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Ozone as an Improved Method to Control Disease in Stored Potatoes.

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Project Summary:

Post-harvest diseases commonly result in the direct loss of more than 10% of the stored potato crop, with further losses associated with reduced consumer acceptance of disease affected product. At present, potato growers use controlled storage conditions coupled with the application of fungicides to manage post-harvest disease. However, losses are still substantial and the fungicides are costly – therefore more effective alternate disease control options are needed. Ozone gas is already widely employed as a surface sterilant – this study evaluated the potential to use ozone to control disease in stored potatoes and also as an alternate seedpiece treatment. A series of experiments showed that ozone applied to a recently harvest potato crop provided only limited disease control, particularly if the storage pathogens were either well established or growing rapidly at the time of treatment. A similar lack of disease control was observed for all post-harvest fungicides approved for use in potatoes. Longer term treatments with relatively low concentration of ozone (2-4 ppm) also provided limited disease control. Ozone treatments tended to produce an undesirable increase in weight loss by the stored crop but they also improved tuber color.

In simplified culture systems, exposure to ozone at recommended treatment levels failed to kill pathogens of stored potatoes. In some cases, the ozone slowed growth and reproduction of the pathogens, but their growth resumed at normal rates as soon as the ozone treatments stopped. Ozone treatment of seed potatoes at load-in, during longterm storage or just prior to planting had few positive effects on the disease levels in the resulting crop and in some cases appeared to reduce the yield potential of the seed.

Trials involving short-term treatment of the potatoes with much higher concentrations of ozone in a commercial scale ozone applicator produced few positive results. Many of the ozone treatments evaluated in these trials actually increases losses to disease, likely due to ozone-induced damage to the tubers.

Consistent application of effective dosages of ozone is complicated by the reactive nature of the ozone molecule and the complexity of the potential interactions between ozone and its surroundings. This study suggests that ozone application does not presently represent a commercially viable alternative disease management strategy in stored potatoes.

1.1 Introduction

The control of potato diseases in storage is a challenging undertaking, involving rigorous control of storage conditions coupled with chemical treatments designed to minimise disease spread and development. Many storage pathogens of potatoes (ie Fusarium and Helminthosporium) are becoming resistant to the limited range of fungicides presently registered for post-harvest use in potatoes. Alternative disease control options in stored potatoes are necessary. Increasing concerns regarding pollution of the environment and food health and safety are also driving the movement towards alternative methods of disease control.

Ozone is a potential alternative treatment for the control of storage diseases of potato and other horticultural crops. However the ability of ozone to control diseases is not fully understood, particularly under the specific environmental conditions encountered during cold storage of a living commodity. For example, the required application rates of ozone for control of various disease problems are not clear. It is also possible that while ozone might have potential as a disease management tool, it may also effect the quality of the tubers. The relative efficacy and cost efficiency of ozone treatments, as compared to current chemical treatments must also be evaluated.

1.2 General Project Objectives

1) To test the efficacy of ozone treatments as pre-storage and/or mid-storage season applications for disease control in potatoes.
2) To compare the efficacy of ozone versus standard commercial fungicidal treatments.
3) To determine whether ozone treatments affect tuber quality.
4) To evaluate the effect of ozone on the physiological processes of the potato such as wound healing, sprouting and seed performance.
5) To evaluate how ozone effects specific post-harvest pathogens.
6) To evaluate ozone as a post-harvest treatment utilizing commercial-scale generation equipment.

Objective 1. Evaluation of ozone applied prior to storage as a disease management tool.

The control of post-harvest diseases of potato has historically been achieved through a combination of storage management and chemical control products applied to the potatoes as they come into storage. Increasing environmental concerns and reduced tolerance of disease in potatoes has necessitated the development of more effective, yet environmentally friendly disease management measures.

The use of ozone (O3), a strongly reactive gas, to control disease spread and development has been suggested as a potentially useful tool in the storage of potatoes. As a chemically reactive compound, ozone exerts significant antibiotic activity on a range of organisms (fungi, bacteria). Ozone does not leave any toxic residues, which is beneficial from a food safety perspective, especially with increasing public concern about food quality and pesticide residues. 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 has also been shown to stimulate plant/fruit defence mechanisms, which can increase the post-harvest life span of commodities (Sarig *et al.*, 1996; Kangasjarvi *et al.*, 1994). With the increasing cost of chemical fungicides, as well as increasing concerns relating to food safety and disease resistance, the development of an alternative post-harvest treatment method would be beneficial.

Specific Objectives

1) To evaluate the efficacy of ozone application in the control of post-harvest pathogens of potato
2) To test the effects of ozone concentration and duration of exposure.
3) To determine if ozone application affects tuber colour and to evaluate any other potentially detrimental effects of ozone treatment.

General Materials and Methods

All trials were conducted on field run potatoes obtained from commercial growers in Saskatchewan. This insured that the handling practices, crop and disease characteristics of the experimental material were representative of "real world" situations. Potatoes of two cultivars (Norland and Russet Burbank) were obtained from Hyland Seed Potato Farms (1999) and one cultivar (Norland) from Barrich Farms (2000). 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 disease resistant 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 bagged into treatments, weighed and treated either with ozone or a standard pre-storage fungicidal treatment. Following treatment, the potatoes were placed into a 4°C cold storage for the duration of the six-month storage period.

Potatoes were sampled immediately following the ozone treatments and again after three and six months in cold storage. The potatoes were evaluated for changes in disease level, physiological quality (skin colour) and weight loss. In 1999/2000, the diseases examined were *Fusarium spp*., *Erwinia carotovora*, and *Rhizoctonia solani*, which are the causal organisms of the potato storage diseases Dry Rot, Soft Rot and Black Scurf. In 2000/2001, *Helminthosporium solani* (Silver Scurf) was added to the list of diseases evaluated.

Background on Diseases Evaluated

Rhizoctonia is a soil borne disease that forms a black scurf on the skins of potatoes. This disease does not spread from tuber to tuber in storage but the lesions may increase in size during storage. Planting of *Rhizoctonia*-infected seed causes stem cankers and seedling diseases. High levels of this disease can reduce marketable yields of potatoes due to grade-out of tubers with excess levels of surface scurf.

Fusarium Dry Rot requires wounds or entry points to infect tubers. *Fusarium* does not spread between tubers in storage but once infection has occurred, the disease can and does increase in the individual tubers in storage.

Erwinia Soft Rot is a bacterial disease that spreads between tubers in storage. One would expect both the incidence and severity of Soft Rot to increase as time in storage increases.

Helminthosporium Silver Scurf is visible as silvery lesions with black-speckles (when conidiophores are present), that can spread through the air in storage when conditions for sporulation are suitable, thereby increasing disease levels.

Weight change was evaluated by weighing each treatment bag before treatment and then prior to washing at all subsequent sampling dates. Tubers were then gently hand-washed to remove dirt and chemical residues, which might interfere with the accuracy of the colour and disease evaluation. Tubers were not scrubbed to the point of skin or surface disease removal. Following washing, tubers were placed on paper towels to air dry.

The number of tubers per treatment bag (total 20 tubers) with Soft rot (*Erwinia sp.*) was recorded at this time and any Soft Rot damaged tubers were then discarded.

In 1999/2000, fifteen potatoes from each treatment bag were then individually scored for percent surface area infested with *Rhizoctonia*, using a predetermined scoring chart from the existing literature (developed by W.C. James; Used by Canadian Food Inspection Agency). Possible scores included zero, one, five, 10 and 15 percent surface area with *Rhizoctonia*. The same tubers were each sliced three times to provide four slices which were rated for the approximate percent of Dry Rot per slice, using a ranking system of zero to six. The ranks corresponded to a range of disease per slice. The amount of Dry Rot per slice was averaged over the four slices. 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 average *Fusarium* rating per tuber was calculated using the average *Fusarium* per slice data.

In 2001, *Erwinia* incidence was determined as in previous evaluations and then ten tubers were rated for percent surface area with *Rhizoctonia* and cut to determine average *Fusarium* rating per tuber. In addition to these evaluations, at least five tubers were set aside in paper bags until further tests could be completed. At least five *Rhizoctonia* sclerotia were isolated from the remaining tubers and placed onto Water Agar (WA) media plates for 24 hours and the percent germination of the sclerotia was determined. If the required five sclerotia could not be obtained, the number of sclerotia were noted and the percentage germination calculated accordingly. Five tubers were then incubated in tubs on water-saturated paper towel for three weeks at room temperature to determine the percent surface area infected with *Helminthosporium* Silver Scurf lesions. Tubers were rated 0, 1, 5, 10, 15, 25, 50, or 75% infected (Using a CFIA key for Scab Severity; similar to % *Rhizoctonia* key)

Skin colour of the tubers was evaluated using a Hunterlab colorimeter (Hunterlab), modified with a semi-soft foam collar to insure a light tight fit between the potato and the sensor. Ten tubers from each treatment bag were analysed. The “L”, “a” and “b” values were recorded and a combined Hue angle was calculated from the “a” and “b” values. Table 1.1 describes the meaning of each colour value.

Table 1.1: 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 higher “a” values denoting more red and a lower “a” value denoting greener colour
B	Refers to the relative amount of yellow versus blue colours, with higher “b” values denoting yellow and lower “b” values denoting blue
Hue angle	Calculated combination of “a” and “b” values, combined in a tangent function of “b” over “a”

Where appropriate, the data were transformed to reduce variability of experimental error and to normalise the data. Typically, straight percentage data were transformed using a Natural Log function, whereas pathological percentage data (*Erwinia, Helminthosporium, Rhizoctonia*) were transformed using square root transformations [SQRT (#+0.5)] or arc sine transformations [ASIN (#+0.5) or @Degrees(ASIN(SQRT(DATA VALUE/100)))]. These manipulations were done to facilitate accurate statistical analysis of results.

2. Pre-storage ozone application trials

1999/2000 Study Period

This experiment was carried out in the fall and winter of 1999/2000, spanning a six-month period. Tuber samples of two locally popular cultivars (Russet Burbank and Norland) were obtained from a local commercial potato grower shortly after harvest. In this way the tubers represented "real world' disease populations. Tubers had not completed their wound healing when received. Following delivery, the potatoes were separated into 20 potato lots in 4.5-Kg bags and then treated with ozone in a controlled environment room at 15°C. The ozone was supplied using corona-type ozone generators supplied by Crystalair (Delta, BC). The tubers were exposed to ozone at rates of 0, 600, 1200 or 2500 mg, for one, seven or 21 days. The rates were determined by multiplying the maximum output potential of a single ozone generator by one, two or four, to achieve an exponential scale. Following the ozone treatment, tubers were sampled according to the procedures outlined in the general materials and methods. The remaining tubers were placed into cold storage (4°C) for 3 or 6 months.

The rates of ozone tested were calculated to be approximately 0, 1.71, 3.4 and 7.14 mg ozone per kilogram of potato per hour. This was calculated based on an estimated average potato weight of eight ounces (227 g). When the actual potato weights (1999) were calculated, it was found that the potatoes weighed approximately five ounces (142 g) on average. This changed the calculated ozone application rates to approximately double the originally calculated rates. This fact was taken into consideration in future experiments.

At the end of the six-month storage period, the collected data were analysed using SAS statistical analysis package (SAS Institute Inc.). Treatment variables were analysed for cultivar differences and then for sample time by treatment interactions.

<u>Summary of Treatments for 1999/2000</u>

Reps: 4
CVs: Norland and Russet Burbank
O3 Concentrations: Control, 600, 1200 and 2500 mg/hr (equivalent to 0, 5, 10 & 20mg/kg/hr)
O3 Exposure Duration: 1 Day, 1 Week, and 3 Weeks
Sample Dates: October, January, and April
20 Freshly harvested potatoes per treatment bag
Average potato weight: 5 oz

2000/2001 Study Period

Based on the limited disease control observed in the 1999/2000 trial, it was decided that the rates of ozone applied should be increased in the 2000/2001 trial. The same exponential rate scale was used, but the rates were shifted to zero, 5, 10 and 20 mgO_3/kg/hr. The same treatment and sampling procedure were followed.

Due to the limited differences between the two potato cultivars observed in the 1999/2000 trial, only the Norland (red-skinned) potato cultivar was used in 2000/2001.

<u>Summary of Treatments for 2000/2001</u>

Reps: 3
CVs: Norland

O3 Rates: 0, 5, 10, 20 mg/kg/hr
O3 Exposure Duration: 1 Day, 1 Week, 3 Weeks
Sample Dates: October, January, April
20 potatoes per treatment bag
Average potato weight: 5 oz

Previous trials focus on a single ozone application during wound healing. It is also possible that continuous application of ozone at a low concentration for the duration of the storage period could be beneficial in the reduction of disease levels. To test this, some of the potatoes that received the previously described pre-storage treatment were also treated with low levels of ozone continuously for the duration of the storage period. The tubers were sampled in January and April (3 and 6 months). The ozone treatments were compared to the controls for disease levels and quality as previously described.

Due to a malfunction in ozone generation equipment during the first long-term ozone exposure period (following the initial curing period treatment), it is unknown whether the continuous ozone application is truly representative. Upon discovery of the problem, the generator was replaced and the application was continued. Tubers were sampled according to previous plans and the differences between pre-storage and continuous treatments were determined.

Continuous Ozone Summary of Treatments for 2000/2001
Reps: 3
CV: Norland
O3 Rates: Pre-storage = Control, ~5, ~10, ~20 mg O_3/kg/hr
Continuous = 400 mg/hr = 1.96 mg O_3/kg/hr
Sample Times: January and April
20 potatoes per treatment replicate (Avg. wt. = 5 oz)

Results and Discussion

1999/2000 Study

Data collected from this study period was analysed for significant interaction effects. Data were analysed for cultivar by treatment interactions and were then analysed separately for other interactions. Data were then tested for significant treatment effects over time (treatment by sample time interaction) and then for treatment interactions for each sample time.

For this report, only a brief summary of 1999/2000 work will be presented, as this data has been presented and discussed in previous reports (See ADF Report April 2001).

Disease Analysis

Ozone treatment effects on disease levels did not vary between cultivars. Cultivars were analysed separately. Average *Fusarium* ratings were significantly affected by ozone treatments over time in Norland potatoes but treatment differences over time were not seen in Russet Burbank potatoes. Ozone treatments did not significantly reduce *Fusarium* levels in potatoes sampled immediately after treatment or when sampled at the completion of the storage period. Ozone treatments did significantly reduce *Fusarium* levels ($p=0.05$) in potatoes sampled at the mid-storage sampling time. The concentration of ozone applied was more important than the duration of exposure in determining the amount of effect on *Fusarium* levels.

Ozone treatments significantly reduced *Erwinia* Soft Rot immediately after treatment, however no differences in Soft Rot levels were detectable after three and six months of storage.

Disease levels decreased with time in storage, which was contrary to expectations. This may be a result of the heterogeneity of disease levels (both Soft Rot and *Fusarium*) in the commercially obtained tubers or due to analysis differences over time (subjective system of rating). Ozone treatments had little effect on disease levels over the storage period. It is possible that ozone treatments slowed disease development resulting in lower incidence and severity ratings in later samples, however one would expect control levels to be higher if this were the case.

Colour Analysis

Potatoes (Norland and Russet Burbank) were tested for changes in their Hunterlab colour values (see Table 1.1 for interpretation) at various intervals over the storage period. Norland and Russet Burbank potatoes differed significantly for A, B and hue angle variables. Both cultivars were significantly changed over time for their lightness values, but not for the other colour variables.

Ozone-treated Norland potatoes were not significantly lighter than the control potatoes immediately after treatment. Ozone-treated tubers were significantly lighter (p=0.05) at the mid-storage sample time but were not lighter at the end of storage. All tubers darkened as time in storage increased.

Ozone-treated Russet Burbank potatoes were significantly lighter (p=0.05) immediately after storage than the controls, however this difference disappeared as storage progressed. As was seen in the Norland potatoes, the tubers darkened over time, however the effect was less pronounced in the Russet Burbank potatoes.

Tuber redness, yellowness and calculated hue angle values were not affected over time by ozone treatments in either Norland or Russet Burbank potatoes.

Ozone treatments had minimal effect on colour variables. Colour changes were often only detectable using the precision equipment and were not apparent to the naked eye.

Weight Loss

The impact of the ozone treatments on weight loss during storage was similar for the two cultivars. Weight loss (√% weight loss) of the ozone treated potatoes differed significantly (p=0.05) over time in Norland but not in Russet Burbank potatoes.

Ozone treatments significantly (p=0.01) increased weight loss in Norland potatoes immediately after treatment and also by the end of storage. As the ozone rate increased weight loss increased. Weight loss increased over time in all treatments.

The relationship between ozone concentration and duration of exposure appears to be quite clear. Low ozone concentrations and longer exposure times would resulted in an application of ozone (total amount) approximately equal to a high concentration and short exposure time application. The data demonstrate that the total amount of ozone applied appears to be the crucial factor, as opposed to any specific ozone concentration or duration of exposure.

2000/2001 Study

Only one potato cultivar was used in this trial. Analysis was carried out to test for treatment (ozone concentration by duration of exposure) interaction effects on the various disease and quality variables at various points during the storage period. Pre-storage applications of ozone with no ozone application during the cold storage period were also compared to similar treatments plus continuous ozone during the cold storage period.

Disease Analysis

Disease data were collected in an identical manner to the 1999/2000 study period, with the exception of the addition of several disease ratings at three months of storage. Five of twenty tubers were incubated and rated for percent surface area with *Helminthosporium solani* (Silver Scurf) and sclerotia of *Rhizoctonia solani* (Black Scurf) were collected and tested for viability.

Ozone treatments had no significant effects on *Fusarium* Dry Rot, *Helminthosporium* Silver Scurf, *Erwinia* Soft Rot or percent *Rhizoctonia* Black Scurf levels over time in the 2000/2001 study. Similarly, *Rhizoctonia solani* sclerotial viability was not significantly affected by ozone treatments.

Pre-storage ozone treatment disease data were compared to continuous ozone application treatment data for the second sample time. No significant differences were detected between the disease control potential of single point versus continuous applied ozone.

Colour Analysis

Ozone treatments (combined ozone concentration and duration of exposure) had no significant effects on Hunter L (lightness), Hunter A (redness) or B (yellowness) values of the Norland potatoes over time. When the data is broken up into individual times some differences appear (Figure 2.3).

