Tetrazolium and germination procedures for six native range species
by Lory Lee Walter-Dye

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
The growing attention in land reclamation has resulted in an increase in native seed sales. As of yet, germination and viability testing methods have not been established for many native species. For efficient marketing and subsequent studies on growth and development of native species, the establishment of optimum testing methods is a prerequisite.

The objective of this paper was to research various treatments and determine which treatment, if any, were optimum for six native species.

Six species indigenous to the Rocky Mountains and Great Plains regions were evaluated for seed viability with 2,3,5-triphenyl-2H-tetrazolium chloride (TZ). Specific staining techniques were developed for each species. Treatments include presoaks, seed coat puncture, seed coat removal, scarification, and seed bisection. Species tested and their viability values are: 1) Achillea millefolium L. (Yarrow) 1977 seedlot 94%, 1981 seedlot 65%; 2) Agropyron spicatum (Bluebunch wheatgrass) 1980 seedlot 61%, 1981 seedlot 88%, 1982 seedlot 91%; 3) Calamovilfa longifolia (Prairie sandreed) 1978 seedlot 64%, 1980 seedlot 88%, 1982 seedlot 85%; 4) Elymus cinereus (Basin wildrye) 1980 seedlot 90%, 1981 seedlot 94%, 1982 seedlot 72%; 5) Ratibida columnifera (Prairie cone flower) 1981 seedlot 89%, 1983 seedlot 85% and 6) Sporobolus airoides (Alkali sacaton) 1981JK seedlot 78%, 1981NK seedlot 70%.

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The objective of this paper was to research various treatments and determine which treatment, if any, were optimum for six native species.

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CHAPTER I

LITERATURE REVIEW

Tetrazolium

Efficient seed production and marketing depends upon the proper evaluation of seed quality. Two methods employed to evaluate quality are purity and germination tests. Results for the former can be obtained in a few hours, whereas the latter may take weeks. The time required for germination tests can hinder seed processing and marketing. This situation necessitated the need to develop a more rapid method for evaluating the germinative potential of seeds.

History

First experiments conducted in the search for a rapid viability test began in the early 1900's. Several scientists explored the possibilities of electrolyte leaching (Darsie et al., 1914), heat liberation (Ching, 1960), and free fatty acid content (Hibbard and Miller, 1928) as a means for indicating rapid viability. These methods were cited as being reliable indicators, but were difficult to evaluate. These tests, at their best, were capable of evaluating viability of a large quantity of seeds as low, medium, or high rather than in percentages.

Researchers recognized that techniques to assess seed quality needed to be based on individual seeds rather than on a quantity of seeds. The vital staining techniques differentially stain live and dead tissue, and were the first to meet this requirement. Dr. Neljubow, in 1925, developed the first reliable

vital staining method (Delouche et al, 1962). The technique was based on the staining of dead tissue with indigo carmine, an aniline dye. Evaluation was based on the relative proportion of colored and uncolored tissue.

Later, vital staining techniques were developed. These techniques were based on the metabolic processes of seeds. More specifically, enzymes were investigated. Since the activity of enzymes can be easily measured, investigators attempted to correlate their activity with seed viability.

Peroxidase (McHargue, 1920) and catalase (Davis, 1926; Legatt, 1938) activity was considered. Methods based upon peroxidase and catalase were later developed to become reliable indicators, but they were limited by their inability to be based on individual seeds. Dr. Turesson, in 1922, was the first to work with the dehydrogenase enzymes (Delouche et al, 1962). These enzymes are involved in the oxidation-reduction reactions of organic compounds. Both the oxidized and reduced forms are distinguishable by their change in color.

Hasegawa, in 1935, reported on a selenium method for determining seed viability (Delouche et al, 1962). The selenium salts produced colored participates on living tissue when reduced. Reduction was believed to result from the presence of active dehydrogenase enzymes. The selenite method was further developed by Eidmann in 1938 (Delouche et al, 1962). Lakon developed the selenite topographical method that stressed the analysis of individual seeds and resulted in improved accuracy of the rapid selenite viability test (Delouche et al, 1962). This method was considered very reliable, but had one serious drawback; selenium's toxicity and its potential danger to the user. Upon hearing of the discovery of tetrazolium salt by two German scientists, Lakon substituted the selenium for the tetrazolium salt. The tetrazolium salt is reduced from a colorless, soluble form, to insoluble, colored formazans in the

presence of living tissue. Lakon concluded that this method allowed the prediction of the germinability of seeds by observation of the stained embryo parts. Several scientists (Cottrell, 1947; Porter et al, 1947; Shuel, 1948) employed this technique and agreed that results from the tetrazolium test did indeed show close agreement with germination tests.

Today the tetrazolium test is a universally accepted method for rapidly evaluating seed viability. It has been adapted to accommodate a wide range of species in evaluating seed quality. Seed industries rely on the tetrazolium test in conjunction with or in place of the standard germination tests.

Dormancy

The inability of viable seeds to germinate under favorable conditions is referred to as dormancy (Villiers, 1972; Copeland, 1976). This is a widespread phenomenon found primarily in the temperate regions and to some extent in tropical and subtropical regions. Domesticated plants show less dormancy than wild plants (Copeland, 1976). Dormancy is biologically advantageous for some species, as it allows the plants growth cycle to adapt to the environmental variations (Villiers, 1972). Agronomically speaking, dormant seeds are unfavorable. A high percentage of dormant seeds would result in nonuniform stand and maturation of plants.

Vegis (1964) defined dormancy as a condition in which normal germination fails to occur regardless of external conditions. This definition seems too restrictive since it does not include artificial manipulation of the seed, such as removal of the seed coat. Others employed the term dormancy to include both environmental and internal conditions that prevent normal germination processes (Pollock and Toole, 1961; Amen, 1968). Because dormancy is an

elusive phenomenon, a precise definition has yet to be developed and universally accepted. A Russian scientist, Nikloaeva, (Villiers, 1972) devised a detailed dormancy classification scheme. He defined four types of dormancy resulting from; a) properties of the outer covers of the embryo, b) immaturity of the embryo, c) physiological condition of the embryo, and d) types of combined dormancy.

A more simplified classification of dormancy types was devised by Crocker (1916) who described dormancy resulting from; a) immature embryo, b) impermeable seed coats to water, c) mechanical resistance of the seed coats to embryo growth, d) low permeability of seed coats to gases, e) dormancy resulting from a metabolic block within the embryo itself, f) any combination of the above, g) secondary dormancy. This classification has provided the basis for comparisons of experimental investigations between dormancy-inducing mechanisms and dormancy-breaking mechanisms.

Germination

A seed contains an embryonic plant in an inactive condition, and germination is its resumption of growth. Germination is said to have occurred when the radicle has burst through its outer structures (Toole et al, 1956). Cell division and elongation starts at this time; and many seed physiologists take these events as the termination of germination and the beginning of seedling development (Bewley and Black, 1985; Berrie, 1984). In some seeds development starts as soon as water is absorbed; however, in other species germination does not take place until other requirements are fulfilled. Three distinct phases of germination are easily measurable within the seed. They are imbibition, cell elongation, and an increase in cell number (Toole et al, 1956).

When dry seeds are exposed to water the initial uptake is rapid, followed by a slower uptake referred to as the lag period. Succeeding the lag period is another burst of rapid water uptake, and then germination proceeds (Berrie, 1984). Measurable increases in respiration, enzyme activity, and protein synthesis begins shortly after the start of imbibition. It is at this time the seed changes from being biologically inert to becoming a fully integrated and actively metabolizing organism. During the lag phase it is thought that the metabolic pathway begins to allow the initiation of the germination process (Berrie, 1984).

Suggestions have been made pertaining to membranes as being the locus for the stimulus of germination (Hendricks and Taylorson, 1976). The nature of the phospholipid bylayers is such that its physical properties can be changed by manipulating the germinating temperature. The phase changes are associated with degree of permeability. At high temperatures permeability is increased because the bylayers are in a liquid state. This results in a greater loss of amino acids which subsequently reduces germination. At low temperatures permeability is low because the bylayers are in a solid state. In the middle temperature range the bylayers exist in a liquid-crystal state (Hendricks and Taylorson, 1976; Murphy and Noland, 1982). Hendricks and Taylorson (1979) suggested that phytochrome may also be involved in changing membrane properties and reactions. Some reports cite that gibberellic acid (GA) acts on the membranes of cells and organelles and that its effect on seed germination is also at this level (Berrie, 1984). The association of these factors with membrane properties have strengthened the view that the membrane may indeed be the locus for the stimulus of germination.

Unlike the imbibition phase where the only morphological change is an increase in volume, the other two phases involve a marked change in the size of the embryonic axis, and the beginnings of development. In parallel with the morphological changes there are also cellular and subcellular changes, especially in storage and regulatory tissues. All the changes are associated with conversion, mobilization, translocation and redistribution of the seeds storage material (Berrie, 1984). Mobilization of the endospermic reserves cannot take place without the embryo and scutellum. The embryo was found to control the hydrolytic capacity of the aleurone and could influence the functioning of that tissue (Berrie, 1984).

