

# Impact of sodium hypochlorite concentration and exposure period on germination and radicle elongation of three annual weed species

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## Summary

This study examined the effect of pre-soaking seeds in sodium hypochlorite (NaOCl) for different periods and at varying concentrations on germination and radicle elongation. Seeds of *Amaranthus powellii*, *Setaria faberi* and *Abutilon theophrasti* were subjected to 0, 0.6 and 6.0% NaOCl for 0, 5, 30 and 60 seconds. After 14 days, *A. powellii* seed germination was highest in the 6.0% NaOCl treatment (80%) and lowest in the control treatment (10%). Germination increased in *A. powellii* as the soaking period increased, but declined for seeds soaking for more than 60 sec. In contrast, germination of *S. faberi* seeds was unaffected by NaOCl: both seeds in the control treatment and seeds soaked in NaOCl had 80% germination. Increases in concentration of NaOCl decreased overall seed germination velocity in *S. faberi*. Finally, *A. theophrasti* exhibited no discernable effect of NaOCl treatment on seed germination. For all three species, radicle elongation of seedlings in the control treatments was greater than seedlings in the NaOCl treatments. The species specific- responses obtained following exposure of seeds to NaOCl in this study suggests that caution should be used when interpreting germination and seedling vigor studies involving seeds soaked in NaOCl solution for periods extending beyond five seconds.

## Introduction

Unexpected seed loss due to microbial infection may have a substantial impact on results of seed germination studies. Natural levels of fungal infection on the seed surface of 0 to 30% have been reported in several studies (Dhingra *et al.*, 2002; Lori and Salerno, 2002; Toma and Boya, 1999). These levels of infection and subsequent seed loss may have significant implications for results of germination related studies depending on the total number of seeds used and level of replication. Researchers have attempted to overcome this limitation by removing harmful fungi and bacteria through surface sterilization of seeds prior to seed storage or use in germination experiments. Baskin and Baskin (1998) recommend that care be taken to ensure that only fully mature seed stocks are used in experiments and further advocate that seeds to be used in germination trials not be pre-treated with disinfecting agents. Despite these calls for caution, many researchers continue to surface sterilize seeds. Sodium hypochlorite (NaOCl), the active ingredient in household bleach, is the most widely used seed-sterilizing product. It is therefore important to determine the impact of seed sterilization using NaOCl on seed

viability, dormancy status and subsequent growth of seedlings. This is especially critical for studies specifically assessing the effect of one or multiple factors on seed germination and seedling growth.

In seed germination studies, NaOCl has been used as either a seed surface sterilant, or to relieve dormancy. Exposure time and concentration of NaOCl in these studies has varied from seconds to hours and at concentrations from 1 to 6% active ingredient, respectively, depending on the specific objectives of the research.

Surface sterilization of seeds with NaOCl has been carried out in numerous studies. For instance, Dhingra *et al.* (2002) immersed seeds of *Anadenanthera macrocarpa* (Benth.) Brenan for 1 min. in 2.5% NaOCl followed by another minute in 70% ethanol in an attempt to remove several seedborne pathogenic fungi. González-Benito *et al.* (1998) used a 10% commercial bleach solution (~0.5% NaOCl) to surface-sterilize seeds of *Centaurea hyssopifolia* Vahl, a species whose seeds exhibit physiological dormancy (Baskin and Baskin, 1998). The bacterial pathogen *Erwinia carotovora* ssp. *carotovora* (Jones) Berez *et al.* was removed from the surface of pepper (*Capsicum annuum* L.) seeds following exposure to 1.0% NaOCl for 30 seconds (Hadas *et al.*, 2001). In all of the above studies, it is especially surprising that no consideration was given to the impact of pre-treating seeds with NaOCl on actual germination levels obtained.

Exposure duration of seeds to NaOCl is quite variable throughout the literature, ranging from seconds to many hours. Hadas *et al.* (2001) surface sterilized pepper seeds with 1.0% NaOCl for 30 seconds which was sufficiently long to remove harmful pathogens from the seed coat. The authors made no comment, however, on the effect this exposure to NaOCl may have had on the resulting percentage germination. McCollum and Linn (1955) found that increased exposure durations of pepper seeds to NaOCl reduced their rate and percentage germination. Exposure times ranging from 1 to 5 minutes were found to significantly increase germination of lentil seeds, by reducing the percentages of infectious fungi found on seed surfaces (Toma and Boya, 1999). Hsiao *et al.* (1979a) tested the viability of *Avena fatua* L. seeds with 6% NaOCl for times ranging from 0 to 24 hours and demonstrated that imbibed seeds were affected almost immediately (<30 min) by the treatment, whereas dry seeds required more than 3 hours of exposure before any influence on the embryo was observed. After 24 hours of exposure to NaOCl the seed hulls were completely degraded. Additionally, Frank and Larson (1970) found that exposure for only 1 hour of *Stipa viridula* Trin. seeds to 3.2% NaOCl was sufficient to increase germination and degrade the lemma and palea.

