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
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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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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.

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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.