et al*., 1986, 1990, 1998). Post-harvest chemicals are typically applied immediately after harvest to increase contact duration and to reduce untreated periods. The interval between infection and treatment does not appear to matter if the infection is deep-seated (Liew and Prange, 1994). Schisler *et al*. (2000) found that the systemic fungicide TBZ was relatively ineffective as a means of disease control in tubers that had been wounded and inoculated with *Fusarium* just 24 hours before application of the fungicide.

Exposing tubers that have been inoculated in a known, reproducible manner with a known quantity of pathogenic propagules at an established interval prior to ozone treatment should make it possible to test whether ozone can control the development and spread of common post-harvest pathogens of potatoes. The objective of this experiment was to evaluate the effect of ozone application on the control of pathogens introduced within 24 hours of the ozone treatment via controlled inoculation at wound depth appropriate to the specific pathogen species.

7.2 Materials and Methods

7.2.1 *Fusarium* Inoculation with Deep Wounding

The inoculation procedures carried out in this experiment were based on techniques found in Platt (1992). The *Fusarium* inoculant was prepared from pure cultures of *Fusarium sambucinum* grown on Potato Dextrose Agar (PDA) at room temperature for four weeks. The *Fusarium* inoculum was prepared by wetting the culture plate with 10 ml of distilled water and gently scraping the surface of the culture

to dislodge the spores and other fungal propagules. The resulting solution was poured into distilled water and the culture plate was rinsed with another 10 ml of distilled water. The number of macroconidia in the resulting solution was established using a two-celled haemocytometer. An inoculation suspension containing 1.5×10^4 macroconidia per ml was prepared.

Russet Burbank potatoes were purchased from a local supermarket chain. The treatment history of these potatoes was unknown, however it was assumed that all tubers had been treated similarly during storage and handling and were therefore uniform from a disease perspective. Holes 2 mm wide by 6 mm deep were punched in the apical end, the stolon end and at the midpoint of the tuber to allow comparison between the various parts of the potato. Twenty tubers were immersed in the *Fusarium* spore suspension for approximately one minute. The control treatments were wounded as previously described but were immersed in distilled water rather than the *Fusarium* spore suspension. Once inoculated, the tubers were moved to a 15°C storage; this simulated conditions during the curing period that follows harvest. The tubers were then treated for 1, 7 or 21 days at ozone rates of 0, 10 or 20 mg O_3/kg/hr. These rates represent ozone levels similar to those used in previous trials (Chapters 4, 5 & 6). Tubers were held at 15°C until all tubers had completed the 3-week treatment period.

After the 3-week treatment period, the tubers were cut along the length of each puncture hole and rated for the degree of spread of *Fusarium* dry rot within the tuber. *Fusarium* causes blackening of the host tissues and some sinking of the tuber surface. The rating scale for *Fusarium* was as follows:

0 = no evidence of any disease

1= trace of disease (1-2 mm width of tissue darkening)

2 = slight disease spread (3-4 mm width of tissue darkening)

3 = moderate disease spread (5-10 mm width of tissue darkening)

4 = severe disease spread (>10 mm width of tissue darkening)

Each treatment was replicated four times and the data were analysed using the GLM procedure of SAS.

7.2.2 Inoculation using Differing Depth Wounding

Based on the results from 7.2.1, the inoculation procedure was modified to further our understanding of ozone efficacy and ozone:pathogen interactions. Norland potatoes of good quality were obtained from a commercial supplier. The tubers were inoculated with *Fusarium sambucinum*, *Phytophthora infestans* or *Helminthosporium solani* using methods which varied in the degree of disruption of the potato surface. All treatments were replicated four times, with ten tubers per treatment replicate.

For the *Fusarium sambucinum* inoculations, 1-month old pure cultures of the fungus were washed and scraped to prepare inoculation suspensions containing 3.0×10^4 macroconidia per ml. The tubers were washed, surface sterilised in a 2% Bleach solution for five minutes, rinsed in distilled water and then air-dried. In the surface application treatment, the tubers were inoculated by spraying the suspension onto unwounded tubers. The spray was applied to surface wetness using a spray bottle and wetting agent. In a lightly wounded treatment, tubers which had been rolled on Ottawa quartz sand were immersed in an agitated spore suspension mixture (Percival *et al*, 1998). The abrasive sand disrupts the integrity of the tuber surface structure, resulting in shallow wounds. In both the surface and light abrasion treatments, control treatments involved soaking or spraying with distilled water. Following inoculation, the tubers were incubated at room temperature for 24 hours and then treated for 1, 7 or 21 days in either ozone or control atmospheres at 15°C. The ozone was applied at approximately 20 mg O_3/kg/hr as in other experiments (Chapters 4, 5 & 6). Following ozone treatment, the tubers were placed in the control environment (15°C) until all tubers had completed the 3-week treatment period. At the completion of this 3-week treatment period, tubers were evaluated for disease incidence (percentage of the tubers in the sample lot expressing disease symptoms) and disease severity (percent tuber rot, 0-100%).

Two isolates of *Phytophthora infestans* (A2 strains isolated on Saskatchewan potato farms) were grown on Clarified Rye Agar (CRA) plates with β-sitosterol added (Forbes, 1997). Spore suspensions were prepared to contain 5.0×10^4 sporangia per ml. The spore suspension was allowed to sit for two hours to create a 50:50 ratio of germinated to non-germinated sporangia (Bjor, 1987). Non-wounded, surface sterilised tubers were sprayed to surface wetness with the *Phytophthora* spore suspension and then

incubated for 24 hours at 15°C. The tubers were treated with ozone as previously described (ozone versus control for 1, 7 or 21 days) and evaluated for disease incidence (%) and tuber rot severity (%) after all tubers had completed the 3-week treatment period.

Helminthosporium solani cultures were grown on PDA for approximately 2 months, following which spores were collected and suspensions containing 2.5 x 10^4 conidia per ml were sprayed onto the tubers (Rodriguez *et al*., 1995). Tubers were incubated for 24 hours at 15°C and then treated with ozone as previously described (ozone versus control for 1, 7 or 21 days). At the completion of the ozone treatment period (3 weeks), the tubers were incubated for two weeks at room temperature in closed plastic tubs containing wet paper towel. The combination of high relative humidity and warm conditions are ideal for *Helminthosporium solani* sporulation. Incubation was stopped after two weeks due to extensive development of bacterial soft rot. Tubers that had broken down completely due to the soft rot were not rated for *Helminthosporium*. The remaining tubers were rated for percent surface area with the silver scurf lesions characteristically caused by *H. solani*. For all experiments, data were analysed using the GLM procedure of SAS as described in Chapter 4.

7.3 Results

7.3.1 Effect of Inoculation on Disease Development

Inoculation generally increased the incidence and severity of all diseases (Table 7.1). Increasing the depth of wounding did not consistently increase the incidence of *Fusarium* dry rot but rot severity was increased as the depth of the wound increased (Table 7.1). To evaluate the ozone treatments as a means for controlling disease, only data from the inoculated potatoes was analysed in detail.

