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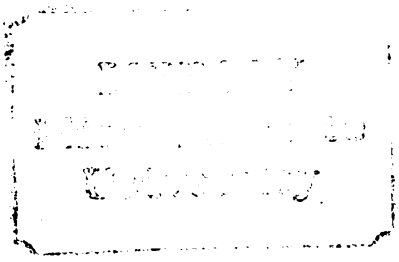

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THE EFFECT OF HYPOCHLORITE WASH TREATMENTS ON THE SUBERIZATION AND FIELD PERFORMANCE OF CUT POTATO SEED.

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Raymond Vander Zaag

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Richard K. Chase

Major professor

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THE EFFECT OF HYPOCHLORITE WASH TREATMENTS ON THE SUBERIZATION AND FIELD PERFORMANCE OF CUT POTATO SEED.

By

Raymond Vander Zaag

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ABSTRACT

THE EFFECT OF HYPOCHLORITE WASH TREATMENTS ON THE SUBERIZATION AND FIELD PERFORMANCE OF CUT POTATO SEED.

By

Raymond Vander Zaag

Hypochlorite wash solutions are used as cut potato seed treatments on some Michigan farms. The effect of chlorine wash solutions on the suberization and field performance of cut seed was investigated.

Histochemical staining showed increasing chlorine rates caused a thicker suberin layer to form. However, increasing chlorine rates caused a less effective water vapour and pathogen barrier to form, as measured by weight lost during drying and decay of tuber disks inoculated with Fusarium sambucinum, respectively. A chemical assay of the lignin-like component of the suberin layer did not show any consistent or large effect of increasing chlorine rates. Washes of 500-1000 ppm Cl did not adversely affect suberization.

In the field, 2000 ppm Cl washes occasionally reduced stands, but produced yields equal to the best treatment, a captan-streptomycin dust. A 500 ppm Cl wash was usually intermediate. Storing chlorine treated seed in various typical on-farm environments for 2-5 days before planting did not adversely affect seed performance.

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INTRODUCTION

Stand problems in potatoes (Solanum tubersum L.) due to seed-piece decay occur throughout North America. The occurance of seed-piece decay varies greatly, depending on seed and soil conditions. Many farmers regularly use chemical seed-piece treatments to guard against the possibility of seed-piece decay. Though it is not a substitute for the proper handling and storage of quality seed, it is considered inexpensive insurance that partially protects against invasion by seed and soil borne microorganisms (62).

Fungicide seed treatments are usually applied as dusts to the cut seed. The anticipated benefits of such treatment include the control of seed-piece decay in the soil (usually caused by Fusarium spp.) when adverse weather (usually cool and wet) follows planting and slows seed-piece suberization, and the control of seed-piece decay when cut seed must be stored for a length of time before planting (62).

In Michigan, approximately 40,000 acres of potatoes are grown annually, having an estimated value of 50 million dollars. In recent years, several potato operations have been using sodium hypochlorite (chlorine bleach) washes to treat cut seed. Hypochlorite is considerably cheaper than the fungicides conventionally used as seed treatments, and

is easier and cleaner to use. However, little information is available on the effectiveness of hypochlorite washes, or guidelines for its use. This study was undertaken to provide some of this information.

The objectives of this study were:

1. to determine the effect of different rates of hypochlorite on tuber wound healing.

2. to determine what rate of chlorine is most effective in preventing seed piece decay and producing good stands.

3. to evaluate the effectiveness of chlorine wash treatments compared to a conventional dust treatment.

4. to determine the effect of storing chlorine-treated seed for various periods of time under various environmental conditions.

CHAPTER 1. LITERATURE REVIEW

Stand problems. The reported severity of stand problems varies greatly from area to area and year to year in North America. In 1967, Cross and Ohms (14) reported that stand reduction in eastern Idaho was due primarily to seed-piece decay. In a four year survey of over 600 farmers they found 18% of the fields had stands of less than 70%, 28% had stands of 70-79%, and 40% had stands of 80-89%. The average yield for these classes was 160, 181, and 197 cwt/a respectively. Vruggink et al (70) reported that the occurance of even 3.5% plants infected with Erwinia reduced yields. This was due not only to plant death, but because some symptomless plants produce smaller tubers. In a study of yield compensation for misses in potato crops, Hirst et al (30) found yield decreased 0.33% (+-0.15%) for every 1.0% of plants removed at emergence.

Other studies have minimized the importance of seed-piece decay in causing stand reductions. James et al (33) found only 2.5% of all misses were caused by rotted or diseased seed-pieces in a 1973 survey of New Brunswick potato fields. There was an average of 32% misses in the fields, the majority due to missing or misplaced seed-pieces. In Washington, Thornton (69) reported that 90% of

misses were due to lack of proper seed-piece placement.

Even though there is considerable variability in the occurance of seed-piece decay between areas and years, the large number of studies investigating the benefits of chemical seed-piece treatments attests to the need to improve stands. Miska and Nelson (48) have compiled a bibliography of 640 papers published between 1930 and 1975 investigating various aspects of seed-piece decay.

Causal organisms of seed-piece decay. Seed-piece decay is typically caused by the dry rot fungus Fusarium solani 'Coeruleum' and/or Fusarium sambucinum, often in association with the soft rot bacteria Erwinia carotovora var. atroseptica (E. c. var. atroseptica) and/or var. carotovora (18, 29, 49, 52). Recently, Davis et al (18) have suggested a synergistic effect between E. c. var. atroseptica and F. sambucinum in causing seed-piece decay in Russet Burbank potatoes in Idaho. When these pathogens were inoculated together, the severity of tuber rot was significantly greater than when either was inoculated alone. Similarily, they also interacted to reduce yield. Blackleg symptoms, however, were observed in less than 1% of the plants. Inoculation with E. c. var. carotovora and F. sambucinum, together or individually, did not reduce yield. Several other studies have reported the control of blackleg with fungicides (17, 50, 67) or the induction of blackleg following inoculation with F. solani (67). Thus, synergistic effects between Fusarium spp. and Erwinia appear

to occur. Corsini and Pavek (11) found that temperature influenced the development of disease caused by two Fusarium species. Fusarium sambucinum caused more disease at 10° C, F. s. 'Coeruleum' caused more disease at 21° C, while both species caused a similar amount of disease 16° C. They also reported that the cultivar Russet Burbank was very susceptible to F. sambucinum.

The environmental conditions that favor development of seed-piece decay are cool (4-6° C), wet soils, which have low oxygen and elevated carbon dioxide concentrations, or very dry soils. Under these conditions suberization of the cut seed-piece is slowed, sprout emergence is delayed and infection by the pathogens is promoted (24, 29, 45, 51).

Seed-borne inoculum appears to initiate most disease development (29, 41, 52, 58) and almost all seed stocks carry some level of Erwinia spp. (57, 58, 59, 67) and Fusarium spp. (52, 53, 64). Thus the benefit of chemical seed treatment depends on the degree of seed contamination (69), and it is important for seed producers to keep these diseases at low levels (14, 70). However, both organisms can overwinter in the soil (19, 29, 52, 64), and soil insects feeding on seed tubers can cause seed infection (23).

Effects of chemical seed treatments. There are many studies which have investigated the benefits of various chemical seed-piece treatments. This review will only summarize some of their findings, since the purpose of the present study was not to evaluate the many chemicals

currently used as seed treatments, but to investigate the use of hypochlorite as a seed treatment. Due to the variable occurance of conditions favoring disease development, chemical seed treatments have had mixed results. Stands and yields are often improved, especially when cool, wet weather follows planting (9, 10, 15, 71), or if cut and treated seed is held several days before planting (8, 20). If seed is cut, treated and planted immediately into soil with conditions favoring rapid emergence, often no benefit is observed (15, 20, 44, 63, 71).

An overall benefit from seed treatment was found in a 1967 study of 936 eastern Idaho fields (14). Fields that received no seed treatment (61%) yielded an average of 183 cwt/a, while treated seed produced an average of 195 cwt/a. Several studies have found no benefit under any of the conditions tested (21, 32).

Seed treatments are generally applied as a dust. However, applying seed treatments as a liquid with a mechanical fog generator has been reported to be as effective as chemicals applied as dusts (40).

Wound healing. The formation of a suberin and wound periderm layer over the cut surface of the seed-piece is of prime importance. Suberin and periderm formation prevents excess water loss from the seed-piece and prevents pathogen entry. Both of these factors are necessary for producing vigorous stands. Numerous studies have shown that resistance to pathogen entry and subsequent decay increased

as suberin and wound periderm formation progressed after wounding (5, 35, 54, 65). O'Brien and Leach (55) reported that the mode of active resistance of potato tubers to F. sambucinum was the formation of a continuous suberin and wound periderm layer around the infection site. They found the cultivar Russet Burbank, which is susceptible to dry rot, tended to form a suberin layer more slowly and less uniformly than a numbered resistant line.

Numerous studies have also been conducted to determine the optimum conditions for wound healing. The optimum temperature is between 20 and 30° C; the rate of healing decreases as the temperature decreases below 20° C (5, 19, 54, 72). High relative humidity (between 70 and 100%) is also required for rapid suberization (3, 5, 54, 72). Oxygen levels lower than, and carbon dioxide levels higher than atmospheric levels also tend to inhibit suberin synthesis and wound periderm development (72).

There are numerous reported methods that have been used to measure the extent of wound healing. Often, thin sections perpendicular to the cut surface are made and the thickness of the stained suberin layer is measured with a microscope (5, 16, 26, 65, 72). The weight of water lost by tuber pieces under a specific drying regime can be measured (34, 36), as the resistance to water loss in potato tissues is directly proportional to the amount of suberin deposited (39). A later study reported evidence that waxes associated with the suberin polymer and not the suberin polymer itself,

formed the major water diffusion barrier (66). Wound healing can also be measured by inoculating healed tissue with Fusarium spores or Erwinia cells, and measuring the extent of decay (35).

Chemical techniques have also been used to identify and measure the compounds deposited during suberization. These studies have shown that suberin molecules are polymers composed of phenolic components, similar to lignin, and aliphatic (lipid) components. Waxes are also deposited in association with the suberin polymer (37, 38, 66). Suberin deposition can be measured by following the amount of certain lipids released after depolymerization with $LiAlH_4$ or BF_3, or by the phenolic aldehydes released by alkaline nitrobenzene oxidation (38). Kolattukudy has successfully applied traditional lignin chemistry techniques, such as alkaline nitrobenzene oxidation, to the study of suberin, and found lignin-like materials were deposited in parallel to suberin aliphatics and increases in diffusive resistance (12, 13, 39). Thus lignin chemistry techniques can be used to measure the lignin-like components of suberin.

One of the factors that suggested the possible usefulness of hypochlorite as a seed treatment was a study by Grigg and Chase (26). They found that increasing rates of hypochlorite to 5000 parts per million (ppm) in the wash water promoted the development of a thicker and more uniform protective layer on the cut surface of potatoes. Tubers washed in water produced a protective layer with an average

thickness of 76 um after six days, while those washed in 100, 500, 1000, and 5000 ppm chlorine had layers 81, 95, 127, and 196 um thick, respectively.

The use of hypochlorite on potatoes. Sodium hypochlorite is one of the most widely used compounds for surface sterilization of seeds and other plant tissues for use in biochemical studies and pathogen isolation (1, 2). Hypochlorite is also widely used as a disinfectant in the wash water in potato packing plants (22, 26). The germicidal efficiency and stability of hypochlorite varies with temperature, pH, concentration and catalyts, though researchers using hypochorite often do not consider these variables (2).

