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Evaluating Seed Surface Disinfection Methods Using a New Apparatus

Alberta
ENVIRONMENTAL CENTRE

Evaluating Seed Surface Disinfection Methods Using A New Apparatus

by

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ACKNOWLEDGEMENTS

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ABSTRACT

Results of germination experiments can be affected by contamination of seeds by fungi and bacteria. Seed surface disinfection is a useful method of reducing seed contamination. M. Pahl of the Alberta Environmental Centre has developed an apparatus to facilitate the disinfection of many seed lots at one time. This experiment was conducted to evaluate several methods of seed disinfection using this apparatus. Four native grass species, broadglumed wheatgrass (*Agropyron violaceum*), *Festuca brachyphylla*, alpine bluegrass (*Poa alpina*), and spike trisetum (*Trisetum spicatum*) were used. Seeds of each species were subjected to surface disinfection using 0.5, 1.0, 1.5, and 2.0 % sodium hypochlorite (NaOCl) solutions, with and without the surfactant Tween 80. Seeds were immersed for 5, 10, or 15 min. One seed lot of each species was not given any treatment and was used as a control. An alcohol pretreatment prior to surface disinfection was also used for some treatments. In addition, several seed lots that were not surface disinfected were treated with the fungicides Vitaflo 280 Fl (14.9% carbathiin and 13.2% thiram), Vitavax Single Solution (23% carbathiin), and Captan (80% captan). Seeds were germinated on filter paper in petri dishes and germination was recorded until day 28. Treatments were arranged factorially within a randomized complete block design. For species studied in this experiment, a concentration of 2% NaOCl for 10 min provided acceptable surface disinfection without affecting germination. The addition of Tween 80 to the NaOCl solution did not improve disinfection. An alcohol pretreatment prior to disinfection with NaOCl reduced contamination but in some cases it had a detrimental effect on germination rate. Fungicides applied to seed reduced germination and/or germination rate even though they effectively controlled contamination in most cases.

1 INTRODUCTION

The Vegetation Branch at the Alberta Environmental Centre (AEC) is involved in a research program aimed at developing native plant species for use in reclamation of disturbed sites. At present, native alpine grasses are our primary target species. As part of our research on these species, germination experiments are used to study seed viability, dormancy, and factors which are conducive to rapid germination. Contamination of seeds by fungi and bacteria can affect results of germination tests. Disinfection of seed surfaces reduces contamination and ensures accurate germination results. Disinfection can be accomplished by placing sodium hypochlorite (NaOCl) solution and seeds together in a flask, beaker, or petri dish and removing the seeds after the prescribed treatment time (Tuite 1969; Dhingra and Sinclair 1985). Recommended concentrations of sodium hypochlorite (NaOCl) vary from 0.2 to 5% and treatment time varies from 1 min to 10 min (Guthrie 1978; Sauer and Burroughs 1986). To facilitate removal of seeds from the solution, small seed lots are often surface disinfected using a "tea ball" or similar perforated container. Leach (1955) developed a simple seed disinfecting apparatus that could treat two lots of seed simultaneously. If large numbers of seed lots are to be tested in a germination experiment, however, seed surface disinfection can become time consuming. To overcome this problem, M. Pahl of AEC developed an apparatus to surface disinfect with NaOCl solution up to 30 small seed lots at one time.

The objectives of this experiment were to use the disinfection apparatus: 1) to determine the optimum concentration of NaOCl and treatment time for four native grass species: alpine bluegrass (*Poa alpina*), broadglumed wheatgrass (*Agropyron violaceum*), *Festuca brachyphylla*, and spike trisetum (*Trisetum spicatum*); 2) to determine if the addition of Tween 80 to a solution of NaOCl improves the effectiveness of seed surface disinfection; 3) to compare the effectiveness of surface disinfection to the use of fungicides to control fungus growth on germinating seeds; and 4) to study the effect of disinfection/fungicide treatments on germination.

2 MATERIALS AND METHODS

2.1 Surface Disinfection Apparatus - Construction

The 15" x 12" x 2 1/2" (38 cm x 30.5 cm x 6.5 cm) apparatus was constructed from 3/8" 8 (9.5 mm) plexiglass in a fixed grid pattern that holds 30 individual 2" x 2" x 2" (5 cm x 5 cm x 5 cm) removable numbered cubes (Fig. 1). The apparatus was constructed with 2" (5 cm) diameter drainage holes in the bottom of the tray and in the lid, above and below each cube. This allows for easier drainage and prevents trapped air. The removable lid is lined on the inner surface with polyethylene monofilament screening (120 x 102 mesh count per inch, 143 µ mesh opening). Silicone was used to glue the apparatus together.

Each cube was made from 1/8" (3 mm) plexiglass with the bottom and top of the cube left open. Polyethylene screening (as above) was attached to the open bottom. The top rim of each cube was lined with 1/32" (0.8 mm) automotive paper gasket to provide a seal between the cubes and the lid. Five wing-nut screws tightly attach the lid to the tray, effectively sealing each cube.

2.2 Germination Studies

Germination and degree of seed contamination in response to various surface disinfection procedures were studied in four native alpine grasses. Seed age varied with each species due to the availability of seed on hand. Seed of *F. brachyphylla* was one year old, alpine bluegrass and broadglumed wheatgrass seed was two years old, and spike trisetum seed was seven years old. Seeds used in the study were produced at Vegreville, Alberta and were composites of various plant collections from the Alberta Rocky Mountains with the exception of *F. brachyphylla.* In this species, seed was taken from a single line derived from one plant from Sugar Loaf Mountain in southwestern Alberta. Each treatment consisted of 22 seeds and was replicated three times in a randomized complete block design.

The seeds were soaked in 0.5, 1.0, 1.5, and 2.0 % NaOCl solution (made from 5% NaOCl commercial bleach), with and without a surfactant for 5, 10, and 15 min. These levels were chosen to bracket recommendations of 1% chlorine for 10 min (Dhingra and Sinclair 1985). One seed lot was not given any disinfection treatment and was used as a control in the experiment. Each seed lot of 22 seeds was placed in a single cube in the surface disinfection apparatus. The