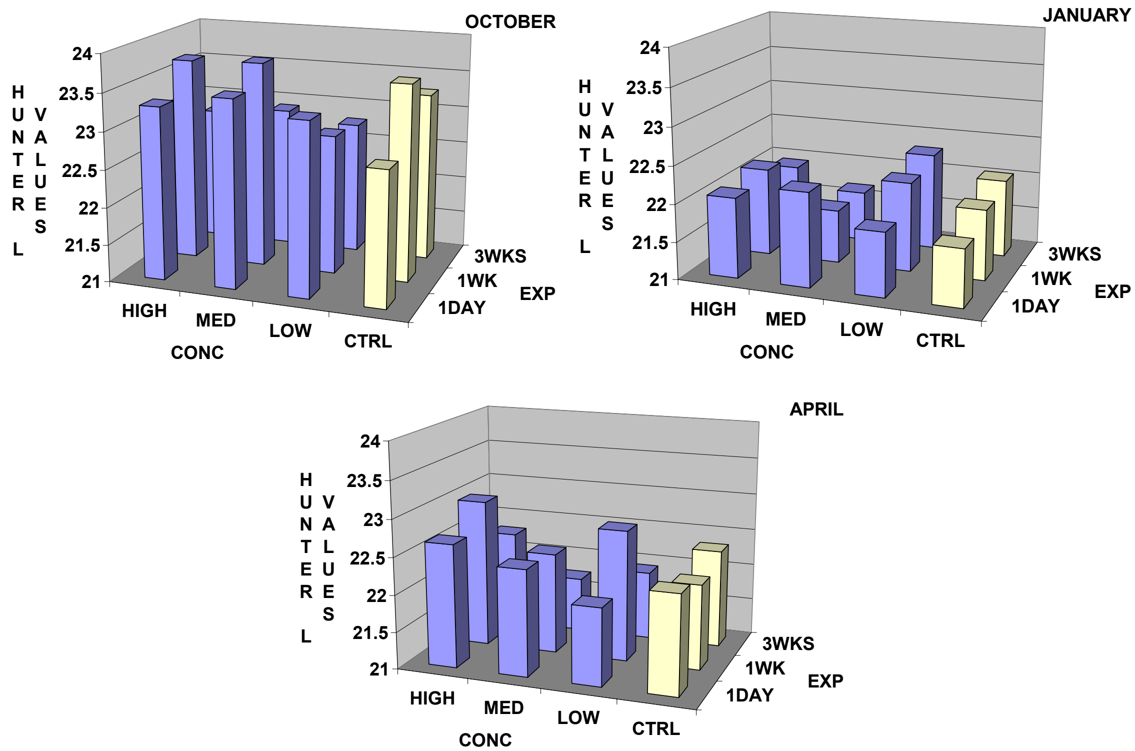

Figure 2.3: Changes in Hunter L (lightness) values during storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods.

Ozone treatments significantly decreased Hunter L (lightness) values of Norland potatoes sampled immediately after treatment. Longer exposure to ozone appears to have decreased lightness, while higher concentrations (with shorter exposures) increased lightness. The combined effects of ozone treatments resulted in lighter potatoes in the second sample time, as compared to the controls. Treatment differences were much less apparent than at the first sample time. Similar results were apparent at the end of the storage period. As in the 1999/2000 study period, potatoes became darker as time in storage passed.

Hunter A (redness) values did not differ significantly between treatments over time. However examination of Hunter A (redness) values at each sample time revealed significant treatment effects for the first two sample times. Treatment differences were less apparent at the end of storage.

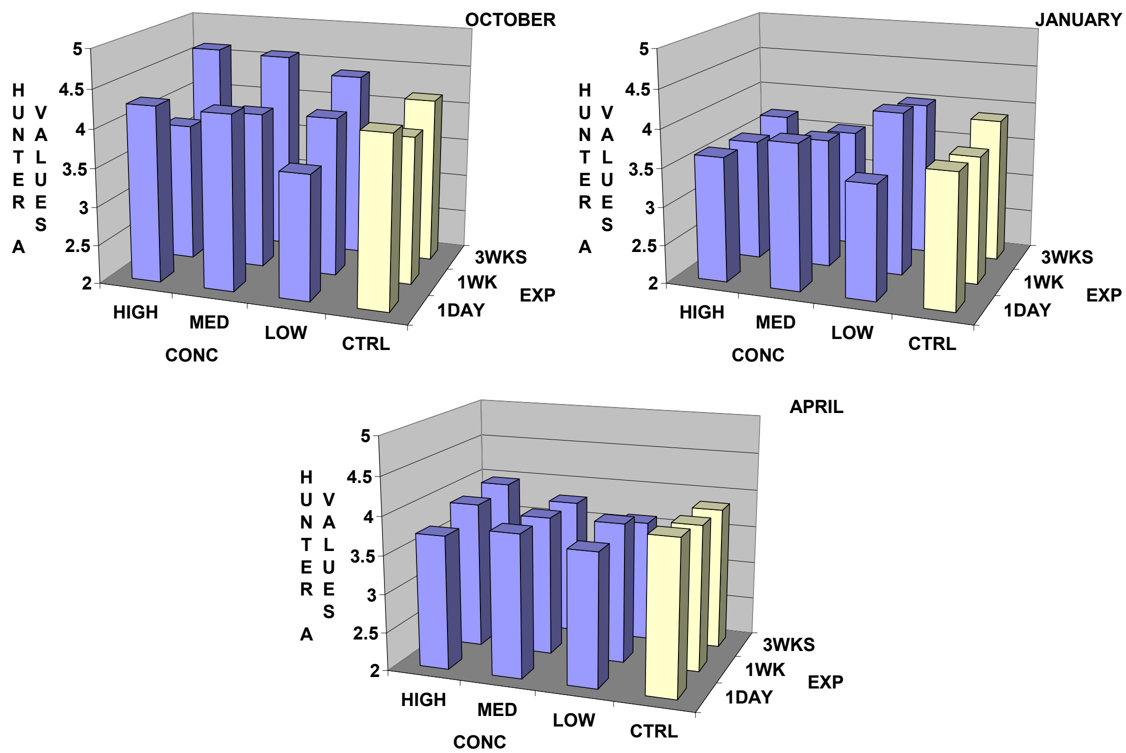

Figure 2.4: Changes in Hunter A (redness) values during storage of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods.

The ozone treatments significantly increased the redness of Norland potatoes immediately after treatment but decreased redness in the second sampling period. Overall, tuber redness decreased with time in storage.

Ozone treatments did not significantly affect Hunter B (yellowness) values, but the tubers did increase in yellowness as the storage duration increased.

The effect of continuous treatment with ozone on Hunter B (yellowness) values differed significantly from pre-storage ozone treatments.

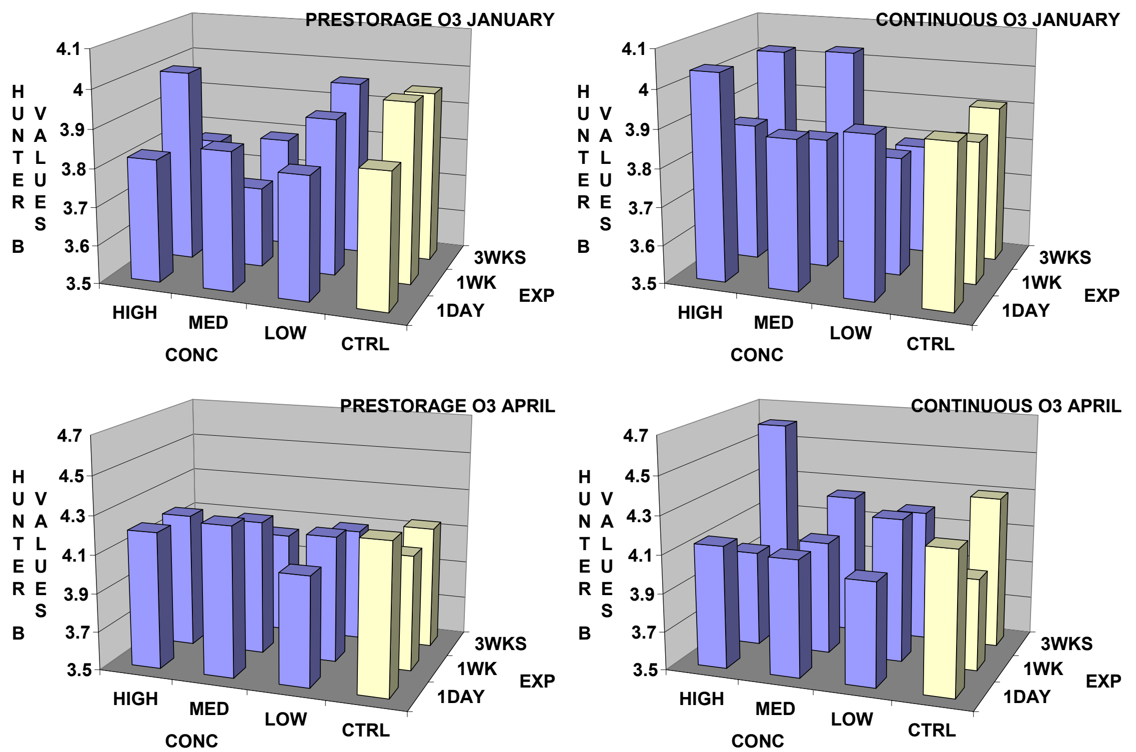

Figure 2.5 Changes in Hunter B (redness) values of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods and then stored in control or continuous ozone long term storage.

Continuous ozone treatments significantly increased tuber yellowness values in Norland potatoes compared to tubers treated with ozone prior to storage. The effect appears to be most prevalent in the highest pre-storage ozone rates. Ozone treatments did not differ significantly in effect on Hunter B (yellowness) in the trials when examined separately. It appears that the greater the amount of ozone applied, the greater the change in tuber Hunter B (yellowness) values. Continuous versus pre-storage ozone treatments did not differ significantly in their effect on any other colour variables.

Weight Loss

Weight loss increased over time in all treatments. Ozone treatment effects were not significant over time, although the ozone treatments did increase the percent weight loss in potatoes slightly compared to the control treatments. Continuous ozone treatments appeared to even out differences between treatments of the pre-storage treated potatoes and weight loss appears to have been slightly higher. The weight loss in continuous ozone treatments was consistently higher than in the pre-storage ozone treatments.

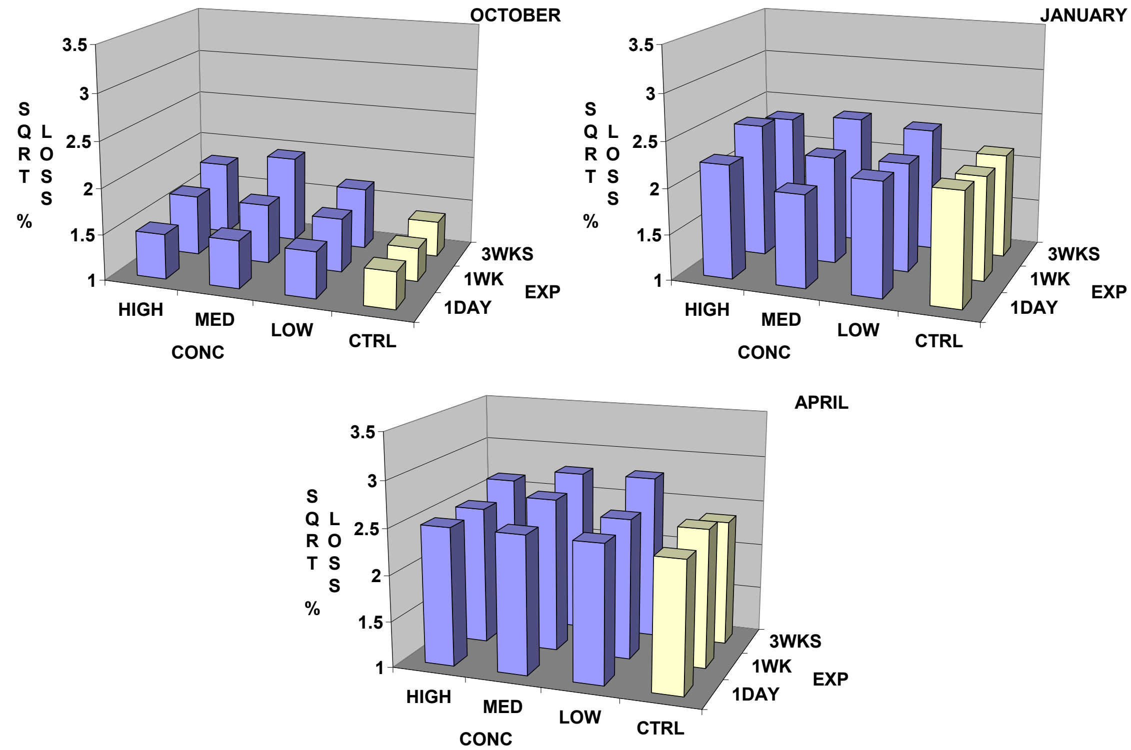

Figure 2.6: Changes over time in √ percent weight loss of Norland potatoes treated prior to storage with ozone (O_3) at varying concentrations and for differing exposure periods.

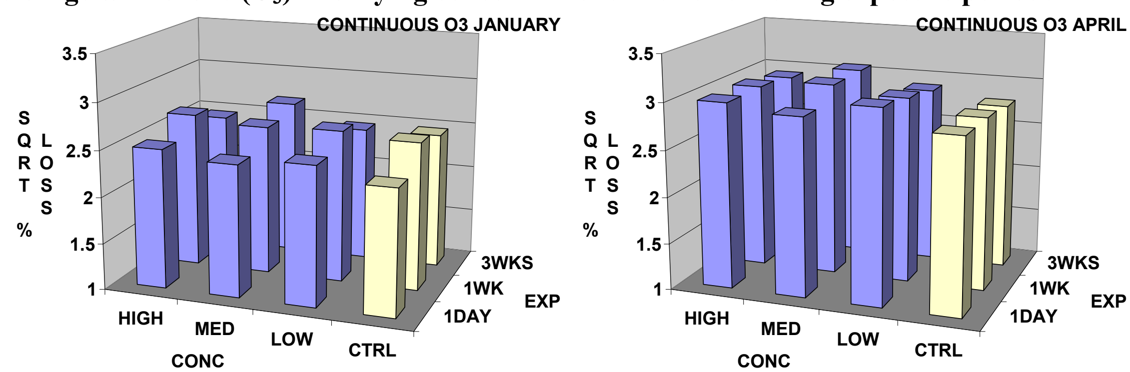

Figure 2.7: Changes over time in √ percent weight loss of Norland potatoes treated continuously with ozone (O_3) (+ pre-storage ozone).

Conclusions

Ozone treatments had limited positive effect in terms of reduction of post-harvest disease in either the 1999/2000 or 2000/2001 study periods. This may be due to the relatively small amounts of disease found irrespective of the treatment received. The tuber quality variables indicated that ozone treatments were not causing significant lasting damage to tubers during the treatment period or following long-term storage.

Comparison of Ozone to Pre-storage Fungicides as Methods to Control disease in Stored Potatoes

Background

The application of fungicides prior to storage has become a common practice in the battle against storage pathogens of potatoes. Unfortunately, pathogens have begun to develop resistance to the registered chemicals and the ability of the chemicals to control the diseases is consequently in question. Current commercially available chemicals must be tested for their continuing efficacy of disease control and new alternatives must be found.

Specific Objectives

1) To test the efficacy of commercially available chemical fungicides in the control of post-harvest diseases of potatoes.

Materials and Methods

Potatoes from the previously outlined sources were treated within a week of harvest with commercial fungicides, prior to the pre-storage curing period. Treatment at this stage represents the industry norm. The fungicides were applied according to product directions and at recommended rates. The fungicides were applied using a CO2 sprayer equipped with a fine spray nozzle (8001). To achieve complete coverage of the tubers, the chemicals were applied to one side of the sample potatoes, the potatoes were turned by hand and the remainder of the spray applied. The treated potatoes were segregated into 20 potato lots in 4.5-Kg nylon mesh bags. Following this treatment, the potatoes were held at 15°C for three weeks in bulk storage to allow curing and then transferred to the cold storage (4°C) for long term storage.

Potatoes were treated with Mertect (Thiabendazole), Dithane F-45 (Mancozeb) and two concentrations of Purogene (Chlorine dioxide [ClO_2]) (100ppm and 400ppm). Mertect and Dithane F-45 are commercially approved fungicides designed to be sprayed onto incoming potatoes during loading of the storage bin. Purogene is a relatively new chemical that is supposed to be fairly “clean” (low residues). Purogene has received interim registration as a method for controlling storage losses in potatoes infected with Late Blight (*Phytophthora infestans*). Purogene can be surface applied to potatoes or can be added through the ventilation system. The two rates used in this study represent the registered Canadian (100PPM) and United States (400PPM) rates for pre-storage application. Potatoes were analysed in the same manner as the ozone treated potatoes, as outlined in the general materials and methods.

Summary of Treatments in the 1999/2000 Trial

Reps: 4
CVs: Norland and Russet Burbank
Chemical Treatments: Control, Mertect, Dithane F-45, Purogene CDN rate (100 PPM), Purogene USA rate (400 PPM)
Sample Dates: October, January, April
20 Freshly harvested potatoes per treatment replicate
Average potato weight: 6-8 oz (actually ~5)

Summary of Treatments in the 2000/2001 Trial

Same as previous year except only Norland potatoes used.

Results and Discussion

1999/2000 Study

As in the ozone treatment trials, treatments were analysed for significant cultivar by treatment interactions and treatment by time interactions before looking at individual treatment differences.

Disease Analysis

Treatment differences did not vary significantly between cultivars for any of the evaluated disease variables, except for the square root of percent surface area with *Rhizoctonia solani*. When cultivars were evaluated separately for all disease variables, it was found that the treatments varied significantly over time in their effect on *Fusarium* levels in Norland potatoes but not in Russet Burbank potatoes

Fungicidal treatments did not significantly affect *Fusarium* levels in Norland potatoes at the first and second sampling periods of the study period. By the end of the storage period, *Fusarium* levels were lowest in the control and Dithane F-45 treated potatoes, with Mertect-treated potatoes having the highest ratings for disease. *Fusarium* levels were variable and do not differ greatly between treatments.

Fungicidal treatments did not have a significant effect on *Erwinia* Soft Rot or *Rhizoctonia* Black Scurf levels in either cultivar in this study.

Colour Analysis

The effect of chemical treatment on tuber lightness (Hunter L) values differed significantly between cultivars. Treatment effects were only significant over time for Hunter A (redness) values in Norland potatoes.

Norland potatoes treated with Dithane F-45 and 100 PPM Purogene were significantly redder three months after treatment than the other treatments at the same time. This does not appear to be due to drastic changes resulting from these treatments but rather a stabilisation or lack of change. Values of all treatments were relatively similar immediately after treatment, but the Hunter A (redness) values for most treatments decreased to the point where significant differences were detected. This appears to be particularly true in the Dithane F-45 treatment. This is perhaps due to the thick paint-like consistency of the applied product, which may serve to reduce light penetration and environmental weathering that may occur in storage. The effects of the 100 PPM Purogene treatment could be similar to what was seen in 2000/2001 ozone treatments, where tubers increased in redness immediately after treatment, but returned to levels seen in other treatments during subsequent storage.

Overall, tuber redness tended to decrease over time in storage in most treatments. This is characteristic of Norland potatoes. Fungicidal chemical treatments had limited negative effects on tuber colour.

Weight Loss

Fungicidal treatments did not significantly affect the percent weight loss of Norland or Russet Burbank potatoes at any sample time. Percent weight loss increased as storage time increased.

2000/2001 Study

This study period was essentially a duplicate of the previous year, with the exception of the use of a single cultivar used in this year. As in the ozone trials, *Helminthosporium* Silver Scurf (% surface area) and *Rhizoctonia solani* sclerotial viability evaluations were also carried out.

Disease Analysis

Fungicidal treatment effects were not all significant over time, however, *Rhizoctonia* levels were significantly lower three months after treatment with Mertect fungicide than all other fungicidal treatments.