The strength of hypochlorite solutions is measured as parts per million available chlorine (ppm available Cl) or percent available chlorine (% available Cl.) Available chlorine is a measure of the oxidizing power of the chlorine solution, not the actual hypochlorite concentration (2).

In addition to the study of the use of hypochlorite in packing plant wash water already mentioned, several other studies involving hypochlorite and potatoes have been reported. In New Zealand, hypochlorite has been used to decontaminate potato tubers bearing nematode cysts (73). 10,000 ppm chlorine solutions destroyed cysts in 30-45 minutes. The rate of destruction was independent of pH above 7.0, while the presence of soil increased the time required for destruction. The amount of Rhizoctonia solani sclerotia adhering to the tubers was also reduced, though

some viable sclerotia remained.

The effect of such chlorine treatments on seed potato dormancy, emergence and yield was also studied (74). Whole, dormant seed of the variety Ilam Hardy was treated by immersion in 10,000 ppm Cl for two hours , rinsing in water and drying. Treated seed emerged faster, produced more stems and tubers and yielded an average 20% more that untreated dormant seed. The length of storage before or after treatment did not affect the treatment benefits. Emergence was reduced when sprouted seed was used because the chlorine burned the sprouts. This reduction was eliminated by treating sprouted seed 3-4 weeks before planting. When seed was treated for longer than two hours or with higher concentrations of NaOCl, emergence and yield were usually reduced. Chlorine treatment (two hours in 10,000 ppm chlorine two weeks before planting) did not influence emergence or yield of three common varieties. However, the emergence of the cultivar Rua and the yield of the cultivar Sebago were reduced.

A second study in New Zealand by Bedi and Genet (7) did not produce such successful results. Dormant tubers of three main crop varieties, Rua, Whitu and Katahdin, harvested 1-2 months previously, were submersed in 10,000 ppm Cl for 1 to 3 hours and planted four months later. Sprouting of Rua was not increased, emergence was decreased, and yield was reduced by 10% compared to water dip or no treatment. Treatment of the cultivar Whitu reduced yield. With

both cultivars, yield was further reduced by longer treatments. The cultivar Katahdin was not affected by any treatment. Thus there appears to be varietal difference in response to hypochlorite treatment.

In Michigan, Grigg and Chase (25) found both pre- or post-storage washing of whole seed tubers with chlorine solutions improved stand counts. The varieties Russet Burbank and Arenac showed a 10-20% increase in stand when washed with 100-1000 ppm chlorine solutions.

Potter has reported a number of seed treatment trials evaluating the use of hypochlorite (unpublished, reported in the 1977-1981 Montcalm Farm Research Reports, Michigan State University). In 1977 with the variety Monona, and in 1978 and 1979 with the variety Sebago, the hypochlorite treatments (2 min. dips) resulted in stands and yields as good (1978) or better (1977 and 1979) than no treatment, and as good or better than other chemical treatments. All treatments were applied the day of planting. In 1980 with the variety Sebago, the hypochlorite treatment (2 min. dip in 500 ppm Cl solution) produced stands and yields better than no treatment and equal to those produced by dust treatments when applied the day of planting. When applied 2 or 14 days before planting, the hypochlorite treatment reduced stand and yield compared to other treatments. For all treatments, stand and yield were reduced as the time between treatment and planting was increased.

In 1981, Potter cut and treated Monona seed 14, 2 and 0

days before planting. For two weeks before planting, all cut and treated or whole and untreated seed was stored at either 4-6° C or 19-21° C at a relative humidity of 90%. As in 1980, stand generally decreased as the time period between cutting/treatment and planting increased. Seed stored at the colder storage temperature produced a poorer stand than seed stored at the warmer temperature, the largest detrimental effect occuring at the longest storage period. The hypochorite dip produced a stand as good as no treatment or the captan-streptomycin treatment at the same temperature-storage period combination. Unlike 1980, it did not reduce stand or yield when applied 2 or 14 days before planting.

Yields did not always correlate with stands in 1981. There was no significant difference between any of the hypochlorite temperature-storage period combinations. For any particular temperature-storage period combination, except seed stored at 19-21° C and cut/treated 0 days before planting, the yield produced from the hypochlorite treatment was as good as that from the captan-streptomycin treatment. In all cases, the hypochlorite treatment was as good or better than no treatment, and better than the water only treatment.

Potter's studies suggest that the 500 ppm Cl hypochorite treatment is as good as a conventional dust treatment in improving stands and yields over no treatment, at least when applied immediately before planting. There

appears to be a possible detrimental effect of hypochorite when applied 2 to 14 days before planting, but this may be due to the method of application, as the hypochorite treatment did not reduce stand or yield compared to a water only dip treatment.

The usefulness of the results of these studies to the present study are limited, however, since the hypochlorite treatments were applied as a 2 minute dip. This is not a practical application method that can readily be adopted in a farm-scale production system. In addition, the 2 min. submersion in a liquid appears to have some detrimental effect on the seed-piece.

Several studies have reported the effectiveness of hypochorite against bacterial pathogens, including potato pathogens. Odlang (56) found a 90% reduction in cell populations for most organisms after a 10 second exposure to hypochlorite solutions of 5 to 15 ppm Cl. Bacterial spores required longer exposures to hypochlorite. Letal et al (43) found a 5 minute exposure to a 5000 ppm hypochlorite solution completely killed all ring rot bacteria (Corynebacterium sepedonicum) on metal, wood and burlap surfaces. This same treatment was also 100% effective against Erwinia carotovora on metal and burlap surfaces, but not completely effective on wood surfaces. Lund and Wyatt (46) inoculated tubers with E. carotovora var. atroseptica and subsequently dipped them in hypochlorite solutions for five min. Hypochlorite concentrations of 600 ppm Cl (pH 9.4)

and 2000 ppm Cl (pH 9.7) considerably reduced the amount of bacterial rot. However, even a 30 min. dip in 1000 ppm (pH 9.4) did not completely prevent decay.

Research in India has shown that Stable Bleaching Powder (SBP) can also effectively control E. carotovora and blackleg (61). A 1000 ppm formulation of SBP inhibited the bacteria both in vitro and on tuber cylinders. Treatment of inoculated seed tubers prior to planting with 1000 ppm SBP gave good germination, disease control and yield as compared to the comparable control. Treated, uninoculated tubers planted in inoculated soil produced poorer results. Soil drenching at 12.5 kg/ha gave better germination, disease control and yield than seed treatment. Lower concentrations of SBP were less effective. The authors report that SBP contains about 35% chorine, but it was not clear if the given concentrations of SBP are in parts per million (ppm) available chlorine or ppm total product.

A comprehensive study into the potential of hypochlorite solutions as a potato seed-piece treatment has never been reported. This appears to be an area where investigation is needed.

CHAPTER 2. LABORATORY EXPERIMENTS

The effect of chlorine on suberization was investigated using several methods reported in the literature.

MATERIALS AND METHODS

All potato tubers used in the laboratory experiments were of the cultivar Russet Burbank, unless otherwise noted. Tubers were stored at 3° C since harvest and were warmed at room temperature 1 to 7 days before use.

Experiment 1. Histochemical measurement of suberization The effect of chlorine on suberin deposition was investigated using histochemical staining techniques. Six to eight ounce tubers were cut in half along their long axis with a sharp knife. Each of the following treatments was applied to 12 half-tubers:

a. 30 sec. dip in water

b. 30 sec. dip in 100 ppm available chlorine.

c. 30 sec. dip in 500 ppm av. Cl.

d. 30 sec. dip in 1000 ppm av. Cl.

e. 30 sec. dip in 5000 ppm av. Cl.

Six half-tubers were left untreated as controls.

Immediately after treatment, the excess liquid was shaken off and the half-tubers were placed on elevated racks in a tub containing an inch of water to provide high

humidity. The tub was covered with a sheet of plastic, and the half-tubers were allowed to suberize at room temperature (20-22^{o} C). After 5 days, 10-20 um sections were made perpendicular to the cut surface of each half-tuber, one-third of its length from the apical end inside the vascular ring, using a Hooker rotary microtome. The sections were extracted and dehydrated in methanol for several days, then stained with 0.01% toluidine blue (28). The thickness of the stained suberin layer was measured using an ocullar micrometer at 3 randomly chosen points on the section, and the values averaged to give the mean suberin layer thickness of that half-tuber.

A logrithmic transformation was used during analysis by Duncan's multiple range test. Means presented are not transformed.

Experiment 2. Resistance to water loss. The effect of chlorine on suberizaton, as measured by resistance to water loss, was investigated using a weight-loss method (34, 36). Six cylinders of tuber tissue, 8.5 mm in diameter and extending 25 mm into the center of the tuber, were cut from the midsection of each tuber, avoiding eyes, using a cork borer. Sufficiently large tubers were used to avoid including the vascular ring of the opposite side of the tuber. Each cylinder was given one of six treatments:

a. no treatment

b. 10 sec. dip in distilled water

c. 10 sec. dip in 200 ppm available chlorine.

d. 10 sec. dip in 600 ppm av. Cl.

e. 10 sec. dip in 1800 ppm av. Cl.

f. 10 sec. dip in 5400 ppm av. Cl.

Immediately after treatment, excess liquid was shaken off and the cylinders were placed on elevated racks in a tub containing an inch of water to provide high humidity. The tub was covered with plastic, and the cylinders were suberized at room temperature (20-22° C). They were then weighed before and after drying for 3 hours at 36-38° C in a convection oven, and the percent weight lost during drying was calculated.

The experiment was repeated 3 times, twice with 5 days of suberization and once with 11 days. Each experiment was arranged as a randomized complete block, with tubers as blocks and 8 replications. An arcsin transformation was used during analysis by Duncan's multiple range test. Means presented are not transformed.

Experiment 3. Resistance to Decay. The effect of chlorine on suberization, as measured by the ability of chlorine-treated tuber tissue to resist invasion by the dry rot fungus, Fusarium sambucinum, was investigated. Six disks of tuber tissue (15 mm in diameter and 4 mm thick) were cut from the center portion of each tuber using a cork borer, avoiding the vascular ring. Each disk was given one of six treatments used previously (see Experiment 2).

Immediately after treatment, excess liquid was shaken off and the 6 disks from the same tuber were placed together

in an inverted Petri plate containing c. 20 ml of 1% water agar (34, 47). The water agar provided high humidity. After two hours, 1 day, 2 days, 4 days or 8 days of suberization at room temperature (20-22^{o} C), 0.1 ml of distilled water containing $2.5*10^{4}$ spores per ml of Fusarium sambucinum was placed on each disk in 8 plates.

Spore suspensions were prepared from 6-10 day cultures by flooding the culture surfaces with distilled water, dislodging the spores with a glass rod, and filtering the suspension through 4 layers of sterile cheesecloth. Final spore concentration was adjusted using a hymacytometer.

After 6 days at room temperature, the disks were rated for decay, using a 1-5 scale (1=0-5% of the disk volume decayed, 2=5-25% of the disk decayed, 3=25-50% of the disk decayed, 4=50-75% of the disk decayed, and 5=75-100% of the disk decayed).

Each suberization period was arranged as a randomized complete block with tubers and petri plates as blocks and 8 replications. The experiment was repeated twice, with a seventh treatment (1 sec. dip in 5400 ppm available Cl.) added the second time, to determine if the time of exposure to chlorine affected suberization.

No statistical analysis was performed because of the non-homogeneous variance in the data, and the clear trends in the results.