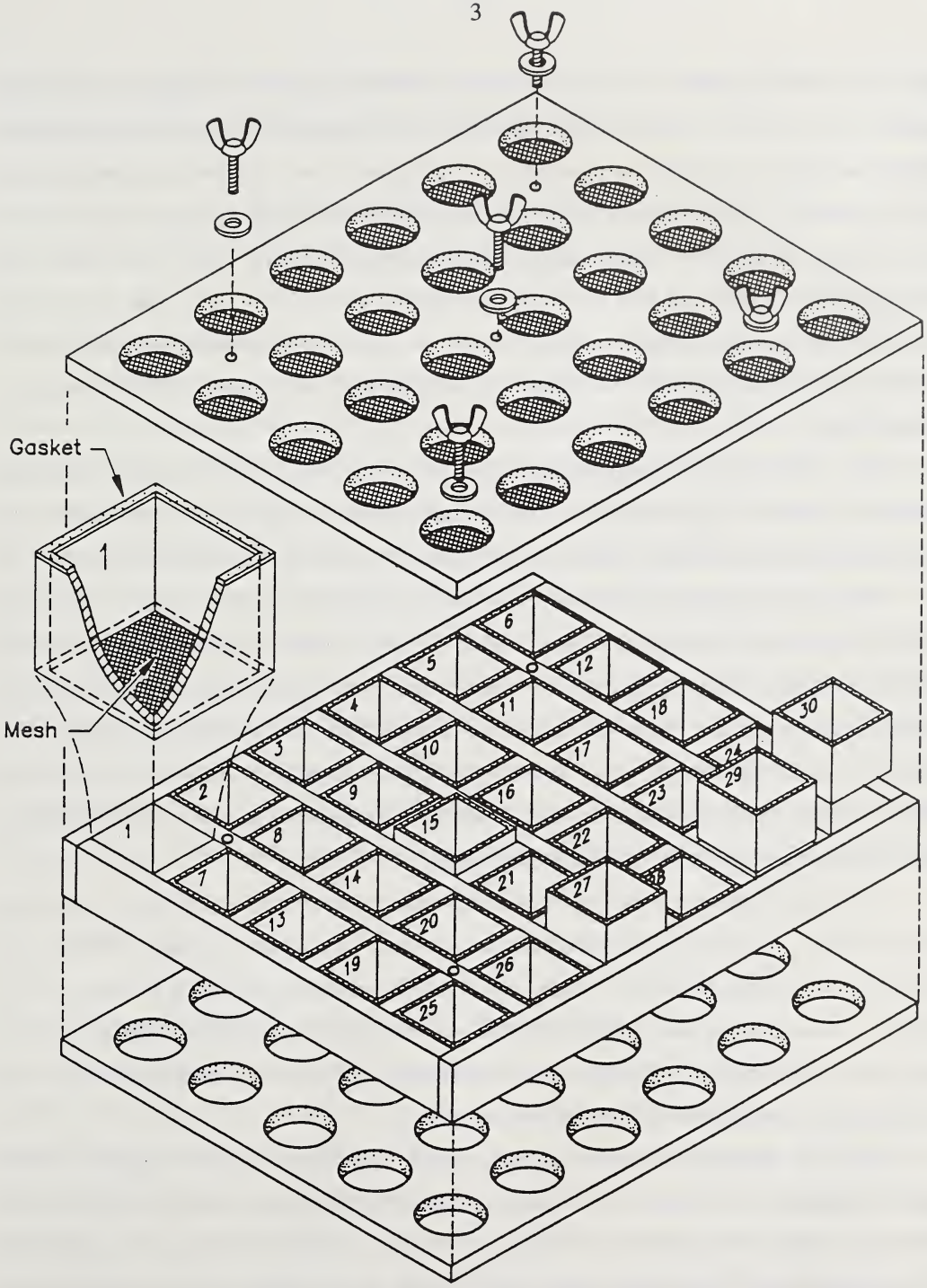

Fig. 1. Seed disinfection apparatus.

apparatus was submerged in 12 L of the treatment solution contained in a large tub. All three replicates were treated simultaneously and this was repeated for each of the prescribed submersion times and NaOCl concentrations. The apparatus was washed and dried between different solutions. While submerged, the apparatus was gently tapped against the bottom of the tub to dislodge air bubbles. Upon removal, seeds, while still in their cubes, were rinsed with sterilized distilled water and then set to dry in a laminar flow hood. Each seed lot was then transferred using sterile technique from the cube to a petri dish containing three sterilized Whatman #2 filter papers. Petri dishes were arranged on trays and watered with 6 ml sterilized distilled water.

For treatments in which a surfactant was used with NaOCl, Tween 80 was added to the solution at a rate of 2 drops per 12 L with an added drop of Zap, an anti-foam agent. All disinfection treatments without surfactant were carried out prior to treatments with Tween 80.

The use of alcohol (95% denatured ethanol) was also tried as a means of improving surface disinfection by increasing contact between the seed surface and the solution. For the alcohol treatments, the entire apparatus containing seeds was immersed first in alcohol and then in NaOCl solution for the prescribed time. The alcohol pretreatment was used in conjunction with 0.5% NaOCl for 5 and 10 min. Because alcohol did not appear to reduce the amount of bubbles attached to the seeds when in NaOCl, further concentrations of NaOCl and submersion times were not tested with the alcohol pretreatment.

Fungicide treatments were also made at this time to another set of petri dishes containing untreated seed. Three fungicide formulations were used as alternative means of controlling contamination during germination: Vitaflo 280 Fl (14.9% carbathiin and 13.2% thiram) at 0.1 % solution, Vitavax Single Solution (23% carbathiin) at 0.13 % percent solution, and Captan (80% captan) at 0.13% suspension. For each fungicide treatment, 6 ml were added to a petri dish with no additional water given at the start of the experiment.

Sterilized distilled water was regularly added to all petri dishes as required. Each replicate consisted of four trays with treatments randomized throughout and replicates blocked within a germination chamber set at 22°C day / 15°C night, 16 h day. Numbers of contaminated and germinated seed were recorded three times weekly for two weeks then every four to five days until day 28. Seeds were discarded as they germinated. Contaminated seeds were considered to be those with fungal growth prior to germination. Germination index (GI), an

indication of germination rate, was calculated using the formula of Acharya (1989). For any day N of the germination experiment, GI is calculated as follows:

$$GI = \frac{\sum_{n=1}^{N}[(No.\ of\ seeds\ germinated\ by\ day\ n) \times n]}{Total\ number\ of\ seeds\ germinated\ at\ day\ N}$$

2.3 Statistical Analysis

The response of percent germination, GI, and percent contamination of each species to NaOCl concentration and treatment time was examined simultaneously using the response surface procedure (Proc RSREG) of the SAS Statistical Package (SAS Institute 1987). When this response surface was significant, the optimum levels (considering only those levels used in the experiment) of NaOCl concentration and treatment time were determined by finding the highest predicted values of percent germination. These optimum levels, or an average of all levels when the response surface analysis was not significant, were then used to compare the use of NaOCl and NaOCl plus Tween 80 to the use of alcohol plus NaOCl and the fungicides Captan, Vitavax, and Vitaflo. For NaOCl with an alcohol pretreatment, the average of the two treatment times was used. An analysis of variance (randomized complete block design) procedure was used to detect differences among treatments and Duncan's new multiple range test was used to compare treatment means. The SAS Statistical Package (Proc ANOVA) was used for these analyses.