Findings from numerous studies have shown that NaOCl pre-treatment of seeds can influence viability, germination and, often relieve dormancy in seeds of many species. Following exposure to NaOCl, physiological dormancy (i.e., seeds possessing a physiological mechanism preventing the radicle from emerging through the seed coat (Baskin and Baskin, 1998)) was relieved and percentage germination increased for species such as *Polygonum convolvulus* L. (Hsiao, 1979b), *Saponaria vaccaria* L. (Hsiao, 1979b), *Thlaspi arvense* L. (Hsiao, 1980), *Stipa viridula* Trin. (Frank and Larson, 1970), *Striga asiatica* (L.) Kuntze (Hsiao and Hanes, 1981), *Capsicum annuum* L. (Fieldhouse and Sasser, 1975) and *Panicum virgatum* L. (Haynes *et al.*, 1997). Nadeem *et al.* (2000) reported a 5-fold increase in germination for *Podophyllum hexandrum* Royle following

exposure to NaOCl, a species with morphophysiological dormancy, a condition where the embryo must increase in size in addition to overcoming physiological dormancy in order for the seed to germinate (Baskin and Baskin, 1998). Interestingly, exposure to NaOCl had deleterious or no effect on other species exhibiting physiological dormancy including *Brassica kaber* (DC.) Wheeler (Hsiao, 1980), *Brassica chinensis* L. (Ferreira and Ranal, 1999), *Sorghastrum nutans* (L.) Nash (Watkinson and Pill, 1998) and *Cypripedium reginae* Walter (Vujanovic *et al.*, 2000).

Subjecting seeds of *Leucaena leucocephala* (Lam.) deWit. to NaOCl had no effect on their germination or dormancy (Mesa *et al.*, 1998). Seeds of this species exhibit physical dormancy.

Pre-treatments with NaOCl to improve germination and to test seed viability have also been developed for many crop species including *Solanum muricatum* Ait. (Prohens *et al.*, 1999), *Capsicum annuum* L. (Hadas *et al.*, 2001), *Spinacia oleracea* L. (Katzman *et al.*, 2001) and *Oryza sativa* L. (Chun *et al.*, 1997).

In contrast to seed germination, the effects of NaOCl concentration and exposure time on radicle elongation have not been extensively examined. Ferreira and Ranal (1999) demonstrated that *B. chinensis* seedlings were sensitive to NaOCl resulting in significant reductions in the length of hypocotyls and roots. Conversely, Chun *et al.* (1997) found a direct stimulation of *O. sativa* seedling growth after exposure of seedlings to 2.6% NaOCl for 3 days.

From a review of the literature, the effects of NaOCl concentration and exposure duration on seed germination and early seedling growth may be species-dependent and linked to dormancy status of seeds. Hence, the specific objectives of this study were to determine the effects of NaOCl concentration and exposure time on percentage seed germination, radicle elongation and germination velocity for three annual agricultural weeds whose seeds exhibit contrasting forms of dormancy, namely non-deep physiological dormancy (*Amaranthus powellii* S. Wats. and *Setaria faberi* Herrm.) and physical dormancy (*Abutilon theophrasti* Medic.)

## Materials and methods

Mature inflorescence samples of *Amaranthus powellii*, *Setaria faberi* and *Abutilon theophrasti* were harvested in August 2001 from plants in a single location near Aurora, NY, USA. Plants from each species had been growing under similar conditions in a fallow area adjacent to a maize field. Samples were immediately stored dry in brown paper bags at a constant 5°C and 50% RH. The samples remained in storage for approximately two months. Upon removal from storage, samples were cleaned by sifting through various sized sieves.

### Seed germination trial

Seeds of each species were randomly separated into samples of 30 seeds. Each seed sample was subsequently enclosed in a single layer of cheesecloth and dipped in either 0, 0.6, or 6.0% (v/v) concentration of sodium hypochlorite for 0, 5, 30 or 60 seconds. The 30 seeds were rinsed with distilled water for 5 seconds and placed in a 9-cm diameter plastic

petri dish on Fisherbrand® filter paper (size P8) and moistened with 5 ml of distilled water. Control treatments (0% sodium hypochlorite) were dipped in distilled water for 0, 5, 30, or 60 seconds, respectively prior to the addition of 5 ml of distilled water. A negative control was also established where 5 ml of 6.0% sodium hypochlorite was added instead of distilled water. Each treatment was replicated five times. The petri dishes from each replicate were then assigned using a randomized complete block design to one of five growth chambers set at an alternating 27/14°C day/night temperature and a 14-hour photoperiod. Germination was recorded daily for 14 days. A seed was considered to have germinated when a radicle of at least 1mm in length had emerged. The experiment was repeated a second time one month later.

#### *Radicle elongation trial*

Germinated seeds from each treatment were removed on the day of germination and transferred to 25 × 37.5 cm (#38) Anchor® germination paper moistened with distilled water. Seeds were placed lengthwise along the germination paper with all radicles arranged with the same orientation. A second sheet of moistened germination paper was placed directly on the top of the first sheet, leaving the seeds between the two sheets. The two sheets of germination paper were gently rolled and placed into a 1000 ml glass beaker with the radicles pointing downward. The beakers were then placed back into the growth chambers used for the germination trial. To maintain germination paper moisture, the beaker was filled to a depth of 2.5 cm with distilled water thus allowing for upward capillary movement of water along the germination paper. Each germination sheet contained germlings of all three species germinating on the same day and subjected to the same soaking time and sodium hypochlorite concentration treatment combination. Germination sheets for all treatments were placed into the same beaker and this was replicated three times. Thus, each set of three beakers included all germlings germinating on the same day. Radicle length for each seedling was measured over a 6-day period from the time of initial transfer.