Experiment 4. Chemical measurement of suberin components. The effect of chlorine on suberization was

measured using a chemical assay of suberin components. Tubers of the cultivars Atlantic and Russet Burbank were cut in half along their longitudinal axis with a sharp knife. One half of each tuber was given one of the following treatments:

a. no treatment
b. 1 sec. dip in distilled water
c. 1 sec. dip in 150 ppm available chlorine.
d. 1 sec. dip in 300 ppm av. Cl.
e. 1 sec. dip in 600 ppm av. Cl.
f. 1 sec. dip in 1200 ppm av. Cl.

A 1 second dip was used since this more accurately reflects exposure times occuring on farms that use spray nozzles to apply chlorine wash treatments.

Immediately after dipping, a plug of tissue (13 mm in diameter) was cut from a random location inside the vascular ring using a cork borer. The top 0.5 mm of the plug was removed with a razor blade and the slice was placed in methanol. The half-tubers were placed in a pan lined with wet paper towel, covered with aluminum foil, and allowed to suberize in a growth chamber at 18^{o} C or 10^{o} C. Water was added as needed to keep the paper towel wet. Relative humidity in the pan, measured using a fan operated pyschrometer, was approx. 70-80% at the higher temperature and 80-85% at the lower temperature.

With the half-tubers suberized at 18^{o} C, plugs of tissue were again cut from inside the vascular ring 1 day, 2

days, 4 days, 8 days and 16 days (cv. Atlantic only) after treatment. The top 0.5 mm of the plug was removed with a razor blade and the slice placed in methanol. The same procedure was carried out 1 day, 2 days, 4 days, 7 days and 11 days after treatment with the half-tubers suberized at 10° C.

The slices were extracted for at least 72 hours in 3 changes of methanol. After extraction, the slices were air dryed and then assayed for lignin-like compounds using a thioglycolic acid procedure (27). Each slice was placed in a test tube and 0.25 ml of thioglycolic acid (Sigma Chemical Co., St. Louis, MO.) and 2.5 ml of 2N HCl added. The tubes were capped with glass marbles or a glass plate and heated at 95° C for 4 hours. After cooling, the liquid was discarded, the slices were washed in 5 ml of water, and the water wash was discarded. The slices were incubated in 2.5 ml of 0.5N NaOH for 16-18 hours in order to solubilize the lignin thioglycolate. The liquid extract was collected into 15 ml conical centrifuge tubes, the slice washed with 2.5 ml of water, and the water wash was combined with the liquid extract. After adding 0.5 ml of concentrated HCl, the acidified solution was held at 4 C for 3.5-4 hours to allow ligninthioglycolic acid precipitation. The precipitated ligninthioglycolic acid was collected by centrifugation (1000xg, 5 min.). The pellet was washed twice by resuspension and centrifugation in 0.1N HCl (2.0 ml per wash). The final pellet was dissolved in 2.5 ml (or multiples thereof,

depending on the amount of ligninthioglycolate present) of 0.5N NaOH. The final solution was centrifuged (1000xg, 3 min.) to remove any insoluble material, and the absorbance of the solution at 280 nm was measured.

Each suberization period was analysed as a separate experiment using Duncan's multiple range test, and the response to increasing chlorine rate was analysed using orthogonal polynomials.

The thioglycolic acid technique was also applied to two treatments from Experiment 3. The top 0.5 mm of tuber disks treated with 200 ppm Cl or 1800 ppm Cl and suberized for 14 days (8 days before inoculation, and 6 days after inoculation-no decay was observed) was removed using a potato peeler, and assayed for lignin-like compound using the above technique. Eight disks of each treatment were used. Results were analysed using a t-test.

RESULTS AND DISCUSSION

Experiment 1. Histochemical measurement of suberization. Increasing rates of chlorine produced a thicker suberin layer (Table 2.1). This is in agreement with the results of a previous study by Grigg and Chase (26).

Experiment 2. Resistance to water loss. Tissue cylinders treated with increasing chlorine rates lost more weight (water) during drying than those treated with water or low rates of chlorine (Figure 2.1). This indicates that high chlorine rates cause a less effective water vapor

Table 2.1. The effect of different wash treatments on the thickness of suberin layer formation.

Treatment[a]	Suberin layer thickness[b] (um)
no treatment	82 a[c]
water	81 a
100 ppm Cl	78 a
500 ppm Cl	94 b
1000 ppm Cl	96 b
5000 ppm Cl	167 c

[a]Thirty second dip.

[b]After 5 days of suberization at 20-22° C. Means are the average of 12 tubers (except no treatment, which is the average of 6 tubers.) The value for each tuber was the average of the thickness of the stained suberin layer at 3 random points on a section taken from inside the vascular ring, one-third of its length from the apical end.

[c]Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

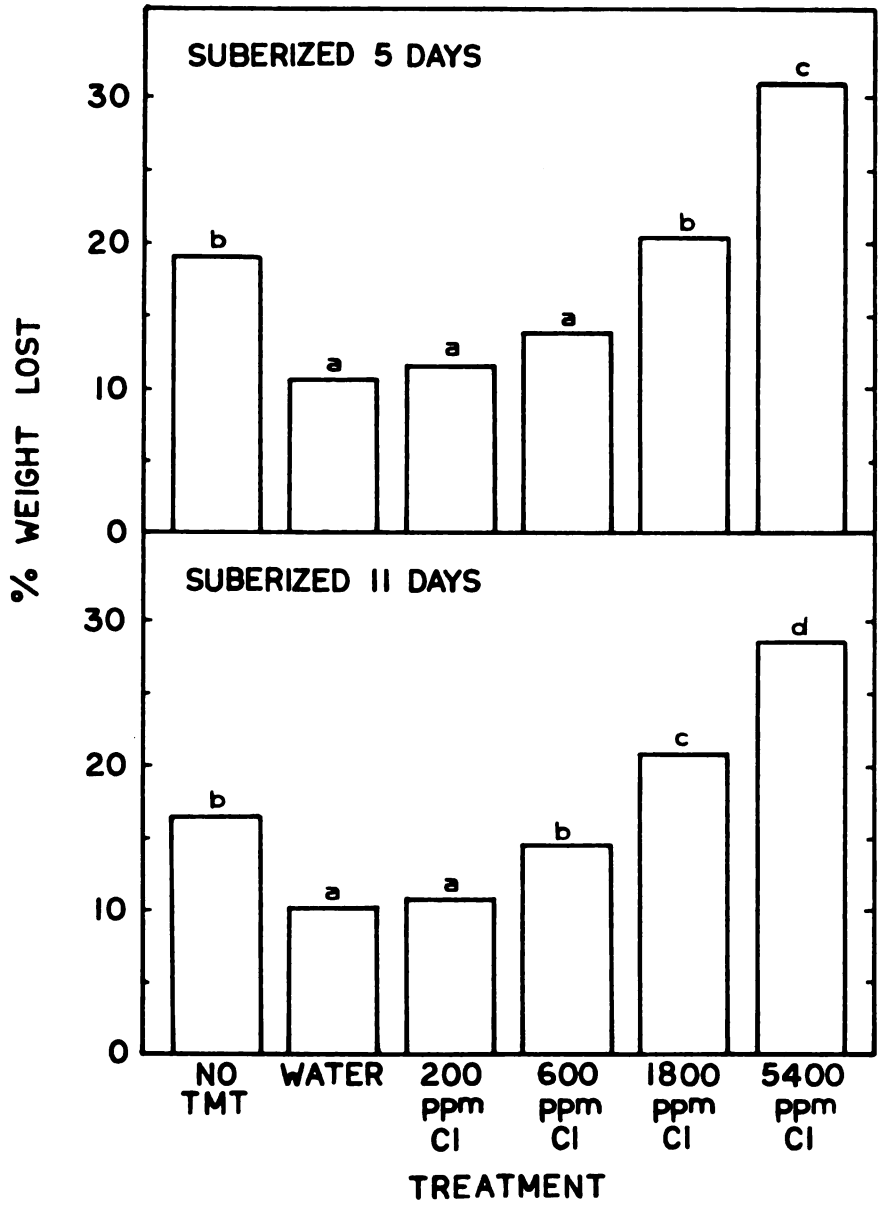

Figure 2.1. Percent weight lost during drying by cylinders tuber tissue treated with different rates of chlorine. Cylinders were suberized 5 or 11 days before drying. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

barrier (suberin layer) to form, possibly due to lack of wax synthesis. Waxes are the vapor barrier in the suberin layer (66). The retardation was evident even after 11 days of suberization (Figure 2.1). However, Grigg and Chase (26) found post-harvest washing of whole tubers with 100 to 1000 ppm Cl wash solutions did not cause any significant difference in weight lost during 4 months of storage, compared to untreated tubers.

Untreated tissue lost more weight than water or 200 ppm Cl treated tissue. There was no significant difference between the water and 200 ppm Cl treatment (and the 600 ppm Cl treatment after 5 days of suberization). This suggests any beneficial effect of treatment compared to no treatment was due to the washing effect of the water, and not the chlorine present in the washes.

Experiment 3. Decay resistance. With disks suberized 2 hours after treatment, increasing rates of chlorine allowed more Fusarium decay than 200 ppm Cl, water or no treatment, which allowed only slight decay (Figure 2.2). As the suberization period increased, the amount of decay decreased and the difference between the high and low chlorine rates became less. After 8 days of suberization, essentially no decay was observed amoung the treatments, except for disks treated with the highest chlorine rate (5400 ppm Cl.) Unlike the inhibition of water-vapor barrier formation, the inhibition of pathogen barrier formation by chlorine was not long lasting.

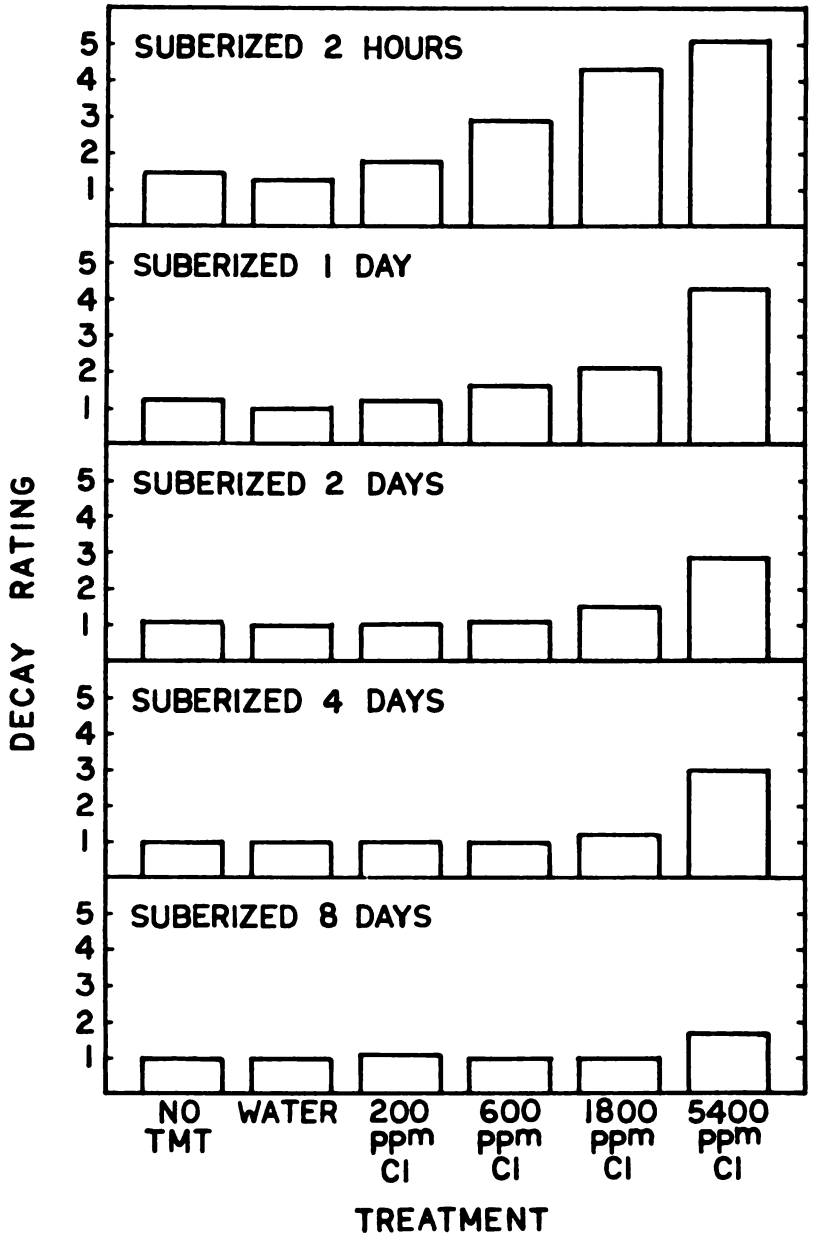

Figure 2.2. Decay ratings of tuber disks treated with different rates of chlorine. After suberization, 0.1 ml of water containing 2.5×10^4 macroconidia per ml of Fusarium sambucinum was placed on each disk. 1=0-5% decay, 2=5-25% decay, 3=25-50% decay, 4=50-75% decay, and 5=75-100% decay.