Table 7.1 Effect of inoculation and depth of wounding on disease incidence and severity in potatoes

WOUND TYPE + FUNGAL SPECIES	SQRT INCIDENCE	SQRT SEVERITY
Deep Wounded + *Fusarium*	N/A	***
Lightly Wounded + *Fusarium*	ns	ns
Surface Inoculated + *Fusarium*	***	***
Surface Inoculated + *Phytophthora*	***	**
Surface Inoculated + *Helminthosporium*	ns	ns

, **, = significant at p=0.10, 0.05 or 0.01 respectively**

SQRT = square root transformed

		MEANS‡	
WOUND TYPE + FUNGAL SPECIES	**INOC[N]**	**% INCIDENCE**	**SEVERITY (Rating 0-4)**
Deep Wounded + *Fusarium*	**CTRL**	-	0.11b†
	INOC[D]	-	0.47a
		% INCIDENCE[a]	**ROT SEVERITY[b] (%)**
Lightly Wounded + *Fusarium*	**CTRL**	68.8	6.41
	INOC[D]	66.3	6.62
Surface Inoculated + *Fusarium*	**CTRL**	42.9b	0.65b
	INOC[D]	70.0a	1.21a
Surface Inoculated + *Phytophthora*	**CTRL**	26.7b	3.00b
	INOC[D]	40.0a	4.67a
		% INCIDENCE[a]	**SEVERITY[b] (%)**
Surface Inoculated + *Helminthosporium*	**CTRL**	10.7	0.18
	INOC[D]	16.7	0.32

‡ Means are original data; † Mean separation is based upon square root transformed data; Values followed by the same letter are not significantly different (p=0.05)
[a] = % of total tubers with disease present; [b] = % of tuber with rot or lesions
INOC[N] = Inoculation; INOC[D] = Inoculated

The purpose of the above table (Table 7.1) was to determine if inoculation could increase disease. The levels of *Phytophthora infestans* recorded for the controls are unreasonably high, as little or no disease would be expected. Disease levels were recorded based on putative symptom evaluations. It is possible that symptoms of physiological damage or other diseases could have been mistaken for the disease. Despite the higher than expected disease in the controls, the difference between the control and inoculated tubers was significant. Only inoculated tuber data is used in ozone treatment comparisons.

7.3.2 Control of Disease Development by Ozone Application

7.3.2.1 Deep Wound Inoculation with *Fusarium*

Disease severity varied significantly among locations on the tuber (Table 7.2). The apical end of the tuber was less susceptible to disease development than the sides and stolon (stem) end of the tuber (Table 7.2). The effect of ozone treatments

(concentration or duration of exposure) did not vary with the inoculation position (Table 7.2).

Table 7.2 Analysis of variance and main effect means for the effect of wound position on the development of *Fusarium* dry rot in deep-wounded potatoes

SOURCE	DF	*Fusarium* SEVERITY
		MS
REP	3	2.08**
INOCULATED (I)	1	25.4***
CONCENTRATION (C)	2	0.45
EXPOSURE (E)	2	4.8***
POSITION (P)	2	10.1***
I*C	2	0.01
I*E	2	0.83
I*P	2	6.26***
C*E	4	2.25**
C*P	4	1.19
E*P	4	0.21
C*E*P	8	0.31
I*C*E*P	20	0.17
ERROR	1023	
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**		
MEANS		
POSITION		**RATING (0-4)**
APICAL		0.13b
STOLON		0.30a
SIDE		0.36a

Values followed by the same letter are not significantly different (*p*=0.05)

The data for the effect of ozone treatments on *Fusarium* dry rot levels in wounds created at the apical, stolon and mid-point of the tubers (side) and averaged over the whole tuber is presented in Table 7.3. Ozone concentration had no effect on disease development at any location (Table 7.3). Exposure to ozone for one week produced the lowest disease levels, although this effect was only statistically significant at the side location, which was the most susceptible to *Fusarium* dry rot infection (Table 7.3).

Table 7.3 Analysis of variance and main effect means for the effect of ozone treatments on the control of *Fusarium* dry rot development in deep-wounded potatoes.

SOURCE	DF	POTATO LOCATION			
		APICAL END	STOLON END	SIDE	AVERAGE
		MS	MS	MS	MS
CONCENTRATION (C)	2	0.32	0.14	0.74	0.10
EXPOSURE (E)	2	0.54	0.41	1.53**	0.90**
C*E	4	0.32	0.03	0.41	0.20
REP	26	0.41*	0.52	0.37	0.22
ERROR	145	0.27	0.45	0.39	0.18

*, **,*** = significant at p=0.10, 0.05 or 0.01 respectively

	MEANS‡			
	APICAL END	STOLON END	SIDE	AVERAGE
CONCENTRATION (mg O_3/kg/hr)	Rating (0-4)	Rating (0-4)	Rating (0-4)	Rating (0-4)
0	0.13	0.42	0.74	0.51
10	0.20	0.10	0.55	0.43
20	0.28	0.48	0.55	0.47
EXPOSURE (DAYS)				
1	0.23	0.48	0.73a	0.55a
7	0.10	0.33	0.43b	0.33b
21	0.28	0.48	0.68a	0.53a

‡ Means are original data; Values followed by the same letter are not significantly different (p=0.05)

7.3.2.2 Shallow Wound Inoculation with *Fusarium*

When tubers were only lightly wounded, the un-inoculated control treatments were extensively damaged by *Fusarium* dry rot and inoculation did not further increase the disease incidence or severity (Table 7.1). This would indicate of a high level of natural inoculum. The ozone treatments had no effect on either the incidence or severity of *Fusarium* dry rot in this trial (Table 7.4).

Table 7.4 Analysis of variance and main effect means for the effect of ozone treatments on *Fusarium* dry rot development in lightly wounded potatoes

SOURCE	DF	SQRT INCIDENCE MS	DF	SQRT SEVERITY MS
CONCENTRATION (C)	1	1488.86	1	0.00
EXPOSURE (E)	2	43.53	2	0.18
C*E	2	1214.93	2	9.79
REP (C*E)			17	4.03
ERROR	18	628.01	217	4.56

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**

SQRT = square root transformed

	MEANS‡	
CONCENTRATION (mg O_3/kg/hr)	**% INCIDENCE[a]**	**ROT SEVERITY[b] (%)**
0	66.0	6.73
45	66.0	6.47
EXPOSURE (DAYS)		
1	60.0	6.73
7	72.5	6.66
21	66.3	6.44

‡ Means are original data; [a] = % of total tubers with disease present; [b] = % of tuber with rot or lesions

7.3.2.3 Surface Inoculation with *Fusarium*

Fusarium incidence and severity were increased by surface inoculating non-wounded potatoes (Table 7.1). Ozone application caused a slight reduction in the incidence of *Fusarium* dry rot compared to the control (Table 7.5). The duration of exposure to ozone significantly affected disease incidence, however the differences between treatments were not substantial and did not follow a discernible trend as a function of duration of exposure (Table 7.5).

Table 7.5 Analysis of variance and main effect means for the effect of ozone treatments on *Fusarium* dry rot development in non-wounded potatoes.

SOURCE	DF	SQRT INCIDENCE MS	DF	SQRT SEVERITY MS
CONCENTRATION (C)	1	54.39*	1	0.37
EXPOSURE (E)	2	50.61*	2	0.59
C*E	2	52.08*	2	0.77
REP(C*E)			10	0.86
ERROR	18	17.28	216	0.72
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**				
SQRT = square root transformed				
		MEANS‡		
CONCENTRATION (mg O_3/kg/hr)		**% INCIDENCE[a]**		**ROT SEVERITY[b] (%)**
0		76.0a†		1.24
45		64.0a		1.14
EXPOSURE (DAYS)				
1		70.0a		0.82
7		69.0a		1.3
21		71.0a		1.46

‡ Means are original data; † Means separation is based on square root transformed data; Values followed by the same letter are not completely different (p=0.05);
[a] = % of total tubers with disease present; [b] = % of tuber with rot or lesions

7.3.2.4 Surface Inoculation with *Phytophthora*

Inoculation with *Phytophthora* increased both the disease incidence and severity (Table 7.1). Ozone treatments did not affect any of the disease development parameters monitored (Table 7.6).