3 RESULTS

3.1 Response to NaOCl Concentration and Treatment Time

Germination of broadglumed wheatgrass seeds responded significantly to changes in NaOCl concentration and treatment time when no surfactant was used (Fig. 2). However, this was not the case when Tween 80 was added to the disinfecting solution (Fig. 3). Response of GI to NaOCl concentration and treatment time was significant for NaOCl alone (Fig. 4) but not for NaOCl plus Tween 80 (Fig. 5). Similarly, a significant response was observed for percent contamination when no surfactant was added (Fig. 6) but not with Tween 80 in the solution (Fig. 7). The optimum treatment was 2% NaOCl alone with the seeds immersed in solution for 10 min. This treatment combination led to the highest predicted percent germination (63.1%).

Although predicted percent contamination was lower at 1.5% NaOCl and 10 min and predicted GI was higher at 2% NaOCl and 5 min, the predicted percent germination was lower at these treatment combinations.

No significant responses to NaOCl concentration and time of immersion in solution were observed for *Festuca brachyphylla* when NaOCl was used alone or in combination with Tween 80 (Fig. 8-13). Germination of seeds of this species was high with values of close to 100% for most treatment combinations (Fig. 8, 9). GI was also high (Fig. 10, 11) and contamination was less than 40% for all treatment levels (Fig. 12, 13).

No significant response surfaces were observed for alpine bluegrass seeds, with and without Tween 80 in the NaOCl solutions (Fig. 14-19). Percent germination and GI were relatively high (Fig. 14-17) and percent contamination was again less than 40% for all treatments (Fig. 18, 19).

For spike trisetum there was no significant germination response to concentration and time when NaOCl was used alone in the solution (Fig. 20). A significant germination response was observed, however, when Tween 80 was included with NaOCl (Fig. 21). The optimum levels, as determined from predicted values of percent germination, were 2% NaOCl and 15 min. GI and percent contamination were not significantly affected by concentration and time, with or without Tween 80 in the surface disinfection solution (Fig. 22-25). Spike trisetum seeds had few contaminants, with percent contamination less than 10% in most cases (Fig. 24, 25).

3.2 Comparison of NaOCl Treatments and Fungicides

Seed surface disinfection or fungicide treatments did not significantly affect percent germination of broadglumed wheatgrass seeds (Fig. 26). However, the coefficient of variation for this character was high (33%) and large differences in means (49% for NaOCl compared to 13% for alcohol plus NaOCl) were not significant. All treatments significantly lowered GI compared to distilled water, with the alcohol plus NaOCl treatment having the lowest index. Seeds receiving only distilled water had a high incidence of contamination (91%) before seed germination (Fig. 26). All other treatments except Captan had significantly lower percent contamination than distilled water. Vitaflo was most effective in controlling seed contamination.

Germination of *Festuca brachyphylla* seeds was greater than 95% for all treatments with no significant differences among treatments (Fig. 27). Vitaflo significantly decreased GI

compared to all other treatments and Captan reduced GI compared to distilled water. All treatments significantly decreased percent contamination compared to distilled water, with Vitaflo completely eliminating seed contamination.

In alpine bluegrass, seeds treated with Vitavax had a lower percent germination (47%) than seeds subjected to all other treatments except Vitaflo (Fig. 28). The use of Vitaflo significantly reduced germination (to 13%) compared to all other treatments. GI, however, was not significantly affected by seed treatment. Vitaflo completely eliminated seed contamination in alpine bluegrass (Fig. 28), and this was significantly better control than the other treatments.

Percent germination of spike trisetum seeds was significantly affected by seed treatment (Fig. 29). Seeds germinated in distilled water and with Captan had significantly higher germination than seeds treated with Vitaflo and Vitavax. Vitaflo and Vitavax also significantly reduced the rate of germination (GI) compared to all other treatments. Percent contamination of spike trisetum seeds, however, was less than 10% for all treatments (Fig. 29).

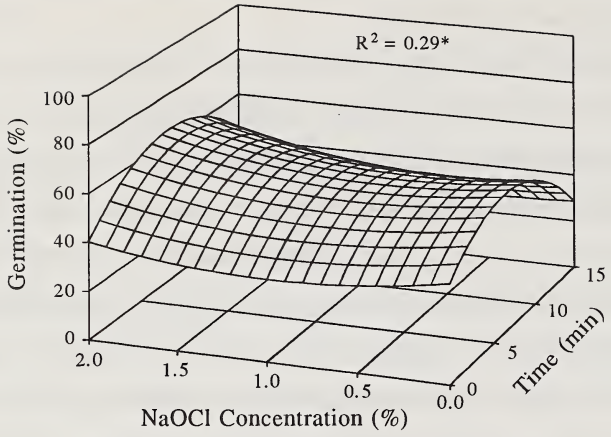

Fig. 2. Germination response of broadglumed wheatgrass seeds to NaOCl concentration and treatment time. No surfactant was added. * = Significant at $P = 0.05$.

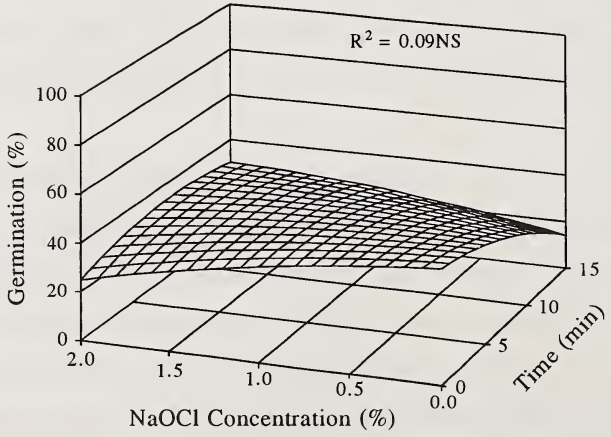

Fig. 3. Germination response of broadglumed wheatgrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

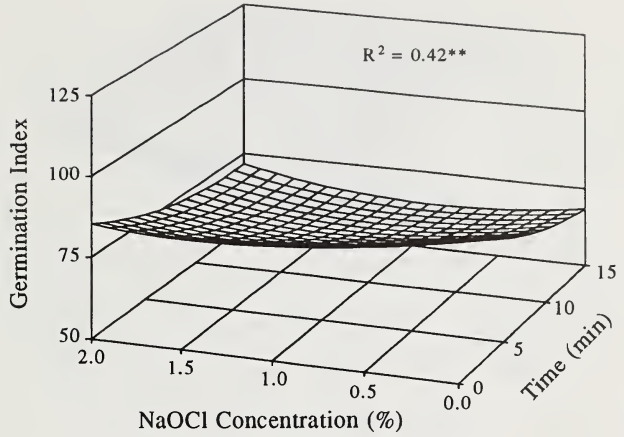

Fig. 4. Response of germination index in broadglumed wheatgrass seeds to NaOCl concentration and treatment time. No surfactant was added. ** = Significant at $P = 0.01$.