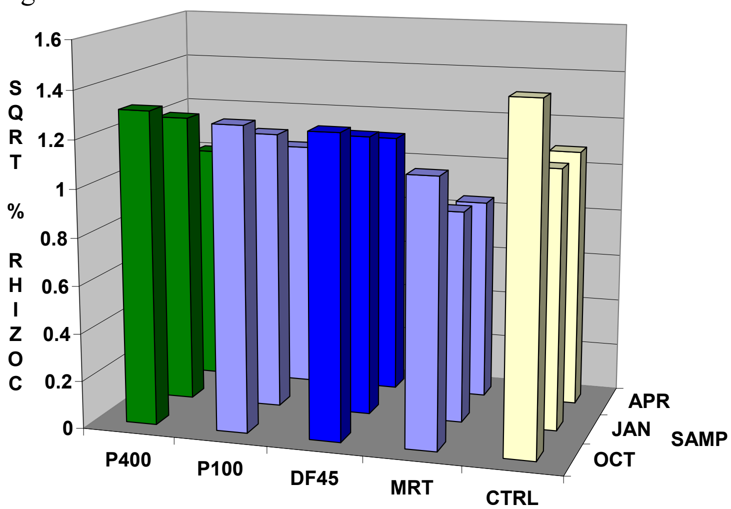

Figure 3.1: Changes in √% surface area covered with *Rhizoctonia* during storage of Norland potatoes treated prior to storage with commercially available fungicides [MRT = Mertect; DF-45=Dithane F-45; P100 & 400 = Purogene 100PPM & 400PPM].

Rhizoctonia levels were not expected to vary over time or in response to the various treatments. However, the fungicidal treatments could affect the viability of the pathogens. To test this, sclerotia were collected from treated tubers and germinated on water agar plates. Figure 3.2 shows the effects of the various treatments on sclerotial viability three and six months after treatment.

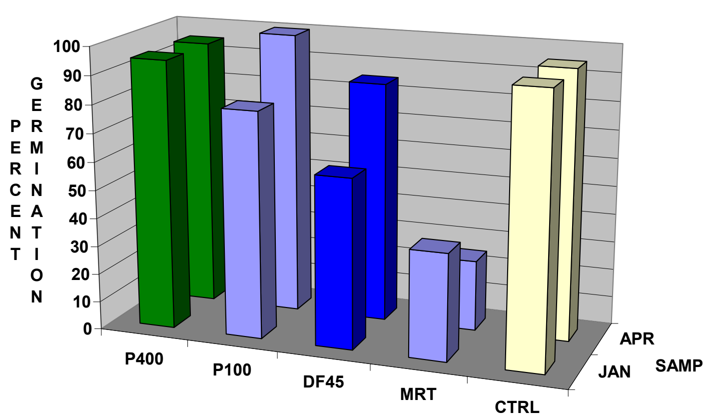

Figure 3.2: Viability of *Rhizoctonia solani* sclerotia [ASIN %GERM] from Norland potatoes treated prior to storage with commercially available fungicides [MRT = Mertect; DF-45=Dithane F-45; P100 & 400 = Purogene 100PPM & 400PPM].

Mertect treatments significantly (p=0.05) reduced the viability of sclerotia compared to the control. Other treatments reduced viability somewhat three months after treatment, but these affects were less apparent by the end of storage. The chemical treatments did not affect *Helminthosporium* Silver Scurf or *Erwinia* Soft Rot levels.

Colour Data

Treatment effects on calculated hue angle differed significantly with storage time. Treatment effects on Hunter A (redness) did not differ significantly immediately after treatment but were significantly lower three and six months after treatment (Figure 3.3). Mertect-treated potatoes were redder than the other potatoes immediately after treatment, however this difference disappeared as storage progressed. Most of the treatments appeared to have maintained red colour over the sample period to date. Apparently obvious large differences between other treatments and Mertect treatments immediately after treatment were not significant, due to variability in colour between tubers in the sample set.

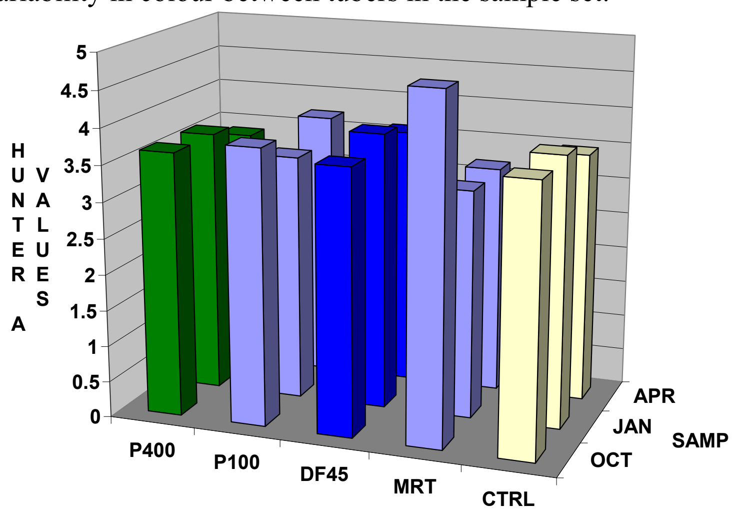

Figure 3.3: Changes in Hunter A (redness) values during storage of Norland potatoes treated prior to storage with commercially available [MRT = Mertect; DF-45=Dithane F-45; P100 & 400 = Purogene 100PPM & 400PPM].

Lightness and yellowness (L and B) values did not differ significantly between treatments. All treatments darkened and became more yellow over time.

Fungicidal treatments had a limited effect on tuber colour change over time. This was also seen in the previous study year as well. Visible differences were often the result of residual colour from the chemicals, which are designed for lasting contact on potatoes to control pathogens.

Weight Loss

All treatments lost weight with time, but at different rates. 100 PPM Purogene applications significantly increased percent weight loss over the control treatments immediately after treatment. Applications of Purogene (400 PPM) and Dithane F-45 significantly increased the percent weight loss immediately after treatment and after three months in storage. Treatment effects did not differ at the completion of storage.

Purogene is a reactive chemical that may have caused damage to the treated tubers, resulting in the increased weight loss over time. The increased concentration of Purogene would perhaps account for differences in percent weight loss between the two Purogene rates, again suggesting a damage response.

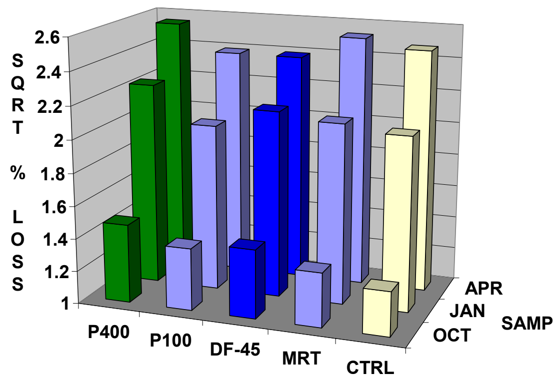

Figure 3.5: Changes over time in √ percent weight loss of Norland potatoes treated prior to storage with commercially available [MRT = Mertect; DF-45=Dithane F-45; P100 & 400 = Purogene 100PPM & 400PPM].

Conclusions

Chemical fungicide treatments should ideally not alter the quality of treated tubers to any significant extent, while reducing the spread and development of storage pathogens. These experiments demonstrated that the fungicide treatments available to Saskatchewan's potato growers were relatively ineffective in reducing disease levels. The treatments were somewhat effective in reducing viability of pathogens (e.g. *Rhizoctonia*). As with the ozone treatments, the chemical treatments appear to be less effective in treating (i.e. stopping) diseases that were already well established at the time of treatment.

Ozone/chemical Application during the Storage Season

During the course of the storage season, the diseases present on tubers can develop and spread to adjacent tubers, thereby reducing post-storage yields and quality. Diseases that persist through the storage season on seed potatoes can contaminate new fields and increase disease incidence in future crops. Low storage temperatures are usually effective in reducing the growth and development of storage pathogens. However, low temperature storage is not an option for processing potatoes and or as temperatures begin to rise in the spring and fall. Even in the middle of the winter, hot spots may also develop in storage due to the heat produced by respiration and decay. Fungicides applied as a spray to the tuber surface during bin loading can create a long-lasting protective barrier to disease infection, provided that coverage is uniform and that subsequent storage conditions are favourable. Chemical control via spray application is not an option once the bin is loaded. A volatile solution may however achieve disease control after the storage is loaded. The use of one or several fumigant type treatments during the storage period could potentially reduce the build up and spread of storage diseases. This type of treatment could also be used to combat the development of isolated pockets of disease. In this way, diseases are not allowed a chance to build to epidemic proportions in storage and growers are more assured of a quality product at the end of the storage period.

Chemicals applied during storage must possess certain characteristics. They must be able to penetrate the mass of potatoes, ensuring an even spread of active ingredients throughout the entire pile. They must also be conducive to application through existing ventilation systems. Purogene (Biocide) and ozone represent two possibilities for mid-season application. Purogene can be applied through the humidification system and ozone can be pumped in through simple ventilation equipment.

Objectives

1) To test the effect of mid-season treatments with fumigants (O_3 and Purogene) on the control of storage diseases of potatoes.

Materials and Methods

Russet Burbank and Norland potatoes from the same source as in previous experiments were stored at 4°C for three months. The potatoes were then treated for one day with either high levels of ozone (~10mg/kg/hr) or with 200 PPM of Purogene, applied with a humidification system. The rates of the Purogene application were according to label recommendations. There is no label recommendation for determining the duration of application for Purogene. 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 quality were evaluated in a manner similar to the other experiments. This experiment was carried out during the 1999/2000 and 2000/2001 storage periods. In 2000/2001, only Norland potatoes were examined.

Results and Discussion

1999/2000 Study

Disease Analysis

The ozone and Purogene treatments significantly reduced average *Fusarium* ratings compared to the control in Russet Burbank potatoes but not for the Norland potatoes. *Rhizoctonia* and *Erwinia* Soft Rot levels for either cultivar were not significantly influenced by the mid-season treatments.

Colour Analysis

The mid-season treatments significantly affected the Hunter A (redness) values for Norland potatoes and the Hunter B (yellowness) values for the Russet Burbank potatoes. The ozone treatment applied to Norland potatoes during storage resulted in significantly higher Hunter A (redness) values than when Purogene was applied. The higher Hunter A (redness) value denotes a redder colour in the ozone treated potatoes. The ozone treatments significantly increased the Hunter B (yellowness) values of Russet Burbank tubers compared to tubers receiving the control or Purogene treatments.

2000/2001 Study

The previous year's study was repeated in an identical manner with the exception of the ozone rate, which was increased to approximately 20 mg/kg/hr, and the use of only Norland potatoes.

Mid-storage ozone and Purogene treatments did not provide any disease reduction or change in quality variables, with the exception of *Helminthosporium solani* Silver Scurf lesions on tuber surfaces. It appears disease levels in 2000/2001 were lower than in 1999/2000.

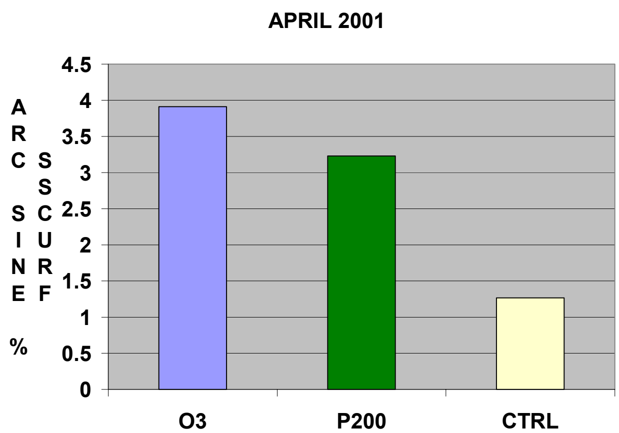

Figure 4.1: Changes in Arc Sine of percent surface area with *Helminthosporium solani* lesions of Norland potatoes treated in mid-storage with ozone or Purogene (200 PPM) treatments.

Ozone treatments significantly increased (p=0.05) silver scurf levels in Norland potatoes compared to the control. This was an unexpected result.

Conclusions

Mid-season applications of Purogene or ozone to stored potatoes in 1999/2000 significantly reduced losses to *Fusarium* in Russet Burbank potatoes, but had no impact on other storage diseases. Colour variables were only marginally affected by the treatments.

The second season data provided only limited information. Mid-season applications of ozone and Purogene do not appear to be any more effective than the pre-season treatments. It is possible that longer exposure time or higher concentrations of these in-season treatments might produce more beneficial treatment responses.

Ozone Treatment of Potatoes Inoculated with Specific Pathogens

Introduction

The amount of disease present at the time of harvest varies with the grower, season, initial seed contamination and other factors. The overall objective of this project is to determine the efficacy of ozone as a method for controlling post-harvest disease. Research of this type is complicated by the variability in the range, level and stage of development of diseases found in samples of potatoes at harvest. Trials with standardised disease loads would help determine whether ozone can reduce the spread and development of storage pathogens.

The location and intensity of pathogen infection is related to the nature of the pathogen, the barriers or resistance that the crop possesses and the method and timing of inoculation. The ability of ozone to counteract deep-seated or well-established infections is unknown. By exposing tubers that have been inoculated in varying manners with a known quantity of pathogenic propagules, it is possible to test whether ozone can control the development and spread of established infections.

Fusarium sambucinum, *Phytophthora infestans* and *Helminthosporium solani* are important diseases of potatoes in storage. The development of these diseases in storage can result in large losses to growers. *Fusarium* requires a wound to enter and infect the host tissues. This often leads to a deep-seated infection. *Phytophthora infestans* is capable of rapid and invasive infection, with no requirement for a wound. *Helminthosporium solani* is capable of spread in storage and can cause significant losses through reduction in quality of marketable product. *Helminthosporium* does not require a wound for infection.

Objective

1) To test the effects of ozone applications on the control of diseases introduced via controlled inoculation treatments.

Materials and Methods

1999/2000 Study Period

The inoculation procedures carried out in this experiment were based on techniques found in Platt (1992). Fungal cultures were prepared using pure cultures of *Fusarium sambucinum* and *Phytophthora infestans*. Cultures were grown on potato dextrose agar at room temperature for four weeks. The *Phytophthora infestans* cultures did not grow (either due to non-optimum growth conditions or to non-viable source cultures) and were therefore not used.

Fungal suspensions of *Fusarium* were 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 scraped solution was poured into 180 ml of distilled water and the culture plate was rinsed with another 10 ml of distilled water, resulting in a total solution volume of 200 ml. The suspension was stirred vigorously and a small sample was taken using a micropipette. The numbers of macroconidia were counted using a two-celled haemocytometer. Using the calculated average for the number of spores, the amount of stock suspension required to make an inoculation suspension containing 1.5×10^4 macroconidia per ml was determined. Twelve litres of this suspension was prepared.

Russet Burbank potatoes of approximately 6 ounces (170g) 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 equally during storage and handling and were therefore uniform. Each tuber was punctured four times, using a nail, leaving a 2-mm wide by 6-mm deep hole. Holes were punched in the apical and stolon ends of the tubers, as well as two holes at the midpoint. This allowed comparison between the various parts of the potato. Following the puncturing procedure, 20 tubers were placed in the agitated/stirred spore suspension for approximately one minute. To determine the background disease levels present in the tubers, control treatments were carried out using identical methods to the inoculation treatments, with distilled water substituted for the spore suspension. Once all inoculation procedures had been completed, tubers were placed into treatment rooms held at 15°C. The tubers were then treated for one day, one week or three weeks at ozone rates of zero or 10 or 20 mg/kg/hr. Following the ozone treatment, tubers were placed in the 15°C control room. After the three-week treatment period, the tubers were held at 2°C for one week until the tubers could be analysed.

Analysis of disease levels involved cutting the tubers along the length of each puncture hole and rating the degree of spread of disease within the tubers. The presence of disease showed as blackening of the host tissues and some sinking of the surface tissues. The rating scale was as follows:

0 = no evidence of any disease
1= trace of disease (1-2 mm)
2 = slight amount of disease spread (3-4 mm)
3 = moderate level of disease spread (5-10 mm)
4 = severe amount of disease spread (>10 mm)

Data were analysed using SAS statistical software.

2000/2001 Study Period

In the 2000/2001 study period, several aspects of the inoculation procedure were modified to increase understanding regarding ozone efficacy and ozone:pathogen interactions. Tubers were inoculated with *Fusarium sambucinum*, *Phytophthora infestans* and *Helminthosporium solani*. Tubers were inoculated using several methods, varying potato surface integrity and inoculum type.

For the *Fusarium sambucinum* inoculations, pure cultures were washed and scraped to prepare inoculation suspensions of 30,000 macroconidia per ml. All tubers were washed, soaked in a 2% Bleach solution for 5 minutes and then air-dried. In one trial, the suspension was applied by spraying the suspension onto tubers using a spray bottle and wetting agent. Tubers were sprayed to surface wetness. In another trial, tubers were immersed in an agitated spore suspension mixture, after having been rolled on Ottawa quartz sand. This treatment disrupted the surface structure integrity of the tubers to some extent, resulting in a deeper wound than the surface application but a shallower wound than the previous punch wounding trial. Control treatments involved a distilled water spray or soak. Following inoculation, tubers were held at room temperature for a 24-hour incubation period. Tubers were then treated for one, seven or 21 days in either ozone or control atmospheres. Ozone rates were determined by tuber weights and calculated to provide a set amount of ozone/mg/kg as reported in other experiments.

Following treatment, tubers were evaluated for disease incidence (presence of disease), percent surface area with visible disease symptoms and disease symptom development (i.e. percent tuber rot).

Phytophthora infestans cultures were grown on Clarified Rye Agar plates with β-sitosterol added. Spore suspensions were prepared using a mix of two isolates and contained 50,000 sporangia per ml. Tubers were sprayed to surface wetness and incubated for 24 hours. Tubers were treated with ozone as in the other trials and then evaluated in a similar manner.

Helminthosporium solani cultures are slow growing and produce few spores. Suspensions containing 2.5 x 10^4 conidia were prepared and sprayed onto the tubers. Tubers were incubated (one day) and treated as in the other trials (three weeks). At the completion of the ozone treatment period, the tubers were incubated for two weeks at room temperature. Incubation was stopped one week earlier than what is normally carried out due to intense rot development and tuber breakdown. Tubers that had broken down completely (due to soft rot organisms) were not rated. Final tuber counts were randomly adjusted to balance the data set. Tubers were rated for percent surface area with silver scurf lesions.

Results and Discussion

1999/2000 - The data collected for this experiment broke the inoculation points (wound location) into the apical, stolon and mid-tuber locations. Inoculated tubers had significantly higher incidence of Dry Rot than the control treatments (See Figure 5.1).

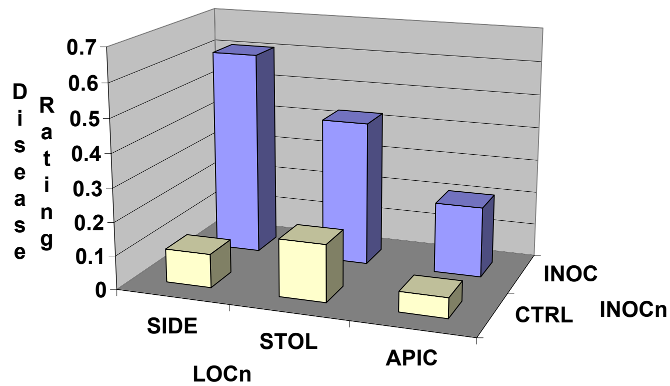

Figure 5.1: Changes in disease level with inoculated versus control treatments (LSD SIDE=0.10, APIC=0.10, STOL=0.14).

The location of the wound appears to have been important in terms of the degree of subsequent infection development. The sides appear to be the most susceptible to infection, followed by the stem end (stolon). The duration of exposure by ozone concentration (rate) treatment combination had no effect on disease levels for any location.

2000/2001

***Fusarium* Surface Inoculation -** *Fusarium* incidence (# potatoes/total) was significantly lower (p=0.1) in ozone treated potatoes than in control treatments. There was no difference between surface area symptoms or in the amount of rot per tuber in the ozone or control treatments.