There was no significant difference at any suberization period in the decay rating of disks dipped for 10 seconds or 1 second in 5400 ppm Cl (data not shown). Grigg and Chase (26) found no difference in the effect of 15 second to 2 minute dips in chlorine (rates of 100 to 1000 ppm Cl) on tuber blackening.

Observation of the disks after several days of suberization showed browning of the disks treated with higher chlorine rates, and some browning of the untreated disks. Disks dipped in water or low chlorine rates remained light yellow. With disks suberized for 4 or 8 days before inoculation, 6 days after inoculation the drop of water containing Fusarium spores remained beaded on the surface of disks treated with water or low chlorine rates (Figure 2.3). Disks treated with high chlorine rates or not treated did not have the water drop remain beaded on their surface. These observations suggest high chlorine rates adversely affect some hydrophobic property or compounds that are part of the suberin layer.

Experiment 4. Chemical measurement of suberin components. The thioglycolic acid assay was originally developed to measure lignin deposition in disease resistance studies (27). Since some of the components of suberin are similar to lignin (38), an attempt was made to use this assay to measure suberization.

The rate of appearance of lignin-like compounds after cutting (averaged over wash treatments) (Figure 2.4) was

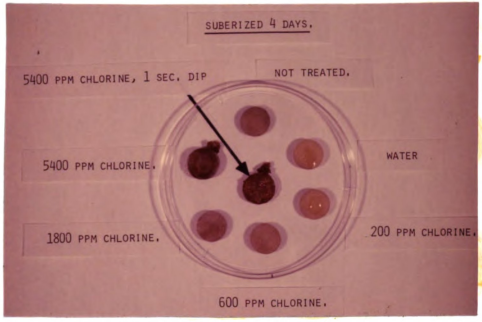

Figure 2.3. Decay of tuber disks given various treatments and suberized 4 days after treatment. Disks were inoculated with 0.1 ml of water containing 2.5×10^4 macroconidia per ml of *Fusarium sambucinum* after suberization and incubated at room temperature for 6 days.

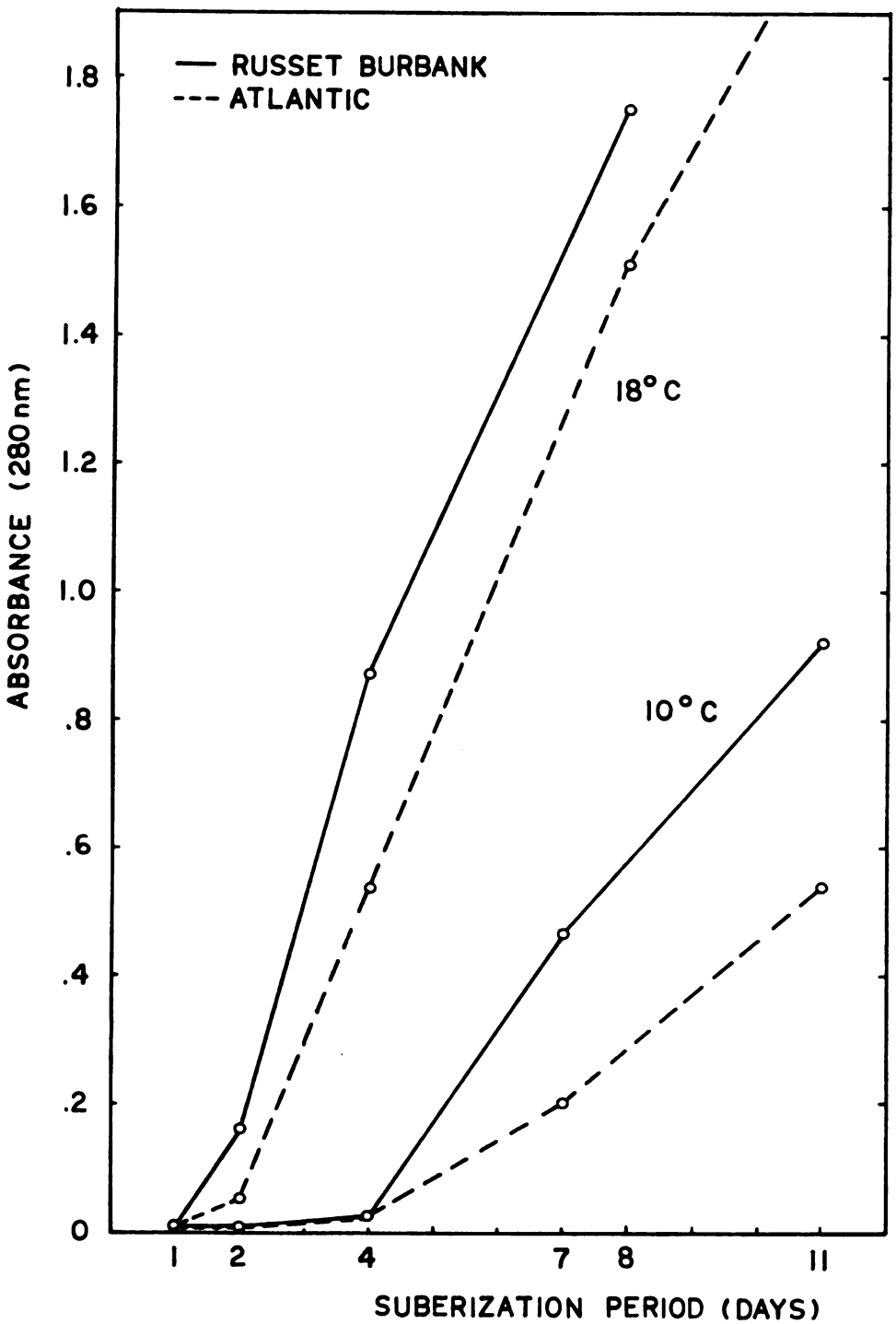

Figure 2.4. Rate of appearance of lignin-like material on the cut surface of potato tubers of two cultivars suberized at 10 and 18° C. Results are the average of all wash treatments.

consistent with published reports (12, 66). Also consistent with reported results were the observations that lignin-like material appeared faster at 18° C than at 10° C (5, 19, 54, 72), and the cultivar Russet Burbank suberized faster than the cultivar Atlantic (42, Hammerschmidt, personnal communication). The consistency of these findings with other researcher's results confirm that this procedure is likely valid for measuring suberin components.

The effect of the wash treatments on the amount of lignin-like material that was deposited was highly variable, with an average coefficient of variability of 43%. There were no significant overall differences (data not shown). Occasional significant differences between individual treatments and occasional significant trends were observed; however, they were not consistent between suberization periods, suberization temperatures and varieties. The large variability was probably due to tuber to tuber variations. Several studies have shown considerable differences in the healing ability of different tubers of the same variety from the same field (4, 72). The procedure of taking disks of suberized tissue randomly from the inside of the vascular ring should not have caused the large variability, since no differences in the rate of healing have been found between areas within the vascular ring (4, 60).

There was no significant difference in the amount of lignin-like material in disks (from Experiment 3) treated with 200 ppm Cl or 1800 ppm Cl. Disks treated with the

higher chlorine rate had 95% as much lignin-like material as those treated with the lower chlorine rate. There was much less variability in the amount of lignin-like material in disks from Experiment 3; the average coefficient of variability was 8.4%. In Experiment 3, the tissue disks were all taken from the center of the tuber, and the experiment was randomized with tubers as blocks. This is further evidence that the large variability in Experiment 4 was due to tuber to tuber differences.

These results suggest that the occasional significant differences observed in Experiment 4 are due to the large variability in its results, and suggests no significant differences in suberin deposition due to chlorine treatment. Further experimentation with greater control of between tuber variability is needed to provide a definite answer to this question.

The two physiological measures of wound healing are undoubtably the most meaningful in terms of the real life effect of chlorine on cut seed potatoes. Prevention of water loss and pathogen entry are the two vital functions of the suberin layer. Contrary to the results of the anatomical and chemical techniques used, which indicated chlorine increased or had no effect on suberin layer formation, the two physiological methods used showed that treatment with increasing chlorine rates cause a less effective water vapor and pathogen barrier to form. This apparent contradiction may be explained by the results of Kolatakudy and coworkers

(66), who found that waxes associated with the suberin polymer, and not suberin itself, form the major water diffusion barrier. These studies support their findings, suggest chlorine inhibits wax deposition, and suggest waxes may also play a role in preventing pathogen entry. However, the effect of chlorine on these two functions is not identical, as the detrimental effect of chlorine on resistance to water loss appeared to be long lasting, while its effect on resistance to decay was not. The chlorine treated disks in Experiment 3 did become resistant to pathogen entry in parallel to the appearance of lignin-like compounds observed in Experiment #4. Possibly waxes are important in preventing pathogen entry until a suberin layer forms.

SUMMARY.

Laboratory experiments indicated increasing chlorine rates caused a less effective water vapor and pathogen barrier to form. Anatomical and chemical measurements showed increasing chlorine rates increased or had no effect on the amount of suberin deposited. This indirectly supports previous findings that waxes are the primary water barrier in the suberin layer and suggests they are also important in preventing pathogen entry and are inhibited by chlorine. Washes of 500 ppm Cl, as might be used by farmers, did not adversely affect suberization.

CHAPTER 3. FIELD EXPERIMENTS

During the 1983 growing season, two field experiments were conducted to evaluate the effect of hypochlorite (chlorine) wash solutions on cut potato seed performance.

MATERIALS AND METHODS.

The experiments were performed at the Montcalm Research Farm, Entrican, MI, on a McBride sandy loam soil.

Experiment 1a. This experiment evaluated the effect of several chlorine wash treatments on 3 varieties of potatoes over a range of spring soil and weather conditions.