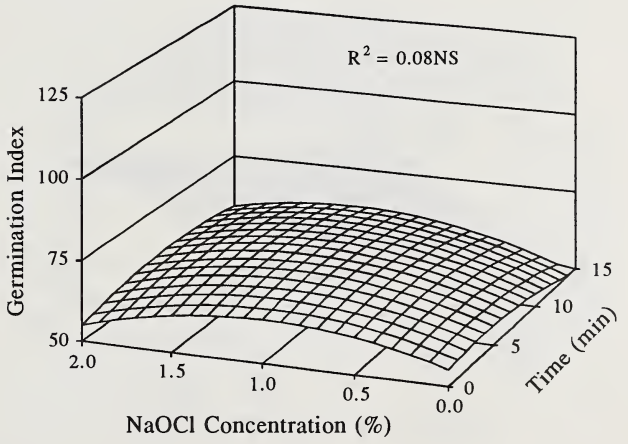

Fig. 5. Response of germination index in broadglumed wheatgrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

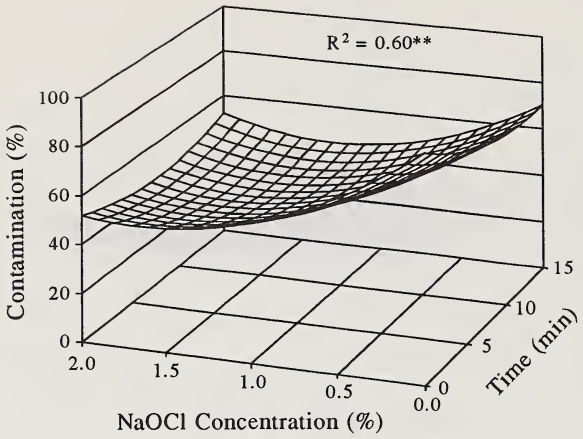

Fig. 6. Response of percent contamination in broadglumed wheatgrass seeds to NaOCl concentration and treatment time. No surfactant was added. ** = Significant at $P = 0.01$.

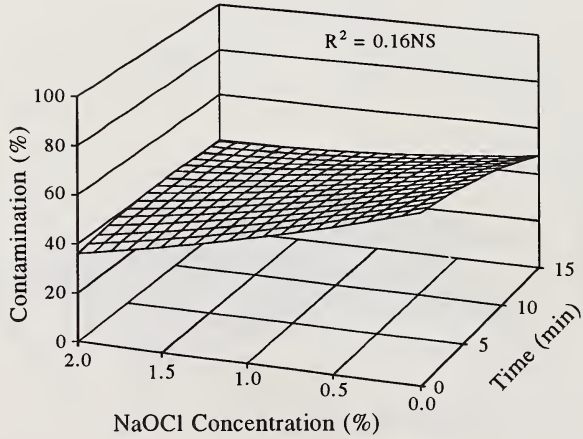

Fig. 7. Response of percent contamination in broadglumed wheatgrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

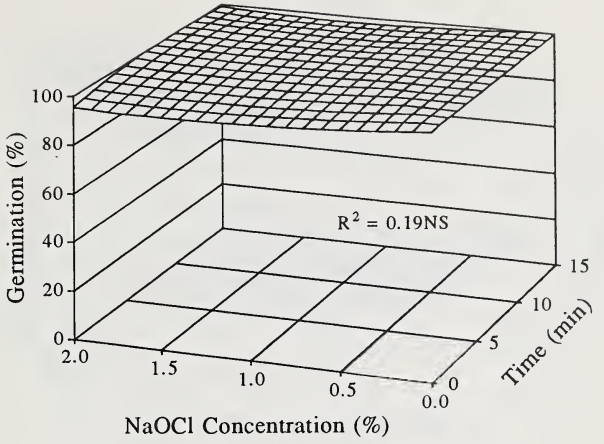

Fig. 8. Germination response of *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

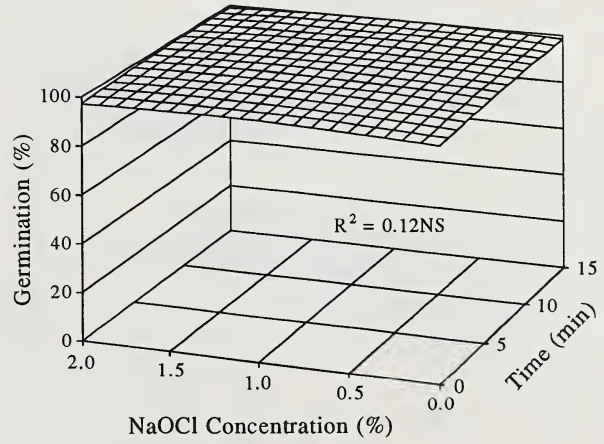

Fig. 9. Germination response of *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

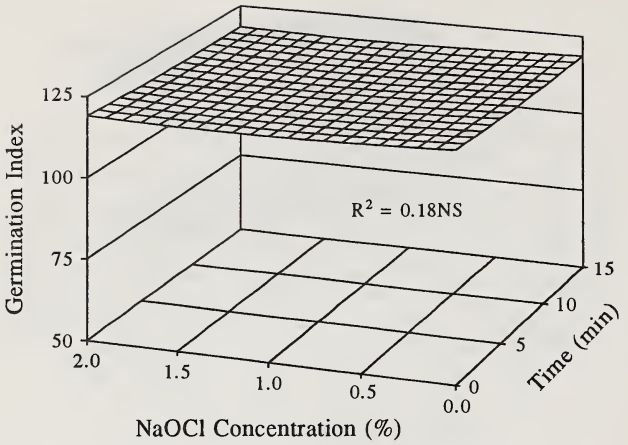

Fig. 10. Response of germination index in *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