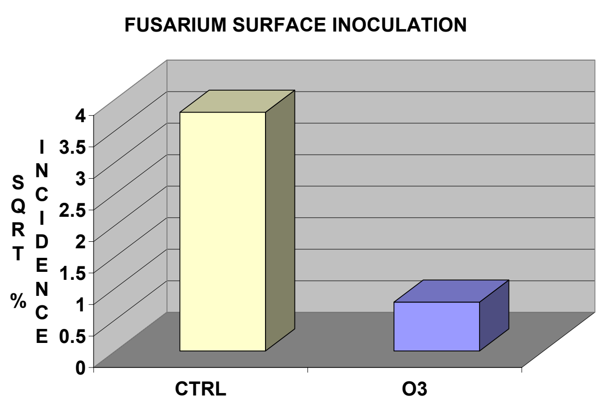

Figure 5.2: Changes in *Fusarium* incidence (√% incidence) in ozone-treated versus control surface-inoculated potatoes.

***Fusarium* Wound Inoculation -** No significant differences were detected between ozone and control treatments in this trial despite the amount of damage that was caused by the sand roll. Inoculation did not significantly increased disease incidence or disease severity. This was unexpected.

***Phytophthora* Surface Inoculation**

Inoculation with *Phytophthora* increased the incidence of disease present in the potatoes. Severity of disease was also significantly increased by inoculation with *Phytophthora*. No significant differences were detected between ozone and control treatments in this trial.

***Helminthosporium* Surface Inoculation**

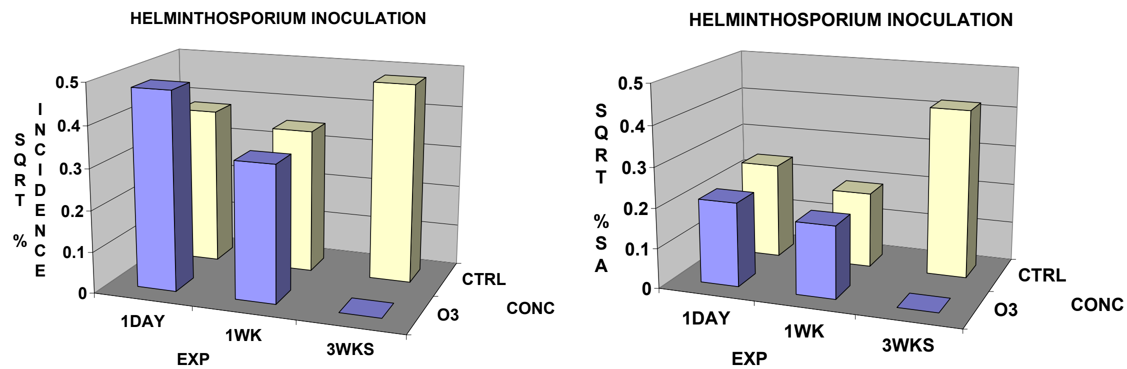

Figure 5.3: Changes in *Helminthosporium* incidence and % surface area in ozone-treated versus control inoculated potatoes.

Ozone-treated potatoes had significantly lower (p=0.05) incidence of *Helminthosporium* than control potatoes. The percent of tuber surface with visible lesions was also significantly lower in ozone treatments than in the controls. Ozone appeared to reduce the ability of this fungus to infect the potato tubers

Conclusions

Ozone applied after the *Fusarium* was established did not control this disease. This type of infection would be encountered in potatoes that had been in storage for an extended period. This disease is commonly introduced by deep wounding and the disease has often had some time to develop, unless treatments commence immediately after harvest. Ozone treatments were somewhat effective against shallow Fusarium infections – this suggests that some benefit may be obtained if ozone can be applied soon after harvest when the Fusarium infections are still relatively new and superficial.

Ozone treatment also had little effect on Phytophthora Late Blight in tubers that had received some degree of wounding prior to inoculation. *Helminthosporium* damage was reduced by ozone treatment. *Helminthosporium* is slow growing relative to fusarium and Phytophthora – this may explain why it is more readily controlled than the more aggressive pathogens.

The results of these trials indicate some potential for the control of certain pathogens with ozone, provided their growth and infection patterns fit into the control capabilities of ozone.

Section 6. Effect of ozone on growth of fungal cultures and spore germination.

Background - The rate of growth of fungal mycelium coupled with the rate of production and germination of propagative spores determine the speed of infection and spread of any pathogen. If either of these processes is adversely affected by a disease management treatment, the ability of the pathogen to cause disease is reduced. The influence of ozone on the growth and reproduction of several of the key pathogens causing disease in stored potatoes was examined in a series of laboratory experiments.

6-A. The effect of ozone on mycelial growth.

Materials and methods:

Pure fungal cultures of *Fusarium sambucinum* and *F. solani* (causal organisms of Dry rot) were established on Potato Dextrose Agar (PDA) plates. Five mm plugs were transferred from the actively growing margins of these cultures to the centre of new plates. These plates were maintained at room temperature for two days, to establish actively growing colonies that were then treated with ozone. The closed plates were placed on wire racks in a dark chamber at 20^0C and ozone was blown from underneath the plates at a rate of 40 mg/plate/hr (20ppm/plate/hour) for zero, two, four, six eight and ten days. The lids were left on the dishes as preliminary experiments had shown that when lids were removed the agar surfaces became overgrown with contaminants, making it impossible to determine the growth rate of the cultures being tested. It is noteworthy that the ozone treatments did control this contamination. A high rate of ozone was used to compensate for the closed plates. Gas exchange into the plates is permitted by the loose-fitting lids, but it was not possible to monitor exactly how much ozone penetrated under the lids and contacted the fungal mycelium. Previous experiments by James et al (1982) have shown that 0.1ppm of ozone applied to open plates reduced the growth rate of *Fomes annosus* when applied at this rate continuously for nine hours daily for three days.

Following the ozone treatment, the plates were incubated at room temperature, in the dark, until evaluations were completed. Colony diameters were measured at the start of the treatment periods and then two, four and six days after the ozone treatment. Typically the cultures grew to the edge of the plate after four to six days. Each treatment consisted of three plates. All treatments were replicated three times on separate occasions.

Results:

When the growth measurements taken at zero, two, four and six days after ozone treatment were combined and averaged, the untreated cultures of *F. sambucinum* grew slightly more than the ozone-treated cultures (Table 6-1).

The growth suppressing effect of the ozone treatments was most evident after four days (Table 6-2), as by day six the majority of colonies had grown to the edge of the plates (88 mm). The continued exposure to ozone for six and ten days had no effect because maximum growth was achieved after six days.

Table 6-1. Effect of length of time of exposure to ozone on colony diameter of *F. sambucinum* averaged over a 10 day treatment period

Exposure to Ozone (days)	Average diam. of colony (mm)
0	67.7 a*
2	64.0 ab
4	63.3 b
6	64.2 ab
8	65.8 ab
10	64.6 ab

*means followed by the same letter are not significantly different. P=0.05

Table 6-2. The effect of length of time of exposure to ozone on colony diameter of *F. sambucinum* measured at four days after treatment.

Exposure to Ozone (days)	Average diam. of colony (mm)
0	85.6 a*
2	77.9 b
4	75.8 b
6	78.3 b
8	79.7 b
10	80.2 b

*means followed by the same letter are not significantly different. P=0.05

The cultures of *F.solani* were measured after zero, two and four days. The cultures grew more slowly than those of *F. sambucinum*. Ozone treatments actually increased the width of the colonies, when measured after four days exposure (Table 1-3).

Table 6-3. The effect of length of time of exposure to ozone on diameter of *F. solani* measured at four days after treatment.

Exposure to Ozone (days)	Average diam. of colony (mm)
0	34.0 a*
2	37.2 ab
4	46.5 b
6	42.0 ab
8	44.1 ab
10	49.0 b

*means followed by the same letter are not significantly different. P=0.05

This effect is opposite to that seen on *F. sambucinum*.

The four, six, eight and ten day treatments had all received four days exposure at this time, and there was no significant difference in the amount of growth on these plates. However the untreated cultures did grow significantly less than some of the plates treated with ozone.

Conclusion:
Ozone treatment did not significantly suppress mycelial growth of the two fungal species under the relatively uniform, controlled conditions of this plate study. Although the actual concentration of ozone achieved in the plates could not be measured, a greater degree of suppression was expected. The fact that ozone was not effective at controlling fungal contamination of the open plates would suggest a limited potential for this concentration of ozone to control vegetative growth of fungal pathogens.

6-B. The effect of ozone on spore germination.

Materials and methods:
Pure fungal cultures of *F. sambucinum*, *F. solani* and *Helminthosporium solani* were grown on PDA for ten days to two months (depending on the growth rate of the colony), until the cultures produced spores. Spores were harvested from the plates by adding 10 ml of distilled water to each plate and then gently scraping the surface of the colony with a plastic scraper. The plates were rinsed once with 10 ml of water, and the suspensions made up to 100 ml. The suspensions were filtered through three layers of cheese-cloth to remove any clumps of mycelium or agar, and one-three drops of Tween 20 were added to the suspensions. The concentrations of spores obtained were:
F. sambucinum - 8 X 10^4 spores/ml
F. solani - 15 X 10^4 spores/ml
H. solani – 2 X 10^4 spores/ml.

One ml of each suspension was placed on a water agar plate and dispersed with a sterile glass spreader. The closed plates were placed in the treatment chamber and exposed to ozone as described in the previous experiment. The plates were exposed for one, two, four and six hours, at 0, 50 and 100 mg O3/plate/hour and spore germination was then evaluated. A drop of dilute lactofuschin stain was placed on the plate immediately after treatment, to prevent further germination occurring, and a coverslip was placed over the stained area. One hundred spores were examined using a compound microscope and the percentage of spores that had germinated was recorded. It was noted that almost 100% germination had occurred in all treatments after approximately two hours.

Results:

The ozone treatment did not affect the percentage spore germination of any of the species tested. By contrast, Kraus and Weidensaul (1978) have shown that germination of conidia of *Botrytis cinerea* was significantly reduced when spores were exposed to ozone at 0.3ppm for two 6-hour periods. However, in the work of Kraus and Weidensaul (1978), ozone was applied to open dishes, and ozone concentrations were monitored constantly to ensure the concentration remained at the established level. The actual levels of ozone to which the *Fusarium* and *Helminthosporium* cultures were exposed could not be determined in this trial, as the petri dish lids were left on the dishes to prevent contamination.

Conclusion:

Treating enclosed plates with ozone provided little in the way of control of either vegetative growth or spore germination of a range of fungi pathogenic to stored potatoes. One potential explanation for the lack of expected control is that the ozone concentrations being utilized were simply inadequate. Although ozone gas would have diffused into the enclosed plates, the actual concentration of ozone inside the plates may have been relatively low. Kim et al., in their review on the use of ozone (1999) point out that the “effectiveness of ozone varies appreciably with minor changes in experimental variables”, and they conclude that it is not feasible to compare results from different sources. This also suggests that ozone may not be a robust control option as its efficacy is affected by temperature and humidity variables that cannot always be controlled under on-farm conditions.

Section 7. Treating Seed Potatoes with Ozone Prior to Planting

Introduction

Potatoes are propagated vegetatively, using sections of tubers as "seed". Fungicides typically are applied to this seed to reduce the disease load and prevent decay after planting. Unfortunately, these treatments are costly, toxic to applicators and may negatively affect the ability of seed tubers to sprout, emerge and grow.

Ozone is a potential means for controlling disease and could represent an alternative seed treatment. However, ozone is a highly reactive substance and may potentially react with tuber buds (eyes) and reduce the degree of sprouting. This could potentially reduce crop emergence, establishment and yield.

Objectives

1) To evaluate the effect of ozone application as a seed treatment on the control of disease at the time of planting.
2) To compare ozone to standard fungicides

Materials and Methods

At the commencement of the 1999, 2000, 2001 and 2002 planting seasons, potato seed pieces were treated with ozone at different concentrations for a period of time just prior to planting.

In 1999, Norland seed pieces heavily infected with *Fusarium* Dry Rot were used. Cut seed pieces (average size ~ 4oz or ~113g) were treated with ozone at 400 (40mg/kg/hr) or 600 mg/hr for a period of eight hours. This treatment represented an initial assay. The control treatment involved treating the seed with a commercial seed treatment fungicide (Metiram). Following treatment, tubers were planted into the University of Saskatchewan Potato Fields (Saskatoon, SK.). Potato plots were managed using standard growing practices (irrigation, fertilisation, etc.). At the end of the season, tubers were mechanically harvested and graded to determine marketable yields as well as non-marketable (oversize and undersize) yields).

In 2000 Elite III seed potatoes of two cultivars, Norland and Russet Burbank (Hyland Seed Potato Farms) were used. Cut seed pieces were treated with three ozone concentrations of ozone (0, ~10 and ~20 mg/kg/hr) for either one or two days. These rates of ozone represent amounts that correspond to the rates used in the other ozone trials conducted in this year. As in the previous year, the control treatment involved treat the tubers with a commercial seed treatment (Metiram). Tubers were planted in mid-May and the emergence data recorded over a period of weeks. The plot was managed using standard crop management protocols. The final tuber yield was determined at harvest and the data was analysed using SAS software.

In 2001, this experiment was repeated with several slight variations on the previous year (2000). Only Norland potatoes were evaluated and a non-treated control was added to contrast the ozone treatments and chemical seed treatment control (Metiram). All other aspects of the study were similar to the previous year.

In 2002 we again evaluated ozone as a seed treatment versus the commonly used fungicidal seed treatment, Polyram 16D (16% metiram). The trial was conducted at a grower site in 2002 using AC Peregrine seed treated one day prior to planting with :

1. fungicide seed treatment, Polyram 16D applied at the recommended rate of 0.65 kg/100 kg seed
2. O_3 applied at 20 mg/kg seed/hr for 24 hours
3. O_3 applied at 200 mg/kg seed/hr for 24 hours
4. untreated control.

The 20 mg/kg/hr rate of ozone represented the standard treatment used in previous trials. The higher rate was designed to test for potential phytotoxic effects of the ozone treatments. The seed used in the 2002 trial was produced in Outlook utilizing standard management practices. The seed had trace levels of Rhizoctonia, Silver surf and common scab – but all disease levels were below the tolerance levels set for seed. The trial was conducted under a pivot irrigation system, on a sandy loam soil at a table potato farm, near Bradwell, Saskatchewan. The trial was planted on June 6 using a mixture of whole and cut seed. Each treatment was replicated four times in a randomized complete block design. Each replicate consisted of a single five metre long row, with plants spaced 23 cm apart within the row. Standard management practices were employed for the duration of the cropping season. The crop was chemically desiccated in early September and harvested in early October. Tubers were sized and tuber numbers and weights in each size category were determined.

Disease levels on the harvested tubers were assessed in mid October. The disease incidence and severity of Rhizoctonia black scurf, common scab and powdery scab were evaluated using a 30-tuber sample for each replicate/treatment. A distinct difference in tuber colour was noted between the tubers harvested from treated seed compared with those harvested from untreated seed. Hunter colorimeter values were determined on 10 tubers in each replicate/treatment. Silver scurf levels were also evaluated on 20-tuber samples in October. Silver scurf levels usually increase after several months in storage so a second evaluation was conducted on stored tubers in January 2003.

In addition to the emergence and yield data collected, seed pieces were set aside for evaluation of sprouting and the development of wound periderm. In each year, the final stand counts were used as a covariant in the yield analysis to remove background differences from treatments.

Results and Discussion

In 1999, ozone treated potatoes did not differ significantly from the control in final marketable or unmarketable yield. Final emergence counts were not significantly correlated with the final yields.

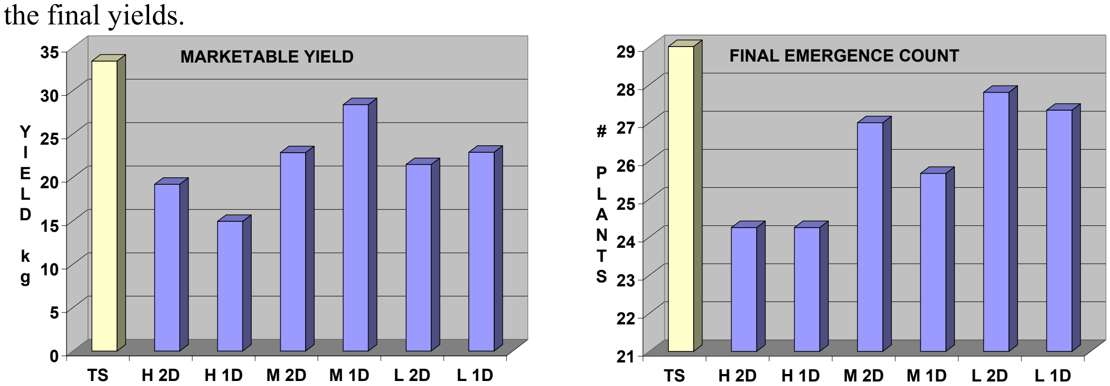

Figure 7.1: Marketable yield and final emergence counts of RB potatoes treated pre-planting with various ozone treatments or a chemical control (MktLSD=9.62).

In 2000, Russet Burbank potatoes treated prior to planting with the higher ozone concentrations had significantly lower marketable yields than the chemical control treatment. This result was only apparent when yields were corrected for plant emergence. The chemical control treatment resulted in significantly higher emergence counts than seen in the highest ozone concentration treatment. Why ozone treatments reduced seed vigour is not known.

In the 2001 trial, the high level of ozone applied for the greatest duration again reduced the stand for Norland potatoes (Table 7-1). This stand reduction produced a corresponding reduction in yields. None of the other seed treatments improved yields relative to the untreated controls. The lack of response to seed treatments in 2001 likely reflects excellent weather both prior to and following planting.

Table 7.1 Effect of treating seed potatoes with ozone on stand and yields of Norland potatoes in 2001

TREATMENT	STAND (%)	MARKETABLE YIELD (T/A)
CONTROL	100 a	16.1 a
FUNGICIDE	98 a	15.6 a
LOW O3 – 1 DAY	97 a	16.0 a
LOW O3 – 2 DAYS	98 a	17.1 a
HIGH O3 – 1 DAY	99 a	15.4 a
HIGH O3 – 2 DAYS	74 a	12.2 b

In 2002 the crop was slow to emerge and appeared to lack vigor throughout the growing season (Table 7-2). The stand was generally poor, even when the seed was treated with fungicides (Table 7-2). Both ozone treatments appeared to negatively affect the stand, but not in a dosage dependant manner. Yields of all treatments were also below normal, with the ozone treatments performing particularly poorly (Table 7-2). The low yields in the ozone treated plots corresponded to the degree of damage to the stand – a good quality stand is critical in potato production.

Table 7-2. Influence of ozone as a seed treatment on the agronomic performance and yields of AC Peregrine seed potatoes.

	Stand %	Vigor (1-5)	Total Yield	Seed Yield	Table Yield
			----------------------T/acre -------------------		
Treatment	**	**	**	*	**
Control	59 ab	2.9 a	10.5 a	9.3 ab	9.3 ab
Fungicide	75 a	3.6 a	13.2 a	12.5 a	11.7 a
O3 @ 20 mg/kg/h	43 b	1.6 b	6.7 b	6.0 b	5.9 b
O3 @ 200 mg/kg/h	48 b	2.6 ab	10.1 a	9.6 b	9.1 ab

Vigor rating 1 = poor, 5 = excellent
*, ** = significant at P=0.05 and 0.01 respectively.
Seed Yield = tubers 33-88 mm diam
Table yield = tubers greater than 55 mm diam

The incidence of disease was assessed as the percentage of tubers falling into three disease categories: no infection, slight infection (1-5% of tuber surface infected) and moderate infection (>5-10% surface infected). There were very few tubers with more than slight infection, so the two categories were combined and total % of infected tubers reported (Table 7-3). There were no significant treatment effects on any of the diseases examined. The incidence of silver scurf was very low and none of the treatments affected the incidence of this disease. It is not uncommon to find low levels of silver scurf early in the storage season - this test will be repeated in January 2003.