On three dates, cut seed of three varieties was given one of 8 seed treatments 2-4 hours before planting. Certified Atlantic seed from New Brunswick, Canada, Foundation Russet Burbank seed from Prince Edward Island, Canada, and Certified Monona seed from Michigan were used. Seed was obtained in mid-April and stored at 3° C. Thirteen days before planting, the seed was moved to 18° C. Seed had 13-25 mm sprouts at the time of treatment. Seed was hand cut to 42-56 gram size. The 8 seed treatments are listed in Table 3.1.

All treatments (except the captan-streptomycin treatment) were applied to the cut seed as a liquid spray

Table 3.1 Seed treatments used in Field Experiment 1.

1. no treatment	inoculated with *Fusarium* macroconidia[a]			
2. water	"	"	"	"
3. 500 ppm chlorine[b]	"	"	"	"
4. 2000 ppm chlorine	"	"	"	"
5 captan-streptomycin[c]	"	"	"	"
6. water	not inoculated			
7. 500 ppm chlorine	"	"		
8. 2000 ppm chlorine	"	"		

[a]Whole tubers were inoculated approx. 18 hrs. before treatment by submersion for 2-3 min. in water containing 1500 spores/ml of *Fusarium sambucinum*. Spore suspensions were obtained by washing 5-7 day old culture plates with distilled water followed by filtering through 4 layers of cheese-cloth. Spore concentrations was determined using a hemacytometer.

[b]The NaOCl source was 5.25% household bleach. Available chlorine was determined by the titration method of Hoffman et al (31). However, by comparing this method with those of commercial test kits obtained later (Taylor Chemicals, 31 Loveton Circle, Sparks, MD. 21152), an error was found. Each ml of arsenite solution required to neutralize 5 ml of chlorine solution equals 2 g/l available chlorine, and not 1 g/l.

[c]Captan-streptomycin dust (1.0 kg/quintal seed) containing 7.5% captan and .01% streptomycin by weight. Hopkins Ag. Chemical, Box 584, Madison, WI. 53701.

solution using a centrifigal pump and flat-fan nozzles as the cut seed passed over a steep decline on a chain conveyor. (This operation caused the seed-piece to roll, providing complete surface coverage.) The spray solution was applied at a rate that wetted the seed-pieces just past dripping (approximately 1.5 l/quintal.) The captan-streptomycin was applied by shaking the dust and seed-pieces together in a plastic bag.

The experimental design was a 3 factor factorial randomized complete block, with one split and 5 replications. Planting date was the whole plot factor, and variety and seed treatment the subplot factors. Each subplot consisted of one 1.83 m row; rows were 0.86 m apart; and each whole plot consisted of 4 15.3 m rows, each with 6 subplots in it.

Eight seed-pieces were hand planted 23 cm apart in each subplot. Plots were separated within each row by 1 or 2 hills of a red-skinned variety, and one red-skinned hill was planted at the end of each row. Planting dates (May 4, May 16 and June 1) were chosen in an attempt to have the treatments undergo typical early, midseason and late spring weather and soil conditions (See Figure 3.1 for weather conditions during this period.) Seed was covered to a depth of 10-13 cm within a half hour of planting. Fertilization was as follows: 280 kg/ha of 0-0-60 at spring plowdown, 560 kg/ha of 20-10-10 banded with the planter (which opened the rows), and 84 kg/ha of 46-0-0 sidedressed twice during the

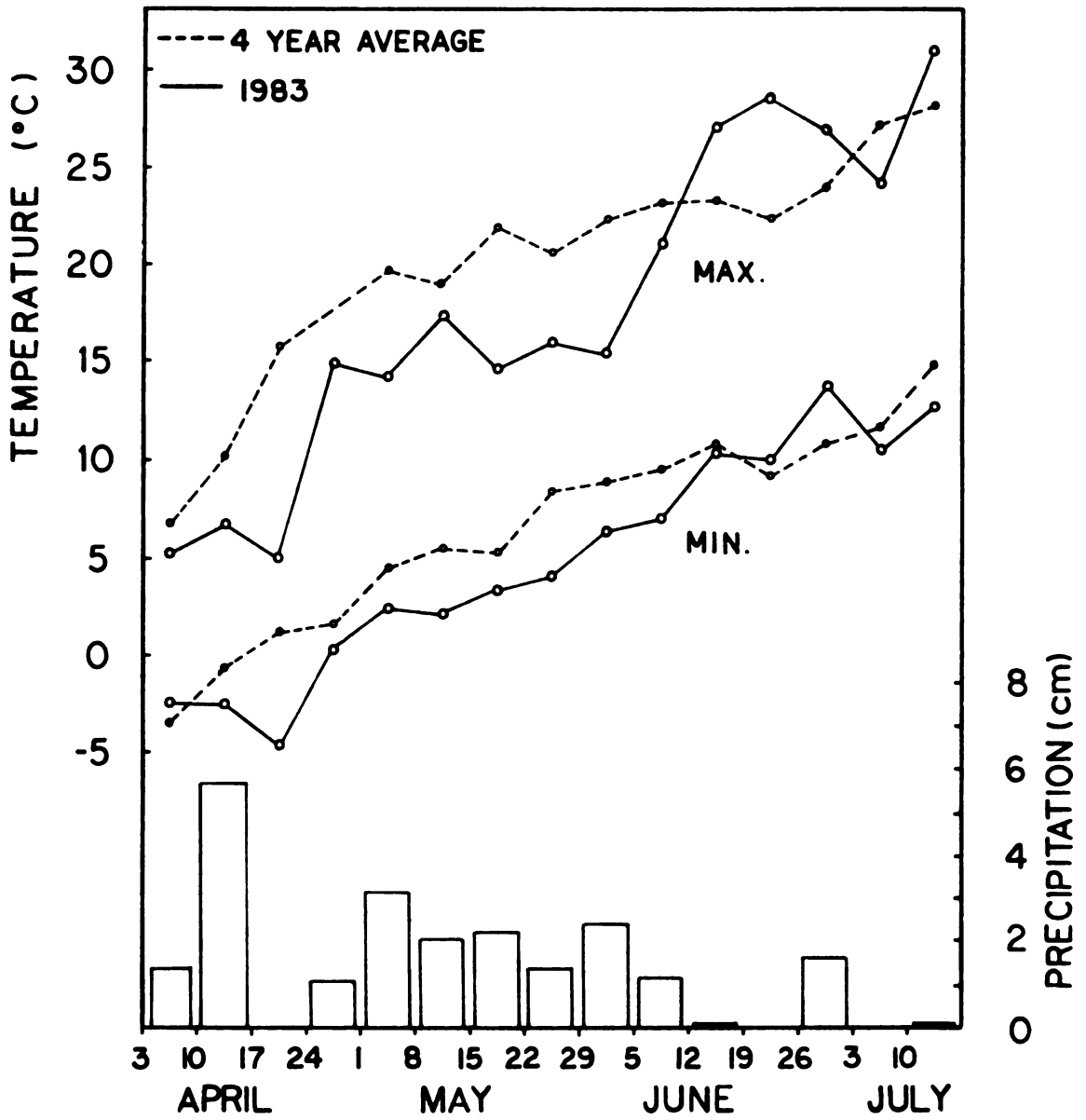

Figure 3.1. Average weekly maximum and minimum temperatures and precipitation at the Montcalm Research Farm during the spring and early summmer of 1983, and the average of the previous four years (temperature only.)

growing season. Aldicarb was also applied at planting at 3.36 kg a.i. per hectare. The previous crop was alfalfa. The plots were sprayed with herbicides, insecticides and fungicides, and irrigated, in accordance with normal potato production practices.

Five to six weeks after planting (see Table 3.2), plots were evaluated for stand and vigor. Plot plant vigor was determined by measuring the above-ground plant height of each hill in a plot and summing. The number of missing hills was also determined. All missing hills were subsequently dug up to determine if the seed-piece was decayed. If the seed-piece was not decayed and appeared blind, or had produced little tubers, it was not counted. Percent stand was calculated from the number of missing hills.

Plots were harvested 125-126 days after planting. A vine desicant was not applied. At harvest, all the cultivars were close to or at maturity, except for the Russet Burbank cultivar at the first two harvest dates. Yield of under 51 mm, 51-83 mm, over 83 mm and No. 2 tubers was determined for each plot. From these, total yield, U.S.#1 yield, and the percent of total yield in each category was calculated. For three seed treatments (dry, 500 ppm and 2000 ppm inoculated), the specific gravity was also determined.

Experiment 1b. At each planting date, a separate planting of only the Atlantic treatments was made. These plots were not allowed to grow to maturity; instead the

Table 3.2 Timetable for field operations performed in Field Experiment 1, and number of days between operations.

	Planting Date	Rating Date	Harvest Date
Exp. 1a	May 4	June 16 (43)[a]	Sept 7 (126)[b]
	May 16	June 27 (42)	Sept 19 (126)
	June 1	July 6 (35)	Oct 4 (125)
Exp. 1b	May 4	June 16 (43)	June 20,22 (47,49)
	May 16	June 27,28 (42,43)	June 29,30 (44,45)
	June 1	July 6 (35)	July 8 (37)

[a]Number of days between planting and rating.

[b]Number of days between planting and harvest.

plots were "harvested" after approx. 6 weeks and the seed-pieces rated for decay. The experimental design was a 2 factor factorial arranged as a randomized complete block, with one split and four replications. Planting date was the whole plot factor, and seed treatment the subplot factor. Each subplot consisted of one 3.7 m row; rows were 0.86 m apart; and each whole plot consisted of 2 15.3 m rows, each containing 4 subplots.

Fourteen seed-pieces were hand planted 25 cm apart in each subplot, and covered with soil to a depth of 10-13 cm within one half hour. Five to six and one half weeks after planting (see Table 3.2), cumulative plot height was determined. Several days later, the plots were "harvested". Total plot vine fresh weight was determined by cutting off all vines at the soil surface and immediately weighing. Each hill was dug up, and the seed-piece was rated for decay. The rating scale used was: 1=0-5% of seed-piece decayed, 2=5-20% decay, 3=20-60% decay, and 4=60-100% decay. Individual ratings were summed to obtain a plot decay rating. The total number of stems per hill was also recorded, and from this the average number of stems per emerged hill was calculated.

Data was analysed using Duncan's multiple range test and a planned comparison between the 3 inoculated and 3 corresponding non-inoculated treatments. When data consisted of small whole numbers, such as for decay ratings or number of missing hills, a square root transformation was

used to increase variance homogeneity. However, all data presented are not transformed.

Experiment 2. This experiment was conducted in cooperation with a Montcalm County potato grower, and was designed to evaluate the effects of a commercial chlorine wash treatment used by that grower, as well as several post-seed-treatment storage conditions. Up to and including the seed cutting operation, Certified Monona seed was handled in bulk in accordance with the grower's regular seed handling practice. Before cutting, the seed had been warmed to 10-13^{o} C for approximately 1 week. At the back of the cutter, one of four seed treatments were applied to the cut seed as it passed over the sliver-removing rollers. The four treatments were: (1) no treatment (dry), (2) water (.7 l/quintal), (3) 2400 ppm chlorine (.7 l/quintal), and (4) captan-streptomycin dust (7.5% plus .01%, .75 kg/quintal of seed). The two liquid treatments were applied with a single cone nozzle over the rollers, and the dust was applied by hand-sprinkling the dust on the seed-pieces as they passed over the rollers. A 1.2 meter auger-conveyor behind the rollers ensured good mixing of the treatments. Four 45 kg burlap bags were filled with each treatment.

Nine post-seed-treatment storage treatments that reflected a series of possible on-farm seed-handling conditions were evaluated. These treatments were:

1. plant treated seed within 4 hours.
2. hold seed for 2 days at 11^{o}C with good ventilation.
3. " " " " " " " poor ventilation.
4. " " " " " " ambient temp. with good vent.