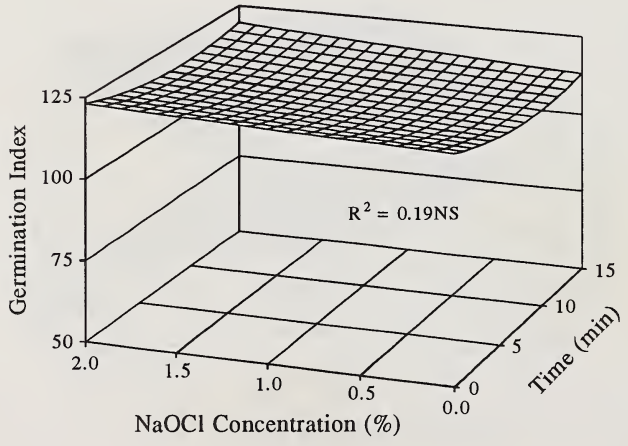

Fig. 11. Response of germination index in *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

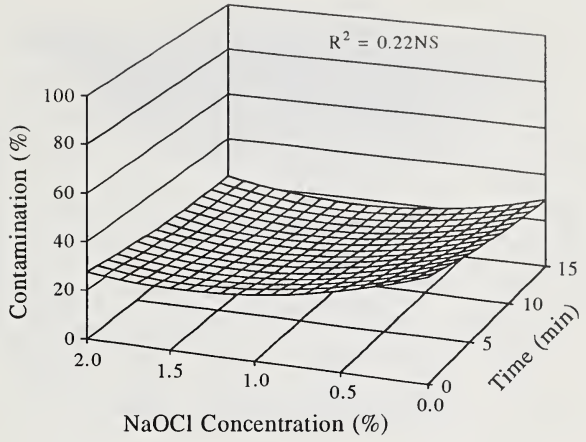

Fig. 12. Response of percent contamination in *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

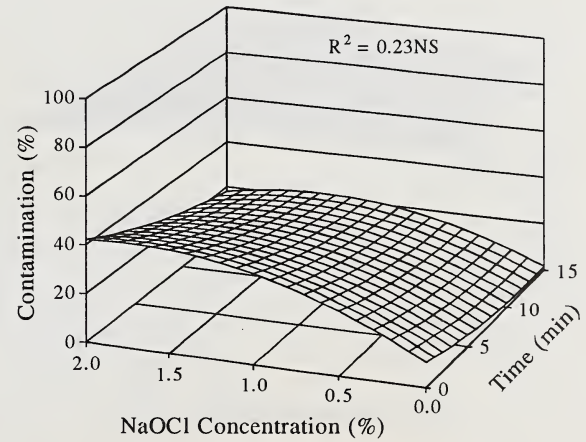

Fig. 13. Response of percent contamination in *Festuca brachyphylla* seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

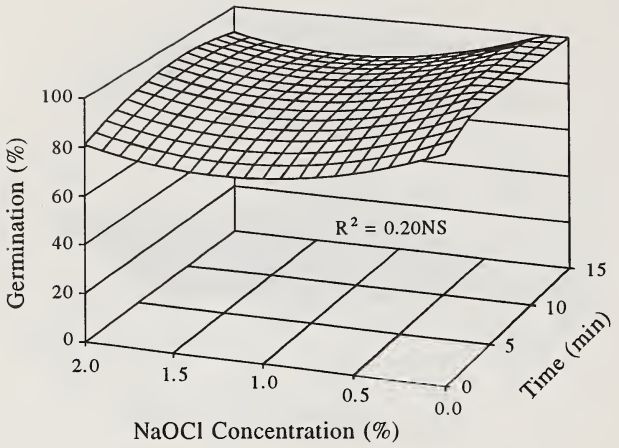

Fig. 14. Germination response of alpine bluegrass seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

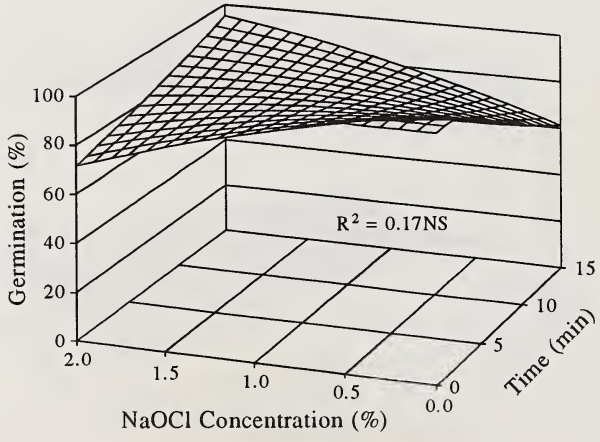

Fig. 15. Germination response of alpine bluegrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

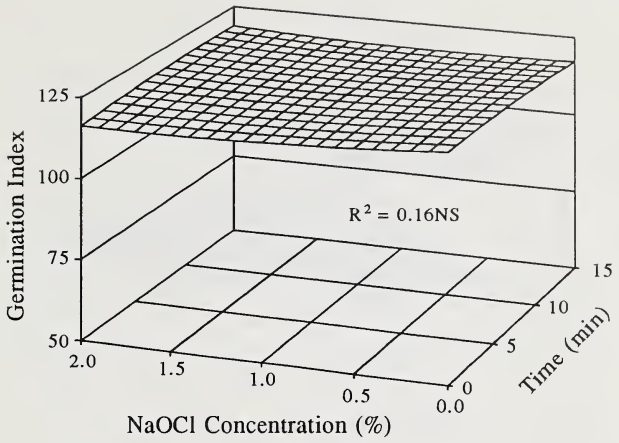

Fig. 16. Response of germination index in alpine bluegrass seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

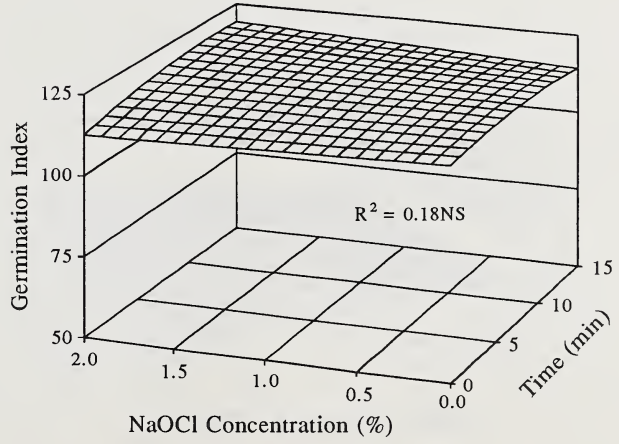

Fig. 17. Response of germination index in alpine bluegrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