Percentage germination and rate of germination were calculated for each species and treatment combination. The rate of germination was calculated using the coefficient of germination velocity (CGV) equation described by Alm *et al.* (1993):

$$CGV = \frac{\sum N_j}{\sum N_j d_j}$$

where N represents the number of germinated seeds, d represents the day of germination and j represents the observation number. Values of CGV range between 0 and 1 with values near 1 indicating a faster velocity of germination. Values of CGV were plotted against time by fitting to either a linear or quadratic regression.

Data for the germination and radicle elongation experiments were analyzed separately by analysis of variance (ANOVA) using PROC GLM in SAS (SAS, 1999). All percentage data from the germination experiment were arcsine square root transformed to homogenize variances. Data from the radicle elongation experiment did not require transformation. Differences between treatments were established at the 5% level of significance by using the LSMEANS function of SAS.

## Results

### *Amaranthus powellii*

Duration of the NaOCl soaking period and concentration had a significant effect ( $P < 0.05$ ) on the cumulative germination of *Amaranthus powellii* seeds after 14 days (figure 1). Control treatments consistently resulted in germination levels between 10 and 20% (figure 1a). Exposure to 6.0% NaOCl for as short as 5 seconds resulted in a 7-fold increase in germination compared with the 0.6% NaOCl and distilled water treatments (figure 1b). The largest increase in germination (70%) was found when seeds were exposed to 6.0% NaOCl for 5 to 60 seconds (figure 1 b,c,d). Moistening the filter paper with 5 ml of NaOCl (i.e., 14-day exposure) resulted in the death of all seeds for both the 0.6 and 6.0% treatments (figure 1e).

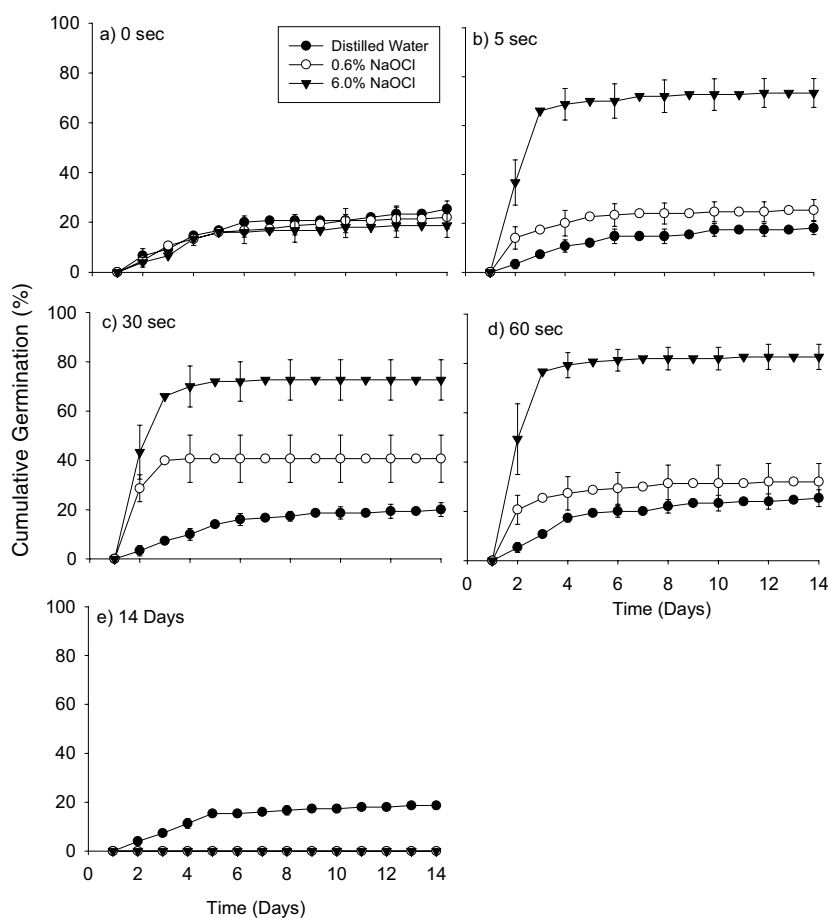


Figure 1. Cumulative germination ( $\pm$ SE) of *Amaranthus powellii* seeds at three NaOCl concentrations and five exposure periods.