Embryo-less seeds cannot accomplish the dissolution of starch. Two enzymes, beta-amylase and alpha-amylase, are responsible for the digestion of starchy material and mobilization of these food reserves to the extending axis (Berrie, 1984). Beta-amylase is present within the seed and appears only after the radicle has emmerged. Early works in the nineteenth century found the embryo and scutellum to be responsible for the release of alpha-amylase and lipase during early stages of germination (Brown and Morris,1890). The amount of alpha-amylase secreted however, was not sufficient to break down the entire endosperm storage material.

Yomo (1960) and Paleg (1961) found that the embryo region of cereal grains secreted GA to the aleurone and that this layer responded by developing increased amounts of the two digestive enzymes. Gibberellic acid promotion of enzyme activity has been found to be dependent on RNA and protein synthesis (Chrispeels and Varner, 1967; Dure, 1979). In contrast to GA, a more recent study suggests that the seeds osmotic potential controls the production of the hydrolytic enzymes (Trewavas, 1982). Where fat is the main storage substance,

lipase is the predominent digestive enzyme (Berrie, 1984). Its activity increases during the course of germination, but the control mechanism for this is unknown. The presence of GA has no effect on increasing the activity of lipase (Berrie, 1984).

Many attempts have been made to pinpoint the triggering stimulus for the onset of germination. As of yet there is no concrete proof that one process alone is responsible for the induction of germination. Rather, a complex involvement of several processes and pathways, each probably in one way or another dependent upon the other, seems to be necessary for germination to occur. It should be noted that external factors such as moisture for rehydration, light, temperature, and oxygen also play a major role in the initiation or prevention of germination.

Temperature

The first study on the influence of temperature on seed germination was that of Lefebure, which was published in 1801 (Lang, 1965). Lefebure interpreted his results in physical concepts and terms which have since been abandoned, but his experimental data was representative of almost all subsequent work done in this area. Thorough investigations on the effects of constant temperature on seed germination of several cultivated plants was studied in 1860 by J. Sachs (Koller, 1972). His studies showed that the seeds germinative ability improved as temperature increased from its lower limit until a certain temperature was reached above which any further increase in temperature resulted in reduced performance (Koller, 1972; Bewley and Black, 1985). Based on these results, he formulated the concept of the three cardinal temperatures; minimum, optimum, and maximum (Lang, 1965).

Most seeds germinate over a wide range of temperatures. Their width and optimum is highly influenced by such factors as seed age, seed coat, prechilling after-ripening, genetic variability, vigor, duration of incubation period, etc. (Evans, 1922; Harrington, 1923; Lang, 1965; Koller, 1972). Such shifts in temperature have been observed in seeds of barley, wheat, asparagus, conifers, and others; although, there are some seeds in which the optimum does remain constant (Lang, 1965). Some seeds have the capacity to germinate equally well with a wide range of temperatures while others have a very definite temperature optimum. For instance, larkspur seed germinates much better at 15C, which represents its optimum, than at 18C; while many cultivated plants germinate with equal completeness between 15C and 30C (Harrington, 1923; Lang, 1965). The time needed for reaching maximum germination varies with temperature. Increasing the temperature means that germination starts earlier and is initially more rapid. In some species, increasing temperature can also cause a rise in percentage germination. However, increasing the temperature above the species maximum will result in reduced performance. This decline in seed germination is the result of thermodormancy and is especially prominent in seeds that are exposed for a prolonged period of time to above optimal temperatures (Harrington, 1923; Koller, 1972; Bewley and Black, 1985).

Frequently, faster germination rates and higher percentages are obtained with fluctuating temperatures. The most striking effect, however, is an increase in germination capacity (Lang, 1965; Koller, 1972). Liebenberg was the first to report the favorable effects of diurnal temperature fluctuations using annual bluegrass. Since then this effect has been confirmed in many crop, grass, ornamental, weed, and native plant seeds (Lang, 1965; AOSA, 1984).

There have been several attempts to account for the rather specific response of seed germination to diurnally alternating temperatures. It is believed that the action of fluctuating temperatures is exerted on the embryo by increasing its growth processes to the extent that it can rupture through the surrounding structures; a feat the embryo is incapable of doing under a regime of constant temperatures. Treatments which change the physical properties of the coats or treatments which remove the seed coat altogether generally result in the loss of the specific requirement for diurnal temperature fluctuations. Seed germination, therefore, can take place over a relatively wide range of constant temperatures (Harrington, 1923; Morinaga, 1926).

The effect of temperature fluctuations is one of those instances where it is difficult to say whether and to what extent dormancy is being affected. In common cattail and bermudagrass, the effect of alternating temperature can be classified as the removal of a dormancy condition (Morinaga, 1926; Lang, 1965). However, seeds of carrot, timothy, awnless bromegrass, and several kinds of flower seeds attain high germination percentages with both constant and diurnal temperature fluctuations (Harrington, 1923). The effect of temperature alteration does not actually remove the specific blocks to germination, but instead provides an increase of the general physiological activity level of the seed (Lang, 1965).

Satisfactory germination percentages can be obtained with alternating regimes of 20-30C or 15-25C. Some suggest that both the higher and lower temperature should act at least for periods between 4.5 and 8 hours per daily cycle (Morinaga, 1926), while others argue that favorable effects can be produced as well by temperature changes without a definite or daily alteration (Harrington, 1923; Koller, 1972). Further research supported both arguments

and found that length and number of the thermoperiod cycles are highly dependent upon the characteristic species. Exposure of seed to diurnal temperature fluctuations produces the best germination results when the temperature differentials are at least ten degrees or greater between the upper and lower limits (Harrington, 1923; Lang, 1965; Koller, 1972).

The optimum upper germination temperature of species is 30C; however, in celery it seems to be 32C, and in johnsongrass 45C. Few seeds are capable of germinating at temperatures below 15C (Bewley and Black, 1985).

Prechill

Low temperatures have frequently been mentioned as a method for overcoming dormancy. Many species, particularly the rose family and woody species, are not capable of germinating until exposed to freezing temperatures.

Generally, prechilling occurs between 1 and 10C with the fastest results obtainable between 2 and 5C (Bewley and Black, 1982). The length of the chilling period is independent among species and may last a few days to several months. In order for the seeds to respond to the cool temperatures, they must be in the imbibed condition (Villiers, 1972).

Prechilling is advantageous for a range of dormancy types. Changes have been found to occur in seed coats that restrict the germination of the embryo (Steinbaur, 1937; Villiers and Wareing, 1964 and 1965; Webb and Wareing, 1972). This is thought to be due to the embryo acquiring greater vigor during the chilling period which enables it to overcome the resistance imposed by the seed coat structures (Villiers and Wareing, 1965). The period of low temperatures is more effective for Fraxinus seeds if it is preceded first by a period of warm

temperature. Fraxinus embryos are immature and must go through this period of warm temperature to develop. The subsequent addition of low temperatures is necessary for removal of the restrictions imposed by its seed coat.

The addition of low temperatures has been found to increase markedly the endogenous concentration of GA (Villiers and Wareing, 1965). Originally it was believed that the inhibitors decreased or disappeared during the chilling period enabling seeds to germinate. Subsequent studies (Frankland and Wareing, 1962; Chen and Varner, 1973) found the concentration of germination promoters increased markedly. Chilled hazel seeds had a 200 time increase in GA concentration over nonchilled seeds (Ross and Bradbeer, 1968). This increase occurs primarily at the end of the chilling period and after the seeds are transferred to their normal germinating temperatures.

Many other changes are reported to occur during prechilling, including increases in acidity, change in lipid organization, transfer of food reserves to the embryo, changes in enzyme activity, water holding capacity, reduction in sugars and respiratory rate (Villiers, 1972; Chen and Varner, 1973; Bewley and Black, 1982).

Light

The light requirement for seeds is a genetic factor (Evenari, 1965). At maturity most seeds have a strong light requirement. Some species maintain this requirement throughout their entire life cycle; but for most, the light requirement can be diminished or eliminated with prolonged dry storage (Evenari, 1965; Koller, 1972).

Seed development and germination can be restricted by the embryo's surrounding structures. Irradiation can overcome the mechanical restriction

imposed by the structures. The embryo, being the primary site of action for the promotive effect of light, secretes cytolytic enzymes when irradiated (Evenari, 1965; Scheibe and Lang, 1965; Koller, 1972; Bewley and Black, 1985). These enzymes cause a weakening of cell membranes allowing embryo development and subsequent germination. There have also been suggestions that light-sensitivity may be linked to gas exchange (Ikuma and Thimann, 1963; Evenari, 1965). This has been found to be true in seeds of evening primrose. An increase in respiration occurred with irradiated seeds that equalled the rise in respiration when the seed coat was pricked or completely removed (Siegel, 1950). This gave impetus to the notion that irradiation is effective via a weakening of cell membranes of surrounding structures. This weakening, in turn, allows an increase in gas exchange to the embryo; thereby promoting the germination process(es) (Siegel, 1950; Ikuma and Thimann, 1963; Evenari, 1965; Scheibe and Lang, 1965; Bewley and Black, 1985).