Table 7-3. Effect of ozone treatment of seed-pieces on incidence of disease in the daughter tubers.

Treatment	Average % of tubers with:				
	Rhizoctonia	Common Scab	Powdery Scab	Soft rot	Silver scurf
Control	59.0	5.8	5.7	6.3	6.2
O3 @ 20 mg/kg/hr	34.0	3.3	3.2	6.3	13.8
O3 @ 200 mg/kg/hr	55.7	1.8	15.9	3.8	1.3
Polyram 16D	27.5	5.0	14.2	5.0	8.8

The colour values for the skin of the harvested tubers were determined using a Hunterlab Colour Meter. For 10 tubers per treatment/replicate a lightness value (100=white, 0 = black), a red/green value (+a=red, -a=green) and a yellow/blue value (+b=yellow, -b=green) was obtained. The average readings are shown in Table 7-4.

Table 7-4. The average skin colour values evaluated 3 weeks after harvest for tubers harvested from ozone treated seed (sample of 10 tubers).

Treatment	Average colour value		
	Lightness	Red/green	Yellow/blue
Control	23.0 a*	4.39 a	4.32 a
O3 @ 20 mg/kg/hr	24.1 ab	5.12 ab	4.39 a
O3 @ 200 mg/kg/hr	23.4 a	4.91 ab	4.28 a
Polyram 16D	25.6 b	5.81 b	5.86 b

*means followed by the same letter are not significantly different. P=0.05

The red/green values are probably the most critical as they illustrate the redness of the tubers, which is a critical marketing feature. All the seed treatments resulted in higher redness values than the control, with the crop arising from Polyram treated seed being significantly redder than the controls. The Polyram treatment also caused harvested tubers to have significantly higher lightness and yellow values, suggesting that these tubers were not only redder but also brighter in colour. These seed treatment effects on the color of the daughter tubers were unexpected and could not be readily explained.

Conclusion:
In 1999 and 2001most of the ozone treatments were comparable to chemical controls in efficacy as determined by total potato yield. There were limited differences between ozone treatments in these trials. In 2000, 2001 and 2002, some of the ozone treatments reduced seed piece viability as indicated by a reduced % emergence. The poor stand translated into reduced final yields. In some cases, the negative effects of ozone on the seed were dosage dependent, while in other cases, the dose appeared to be irrelevant. Adverse field conditions encountered after planting could have played a role in the reduction in plant stand observed in the ozone treated plots. The

ozone treatments would have provided no residual protection to the seedpieces after planting – while the fungicidal products are designed to protect the seedpieces against decay causing organisms. Under adverse conditions the added protection provided by the fungicidal treatments may be crucial to getting a good stand. In the 2001 trial, high levels of ozone actually produced a poorer stand that untreated seed – this suggests that the ozone may have been actually injuring the seed. The concept of short-term ozone treatment injuring seed runs contrary to previous research and merits further investigation.

In the 2002 trial, both ozone and fungicidal seed treatments appeared to improve the skin color of the red-skinned cultivar used in this trial. This effect is similar to that observed following treatment of stored potatoes with ozone. Skin colour would be improved by reducing the incidence of superficial skin diseases, such as silver scurf and black scurf. However there were no obvious reductions in these diseases in this trial. The improvements in color observed in this trial were great enough that they would have enhanced the marketability of the crop. As the skin color of red potatoes tends to fade in storage, the influence of the per-planting treatments on skin color should be monitored through the storage period. It is noteworthy that ozone effects on skin coloe were not observed in the 1999-2001

Influence of Ozone Treatments on Wound Periderm Formation

Introduction

Potatoes have the ability to heal wounds that occur during the harvest, grading and preliminary storage activities. To speed this healing, tubers are held at 15°C for several weeks after harvest. During wound healing, the tubers form protective tissue layers that serve as a barrier against invasion by disease pathogens. The thickness of this suberin layer or wound periderm layer may represent the difference between infection and resistance to disease.

Ozone has been proposed as a potential alternative to chemicals for the protection of potatoes from infection by disease during the curing period. The higher temperatures used to enhance potato curing also accelerate disease growth and development. Activity of ozone on the wounded surfaces could alter the healing process.

Objective:

1) To test the effect of ozone applications on the formation of wound periderm in cut seed pieces.

Materials and Methods

In 2000, Russet Burbank potatoes (avg. wt. = 6-8oz), were cut in half, then the tubers were exposed to ozone at approximately 22 mg/kg/hour at 20°C for either one or two days. Following ozone treatment, tubers were held at room temperature 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. Data were analysed using SAS software.

In 2001, this experiment was carried out using Norland potatoes. Tubers were surface sterilised in a 2% Chlorine bleach solution for five minutes and then rinsed in distilled water. Tubers were then cut in half and exposed to ozone for one day, one week or three weeks. Following ozone treatment, two thin slices were cut from the centre of each tuber piece and placed in a killing solution (FAA) for one hour. The FAA solution contained 10-ml formaldehyde (38 - 40%); 5-ml glacial acetic acid, 50 ml 95% ethyl alcohol and 35 ml distilled water in every 100 ml of solution. Once this killing treatment was completed, slices were transferred to a stain solution (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 (15-minute minimum) and held in distilled water until periderm layers could be measured. This paralleled a procedure outlined in Nielsen (1973). An ocular micrometer was placed in the eyepiece of a dissecting microscope and calibrated for different magnification settings. Wound periderm thickness was determined using 12X magnification. 10 of the 20 sample slices were measured in two places and the resulting ocular units were averaged and converted to µm. Data were analysed using SAS software.

Results and Discussion

In 2000, preliminary trials indicated that ozone treatment might increase wound periderm thickness. The trial was repeated in 2001 with greater precision.

In 2001, analysis of the data indicated that the wound periderm thickness was significantly decreased by ozone treatment, however longer exposure to ozone did appear to increase periderm thickness slightly (See Figure 7.1).

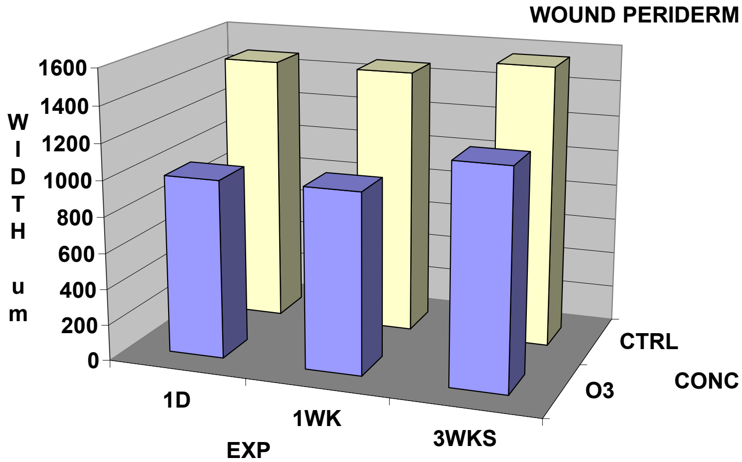

Figure 7.1: Effect of ozone treatments on potato wound periderm thickness (μm).

It is likely that the ozone treatment differences are a demonstration of the natural reactions of ozone, where ozone molecules react and oxidise compounds. Reaction of the ozone with cell tissues may have resulted in a reduction in the formation of the wound periderm tissues.

Conclusions

Ozone treatment caused a significant reduction in periderm thickness in wounded potatoes. Any inhibition of wound periderm formation would represent a problem from a storage management standpoint, as the periderm is critical to safeguarding wounded potatoes against diseases and excessive moisture loss. The ozone treated tubers did not appear to have been adversely affected in this trial – but the observation period was relatively short.

Influence of Ozone Treatments on Sprouting of Seed Potatoes

Introduction

The ability of seed potatoes to sprout and emerge has a direct impact on the vigour and yield potential of a crop. The control of sprouting in storage is also important to potato producers. Ozone applied prior to or during storage may affect the sprouting ability and vigour of seed tubers.

Objectives

1) To test the effect of ozone treatments on the amount of sprouting and sprout vigour (as measured by the size of sprouts) in seed potatoes.

Materials and Methods

In 2000, tubers that had received the pre-planting seed treatments (O_3 or Tuberseal [Metiram]) were evaluated for differences in sprouting. Sprout counts and final sprout weights were collected for Norland and Russet Burbank potatoes.

In 2001, de-sprouted Norland potatoes were treated with a range of increasing ozone concentrations for two days. 10 tubers were exposed to 0, 20, 40, 80, 160 and 320 mg/kg/hr ozone for the two-day treatment period. 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. The overall final sprout weight was collected and the final sprouting percentage was calculated. The data was analysed using SAS software.

Results and Discussion

Preliminary experiments in 2000 indicated that ozone treatments had no negative effects on tuber sprouting.

In 2001, the percentage of eyes that had sprouted at four days after the start of the treatment differed significantly between the various ozone treatments (See Figure 8.1). Ozone treatments did not differ significantly from the control for any of the other times or in total sprout weight.

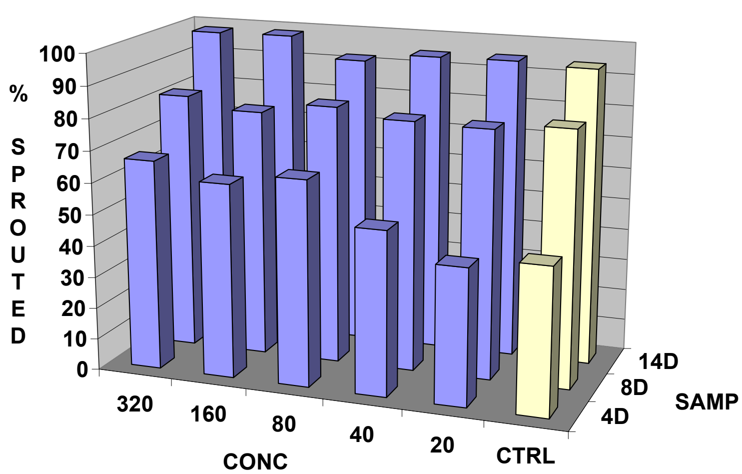

Figure 8.1: Changes in % sprouting over time in Norland potatoes treated with various ozone concentrations.

At four days after the commencement of treatment, potatoes that received 320 or 80 mg O_3/kg/hour had significantly higher percentages of eyes sprouted than control or 20 mg O_3/kg/hr treatments. It is possible that this was caused by a stressing of the eyes or could represent some subjective error. No difference between treatments was apparent at any other time in the experiment.

Conclusions

Ozone treatments did not alter the sprouting capability of the potatoes.

Ozone Treatment of Pure Fungal Cultures

Introduction

Disease causing pathogens vary in their resistance to chemical and other treatments, including ozone. The effects of ozone on sporulation, mycelial development and general pathogen viability after treatment will help illuminate the potential usefulness of ozone in controlling various diseases. The use of pure fungal cultures in these assays eliminates the influence of external factors, such as host responses and background infection levels on response assays.

Objectives

1) To test the efficacy of ozone in controlling mycelial development (as measured by culture growth rates) of a range of pathogenic fungi.
2) To test the efficacy of ozone in controlling fungal sporulation (as measured by the number of spores counted after treatment) of a range of pathogenic fungi.
3) To test the efficacy of ozone in reducing culture viability of fungal pathogens.

Materials and Methods

2001 Trials

Pure fungal cultures of *Fusarium sambucinum, Fusarium solani, Phytophthora infestans* and *Helminthosporium solani* were prepared on Potato Dextrose Agar (PDA), PDA, Clarified Rye Agar (CRA) and PDA respectively. *Rhizoctonia solani* and *Sclerotinia sclerotiorum* sclerotia were generated on PDA plates.

Fusarium sambucinum and *Fusarium solani* cultures for ozone treatment were generated using 5-mm plugs transferred from the actively growing margins of pure culture plates. Plates were grown at room temperature for two days and then treated with either zero or approximately 45 mg ozone per plate for one or two days in the dark. Following the two days of treatment, plates were maintained at room temperature in the dark until evaluations were completed. Initial colony diameter was measured at the start of the treatment period. Colony diameter was measured for each plate on a daily basis for four to five days. Each treatment consisted of three replicate sample plates. All treatments were replicated three times. Each replicate consisted of a separate set of treatment days.

Cultures were also started and treated to evaluate ozone treatment effects on the sporulation of *Fusarium* cultures. Following treatment, cultures were placed in the dark for three days and then spores were harvested. Each culture was washed and gently scraped with a plastic tool and 10 ml of distilled water, which was poured off into a beaker containing 80 ml of distilled water. Each plate was again rinsed with 10 ml of distilled water and the final suspension was stirred vigorously. Two 1-ml samples were removed and placed into separate microcentrifuge tubes containing a 0.5-ml dilute lacto-fuschia stain. This ensured maintenance of spores in storage. 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 *Fusarium solani* cultures.

This procedure was completed for each of the culture pathogens (*Phytophthora, Helminthosporium*). *Helminthosporium* grows much more slowly than the other pathogens and therefore treatments was conducted on two to three week old cultures and measurements were taken at two-day intervals. Sporulation measurements were tried, however spore production was very limited and accurate samples could not be collected.

Phytophthora infestans sporulation treatments were carried out on CRA media modified with 0.05g B-sitosterol (40% from soybean; Sigma-Aldrich Canada Inc.) per litre of media. Mycelial growth rates were conducted on standard CRA media.

When spore collection procedures were carried out on *Phytophthora* cultures, colony growth and sporulation were such that the final amount of spore solution was seven ml rather than 100 ml as in the *Fusarium* trials. Plates were washed and rinsed with two and five ml of distilled water and two samples were collected in the other trials.

To test the effects of ozone treatment on fungal viability of resting bodies, sclerotia of *Sclerotinia* were generated in culture. 10 *Sclerotinia* sclerotia (large) were placed on Water Agar (WA) plates and treated with the standard ozone treatments. Germination was determined on a daily basis until 100 percent germination was achieved in the controls (or five days). A similar trial was supposed to be conducted on *Rhizoctonia* sclerotia, however *Rhizoctonia* colonies are extremely variable in their growth characteristics and no useful sclerotia were produced.

2001 Trials - Results and Discussion

Sporulation - Ozone treatments had no effect on *F. sambucinum* sporulation, but appeared to stimulate sporulation in *F. solani* cultures. This may have been a stress response. Applying ozone for 2 days appeared to decrease sporulation in *P. infestans.*

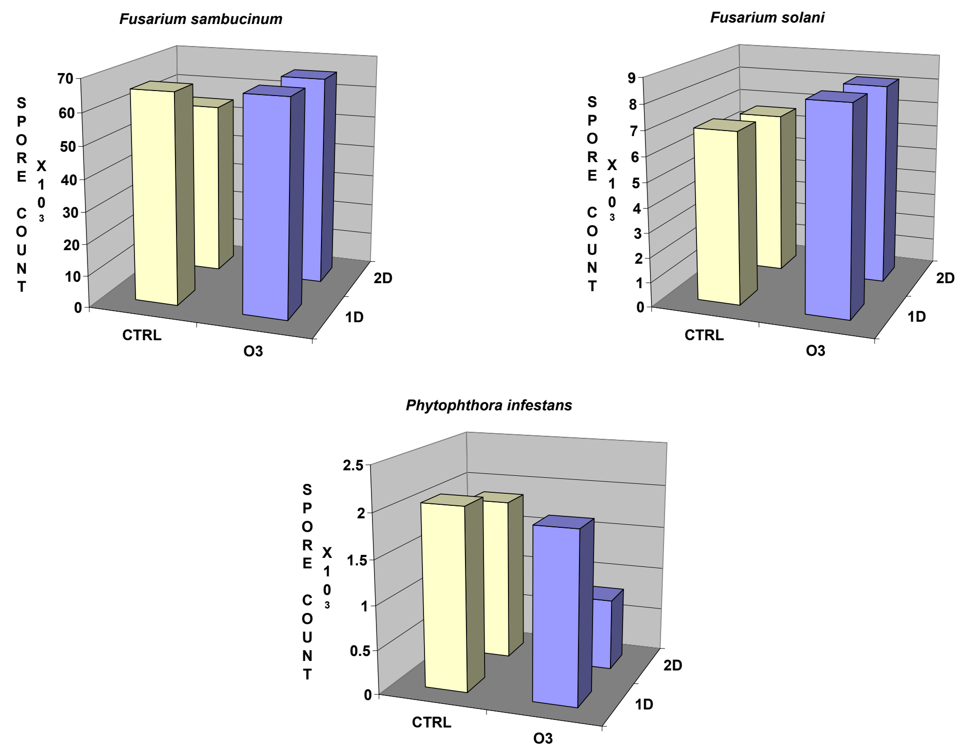

Figure 9.1: Differences in spore counts (x 10^3) from *Fusarium sambucinum, F. solani* and *Phytophthora infestans* pure cultures grown in various ozone treatments.

Mycelial Growth

This experiment was designed to evaluate the effects of ozone on colony growth rates and the ability of the colony to withstand and recover from ozone treatment. Colonies were measured to determine daily colony diameters for comparison of growth rates during and after treatment. Figure 9.2 shows the effects of ozone treatments on overall daily colony diameter and Figure 9.3 shows the effect of treatment on growth rate. It is expected that control treatments will have a steady growth rate over time.

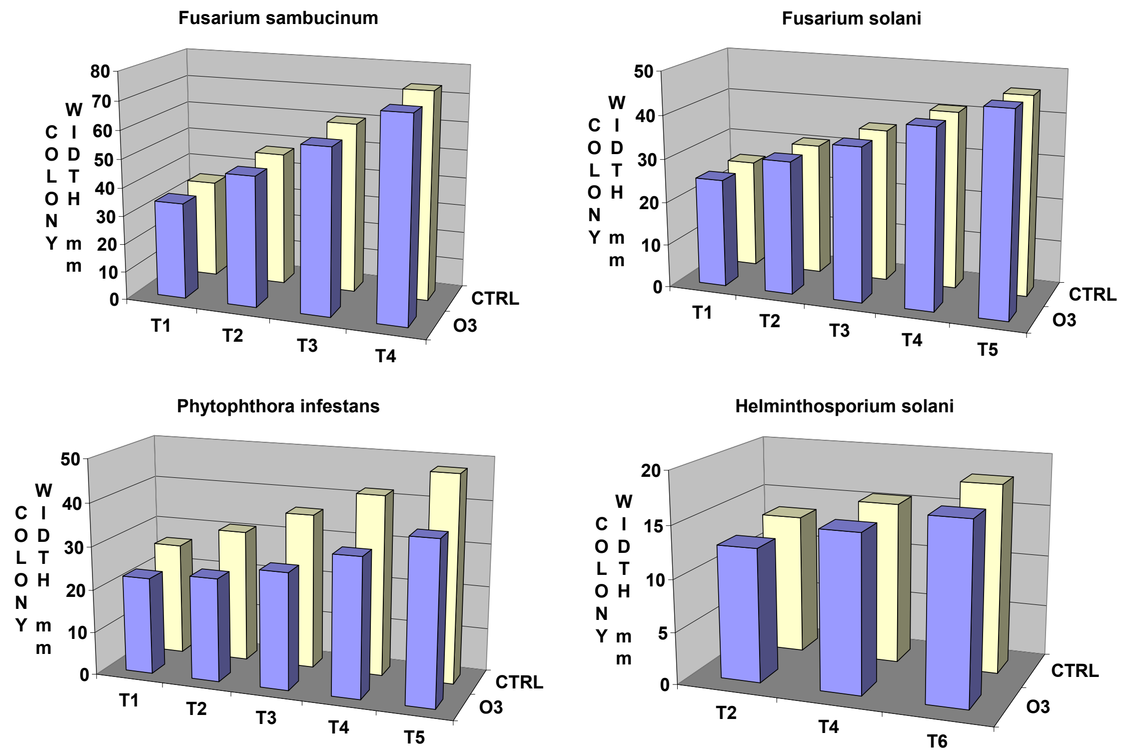

Figure 9.2: Changes in fungal colony width (mm) over time in *F. sambucinum, F. solani, P. infestans* and *H. solani* cultures.