Table 7.6 Analysis of variance and main effect means for the effect of ozone treatments on *Phytophthora* rot development in non-wounded potatoes

SOURCE	DF	SQRT INCIDENCE MS	DF	SQRT SEVERITY MS
CONCENTRATION (C)	1	0.01	1	1.35
EXPOSURE (E)	2	0.03	2	5.04
C*E	2	0.00	2	3.85
REP (C*E)			18	4.99
ERROR	18	0.01	216	3.73
, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**				
SQRT = square root transformed				
		MEANS‡		
CONCENTRATION (mg O_3/kg/hr)		**% INCIDENCE[a]**		**ROT SEVERITY[b] (%)**
0		38.0		3.61
45		42.0		5.72
EXPOSURE (DAYS)				
1		43.8		6.71
7		45.0		3.79
21		31.3		3.48

‡ Means are original data; [a] = % of total tubers with disease present; [b] = % of tuber with rot or lesions

7.3.2.5 Surface Inoculation with *Helminthosporium*

Inoculation did not significantly increase *Helminthosporium* incidence or severity (Table 7.1). Ozone-treated potatoes appeared to have a lower incidence of *Helminthosporium* than control potatoes, while the percent of tuber surface with visible lesions was significantly (p=0.10) reduced by the ozone treatments relative to the controls (Table 7.7).

Table 7.7 Analysis of variance and main effect means for the effect of ozone treatments on *Helminthosporium* silver scurf lesion development in non-wounded potatoes

SOURCE	DF	SQRT INCIDENCE MS	DF	SQRT SEVERITY MS
CONCENTRATION (C)	1	0.11	1	0.93*
EXPOSURE (E)	2	0.07	2	0.02
C*E	2	0.18**	2	0.76*
REP (C*E)			18	0.24
ERROR	18	0.05*	144	0.27
, **, = significant at p=0.10, 0.05 or 0.01 respectively**				
SQRT = square root transformed				
		MEANS‡		
CONCENTRATION (mg O_3/kg/hr)		**% INCIDENCE[a]**		**SEVERITY[b] (% SURF AREA)**
CONTROL		20.0		0.48a†
45		10.0		0.15a
EXPOSURE (DAYS)				
1		16.3		0.29
7		15.0		0.23
21		13.8		0.43

‡ Means are original data; † Mean separation is based on square root transformed data; Values followed by the same letter are not significantly different (p=0.05);
[a] = % of total tubers with disease present; [b] = % of tuber with lesions

The highest amount of applied ozone (highest concentration and longest duration of exposure) dramatically reduced silver scurf severity and incidence as compared to the controls (Fig. 7.1).

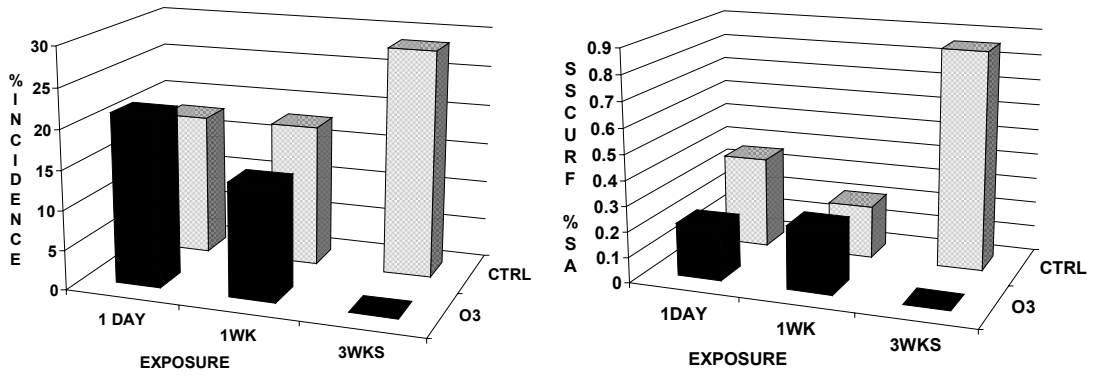

Figure 7.1 Changes in *Helminthosporium* incidence and percent surface area in ozone-treated versus control inoculated potatoes [LSD %Incid=34.0; %SA=0.28]

7.4 Discussion and Conclusions

Post-harvest diseases are often introduced during harvest and grading operations, via wounds or contact between healthy and infected tubers. Some post-harvest pathogens can spread from tuber to tuber during storage (e.g. *Helminthosporium*), while others do not spread, but can increase in severity during storage (e.g. *Fusarium*). The extent and depth of wounds and the degree of infection by disease influence the potential for post-harvest losses and efficacy of disease control treatments.

Fusarium is commonly introduced by wounding at harvest and is best controlled if the tubers are treated immediately after infection (Carnegie *et al*., 1986, 1990, 1998). Commercial fungicides are typically applied to freshly harvested potatoes during loading into storage (Burton, 1966). This ensures early contact between the treatment and the pathogen. Schisler *et al*. (2000) showed that the standard fungicide (TBZ) applied within 24 hours of harvest provided some control of *Fusarium* dry rot. In this experiment, ozone applied within 24 hours of exposing the potatoes to *Fusarium* was ineffective at controlling the development of *Fusarium* dry rot. The lack of response to ozone, even when the *Fusarium* dry rot infection was recent and therefore not well established would suggest that the potential to use ozone to control this disease is limited. Further shortening of the time between ozone treatment and infection might be possible, for example by treating the potatoes during the loading process. However, this involves an extremely short time period (< 1min), therefore very high ozone concentrations would be required.

Increasing the depth at which the *Fusarium* is introduced generally reduces efficacy of control treatments. Liew and Prange (1994) inoculated carrots with 1.0 cm plugs of *Botrytis cinerea* and *Sclerotinia sclerotiorum*, resulting in a deep infection. Exposing the inoculated carrots to a range of ozone treatments shortly after inoculation suppressed mycelial growth, however, the pathogen was not killed. When the carrots were moved into an ozone-free atmosphere, the infection resumed a normal rate of growth.

Ozone did not control *Phytophthora infestans*, which is an aggressive post-harvest pathogen capable of infecting even healthy tubers in storage if inoculum is introduced onto the tubers. Currently there are no fungicides registered for the post-harvest control of *Phytophtora* in potatoes, although the fumigants Purogene® and Oxidate claim to be capable of controlling it. Ozone treatments (concentration and duration of exposure) were more effective for reducing the incidence and severity of silver scurf caused by *Helminthosporium*. This may reflect a greater sensitivity of this pathogen to ozone. Alternatively it may be related to the relative aggressiveness, rate or nature of growth/spread of the pathogen. *Phytophthora* grows rapidly compared to *Helminthosporium*, however *Phytophthora* does not spread in storage, while *Helminthosporium* produces spores and can spread throughout storage. In this case, ozone is likely controlling the spore production by the *Helminthosporium*, while it was not capable of controlling the deep-seated rot development characteristic of *Phytophthora*.

Standard fungicides persist on tuber surfaces and provide residual protection against developing post-harvest pathogens. A treatment such as ozone does not provide any residual activity and must therefore completely kill the pathogen – otherwise it will continue to spread upon cessation of the treament. Liew and Prange (1994) observed this effect in inoculated carrots. *Helminthosporium* would likely resume growth and sporulation, unless the ozone treatments were repeated or applied continuously. Continuous ozone treatments in stored onions reduced airborne spore production in storage but did not remove deep-seated infections.

This experiment was designed to evaluate the effect of ozone applications on several common post-harvest disease organisms of potatoes introduced via controlled

inoculation just prior to the ozone treatment. This allowed for controlled observation of ozone:pathogen and wound:pathogen:ozone dynamics. These trials indicate only limited potential for the control of common pathogens via ozone application, even under near-ideal treatment conditions.