The promotive effect of light is controlled by a photoreversible pigment called phytochrome (Evenari, 1965; Hendricks and Taylorson, 1967; Koller, 1972; Bewley and Black, 1985). Phytochrome exists in two forms: phytochrome red (PR) and phytochrome far-red (PFR). The physiologically active form is PFR which is involved in the breaking of dormancy.

Irradiation with red light results in a conversion of inactive PR to active PFR. During this conversion a series of events are triggered that eventually lead to the elimination of dormancy and the onset of germination (Copeland, 1976; Bewley and Black, 1985). Irradiation with far-red light reverses the reaction. Dormancy is maintained because of far-red lights lower energy status which cannot participate in the reactions leading to germination. With

alternate applications of red and far-red light to seeds, the elimination or retention of dormancy is determined by the last irradiation form given (Copeland, 1976; Bewley and Black, 1985).

The duration of photoperiod for obtaining maximum germination varies among species. Fiddleneck seeds (Borthwick et al, 1954) requires a critical photoperiod of 12 hours or less; whereas eastern hemlock, european white birch, and wood fern (Koller, 1972; Baskin and Baskin, 1976) require a photoperiod of greater than 12 hours. Some species do not require a critical photoperiod. Instead germination steadily increases as the photoperiod duration increases (Evenari, 1965; Bewley and Black, 1985). Germination of tomato, cucumber and some amaranthus species are inhibited when exposed to any form of light (Mancinelli et al, 1967; Mancinelli and Tolkowsky, 1968; Taylorson and Hendricks, 1969). Dark germination, like light germination, is also phytochrome controlled and the capacity for the embryo to germinate in the dark is dependent upon the seeds phytochrome content. Dark germinating seeds contain more active phytochrome in their embryo than light-requiring seeds (Evenari, 1965; Koller, 1972; Bewley and Black, 1985).

Temperature Influences on Photoperiod.

Temperature has the greatest impact on a seeds photorequirement. As temperature varies so does the relation of germination to its photoperiod. Generally, a photoperiod, which produces optimum germination levels at one temperature, produces levels which are excessive at another (Stearns and Olson, 1958; Elliot and French, 1959). The most detailed study conducted on the interaction of temperature and light has been with lettuce seed germination (Borthwick et al, 1954; Elliot and French, 1959; Ikuma and Thimann, 1963;

Scheibe and Lang, 1965). Grand rapid lettuce seeds are capable of maximum germination in total darkness at low temperatures. Between 20C and 30C germination is best with irradiation. At temperatures higher than 30C no germination ensues. The presence of high temperatures generally results in deleterious effects with most seeds. The light response is such that it can overcome some of the injurious effects and promote germination (Siegel, 1950; Koller, 1972).

Where increases in temperature causes an increase in the photorequirement for lettuce seed germination, in seeds of tomato and cucumber temperature increases results in an escape from photocontrol (Mancinelli et al, 1967; Mancinelli and Tolkowsky, 1968) . Measurable increases in phytochrome during dark germination of cucumber seeds has been cited and that the rate of this increase is temperature dependent (Mancinelli and Tolkowsky, 1968). At higher temperatures, less PFR seemed to be required for germination. It is not established, yet, if this is due to the activation of a system different from phytochrome or to an increased rate of phytochrome reaction.

The number of photoperiods required to trigger seed germination changes dramatically with temperature. Low temperatures can reduce or completely eliminate most seeds red light requirement by delaying or preventing the transformation of PFR to PR, thus making dark germination possible for light-requiring seeds (Siegel, 1950; Stearns and Olson, 1958; Evenari, 1965; Schiebe and Lang, 1965). Several exposures to light necessitate the initiation of the germination processes when these seeds are preconditioned at higher temperatures. European white birch seeds are an exception. They become more light sensitive the longer they are exposed to moist chilling. Several long

exposures to light interrupted by dark periods is necessary for terminating dormancy. At higher temperatures the seeds light requirement is still present; but only a single exposure to light, either long or short, is necessary to start the chain of germination processes (Bewley and Black, 1985)

CHAPTER II

TETRAZOLIUM VIABILITY PROCEDURES FOR SIX NATIVE RANGE SPECIES

Introduction

The increasing disturbance of large acreages due to surface mining has increased the commercial sale of native species seed for reclamation. Viability testing of this seed has not been practical due to the lack of known testing procedures.

The need for a rapid method to assess viability has led to the introduction and acceptance of staining seed with 2,3,5-triphenyl-2H-tetrazolium chloride (TZ) (Smith and Throneberry, 1951; Delouche et al., 1962; Moore, 1969). Seeds that are difficult to test with conventional germination procedures have successfully been evaluated for viability using the TZ technique. Our objective was to develop TZ techniques for six species: Achillea millefolium L. (Yarrow); Agropyron spicatum (Bluebunch wheatgrass); Calamovilfa longifolia (Prairie sandreed); Elymus cinereus (Basin wildrye); Ratibida columnifera (Prairie coneflower); Sporobolus airoides (Alkali sacaton).

Correlations between specific staining patterns and actual germination can only be obtained after considerable testing experience. It is not the objective of this paper to provide this information.

Materials and Methods

Seed samples were contributed by various seed companies varying in geographical location. Seedlots varied in age from one to seven years.

Two replicates of 100 seeds were used for tetrazolium (TZ) viability tests. Seeds were preconditioned by placing in beakers filled with tap water or between blotters moistened with tap water. Seeds were preconditioned at room temperature for 4 to 16 hours. Length of water soak prior to TZ staining is not often critical to successful staining, and can be adjusted for laboratory convenience.

Preparation of the seeds involved bisecting bluebunch wheatgrass and basin wildrye longitudinally and prairie sandreed horizontally using dissecting knives. The seed coat of yarrow was pierced with a needle, whereas, the seed coat of prairie coneflower was completely removed. Alkali sacaton seeds were prepared for staining by mechanical scarification with a Forsberg laboratory scarifier, for 0, 1, 2, and 3 seconds before preconditioning between moist blotters. A stereoscopic microscope with 10 and 20 power magnification and top and bottom illumination was used to aid in preparation and interpretation. The preparation methods used were similar to the guidelines as outlined in the Tetrazolium Testing Handbook (Grabe, 1970).

After preparation, seeds were placed in Syracuse watchglasses and covered with TZ solution. Seeds were stained in the dark at 32C. Tetrazolium solutions were prepared using 2,3,5-triphenyl-2H-tetrazolium chloride (Smith, 1951; Grabe, 1959 and 1970) at concentrations of: 0.1% for bisected embryos and 1.0% for intact seeds and embryos. Duration of TZ soak ranged from 3 hours to 56 hours, depending on the species. Upon completion of staining, the

seed coats of yarrow and alkali sacaton were cleared using lactophenol solution. Seeds were allowed to clear for a minimum of one hour at 7C. Tetrazolium was removed with pipette and blotting paper before covering seeds with lactophenol.

Results and Discussion

Tetrazolium Testing Procedures

Yarrow. Seeds were preconditioned between moist blotters overnight (Table 1). Seed coats were punctured at the broad distal end and placed in 1% TZ for eight hours. Seed stained for six hours or less was understained. Minor change in staining intensity resulted from extending the staining time to 12 hours. Seed coats need to be cleared with lactophenol solution for a minimum of one hour to aid in interpretation (Table 2). Stained yarrow seeds were interpreted as a dicotyledon.

Bluebunch Wheatgrass. Seeds were preconditioned by soaking in water or between moist blotters (Table 1). After preconditioning, seeds were bisected longitudinally. Optimum staining occurred when one-half of each seed was placed in 0.1% TZ for four hours. Understaining occurred when bluebunch wheatgrass seeds were stained for less than three hours. Seven hours or more of staining resulted in overstaining. Lactophenol is not needed because a bisected surface of the embryo is exposed for interpretation (Table 2). Stained bluebunch wheatgrass seeds were interpreted as a monocotyledon.

Prairie Sandreed. Seeds were preconditioned by placing between moist blotters overnight (Table 1). Longitudinal bisecting was impossible due to the

oblong shape of the seed. Seeds were bisected horizontally at the periphery of the embryo after the seed coat was removed. The embryonic axis was placed in 1% TZ for 10 to 16 hours (overnight). Insignificant change occurred in staining intensity between 10 and 24 hours. Optimum TZ staining ranged from 10 to 24 hours. The staining time used would depend upon convenience in laboratory routine. Lactophenol is not necessary for interpretation (Table 2). Stained prairie sandreed seeds were interpreted as a monocotyledon.

Basin Wildrye. Seeds were preconditioned by soaking in water or between moist blotters (Table 1). After preconditioning, seeds were bisected longitudinally and one-half of each seed was placed in 0.1% TZ for four hours. Less than three hours resulted in understaining of embryos, whereas, seven hours or longer resulted in overstaining. Lactophenol is not needed because a bisected surface of the embryo is exposed for interpretation (Table 2). Stained basin wildrye seed were interpreted as a monocotyledon.