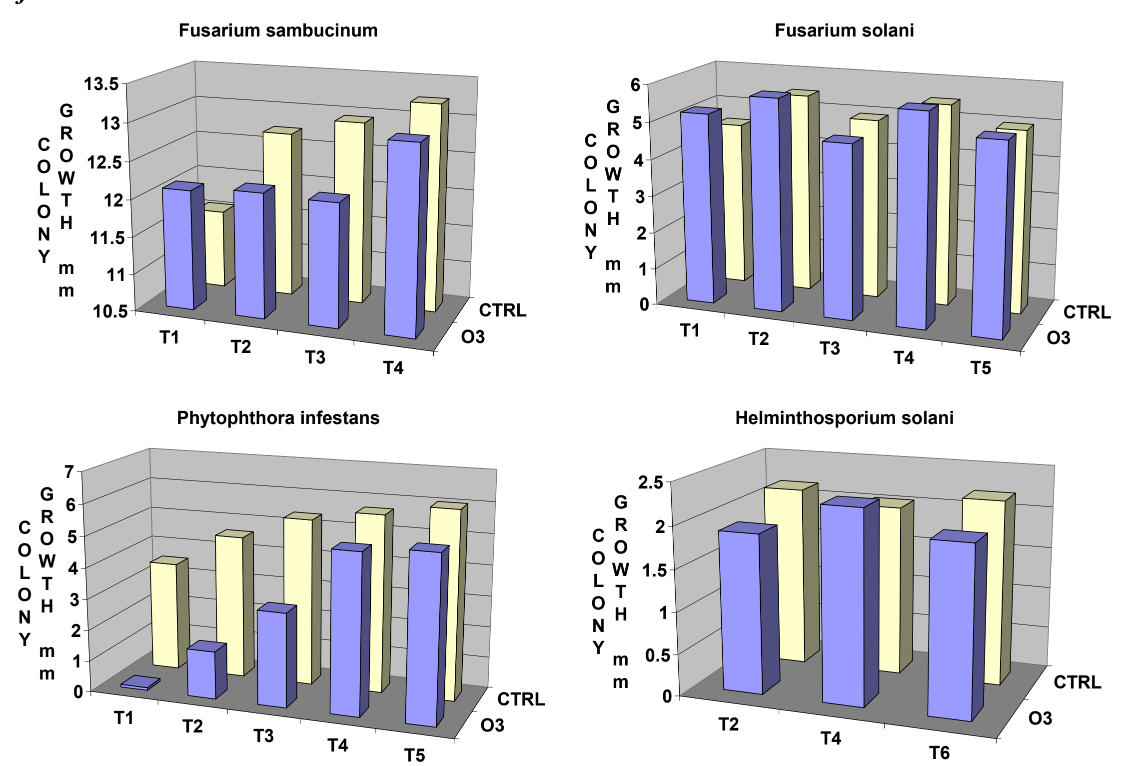

Figure 9.3: Changes in fungal colony growth rate (mm/day) of *F. sambucinum, F. solani* and *P. infestans* cultures grown in various ozone treatments.

F. sambucinum, *F. solani* and Helminthosporium solani cultures treated with ozone did not differ significantly from the controls in colony diameter or the colony growth rate. The ozone treatments decreased *Phytophthora infestans* colony growth while the cultures were in the ozone atmosphere. Colony growth was essentially stopped for the two days of ozone treatment, however when removed from the ozone treatment area the *Phytophthora* colonies recovered and resumed a standard growth rate equivalent to the controls for the remainder of the study period. Figure 9.2 shows how the ozone-treated *Phytophthora* colonies have a consistent lag in diameter that is not overcome. Figure 9.3 shows the altered growth rate early in the treatment, with a resumption of growth rate shortly after treatment. Figure 9.4 shows the ozone treatments broken up into the two exposures, demonstrating the effect of the two day versus the one day treatments.

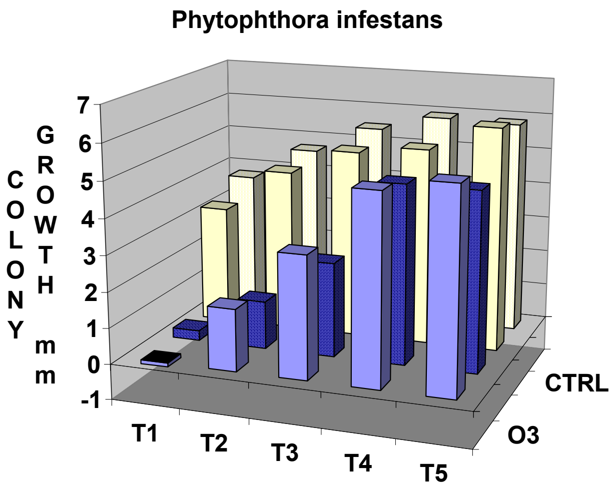

Figure 9.4: Effect of ozone treatments (concentration vs. duration of exposure) on the growth rate (mm/day) of *P. infestans* cultures.

Longer exposure to ozone caused a slight decrease in *Phytophthora* colony growth rate following treatment, however this difference is not significantly different from the other duration of exposure treatment.

Sclerotinia Viability - Ozone treatments significantly reduced the germination of mature, culture-grown sclerotia compared to the controls. Germination percentage for the ozone treatments lagged behind the controls, although the inhibitory effects of the ozone were overcome as time after treatment increased. It is likely that all sclerotia would have germinated given sufficient time. This recovery effect was also apparent in the *Phytophthora infestans* cultures.

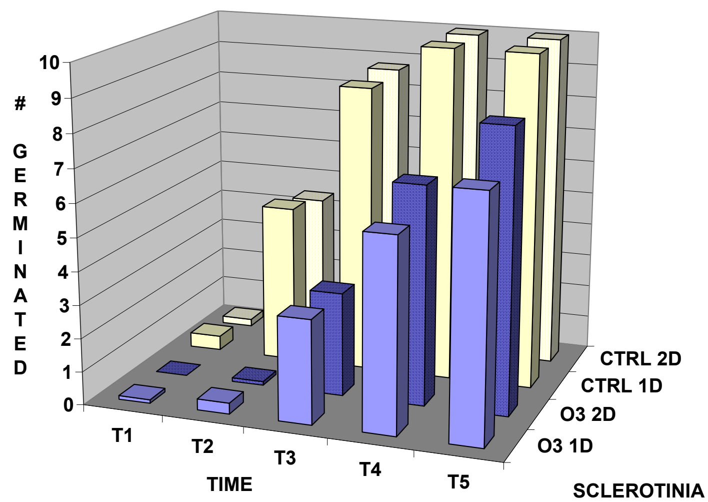

Figure 9.5: Effect of various ozone treatments on *Sclerotinia* sclerotia germination over time.

Conclusions

Exposure to ozone reduced colony function and growth in *Phytophthora* cultures, however, this effect was not permanent. Similarly, ozone treatment temporarily slowed sclerotial germination of *Sclerotinia*. Ozone treatment did not significantly effect the growth, development or sporulation of the other fungal species tested.

2002 Trials

2002-1: The effect of ozone on mycelial growth.

Materials and methods:

Pure fungal cultures of *Fusarium sambucinum* and *F. solani* (causal organisms of Dry rot) were established on Potato Dextrose Agar (PDA) plates. Five mm plugs were transferred from the actively growing margins of these cultures to the centre of new plates. These plates were maintained at room temperature for two days, to establish actively growing colonies that were then treated with ozone. The closed plates were placed on wire racks in a dark chamber at 20^0C and ozone was blown from underneath the plates at a rate of 40 mg/plate/hr (20ppm/plate/hour) for zero, two, four, six eight and ten days. The lids were left on the dishes as preliminary experiments had shown that when lids were removed the agar surfaces became overgrown with contaminants, making it impossible to determine the growth rate of the cultures being tested. It is noteworthy that the ozone treatments did control this contamination. A high rate of ozone was used to compensate for the closed plates. Gas exchange into the plates is permitted by the loose-fitting lids, but it was not possible to monitor exactly how much ozone penetrated under the lids and contacted the fungal mycelium. Previous experiments by James et al (1982) have shown that 0.1ppm of ozone applied to open plates reduced the growth rate of *Fomes annosus* when applied at this rate continuously for nine hours daily for three days.

Following the ozone treatment, the plates were incubated at room temperature, in the dark, until evaluations were completed. Colony diameters were measured at the start of the treatment periods and then two, four and six days after the ozone treatment. Typically the cultures grew to the edge of the plate after four to six days. Each treatment consisted of three plates. All treatments were replicated three times on separate occasions.

Results:

When the growth measurements taken at zero, two, four and six days after ozone treatment were combined and averaged, the untreated cultures of *F. sambucinum* grew slightly more than the ozone-treated cultures (Table 9-1).

The growth suppressing effect of the ozone treatments was most evident after four days (Table 9-2), as by day six the majority of colonies had grown to the edge of the plates (88 mm). The continued exposure to ozone for six and ten days had no effect because maximum growth was achieved after six days.

Table 9-1. Effect of length of time of exposure to ozone on colony diameter of *F. sambucinum* averaged over a 10 day treatment period

Exposure to Ozone (days)	**Average diam. of colony (mm)**
0	67.7 a*
2	64.0 ab
4	63.3 b
6	64.2 ab
8	65.8 ab
10	64.6 ab

*means followed by the same letter are not significantly different. P=0.05

Table 9-2. The effect of length of time of exposure to ozone on colony diameter of *F. sambucinum* measured at four days after treatment.

Exposure to Ozone (days)	**Average diam. of colony (mm)**
0	85.6 a*
2	77.9 b
4	75.8 b
6	78.3 b
8	79.7 b
10	80.2 b

*means followed by the same letter are not significantly different. P=0.05

The cultures of *F.solani* were measured after zero, two and four days. The cultures grew more slowly than those of *F. sambucinum*. Ozone treatments actually increased the width of the colonies, when measured after four days exposure (Table 9-3).

Table 9-3. The effect of length of time of exposure to ozone on diameter of *F. solani* measured at four days after treatment.

Exposure to Ozone (days)	Average diam. of colony (mm)
0	34.0 a*
2	37.2 ab
4	46.5 b
6	42.0 ab
8	44.1 ab
10	49.0 b

*means followed by the same letter are not significantly different. P=0.05

This effect is opposite to that seen on *F. sambucinum*.

The four, six, eight and ten day treatments had all received four days exposure at this time, and there was no significant difference in the amount of growth on these plates. However the untreated cultures did grow significantly less than some of the plates treated with ozone.

Conclusion:
Ozone treatment did not significantly suppress mycelial growth of the two fungal species under the relatively uniform, controlled conditions of this plate study. Although the actual concentration of ozone achieved in the plates could not be measured, a greater degree of suppression was expected. The fact that ozone was not effective at controlling fungal contamination of the open plates would suggest a limited potential for this concentration of ozone to control vegetative growth of fungal pathogens.

2002-2: The effect of ozone on spore germination.

Materials and methods:
Pure fungal cultures of *F. sambucinum*, *F. solani* and *Helminthosporium solani* were grown on PDA for ten days to two months (depending on the growth rate of the colony), until the cultures produced spores. Spores were harvested from the plates by adding 10 ml of distilled water to each plate and then gently scraping the surface of the colony with a plastic scraper. The plates were rinsed once with 10 ml of water, and the suspensions made up to 100 ml. The suspensions were filtered through three layers of cheese-cloth to remove any clumps of mycelium or agar, and one-three drops of Tween 20 were added to the suspensions. The concentrations of spores obtained were:
F. sambucinum - 8 X 10^4 spores/ml

F. solani - 15 X 10^4 spores/ml
H. solani – 2 X 10^4 spores/ml.

One ml of each suspension was placed on a water agar plate and dispersed with a sterile glass spreader. The closed plates were placed in the treatment chamber and exposed to ozone as described in the previous experiment. The plates were exposed for one, two, four and six hours, at 0, 50 and 100 mg O3/plate/hour and spore germination was then evaluated. A drop of dilute lactofuschin stain was placed on the plate immediately after treatment, to prevent further germination occurring, and a coverslip was placed over the stained area. One hundred spores were examined using a compound microscope and the percentage of spores that had germinated was recorded. It was noted that almost 100% germination had occurred in all treatments after approximately two hours.

Results:

The ozone treatment did not affect the percentage spore germination of any of the species tested. By contrast, Kraus and Weidensaul (1978) have shown that germination of conidia of *Botrytis cinerea* was significantly reduced when spores were exposed to ozone at 0.3ppm for two 6-hour periods. However, in the work of Kraus and Weidensaul (1978), ozone was applied to open dishes, and ozone concentrations were monitored constantly to ensure the concentration remained at the established level. The actual levels of ozone to which the *Fusarium* and *Helminthosporium* cultures were exposed could not be determined in this trial, as the petri dish lids were left on the dishes to prevent contamination.

Conclusion:
Treating enclosed plates with ozone provided little in the way of control of either vegetative growth or spore germination of a range of fungi pathogenic to stored potatoes. One potential explanation for the lack of expected control is that the ozone concentrations being utilized were simply inadequate. Although ozone gas would have diffused into the enclosed plates, the actual concentration of ozone inside the plates may have been relatively low. Kim et al., in their review on the use of ozone (1999) point out that the "effectiveness of ozone varies appreciably with minor changes in experimental variables", and they conclude that it is not feasible to compare results from different sources. This also suggests that ozone may not be a robust control option as its efficacy is affected by temperature and humidity variables that cannot always be controlled under on-farm conditions.

Section 10. Ozone in Stored Potatoes – Trials with a Commercial-Scale Ozone Generator

Background:

Previous research into the effectiveness of ozone as a control agent for potato storage diseases, conducted at the University of Saskatchewan, had proven somewhat inconclusive. Applying ozone at a range of concentrations and for varying durations of time to tubers, and to cultures of fungi, did not produce consistent or persistent disease control. There have been numerous studies that have demonstrated the efficacy of ozone as a sterilizing agent in the food industry. However duplication of effective treatments from these studies is complicated by the different ways that ozone is applied and measured. Ozone has a very short half-life (15 minutes) and is also very reactive, so that the measurement of exactly how much ozone is present during a treatment is complicated. The relative merit of measuring the amount of ozone generated by a system, the concentrations of ozone in the treatment chamber, and the amount of residual ozone leaving the chamber is unknown. The length of exposure to ozone is also an important variable. Liew and Prange (1994) considered that it was not appropriate to use residual ozone concentration as a measure of treatment, because it was affected by both temperature and the reactivity of the exposed material. This exposed material would include not only the food commodity being treated but also the storage structure surrounding the product. They concluded that ozone efficacy should be assessed on individual commodities under ideal storage conditions, and that both the quantity of produce and the dispensing and measuring system for the ozone treatment should be considered. In other words, ozone efficacy needs to be determined on a case-by-case basis.

The opportunity to assess the effect of applying higher levels of ozone, in an on-site situation, became available as a Saskatchewan potato producer purchased an industrial scale ozone generation system (O3Co. Aberdeen, Idaho). This unit applies ozone in a tunnel system constructed on the conveyor that takes the freshly harvested potatoes from trucks into the storage. Levels of ozone approaching 500 ppm (1000mg/m^3) occur in the tunnel. These levels are much higher than those employed in research studies previously reported (up to 3.25ppm, Schomer and McCollogh, 1948) or utilized in the studies dealt with in previous sections of this report. However the duration of exposure to these very high concentrations of ozone is also very brief (16 seconds).

In an effort to evaluate this ozone treatment system, tubers were treated in the tunnel, under different conditions, and were sampled and evaluated for disease levels and physiological changes. Ozone treated samples were compared with untreated samples at varying intervals after treatment. In some cases, low levels of ozone (up to 2.5 ppm) were also applied to the potatoes on a continuous basis throughout the winter storage period. The effectiveness of this treatment was also monitored.

Objectives: to test effectiveness of ozone to control diseases of potatoes, using two types of exposure:

A) **high concentration of ozone (up to 500ppm) for short periods of time (16 secs) as potatoes moved along conveyor before going into storage (belt treatment)**
B) **low concentrations of ozone (2.5ppm) for extended periods (up to 6 months) during storage (storage treatment).**

Experimental details:

A number of variables including disease levels, skin colour, physiological age and sprouting vigour of tuber may be influenced by ozone treatments.

Assessment of disease levels were carried out as follows:

Fusarium dry rot (FDR) – the percentage of infected tubers, and the internal surface area affected by rot were evaluated.
Rhizoctonia black scurf – the viability of sclerotia, taken from the surface of the tubers, was determined by plating a sample of sclerotia on Potato Dextrose agar.
Silver scurf – tubers were incubated in humid conditions for three weeks and the percentage of infected tubers, and the severity of disease was assessed.
Bacterial soft rot (BSR)– the percentage of infected tubers, after incubation at room temperature and high humidity, was recorded.

The effects of the ozone treatments, on skin colour and sprouting, were also evaluated.

The majority of tests were carried out on two cultivars, one red (Sangre or Norland) and one white (Yukon Gold).

Trial 10-1 Evaluating the efficacy of ozone treatment (high concentration, low duration during loading into storage) for control of diseases of potatoes in storage

These experiments were conducted using one rate (maximum concentration of ozone available) and one exposure time (length of time taken for tubers to move along the loading belt through the treatment zone = 16 seconds). Ozone treated and untreated tubers were compared. After treatment, tubers were cured by exposing them to a high airflow for several weeks, at 15^0C. Once curing was complete the tubers were stored in a cold room (4^0C) prior to disease assessments. These conditions simulate standard grower practices.

10.1.1 Control of Fusarium dry rot (FDR)

Materials and methods:

FDR development is more likely to occur in potatoes wounded during harvest. In this experiment, half the tubers used were wounded by puncturing them on two sides with a set of three nails protruding one cm from a flat piece of wood. The wounded tubers were then inoculated by dipping in a spore suspension of *Fusarium sambucinum*. The *Fusarium* isolate used to make the spore suspension was obtained from a sample of diseased tubers collected from a Saskatchewan potato farm in 1997. This isolate is resistant to the commonly employed fungicide Mertect. The spore suspension contained 9 x 10^4 macrospores/ml. *Fusarium* inoculated tubers were dried overnight before ozone treatment. Field run tubers that were exposed to normal handling and to inoculum present in the field, were also evaluated. They were removed from the conveyor system just prior to the ozone tunnel (treatment four), or just after the tunnel (treatment three) before being piled in the storage. One hundred and twenty tubers were used in each treatment. Wounded, inoculated tubers exposed to ozone (treatment one) were run through the tunnel in three lots, of 40 tubers each. The ozone levels recorded on the analyzer sampling air from the tunnel were noted for each run. Ozone levels ranged from 360-506 ppm over the various treatment runs – this variance likely reflects differences in the amount of potatoes and soil in the treatment chamber. After treatment, the tubers from each run were stored separately in paper bags.