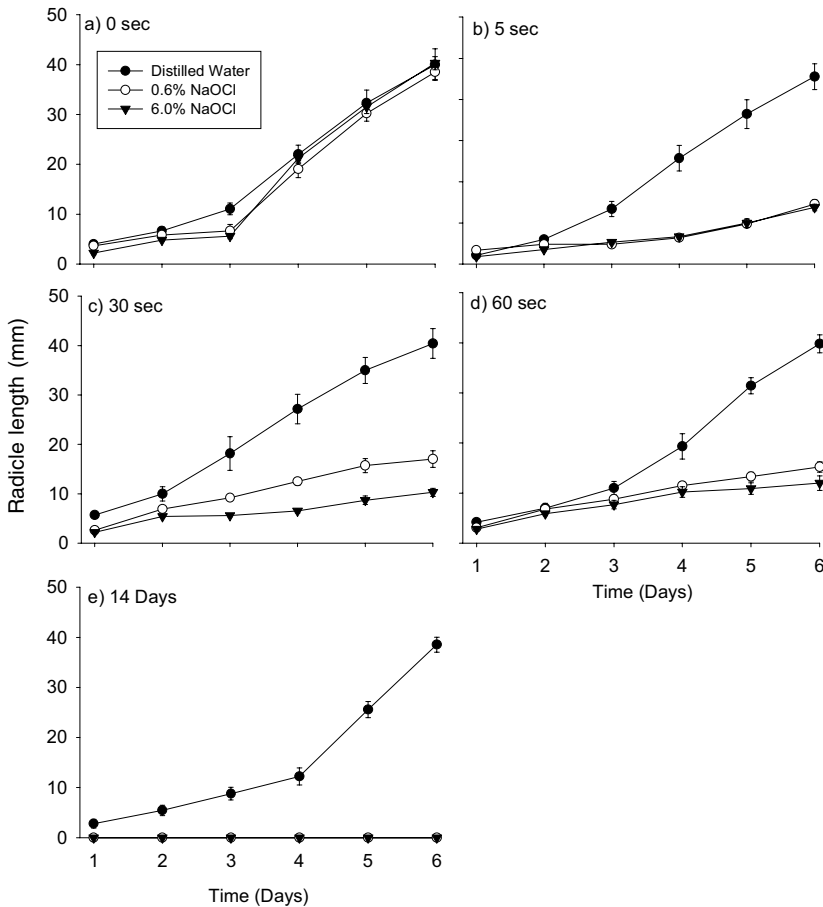


Figure 2. Radicle elongation ( $\pm$ SE) of *Amaranthus powellii* seedlings originating from seeds subjected to three NaOCl concentrations and five exposure periods.

Radicle elongation of *A. powellii* seedlings was decreased for seeds exposed to NaOCl (figure 2). On average, the control radicle length in this species was 40 mm after 6 days of growth. Exposure to either 0.6 or 6.0% NaOCl caused a 4-fold decrease in the length of radicles (figure 2 a,b,c,d). No germlings were available for the 14-day soaking treatment in 0.6% and 6.0% NaOCl because no germination occurred in this treatment (figure 2e).

#### *Setaria faberi*

Concentration and soaking duration did not have any effects on percentage germination of this species (figure 3). Control treatments resulted in over 90% germination of seeds as did treatments where seeds were exposed to 0.6% and 6.0% NaOCl concentrations for periods ranging from 5 to 60 seconds. No germination was recorded for seeds exposed to the two NaOCl concentrations for 14 days (figure 3e).

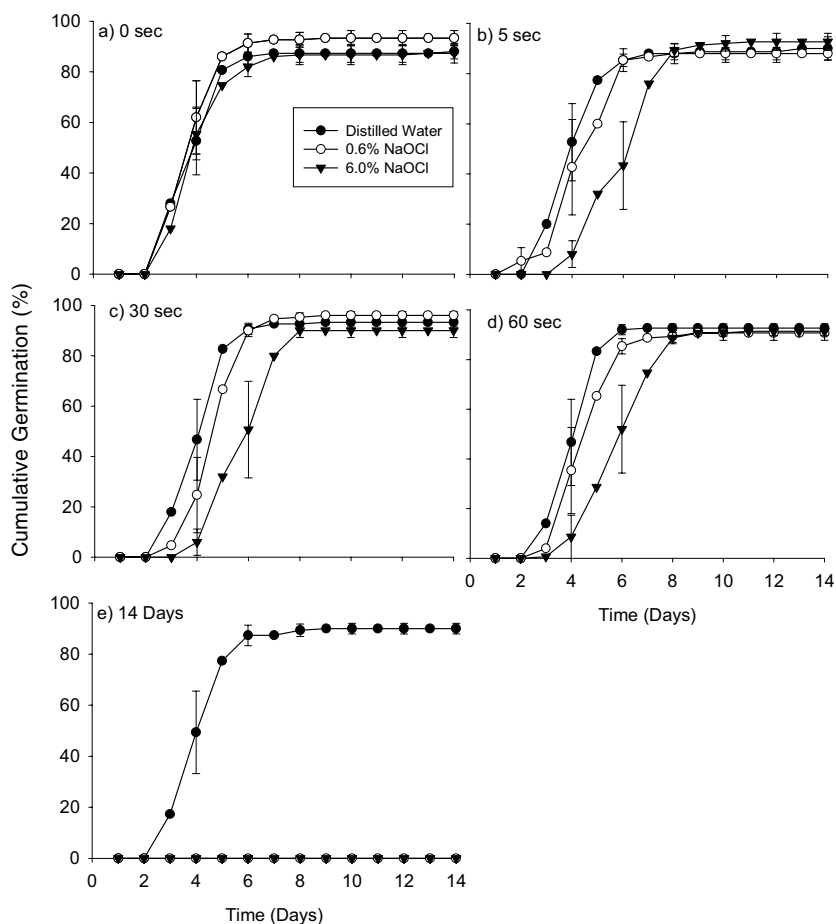


Figure 3. Cumulative germination ( $\pm$ SE) of *Setaria faberi* seeds at three NaOCl concentrations and five exposure periods.