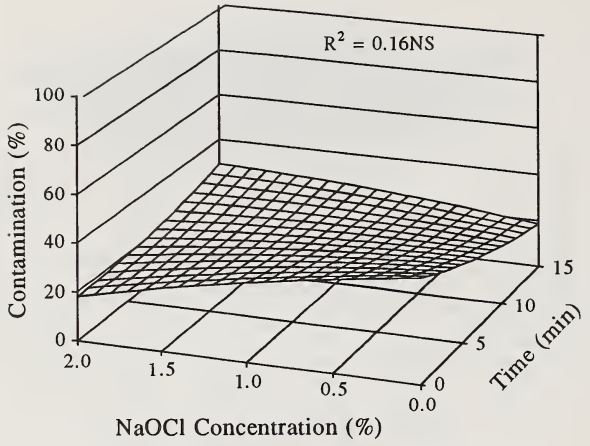

Fig. 18. Response of percent contamination in alpine bluegrass seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

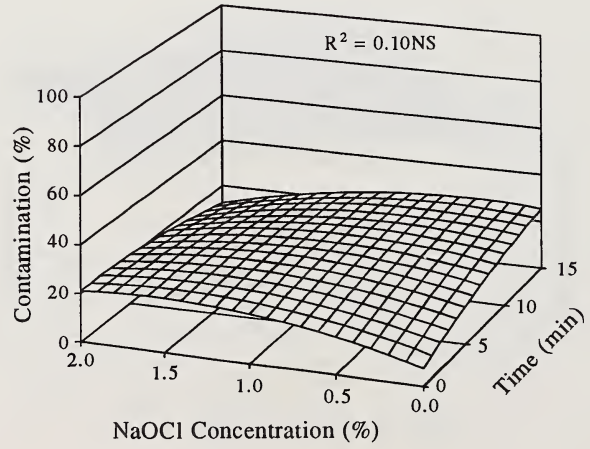

Fig. 19. Response of percent contamination in alpine bluegrass seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

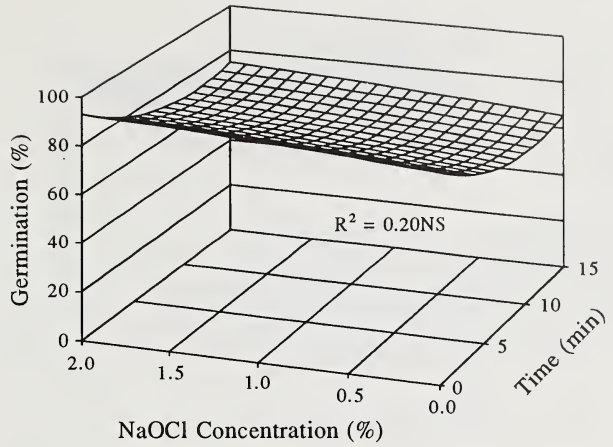

Fig. 20. Germination response of spike trisetum seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

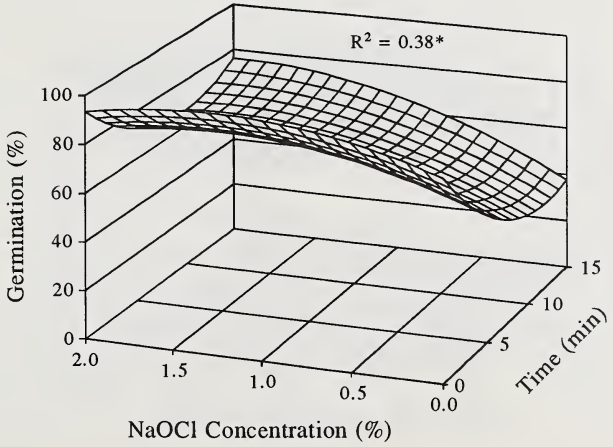

Fig. 21. Germination response of spike trisetum seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. * = Significant at $P = 0.05$.

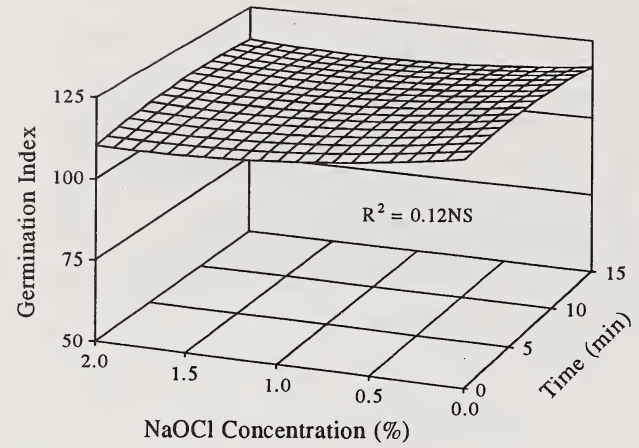

Fig. 22. Response of germination index in trisetum spicatum seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

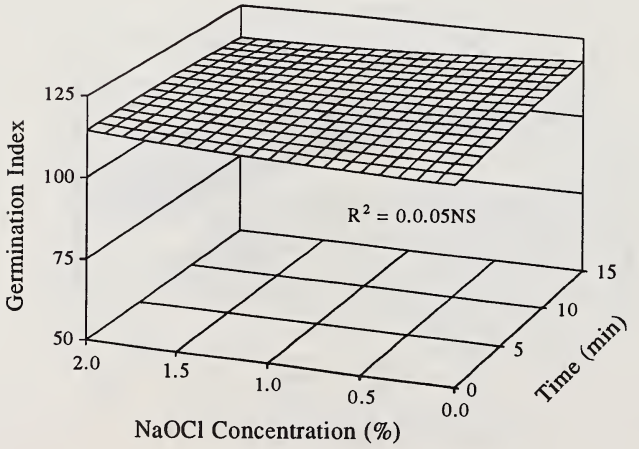

Fig. 23. Response of germination index in spike trisetum seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

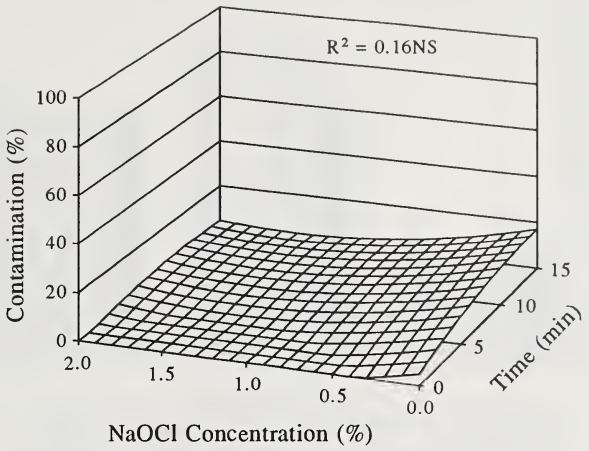

Fig. 24. Response of percent contamination in spike trisetum seeds to NaOCl concentration and treatment time. No surfactant was added. NS = Not significant at $P = 0.05$.