5. hold seed for 2 days at ambient temp. with good vent.
6. hold seed for 5 days at 11°C with good ventilation.
7. " " " " " " " poor ventilation.
8. " " " " " " ambient temp. with good vent.
9. " " " " " " " " " poor vent.

Forty 42-56 g. seed-pieces of each seed treatment were randomly selected immediately after treatment and planted the same day. The four bags of each seed treatment were then randomly distributed , one to each of the four temperature-ventilation conditions. The 11° C temperature treatment was performed in one of the insulated storage bins of the cooperating grower. The ambient air temmperature treatment was performed in an uninsulated drive-in shed (the doors were closed at night.) The temperature in the drive-in shed fluctuated with the diurnal temperature changes: consecutive daily highs were 20, 20, 22, 14 and 15° C, and daily lows were 8, 10, 12, 6 and 3° C. Temperatures at both locations were recorded using a recording thermograph. The poor ventilation treatment was achieved by sliding a plastic garbage bag, which had the bottom cut open, over the outside of the burlap bag, while the good ventilation treatment was achieved by leaving the burlap bag uncovered.

After 2 days, 40 42-56 g. seed-pieces were taken from the center of each bag (2/5 of the bag height down from the top of the bag) and planted. Three days later, 40 more seed-pieces were taken from the center of each bag (3/5 of the bag height down from the top of the bag) and planted. If decayed seed-pieces were selected, they were graded out, and the percent rotted seed-pieces in that treatment was

calculated.

The experimental design was a 2 factor randomized complete block, with one split and four replications. The whole plot factor was storage treatment, and the subplot factor was seed treatment. Each subplot consisted of one 3.0 m row; rows were 0.86 m apart. Each whole plot consisted of two 6.0 m rows, with 2 subplots in each row.

Ten seed-pieces were hand-planted 30 cm apart in each subplot. All plots were separated by 2 hills of a red-skinned variety, and one red-skinned hill was planted at the end of each row. Planting dates were May 11, 13 and 16. Seed was covered within a half hour of planting to a depth of 10-13 cm. Fertilizer and aldicarb application was the same as in Exp. 1. The previous crop was alfalfa, and herbicide, insecticide and fungicide, and irrigation applications were made as required.

On June 28 and 29, six to seven weeks after planting, plots were evaluated for stand and vigour, using the method described in Experiment 1. Plots were harvested on Sept. 15, 122-127 days after planting, and yield of under 51 mm, 51-83 mm, over 83 mm and No. 2 tubers was determined for each plot. From these, total yield and U.S.#1 yield was calculated, as well as percent of total yield in each category.

Data was analyzed using Duncan's multiple range test. Storage treatment means were analysed using planned comparisons, to allow determination of the main effects of storage

period, ventilation and length. When data consisted of small whole numbers, a square root transformation was used to increase variance homogeniety. All data presented are not transformed.

RESULTS AND DISCUSSION

Experiment 1. There were no significant differences between the inoculated and non-inoculated treatments in almost all cases in both Experiment 1a and 1b. Any significant differences observed were not consistent. Therefore new means, averaged over inoculation treatments, were calculated for the 3 seed treatments that were both inoculated and non-inoculated. Duncan's multiple range test, adjusted for the unequally replicated means (68), was used to compare means.

In the Atlantic planting (Exp. 1b), different responses to seed treatment occured at each planting date. In the May 4 planting, the captan-streptomycin treatment produced a better stand than the water, 500 ppm Cl and 2000 ppm Cl treatments, and untreated seed produced a better stand than the water treated seed, which had only 80% stand (Figure 3.2). The captan-streptomycin and 500 ppm Cl treatments had a lower plot decay rating than the water and 2000 ppm Cl treatments (Figure 3.3). Under the wet, cool soil conditions following the May 4 planting, the captan-streptomycin treatment was clearly the superior treatment, and the water and 2000 ppm Cl treatments were the poorest.

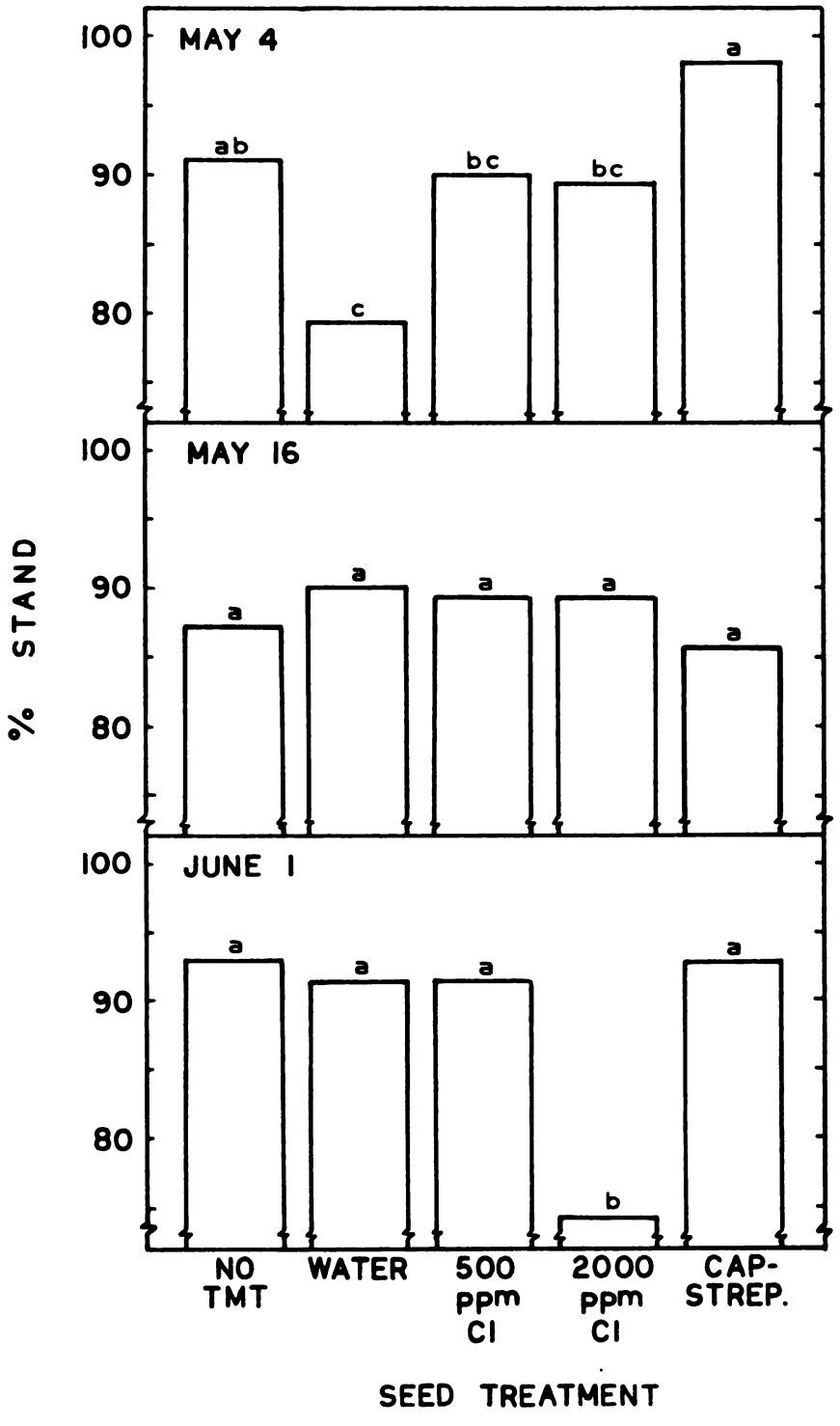

Figure 3.2. Percent stand produced by Atlantic seed (Exp. 1b) treated with one of 5 seed treatments and planted on 3 dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

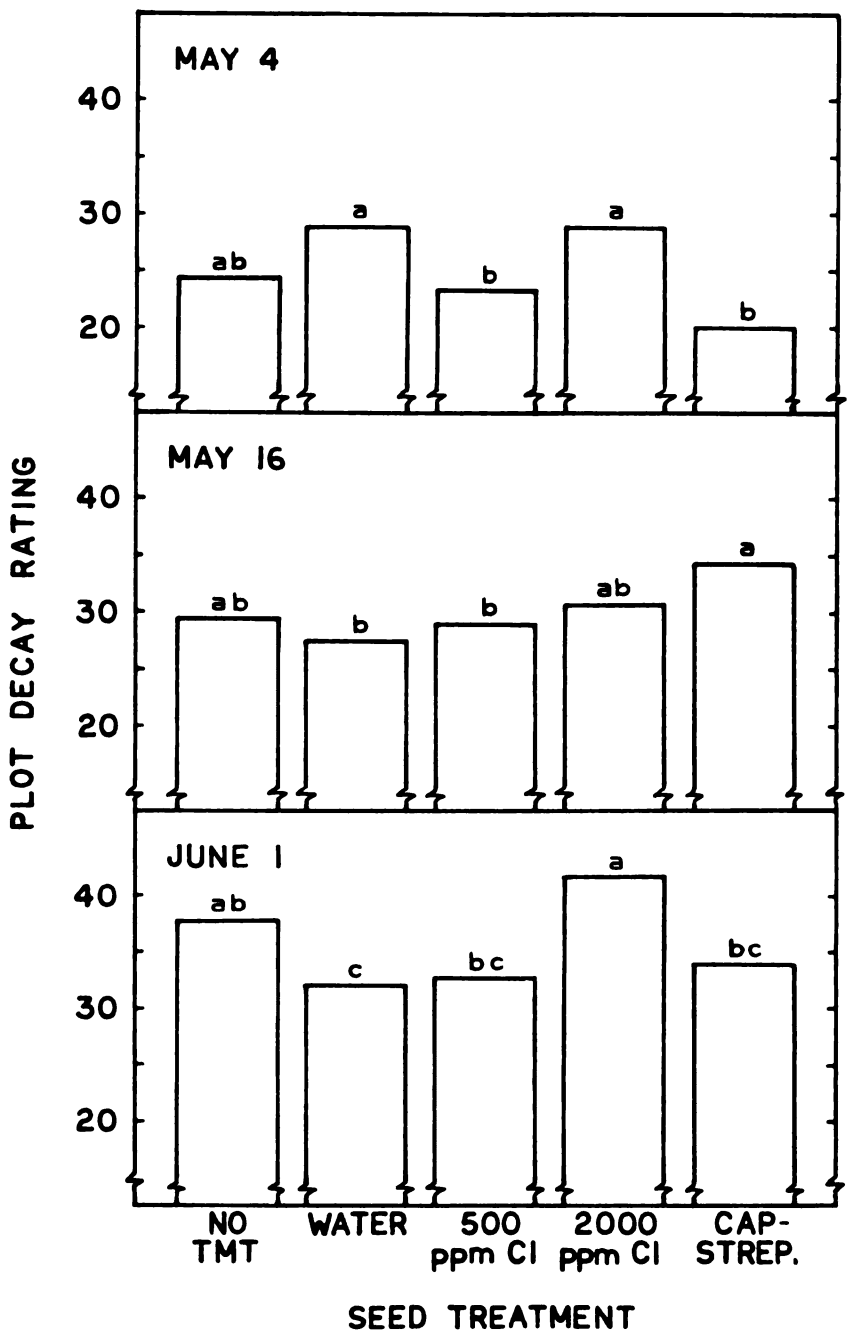

Figure 3.3. Plot decay rating of Atlantic seed (Exp. 1b) treated with one of 5 seed treatments and planted on 3 dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

In the May 16 planting, there were no significant differences in stand. Untreated seed and seed treated with 500 ppm Cl had a lower plot decay rating than seed treated with captan-streptomycin.