Ozone does not appear capable of controlling either deep-seated infections or rapidly developing aggressive pathogens, however it did reduce, but not eliminate, a slower moving, surface-type disease such as *Helminthosporium* silver scurf. Ozone might potentially work for similar diseases, such as *Penicillium* rot or *Botrytis* grey mold. These results raise the the question of whether the observed differences in control of different potato pathogens reflect differences in ozone sensitivity or differences in their ability to rapidly establish deep-seated, treatment-resistant infections. Studies evaluating the effect of ozone on various pathogens under controlled conditions (i.e. *in vitro*) may help to provide a better understanding of the capabilities of ozone to control specific disease organisms.

8. OZONE TREATMENT OF POTATO PATHOGENS (*IN VITRO*)

8.1 Introduction

Exposure to ozone has shown potential as a treatment to reduce or alter the growth, development and viability of pathogens of a number of crops (Hibben and Stotzky, 1969; Treshow *et al*., 1969; James *et al*., 1982). Ozone rates and treatment efficacy have varied greatly, depending on the crop and treatment method employed. However, previous studies presented in this thesis (Chapters 4, 5, 7) indicated that ozone was relatively ineffective in reducing disease development in stored potatoes under the conditions used in the experiments. The responses to ozone were variable depending on the ozone treatment, experimental conditions, the pathogen and the response variable being examined. Disease-causing pathogens are known to vary in their resistance to chemical treatments, including ozone (Hibben and Stotsky, 1969; Kawchuk *et al*., 1994; Bains *et al*., 2002). Testing the efficacy of any treatment on a pathogen is complicated by interactions between the pathogen, the treatment and the environment. This relationship is further complicated by treatment variables such as the position of the infection, host age, type and vigour, the amount of pathogen present, as well as the presence of contaminants which might interfere with the function of the disease control treatment. Using pure fungal cultures in response assays eliminates many of these external factors and may allow a clearer understanding of the capabilities of any disease control agent.

The objective of this experiment was to evaluate the effect of ozone on germination, mycelial development and sporulation of a range of pathogenic fungi under controlled environmental conditions.

8.2 Materials and Methods

Pure fungal cultures of a number of post-harvest pathogens of stored potatoes (*Fusarium sambucinum, Fusarium solani, Phytophthora infestans* and *Helminthosporium solani*) were prepared on 88 cm diameter petri dishes containing Potato Dextrose Agar (PDA), PDA, Clarified Rye Agar (CRA) and PDA respectively.

Fusarium sambucinum and *Fusarium solani* cultures were established using 5-mm plugs transferred from the actively growing margins of a pure stock culture. Plates were grown at room temperature for two days and then treated in the dark with either 0 or ~45 mg O_3/plate/hr for 1 or 2 days. The amount of ozone is based on the number of plates present in the room at the time of treatment. This rate of ozone corresponds to approximately double the maximum rate used in the previously discussed potato trials and represented an initial, high dosage screening treatment. During the treatment period, plates were closed, to prevent contamination by other pathogen species. It was assumed that some air exchange was possible.

Following the two days of treatment, the plates were maintained at room temperature in the dark. Colony diameters were measured before the ozone treatment and then on a daily basis for up to five days (including the treatment period). Fungal growth rates were determined by calculating the increase in diameter (mm) for each day, using the measurement from the previous day as the base. Each treatment consisted of three sample plates, with the complete treatment set replicated three times.

The effect of ozone treatment on spore production by *Fusarium* cultures was also evaluated. *Fusarium* cultures were established as in the mycelial growth rate trials and exposed to the same ozone treatments. Following treatment with ozone, the *Fusarium* cultures were grown in the dark for three days and the spores were then harvested. Spores were collected by spreading 10 ml of distilled water over each plate, at which time the cultures were gently scraped with a plastic tool to dislodge the spores. The resulting spore suspension was poured into 80 ml of distilled water and an additional 10ml of distilled water was added to rinse any remaining spores from the cultures. This rinse was added to the suspension and the final suspension was stirred vigorously. Two 1-ml samples from this suspension were placed into microcentrifuge tubes containing 0.5-ml of dilute Lacto-Fuschin stain as a preservative. Tubes were refrigerated until they could be counted using a two-celled haemocytometer. Each sample was counted twice to ensure accuracy and the average number of spores per ml determined. The same procedure was used for the *Fusarium solani* cultures.

Helminthosporium solani grows slowly and therefore two to three week old cultures were used in this trial. *Helminthosporium* cultures were treated with ozone for

1 or 2 days as previously described. Post-treatment growth rates were measured at 2-day intervals over 1 week. Spore production by the *Helminthosporium* cultures was too limited to be evaluated.

Trials to evaluate the effects of ozone on *Phytophthora infestans* sporulation were carried out on CRA media modified with 0.05g β-sitosterol/L (40% from soybean; Sigma-Aldrich Canada Inc.) (Forbes, 1997). Mycelial growth rates were evaluated on the standard CRA media. Cultures were treated with the previously described ozone rates for one or two days and then the cultures were allowed to grow for five days. After treatment and growth, the plates were washed and rinsed with 2 and then 5 ml of distilled water to collect spores. The spore suspension samples were stained and counted as in the other trials.

Sclerotia are masses of condensed hyphae, which can remain viable for long periods of time. To test the effects of ozone treatment on the viability of the fungal resting bodies, the generation of sclerotia of *Rhizocotonia solani* was attempted on PDA plates. Due to isolate variability (Sabina Banniza, Personal Communication), no sclerotia were produced. Sclerotia of *Sclerotinia sclerotiorum* were produced on PDA plates from a pure source culture (Isolated from an unknown host, Saskatchewan). Ten sclerotia were placed on Water Agar (WA) plates and treated for one or two days with ozone at the rates previously described. Germination percentage was observed on a daily basis until 100 % germination was achieved in the controls (after five days).

All experiments were treated as Split-Plot Designs with measurements over time (Split-Split Plot). Ozone concentrations represented the main plots, with the duration of exposure (one or two days) representing the subplot treatment. Data were analysed accordingly, using the GLM procedure of SAS.

8.3 Results

8.3.1 Effect of Ozone Treatments on Sporulation

Ozone treatments did not significantly influence spore production by any of the fungal species tested (Table 8.1). Plate to plate variability in the spore counts was high (Table 8.1).

Table 8.1 Analysis of variance and main effect means for the effect of ozone treatments on sporulation by various fungal pathogens

SOURCE	DF	*Fusarium sambucinum*	*Fusarium solani*	*Phytophthora infestans*
		MS	MS	MS
REP	2	765.21	3.25	9.19
CONCENTRATION (C)	1	332.45	19.51	3.18
REP*C	2	567.30	10.82	19.38**
EXPOSURE (E)	1	327.61	0.61	3.93
C*E	1	241.28	0.05	1.82
REP (C*E)	4	268.43***	6.69**	2.35***
ERROR	24	46.55	2.31	0.31

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**

	MEANS		
	F. sambucinum	***F. solani***	***P. infestans***
CONCENTRATION (mg O_3/plate/hr)	**Spores/ml (x 10^4)**	**Spores/ml (x 10^4)**	**Spores/ml (x 10^4)**
0	60.24	6.77	1.96
45	66.32	8.24	1.37
EXPOSURE (DAYS)			
1	66.29	7.63	1.99
2	60.26	7.37	1.33

8.3.2 Mycelial Growth

Fusarium spp. colony size increased with time, irrespective of the ozone treatment. Growth rates were not noticeably suppressed during the ozone treatment period (Table 8.2). Ozone-treated cultures of *Fusarium solani* had slightly higher average growth rates compared to the control. Otherwise, the ozone treatments had no significant impact on the rate of growth or final colony size of either species of *Fusarium*. Significant exposure by time interactions appear to be largely influenced by the time variable, with some slight reduction in colony width caused by longer exposure.