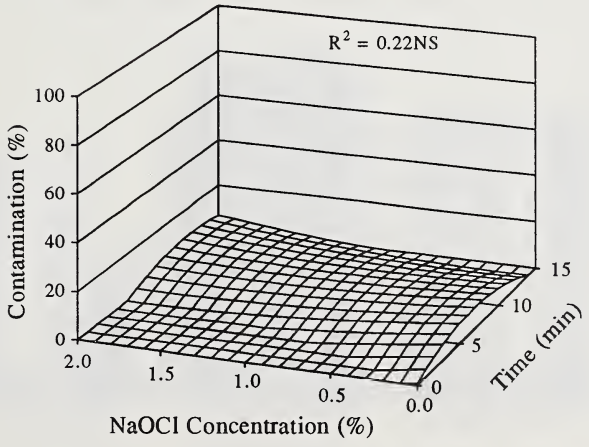

Fig. 25. Response of percent contamination in spike trisetum seeds to NaOCl concentration and treatment time. Tween 80 was added as a surfactant. NS = Not significant at $P = 0.05$.

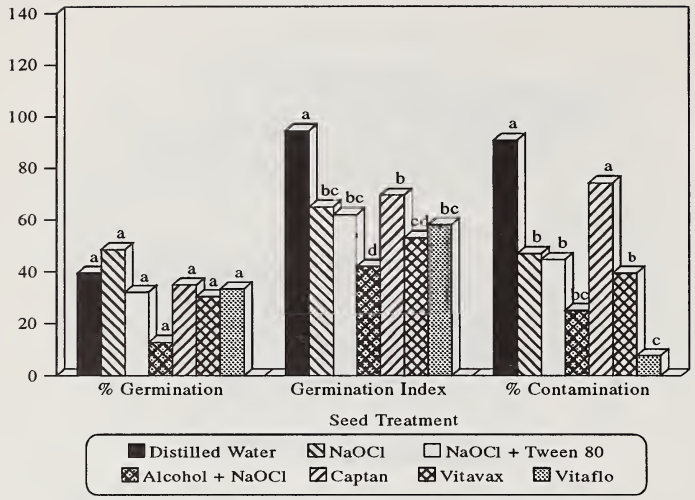

Fig. 26. Effect of seed treatment on germination, germination index, and contamination of broadglumed wheatgrass seeds. Within groups, bars with the same letter are not significantly different by Duncan's New Multiple Range Test.

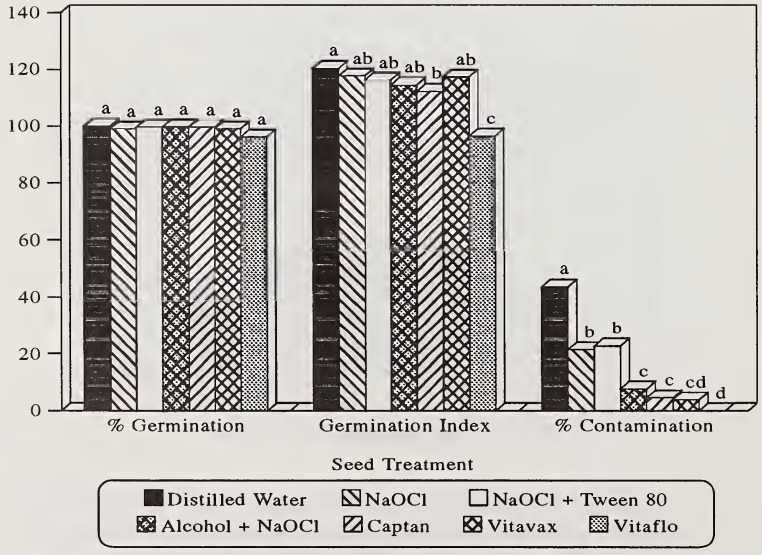

Fig. 27. Effect of seed treatment on germination, germination index, and contamination of *Festuca brachyphylla* seeds. Within groups, bars with the same letter are not significantly different by Duncan's New Multiple Range Test.

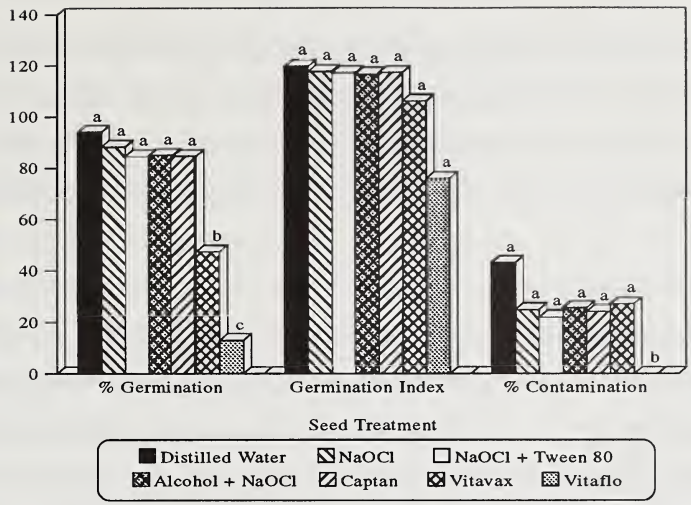

Fig. 28. Effect of seed treatment on germination, germination index, and contamination of alpine bluegrass seeds. Within groups, bars with the same letter are not significantly different by Duncan's New Multiple Range Test.

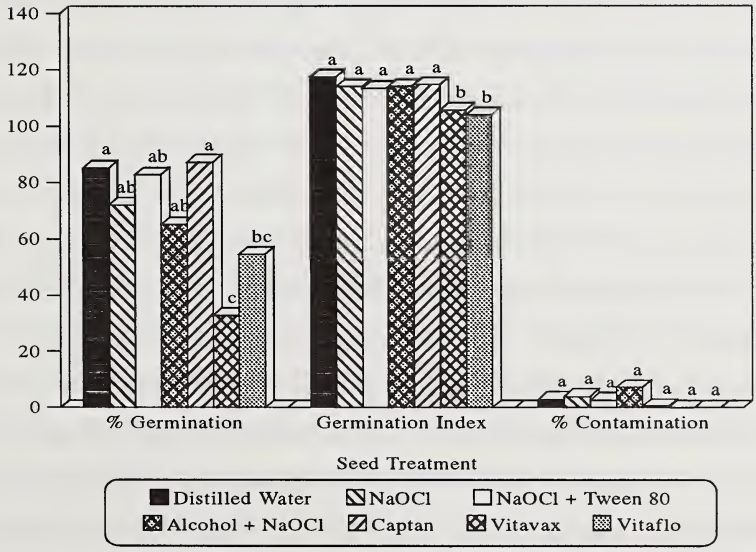

Fig. 29. Effect of seed treatment on germination, germination index, and contamination of spike trisetum seeds. Within groups, bars with the same letter are not significantly different by Duncan's New Multiple Range Test.