Radicle elongation in *S. faberi* decreased up to 5-fold following prolonged exposure to NaOCl (figure 4 a,b,c,d) and no radicle growth occurred after 14 days exposure (figure 4e).

#### *Abutilon theophrasti*

Seeds of *Abutilon theophrasti* were not affected by either NaOCl concentration or soaking duration (figure 5). All control treatments had cumulative germination between 30 and 40% (figure 5a). Unlike, *A. powellii* and *S. faberi*, seeds of *A. theophrasti* survived the negative control treatment of 5 ml 0.6% NaOCl added to the petri dish for 14 days (figure 5e), but no seeds of *A. theophrasti* germinated when exposed to 6% NaOCl for 14 days.

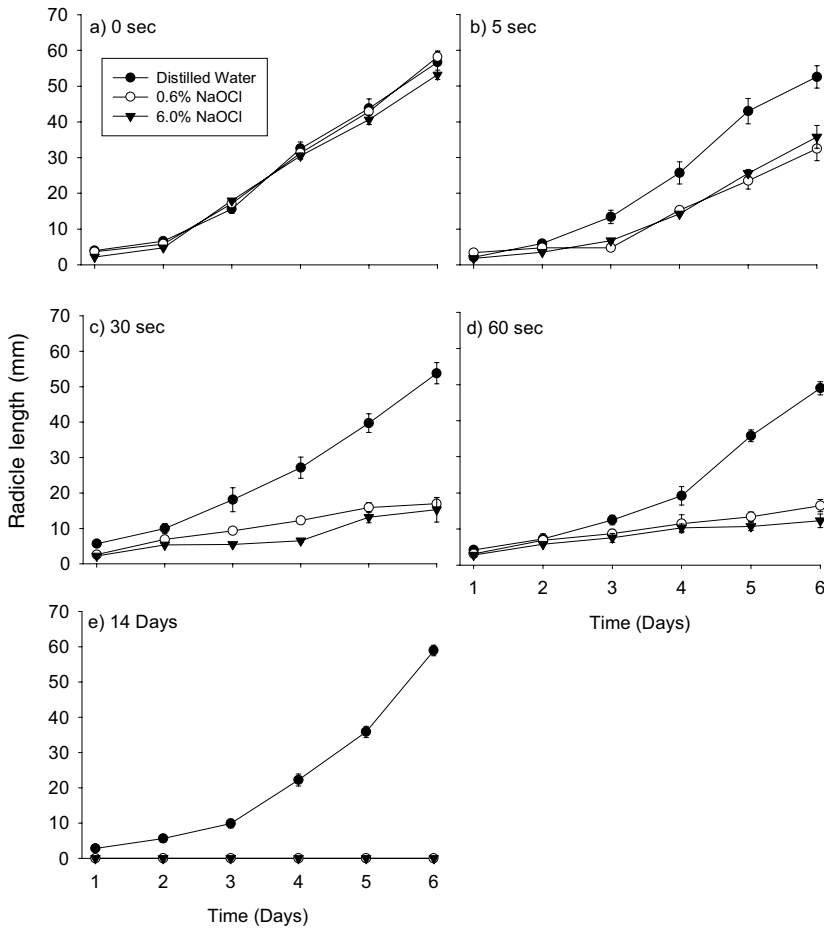


Figure 4. Radicle elongation ( $\pm$ SE) of *Setaria faberi* seedlings originating from seeds subjected to three NaOCl concentrations and five exposure periods.

In general, soaking period and concentration of NaOCl did not affect radicle elongation of *A. theophrasti* seedlings (figure 6). The only exception was enhanced radicle elongation from 90 to 130 mm when seeds were exposed to 0.6 % NaOCl for 14 days. However, seedlings from the control treatment for this concentration (figure 6e) produced shorter radicles (80 mm) in comparison to other control treatments (120 mm) (figure 6a).

#### Velocity of germination

The coefficient of germination velocity (CGV) for *A. powellii* seeds averaged 0.23 for the control treatments (figure 7a). Values of CGV peaked at 0.44 when seeds were exposed to 0.6% NaOCl for 30 seconds. Exposure times of 5 and 60 seconds did not differ from the