Table 8.2 Analysis of variance and main effect means for the effect of ozone treatments on final colony diameter and the colony growth rates of *F. sambucinum* and *F. solani*

SOURCE	DF	*Fusarium sambucinum*		*Fusarium solani*	
		Colony Diameter	Growth Rate	Colony Diameter	Growth Rate
		MS	MS	MS	MS
REP	2	236.58	0.73	49.51	1.72*
CONC (C)	1	84.03	2.64	1.17	2.57**
REP*C	2	87.37	7.04	8.63*	0.14
EXPOSURE (E)	1	171.17	16.34	0.73	0.01
C*E	1	38.03	0.29	4.20	0.31
REP (C*E)	4	49.41***	0.23	1.56**	0.08
TIME (T)	3	9653.48***	9.86**	2480.53***	5.40***
C*T	3	5.06	3.15	0.38	0.91
E*T	3	9.99***	1.80	0.43	0.70
C*E*T	3	0.05	0.38	0.22	0.05
REP (C*E*T)	24	2.34	2.82**	0.57	0.99***
ERROR	96	7.04	1.65	0.49	0.25

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively; CONC =Concentration**

MEANS

	F. sambucinum		*F. solani*	
CONCENTRATION (mg O_3/plate/hr)	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**
0	54.1	12.6	35.5	5.06a
45	52.6	12.4	35.7	5.29a
EXPOSURE (DAYS)	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**
1	54.4	12.8	35.7	5.17
2	52.2	12.2	35.6	5.18
TIME (DAYS)	**Daily Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Daily Diameter (mm)**	**Avg. Growth Rate (mm/day)**
1	34.4d	11.8b	25.0e	4.85b
2	47.0c	12.4ab	30.6d	5.60a
3	59.4b	12.5ab	35.4c	4.82b
4	72.5a	13.1a	41.0b	5.58a
5			46.0a	5.03b

Values followed by the same letter are not significantly different (*p*=0.05)

Growth of the *Helminthosporium solani* cultures was unaffected by the ozone treatments (Table 8.3). The application of ozone significantly reduced growth of the *Phytophthora* colonies during the treatment period, however growth rates returned to normal upon removal from the ozone atmosphere (Table 8.3; Fig. 8.1). The duration of exposure to ozone did not affect colony growth of *Phytophthora* (Table 8.3). Some differences in the effect in ozone exposure on *Helminthosporium* colony diameter and growth rate were visible over time, however these effects appear to influenced largely by the time variable (Table 8.3).

Table 8.3 Analysis of variance and main effect means for the effect of ozone treatments on final colony diameter and the colony growth rates of *P. infestans* and *H. solani*

SOURCE	DF	*Phytophthora infestans*		*Helminthosporium solani*	
		Colony Diamter	Growth Rate	Colony Diameter	Growth Rate
		MS	MS	MS	MS
REP	2	105.79***	1.21	3.03	0.25
CONC (C)	1	2868.01***	190.14***	6.72	0.11
REP*C	2	0.55	0.17	3.09	0.51
EXPOSURE (E)	1	0.07	0.09	1.13	0.15
C*E	1	0.17	3.76*	24.50	0.38
REP (C*E)	4	12.99***	0.76	6.57***	0.68
TIME (T)	3	1940.38***	92.95***	109.45***	0.11
C*T	3	69.47***	15.30***	0.11	0.49
E*T	3	0.61	1.05	0.95**	1.84**
C*E*T	3	3.21	0.31	0.22	0.05
REP (C*E*T)	24	1.63	2.96***	0.18	0.34
ERROR	96	3.90	1.61	0.81	0.40

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively; CONC = Concentration**

MEANS

	P. infestans		*H. solani*	
CONCENTRATION (mg O_3/plate/hr)	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**
0	35.6a	5.04a	15.6	2.14
45	28.6b	2.99b	15.0	2.06
EXPOSURE (DAYS)	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Avg. Diameter (mm)**	**Avg. Growth Rate (mm/day)**
1	32.6	4.03	15.2	2.06
2	32.6	3.99	15.4	2.15
TIME (DAYS)	**Avg. Daily Diameter (mm)**	**Avg. Growth Rate (mm/day)**	**Avg. Daily Diameter (mm)**	**Avg. Growth Rate (mm/day)**
1	24.4e	1.81d		
2	27.4d	3.67c	13.2c	2.03
3	31.6c	4.18b		
4	37.0b	5.39a	15.3b	2.17
5	42.6a	5.64a		
6			17.4a	2.10

Values followed by the same letter are not significantly different (*p*=0.05)

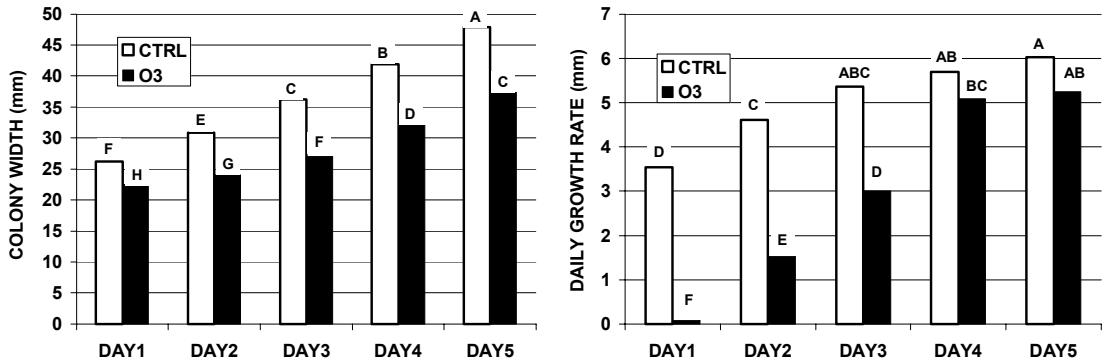

Figure 8.1 Effect of ozone treatments on *Phytophthora infestans* daily colony width and average mycelial growth rate over time [Within each graph, columns with the same letter are not significantly different, p=0.05]

8.3.3 Ozone Effect on Viability of *Sclerotinia* sclerotia

Ozone treatments significantly reduced the germination of mature, culture-grown sclerotia compared to the controls. The effect was apparent within one day of treatment and continued over the remainder of the evaluation period, although the inhibitory effects of the ozone treatments diminished as time after treatment increased (Table 8.4; Figure 8.2). It appears likely that all sclerotia would have germinated given sufficient time. The duration of exposure to ozone did not significantly affect germination of the sclerotia (Table 8.4).