In summary, the four treatments were:

1. wounded, inoculated and treated with ozone
2. wounded, inoculated and not treated with ozone
3. field run - treated with ozone
4. field run - not treated with ozone.

The first assessment of dry rot development was made five weeks after harvest. Tubers were selected randomly from the four treatments, and were sliced open in three equally spaced regions. The three exposed faces were examined for dry rot infection, and the maximum lesion depth and width were measured for each infection site on the face. These numbers were multiplied to give the surface area infected, and the total surface area affected per slice was recorded. The totals for the three slices were then added to give an infection value for each tuber. These infection values were then compared. Thirty tubers were selected randomly from the two treatments that were not exposed to ozone. The exposed tubers had been subdivided into bags of 40 tubers, for each separate run through the tunnel. Ten tubers were sampled from each bag, to give a total of thirty tubers per ozone treatment. The two cultivars used were Norland and Yukon Gold. Neither cultivar had been exposed to frost. The Norland tubers were severely skinned as the crop was skins still immature at harvest-time. The Yukon Gold tubers were hand dug and were not wounded.

Results and discussion:
Only the inoculated tubers developed dry rot. (Table 10.1.1.)

Table 10.1.1. Influence of ozone treatments on the extent of dry rot infection developed in tubers treated at a commercial storage, September 2002.

Treatment	**Average infection value for 30 tubers (cm^2) of each cultivar**	
	Norland	**Yukon Gold**
Inoculated + O3	14.1	1.6
Inoculated – O3	13.6	1.3
Field run + O3	0	0
Field run – O3	0	0

The inoculated Norland tubers developed significantly more dry rot than Yukon Gold tubers. This likely reflects the poor skin set in the Norland tubers, which resulted in extensive skinning. Wounds allow entry of the *Fusarium* spores. There were no dry rot infections in uninoculated tubers, and no difference in dry rot levels between ozone-treated and untreated tubers.

Conclusions:

Ozone treatment using high concentrations for brief period during load-in did not reduce the amount of dry rot developing in inoculated tubers. This finding is in agreement with the previous trials conducted in this study and with the study conducted by Schomer and McColloch (1948). They concluded that ozone did not control decay of apples, nor did it reduce infection of inoculated wounds. Ozone does not appear to be effective in preventing fungal growth when the pathogen is protected by plant tissue. Once *Fusarium* spores have penetrated into wounds, short-term exposure to high concentrations of ozone is not effective as a means of controlling FDR.

Trial 10.1.2 Effectiveness of short-term high concentration ozone treatment versus fungicide treatment, for control of FDR.

A comparison of the effectiveness of ozone for the control of FDR versus two post-harvest fungicides available to seed growers was conducted using wounded, inoculated tubers. Mertect fungicide, applied as a mist on tubers going into storage, has commonly been used by both seed and table potato producers for control of FDR. However, isolates of *F. sambucinum* resistant to this fungicide have become widespread in Saskatchewan. Dithane is also registered for application on seed tubers.

Materials and methods:
Tubers of two cultivars (Norland and Yukon Gold) were wounded and inoculated with *F. sambucinum* as in experiment one. The tubers were divided into four lots of 120 tubers each, and four treatments were applied.

The tuber treatments were:

1. wounded, inoculated and treated with O3
2. wounded, inoculated and not treated with O3
3. wounded, inoculated and treated with Mertect
4. wounded, inoculated and treated with Dithane.

Ozone levels in the tunnel, for treatment one, ranged from 378 – 530 ppm over the 16 second treatment period. The fungicides were applied with a hand sprayer using the recommended rate of product but in larger than normal volumes of water, as this would insure thorough coverage The tubers in treatments 3 and 4 were sprayed on both sides with the fungicide and then allowed to dry before bagging. Two cultivars, Norland and Yukon Gold, were tested.

Dry rot development was evaluated as in Experiment 1, five weeks after treatment.

Results and discussion:

In this experiment all tubers were wounded and inoculated. The majority of tubers developed dry rot, at similar levels to those observed in the first experiment for both Norland and Yukon Gold (Table 3.1.2). The highest level of FDR infection in the Norlands was observed in the tubers that received neither the ozone nor the fungicide treatments. Ozone and Mertect treatments both reduced disease levels slightly, with Dithane providing the most disease control.

Table 10.1.2. The extent of dry rot infection developed in tubers treated with fungicides and ozone in a commercial storage.

Treatment	Average FDR lesion area (cm^2)	
	Norland	**Yukon Gold**
Inoculated + O3	13.7	1.4
Inoculated – O3	16.4	1.9
Inoculated + Mertect	11.5	3.5
Inoculated + Dithane	6.0	0.3

N=30.

As seen in Experiment 10.1.1, dry rot levels were lower in the Yukon Gold tubers than in the Norlands. Mertect treated Yukon Gold tubers developed the most FDR, while the least FDR was again observed in the Dithane treated tubers. This suggests that the Fusarium isolate used was still resistant to Mertect, that ozone treatment may have slightly reduced damage FDR, but that it was not as effective as Dithane.

Conclusions:

Ozone acts primarily on surface infections and does not appear to control infections within the tubers. Fusarium spores germinate and enter into the tuber through small wounds. Once inside the tuber the pathogen is not apparently affected by ozone. Infection by the FDR pathogen is completed within 12 hours, and tubers in this experiment were treated with ozone approximately 12 hours after inoculation. The fungicide Dithane was able to reduce the amount of dry rot, even though the fungicide treatment was applied more than 12 hours after inoculation. This is relate to the mode of action of Dithane – it is a long last, systemic product.

Trial 10.1.3 Evaluating short-term high concentration treatments with ozone for control of *Rhizoctonia*.

In previous trials, treatment of potatoes with ozone had little effect on the incidence or severity of Rhizoctonia (black scurf). This was not surprising as the sclerotia of this fungus are very slow growing and highly resistant to many forms of stress. There were however, some indications that the ozone treatments reduced the viability of the sclerotia. This trial evaluated the impact of short-term exposure to high concentrations of ozone on Rhizoctonia.

Tubers with significant levels of black scurf (Rhizoctonia) were selected for use in this experiment. The survival of sclerotia on the surface was assessed as this infection structure represents the initial inoculum source in the spring. As the amount of dirt present on the tubers may affect the ability of ozone to kill the sclerotia, a comparison of the impact of ozone on washed and unwashed tubers was made.

Materials and methods:

Tubers of Norland and Yukon Gold, which were significantly infected by *Rhizoctonia* were selected for treatment. The following treatments were carried out on Sept 25th 2002:

1. tubers washed, dried, treated with O3
2. tubers unwashed, treated with O3
3. tubers unwashed, not treated with O3.

The ozone treatments were as previously described in Experiment 10.1.1. The same treatments were carried out on Sangre tubers on October 3rd 2002. Two to five days after treatment with ozone, the viability of the *Rhizoctonia* sclerotia was assessed by plating small pieces of sclerotial tissue onto PDA agar plates. The ozone treated potatoes were separated into three runs, and five tubers were selected from each run, to give a total of 15 tubers evaluated. The tubers from treatments two and three were not separated into runs, 15 tubers were selected at random from each of these treatments. Two plates, each containing four sclerotial pieces, were prepared from each tuber. The plates were incubated at room temperature (22^0C) for three to four days. Viability of the sclerotia was then determined by examining the agar in the vicinity of the sclerotia for the presence of hyphal strands of Rhizoctonia. The presence of other fungi, and of bacteria was also noted.

Results and discussion:

The average percentage of viable sclerotia, obtained from the 15 tubers was recorded (Table 10.1.3). There was no significant reduction in sclerotial viability by the ozone treatment. This corresponds with previous findings reported elsewhere in this study.

Table 10.1.3. Influence of ozone treatments on percentage viable sclerotia September/October 2002.

Treatment	**Average percentage viable sclerotia on 15 tubers**		
	Norland	**Yukon Gold**	**Sangre**
Washed + O3	79.0	62.2	66.6
Unwashed + O3	67.5	75.8	67.5
Unwashed – O3	57.0	74.1	62.5

The resistance of fungal spores to ozone damage increases with the size, thickness and presence of pigment in the spore walls. Sclerotia of *Rhizoctonia* are large, hard, black structures capable of surviving inclement conditions, thus ozone may not affect their viability. The overall vigour of the pathogen was not evaluated in the plate test. This factor will be evaluated in a greenhouse study. Tubers will be germinated in pots under cool, damp conditions conducive to disease, and the amount of *Rhizoctonia* damage on roots and stolons will be evaluated.

The possibility was evaluated that ozone would be more effective in limiting *Rhizoctonia* survival when there was no covering layer of dirt to shield the fungus from the effects of the ozone. However in two of the three cultivars examined, the viability of sclerotia from washed tubers was higher than from unwashed.

Conclusions:

Short term exposure to high concentrations of ozone is not effective in killing *Rhizoctonia* sclerotia on the surface of tubers. This would suggest that ozone treatments have limited potential to eliminate the seed-borne Rhizoctonia that attacks emerging shoots of the potato crop. The possibility that the ozone treatments might reduce the ability of the pathogen to cause disease on the roots and stolons of developing plants is being evaluated.

Experiment 10.1.4. Control of Soft Rot by short-term exposure to high concentrations of ozone.

Bacterial soft rot (BSR) generally occurs after tubers have been wounded during harvest or damaged by frost. When tubers are invaded by *Erwinia* and *Clostridium* bacteria, a soft, decaying mass of tissue with a distinctive, unpleasant odour results. In storage, these rotting tubers decompose into a slimy ooze that can then infect other adjacent tubers in the pile. When soft rot occurs in storage the only treatment possible is to apply sufficient dry air to cause the rotting tubers to mummify. This may prevent the spread of breakdown in the pile, but this treatment accelerates weight loss from any healthy tubers in storage.

Ozone is a surface sterilant and may have the capability to kill bacteria on the surface of tubers. Ozone has been reported to inactivate gram-negative and gram-positive bacteria in both vegetative and spore forms (Kim et al., 1999). These authors also state that "it is not feasible to compare the sensitivity of bacteria to ozone using results from different sources; effectiveness of ozone varies appreciably with minor details in experimental variables". Temperature, humidity and length of exposure time all influence the efficacy of the ozone treatment.

Several experiments were conducted, using both unfrozen tubers that were wounded prior to treatment and tubers harvested after a significant frost had occurred, to evaluate the effectiveness of ozone in reducing BSR damage under typical storage conditions at a commercial site.

Materials and methods:
In experiments 10.1.4 A-D, tubers were harvested from hills exposed to temperatures low enough to cause frost damage. The tubers were then put through the ozone tunnel on the conveyor system and exposed to the previously (Experiment 10.1.1) described high concentration of ozone for 16 seconds. A control set of tubers was not treated with ozone for each experiment.

In experiment 10.1.4 A, 70 Sangre tubers removed after unloading at the storage were inoculated with BSR by rubbing the test tubers against tubers that were decomposing because of soft rot. Half of the bacterial-infested tubers were then treated with ozone before storage.

In experiment 10.1.4 B, samples were taken from two large storage boxes of Norland potatoes. The tubers in one box had been treated in the ozone tunnel before being stored in the box, the tubers in the other box were untreated.

In experiment 10.1.4 C, 75 field-run Sangre tubers were collected both before and after treatment in the ozone tunnel.

In experiment 10.1.4 D, samples of Yukon Gold and AC Ptarmigan were treated with ozone and compared with untreated tubers.

In Experiment 10.1.4 E, tubers of Yukon Gold and Norland not exposed to frost were used. Half of the tubers were wounded by patting twice with a board that had 3 nails protruding (as in dry rot experiment). The remainder of the test material (non-wounded controls) was taken off the conveyor system.

The four treatments in experiment 10.1.4 E were:

1. wounded and treated with O3
2. wounded and not treated with O3
3. field run treated with O3
4. field run not treated with O3.

After treatment, the tuber samples were placed in mesh bags and were cured for several weeks by exposing them to high airflow at 15^{0}C. This temperature is conducive to development of BSR. Soft rot damage was assessed after the curing period. Thirty tubers were selected randomly from each treatment and were stored in plastic boxes at room temperature and high humidity for three weeks. These conditions are ideal for the development of BSR. The tubers were examined weekly and any rotting tubers were removed to reduce spread of rot from tuber to tuber. The percentage of rotted tubers, including ones removed, was recorded after three weeks.

Results and discussion:
In experiment 10.1.4 A**,** 94% of the ozone-treated frost-damaged tubers developed BSR while only 60% of the untreated frost-damaged tubers rotted.

In experiment 10.1.4 B, 60 % of the ozone-treated frost-damaged tubers developed BSR while only 37% of the untreated frost-damaged tubers rotted.

In experiment 10.1.4 C, 13% of the ozone-treated frost-damaged tubers developed BSR while only 7% of the untreated frost-damaged tubers rotted.

In experiment 10.1.4 D, 17% of the ozone-treated frost-damaged AC Ptarmigan tubers developed BSR compared to 13% of the untreated frost-damaged tubers. Six percent of the ozone-treated Yukon Gold tubers developed BSR but none of the untreated tubers rotted.

In experiment 10.1.4 E, there was no soft rot development in any of the Yukon Gold tubers, or in any of the Norland tubers treated with ozone (Table 10.1.4). By comparison, both the wounded and field run samples of Norland developed significant amounts of BSR if they were not treated with ozone.

Table 10.1.4. Influence of wounding and ozone treatments on percentage of tubers developing BSR (Experiment 10.1.4 E).

Treatment	**Average % BSR**	
	Norland	**Yukon Gold**
Wounded + O3	0	0
Wounded – O3	36	0
Field run + O3	0	0

Field run – O3	25	0

N=30

In the first four experiments all the frost-damaged tubers treated with ozone developed more BSR than the untreated samples. The tubers exposed to the bacterial slime in experiment 10.1.4 A developed the highest levels of soft rot, indicating that the disease was spread by the slime. In many of the samples over 10% of the tubers developed soft rot, a level that would make storage of the tubers challenging.

In the last experiment treating tubers free of frost damage with ozone significantly reduced losses to BSR.

Conclusions:
In the experiments using tubers damaged by frost, ozone treatment was not effective in controlling soft rot, and actually increased the amount of damage. The ozone levels may have been high enough to cause tissue damage, which would accelerate breakdown due to soft rot. However when tubers undamaged by frost were wounded and then treated with ozone immediately, problems with soft rot were controlled by the ozone treatment. This suggests that in the frozen tubers the infection by the bacteria may have already been well established prior to the ozone treatment. Ozone treatments are relatively ineffective against deep-seated disease problems. By acting as a surface sterilant, ozone may be useful as a means of slowing the spread of soft rot in stored potatoes.

Experiment 10.1.5. Effect of length of exposure to ozone on development of soft rot.

In Experiment 10.1.4, the length of time the tubers were exposed to ozone was governed by the speed of the belt moving them through the ozone tunnel. At normal speed, tubers were exposed to high concentrations of ozone for 16 seconds. As few positive effects of this treatment were noted when the test material was frost damaged prior to treatment, increasing durations of exposure to ozone were evaluated. By stopping the belt for a period of time, the duration of exposure to high concentrations of ozone was altered in this trial. It was hypothesized that increasing the duration of exposure might increase the efficacy of the ozone treatments. This might be particularly important in material damaged by field frost as bacterial and fungal infections of this material can already be well established by the time the crop is harvested.

Materials and methods:
Sangre tubers, harvested a week after a significant frost in the field, were exposed to ozone for a range of exposure times by stopping the conveyor belt in the ozone tunnel. The exposure times were 16 seconds (standard), 30 seconds, 1 minute, and 4 minutes. The ozone levels in the tunnel during the times ranged from 108 – 350ppm, with increasing levels of ozone building up with increasing exposure time. After five weeks of post-treatment storage (two weeks at 15^{0}C followed by three weeks at 4^{0}C), samples of 30 tubers were taken from each treatment. The samples were incubated for a further three weeks at room temperature and high humidity, to stimulate development of BSR. The tubers were then evaluated for BSR as previously described.

A second sampling was done after a further six weeks storage at 4^{0}C.

Results and discussion:

Tubers were checked for soft rot each week during the incubation period and the final percentage of rotted tubers was recorded after three weeks incubation. After five weeks in storage, there was no difference in the amount of BSR in tubers exposed to ozone for 30 seconds, one minute and no ozone (Table 10.1.5). The samples exposed to ozone for either 16 seconds or four minutes developed significantly more BSR. After 11 weeks, the levels of soft rot in the zero ozone and 30 second treatments were low, but high levels of rot occurred in the 16 second, one and four minute treatments.

Table 10.1.5. Development of BSR in frost-damaged tubers exposed to high concentrations of ozone for varying lengths of time.

Treatment (length of time exposed to ozone)	% tubers with BSR after storage for:	
	5 weeks	11 weeks
0	13	3
16 seconds	53	27
30 seconds	10	7
1 minute	17	50
4 minutes	43	43

Conclusions:
Treatment with ozone did not control soft rot in frost-damaged tubers, and in some cases the ozone treatments appeared to increase the percentage of rotted tubers. Again these tubers were likely infected with soft rot bacteria as soon as the frost damage occurred and ozone is not effective in killing microbes already inside the tubers. Schomer and McColloch (1948) report that experiments conducted in 1942 by Elford and Van den Ende show that while 330ppm of ozone killed bacteria on the surface of agar there was no appreciable penetration of this sterilizing action below the surface of the agar plate. The duration of exposure to ozone did not increase its efficacy in this trial. The increase in levels of BSR observed following some ozone treatments was of concern. The possibility that the ozone treatments damaged tuber tissues, increasing their susceptibility to disease should be considered.

Experiment 10.1.6. The effect of varying durations of ozone application on development of BSR.

Large wooden boxes, capable of holding 900 lbs of potatoes are used by some growers to store smaller lots of tubers. The possibility of treating tubers in these boxes with a high level of ozone was evaluated.

Materials and methods:

Four boxes were filled with Norland and four with Yukon Gold tubers. The tubers were harvested a week after the crop was exposed to a damaging frost. The ozone was applied to three of boxes per cultivar. The ozone was applied through a hole in the cover of the box, and an exhaust fan was used to draw the gas through the tubers. The level of ozone at the top of the box, and exiting through the exhaust was measured. It took approximately one minute for the level of ozone at the top of the box to reach 200 ppm. Ozone was applied for one to ten minutes, and at the end of the treatment time the generator was turned off and the box cover removed. It took 30 seconds after the removal of the cover for the ozone reading at the surface of the tubers to drop to 0.2 ppm, and one minute for the exhaust reading to drop to 0.3 ppm.

The treatments applied to the boxes were:

Yukon Gold

1) no ozone applied
2) ozone applied at 410 ppm for 1 minute
3) ozone applied at 379 ppm for 2 minutes
4) ozone applied at 1005 ppm for 4 minutes
5) ozone applied at 7-9 ppm for 3 minutes and 566-613 for the next 7 minutes

Norland

1) no ozone
2) ozone applied at 423 ppm for 1 minute
3) ozone applied at 387-467 ppm for 2 minutes
4) ozone applied at 452-704 ppm for 4 minutes.

Samples were evaluated as previously described for the development of BSR three and six weeks after treatment.

Results and discussion:

After three weeks of storage, only the tubers treated with ozone for the longest periods developed soft rot (Table 10.1.6). After six weeks there was no soft rot in the untreated tubers, and the tubers treated for the longest periods had developed the most soft rot. It appears that ozone treatment for more than two minutes at these high concentrations may increase damage by soft rot bacteria. Schomer and McColloch (1948) report that when ozone was applied to apples in storage, at 10.25 ppm for 1-2 hours daily for seven months, injury occurred on every variety in the storage. Brown, sunken areas developed around the lenticels, and the skin of some varieties also became sticky. As most lenticels contain bacteria, any damage to the lenticels by ozone would enhance bacterial penetration into the tissues. The levels of ozone used in this trial were high, and it is not recommended that plant tissue be treated at this high level for more than a brief period.