Prairie Coneflower. Seeds were preconditioned by placing between moist blotters overnight (Table 1). After preconditioning, the seed coat was removed by applying pressure at the broad, distal end of the seed. This forces the embryo out of the seed coat. The embryo was then placed in 1% TZ for four hours. This procedure will damage the tip of the cotyledon and must be ignored when interpreting the staining. Seeds were understained when soaked in TZ for two hours or less. Greater than seven hours of staining overstained the embryo. No lactophenol solution is necessary for interpretation of stained seed (Table 2). Stained prairie coneflower seeds were interpreted as a dicotyledon.

Alkali Sacaton. This species has a high percentage of hard seed and should be scarified for two seconds with a mechanical scarifier in order for seeds to stain with TZ (Table 3). Scarified seed were preconditioned overnight between moist blotters and placed in 1% TZ for 24 hours. Without scarification, staining time needs to be increased to 48 hours (Table 4). Swelling of seed coat, generally, did not occur until 20 hours of TZ staining with scarified seed and 40 hours with nonscarified seed. Radicle protrusion was observed at both staining times. Alkali sacaton seed could not be bisected because of the presence of a mucilagenous compound that is present on wet seeds and due to the size and shape of the seed (Table 1). Stained seed was cleared with lactophenol for approximately one hour. Clearing permitted interpretation of staining with the aid of magnification and using top and bottom illumination (Table 2). Stained but ungerminated alkali sacaton seed was interpreted as a dicotyledon.

Table 1. Tetrazolium viability procedures for six native range species.

Species	Precondition	Preparation	TZ Conc. (%)	TZ Stain* (Hours)	Remarks
Yarrow	Between moist blotters overnight**	Puncture seed coat	1	8	
Bluebunch wheatgrass	Soak in water*** or between moist blotters overnight	Bisect longitudinally	0.1	4	
Prairie sandreed	Between moist blotters overnight	Bisect laterally	1	10 or overnight	Remove outer coverings
Basin wildrye	Soak in water or between moist blotters overnight	Bisect longitudinally	0.1	4	
Prairie coneflower	Between moist blotters overnight	Remove seed coat	1	4	
Alkali sacaton	Between moist blotters overnight	Mech. scarif. 2 seconds	1	24	Mucilagenous seed coat
		or 0 seconds	1	48	Clear 1 hour with lactophenol

* at 32C ** approximately 16 hours *** approximately 4 hours

Table 2. Percentage viability, effect of lactophenol, effect of seed coat preparation on staining, and tetrazolium staining time evaluation for six native range species.

Species	Viability (%)	Lactophenol Effect	Stained With Seed Coat Intact	TZ Stain Time (hrs)		
				Understain	Optimum	Overstain
Yarrow		+*	-	6	8	NC ***
1977	94%					
1988	65%					
Bluebunch wheatgrass		-**	+	3	4	7
1980	61%					
1981	88%					
1982	91%					
Prairie sandreed		-	-	8	10	NC
1978	64%					
1980	88%					
1982	85%					
Basin wildrye		-	+	3	4	7
1980	90%					
1981	94%					
1982	72%					
Prairie coneflower		-	-	2	4	8
1981	89%					
1983	85%					
Alkali sacaton		+	slow	20	24-48	NC
1981NK	70%					
1981JK	78%					

* Positive (+) effect ** Negative (-) effect *** NC = No change with increased staining time

Table 3. Percentage viability, determined by TZ staining, for four scarification treatments when averaged over two alkali sacaton seedlots. Seeds were TZ stained for 24 hours.

Scarification Treatments (Seconds)	Percentage Viability 24 Hours TZ Staining (%)
0	48 a
1	59 b
2	72 c
3	61 b
LSD	3.32

Means in column followed by letters in common are not significatly different at the 5% level of probability.

Table 4. Percentage viability at 0 and 2 seconds of scarification with TZ staining time. Percentages are the average of two alkali sacaton seedlots.

TZ Staining (Hours)	Percentage Viability	
	Scarification Treatments (Seconds)	
	0	2
8	1 a	26 a
16	15 b	64 b
24	50 c	74 c
36	57 d	74 c
48	72 e	74 c
LSD	4.59	2.69

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

CHAPTER III

GERMINATION PROCEDURES FOR SIX NATIVE RANGE SPECIES

Introduction

Achillea millefolium L. (Yarrow) and Ratibida columnifera (Prairie coneflower) are perennial forbs distributed in the Great Plains and adjacent areas. Yarrow is a cool season plant that has high potential for alpine and subalpine revegetation, but its palatibility is low. Yarrow thrives in cool, moist climates and reproduces by rhizomes and seeds (U.S. Forest Service, 1937; Harrington, 1964).

Prairie coneflower is a warm season plant with shallow taproots and branch roots. Because of its aesthetic qualities, prairie coneflower is used to vegetate roadsides, rest areas, gardens, parks, recreation areas and prairies. Prairie coneflower is a weakly competitive species that cannot tolerate shade or intense grazing. Palatibility to livestock is high prior to heading (Harrington, 1964).

Agropyron spicatum (Bluebunch wheatgrass) and Elymus cinereus (Basin wildrye) are cool-season bunchgrasses common to the western United States. Bluebunch wheatgrass is used for seeding abandoned cropland, depleted range and disturbed land. This species is drought tolerant and winterhardy. It provides good palatible forage for elk and fair palatible forage for deer and

livestock. This species is superior in forage nutrient value to crested wheatgrass. Poor seed fill and stand establishment are the primary limitations of bluebunch wheatgrass (Plummer et al, 1955).

Basin wildrye is adaptable to drainage basins and high elevations. It is a very strong competitor, well suited for disturbed soils and drainage ditches. Basin wildrye also provides excellent cover to game birds and forage for big game animals (Vallentine, 1971; Shaw and Cooper, 1973).

Calamovilfa longifolia (Prairie sandreed) and Sporobolus airoides (Alkali sacaton) are warm-season, winterhardy, drought tolerant species. Native stands of prairie sandreed are used for grazing and hay. Prairie sandreed is commonly used for stabilization of sandy soils because of its coarse fibrous root system which is augumented by rhizomes (Shaw and Cooper, 1973).

Alkali sacaton has a dense fibrous root system that is ideal for use in erosion control. It provides cover and food for birds and small mammals. Alkali sacaton has average palatibility for livestock, when green. Alkali sacaton is highly salt tolerant (Leithead et al, 1971; Vallentine, 1971).

Neither the Association of Official Seed Analysts nor the International Seed Testing Association have established methods for laboratory seed germination testing of these species.

Materials and Methods

Two seedlots each of alkali sacaton, prairie coneflower, and yarrow; and three seedlots each of bluebunch wheatgrass, basin wildrye, and prairie sandreed were evaluated. Seedlots ranged from one- to seven-years old. Four 100-

seed replications of each seedlot were tested for each treatment. The seeds were selected at random without regard to size or color, but only fully developed, undamaged seeds were tested.

Seedlots of these six species were moist prechilled for 0, 5 and 10 days at 5C. After prechilling, seedlots were germinated at constant temperatures of 20C, 25C, and 30C, and at an alternating temperature of 15-25C. The alternating temperature was programmed for a daily cycle of 16 hrs. at the low temperature and 8 hrs. at the high temperature. The light treatments for the alternating 15-25C and constant 25C temperatures were programmed for 16 hrs. darkness and 8 hrs. light during a 24 hr. period. For the alternating germination temperature, the light period coincided with the high temperature period. The constant 20C temperature was evaluated using two light treatments. One light treatment was programmed for 16 hrs. darkness and 8 hrs. light during a 24 hr. period. The second light treatment was programmed for 24 hrs. of dark. The constant 30C temperature was programmed for 24 hrs. of light. Standard cool white flourescent tubes were used as the light source. Seeds were placed in plastic germination boxes on top of two standard blue germination blotters moistened with distilled water. Seeds were then placed in the prechill chamber for various time periods and then transferred to the germination chamber. Germination tests were organized so the two prechill treatments and the control treatment were placed in the germinator at the same time. When necessary during germination, boxes were watered with distilled water.

Germination tests were conducted for 38 days. Counts were made weekly during the first month and at five day intervals, thereafter. A seed was considered germinated when both radicle and plummule had attained a length

one-half the size of the seed. In the case of alkali sacaton, where seeds are extremely small, seeds were considered as being germinated when both radicle and plummule reached 3 mm in length. Germinated seeds were removed from the germination boxes at each count. These experiments were designed as a randomized block design.

Results and Discussion

Yarrow

The viability of the 1977 and 1981 seedlots was 94% and 65%, respectively, as determined by tetrazolium (TZ). Poor viability of the 1981 seedlot was due to poor seed fill and immature embryos. Low viability may also be associated with improper environmental conditions during seed development and poor storage conditions (Barton, 1961; Harrington, 1972; Justice and Bass, 1978).

No additional germination occurred after 14 days of germination for both seedlots (Table 5). Sorensen and Holden (1974) reported yarrow germination occured in 2 to 8 days. Our data suggest that more time for germination is necessary.