Table 8.4 Analysis of variance and main effect means for the effect of ozone treatments on the viability of sclerotia of *Sclerotinia sclerotiorum*

SOURCE	DF	*Sclerotinia sclerotiorum* MS
REP	2	1.24
CONCENTRATION (C)	1	476.94***
REP*C	2	4.74
EXPOSURE (E)	1	0.67
C*E	1	2.01
REP (C*E)	4	2.06*
TIME (T)	4	498.59***
C*T	4	38.74****
E*T	4	1.26
C*E*T	4	0.70
REP (C*E*T)	32	0.81
ERROR	120	1.20

, **, = significant at *p*=0.10, 0.05 or 0.01 respectively**

	MEANS[‡]
CONCENTRATION (mg O_3/plate/hr)	**% Germination**
0	67.3a
45	34.8b
EXPOSURE (DAYS)	**% Germination**
1	50.4
2	51.7
TIME (DAYS)	**% Germination**
1	1.90e
2	24.2d
3	59.2c
4	80.8b
5	89.2a

Values followed by the same letter are not significantly different (*p*=0.05)

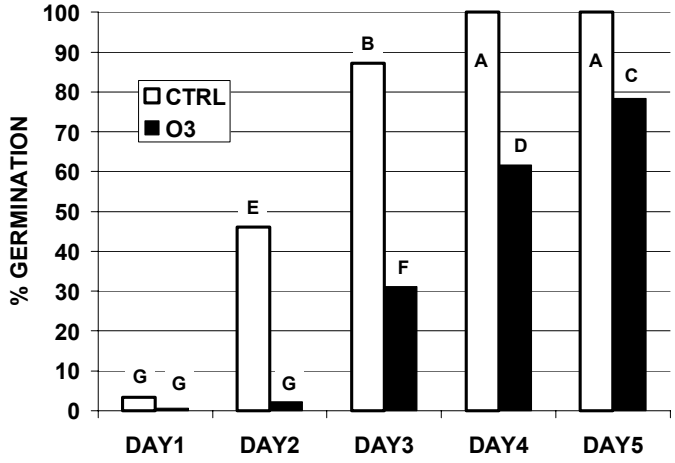

Figure 8.2 Changes in germination over time of sclerotia of *Sclerotinia sclerotiorum* treated with ozone [Columns with the same letter are not significantly different, *p*=0.05].

8.4 Discussion and Conclusions

The control of disease in storage can be achieved by reducing the amount of disease entering the storage, followed by correct management of storage conditions. Ozone has been suggested as a potential replacement for post-harvest chemicals commonly used to control disease in potato storage. Ozone has strong biocidal capabilities (Horváth *et al*., 1985; Hodge, 1998; Kim *et al*., 1999b) and a number of unique and useful characteristics (e.g. no residues, gaseous form, etc.). Ozone treatments have been shown to effectively control disease in a number of crops (Horváth *et al*., 1985; Barth *et al*., 1995; Xu, 1999), but results are variable and the suitability of ozone must be determined for each application situation. For ozone (or any post-harvest treatment) to be useful, it must effectively control any diseases present at the time of treatment and potentially reduce or suppress further development by the pathogens. Treatment responses to any pesticide *in vivo* are variable, due to non-homogeneous disease levels and the influence of environmental factors. *In vitro* studies ideally remove these confounding factors and allow for the selection of effective treatment types and rates. Similarly, a clearer understanding of pathogen and treatment interactions could be developed.

A number of studies have documented the effect of ozone treatments on the growth patterns of fungal pathogens. Heagle and Key (1973) and Heagle and Strickland (1972), in studies on wheat stem rust (*Puccinia graminis* Pers.) and powdery mildew (*Erysiphe graminis f. sp. hordei* DC.), found that ozone treatments significantly reduced *Puccinia* hyphal growth while increasing *Erysiphe* colony length. Both studies were conducted *in vivo*. Hibben and Stotzky (1969) observed that the effect of ozone treatments on germination of spores of a range of fungal species varied with the species and treatment rates. They also observed abnormal growth in fungal colonies maintained in an ozone atmosphere. Treshow *et al*. (1969) ozonated culture plates of a number of fungal species and observed subsequent vegetative and reproductive growth. Treating *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara with ozone (10 or more pphm) suppressed radial growth. More resistant species were not affected by ozone treatments of up to 60 pphm. Ozone-sensitive species showed reduced spore production following exposure to ozone compared to resistant species. However, in

some species, spore production was actually increased by ozone treatment, likely reflecting a stress response. A similar observation was made in this experiment, where ozone-treated *F. solani* cultures appeared to have a slightly higher (but not significant) spore count than the untreated cultures (Table 8.1). Krause and Weidensaul (1978a) observed that ozone significantly inhibited sporulation of *Botrytis cinerea*, *in vivo* and *in vitro* and also reduced the viability of any conidia that were produced. James *et al*. (1982) found that low ozone concentrations significantly reduced conidial production and germination as well as colony growth rates in *Fomes annosus* cultures. Harding Jr. (1968) observed that ozone treatments reduced sporulation of *Penicillium* in inoculated oranges, although mycelial development was not reduced. In this experiment, ozone did not reduce sporulation by any of the tested species. The effect of ozone on sporulation appears to depend on the species being examined and on the rate of ozone applied.

Many studies have shown that the viability of fungal propagules can be reduced by ozonation. Hibben and Stotzky (1969) found large, pigmented spores were unaffected by ozonation, whereas small, hyaline spores were highly sensitive. This suggested either a size effect or resistance relating to pigmentation. A large spore surface area or the presence of pigments may increase the reactive surface quantities of the spores, reducing the effect of the ozone. A study by an ozone generator company (www.airpure.com/mold.html) stated that ozone treatment could effectively eliminate pathogens, however subsequent recovery of the pathogen was possible if the ozone treatment was not complete and thorough. In this study, similar effects were observed, where pathogens showed normal growth or germination following removal to an ozone-free atmosphere. This suggests that the ozone treatments were not of sufficient duration to kill or permanently inhibit growth and would therefore provide a delaying action at best.

This experiment demonstrated the variability in effectiveness of ozone treatments depending on the fungal species and the growth or developmental process being observed. The ozone treatments used in this trial were generally ineffective at killing or even inhibiting growth or reproduction of a range of post-harvest pathogens even under "ideal" conditions. Higher ozone concentrations and longer exposures might be required but whether this might be practical is questionable, as the window for effective control is

relatively narrow, as demonstrated in Chapter 7. Alternatively, ozone could be recognised as a limited alternative, to be used for moderate and temporary suppression until such time as additional control measures could be implemented. The conditions under which this type of control would be possible would be highly specific, with special considerations made for environmental conditions, the predominant pathogen, and the ozone generation capabilities of the system.

9. GENERAL DISCUSSION AND CONCLUSIONS

This project evaluated the efficacy of ozone gas (O_3) as an alternative post-harvest treatment for disease control in stored potatoes. Dosage effects on tuber quality and seed potato performance, and pathogen:treatment interactions were examined.

Ozone is a strong oxidizer and has been used for many years as a disinfectant for water. Post-harvest treatment with ozone has also been shown to effectively control a range of post-harvest pathogens on a variety of crops (Horváth *et al*., 1985; Kim *et al*., 1999b). The ozone treatments used for management of post-harvest disease vary widely in the rate and method of application, as well as in their effectiveness (Horváth *et al*, 1985; Kim *et al*, 1999b; Xu, 1999). Given this variation in efficacy and method of application, determining an appropriate combination of ozone dose, delivery method and treatment conditions is crucial for each host:pathogen combination. The first phase of this project evaluated ozone treatments of varying concentrations and durations of exposure as a means of controlling several of the most common post-harvest diseases of potatoes. The effect of these ozone treatments on quality of the potatoes was also examined. The treatment combinations used were based on; a) the ozone generation capacities of the available equipment, b) preliminary evaluations and c) treatments used by other researchers. For practical reasons, only a limited number of treatment combinations could be tested. The ozone treatments were applied just after harvest, as this represents the point at which post-harvest diseases are becoming established. Populations of the pathogen are low and therefore more readily managed and significant damage to the commodity has yet to occur. For these reasons, this is the time when commercial fungicides are typically applied to stored potatoes. The efficacy of mid-storage and continuous ozone treatments (during long-term storage) were also evaluated.