Table 10.1.6. Level of soft rot in frost damaged tubers treated in boxes with high concentrations of ozone for differing periods of time.

% of tubers with soft rot (30 tuber sample)					
Yukon Gold			Sangre		
Exposure time (minutes)	3 weeks after treatment	6 weeks after treatment	Exposure time	3 weeks after treatment	6 weeks after treatment
0	0	0	0	0	0
1	0	3	1	0	3

2	0	0	2	0	3
10	23	20	4	7	17

Conclusions:
It appears that treating frost-damaged tubers for more than a brief period with high levels of ozone may increase problems with soft rot. The ozone may be damaging the surface tissues of the tubers facilitating entry of the bacteria causing BSR. As this was a non-replicated trial and it is also possible that the high levels of BSR observed in the treatments exposed to ozone for the greatest period of time only reflected random variability in the incidence of BSR inoculum. Nonetheless, the results suggest that growers proceed with caution when considering ozone treatment of a frost-damaged crop.

Experiment 10.1.7. Effect of increasing numbers of tubers during ozone treatment on the development of soft rot.

When the tubers are moving through the ozone tunnel during the standard 16 second treatment they normally form a continuous single layer of tubers on the belt. At this rate, 50 000 lbs of crop can be treated per hour. The number of potatoes on the belt at any one time may influence the ability of the ozone to interact with all tuber surfaces. In this experiment the density of tubers on the belt was adjusted, and the efficacy of ozone in preventing BSR was determined.

Materials and methods:
Approximately 80 Norland tubers, not exposed to frost but skinned during harvesting, were collecting from the conveyor system before ozone treatment, as a control. A similar sample was collected after ozone treatment at three different tuber densities. The treatments were:

1. no ozone applied
2. low loading rate = ozone applied with tubers loading in at 28 000 lbs/hour (200 ppm O3 in tunnel)
3. standard loading rate = ozone applied with tubers loading in at 50 000 lbs/hour (220 ppm O3 in tunnel)
4. high loading rate = ozone applied with tubers loading in at 65 000 lbs/hour (175-200 ppm O3 in tunnel).

At the 50 000 lbs/hr loading rate there was a continuous, single layer of tubers on the conveyor belt. At 28 000 lbs/hr the tubers were well spaced out and were not touching each other, making the tuber surfaces more available to the ozone. At 65 000 lbs/hr there were often two layers of tubers on the belt, making the lower tubers less accessible to ozone.
The tubers were stored for three months after treatment (as described previously), and 30 tuber samples for each treatment were then incubated for three weeks before determining soft rot levels, as described in Experiment 10.1.4. A sample of ten tubers was also examined for dry rot development. The tubers were cut open in three places and the presence or absence of dry rot was recorded. The dry rot typically started as a dark brown rot, with cavities in the rotten area filled with mycelium.

Results and discussion:
The levels of soft rot in tubers not treated with ozone were the same as those treated while loading in at the two higher rates (Table 10.1.7). The tubers treated with ozone while loading in at

the lowest rate developed the most soft rot. Tubers that were more spread out might receive ozone at higher concentrations all over the surface. This appeared to make the tubers more prone to attack by BSR.

Table 10.1.7. Effect of rate of loading of tubers in ozone tunnel on development of *Fusarium* dry rot.

Treatment	% tubers with FDR (sample of 10)	% tubers with BSR (sample of 30)
No ozone	40	20
Tubers at 28 000lbs/hour	90	53
Tubers at 50 000lbs/hour	80	17
Tubers at 65 000lbs/hour	80	17

The incidence of dry rot was also increased by all the ozone treatments (Table 10.1.7). The lowest level of dry rot (40%) was found in the untreated tubers. This was still an extremely high level of FDR. The Norland tubers used in this trial were badly skinned during harvest which would provide wounds for entry for the *Fusarium* organism. Increasing the density of tubers in the tunnel during the ozone treatment had no effect on the incidence of FDR.

Conclusions:
Ozone treatment did little to reduce soft rot development in tubers skinned at harvest but free from frost damage. At the lowest load-in rate there appeared to be an increase in soft rot development, again suggesting that ozone may be damaging the tubers. The high levels of dry rot observed in this trial can be attributed to extensively skinning at harvest. As had been noted in previous trials, ozone treatments had little potential to control FDR – in fact in this trial, the untreated controls had the least FDR. These results again suggest that the ozone treatment is actually damaging the tubers.

Experiment 10.1.8. Effect of short-term, high concentration ozone treatments on silver scurf.

Previous reports have shown that silver scurf levels in potato storages are reduced by application of Oxidate (H2O2) during storage (Al-Mughrabi, 2002). Oxidate and other chemicals such as Purogene (chlorine dioxide) are applied through the humidification system at intervals during the storage period. This repeated treatment suppresses sporulation by the fungus causing silver scurf (*Helminthosporium solani*). Reduction of the initial pathogen levels on tubers going into storage should further problems with this disease later in the season. The efficacy of a short-term, high concentration ozone treatment to reduce silver scurf levels was evaluated.

Materials and methods:
A sample of 20 tubers was taken from treatments three and four in Experiment 10.1.1. These two treatments involved field run Yukon Gold and Norland tubers either untreated or treated with ozone on the conveyor belt (see Experiment 10.1.1 for treatment details). The tubers were washed, and placed in plastic boxes at room temperature and high humidity to encourage

sporulation by the *H. solani*. After 3 weeks, the tuber were assessed for the percentage surface area affected by silver scurf.

Results and discussion:
The incidence and severity of silver scurf infection was low (Table 10.1.8), with less than 1% of the surface area infected per tuber. These low levels are not surprising as typically high levels of infection are not recorded until the end of the storage season. The higher incidence of disease in the ozone-treated Norland tubers compared to the untreated controls suggests that treatment of tubers at load-in does not significantly reduce silver scurf levels.

Table 10.1.8. Extent of silver scurf infection after a month of storage on tubers treated with ozone at load in.

Treatment	% of tubers infected with silver scurf	
	Norland	Yukon Gold
Field run + O3	30	20
Field run – O3	0	20

N=20

Conclusion:
Silver scurf is a slow growing fungal disease that gradually causes a silvery discolouration of the surface of stored tubers. Tubers are initially infected with the silver scurf pathogen in the field, therefore eliminating the inoculum on the tuber surface as potatoes are loaded into storage is a logical approach to control this disease problem. However, treatment of tubers with a high concentration of ozone for a short period as they entered storage did not to reduce the incidence of silver scurf in this trial. The levels of this disease at the end of the storage period will be evaluated after in-storage exposure to ozone, as this treatment is more likely to reduce sporulation and spread of the pathogen.

Trial 10.2. Evaluating the efficacy of continuous application of ozone during storage for control of diseases of potatoes

Treatment of tubers with ozone as they are being loaded into storage represents an extremely brief window for exposure. Unless this treatment completely eliminates inoculum the odds are great that the disease will become established at some later point in the storage season. An alternative or additional approach is to apply disease control agents to the crop during the main storage period. Potato storages are equipped with ventilation systems designed to circulate air through the piled product. Gaseous disease control agents such as ozone can be applied through this ventilation system – either sporatically or continuously throughout the storage period. Continued treatment with disease control agents such as ozone may be particularly useful in the control of diseases that spread within the storage. In particular silver scurf, which sporulates profusely in storage towards the end of the season, is reported to be controlled by ozone application during long-term storage.

Experiment 10.2.1. Evaluating the efficacy of long-term application of ozone for control of soft rot in storage.

Materials and methods:
Norland and Yukon Gold tubers that had not been exposed to frost were divided into two lots. Half were treated with ozone on the conveyor belt system and half were not. The treated tubers were placed in four 50lb mesh bags. Two of these bags were left in the commercial storage, where they were treated with 2.5 ppm of ozone introduced via the ventilation system throughout the storage season. The other two bags were stored in an ozone-free cold room at the University of Saskatchewan. Similarly the four bags of tubers that did not receive ozone treatment on the conveyor were also divided so that two bags remained in the ozone-treated storage while the other two bags were stored at the University of Saskatchewan without long-term ozone treatment. Disease development on the tubers was compared for identical lots of tubers stored at the commercial storage and at the University of Saskatchewan.

The four treatments were:
1. O3 on belt, treated with O3 in storage
2. O3 on belt, not treated with O3 in storage
3. no O3 on belt, treated with O3 in storage
4. no O3 on belt, not treated with O3 in storage.

Samples of 30 tubers were removed at random from the bags in storage at both locations and were evaluated for soft rot development, after ten weeks in storage.

Results and discussion:
For the cultivar Norland, the lowest levels of soft rot occurred in tubers not treated with ozone either during load-in or during long-term storage (Table 10.2.1). In Yukon Gold, treating the tubers with ozone during long-term storage increased BSR.
Additional samples will be taken to determine the progress of both soft rot and silver scurf during storage with or without added ozone.

Table 10.2.1. Influence of ozone treatments on development of soft rot in stored potato tubers.

Treatment	% tubers with soft rot after storage for 10 weeks	
	Norland	Yukon Gold
O3 on belt + O3 in storage	20	20
O3 on belt - no O3 in storage	30	0
no O3 on belt + O3 in storage	27	20
no O3 on belt - no O3 in storage	7	0

Conclusions:
Application of ozone either at high levels during load-in or at low concentrations during longterm storage appeared to increase the levels of soft rot in the stored tubers. This conforms to the results observed in previous trials.
Levels of silver scurf will also be evaluated at the end of the storage season.

Experiment 10.2.2. To evaluate the effect of longterm ozone treatment on the colour of tubers.

Red-skinned tubers tend to fade in color during storage. This is undesirable, as consumers prefer tubers that are a bright red colour. Tubers exposed to bleaching agents, such as Purogene (chlorine dioxide), may also show a loss in colour. The effect of ozone on colour of both red and white-skinned tubers was evaluated by taking measurements of skin colour of Norland and Yukon Gold tubers exposed to ozone.

Materials and methods:
Ten tubers from the treatments in Experiment 10.2.1. were sampled three months after harvest and their colour values were determined using a Hunterlab Colour Meter. For each of the 10 tubers per treatment a lightness value (100=white, 0 = black), a red/green value (+a=red, -a=green) and a yellow/blue value (+b=yellow, -b=green) was obtained.

Results and discussion:
The red/green values are probably the most critical measurement for Norland tubers as they indicate the redness of the tubers, which is a critical marketing feature. The ozone treatments had no significant effect on the colour values for the Norland tubers (Table 10.2.2).

Table 10.2.2. Average skin colour values for ozone treated Norland tubers.

Treatment	Average colour value		
	Lightness	Red/green	Yellow/blue
O3 on belt + O3 in storage	25.2	5.7	5.0
O3 on belt - no O3 in storage	25.4	5.5	4.9
No O3 on belt + O3 in storage	25.8	5.4	5.0
No O3 on belt - no O3 in storage	25.1	5.9	4.8

Similarly there were no obvious ozone effects on the colour values for the Yukon Gold tubers (Table 10.2.3).

Table 10.2.3. Average skin colour values for ozone treated Yukon Gold tubers.

Treatment	Average colour value		
	Lightness	Red/green	Yellow/blue
O3 on belt + O3 in storage	33.2	3.6	10.5
O3 on belt - no O3 in storage	30.8	3.4	9.4
No O3 on belt + O3 in storage	31.0	3.2	9.6
No O3 on belt - no O3 in storage	31.7	3.4	9.6

Conclusions:
Neither brief exposure to high concentrations of ozone, nor longterm exposure to lower ozone treatments appeared to affect the colour of the two cultivars tested.

Trial 10.3 Effect of ozone on sprouting of seed potatoes – a comparison of short-term high concentration treatments with long-term low dosage exposure

Before ozone can be adopted as a disease abatement measure in stored potatoes, growers will need to be confident that the treatment has no detrimental side effects on the quality of the stored crop. In previous sections, we reported on the impact of ozone treatments on the key quality of table potatoes (color and moisture loss). In section 1 we also reported that short-term treatment of seed potatoes with low levels of ozone just prior to planting had little impact on the field performance of the seed. However, as mentioned in Section 10.1 the levels of ozone being applied by the commercial grower during load-in are much higher (but for a shorter duration) than those explored in section 1. The grower is also continuously exposing the crop to low levels of ozone throughout the storage period. The combination of short-term exposure to high concentrations of ozone during load-in coupled with continuous exposure to low levels of ozone throughout the storage period would represent a very large cumulative exposure.

This trial evaluated the sprouting ability of seed potatoes treated with ozone going into storage and/or during storage.

Materials and methods:

Sound, disease-free, Elite 3 Norland and Yukon Gold tubers were divided into two lots after harvest. Half were treated with ozone on the conveyor belt system as described in Section 10.1. The other half of the potatoes were not treated with ozone at load-in. The treated tubers were placed in four 50lb mesh bags. Two of these bags were left in the commercial storage, where they were continuously treated throughout the storage season with 2.5 ppm of ozone introduced via the ventilation system. The other two bags were stored in an ozone-free cold room at the University of Saskatchewan. Similarly, the four bags of tubers that did not receive ozone treatment on the conveyor during load-in were also divided so that two bags remained in the ozone-treated commercial storage while the other two bags were stored at the University of Saskatchewan without long-term ozone treatment.

In summary, the four treatments were:

1. O3 on belt, treated with O3 in storage
2. O3 on belt, not treated with O3 in storage
3. No O3 on belt, treated with O3 in storage
4. No O3 on belt, not treated with O3 in storage.

Four months after harvest (mid-December), samples of 10 tubers were removed at random from the bags in storage at both locations for evaluation of their sprouting potential. The tubers were placed in plastic containers at room temperature under low light for approximately three weeks. The number of sprouts initiated was measured for each tuber. After one more week of growth the number of sprouts > 1 cm in length was recorded and the total weight of sprouts/tuber was determined.

Results and discussion:

For the cultivar Norland, the ozone treatments had no significant effect on the number of sprouts produced (Table 10.3.1.). An average of ten sprouts developed on each tuber. The weight of sprouts produced was lowest on the tubers that had not received any ozone treatment, but this value was not significantly different from the treatments that had received the most ozone (applied both during load in and during storage).

Table 10.3.1. Effect of ozone treatment on sprouting of Norland tubers, evaluated 5 months after harvest (February 2003).

Treatment	**Total # sprouts per tuber**	**# sprouts per tuber > 1cm long**	**Total sprout wt (g) per tuber**
O3 on belt + in storage	10.6 a*	6.8 a	2.5 ab
O3 on belt	10.9 a	7.0 a	3.3 c
O3 in storage	10.4 a	6.4 a	2.7 bc
No O3	10.7 a	5.8 a	2.0 a

*means followed by the same letter are not significantly different. P=0.05

For the cultivar Yukon Gold, the greatest total number of sprouts, number of sprouts > 1cm long and weight of sprouts was produced on tubers that received no ozone (Table 10.3.2.). Tubers treated with ozone both going into and during storage produced the same number and weights of sprouts as tubers not treated with ozone at either time.

Table 10.3.2. Effect of ozone treatment on sprouting of Yukon Gold tubers, evaluated 5 months after harvest (February 2003).

Treatment	**Total # of sprouts per tuber**	**# sprouts per tuber > 1 cm long**	**Total sprout wt (g) per tuber**
O3 on belt + in storage	5.6 ab*	3.3 ab	1.1 ab
O3 on belt	5.3 ab	2.1 bc	0.6 b
O3 in storage	3.6 a	1.7 c	0.6 b
No O3	6.3 b	4.1 a	1.6 a

* means followed by the same letter are not significantly different. P=0.05

Conclusions:
The sprouting ability of the tubers treated with ozone going into and/or during storage was not consistently affected by the ozone treatments. This would suggest that ozone treatments could be used as a disease abatement tool without undue concern as to impact of the ozone treatments on seed performance. Nonetheless, the impact of the ozone treatments on field performance of the seed should be monitored before this treatment is widely recommended.

Outline of Project Related Extension Activities

- Mr. Rob Spencer (MSc Candidate) travelled to Fargo, North Dakota on March 23-25, 2001 to participate in the 2001 Plant Sciences Graduate Student Symposium. This event is held annually as a co-operative collaboration between the University of Saskatchewan, the University of Manitoba and North Dakota State University. Mr. Spencer presented a 10-15 minute presentation on his research, with the focus being on the 1999/2000 Ozone treatment component of this study. The presentation was given to other graduate students and some local faculty members.

- Provided Crystalair with copy of April Progress report each year
  - Contact with company to refine techniques

- D. Waterer presented project results at the annual Meetings of the Saskatchewan Vegetable Growers Association and the Saskatchewan Seed Potato Growers Associations

- J. Thomson and M.L. McArthur worked closely with the commercial grower who owns the large-scale ozone generating equipment.

Project Personnel

Rob Spencer	Graduate Assistant (full time)	1999-2002
J. Thomson	Research Scientist (part time)	2002
M.L. McArthur	Research Technician (part time)	2002
T. Sander	Student Assistant (part time)	2001 and 2002

Budget

Total funds allocated to the Project ……… $ 103,000
Expenditures to December 31, 2002 …….. $ 103,000
Unexpended balance …………………….. $ 0
Capital Expenditures …………………….. $ 0

The office of Research Accounts of the University of Saskatchewan has developed a detailed breakdown of expenditures on this project. This document will be electronically forwarded to SDAF along with this report.

Literature Cited

Al-Mughrabi, K. 2002. Oxidate potato storage trial. New Brunswick Department of Agriculture, Fisheries and Aquaculture, Canada, 19 pages. (Report.)

Dickson, R.G., Kays, S.J., Law, S.E., and Eiteman, M.A. 1992. Abatement of ethylene by ozone treatment in controlled atmosphere storage of fruits and vegetables. American Society of Agricultural Engineers 1992 Winter Meeting Nashville, TN #92-6571:1-8.

James, R.L., F.W. Cobb Jr. & J.R. Parmeter Jr. 1982. Effects of ozone on sporulation, spore germination, and growth of *Fomes annosus*. Phytopathology 72: 1205-1208.

Kangasjarvi, J., Talvinen, J., Utriainen, M., and Karjalainen, R. 1994. Plant defence systems induced by ozone. Plant, Cell, and Environment. 17:783-794.

Kim, Jin-Gab, A.E. Yousef & S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. Journal of Food Protection 62 (9): 1071-1087.

Krause, C.R. & T.C. Weidensaul. 1978. Effects of ozone on the sporulation, germination and pathogenicity of *Botrytis cinerea*. Phytopathology 68: 195-198.

Mehta, A. and Kaul, H.N. 1994. Wound healing in potato tuber tissue: cultivar variation. Journal of the Indian Potato Association. 21(3-4):247-250.

Nielsen, N.K. 1973. A quick microtechnique for inspection of potato periderm or wound periderm formation. Potato Research. 16:180-182.

Platt, H.W. 1992. Cultivar response to Fusarium storage rot as affected by two methods of seed origin propagation; clonal selection and *in vitro* culture. American Potato Journal. 69:179-186.

Sarig, P., Zahavi, T., Zutkhi, Y., Yannai, S., Lisker, N., and Ben-Arie, R. 1996. Ozone for control of post-harvest decay of table grapes caused by *Rhizopus stolonifer.* Physiological and Molecular Plant Pathology. 48:403-415.

Schomer, H.A. & L.P. McColloch. 1948. Ozone in relation to storage of apples. Circular 765, U.S. Department of Agriculture.