When specific germination temperatures and light combinations were evaluated length of germination varied (Table 6). The 20C treatment without light (20C,0L) and the 20C with 8 hrs. of light treatment (20C,8L) were the only two temperature and light combinations which required 14 days to complete germination. All other temperature and light combinations required seven days. However, for the 1977 seedlot, the germination percentages did not reflect the

seedlot viability until 14 days of germination had occurred for the 20C,8L combination (Table 7). This temperature and light combination was the only treatment which equalled viability of the 1977 seedlot.

Table 5. Percentage germination of two yarrow seedlots with time when averaged over five temperature and light combinations.

	Percentage Germination				
	Length of Germination (Days)				
Seedlots	7	14	21	38	LSD
1977	74 a	77 b	77 b	77 b	2.39
1981	50 a	55 b	56 b	56 b	2.01

Means in rows followed by letters in common are not significantly different at the 5% level of probability.

The 15-25C,8L, 20C,8L, and 25C,8L gave the highest germination percentages for both seedlots at 14 days of germination; whereas, the 20C,0L treatment had the lowest percentage germination (Table 7). Only the 20C,8L treatment equalled the 1977 seedlot's percentage viability, whereas the 15-25C,8L, 20C,8L and 25C,8L had equalled the 1981 seedlot's percentage viability. Eddleman (1979) found that a fluctuating 10-20C resulted in optimum yarrow germination. Other reports state 17-12C (McDonough, 1969) and 20-30C (Maguire and Overland,1959) provide optimum germination. Sorensen and Holden (1974) found optimum germination occurs with continuous exposure to moisture at 20C. The 20C,8L treatment was the only temperature and light combination that resulted in germination prcentages equivalent to both seedlot's viability; therefore, it can be concluded that optimum germination of yarrow is obtained with 14 days of germination at a constant 20C temperature with 8 hrs. of light.

Table 6. Percentage germination of two yarrow seedlots with time when germinated at various temperature and light combinations.

Seedlot	Length of Germination (Days)	Percentage Germination: Temperature and Light Treatments				
		15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1977	7	83 a	33 a	85 a	86 a	80 a
	14	85 a	39 b	90 b	87 a	82 a
	21	85 a	39 b	90 b	87 a	82 a
	38	85 a	39 b	90 b	87 a	82 a
	LSD	5.18	4.71	3.35	2.40	2.93
1981	7	60 a	27 a	56 a	62 a	42 a
	14	62 a	39 b	65 b	63 a	46 a
	21	63 a	39 b	66 b	63 a	46 a
	38	63 a	40 b	67 b	63 a	46 a
	LSD	3.83	4.70	1.95	3.46	4.05

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light.

Table 7. Comparison of percent viability with five temperature and light combination's percentage germination for two yarrow seedlots.

Temperature & Light Treatments	Percentage Germination 14 Days of Germination	
	1977	1981
15-25C,8L*	85 bc	63 c
20C,0L	39 a	39 a
20C,8L	90 cd	65 c
25C,8L	87 bc	63 c
30C,24L	82 b	46 b
Viability	94 d	65 c
LSD	5.77	4.25

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

*8L; hours of light

Germination of yarrow seeds appears to be positively photoblastic since optimum germination was achieved with the 20C,8L treatment and not the

20C,0L treatment. In contrast to these findings Eddleman (1979), Sorensen and Holden (1974), and Maguire and Overland (1959) report favorable germination in continuous dark. McDonough (1969) reports 8 hrs. light treatment as optimum.

Prechill did not improve the germination rate of yarrow (Table 8). Fourteen days was still the maximum time required for optimum germination of yarrow, regardless of the length of the prechill treatment.

Table 8. Percentage germination of three prechill treatments with time. Percentages are averaged over two yarrow seedlots and five temperature and light combinations.

Length of Germination (Days)	Percentage Germination		
	Prechill Treatments (Days)		
	0	5	10
7	63 a	63 a	59 a
14	68 b	66 b	63 b
21	69 b	67 b	63 b
38	69 b	67 b	63 b
LSD	1.99	2.49	3.30

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

Evaluation of individual seedlots at 14 days of germination showed no improvement in percent germination with prechill, regardless of the temperature and light combination (Table 9). These data indicate that yarrow does not respond to prechill. Sorensen and Holden (1974) are in agreement.

Table 9. Prechill effect on two yarrow seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations for 14 days.

Seedlots	Prechill Treatments (Days)	Percentage Germination 14 Days of Germination Temperature & Light Treatments 15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1977	0	92 b	42 a	94 a	89 a	81 a
	5	90 b	41 a	87 a	86 a	82 a
	10	73 a	33 a	89 a	87 a	82 a
	LSD	12.83	11.69	7.93	5.95	7.14
1981	0	69 b	39 ab	67 a	63 a	51 a
	5	61 ab	46 b	63 a	64 a	42 a
	10	59 a	32 a	64 a	63 a	46 a
	LSD	6.12	10.89	5.35	8.52	9.20

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

*8L; hours of light.

Bluebunch Wheatgrass

The viability of the 1980, 1981, and 1982 seedlots was 61%, 88%, and 91%, respectively. Mean percentage viability decreased as seedlots increased in age. The duration for good viability is reported to be five years, where 85% to 90% germination capacity can be expected (Hull and Pearse, 1943). The low viability, as exhibited in the 1980 seedlot, may be due to environmental conditions during maturation, harvest damage, unfavorable storage conditions, ageing, or a combination of all factors (Hull and Pearse, 1943; Barton, 1961; Harrington, 1972; Justice and Bass, 1978).

Compared to seven days of germination, 14 days increased percentage germination significantly for each seedlot. The 1982 seedlot had the greatest

increase in percentage germination (Table 10). Hafenrichter et al (1968) also reports complete germination in 14 days.

Table 10. Percentage germination of three bluebunch wheatgrass seedlots with time when averaged over five temperature and light combinations.

Length of Germination (Days)	Percentage Germination		
	Seedlots		
	1980	1981	1982
7	52 a	78 a	64 a
14	58 b	84 b	86 b
21	58 b	84 b	88 b
38	59 b	85 b	88 b
LSD	2.07	1.69	3.88

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

Comparison of specific temperature and light combinations shows the 20C,0L treatment resulted in the most rapid germination. This treatment completed germination within 7 days of testing for all seedlots (Table 11). The 25C,8L treatment completed germination in 7 days but only for the 1980 and 1981 seedlots. Even though these treatments completed germination at a fast rate, the highest percentage germination for all seedlots was obtained with a temperature and light treatment that required 14 days of germination (Table 12).

The 20C,8L treatment was the only temperature and light combination to consistently give the highest germination percentages and equal percentage viability for all seedlots. There were other temperature and light treatments that gave equivalent germination and viability percentages but they were not consistent among seedlots. A fluctuating temperature of 20-30C or 15-30C was found to be optimum for Haferkamp and McSwain (1951). Our germination

response with the 15-25C, 8L treatment was equivalent to the constant 20C, 8L treatment for the 1981 and 1982 seedlots but not for the 1980 seedlot (Table 12). The fluctuation of warm and cold temperatures could have stressed the older seed which resulted in a significant reduction in germination percentages.

Table 11. Percentage germination of three bluebunch wheatgrass seedlots with time when germinated at various temperature and light combinations.

		Percentage Germination				
		Temperature & Light Treatments				
Seedlots	Length of Germination (Days)	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1980	7	29 a	52 a	47 a	46 a	37 a
	14	36 b	54 a	59 b	47 a	42 b
	21	37 b	54 a	59 b	47 a	43 b
	38	37 b	54 a	59 b	48 a	44 b
	LSD	4.84	4.37	4.19	3.76	4.18
1981	7	77 a	85 a	82 a	80 a	67 a
	14	88 b	86 a	91 b	82 a	78 b
	21	88 b	88 a	91 b	82 a	82 b
	38	88 b	88 a	91 b	83 a	83 b
	LSD	4.21	3.17	2.77	3.89	5.95
1982	7	76 a	90 a	85 a	81 a	48 a
	14	94 b	90 a	92 b	87 b	72 b
	21	94 b	91 a	93 b	88 b	72 b
	38	94 b	91 a	93 b	88 b	72 b
	LSD	4.27	2.11	2.95	3.33	6.12

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Harrington (1923) and Koller (1972) cite thermodormancy is the result of seed being exposed to prolonged periods of above optimum temperature, causing a decline in percentage germination. There was a trend for the 30C,24L treatment to be lower than the rest of the temperature and light combinations. Whether this was a result of thermodormancy and, or the effect of the

continuous light treatment, is not clear. A continuous dark study at the 30C temperature would need to be conducted to determine the effect of light on bluebunch wheatgrass percentage germination.

Table 12. Comparison of percent viability with five temperature and light combination's percentage germination for three bluebunch wheatgrass seedlots.