The most common diseases found in or on stored potatoes grown on the Canadian prairies include *Fusarium* dry rot, *Erwinia* soft rot, *Rhizoctonia* black scurf and *Helminthosporium* silver scurf. None of the ozone treatment combinations resulted

in a consistent, significant reduction in the levels of *Fusarium*, *Erwinia*, *Helminthosporium* or *Rhizoctonia*, based on samples taken at several intervals after the initial ozone treatment. Application of ozone at the mid-point of the storage period also provided little disease control. Increasing the amount of ozone applied to the crop by increasing the ozone concentration or by applying ozone for longer periods did not significantly improve treatment efficacy. This lack of beneficial effects of ozone treatments was likely due to; a) intrinsically low levels of disease in the commercial stock potatoes used in these trials and b) some of the diseases may have been well established prior to treatment or may have been introduced via deep wounds caused at harvest. To be effective, ozone must directly contact the pathogen. Ozone treatments are less effective on deep-seated or well-established disease infections, as opposed to surface infections (Klotz, 1936 cited in Harding Jr., 1968; Spotts and Cervantes, 1992, Liew and Prange, 1994). The potential for ozone to diffuse to deep-seated disease problems is limited, as the ozone molecule has a very short half-life. Other researchers have noted that deep infections are not controlled by ozone, although the rate of development and spread of these infections may be restricted by ozone treatment (Liew and Prange, 1994). The maximum interval between infection and ozone treatment that would still allow effective control is not known. Reducing the time between initial infection and the onset of the ozone treatment should reduce the opportunity for the pathogen to become established prior to treatment, thereby increasing the potential efficacy of the ozone treatment. In this study, the interval between harvest and the onset of ozone treatments was at least one day, which provided at least some opportunity for pathogen establishment. Temperatures both prior to and following ozone treatment were warm enough (15°C) to allow fairly rapid development of most post-harvest pathogens. In grower operations, it may be possible to begin treatments within hours of harvest. Ideally, ozone treatment could commence as freshly harvested potatoes are loaded into storage.

As disease levels on commercial potato stocks were found to be too low or unpredictable to be able to effectively evaluate ozone treatments, potatoes were inoculated with specific pathogens prior to ozone treatment. This ensured significant levels of disease and allowed control of the time period between infection and the onset

of the ozone treatments. Minimizing the interval between inoculation and treatment should, in theory, increase treatment efficacy by preventing the pathogens from becoming established beyond the zone of control exerted by ozone. Ozone treatments' capacity to control pathogens introduced via different wounding methods were also examined. Ozone treatments did not control *Fusarium*, irrespective of wound depth or the fact that the ozone treatments commenced within hours of the introduction of the pathogen. Similarly, ozone treatments also failed to control *Phytophthora* introduced by surface inoculation. Aggressive pathogens such as *Phytophthora* infect tissues rapidly, apparently making surface treatments like ozone relatively ineffective. Although *Fusarium* is slower growing, the lack of control observed in this study indicates that either the time between inoculation and treatment was still too great or that the ozone treatments employed were not capable of controlling this particular pathogen. Ozone treatments reduced *Helminthosporium* incidence and severity in surface-inoculated potatoes. *Helminthosporium* is a slower growing organism that spreads via spores (Howard *et al.*, 1994; Stevenson *et al.*, 2001). Spores are known to be sensitive to ozone as they are small and can be targeted in the air, away from the high ozone demand of the tuber surface (Hibben and Stotzky, 1969).

In the preliminary trials, where the capabilities of the ozone generators were tested and the generation/degradation profiles were detailed, the addition of reactive materials such as potatoes and soil in the treatment environment substantially reduced residual ozone levels. This complicated attempts to evaluate the efficacy of ozone as a biocide. *In vitro* trials are commonly used to control interference by aspects of the treatment environment. Ozone treatments have demonstrated efficacy against a range of pathogens in very simple environments or *in vitro* (Hibben and Stotsky, 1969). However, in a series of agar plate studies, exposing pure cultures of *Fusarium sambucinum*, *F. solani* and *Phytophthora infestans* to ozone at varying concentrations and durations did not reduce (control) sporulation of any of these organisms. Mycelial growth of *Fusarium sambucinum*, *F. solani* and *Helminthosporium solani* were also not suppressed by the ozone treatments. Ozone treatments did suppress vegetative growth of *Phytophtora infestans* during the treatment period, however the cultures resumed a normal growth rate when returned to ozone-free conditions. This suggests that the

Phytophthora cultures were just suppressed by the ozone treatment and ozone would have to be added continuously to maintain control of this pathogen. The germination of *Sclerotinia sclerotiorum* sclerotia was also suppressed during the ozone treatment but germination rates again returned to normal once the ozone treatments ceased. Even under the simple, interference-free conditions presented by the agar plate system, the ozone treatments were not of sufficient duration or intensity to kill or permanently damage the fungi that commonly cause damage to stored potatoes. At best, the ozone treatments slowed pathogen growth during the treatment period. Liew and Prange (1994) observed a similar phenomenon in carrots inoculated with *Botrytis* and *Sclerotinia*. Vegetative growth of these pathogens stopped during ozone treatment, but resumed once the treatment ended. This would indicate that continuous exposure to ozone may be necessary to provide consistent, long-term disease control. The implications of a reliance on continuous ozone treatment would be; a) a potential increase in cost (electricity to run generators) b) increased wear on equipment and storage facilities by longterm exposure to ozone and c) increased potential for ozone damage to the commodity.

While disease control was the principal focus of this project, the effect of ozone application on quality of the crop was also determined, as any treatment-induced reduction in quality could reduce marketability. Skin colour and firmness of the tuber are the primary quality determinants in fresh-market potatoes. Liew and Prange (1994) found that ozone treatments caused bleaching of carrots, which is consistent with the oxidizing action of ozone, but ozone treatments did not affect the colour of blackberries (Barth *et al.*, 1995). In a number of the trials conducted in this project, ozone treatments had consistent but subtle effects on skin colour of the treated tubers based on Hunter Colorlab assessments. Norland tubers were typically more red (Hunter "a") following ozone treatment, while Russet Burbank potatoes were more yellow (Hunter "b"). The effects were most apparent immediately after treatment and increased in a dosage-dependent manner. These colour changes were not easily detected with the naked eye. None of the differences would have a negative impact on marketability, as red colour is desirable in Norland potatoes, while yellow colour is preferred in Russet Burbank potatoes. The observed effects of ozone treatment on skin colour may reflect

stabilisation of the colour generating-compounds in the skin of the potato, as skin colour typically fades during storage. Bleaching of the tubers due to the oxidizing activity of ozone was not observed, although lightness (Hunter L) values did tend to increase following ozone treatment.

Weight loss in stored potatoes is generally associated with moisture loss through the skin and wound sites. Potato growers strive to minimise moisture loss during storage by maintaining high relative humidity and low storage temperatures, by minimising harvest and post-harvest damage and by allowing tubers to form periderm layers over wound sites. Ozone treatments have the potential to increase moisture loss, as ozone is known to react with cell membranes, disrupting cellular function (Kim *et al*., 1999b; Hodge, 1998). Liew and Prange (1994) observed increased electrolyte leakage in ozone-treated carrots suggesting that ozone treatments have the potential to increase water loss. In the trials presented in this thesis, ozone treatments increased weight loss in potatoes in a dosage-dependent manner, particularly if ozone was applied continuously for the duration of the storage period. The observed degree of weight loss did not result in enough wrinkling to negatively effect the visual appeal of the crop, however weight loss in ozone-treated tubers was significantly higher than in control tubers. This increase in post-harvest weight loss would represent an additional cost to the growers, as potatoes are sold on a weight basis.