4 DISCUSSION

Seed contamination can adversely affect germination results and reduce seedling vigour. Although it can be a time consuming procedure, seed surface disinfection reduces seed contamination and improves accuracy in germination experiments. The apparatus developed at AEC reduces the time required for this procedure. In many native plant species and domestic perennial forages, whole seeds must be selected and counted using a seed light table. Using the 'tea ball' method of disinfection (one seed lot at a time), seeds were selected and counted using the light table, grouped together, disinfected, and then recounted within the laminar flow hood into petri dishes or other containers for treatment. Using the disinfection apparatus, seeds are counted only once directly into cubes from the seed light tables, surface disinfected and then easily transferred within the laminar flow hood to their treatment containers. This also decreases the chance of recontaminating the seeds by reducing handling. The apparatus also allows for numerous seed lots (different species, varieties, harvest dates, fields, locations, etc.) to be surface sterilized simultaneously. This decreases not only the time required for the procedure but also the variability among seed lots.

In all four species, the use of NaOCl, alone or in combination with Tween 80 or alcohol, did not significantly affect percent germination compared to distilled water. Other researchers have also shown that NaOCl did not adversely affect seed germination (Mohapatra et al. 1986; Basky and Aponyi 1987; Elmer and Stephens 1988). In broadglumed wheatgrass, however, GI was negatively affected by NaOCl treatments. Although rate of germination may have been reduced by the use of NaOCl, disinfection could still be beneficial as seedling vigour may have been affected by seed contamination. Seed contamination in broadglumed wheatgrass was very high (greater than 90% in distilled water) but the analysis of variance procedure showed that the use of NaOCl, alone or in combination with Tween 80 or alcohol, reduced contamination to less than 50%. In *Festuca brachyphylla*, NaOCl treatment reduced percent contamination from 43% to less than 23%. The alcohol plus NaOCl treatment was the most effective of the NaOCl treatments, reducing contamination to less than 8%. In seed lots such as the spike trisetum seed where seed contamination was very low (less than 10%) and therefore would have little effect on germination or seedling vigour, the use of seed surface disinfection may not be necessary.

Trapped air bubbles can be a problem when surface disinfecting seeds, especially seeds with hulls. A partial vacuum can be applied to reduce air bubbles. When the vacuum procedure is impractical, an ethanol pretreatment can replace the procedure with similar results (Sauer and Burroughs 1986). In this experiment, the use of an alcohol pretreatment did not seem to reduce the number of air bubbles. The alcohol treatment also had a negative effect on GI in broadglumed wheatgrass. Only in *Festuca brachyphylla*, did it reduce contamination beyond that of NaOCl alone.

Surfactants can also be used to reduce air bubbles and improve contact between seed surface and NaOCl (Elmer and Stephens 1988). However, a surfactant such as Tween 80 may inhibit or stimulate plant growth (McWhorter 1985). Dunaeva et al. (1991) reported that soaking wheat seeds in a 2% solution of Tween 80 stimulated germination by increasing the rate of water uptake by the seeds. Hurtt and Hodgson (1987) found that Tween 20 and Tween 80 stimulated germination of barnyardgrass seeds at concentrations as low as 0.05% but inhibited germination of redroot pigweed seeds. In the present experiment, very low concentrations of Tween 80 were used and there were no significant differences between NaOCl plus Tween 80 and NaOCl alone for percent germination, GI, and percent contamination in all four species.

Response to NaOCl concentration and seed immersion time in NaOCl solution varied with species and with the presence or absence of Tween 80. In broadglumed wheatgrass, there were significant responses to NaOCl concentration and time when NaOCl was used alone but not when Tween 80 was added to the disinfecting solution. This may have been a result of increased variability when Tween 80 was added. The optimum concentration, based on germination response, was 2% NaOCl and optimum time was 10 min. In spike trisetum, percent germination responded significantly to concentration and time when Tween 80 was included but not when NaOCl was used alone. The optimum concentration was 2% NaOCl plus Tween 80 with seeds immersed for 15 min. Concentrations of 0.2 to 5% NaOCl and treatment times of 1 to 10 min are commonly used for disinfection of seed surfaces (Guthrie 1978; Dhingra and Sinclair 1985; Sauer and Burroughs 1986). The optimum values for broadglumed wheatgrass and spike trisetum fall within this range but we have no explanation for the apparent contradiction in the effect of Tween 80 on these species. For alpine bluegrass and *Festuca brachyphylla*, no significant responses to NaOCl concentration and treatment time were observed. Lack of significant differences in these species may have been due to high variability within the experiment since

only three replications were used. Higher concentrations of NaOCl than were used in this experiment may reduce germination in these species.

Sodium hypochlorite has been shown to be effective in releasing dormancy in wild oat seeds (Hsiao and Quick 1984) and in green needlegrass (Frank and Larson 1970). In this study, however, NaOCl did not increase germination compared to seeds treated with water in any of the four species. This may be related to our use of lower concentrations of NaOCl and shorter immersion times compared to the above studies. Although 1% NaOCl for 5 min (similar to the concentrations and times used in this experiment) stimulated germination of pepper seed (Fieldhouse and Sasser 1975), the authors did not indicate if this was related to dormancy or some other factor.

The effect of fungicides on contamination varied with species and fungicide. Vitaflo was most effective in controlling contamination but it significantly reduced germination and/or GI in some species. With the exception of *Festuca brachyphylla*, Vitavax did not control contamination better than the NaOCl treatments and it negatively affected germination and/or GI in three species. Captan controlled contamination in only *Festuca brachyphylla* and reduced GI in two species. The phytotoxic effect of fungicides as observed in this experiment is common when fungicides are used to control seed-borne fungi when germinating seeds on filter paper (de Tempe 1970). Injury from such fungicides is reduced when seeds are germinated in soil because the fungicide is diluted and adsorbed by the soil.

5 SUMMARY

The seed disinfection apparatus developed at AEC is effective in simultaneously exposing many seed lots to a disinfecting solution and can save much time when preparing large germination experiments. The apparatus may also be useful in plant pathology when disinfecting seed surfaces to study microflora within seeds. For the species studied in this experiment, immersion of seeds in a solution of 2% NaOCl for 10 min provided acceptable seed surface disinfection without affecting germination. The addition of Tween 80 to the NaOCl solution did not improve disinfection. Alcohol in combination with NaOCl did reduce contamination but in some cases it also had a negative effect on germination index. Fungicides applied to the seed

reduced germination and/or germination index even though they effectively controlled contamination in most cases.

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