Temperature & Light Treatments	Percentage Germination 14 Days of Germination		
	Seedlots		
	1980	1981	1982
15-25C,8L*	36 a	88 cd	94 c
20C,0L	54 d	86 c	90 bc
20C,8L	59 e	91 d	92 bc
25C,8L	47 c	82 b	87 b
30C,24L	42 b	78 a	67 a
Viability	61 e	88 cd	91 bc
LSD	4.40	3.67	5.20

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

The continuous dark treatment at 20C resulted in significantly lower percentage germination than did the 8 hrs. of light treatment, with the exception of the 1982 seedlot (Table 12). It is possible the germination that occurred during the first week with the continuous dark treatment was a result of phytochrome far-red (PFR) existing in the seed. By the end of the first week the PFR content was exhausted, and germination was not possible during the second week (Table 11). The eight hours of light treatment provided enough PFR to allow maximum germination to continue through the second week of germination. Haferkamp and McSwain (1951) have found bluebunch wheatgrass germination to be light requiring. The environment in which the seed was

developed and stored can have a significant effect on the seed's requirement for light. This may explain why the 1982 seedlot produced optimum germination with the continuous dark treatment. A seed's requirement for light is a genetic factor which can be modified by the environment. A favorable environment can eliminate the seed's light requirement, while an unfavorable environment can strongly amplify the seed's light requirement (Barton and Garman, 1946; Evenari, 1965; Koller, 1972; Justice and Bass, 1978).

The 20C,8L treatment resulted in germination percentages that were equal to viability of the seedlots, making this temperature and light treatment the optimum procedure for germinating bluebunch wheatgrass. This temperature and light treatment required 14 days to reach maximum germination. Ungerminated seeds remaining after 14 days became moldy. A common practice in seed testing laboratories is to conduct a tetrazolium test on ungerminated seeds at the conclusion of the germination period to determine if those seeds are dormant. For bluebunch wheatgrass, this test should be conducted at the end of 14 days of germination because of the mold problem which kills dormant seeds.

The requirement for prechilling of bluebunch wheatgrass seed is dependent upon the temperature and light treatment used (Table 13). At 14 days of germination the 20C,8L and 15-25C,8L treatments did not require prechill to promote maximum germination. Ten days of prechill did promote maximum germination with the other three temperature and light treatments.

Table 13. Prechill effect on percentage germination for five temperature and light combinations. Percentage germination was averaged over three bluebunch wheatgrass seedlots.

Prechill Treatment (Days)	Percentage Germination 14 Days of Germination				
	Temperature & Light Treatments				
	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
0	71 a	77 b	80 a	65 a	57 a
5	73 a	73 a	80 a	73 b	57 a
10	73 a	81 c	81 a	77 b	72 b
LSD	4.22	3.41	2.54	2.92	7.05

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

The greatest increase in percentage germination occurred with the ten day prechill treatment for the 30C continuous light treatment. It is possible that prechill delayed the onset of thermodormancy. The cold treatment plus the light treatment may have acted cumulatively to increase the endogenous gibberillic acid (GA) concentration to promote seed germination (Villiers and Wareing, 1965; Frankland and Wareing, 1962; Chen Varner, 1973). The five day prechill treatment, however, was not long enough to overcome the inhibitory effect this above optimum temperature may have caused. For the 25C,8L treatment, the five day prechill treatment increased percentage germination significantly over the control indicating that germination of bluebunch wheatgrass is not as strongly inhibited by the 25C,8L treatment as with the 30C,24L treatment. The percentage germination of the non-prechilled treatment at 20C,8L was greater than or equal to the percentage germination obtained with the 5 or 10 day prechill treatments for the other temperature and light combinations.

Seedlots varied in their requirement to prechilling when germinated at five temperature and light combinations. Only the 20C,8L and 15-25C,8L treatments consistently did not require prechilling for all seedlots (Table 14). There were other temperature and light treatments that did not require prechill, but their responses were not consistent over seedlots.

Table 14. Prechill effect on three bluebunch wheatgrass seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations.

		Percentage Germination 14 Days of Germination				
	Prechill Treatments	Temperature & Light Treatments				
Seedlots	(Days)	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1980	0	30 a	55 b	58 a	38 a	31 a
	5	38 a	43 a	58 a	47 b	41 a
	10	39 a	65 c	61 a	56 c	54 b
	LSD	10.29	8.27	7.15	5.55	10.51
1981	0	88 a	86 a	92 a	76 a	77 ab
	5	86 a	87 a	91 a	85 b	72 a
	10	89 a	87 a	92 a	86 b	86 b
	LSD	7.34	5.43	4.61	5.29	11.04
1982	0	93 a	91 a	91 a	83 a	63 a
	5	96 a	89 a	93 a	88 ab	63 a
	10	92 a	91 a	93 a	90 b	75 a
	LSD	6.50	5.14	4.32	5.68	19.81

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Prairie Sandreed

The viability of the 1978, 1980, and 1982 seedlots was 64%, 88%, and 85%, respectively. Stefferud (1948) reported an average of 75% in germinative capacity for prairie sandreed. Seed viability declines noticeably as the seed is

stored beyond five years. Reports indicate that reduced viability is a result of improper storage methods, unfavorable conditions during development and harvest, or natural ageing (Barton, 1961; Floris, 1970; Justice and Bass, 1978). Since the history of these seedlots is unknown, we cannot speculate further on what caused the viability decline for the 1978 seedlot.

Various temperature and light combinations affect time required for maximum germination. For all seedlots, germination was complete in 7 days when germinated at 25C,8L and 30C,24L treatments (Table 15). The largest germination increase between 7 and 14 days occurred with the 20C, 8L treatment.

Table 15. Percentage germination of five temperature and light combinations with time. Percentages were averaged over three prairie sandreed seedlots.

Length of Germination (Days)	Percentage Germination				
	Temperature & Light Treatments				
	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
7	66 a	63 a	52 a	75 a	67 a
14	74 b	73 b	74 b	78 a	69 a
21	76 b	74 b	74 b	78 a	69 a
38	76 b	74 b	75 b	78 a	69 a
LSD	2.51	1.85	2.63	3.23	2.38

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

The 25C,8L treatment was the only temperature and light treatment to produce a percentage germination during the first week of germination that was equal to viability of all seedlots (Table 16). After 14 days of germination, several other temperature and light treatments had germination percentages equal to the 25C,8L treatment, but the results were not consistent among

seedlots. Haferkamp and McSwain (1951) reported a fluctuating 20-30C as optimum. However, this temperature required 21 days for complete germination. These data suggest that optimum procedure for seed germination of prairie sandreed is the 25C,8L treatment with 7 days of germination.

Table 16. Comparison of percent viability with five temperature and light combination's percentage germination for three prairie sandreed seedlots.

Length of Germination (Days)	Temperature & Light Treatments	Percentage Germination		
		Seedlots		
		1978	1980	1982
7	15-25C,8L*	53 b	79 b	66 c
	20C,0L	50 b	79 b	60 b
	20C,8L	43 a	68 a	44 a
	25C,8L	62 c	85 c	79 d
	30C,24L	54 b	79 b	70 c
	Viability	64 c	88 c	85 d
	LSD	5.72	4.73	4.29
14	15-25C,8L	58 a	83 a	83 b
	20C,0L	58 a	85 b	75 a
	20C,8L	58 a	85 b	77 a
	25C,8L	63 b	85 b	83 b
	30C,24L	55 a	80 a	73 a
	Viability	64 b	88 b	85 b
	LSD	4.21	3.69	4.51

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Severe fungal activity was observed on the seeds after 14 days of germination. The fungal contamination is another reason why 7 days of germination is more optimum than 14 days. Fungal activity would not allow for tetrazolium evaluation to detect dormancy in ungerminated seeds present at the end of the germination testing period (AOSA, 1984).

The effect of an eight hrs. light treatment versus a continuous dark treatment on germination of prairie sandreed varies depending on which temperature treatment is used. At the established optimum germination temperature of 25C, the rate and percentage of seed germination are equivalent for both light and dark treatments (Table 17). However, at a below optimum temperature of 20C, a slower germination rate occurred with the light treatment. By 14 days percentage germination was equivalent between the light and dark treatments.

Table 17. Comparison of percentage germination among continuous dark and 8 hrs. light treatments when averaged over three prairie sandreed seedlots. Seeds were germinated at 25C and 20C.

Light Treatments (Hours)	Percentage Germination			
	25C		20C	
	7*	14	7	14
0	72 a	74 a	63 a	77 a
8	73 a	74 a	52 b	77 a
LSD	1.86	1.86	2.37	1.94

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* Days of germination

The prechill requirement for prairie sandreed germination appears to be dependent upon the temperature and light treatment. Only the 25C,8L and 20C,0L treatments did not have increased germination with prechill for all seedlots (Table 18). The other temperature and light treatments had a varying response among the seedlots.

Table 18. Prechill effect on three prairie sandreed seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations for 7 days.