The increase in weight loss caused by ozone treatments was potentially the result of the ozone interfering with the formation of wound periderm layers. The formation of wound periderm at wound sites incurred during harvest and post-harvest handling reduces post-harvest water loss and enhances resistance to post-harvest diseases. Wound periderm layers consist of several layers of suberin and lignin formed on healthy cells adjacent to damaged cells. Ozone has been shown to increase formation of phenylpropanoid compounds, such as suberin and lignin, in soybean (Booker and Miller, 1998). In contrast, ozone has also been used to enhance the degradation of lignin-rich tissues, such as wood pulp (Kaneko *et al*., 1983; Kang *et al*., 1995; Korai *et al*., 2001). In this study, ozone application to recently wounded potatoes appeared to decrease the thickness of the wound periderm layer. Thinning of this protective layer could impair natural disease defences while increasing water loss during storage. Any impairment of

healing by the ozone treatments would potentially exacerbate problems with wound pathogens, such as *Fusarium* dry rot. This effect may contribute to the limited *Fusarium* dry rot control reported in this thesis and would weigh against adoption of ozone as a standard disease control method for stored potatoes.

In a vegetatively propagated crop like potato, seed quality has a significant impact on yield and quality of the crop. Diseases present on or within the seed tubers may be passed onto the resulting crop. Good quality seed produces a complete, uniform stand and good marketable yields at the end of the growing season. At present, seed growers rely on crop health and storage management practices to reduce disease in their seed. Seed potatoes are also typically treated with fungicides just before planting. Ozone was also tested as a potential pre-planting seed treatment. Ozone does not leave any residues on treated produce, and therefore ozone treatments applied to seed tubers would have no effect on soil-borne diseases. In contrast, fungicidal seed treatments control both surface pathogens on the seed, as well as soil-borne pathogens adjacent to the seed (Schaupmeyer, 1992). In several trials, crop stand and yields obtained using ozone-treated seed tubers were comparable to standard fungicidal seed treatments. It should be noted that the seed lots used in these trials were of high quality and were planted into near-ideal field conditions. Disease pressure would have been low under these conditions, therefore the absence of treatment effect would be expected and acceptable. In one trial, plant stand was reduced in one cultivar by the highest rates of pre-planting ozone. This resulted in a corresponding reduction in marketable yields. As discussed, ozone is not capable of eradicating well-established or deep-seated infections caused by many common post-harvest pathogens of potato. Any diseases affecting a seed lot would be well established after six months of storage. Therefore, the potential efficacy of ozone as a seed treatment would appear limited. The dosage-related problems observed with the performance of ozone-treated seed would also mitigate against increasing ozone dosage as a means of increasing disease control in seed. Increasing ozone application rates in an effort to improve control of these pathogens would also not reduce the threat of soil-borne disease. Based on the occasional performance problems and the lack of residual protection from disease, ozone treatments do not appear to represent viable pre-planting treatment for seed tubers.

The previous studies showed that pre-planting ozone treatments were potentially damaging the vigour and yield potential of seed potatoes. The basis for this treatment effect was unknown. Daniels-Lake *et al*. (1996) had previously found that ozone treatments did not reduce sprouting in Russet Burbank potatoes. In studies performed in this thesis, ozone treatments applied to seed potatoes prior to planting did not significantly alter sprout growth, although exposure to ozone (80 or 320 mg O_3/kg/hr) slightly accelerated the onset of sprouting. The ozone treatments may have been aging the tubers slightly, resulting in accelerated sprouting (Burton, 1966). Ozone-related acceleration of weight loss in stored potatoes may also contribute to an aging effect.

In summary, the ozone treatments utilised in this project were not effective in reducing or controlling the development of fungal diseases in stored potatoes to a level that would be considered commercially acceptable or economically viable. The impact of the ozone treatments tested on tuber quality and seed performance was minimal, except for a slight increase in weight loss. The ozone treatments tested did not appear to have any negative effect on the storage facilities, in terms of accelerated weathering of equipment or degradation of materials. This is a concern with some other disease control products (e.g. Chlorine dioxide). The storages had a fresh scent following ozone treatment, which might indicate some control of odour-causing bacteria or the degradation of the volatile metabolites produced by these decay organisms. Odour or specifically the absence of odour arising from decay is a means whereby potential buyers evaluate a load of potatoes. Using ozone to eliminate these odours may increase marketability of the treated potatoes, however the data suggests that the actual disease-causing organisms are not being controlled. The ozone treatments may simply be covering up problems.

Overall, ozone was not effective as a post-harvest treatment for the management of disease or enhancement of produce quality. The question arises as to whether ozone is simply ineffective for these objectives or whether the lack of positive responses is a function of ineffective rates or modes of delivery. The ozone rates and modes of delivery were selected based on a balance of practicality and reasonable costs factoring in levels of risk of damage to the commodity or injury to operators or storage equipment. There were indications from previous research that comparable dosages had been

effective at controlling various pathogens in other crops. Based on these criteria, the use of ozone as a post-harvest treatment for the control of fungal disease on stored potatoes cannot be recommended until a significant increase in efficacy is achieved. One potential method to increase efficacy of ozone treatments is to apply much higher dosages over a very short time just as potatoes are coming into storage. Currently, one Saskatchewan potato grower is utilising a system in which tubers are exposed to 500 ppm of ozone as they are unloaded from the field into storage. This ozone concentration is approximately 4-5 times the maximum concentration evaluated in this thesis project. However, the associated duration of treatment is approximately 15 seconds compared to one day as the shortest duration of treatment in this thesis project. This initial application is combined with the use of a continuous application of low levels of ozone (up to 2.5 ppm) for the duration of storage. Treatment with extremely high concentrations of ozone has the potential to be toxic to surface pathogens while the extremely short treatment period would potentially prevent reduction in commodity quality. Residual levels of disease escaping this initial treatment could potentially be suppressed by the continuous ozone treatments. There is, however, potential for damage to the commodity from the extreme ozone dosages. There is also increased potential for weathering of the equipment by ozone. Thomson and Waterer (2003) actually observed increased levels of some diseases (*Fusarium, Erwinia, Helminthosporium*) in the potatoes treated with either the high ozone concentrations at load in or the continuous ozone treatment. The potential for these ozone treatments to actually increase disease incidence and severity was most obvious when ozone was applied to tubers that had been damaged by frost. This would indicate that ozone is actually increasing the level of damage to the commodity.

Recommendations for Ozone Use

- Ozone may be of use in post-harvest control of slow-growing, superficial diseases of potato such as silver scurf
- Ozone cannot be considered a curative treatment for more aggressive fungal diseases attacking potatoes in storage. However, the use of ozone in an integrated management program, combined with appropriate management of storage

temperature, supplemented by application of effective fungicides, could potentially improve overall product quality by controlling disease and minimising damage

- Ozone cannot be recommended as a replacement for fungicides as a pre-planting seed treatment

Future Directions/Research

The ozone treatments tested were largely ineffective against rapidly growing, wound-introduced diseases of potatoes, however treatments showed some control of slower growing species, such as *Helminthosporium solani*.

Future work should focus on;

- *In vitro* studies to determine effective dosages for problematic fungal species. This data to could be used to determine if commercial-scale control of these pathogens is possible
- Fine-tune ozone treatments to maximize control of the growth and spread of *H. solani* within storage
    - Evaluate the commercial feasibility of ozone concentrations proven to be effective against *H. solani*
    - Evaluate the influence of these effective dosages on quality issues, including taste, processing quality and seed vigour
- Further compare the effects of short-term exposure to high dosages versus long-term treatment with lower concentrations of ozone
    - Evaluate the impact of these variables on disease control, product quality and damage to the commodity and facilities
- Evaluate the potential to use ozone along with existing fungicides in an integrated control program
        - Determine potential synergistic/antagonistic effects between ozone and fungicides
        - Determine whether using multiple control methods reduces selection pressure for pesticide resistance.

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