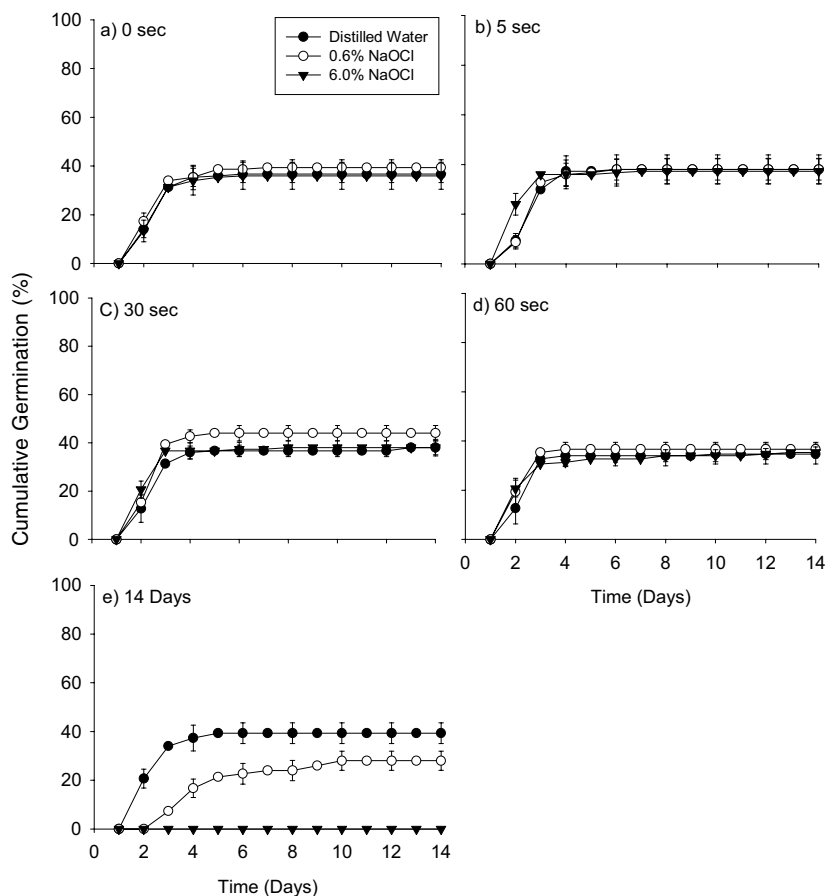


Figure 5. Cumulative germination ( $\pm$ SE) of *Abutilon theophrasti* seeds at three NaOCl concentrations and five exposure periods.

distilled water control ( $P > 0.05$ ). When the concentration of NaOCl was increased to 6.0%, values of CGV were highest at a dipping time of 30 seconds. No germination occurred in the negative controls and therefore the CGV was zero.

Velocity of germination of *S. faberi* decreased with increasing NaOCl concentration (figure 7b).

Overall, CGV for *A. theophrasti* seeds was not significantly affected by the soaking durations tested (figure 7c). CGV values decreased significantly, however, when seeds of *A. theophrasti* were exposed to 0.6 and 6.0% NaOCl for a period of 14 days (data not shown).

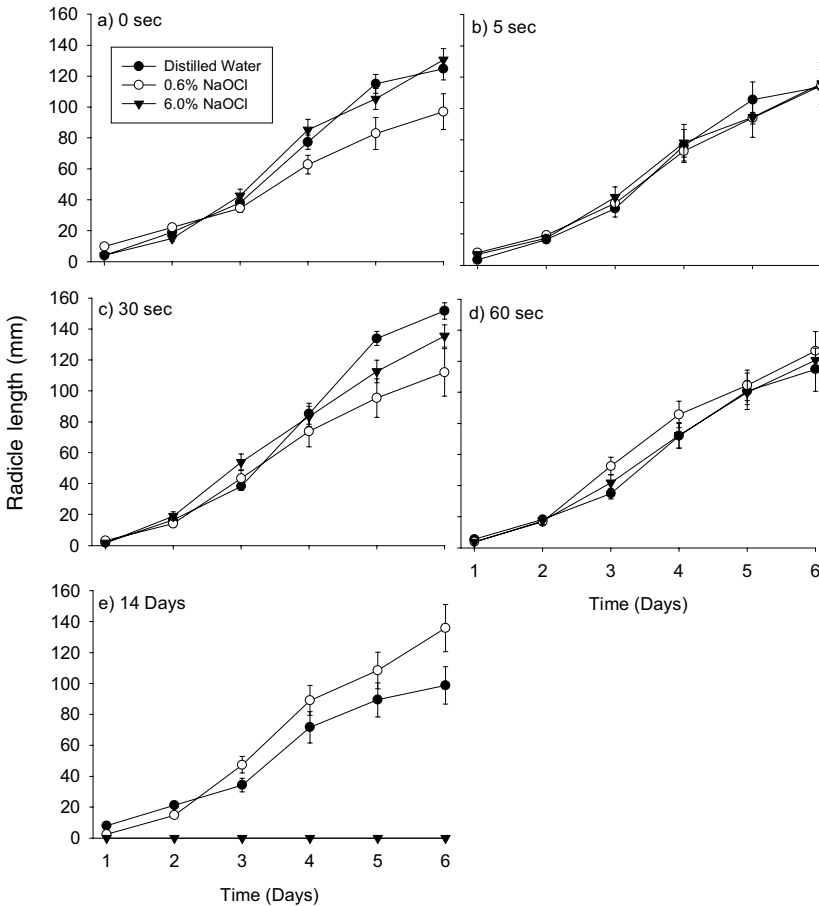


Figure 6. Radicle elongation ( $\pm$ SE) of *Abutilon theophrasti* seedlings originating from seeds subjected to three NaOCl concentrations and five exposure periods.