Seedlots	Prechill Treatments (Days)	Percentage Germination 7 Days of Germination Temperature & Light Treatments 15-25C,8L	20C,0L	20C,8L	25C,8L	30C,24L
1978	0	45 a	54 a	21 a	60 a	58 a
	5	54 ab	60 a	50 b	70 a	62 ab
	10	59 b	63 a	62 c	65 a	66 b
	LSD	11.86	28.13	9.92	18.48	7.34
1980	0	70 a	62 a	50 a	67 a	83 a
	5	85 b	65 a	64 b	70 a	87 a
	10	82 b	71 a	67 b	73 a	86 a
	LSD	8.06	23.48	5.45	20.93	6.73
1982	0	63 a	60 a	63 a	62 a	74 a
	5	60 a	64 a	60 a	73 a	82 b
	10	77 b	68 a	77 b	69 a	81 ab
	LSD	9.93	19.73	9.93	23.06	7.98

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

Basin Wildrye

The viability of the 1980, 1981, and 1982 seedlots was 90%, 94%, and 72%, respectively. Stefferud (1948) reports an average germinative capacity of 83% for basin wildrye. The 1982 seedlot has a lower percentage viability than the 1980 and 1981 seedlots. This decline may be attributed to unfavorable conditions during development, harvest, or storage that resulted in faster seed deterioration (Barton and Garman, 1946; Harrington, 1972; Justice and Bass, 1978).

The 15-25C,8L, 20C,0L, and 25C,8L treatments required 14 days for maximum germination; whereas, the 20C,8L and 30C,24L treatments required 21 days (Table 19). The 30C,24L treatment gave the lowest percentage

germination. Extending the testing period did not improve germination at this temperature and light treatment.

Table 19. Percentage germination of five temperature and light combinations with time. Percentages are averaged over three basin wildrye seedlots.

Length of Germination (Days)	Percentage Germination				
	Temperature & Light Treatments				
	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
7	64 a	55 a	62 a	65 a	61 a
14	82 b	80 b	78 b	84 b	73 b
21	83 b	80 b	84 c	85 b	75 bc
38	83 b	81 b	85 c	85 b	76 c
LSD	3.68	2.24	3.29	3.68	2.16

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

The 15-25C,8L and 25C,8L treatments consistently provided the highest germination percentages for all seedlots, and these percentages were equal to seed viability (Table 20). Harrington (1923), Koller (1972) and Bewley and Black (1985) cited that higher temperatures will result in more rapid germination and greater percentages. Liebenburg (Lang, 1965) was the first to report the favorable effects of diurnal temperature fluctuation. A significant increase in germination rate and capacity was reported in seeds of most species. Vallentine (1971) cites alternating temperatures optimum for basin wildrye germination. These data suggest that 25C,8L and 15-25C,8L treatments were optimum for germination of basin wildrye.

The 15-25C,8L, 20C,0L, and 25C,8L treatments showed no response to prechilling and percentage germination was high for all treatments except 20C,0L (Table 21). The 20C,8L and 30C,24L treatments did respond to

prechilling but required at least 14 days of germination. The 30C temperature appears to be above optimum for basin wildrye germination as maximum germination was not reached even with prechill.

Table 20. Comparison of percent viability with five temperature and light combination's percentage germination for three basin wildrye seedlots.

Temperature & Light Treatments	Percentage Germination 14 Days of Germination		
	Seedlots		
	1980	1981	1982
15-25C,8L*	88 c	92 c	67 ab
20C,0L	86 c	87 b	65 a
20C,8L	81 b	87 b	65 a
25C,8L	88 c	92 c	73 b
30C,24L	75 a	81 a	63 a
Viability	90 c	94 c	72 b
LSD	4.01	3.90	6.50

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Table 21. Prechill effect on percentage germination for five temperature and light combinations. Percentages were averaged over three basin wildrye seedlots.

Prechill Treatments (Days)	Mean Percentage Germination 14 Days of Germination				
	Temperature & Light Treatments				
	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
0	82 a	78 a	70 a	85 a	64 a
5	82 a	78 a	81 b	82 a	75 b
10	84 a	82 a	83 b	85 a	79 b
LSD	6.28	4.08	6.02	4.25	4.10

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Specific evaluation of individual seedlots with each temperature and light treatment shows no prechill effect with the 15-25C,8L and 25C,8L treatments, but the prechill effect varied with the other temperature and light treatments (Table 22). The application of prechill to basin wildrye seed when germinating at 15-25C,8L or 25C,8L treatments will increase the total testing time and not the percentage germination.

Table 22. Prechill effect on three basin wildrye seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations for 14 days.

		Percentage Germination 14 Days of Germination				
	Prechill Treatments	Temperature & Light Treatments				
Seedlots	(Days)	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1980	0	90 a	85 a	68 a	87 a	57 a
	5	88 a	86 a	87 b	87 a	80 b
	10	87 a	88 a	89 b	89 a	86 b
	LSD	6.93	8.80	7.31	8.14	7.65
1981	0	94 a	84 a	82 a	89 a	78 a
	5	88 a	85 a	90 b	89 a	85 a
	10	95 a	93 b	91 b	96 a	80 a
	LSD	10.30	6.26	6.53	7.02	7.10
1982	0	78 a	65 a	60 a	78 a	58 a
	5	70 a	64 a	66 a	72 a	60 a
	10	70 a	67 a	69 a	69 a	70 b
	LSD	16.65	8.08	17.33	9.06	8.63

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Prairie Coneflower

The viability of the 1981 and 1983 seedlots was 89% and 85%, respectively. No decline in viability percentage was evident with seedlot age. Sorensen and Holden (1974) and Eddleman (1977) both reported a high viability percentage of 99%.

Eddleman (1977) reports prairie coneflower requires 14 days of germination. Based on our studies the duration of germination for prairie coneflower seedlots varied with the temperature and light combinations (Table 23). Three temperature and light treatments required seven days of germination, whereas two temperature and light treatments required 14 days to reach maximum germination.

Table 23. Percentage germination of two prairie coneflower seedlots with time when germinated at various temperature and light combinations.

		Percentage Germination				
		Temperature and Light Treatments				
Seedlots	Length of Germination (Days)	15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1981	7	88 a	90 a	88 a	88 a	49 a
	14	92 b	91 a	89 a	89 a	55 b
	21	92 b	91 a	89 a	89 a	57 b
	38	92 b	91 a	89 a	89 a	57 b
	LSD	1.65	1.76	5.20	2.33	3.68
1983	7	77 a	59 a	81 a	71 a	11 a
	14	87 b	72 b	89 a	75 a	15 a
	21	90 b	73 b	90 a	76 a	17 a
	38	90 b	73 b	90 a	76 a	19 a
	LSD	3.57	5.13	9.93	5.19	7.03

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

The 20C,8L treatment was the only temperature and light treatment that was equal to viability of both seedlots at seven days of germination (Table 24). The 15-25C,8L treatment was equal to the viability of the 1981 seedlot at seven days of germination and was equal to the 1983 seedlot after 14 days of germination. Although the 25C,8L treatment completed germination in seven days, it did not equal the viability of the 1983 seedlot. The 30C,24L treatment was an inferior treatment for germination of both seedlots. However, with seed age, the seedlot's specific requirement to light and temperature disappear so at least 50% of the seeds are able to germinate at 30C,24L.

Table 24. Comparison of percent viability with five temperature and light combination's percentage germination for two prairie coneflower seedlots.

Temperature & Light Treatments	Percentage Germination			
	Seedlots			
	7 Days of Germination		14 Days of Germination	
	1981	1983	1981	1983
15-25C,8L*	88 b	77 d	92 b	87 c
20C,0L	90 b	59 b	91 b	72 b
20C,8L	88 b	81 d	89 b	89 c
25C,8L	88 b	71 c	89 b	75 b
30C,24L	49 a	11 a	55 a	15 a
Viability	89 b	85 d	89 b	85 c
LSD	3.18	5.13	3.18	6.37

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

These data suggest that optimum germination of prairie coneflower can be achieved by germinating for 7 days at 20C,8L. Optimum germination may

also be obtained with the 15-25C,8L treatment, but 14 days is required. Eddleman (1977) reported optimum germination of prairie coneflower to occur at 20C.

The 1983 seedlot exhibited a significant reduction in percentage germination with the dark treatment as compared to the 8 hrs. light treatment at 20C (Table 24). Increasing the testing duration did not alter this effect. The 1981 seedlot showed no effect due to light or dark treatments. Sorensen and Holden (1974) and Eddleman (1977) reported optimum germination of prairie coneflower occurred in a dark environment. Evenari (1965) and Koller (1972) report that with an increase in seed age many specie's light requirements can be diminished or completely eliminated. Bewley and Black (1985) state dark germination is phytochrome controlled and their embryo contains more active phytochrome than light-requiring seeds. The 1983 seedlot's low response to the 20C,0L treatment may be a result of a germination block within the seed that can be overcome with the addition of light.

Prechilling for 5 and 10 days did not improve germination of the seedlots regardless of the temperature and light treatment used. For some temperature and light treatments, prechill lowered the percentage germination of seedlots (Table 25). Based on these data, it can be concluded that prechilling of prairie coneflower will not increase germination percentages; and if used, could result in lower than optimum germination percentages.

Table 25. Prechill effect on two prairie coneflower seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations for 7 days.