The 2000 ppm Cl treatment produced a poorer stand than the other treatments in the June 1 planting. It produced a higher plot decay rating than the water, 500 ppm Cl and captan-streptomycin treatments. Untreated seed produced a higher plot decay rating than the water treated seed. It appears the high chlorine treatment significantly damaged the seed under the warm dry conditions that occured after June 1 (see Figure 3.1).

The effect of seed treatment on plant height and vine fresh weight was very similar to its effect on percent stand and plot decay rating; therefore data for plot height and vine fresh weight are not presented.

Seed treatment had no effect on the number of stems per hill on the first two planting dates. On the third planting date, seed treated with captan-streptomycin produced fewer stems per hill than untreated seed. The rates of chlorine used do not appear to effect the number of stems treated seed-pieces produce.

In the main planting (Exp. 1a), the Russet Burbank and Monona varieties produced excellent stands (averaging over 99%), and there was no significant seed treatment effect on stand. Therefore, stand data for the Atlantic plots was analyzed separately. There were no planting date x seed

treatment interactions. Averaged over the 3 planting dates, the captan-streptomycin treatment produced a better stand than the water and 2000 ppm Cl treatments (Figure 3.4). These results are similar to those found in the Atlantic planting.

There was no significant effect of the seed treatments on plant height with the cultivar Russet Burbank. The captan-streptomycin treatment produced a larger plant height than the 500 ppm Cl and 2000 ppm Cl treatments with the cultivar Monona, and a larger plant height than the water, 500 ppm Cl and 2000 ppm Cl treatments with the Atlantic cultivar (Figure 3.5).

The effect of seed treatment on yield did not interact significantly with the effect of variety or planting date. The overall yields produced by the seed treatments, averaged over varieties and planting date, are shown in Figure 3.6. The captan-streptomycin dust treatment produced the highest total and U.S.#1 yield. This was better than all treatments except the 2000 ppm Cl treatment. The 2000 ppm Cl treatment frequently had the most detrimental effect on seed performance (see above), but this effect is not reflected in the yields (Figure 3.6). The yield from the water-treated seed was significantly lower than the captan-streptomycin and 2000 ppm Cl treated seed. From this experiment, it appears that any benefit of the chlorine wash treatments is not due to the washing effect, but rather to the chlorine in the wash solution, since the water treatments often produced the

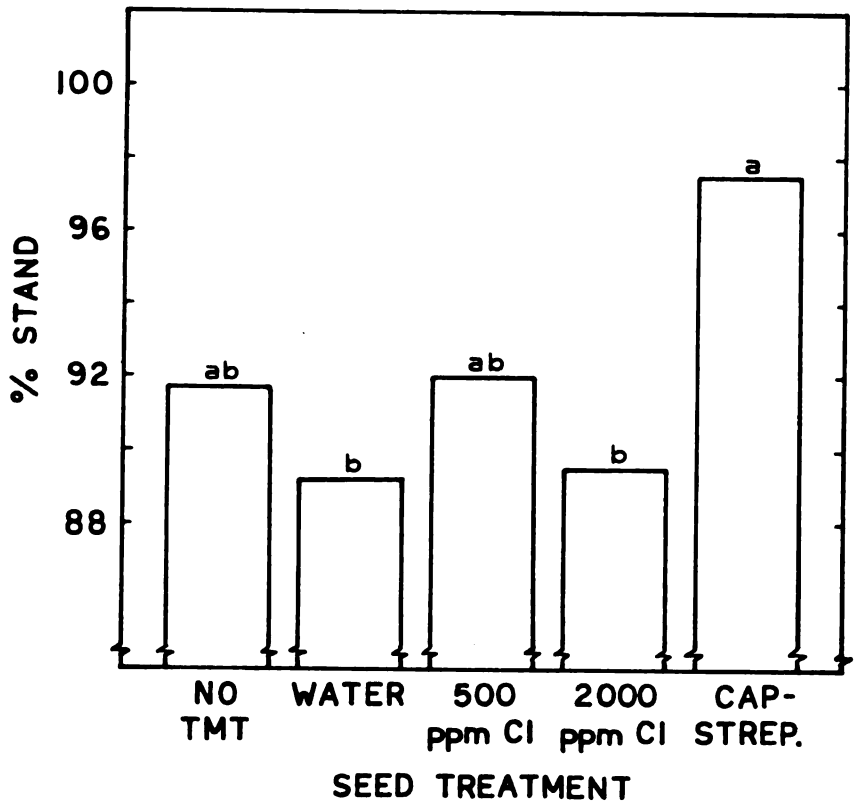

Figure 3.4. Percent stand produced by Atlantic seed (Exp. 1a) treated with one of 5 seed treatments , averaged over 3 planting dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

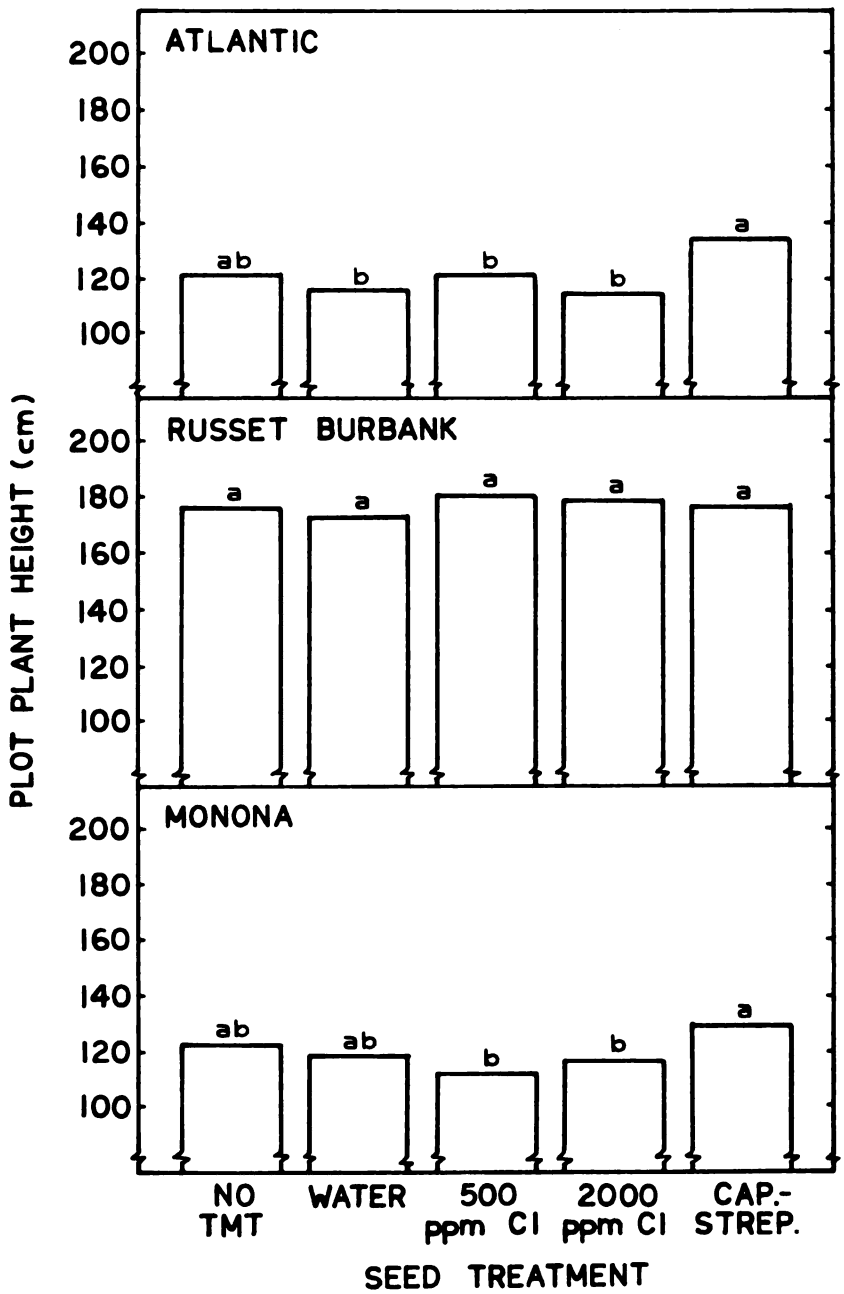

Figure 3.5. Plot plant height produced by 3 varieties treated with one of 5 seed treatments, averaged over 3 planting dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

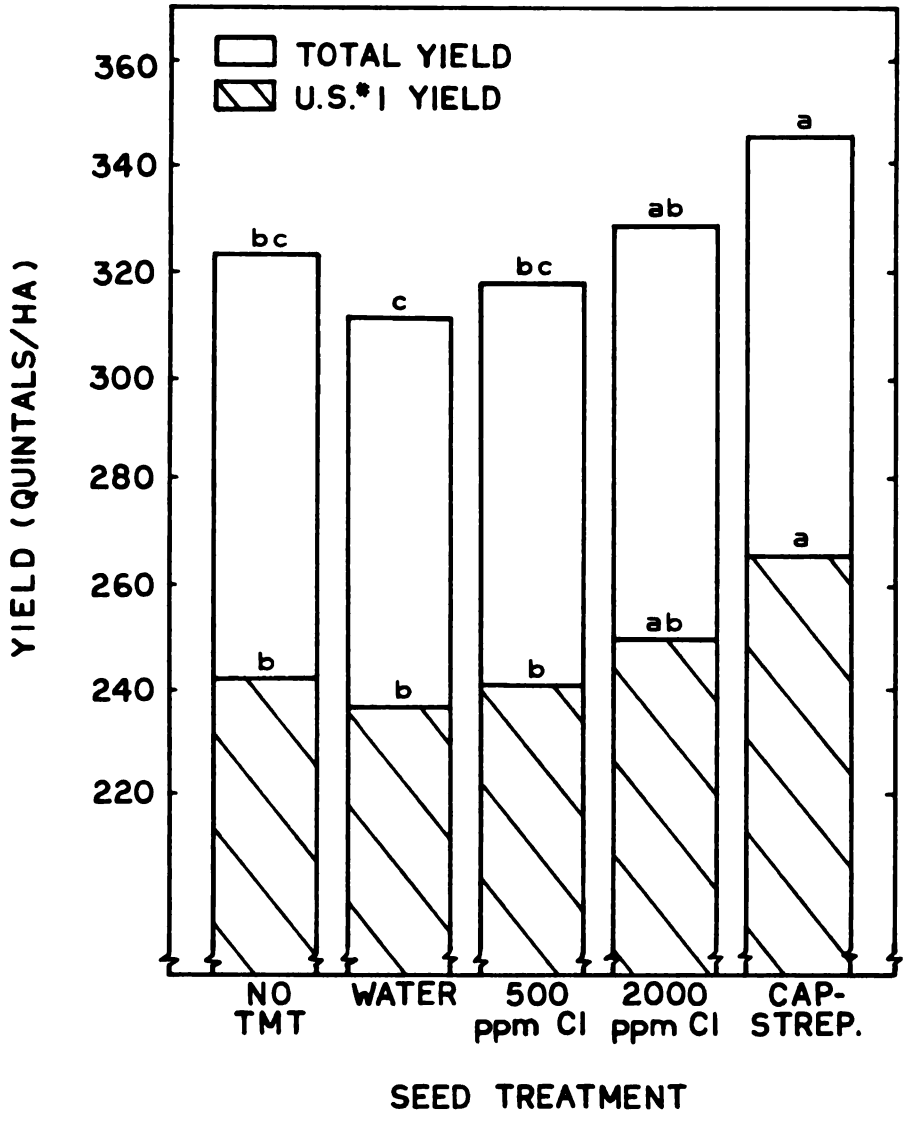

Figure 3.6. Total and U.S. #1 yield produced by seed treated with one of 5 seed treatments. Means are the average of 3 varieties and 3 planting dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

poorest stand and resulted in the poorest yield.