## Discussion

The use of sodium hypochlorite (NaOCl) to surface sterilize seeds may impact results of seed germination trials. Findings from this study indicate that NaOCl concentration and exposure time can alter the dormancy status of seeds and strongly suggest that it is a species-specific phenomenon. Germination studies that involve seeds possessing physiological dormancy (i.e. *A. powellii* and *S. faberi*) may be more affected by pre-treatments using NaOCl than seeds with physical dormancy. Apparently, species with hard seed coats (e.g. *A. theophrasti*) may be treated with NaOCl for short periods with little effect on germination levels and speed of germination. However, it is recommended

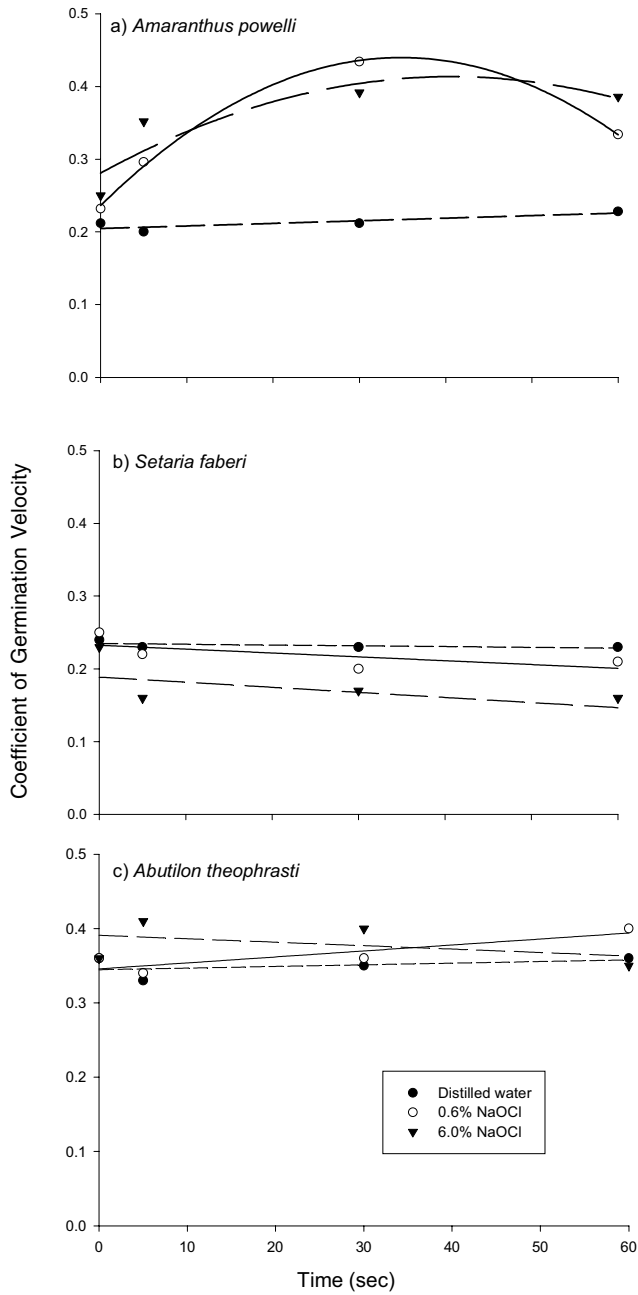


Figure 7. Relationship between the coefficient of germination velocity (CGV) and NaOCl concentration for seeds of a) *Amaranthus powellii*, b) *Setaria faberi*, and c) *Abutilon theophrasti* for five exposure periods.

that if surface sterilization of seeds is necessary, that an *a priori* assessment of the effects of NaOCl pre-treatment be carried out on each species to determine optimal exposure periods and concentrations that will minimally affect germinability.

The seed coat plays an especially important role in physiological dormancy as shown by *A. powellii* and *S. faberi* because it may possess chemical inhibitors, may inhibit oxygen uptake and/or may physically restrict embryo growth. Physiological seed dormancy may also be due to chemicals within the embryo or endosperm that hinder the onset of germination (Baskin and Baskin, 1998). Seeds of *A. powellii* may require an after-ripening period of several months following harvest to relieve seed dormancy (Weaver and McWilliams, 1980). The factors regulating seed dormancy in this species are complex and varied, but may involve the inhibition of embryo growth by the seed coat as well as phytochrome regulation of the germination process (Weaver and McWilliams, 1980). Bewley and Black (1994) suggest that almost all seeds requiring light for germination have coat imposed seed dormancy. Evans (1922) reported that maximum germination vigor was achieved in a closely related species (*Amaranthus retroflexus* L.) when seed coats were removed from freshly collected seeds. In our study, exposure of *A. powellii* seeds to NaOCl for only 5 seconds was sufficient to increase germination 7-fold and decrease radicle length 5-fold. Thus, even short exposure periods of seeds to NaOCl appear to have altered the structural integrity or chemical composition of the seed coats or embryo such that any physiological obstacle to germination was relieved. However, the specific process by which NaOCl alleviates dormancy in this species is still unclear. Similarly, Hsiao (1979b) demonstrated that prolonged exposure (30 and 60 minutes) of seeds of two weed species *Polygonum convolvulus* L. and *Saponaria vaccaria* L. to 6.0% NaOCl removed physiological dormancy of seeds in both species. It was hypothesized that NaOCl softened the seed coat, allowing increased water and gas uptake in *P. convolvulus* and increased seed coat porosity in addition to possibly removing inhibitory chemicals in *S. vaccaria*.