Seedlots	Prechill Treatments (Days)	Percentage Germination 7 Days of Germination Temperature and Light Treatments 15-25C,8L*	20C,0L	20C,8L	25C,8L	30C,24L
1981	0	91 a	91 a	89 a	90 a	63 b
	5	92 a	91 a	88 a	88 a	65 b
	10	93 a	90 a	91 a	89 a	39 a
	LSD	3.38	3.35	10.20	4.63	6.57
1983	0	87 b	70 a	86 a	80 b	17 a
	5	78 a	69 a	84 a	66 a	11 a
	10	89 b	63 a	90 a	71 ab	17 a
	LSD	6.26	8.90	20.07	9.67	12.33

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Alkali Sacaton

Seedlot viability, as determined by TZ, was 78% for the 1981JK seedlot and 70% for the 1981NK seedlot. Previous studies (Stefferud, 1948; Hafenrichter et al., 1968) have found that alkali sacaton seed, four years old or less, contained approximately 80% viable seed. Knipe (1969) indicated that alkali sacaton remains viable under good storage for over four years. Seedlots used in this study were four years old. Viability of these seedlots were lower than that encountered with Hafenrichter et al. (1968) and Stefferud (1948).

Germination of alkali sacaton was rapid. Seeds in four of the temperature and light treatments had completed germination within seven days (Table 26). Only the 20C,8L treatment required 14 days and its total germination

percentage was the lowest. Wasser (1982) also found complete germination to occur in 5 to 10 days. Toole (1941) had complete germination in a minimum of 21 days. Our results showed extreme seed decay due to fungi after 14 days.

Table 26. Percentage germination of five temperature and light combinations with time. Percentages are averaged over two alkali sacaton seedlots.

Length of Germination (Days)	Percentage Germination				
	Temperature and Light Treatments				
	15-25C,8L*	20C,0L	20C,8L	30C,24L	25C,8L
7	71 a	40 a	4 a	58 a	66 a
14	74 a	42 a	13 b	61 a	66 a
21	74 a	42 a	14 b	61 a	66 a
28	74 a	42 a	14 b	62 a	66 a
38	74 a	42 a	14 b	62 a	66 a
LSD	3.49	3.35	2.33	6.23	4.02

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

*8L; hours of light

The 15-25C,8L treatment resulted in maximum germination for both alkali sacaton seedlots (Table 27). The 25C,8L treatment also gave maximum germination for the 1981NK seedlot. Low percentage germination occurred with both light and continuous dark treatments at 20C, with the light treatment being significantly lower in percentage germination than the continuous dark treatment (Table 27). Wilson (1931) and Knipe (1967) reported significantly higher germination percentages with 20-30C or 30-40C temperature than with 15-25C temperature. These two temperatures were not included in this study because the percentages obtained with the 15-25C treatment were equal to the viability of both seedlots.

Table 27. Comparisons of percent viability with five temperature and light combination's percentage germination for two alkali sacaton seedlots.

Temperature and Light Treatments	Percentage Germination 7 Days of Germination	
	Seedlots	
	1981NK	1981JK
15-25C,8L*	67 d	75 e
20C,0L	28 b	52 b
20C,8L	3 a	5 a
25C,8L	67 d	66 d
30C,24L	56 c	60 c
Viability	70 d	78 e
LSD	7.82	5.27

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Knipe (1967) showed that percentage germination was lower with the 20C and 25C temperatures than with the 30C temperature. Our data for the 20C temperature, with both light and dark conditions, agrees with Knipe (Table 27). Percentage germination of both light and dark treatments at 20C was significantly lower than the percentage germination at 30C. The 25C,8L treatment, however, showed a significant increase in percentage germination as compared to the 30C,24L treatment. The lower germination of the 30C,24L treatment could be attributed to the inhibitory action imposed by the continuous light. Percentage germination of alkali sacaton was found to decrease significantly if seeds were exposed to light for 12 hrs. or longer (Knipe, 1971).

At the below optimum temperature of 20C, the presence of light inhibits both rate (Table 26) and percent germination (Table 28). The low constant temperature, plus the presence of light, may be the reason for low percentage

germination. Apparently, 8 hrs. light treatment is more inhibitory on germination of alkali sacaton at 20C than at 15-25C. The 1981JK seedlot exhibited a greater germination capacity than did the 1981NK seedlot.

Table 28. Comparison of percentage germination among continuous dark and 8 hrs. light treatments for two alkali sacaton seedlots. Seeds were germinated at 15-25C and 20C for 7 days.

	Percentage Germination 7 Days of Germination			
	15-25C		20C	
Light Treatments	1981NK	1981JK	1981NK	1981JK
0 hrs	66 a	77 a	28 b	52 b
8 hrs	65 a	74 a	3 a	5 a
LSD	2.83	4.06	7.82	5.27

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

Comparisons were made between seeds germinated in 8 hrs. of light and continuous dark for 7 days at 15-25C (Table 28). Germination at the optimum temperature of 15-25C showed no significant difference in percentage germination between the light and dark conditions. Seeds that had germinated in the dark germinated faster because the length of the epicotyls from the dark treatment were approximately 3.75 cm as compared to 1.75 cm length for the light treatment.

Prechill did not increase alkali sacaton percentage germination except for the 20C,0L treatment (Table 29). Five days of prechill enhanced the germination of this treatment for both seedlots, but ten days of prechill significantly reduced the germination of the 1981NK seedlot. Prechill did not increase germination of the 20C,8L treatment. This may be due to a strong

inhibitory effect with light on alkali sacaton germination. Dormancy in alkali sacaton seed is uncommon (Wilson, 1931; Toole, 1941; Wasser, 1982) and germination is normally rapid; therefore, prechill would not be expected to improve germination.

Table 29. Prechill effect on two alkali sacaton seedlot's percentage germination. Seedlots were germinated at five temperature and light combinations for 7 days.

		Percent Germination 7 Days of Germination				
	Prechill Treatments	Temperature and Light Treatments				
Seedlots	(Days)	15-25C,8L*	20C,0L	20C,8L	30C,24L	25C,8L
1981NK	0	68 a	29 b	1 a	57 a	63 a
	5	69 a	41 c	4 a	49 a	70 a
	10	65 a	14 a	6 a	63 a	67 a
	LSD	11.45	9.70	5.64	28.24	7.81
1981JK	0	79 b	45 a	4 a	53 a	62 a
	5	65 a	57 b	4 a	66 a	71 a
	10	80 b	52 ab	6 a	62 a	65 a
	LSD	10.06	7.31	8.15	21.10	13.64

Means in columns followed by letters in common are not significantly different at the 5% level of probability.

* 8L; hours of light

Summary

Rarely do seed testing laboratories know the age and environmental conditions of a seedlot. When germinating seeds it is important that the procedure used does not discriminate among the diversity that can occur between seed of the same species. When choosing the optimum germination procedure for a species, these diversities need to be considered. Maximum germination needs to be attained in the shortest possible time. No unnecessary steps should be used which would increase the time in preparation or

germination. The most important consideration is that the samples viability must be expressed in the percentage germination. The procedures that meet these criteria for the six native range species are summarized.

Yarrow. Optimum germination of yarrow occured when seeds were germinated for 14 days at 20C with 8 hrs. of light. Viability, as determined by TZ, and the percentage germination were equal for both seedlots. Neither seedlot required prechilling to attain maximum germination.

Bluebunch Wheatgrass. Optimum germination of bluebunch wheatgrass seedlots was obtained by germinating at 20C with 8 hrs. of light. Prechilling was unnecessary and if applied would increase the duration of the test period. Bluebunch wheatgrass seed should be germinated for 14 days. Ungerminated seeds should be checked for dormancy with TZ. Exposing the seeds to germination for longer than 14 days resulted in increased fungi activity and eventual death of viable seeds. Percentage viability of all seedlots was obtained with this germination procedure.

Prairie Sandreed. Optimum germination for three prairie sandreed seedlots was attained by germinating at 25C for 7 days in 8 hrs. light or continuous dark conditions. Prechilling, prior to germination, was not necessary. Percentage germination by this procedure was equal to TZ viability percentages.

Basin Wildrye. Basin wildrye will germinate over a wide range of temperature and light combinations. Some of these treatments provide faster germination than others. The most rapid procedure for determining germination was the 15-25C,8L and the 25C,8L treatments. Seeds need to be

germinated for 14 days. Prechill did not improve germination at these temperatures. Percentage germination of seedlots was equal to percentage viability. A TZ test may be conducted to detect possible dormancy if firm ungerminated seeds remain at the end of the testing period.

Prairie Coneflower. Rapid and maximum germination occured when prairie coneflower seeds were germinated for seven days at 20C with 8 hrs. of light. Maximum germination was also attained with the 15-25C,8L treatment, but 14 days of germination was required. Both of these temperature and light treatments provide germination percentages equal to the percentage viability of the seedlots. Prechilling appeared to stress the seeds which resulted in lower percentage germination.

Alkali Sacaton. Maximum germination of alkali sacaton was obtained when seeds were germinated for seven days at 15-25C with 8 hrs. of light or in continuous dark. Prechilling is not necessary at this temperature to obtain optimal germination. This procedure reflects the maximum viability of the alkali sacaton seedlots.

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