The average specific gravity increased slightly as the chlorine concentration increased (Figure 3.7). This increase is not likely due to any direct effect of the chlorine treatments, but possibly to more vigorous stands produced by the chlorine-treated seed.

Experiment 2. After storage, untreated seed stored 5 days at ambient air temperature with poor ventilation had 13% rotted seed-pieces by weight. Under the same storage conditions, seed washed with water or 2400 ppm Cl, or dusted with captan-streptomycin did not decay, suggesting all 3 of these treatments were beneficial. The decayed seed-pieces were graded out before planting; this should be considered when evaluating subsequent stand and yield data. This was the only treatment in which storage decay was observed.

Results for stand and yield are presented in Table 3.3 and Figures 3.8 and 3.9. There were no seed treatment x storage treatment interactions. Therefore only seed treatment means, averaged over storage treatment, and storage treatment means, averaged over seed treatment, are presented.

Effect of seed treatment. The captan-streptomycin and chlorine wash seed treatments produced equivalent stands, and total and U.S.#1 yields (Figure 3.8 and 3.9). Both increased stands and yields compared to untreated seed. There was no significant difference in stand or yield between the water and chlorine wash treatments, suggesting

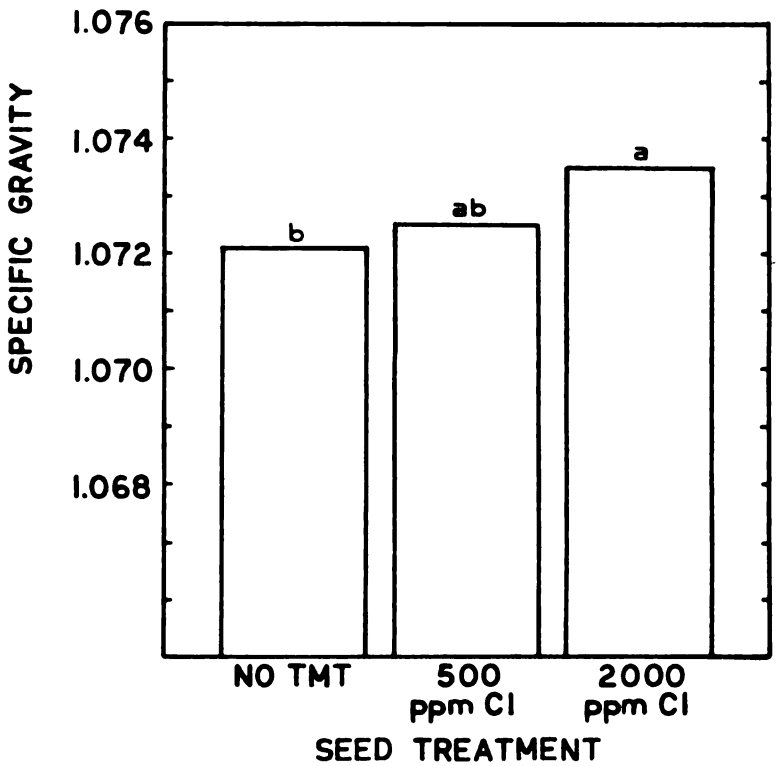

Figure 3.7. Specific gravity of tubers produced by seed treated with one of 3 seed treatments. Means are the average of 3 varieties and 3 planting dates. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 3.3. Percent stand and yield produced by seed given various storage treatments.

Treatment	Stand (%)	Total Yield (quintals/ha)	U.S.#1 Yield (quintals/ha)
Storage Period			
0 days	98.7 a[a]	318 ab	293 ab
2 days	97.6 a	309 b	284 b
5 days	98.7 a	335 a	309 a
Storage Temperature			
11° C	97.0 a	324 a	300 a
ambient air temp.	98.9 a	319 a	293 a
Storage Ventilation			
good	98.1 a	321 a	295 a
poor	97.8 a	321 a	299 a

[a]Means are averaged over seed treatment and other storage treatments. Means, within the same column and storage treatment, followed by the same letter are not significantly different at the 5% level.

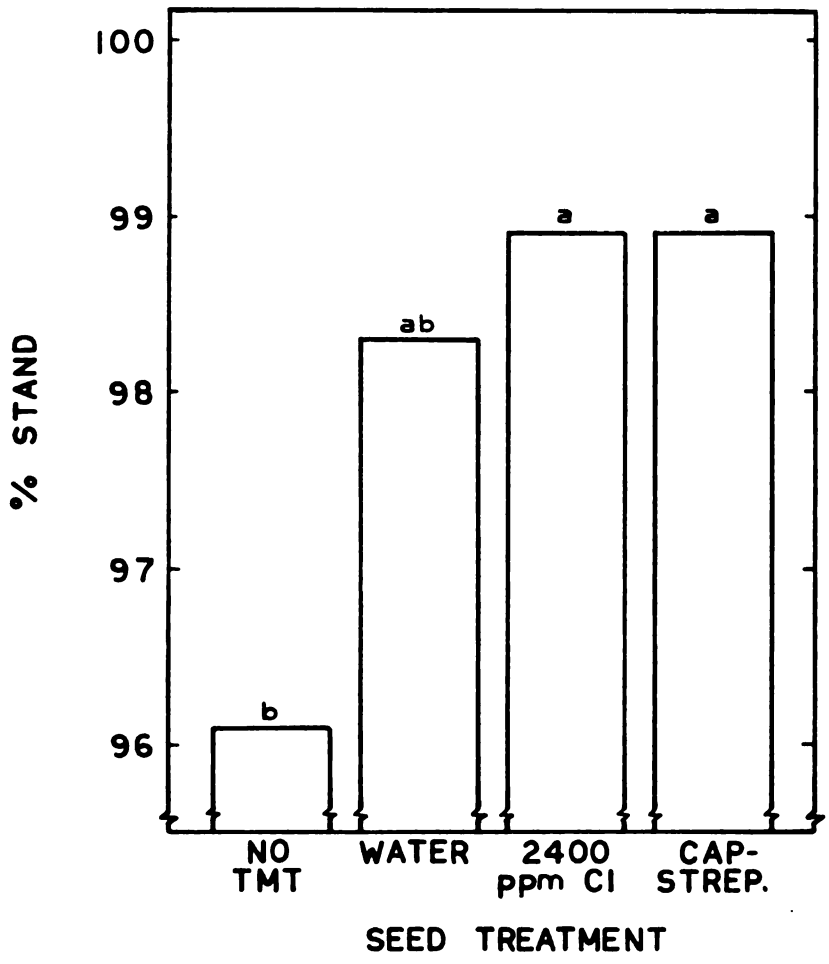

Figure 3.8. Percent stand produced by seed treated with one of 4 seed treatments. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

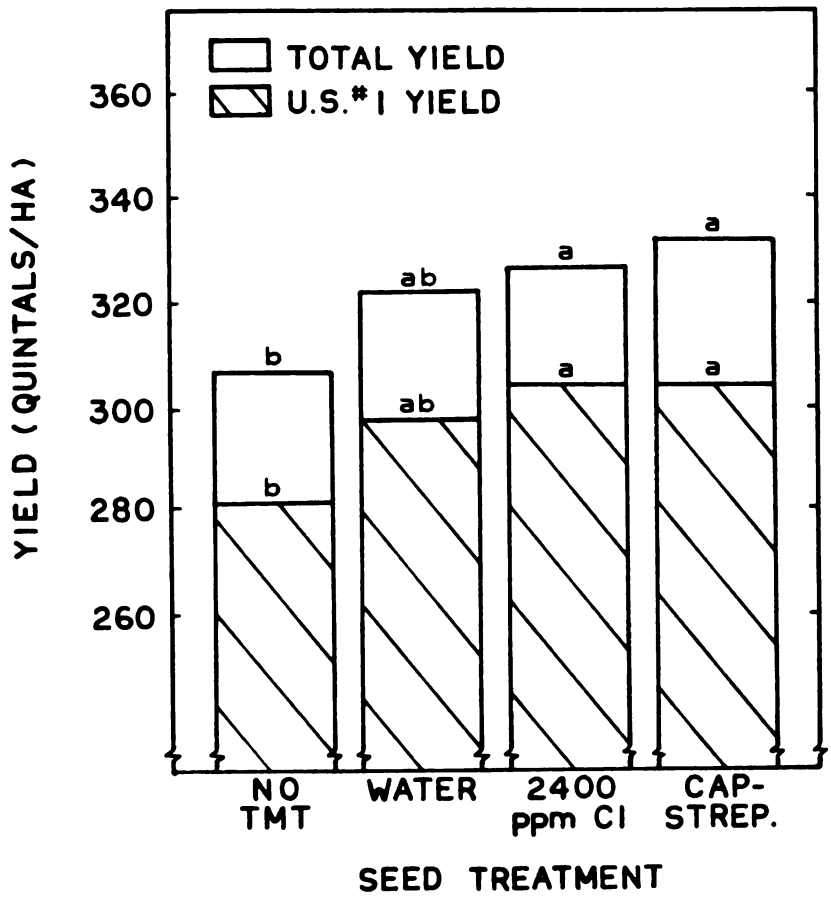

Figure 3.9. Total and U.S. #1 yield produced by seed treated with one of 4 seed treatments. Treatments with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

that in this experiment the washing action of the water was responsible, in part, for the beneficial effect on seed performance of the chlorine wash treatment. There was no significant effect of seed treatment on plant height.

The captan-streptomycin treatment caused a shift in the tuber size distribution, producing a smaller percentage of 51 to 83 mm tubers than the other treatments and a larger percentage of over 83 mm tubers than the untreated and water-treated seed.

Effect of storage treatment. Percent stand, plant height and yield produced by the nine storage treatments were not significantly different. When the experiment was analyzed using planned comparisons to determine main effects of the 3 storage variables, the only significant effect found was due to storage period (Table 3.3). Seed stored 5 days between treatment and planting produced larger total and U.S.#1 yields than seed stored 2 days. The reason for this is not clear; the longer storage period may have allowed the cut surfaces to heal more completely before planting. The observation of no significant effect of storage ventilation suggests that: 1) the plastic bags did not severely restrict ventilation; 2) the seed carried low levels of disease inoculum; 3) the holding period was too short. However, the long storage period and poor ventilation treatment did result in 13% seed-piece decay during storage of untreated seed held at ambient air temperature. A second reason caution must be used in interpreting the

effects of storage treatment is that the storage treatments were not replicated, due to the large amount of seed that would have been required.

SUMMARY.

Under some field conditions, the water and 2000 ppm Cl washes produced poorer stands than the best treatment (usually the captan-streptomycin treatment.) However, the 2000 ppm Cl chlorine wash treatment always resulted in yields as good as the captan-streptomycin treatment. The 500 ppm Cl wash was usually intermediate. Storing chlorine treated seed for up to five days under various environmental conditions did not adversely affect seed performance.

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