Although seeds of *S. faberi* also exhibit physiological seed dormancy, the cumulative percentage germination for *S. faberi* was not significantly increased even when seeds were exposed to NaOCl for 60 seconds. However, NaOCl treatment did decrease their rate of germination. The high level of germination in the control treatment for this species suggests that the seeds used in this study were not dormant, which may have influenced the response of this species.

Although the thickness of *A. theophrasti* seed coats was not determined following exposure to NaOCl in this study, similar seed coat degradative processes found in seeds with physiological dormancy were not sufficient to relieve dormancy in this hardseeded species after short exposure periods to NaOCl. Dormancy in *A. theophrasti* seeds is regulated by a seed coat that is impermeable to water and by expansion and contraction of the chalazal opening (Mulliken and Kust, 1970; Warwick and Black, 1988). Therefore, fractures in the chalazal region (LaCroix and Staniforth, 1964) as well as changes in relative humidity may relieve dormancy in these seeds by causing the chalazal region to become permeable to water (Steinbauer and Grigsby, 1959). Short exposure periods of *A. theophrasti* seeds to hot water (Horowitz and Taylorson, 1984) and sulfuric acid ( $H_2SO_4$ ) (Horowitz and Taylorson, 1985) have been shown to increase permeability of the

seed coat, probably through the removal of tannins, lipids or other structural barriers. In this study, similar exposure times to NaOCl did not have the same effect on removal of these seed coat permeability barriers. Interestingly, exposure to 0.6% NaOCl for 14 days did not result in the loss of viability of all seeds, although cumulative germination was reduced in this treatment. However, if seeds were left in NaOCl for long periods of time (i.e., 14 days) under optimal germination conditions, seeds did imbibe the NaOCl and at concentrations of 6.0%, the viability of seeds was lost. It is possible that the NaOCl was not imbibed through the *A. theophrasti* seed coat but rather the solution gained entry into the seed interior via the chalazal opening. Similarly, Horowitz and Taylorson (1985) found that dormancy was not relieved in *A. theophrasti* after seeds had been soaked in 5% NaOCl for 1 and 2 hours.

Sodium hypochlorite, the active ingredient in household bleach (i.e., Clorox®), is a strong oxidizing agent. This compound is inherently unstable and slowly decomposes liberating chlorine gas (Cl<sub>2</sub>). The oxidation number of chlorine in NaOCl is +1; however, this molecule quickly gains electrons yielding chlorine with a -1 oxidation number and water as products (Cotton and Wilkinson, 1980). Thus, constituent compounds of the seed coat or seed interior with reducible carbon such as enzymes, carbohydrates, lipids and proteins are at risk of being oxidized by NaOCl. Hypochlorites are known eye and skin irritants due to their production of active (nascent) oxygen (Manahan, 1994). It is possible that the nascent oxygen also plays a role in the oxidation of compounds within the seed coat.

The effect of NaOCl on radicle growth in our study was species dependent. Radicle length in *A. powellii* was negatively affected after exposure of seeds to NaOCl for as little as 5 seconds even though NaOCl relieved dormancy in this species. In addition to the reduction in radicle length, *A. powellii* radicles lost pigmentation. Similarly, radicle length was also reduced in *S. faberi* germlings. The hardseeded species *A. theophrasti* showed no relationship between radicle elongation and exposure duration and concentration of NaOCl. These findings suggest that species which readily imbibe water also transport NaOCl more quickly into seed interiors, thus affecting embryo integrity and ultimately seedling vigor. Ferreira and Ranal (1999) found that hypocotyl and root length was reduced in *Brassica chinensis* and attributed this result to seed scarification by the NaOCl solution. They did not however, provide possible physiological reasons for this reduction in growth. Chun *et al.* (1997) attributed increases in the seedling growth of rice to the removal of germination inhibitors within the seed coat by NaOCl.

In conclusion, our findings revealed that exposure period and concentration of NaOCl influenced the viability and germinability of seeds tested and was species specific. The type and status of dormancy exhibited by seeds used in this study also affected results as seeds of a species possessing physiological dormancy (i.e., *A. powellii* and *S. faberi*) were affected to a greater extent by the NaOCl treatments than seeds of species possessing physical dormancy (i.e., *A. theophrasti*). Results of previous work using NaOCl as a seed pre-treatment have been highly variable, with dormancy relieved in some species, thus increasing germination, whereas seed viability was reduced in other species. Clearly, caution should be used when carrying out germination trials where seeds are pre-treated with sodium hypochlorite. In cases where pretreatment of seeds using NaOCl is

necessary, we strongly recommend that *a priori* tests using dilute NaOCl concentrations (i.e., 0.6% or less) at short exposure periods (i.e., 30 sec) be performed on an individual species basis to determine the impact of the pre-treatment on viability and germinability of seeds tested.

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